

Aggression in imported fire ants: an explanation for shifts in their spatial distributions in Southern United States?

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Abstract. 1. The imported fire ants, *Solenopsis invicta* Buren (red), *S. richteri* Forel (black), and their hybrids (*S. invicta* × *S. richteri*) are sympatric congeners with overlapping but shifting spatial distributions in northern Alabama and Mississippi, United States.

2. The abilities of workers of the three ant forms (or species) to recognise non-nestmate conspecifics and heterospecifics were compared in separate aggression bioassays using three group sizes of competitor numbers (battles): 1-1, 5-1, and 5-5 resident–intruder battles.

3. Workers of all three forms showed significant aggression toward non-nestmate conspecifics and heterospecifics. The results of the intra-specific aggression bioassays showed that *S. invicta* had the lowest aggression threshold, whereas *S. richteri* was the least aggressive. Survival rates 1 day after intra-specific encounters were higher for *S. richteri* than for *S. invicta* or hybrids, consistent with its lower aggressiveness.

4. In inter-specific interactions, *S. invicta* workers showed the greatest aggression and were more aggressive towards *S. richteri* than to hybrids. Furthermore, survival 1 day after inter-specific encounters was highest for *S. invicta* workers, whether they were the intruders or residents. The hybrid form was intermediate between both parental forms in aggression and post-aggression survival.

5. The live 1-1 and 5-5 battles produced similar and consistent results, but differences were amplified in the 5-5 battles, suggesting an effect of group size on aggression. These results may explain the observed shifts in the spatial distributions of the three forms and range restriction of *S. richteri* in southern United States.

Key words. aggression, imported fire ants, nestmate recognition, *Solenopsis invicta*, *Solenopsis richteri*, hybrids.

Introduction

Ants and other social insects are known to exhibit aggressive behaviour towards conspecifics from different colonies (intra-specific aggression) and heterospecifics (inter-specific aggression) (Wilson, 1971; Hölldobler & Michener, 1980). Aggressive behaviour in social insects is regarded as an adaptation for nestmate recognition and protection of the colony against threats

and intrusion, such as robbery, predation, parasitism, and competition (Wilson, 1971).

A proposed mechanism for nestmate recognition in social insects is phenotype matching (or template-label matching), which involves matching the label of an encountered conspecific or heterospecific to the individual's inner template acquired through the learning of chemical cues (Sherman & Holmes, 1985; Obin & Vander Meer, 1989a; Reeve, 1989; Liang & Silverman, 2000; Ozaki *et al.*, 2005), which may be heritable or environmentally acquired (Hölldobler & Michener, 1980; Obin, 1986). Phenotype matching consists of three components: expression, perception, and action (Sherman & Holmes, 1985; Reeve, 1989). Nestmate recognition can vary with several

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factors including colony size, queen number (monogyny versus polygyny), and stage (Stuart, 1991). Thus, the action component of nestmate recognition is context dependent and plastic (Reeve, 1989).

The ability of social insects to discriminate nestmates from alien conspecifics is probably mediated by chemicals on the cuticle, which are shared by members of the same colonies (nestmates) but vary between colonies and species (Wilson, 1971; Hölldobler & Michener, 1980; Vander Meer, 1988; Howard, 1992). Several studies have suggested the involvement of cuticular hydrocarbons in nestmate recognition in ants (Provost *et al.*, 1994; Lenoir *et al.*, 1999). This proposition is supported by a growing number of studies which showed direct evidence for cuticular hydrocarbons as nestmate recognition cues in many ant species (Lahav *et al.*, 1999; Liang & Silverman, 2000; Wagner *et al.*, 2000; Akino *et al.*, 2004; Ozaki *et al.*, 2005; Vasquez *et al.*, 2008). In contrast, Obin (1986) reported that the cuticular hydrocarbon profile was a poor predictor of aggressive behaviour in a laboratory colony of red imported fire ants. Furthermore, two recent studies have also implicated the involvement of volatile chemical cues (non-cuticular hydrocarbons) that exist in the nest in ant nestmate recognition (Errard *et al.*, 2008; Katzav-Gozansky *et al.*, 2008). Thus, the exact nature and identity of the chemicals which mediate nestmate discrimination in ants remains controversial.

Nestmate recognition and aggression towards non-nestmates and heterospecifics can be used as a measure of competitive ability and fitness among sympatric congeneric species/forms (Hölldobler, 1983; Obin & Vander Meer, 1989b). In inter-specific encounters, species with the greater aggression scores are usually predicted to have a competitive advantage (Obin & Vander Meer, 1989b; Tsutsui *et al.*, 2003).

Two species of imported fire ants, *Solenopsis* spp. (Hymenoptera: Formicidae) were accidentally introduced from South America into southern United States (U.S.) in the past century. The black imported fire ant, *S. richteri* Forel was introduced in the 1910s followed by the introduction of the red imported fire ant, *S. invicta* Buren in the early 1930s. Both species are believed to have entered the U.S. through the port of Mobile, Alabama, spreading northward in Alabama and into neighbouring states (Wilson, 1958). While *S. invicta* is now widely distributed throughout southeastern U.S. (Callcott & Collins, 1996; Porter, 2000), *S. richteri* is restricted in distribution to a relatively small area around the northern border of Alabama, Mississippi, and southern Tennessee (Obin & Vander Meer, 1989b; Streett *et al.*, 2006). Hybridisation between *S. invicta* and *S. richteri* has been documented in Alabama, Mississippi, Georgia, and Tennessee (Vander Meer *et al.*, 1985; Ross *et al.*, 1987a, b; Diffie *et al.*, 1988; Vander Meer & Lofgren, 1988; Shoemaker *et al.*, 1996). However, hybrid imported fire ants are known to occur parapatrically with both parent types only in the Mississippi hybrid zone, which consists of areas in northern Mississippi and Alabama (Vander Meer & Lofgren, 1988; Streett *et al.*, 2006). The restricted range of *S. richteri* has been attributed to displacement by *S. invicta*, as well as the temporal stability and continued range expansion of the hybrid form (Tschinkel, 1993). In northern Alabama and Mississippi where the three types of imported fire ants (referred to as 'forms' in this paper) occur

sympatrically, population decrease and range restriction of *S. richteri* is being observed, whereas population increases and range expansion are recorded for *S. invicta* and hybrids (Streett *et al.*, 2006).

