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How to coexist with fire ants: The roles of behaviour and cuticular compounds

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A B S T R A C T

Because territoriality is energetically costly, territorial animals frequently respond less aggressively to neighbours than to strangers, a reaction known as the “dear enemy phenomenon” (DEP). The contrary, the “nasty neighbour effect” (NNE), occurs mainly for group-living species defending resource-based territories. We studied the relationships between supercolonies of the pest fire ant *Solenopsis saevissima* and eight ant species able to live in the vicinity of its nests plus *Eciton burchellii*, an army ant predator of other ants. The workers from all of the eight ant species behaved submissively when confronted with *S. saevissima* (dominant) individuals, whereas the contrary was never true. Yet, *S. saevissima* were submissive towards *E. burchellii* workers. Both DEP and NNE were observed for the eight ant species, with submissive behaviours less frequent in the case of DEP. To distinguish what is due to chemical cues from what can be attributed to behaviour, we extracted cuticular compounds from all of the nine ant species compared and transferred them onto a number of *S. saevissima* workers that were then confronted with untreated conspecifics. The cuticular compounds from three species, particularly *E. burchellii*, triggered greater aggressiveness by *S. saevissima* workers, while those from the other species did not.

Keywords:

Aggressiveness
Cuticular hydrocarbons
Dear enemy phenomenon
Nasty neighbour effect
Species coexistence
Supercoloniality

1. Introduction

The territoriality of a species is regulated by the cost of defending a territory from both neighbours and “strangers” (i.e., individuals from distant territories) and by benefits that can include access to resources such as food and nesting sites. Strangers can represent the greatest threat as they might seek to take the territory from the current holders, while neighbours already possess a territory and so have less to gain from engaging in conflict. This result in less aggressive responses towards neighbours than towards strangers which is known as the “dear enemy phenomenon” (DEP) (Fisher, 1954; Temeles, 1994). Yet, the opposite of DEP, or the “nasty neighbour effect” (NNE) with greater aggressiveness towards neighbours than towards strangers, occurs particularly for group-living species defending resource-based territories (Müller and Manser, 2007).

Both DEP and NNE have been noted in ants (Temeles, 1994) whose recognition of colony mates and rejection of aliens is based on the chemical cues (CCs) encoded in a complex mixture of low-volatile cuticular hydrocarbons that constitute a genetically determined “colony odour”. The latter can be influenced by environmental factors such as diet and nesting material (d’Ettorre and Lenoir, 2010). DEP, which limits the escalation of fighting during the frequent encounters between homo- and heterospecific neighbours, is based on habituation related to learning the colony odour of encountered individuals and/or their behaviour (Jutsum et al., 1979; Heinze et al., 1996; Dimarco et al., 2010; Pirk et al., 2001). NNE, noted intra- and interspecifically, can occur when an alien ant from a species representing a particularly high level of threat belonging to a neighbouring colony is perceived as the vanguard of a competitive force. This leads to a higher level of aggressiveness than for a stranger (Knaden and Wehner, 2003; Sanada-Morimura et al., 2003; Boulay et al., 2007; Thomas et al., 2007; van Wilgenburg, 2007; Newey et al., 2010). Escalating conflicts leading to injury or death are limited through avoidance, ritualized fights or when ant species with different rhythms of activity share food sources (Hölldobler and Wilson, 1990; Orivel and Dejean, 2002; van Wilgenburg et al., 2005). Also, by frequently

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displaying submissive behaviour or feigning death (thanatosis), dominated workers from species coexisting with very aggressive dominant ants suffer only brief and less harmful attacks (Langen et al., 2000; Grangier et al., 2007; Abril and Gomez, 2009; Menzel et al., 2010).

As opposed to typical, multicolonial ant species with “closed” colonies, “unicolonial” invasive species are “ecologically dominant” (i.e., both numerically and behaviourally dominant) and develop “open” supercolonies extending over hundreds of kilometres in their introduced range (Hölldobler and Wilson, 1990; Davidson, 1998; Holway et al., 2002). Intraspecific aggressiveness occurs along the border between the territories of two supercolonies because here, too, outsiders are distinguished from colony mates through CCs (Thomas et al., 2007; Moffett, 2012a). Certain of these species also present the supercolony syndrome in their native range, but here the expansion of their territories is much lower due to intra- and interspecific competition (Orivel et al., 2009; Fournier et al., 2012; Moffett, 2012a,b).