Several factors may account for the observed population dynamics and shifts in spatial distributions of the three forms of imported fire ants in southern U.S., including genetic, ecological, and other factors. For instance, it is possible that hybrids simply preferentially backcross with *S. richteri* (rather than with *S. invicta*), thus leading to an erosion of pure *S. richteri* populations. However, inter-specific competition through exploitative competition and/or interference mechanisms, such as inter-specific aggression, is the most probable factor (Wilson, 1971; Holway & Suarez, 1999; Calcaterra *et al.*, 2008). Several authors have attributed the ecological dominance of *S. invicta* in southern U.S. to its superior competitive ability (Jones & Phillips, 1987; Tschinkel, 2006). However, the exact mechanisms which mediate its competitive advantage remain unclear. In southern U.S., *S. invicta* colonies occur in two social forms, monogyne (single queen) and polygyne (multiple queen) colonies (Ross *et al.*, 1996; Tschinkel, 1998). Polygyne colonies of *S. invicta* typically show reduced nestmate discrimination compared with monogyne colonies, possibly as a result of increased queen number and population density (Vander Meer *et al.*, 1991; Holway & Suarez, 1999). Thus, the imported fire ant complex in southern U.S. presents a good model system for investigating the mechanisms of inter-specific competition, in particular the connection between aggression (nestmate discrimination) and competitive superiority.

In perhaps the only published study of nestmate discrimination among the three imported fire ant forms, Obin and Vander Meer (1989b) reported no significant differences in the capabilities of *S. invicta*, *S. richteri*, and their hybrids to display aggression towards heterospecific competitors. However, our observations of nestmate discrimination in laboratory populations and recent shifts in the distributions of the three forms in southern U.S. suggest the possibility of differences in their aggressive behaviours. Thus, the present study was conducted to re-investigate nestmate discrimination and compare aggression of the three imported fire ant forms towards conspecifics and heterospecifics. The main aim was to investigate whether or not the observed shifts in the populations and spatial distributions of the three forms can be explained by aggression. Based on the range expansion and wider distribution of *S. invicta* and hybrids relative to *S. richteri*, we hypothesised that *S. invicta* and hybrids will demonstrate a lower aggression threshold than *S. richteri*.

Methods

Sources and maintenance of ant colonies

Three colonies of *S. invicta*, and six colonies each of *S. richteri* and hybrid (*S. invicta* × *S. richteri*) ants were tested in this study. Colonies of *S. richteri* and hybrid fire ants were collected in northern Alabama and western Tennessee in spring 2008. Colonies of *S. invicta* were collected in Auburn, Alabama, approximately 200–300 miles (322–483 km) from *S. richteri* and

hybrid collection sites. All colonies were considered monogyne based on colony size, worker size, and presence of only one queen during collection (Morel *et al.*, 1990). Ant workers were collected by transferring approximately 1 litre of soil (containing workers, broods and the queen) from each mound into 1-gallon (3.785-litre) Rubbermaid plastic jars coated with Fluon® (ICI, Wilmington, DE) to prevent escape. Workers collected from each colony (mound) ranged from 1,000 to 5,000 and were maintained in their nest soil in the laboratory at 25 ± 1 °C, $50 \pm 10\%$ relative humidity, on a LD 14:10 h cycle and fed 10% sugar water and crickets. The colonies were maintained for 1–2 months after collection before the tests. Major workers from each colony were used for aggression tests, as they are the workers likely to be involved in foraging and colony defence (Moffett, 1985). The average weight of the major workers of *S. invicta*, hybrid, and *S. richteri* used for the tests were 212.9 ± 4.7 , 212.6 ± 5.7 , and 210.3 ± 6.1 mg per 50 workers (five replicates per form), respectively.

Species (form) identification by cuticular hydrocarbon profiling

Colonies were separated and identified as *S. invicta*, *S. richteri*, and hybrid by analysis of worker venom alkaloids and cuticular hydrocarbons profiles using gas chromatography (GC). This is the standard method for identification of *Solenopsis* fire ants (Vander Meer *et al.*, 1985; Ross *et al.*, 1987a, b). Briefly, approximately 50 workers from each colony were soaked in hexane for 24 h to extract their chemical compounds. GC analyses were performed on a Shimadzu GC17A equipped with a Rtx®-1MS column (30 m \times 0.25 mm i.d., 0.25 μ m, Restek, Bellefonte, PA). The oven was programmed at 15 °C/min from 90 °C to 270 °C with a 2-min initial time and a 16-min final holding time.

Aggression bioassay protocols

The bioassay used in this study measured aggression by nested (resident) worker ants against non-nestmate intruders of the same (intra-specific aggression) or different (inter-specific aggression) species/forms. The bioassay consisted of a white plastic cup (500 ml) coated inside with Fluon to prevent ant escape. Worker ants were individually placed in the bottom of the cup with a soft forcep. The workers which were first placed in the cup and allowed to acclimate for 30 min were considered resident ants, whereas those placed secondarily in the cup were regarded as intruders. Intruder workers from different colonies of the same form (intra-specific aggression) or a different form (inter-specific aggression) were placed in an empty space in the bottom of the cup.

To differentiate between intruders and resident ants, intruder workers were marked by placing a small dot of non-toxic white acrylic paint on their abdomen (van Wilgenburg *et al.*, 2007). Intruder ants were first chilled in a container at 4 °C for 10 min to reduce activity, marked, and allowed to acclimate to ambient conditions for 30 min before they were introduced with a soft forcep into the cup containing resident ants. No effect of mark-

ing or chilling was observed on the behaviour and survival of ant workers in this study (see results of intra-specific aggression tests). The reaction of resident ants to intruders (and vice versa) was observed for 10 min. The ants were then kept together in the same cup for 24 h at the end of which the survival of both intruder and resident ants was recorded.

Aggressive responses of resident ants to intruders were scored on a scale of 0–4 similar to Suarez *et al.* (1999) and Tsutsui *et al.* (2000) in which 0 = ignore (contacts between resident and intruder ants in which neither showed any interest, i.e. no antennation or aggression), 1 = touch (contacts with antennation), 2 = avoid (contacts that resulted in intruder and/or resident ants retreating in opposite directions), 3 = aggression (including lunging, and brief bouts of biting or pulling), and 4 = fighting (prolonged aggression, and often consisted of one or both opposing ants locking their mandibles onto a body part of the other, carrying the other with its mandibles, or grappling). Approximately 1 h after the test, a 1-cm³ sugar-agar block (10% sugar water + 1% agar after boiling and chill) was placed in the centre of the bottom of the cup to allow feeding. The survival of the ants was recorded 24 h after the test. The above protocol was used to test for intra-specific and inter-specific aggression in colonies of red, black, and hybrid fire ants.

Intra-specific aggression

A pilot test was first conducted which showed that workers of the three ant forms displayed no aggression towards nestmates. The results of this pilot test also confirmed very high survival rates (>98% survival after 24 h) for workers which did not engage in aggression. For non-nestmate intra-specific aggression tests, three colonies of *S. invicta* were tested in six different pairwise combinations of residents and intruders (10 replicates per pairwise combination \times 6 combinations for a total of 60 replicates). For *S. richteri* and hybrid ants, six colonies of each were tested in six different pairwise combinations of residents and intruders (10 replicates per pairwise combination \times 6 combinations for a total of 60 replicates). Based on a recent study which reported an effect of group size on aggression in ants (Tanner, 2006), three group sizes of competitor numbers (battles) were tested in all aggression bioassays: 1-1, 5-1, and 5-5 resident–intruder battles.

For treatments using multiple individuals as residents, the response of all residents ($n=5$) within each cup (replicate or set) was averaged and used to score aggression for that set. Similarly, the response of all intruders in the 5-5 battles was averaged. Since preliminary data analysis showed no significant effect of colony on aggression, mean aggression score for each form was calculated by pooling the data for the six pairwise (inter-colony) combinations ($n=60$ replicates). Data from this and the second experiment (below) were not normally distributed and transformations did not adequately correct this anomaly. Thus, these data were analysed using non-parametric statistics. Data for each resident–intruder battles were first analysed separately and significant differences in intra-specific aggression among the three forms were established using the Kruskal–Wallis test ($P < 0.05$; JMP® 7.0.1, SAS Institute, 2007). In this and other data analysed with the Kruskal–Wallis test, designations of significantly

different pairs of treatment means were made based on consensus between the Tukey–Kramer HSD (honestly significant difference) parametric test and a simultaneous test using the non-parametric Wilcoxon signed-rank test for each paired comparison, similar to the procedure used by Obin and Vander Meer (1989b). Only the treatment pairs shown to be significant in both tests were designated significant in the results section. For each ant form, aggression scores were also compared among the three resident–intruder battles using the same test. Survival data were generated for resident and intruder ants only for experiments using the 1-1 and 5-5 resident–intruder battles, because both battles have been reported to produce consistent results (Roulston *et al.*, 2003). Percentage survival for the 5-5 battles was calculated by averaging the mortalities of individual ants in the cups. Survival data for residents or intruders were compared among the three forms using the Kruskal–Wallis test ($P < 0.05$). Significant differences between the survival of resident versus intruder workers in each battle were established using the Matched pairs test ($P < 0.05$; JMP® 7.0.1, SAS Institute, 2007).

Inter-specific aggression

For inter-specific aggression tests, the three ant forms were tested in all pairwise combinations of residents and intruders at the resident–intruder battles of 1-1, 5-1, and 5-5 (six pairwise combinations for each battles) using the procedure described above for intra-specific aggression. Each pairwise combination treatment (set) was replicated 10 times using ant workers from the same colony. The experiment was repeated three times (three sets) each time with a different colony of each ant form (i.e. 10 replicates per colony per pairwise combination \times 3 colonies for a total of 30 replicates). Analysis of the data by colony showed no significant colony effect. Thus, data obtained for the different colonies (3 per form) were pooled for further analysis. Mean aggression scores for the different battles were calculated for each form by averaging the scores for the 30 replicates. Differences in inter-specific aggression among the six pairwise combinations were established for each battle using the Kruskal–Wallis test ($P < 0.05$). As an additional measure of aggressiveness, the percentage of attacks initiated by intruder ants (at the 1-1 battles) was calculated and compared between the two pairwise combinations of the same two ant forms using the Wilcoxon signed-rank test ($P < 0.05$; JMP® 7.0.1, SAS Institute, 2007). As in the intra-specific aggression test, survival data were generated for resident and intruder ants only for the experiments using 1-1 and 5-5 battles. Significant differences between the survival of residents versus intruders in each pairwise combination of the different forms were established using the Matched pairs test ($P < 0.05$; JMP® 7.0.1, SAS Institute, 2007).