In this study, we focused on the relationships between the red fire ant *Solenopsis saevissima* (Smith) and its sympatric ant species in forest edges in French Guiana, a part of its native range that extends from Suriname to northern Amazonia. This species belongs to an assemblage of recently diverged taxa, including the well-known invasive fire ants *S. invicta* (Buren), *S. richteri* (Forel) and *Solenopsis geminata* (Fabricius) (Ross et al., 2010). It has been little studied although considered a major pest in human-disturbed areas of its native range where it forms supercolonies that displace other ant species (Trager, 1991; Taber, 2000; Martin et al., 2011). Yet, still other species resist, competing for food and/or nesting sites. Moreover, *S. saevissima* is, like many ant species in the Neotropics, under predation pressure from the army ant *Eciton burchellii* (Westwood) (Gotwald, 1995).

The aim of this study was to identify how workers from certain ant species are able to coexist with *S. saevissima* supercolonies. We hypothesized that during encounters between *S. saevissima* and workers from these ant species, DEP or NNE can occur along with submissive behaviour. We investigated this question through confrontation tests between *S. saevissima* workers and those from sympatric species and by manipulating the ants' CCs (see Roux et al., 2009). We also conducted confrontations using the army ant *E. burchellii* because it is prevalent in French Guiana (Delabie et al., 2009) and has been successfully observed raiding *S. saevissima* colonies.

2. Methods

2.1. Sampling *S. saevissima* and other ant species

This study was conducted in French Guiana between March and June 2007. The ants were collected from forest edges near Petit Saut (4°59' N; 53°08' W) and the city of Sinnamary (5°22' N; 52°57' W) corresponding to parts of the territories of two different *S. saevissima* supercolonies. Supercolony “A” extended over 54 km from Petit Saut to Paracou (05°16' N; 52°55' W), while supercolony “B” extended from Paracou to Sinnamary (Martin et al., 2011).

During studies related to the breadth of the *S. saevissima* supercolonies (Martin et al., 2011), we noted that only eight ant species nest within a radius of 3 m from a *S. saevissima* nest, namely: *Atta sexdens* (Linnaeus), *Crematogaster tenuicula* (Forel), *Pheidole fallax* (Mayr), *S. geminata* (Fabricius) and *Wasmannia auropunctata* (Roger) (all Myrmicinae); *Camponotus melanoticus* (Emery) and *Camponotus blandus* (Smith) (both Formicidae); and *Dorymyrmex pyramicus guianensis* (Santschi) (a Dolichoderinae). *A. sexdens*, frequent along roads and tracks, is a leaf-cutting, fungus-growing species that, therefore, competes with *S. saevissima* only for nesting space, while other species compete for both nesting space and food.

To collect individuals from the *S. saevissima* colonies, we used a shovel to gather the upper parts of the mound-shaped nests. For the other species, we first located the nests or the nest entrances that were within a ca. 3 m radius from a *S. saevissima* mound, and then we again used a shovel to gather the ants. Because we gathered them the day before the confrontation tests, we put them and the earth into several large plastic boxes whose walls were coated with Fluon® to prevent the workers from climbing out and then placed two small test-tubes in each box, one of which contained cotton imbibed with water and the other cotton imbibed with honey. The plastic boxes were then transported to the laboratory. We left the ants overnight to allow them to calm down prior to the experiments.

E. burchellii is prevalent in the area studied (Delabie et al., 2009) and able to attack *S. saevissima* (J-MM, AD, pers. com.). We followed columns at dusk that had foraged within the range of the *S. saevissima* supercolonies until we located the bivouac and then proceeded in the same manner as above. Using a shovel, we gathered part of the workers as well as some of the ground around the bivouac. We then put the earth and the ants into large plastic boxes whose walls were coated with Fluon® and placed a small test-tube containing cotton imbibed with water into each plastic box.

2.2. Confrontations between *S. saevissima* workers and those from the other ant species

Our aim was to determine if the relationships between *S. saevissima* workers from each supercolony and the neighbours and strangers collected from the eight species able to nest in its vicinity were based on DEP or on NNE. The experiments were set up so that *S. saevissima* supercolony A served as a reference (the colonies from the eight other species situated within a radius of 3 m from its nests are “neighbours”), while supercolony B served as a reservoir of “strangers” for the first 30 confrontation tests (the colonies from the eight other species situated within a radius of 3 m from its nests are “strangers” for the *S. saevissima* workers from supercolony A). The contrary set up was used for the 30 other tests (60 tests in each case). Note that for each of these eight species, workers were taken from six different nests situated in the vicinity of six different *S. saevissima* mounds (10 workers tested for each *S. saevissima* mound).

We employed the standard behavioural tests commonly used in studies dealing with the relationships between ant individuals whether they are conspecific or not (Suarez et al., 1999; Tsutsui et al., 2003). Two workers were placed together for 5 min in a neutral arena (a Petri dish; 60 mm in diameter and 20 mm deep) whose walls were previously coated with Fluon®. Each worker was used only once. We observed the interactions and scored them as follows: 1 = antennal contact (physical contact without an aggressive response; may include trophallaxis), 2 = retreat (brief contact inducing a sudden U-turn and one or both of the ants quickly running away; the confronted individual is likely recognized as a non-colony mate), 3 = aggressiveness (brief biting by one or both of the workers; may include jerking and opening the mandibles), and 4 = fighting (prolonged aggressiveness, including prolonged biting and stinging or venom spraying) (see Fig. 1). Each time, we noted the maximal level reached (regardless of the species). The species retreating (level 2) or initiating the aggressive acts (levels 3 and 4) was also noted. All of the possible dyadic interactions between *S. saevissima* and the eight ant species able to nest in its vicinity were examined (16 kinds of confrontations). In addition, we set up confrontations between *S. saevissima* and *E. burchellii* workers. *E. burchellii* can neither be considered a neighbour nor a stranger due to the nomadic behaviour of the species (Gotwald, 1995). Control confrontations were set up between *S. saevissima* nestmates (taken from the nests of supercolony A for 30 cases and supercolony B

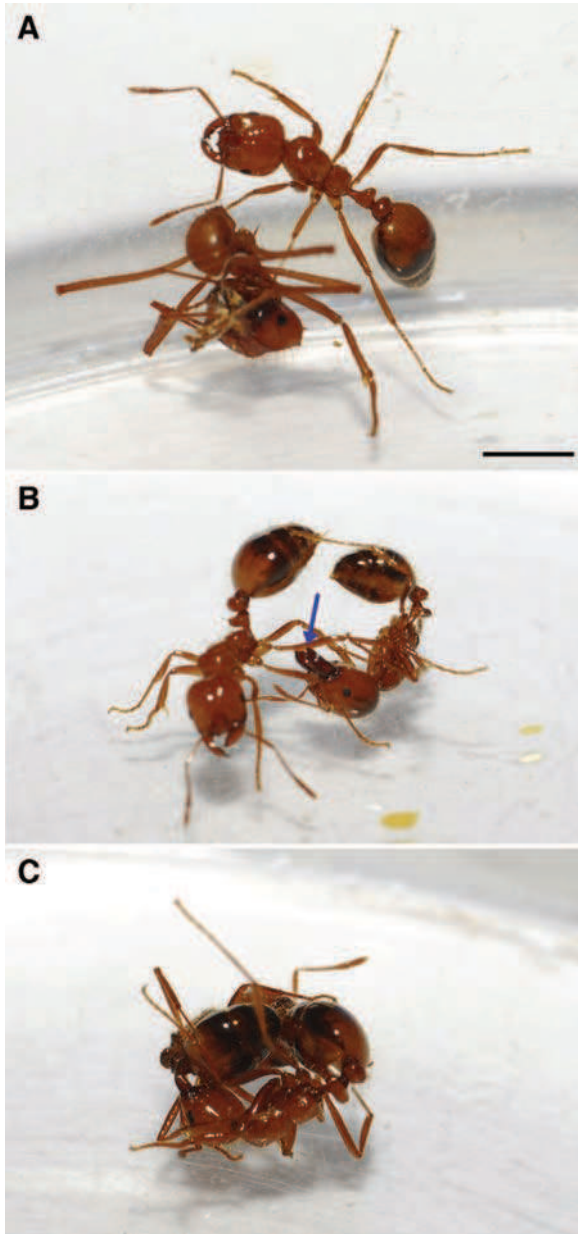


Fig. 1. Some of the behaviours noted during confrontations. (A) An *A. sexdens* media worker feigning death (thanatosis) during a confrontation set up with a *Solenopsis saevissima* worker. The scale bar represents 1 mm. (B) Fighting between two *S. saevissima* workers; one of them is biting a leg (arrow) of its opponent. (C) Reciprocal full attack between two *S. saevissima* workers (they bite and sting each other).