Results

Species (form) identification by cuticular hydrocarbon profiling

Gas chromatographic traces of whole body extracts of the three ant forms showing their typical alkaloid and cuticular hydrocarbon profiles are shown in Fig. 1. Note that the hybrid pro-

file is intermediate between its parental forms. These profiles are consistent with published profiles for the three forms (Vander Meer, 1986), and have been used by various authors as a taxonomic character for identification of *Solenopsis* fire ants (Vander Meer *et al.*, 1985; Ross *et al.*, 1987a, b). No notable differences were detected in the cuticular hydrocarbon profiles of conspecific fire ants from different colonies, suggesting that this character is conserved within each form.

Intra-specific aggression

Solenopsis invicta, *S. richteri* and their hybrids showed no aggression towards nestmates, but did display considerable aggression towards non-nestmate conspecifics. Significant differences were recorded among the three forms in the level of intra-specific (non-nestmate) aggression (Fig. 2). Intra-specific aggression ratings for *S. invicta* were significantly greater than ratings for hybrids and *S. richteri* at the 1-1 ($\chi^2_2 = 57.21$, $P < 0.0001$) and 5-5 ($\chi^2_2 = 80.72$, $P < 0.0001$) battles. At the 5-1 battles, intra-specific aggression was highest for hybrid ants followed by *S. invicta* ($\chi^2_2 = 54.48$, $P < 0.0001$). In all cases, the lowest intra-specific aggression level was recorded for *S. richteri* (Fig. 2). No significant effect of group size (battles) was recorded for *S. invicta* ($\chi^2_2 = 0.44$, $P = 0.80$). In contrast, significantly higher aggression scores were recorded for *S. richteri* at the 5-1 and 5-5 battles compared with the 1-1 battles ($\chi^2_2 = 19.16$, $P < 0.0001$), possibly suggesting an effect of group size on aggression. Similarly, higher aggressive responses ($\chi^2_2 = 40.44$, $P < 0.0001$) were recorded for hybrids at the 5-1 than at the 1-1 and 5-5 battles (Fig. 2).

In order to determine the impact of intra-specific aggression on survival of the three ant forms, the percentage of resident or intruder ants that were alive 24 h after aggression tests were compared among the three forms at the 1-1 and 5-5 battles. The percentages of alive resident ants were significantly higher for *S. richteri* than for *S. invicta* and hybrids at both the 1-1 ($\chi^2_2 = 23.19$, $P < 0.0001$) and 5-5 ($\chi^2_2 = 52.57$, $P < 0.0001$) battles (Fig. 3). Similar results were recorded among intruder ants of the three forms: percentage of intruder ants alive after 24 h was significantly greater for *S. richteri* at both battles (1-1 battles: $\chi^2_2 = 50.91$, $P < 0.0001$; 5-5 battles: $\chi^2_2 = 56.63$, $P < 0.0001$) (Fig. 3). These results showing that *S. richteri* suffered the least mortality 24 h after intra-specific encounters, are not surprising given the comparatively lower intra-specific aggression ratings recorded for this form. No significant effect of group size (battles) was recorded on the survival of *S. invicta* and hybrids. However, lower survival rates were recorded for *S. richteri* at the 5-5 compared with the 1-1 battles (Fig. 3). In general, no significant differences were recorded in the survival of resident versus intruder workers at the 1-1 and 5-5 battles. However, the survival of resident workers was greater than that of intruders at the 5-1 battles, which was not surprising.

Inter-specific aggression

Significant differences were recorded in inter-specific aggression ratings among the six pairwise combinations of *S. invicta*

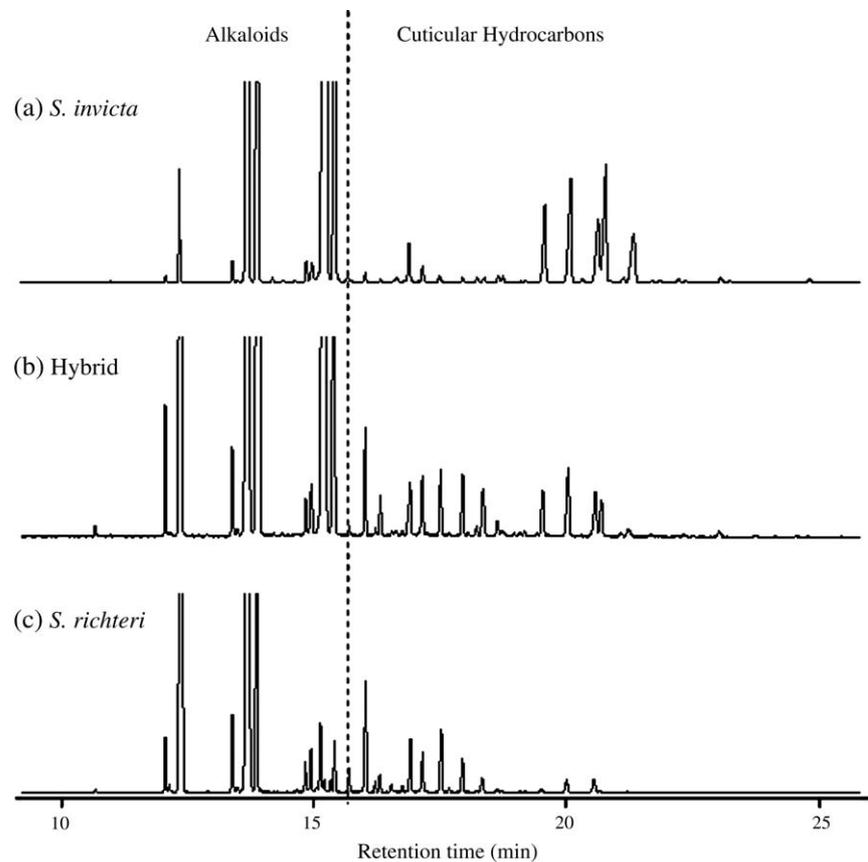


Fig. 1. Gas chromatographs of whole body extracts of imported fire ant workers showing their alkaloid and cuticular hydrocarbon profiles: (a) *Solenopsis invicta*, (b) Hybrid (*S. invicta* × *S. richteri*), and (c) *S. richteri*.

(R for red, *S. richteri* (B for black), and hybrid (H) ants at the 5-5 battles ($\chi^2_5 = 84.92$, $P < 0.0001$). The highest inter-specific aggression ratings were recorded for the two pairwise combinations of *S. invicta* and *S. richteri* (R-B and B-R pairwise combinations), suggesting intense competition between both forms (Fig. 4b). In contrast, the least inter-specific aggression ratings were recorded for the two pairwise combinations of *S. richteri* and hybrids (B-H and H-B pairwise combinations) (Fig. 4b). However, these differences were not significant at the 1-1 battles ($\chi^2_5 = 0.37$, $P = 0.99$; Fig. 4a). Further analysis of the aggression data for the 1-1 battles by comparing the percentage of attacks initiated by intruder ants between the two pairwise combinations of the same two ant forms, revealed some important differences (Fig. 5). For the pairwise combinations of *S. invicta* and *S. richteri* (R-B versus B-R), the percentage of attacks initiated by intruder ants was significantly higher when *S. invicta* workers were the intruders than when *S. richteri* were the intruders ($\chi^2_1 = 3.97$, $P = 0.04$; Fig. 5). However, no such differences were recorded in the interactions between *S. invicta* and hybrids (R-H versus H-R) ($\chi^2_1 = 0.43$, $P = 0.51$; Fig. 5) or between *S. richteri* and hybrids (B-H versus H-B) ($\chi^2_1 = 0.006$, $P = 0.94$; Fig. 5). These results further confirmed that *S. invicta* workers were generally more aggressive towards *S. richteri* workers than they were to hybrid workers. In general, the levels of inter-specific aggression recorded among the three forms were similar to their levels of intra-specific aggression.

Significant differences in the post-aggression survival of residents versus intruders were recorded in the pairwise combinations of *S. invicta* versus hybrids, and *S. richteri* versus hybrids at the 1-1 and 5-5 battles (Fig. 6a,b). In the interactions between *S. invicta* and hybrid ants, significantly lower survivals were recorded for hybrid ants, irrespective of whether they were the residents (at 1-1 battles: $t = 3.61$, $P = 0.001$; at 5-5 battles: $t = 4.58$, $P < 0.0001$) or the intruders (at 1-1 battles: $t = 3.01$, $P = 0.004$; at 5-5 battles: $t = 8.97$, $P < 0.0001$) (Fig. 6a,b). Significant differences in survival of residents versus intruders were also recorded in the 5-5 interactions between *S. invicta* versus *S. richteri*. When *S. invicta* was the resident, survival of *S. richteri* intruders was significantly lower than survival of *S. invicta* residents (Fig. 6b).

Discussion

The results of the intra-specific and inter-specific aggression bioassays showed that *S. invicta* was the most aggressive of the three forms, whereas *S. richteri* was the least aggressive. Workers of *S. invicta* were not only highly aggressive towards their congeners, but also displayed high aggressive responses towards non-nestmate conspecifics, suggesting a low aggression threshold. These results were confirmed by our data showing significantly lower post-intra-specific aggression mortality

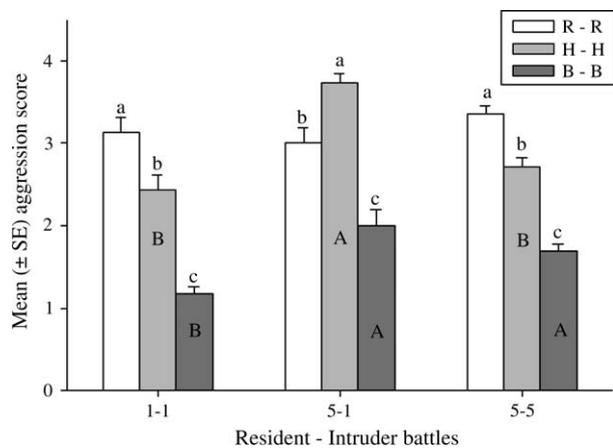


Fig. 2. Aggression of *Solenopsis invicta* (red), *S. richteri* (black) and hybrid imported fire ants towards non-nestmate conspecifics at 1-1, 5-1, and 5-5 resident-intruder battles. For this and Fig. 3, R - R = intra-specific encounters between red workers; H - H = intra-specific encounters between hybrid workers; and B - B = intra-specific encounters between black workers. Small letters indicate significant differences among the three ant forms at each resident-intruder battle (Kruskal-Wallis test, $P < 0.05$, $n = 60$). Capital letters indicate significant differences among the three battles for each ant form (Kruskal-Wallis test, $P < 0.05$, $n = 60$).