for the 30 remaining cases) as well as between “strangers” (one taken from a nest of supercolony A, the other from supercolony B).

Furthermore, during these experiments, each time we noted what worker displayed any clearly submissive behaviour (i.e., an all or nothing response: a curled-up position with the legs and antennae folded back against the body, thanatosis; see Fig. 1) and recorded the number of interactions that resulted in at least one submissive behaviour.

The species were defined as responding according to DEP when the level of aggressiveness was lower during confrontations between neighbours than between “strangers” (non-neighbours). Conversely, species were defined as responding according to NNE when the level of aggressiveness was higher in interactions between neighbours than between “strangers”.

2.3. Testing the effect of semiochemicals from sympatric ant species on the behaviour of *S. saevissima* workers

To suppress the behavioural component of the responses in the above-mentioned experiments, we set up confrontations between pairs of *S. saevissima* workers, one of which was “made-up” with CCs taken from heterospecific workers belonging to colonies situated within a 3 m radius from a *S. saevissima* mound. The “make-up” was created using a water-based method consisting in emulsifying the CCs of workers from the other ant species and applying them onto the cuticle of the *S. saevissima* workers after the latter were themselves rinsed with water to reduce the quantity of their own CCs (the details concerning *Ca. blandus* are provided in Roux et al., 2009). About 70 individuals from each species were rinsed separately in a 30 mL glass vial containing 20 mL of ultra pure water (at room temperature or ca. 27 °C) and vigorously shaken by hand for 5 min with the aim of emulsifying the CCs. The ants were then removed. To apply the CC emulsion (i.e., the “make-up”), we used *S. saevissima* workers having undergone a similar treatment as above, but they were then placed into glass containers whose floor was lined with absorbent paper on which they dried. These rinsed workers were then placed directly into the CC emulsion obtained from one of the neighbour ant species, shaken for 10 s, and then left in the emulsion for 5 min. They were then removed and returned to a glass container where they again dried for 30 min before the behavioural confrontations were conducted.

Note that this method is possible because many ground-nesting ant species are adapted to flooding and do not drown when they are immersed (Mlot et al., 2011) and because ants are able to discriminate the presence of low levels of heterocolonial cuticular hydrocarbons (Ichinose and Lenoir, 2010). This permitted us to investigate if the CCs from the tested ant species can influence the behaviour of *S. saevissima* workers during confrontations between two strangers that usually result in a high level of aggressiveness.

Two types of confrontations were conducted in order to determine if some CCs can reduce or, on the contrary, increase the aggressiveness of *S. saevissima* workers.

First, to search for the CCs potentially responsible for low aggressive responses, we set up 60 confrontations between two *S. saevissima* workers from different supercolonies (strangers), one of them being untreated, the other being “made-up”. The first control tests concerned untreated *S. saevissima* workers from different supercolonies to evaluate their natural aggressiveness level. In the second control tests, we intended to reproduce the stress inflicted on workers when they were “made-up”. Here, an untreated worker was confronted with a conspecific stranger that, after being rinsed, was placed into water (rather than into the CC emulsion obtained from another species). All of the behavioural tests were conducted and scored as previously described.

Second, to search for the CCs potentially responsible for highly aggressive responses, we set up confrontations between two *S. saevissima* workers from the same nests (30 cases for supercolony A; 30 other cases for supercolony B); indeed, nestmates do not attack each other. Here, too, one of them was “made-up” with CCs from workers belonging to another neighbour ant species as previously described. Two controls were conducted as previously, but this time with nestmates.