for *S. richteri* than for *S. invicta* or hybrids: lower aggression resulted in higher survival. Data from the inter-specific aggression tests showed intense aggression between *S. invicta* and *S. richteri* with most of the attacks initiated by the former, irrespective of which form the intruder was, and may suggest that *S. invicta* workers are generally more aggressive toward *S. richteri* workers than they are to hybrid workers. Alternatively, *S. richteri* workers may have a greater tendency than hybrids to escalate interactions with *S. invicta*. The data also showed that in the interactions between *S. invicta* and *S. richteri* or hybrids, *S. invicta* workers suffered the least mortality as intruders or residents.

Our results are consistent with those reported by Jones and Phillips (1987). The authors compared the aggressive behaviour of *S. invicta* and three native ant species and reported that *S. invicta* was more aggressive than two of the native species. Similarly, *S. invicta* also showed greater aggressive interactions than the Argentine ant, *Linepithema humile* (Mayr) (Kabashima *et al.*, 2007). However, our results are not consistent with those from an earlier study by Obin and Vander Meer (1989b) in which no differences were detected among *S. invicta*, *S. richteri* and their hybrids in aggression towards heterospecific intruders. The differences between our data and those of Obin and Vander Meer (1989b) may be as a result of several factors including differences in ant populations and handling protocols. For instance, the ant colonies tested by Obin and Vander Meer (1989b) were obtained from variable sources: *S. invicta* workers were reared from newly mated queens, whereas workers of *S. richteri* and the hybrid were field collected. Second, the colonies had been maintained in the laboratory 1-2 years before the tests. In contrast, monogyne colonies of the three forms collected around

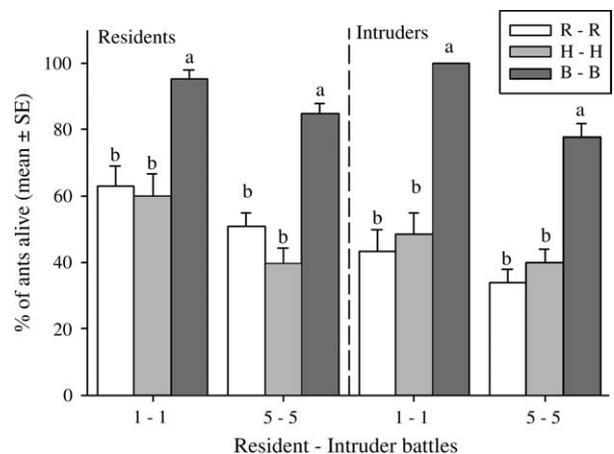


Fig. 3. Percentage of resident and intruder workers of *Solenopsis invicta* (red), *S. richteri* (black) and hybrid imported fire ants alive 24 h after intra-specific aggression encounters at 1-1 and 5-5 resident-intruder battles. Keys (see Fig. 1 for detailed keys): R = red; B = black; H = hybrid. Letters indicate significant differences among the three ant forms at each battle (Kruskal-Wallis test, $P < 0.05$, $n = 60$).

the same time and held in the laboratory for a similar length of time before testing, were tested in the present study.

Many authors have reported on the use of cuticular hydrocarbons as nestmate recognition cues by ants (Provost *et al.*, 1994; Lahav *et al.*, 1999; Lenoir *et al.*, 1999; Liang & Silverman, 2000; Wagner *et al.*, 2000; Akino *et al.*, 2004; Ozaki *et al.*, 2005; Vasquez *et al.*, 2008). While this study was not designed to identify nestmate recognition cues, our data suggest that cuticular hydrocarbons are unlikely to be the sole or main nestmate recognition cue in imported fire ants. This supposition is based on our data which showed that all three fire ant forms displayed no aggression towards nestmates, but high aggression towards non-nestmate conspecifics. However, we did not detect significant variations in the cuticular hydrocarbon profiles of conspecific fire ants from different colonies, a finding in agreement with a previous report that cuticular hydrocarbon profile was a poor predictor of aggressive behaviour in a laboratory colony of *S. invicta* (Obin, 1986). Nevertheless, these results must be interpreted with caution in the light of a few recent papers, which suggest that nestmate discrimination in ants may be mediated by even subtle differences in the ratios of saturated and unsaturated components of their cuticular hydrocarbons (Akino *et al.*, 2004; Vasquez *et al.*, 2008). It is plausible that nestmate recognition in imported fire ants is mediated (either solely or in combination with cuticular hydrocarbons) by non-cuticular hydrocarbon contact chemicals possibly from the nest, which are shared by nestmates, but which vary between colonies and species (Wilson, 1971; Hölldobler & Michener, 1980; Vander Meer, 1988; Howard, 1992). Further studies are necessary to determine the cues mediating nestmate discrimination in imported fire ants.

In general, similar aggression and survival rates were recorded in the 1-1 and 5-5 resident-intruder battles, although differences between forms and residents versus intruders were

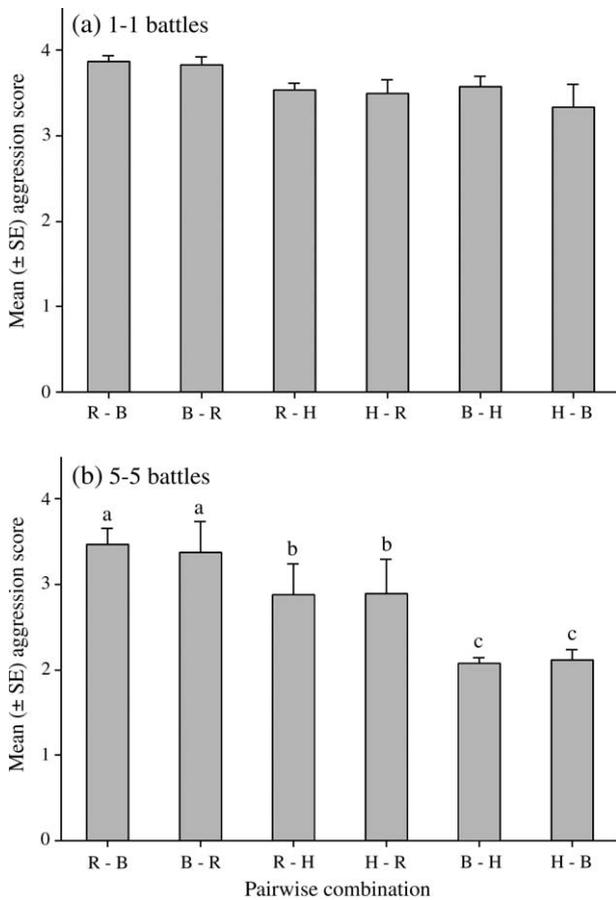


Fig. 4. Aggression of workers of *Solenopsis invicta* (red), *S. richteri* (black) and their hybrids toward heterospecifics at (a) 1-1 and (b) 5-5 resident-intruder battles. For this and subsequent figures, **R - B** = inter-specific encounters between resident red (R) and intruder black (B); **B - R** = inter-specific encounters between resident black (B) and intruder red (R); **R - H** = inter-specific encounters between resident red (R) and intruder hybrids (H); **H - R** = inter-specific encounters between resident hybrids (H) and intruder red (R); **B - H** = inter-specific encounters between resident black (B) and intruder hybrids (H); **H - B**: inter-specific encounters between resident hybrids (H) and intruder black (B). Note: the intruder in each pairwise combination is indicated in bold. Letters indicate significant differences among the six pairwise combinations of the three ant forms (Kruskal-Wallis test, $P < 0.05$, $n = 30$).

slightly amplified in the 5-5 battles. These results are consistent with those of a recent paper on the effect of bioassay on aggressive behaviour in *L. humile*, which showed that the live 1-1 and 5-5 battles correlated with each other and provided consistent results (Roulston *et al.*, 2003). However, the 5-1 battles offered an advantage to resident ants and may not represent a fair measure of aggression. The lower survival recorded for *S. richteri* at the 5-5 compared with the 1-1 battles was a direct reflection of their relatively higher aggression score at the 5-5 battles, and may suggest that this form displayed higher aggression as a group than as individuals. A similar result was reported for the ant, *Formica xerophila* Smith, whose workers acted more aggressively towards a competitor when in a large group than as

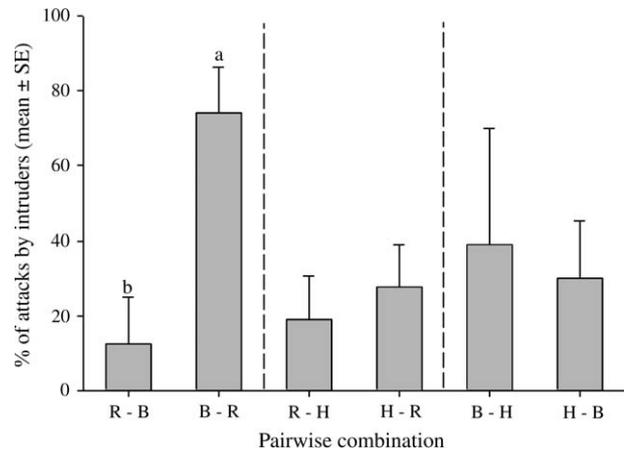


Fig. 5. Percentage of attacks initiated by intruder workers of *Solenopsis invicta* (red), *S. richteri* (black) and their hybrids during inter-specific aggression encounters at 1-1 resident-intruder battles. Keys (see Fig. 4 for detailed keys): **R - B** (red = resident, black = intruder); **B - R** (black = resident, red = intruder); **R - H** (red = resident, hybrid = intruder); **H - R** (hybrid = resident, red = intruder); **B - H** (black = resident, hybrid = intruder); **H - B** (hybrid = resident; black = intruder). Note: the intruder in each pairwise combination is in bold. Dotted lines are used to separate data for the different combinations of the three ant forms. Letters indicate significant differences between two pairwise combinations of the same two ant forms (i.e. **R - B** versus **B - R**; Wilcoxon's signed-rank test, $P < 0.05$, $n = 30$).

isolated individuals (Tanner, 2006). The author reported in a follow-up study that the correlation between group size and aggressive behaviour in *F. xerophila* is coordinated by direct nestmate contact (Tanner, 2008).

No significant size differences were recorded among the major workers of the three ant forms, suggesting that the superior aggressiveness of *S. invicta* and hybrids is not because of their larger size. The data which showed relatively lower intra-specific aggression for *S. richteri* are consistent with a previous report of lower genetic diversity in this form compared with *S. invicta* and hybrids (Ross *et al.*, 1987a, b). Higher genetic variability may thus explain the higher intra-specific aggression recorded in the present study for *S. invicta* and hybrids. Our data showed that the hybrid is intermediate between both parental forms in aggressiveness and post-aggression survival, and thus is not entirely in consonance with the 'hybrid superiority' theory proposed by Moore (1977).