2.4. Statistical analysis

To compare the levels of aggressiveness between *S. saevissima* workers and workers from the sympatric species, we firstly modelled the link between the scores assigned to each confrontation taking into account if the workers were neighbours or strangers using a generalized linear model (GLM) with an ordinal probit link (Agresti, 2002). This model respected the ordinal qualitative

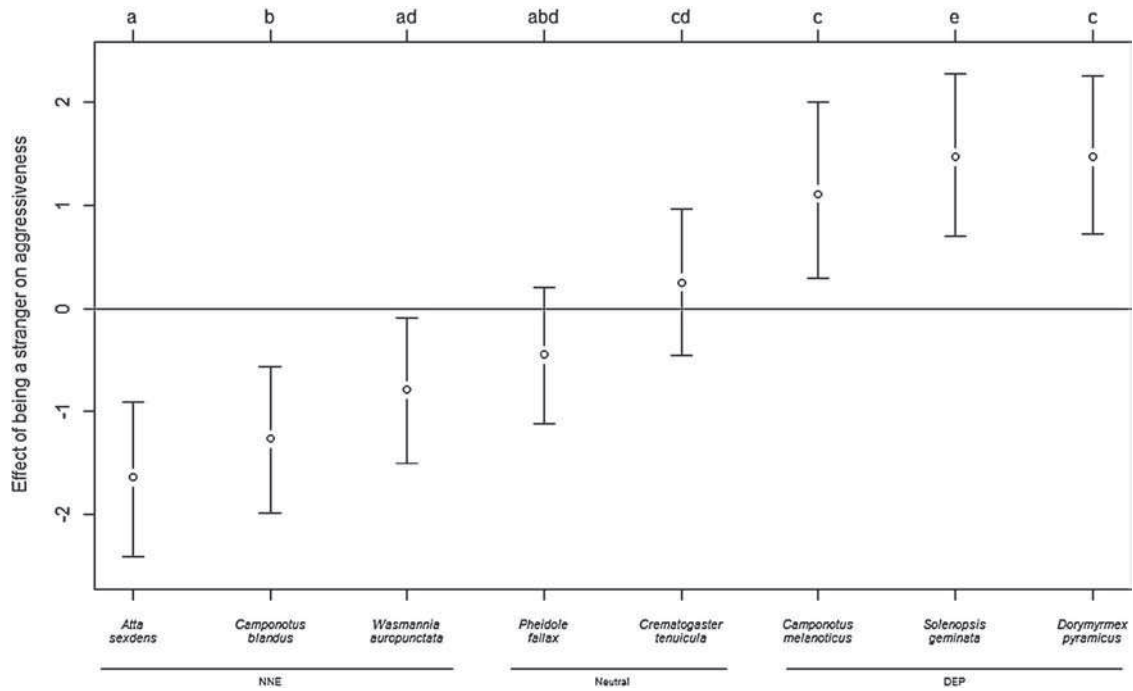


Fig. 2. Interactions between *S. saevissima* workers and those from the eight ant species able to live in the vicinity of *S. saevissima* nests. The values presented on the Y-axis correspond to the effect of “being a stranger” on the inverse probit of the scores (plots show means and 95% CI). As the inverse probit is an increasing function, positive values imply higher probabilities for higher scores and negative values imply higher probabilities for lower scores. Statistical comparisons: different letters indicate significant differences at $P < 0.05$. NNE: greater aggressiveness towards familiar ants (neighbours). DEP: greater aggressiveness towards unfamiliar ants (strangers).

nature of the scores which were equal to 1, 2, 3 or 4 depending on the level of aggressiveness of the confrontation. The significance of being a neighbour or a stranger was assessed through likelihood ratio effect tests. Secondly, to identify homogeneous groups of species according to the level of aggressiveness noted during the confrontations, we carried out likelihood ratio effect tests on pairs of species to assess their differences. For each pair of species, we compared the general model with the GLM probit, integrating

the variable “neighbour” or “stranger” and assuming (1) that each species had a different effect, and (2), on the contrary, that the two species had an identical effect. Multiple comparisons were adjusted using the false discovery rate (FDR) (“BY” correction; Benjamini and Yekutieli, 2001; Fig. 2). We did not find a colony effect.

To compare the percentage of submissive behaviours by neighbours and strangers for each species tested, we conducted proportion tests (Fig. 3).

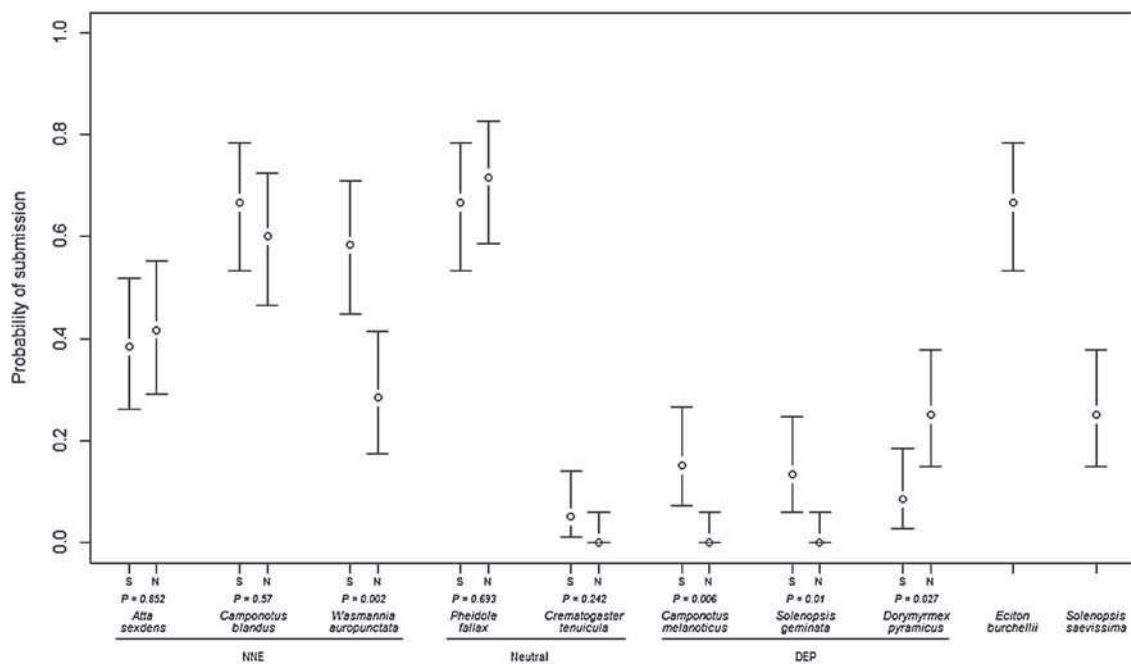


Fig. 3. Probability of submissive behaviours being displayed by the different ant species during confrontations with *S. saevissima* (plots show means and 95% CI). Note that *S. saevissima* workers presented submissive behaviours only during intraspecific confrontations or when they were confronted with *E. burchellii* individuals. Statistical comparisons between neighbouring colonies of an *S. saevissima* nest (*n*) and “stranger” colonies (*s*): the *P*-values are indicated above the name of the ant species.