Although the results of this laboratory aggression study may not entirely reflect field situations, they may provide insights into the interactions between *S. richteri* and *S. invicta* in the early years after their introductions into Alabama in the early 1900s (Wilson, 1958). The aggressiveness and superior combative ability of *S. invicta* and hybrids may contribute, at least in part, to the continuing range expansion of both forms in southern U.S. In contrast, the relatively lower aggression and competitive ability of *S. richteri* may, in part, explain the range restriction and dwindling populations of this form, which is now limited to a relatively small area in northwest Alabama, northern Mississippi and southern Tennessee (Streets *et al.*, 2006).

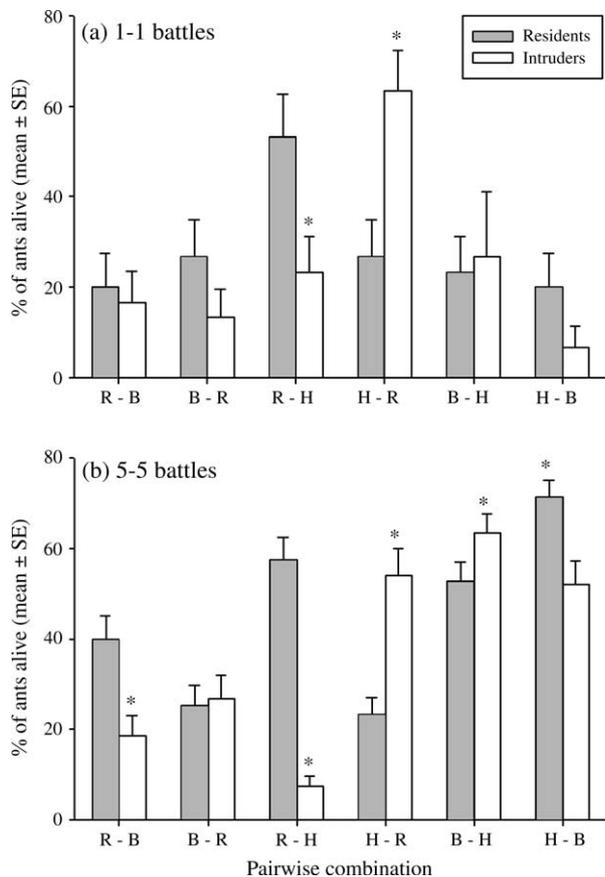


Fig. 6. Percentage of resident and intruder workers of *Solenopsis invicta* (red), *S. richteri* (black) and their hybrids alive 24 h after inter-specific aggression encounters at (a) 1-1 and (b) 5-5 resident-intruder battles. Keys (see Fig. 4 for detailed keys): **R - B** (red = resident, black = intruder); **B - R** (black = resident, red = intruder); **R - H** (red = resident, hybrid = intruder); **H - R** (hybrid = resident, red = intruder); **B - H** (black = resident, hybrid = intruder); **H - B** (hybrid = resident; black = intruder). Note: the intruder in each pairwise combination is in bold. Asterisks (*) indicate significant differences between resident and intruder ants in each pairwise combination (Matched pairs test, $P < 0.05$, $n = 30$).

It has been proposed that inter-specific competition in ants may be explained by Lanchester's laws of combat (Lanchester, 1916), which describe casualty rates in two opposing armies as functions of the numbers of individuals and their fighting abilities (McGlynn, 2000). However, a recent study by Plowes and Adams (2005) did not provide strong support for this proposition. The authors reported that casualties incurred by two fighting groups of *S. invicta* were not strongly affected by group sizes and that individual prowess was the main factor which determined the outcomes of the battles (Plowes & Adams, 2005). Other factors such as exploitative competition may also contribute to the ecological dominance of *S. invicta*, and the range restriction of *S. richteri* in southern U.S. In addition to inter-specific aggression (Holway, 1999; Rowles & O'Dowd, 2007), exploitative competition was reported as a major mechanism underlying

the displacement of native ants by *L. humile* (Holway, 1999). However, the role of this mechanism in mediating shifts in the population densities and distributions of the three forms of imported fire ants in southern U.S. remains uninvestigated. Climatic requirements may also play a role: *S. richteri* has a more temperate distribution than *S. invicta* in its native South America (Shoemaker *et al.*, 1996), and was found to be more cold-tolerant than *S. invicta* in a laboratory test (James *et al.*, 2002). Climatic conditions in the native range of *S. richteri* are similar to those in northern Alabama, Mississippi and southern Tennessee, where this form is currently restricted in the U.S. The combination of climatic requirements, inferior aggression and competitive ability will make it difficult for *S. richteri* to compete with *S. invicta* in the warmer parts of their introduced range and may explain the current restricted range of *S. richteri*.

These results suggest that the hybrid form, which has an overlapping range with its parental forms in Alabama and Mississippi (Streett *et al.*, 2006), is likely to continue to expand its range northward and southward and may pose a threat to the survival of *S. richteri* in southern U.S. Based on their recent data showing major shifts in spatial distributions of the three ant forms in Mississippi, Streett *et al.* (2006) predicted that *S. richteri* will eventually be replaced by *S. invicta* and hybrid populations in the state and perhaps in the entire country. While temperature and other climatic factors (e.g. isoclines) may prevent a total replacement of *S. richteri* by its congeners in the northernmost part of their range, these results suggest the likelihood of a more restricted and narrower range for *S. richteri* in the future.

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