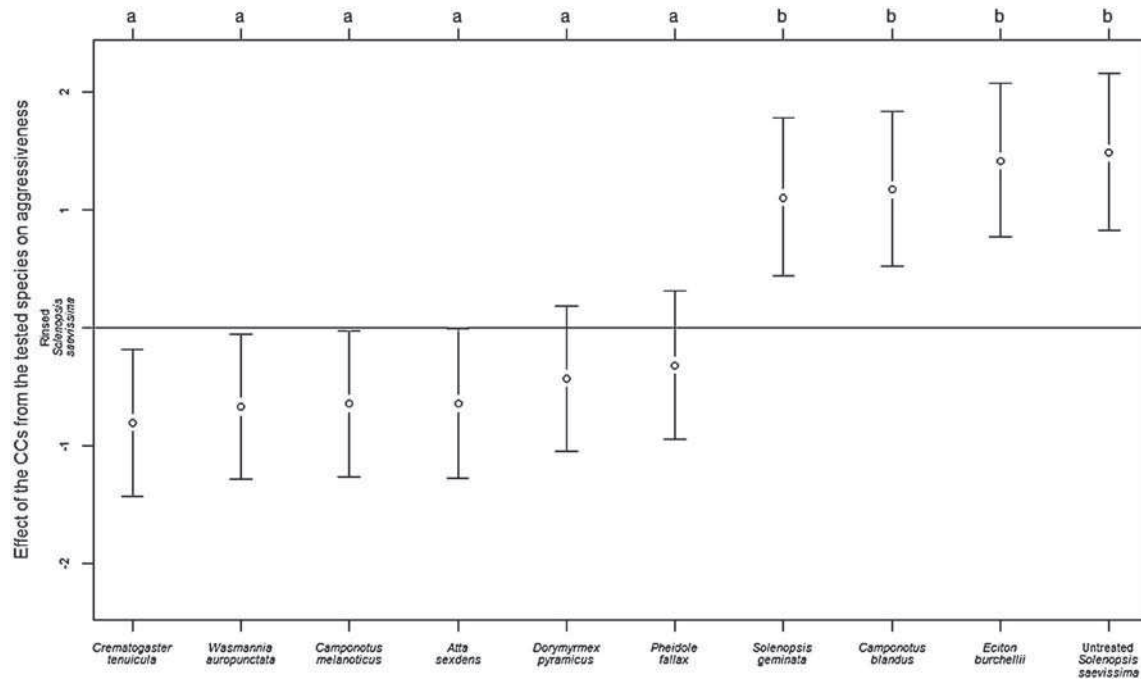


Fig. 4. Aggressiveness noted during interactions between “stranger” *S. saevissima* workers (plots show means and 95% CI). The values presented on the Y-axis correspond to the effect of the CCs from the tested ant species on the inverse probit of the scores (see also the legend for Fig. 2). The first control group corresponds to the interactions between untreated *S. saevissima*. The horizontal line corresponds to the second control group, or confrontations between alien *S. saevissima* workers, one of them having been rinsed. The experimental groups correspond to confrontations between alien *S. saevissima* workers, one of them having been “made-up” with the cuticular extracts from a sympatric ant species. Statistical comparisons: different letters indicate significant differences at $P < 0.05$.

For confrontations between *S. saevissima* workers when one of them was “made-up” with CCs from a sympatric ant species (and for the controls), we also modelled the link between the scores assigned to each confrontation between two workers and the ant species using a GLM with an ordinal probit link. To identify homogeneous groups of species according to the impact of their CCs on the level of aggressiveness during these confrontations, we carried out likelihood ratio effect tests on the CCs of pairs of species to assess their differences. For each pair of species, we compared the general model with the GLM probit and assumed (1) that the CCs of each species had a different effect, and (2), on the contrary, that the CCs of the two species had an identical effect. Here, too, multiple comparisons were adjusted using the BY FDR correction (Fig. 4).

The statistics were carried out using R (v. 2.14.2) software (R Development Core Team, 2011). The ordinal probit regression was carried out using the package MASS.

3. Results

3.1. Confrontations between *S. saevissima* workers and those from the other ant species

Among the eight ant species able to live in close vicinity to *S. saevissima* mounds, only *Ca. melanoticus*, *S. geminata* and *D. pyramicus* workers elicited a significant DEP during aggressiveness tests as interactions between neighbours resulted in a lower level of aggressiveness than interactions between non-neighbours (values above 0 in Fig. 2). The contrary was true, illustrating NNE, for *A. sexdens*, *Ca. blandus* and *W. auropunctata* (values above 0 in Fig. 2), while interactions with *P. fallax* and *Cr. tenuicula* revealed the same level of aggressiveness regardless of whether the workers were neighbours or “strangers”.

During dyadic confrontations with these eight ant species, *S. saevissima* workers, which never escaped (level 2 of scoring) and never displayed submissive behaviours, were always the aggressor when

scoring levels 3 and 4 were noted. Among the eight confronted ant species three trends appeared (Fig. 3): (1) only *D. pyramicus* workers displayed significantly more submissive behaviours when they were neighbours than when they were “strangers”; (2) *A. sexdens*, *Ca. blandus*, *P. fallax* and *Cr. tenuicula* displayed a similar rate of submissive behaviours whether they were neighbours or “strangers”; and (3) *W. auropunctata*, *Ca. melanoticus* and *S. geminata* displayed significantly more submissive behaviours when they were “strangers” (Fig. 3). Overall, species eliciting DEP tended to display fewer submissive behaviours than others regardless of whether they were neighbours or “strangers” (Fig. 3).

Contrarily to the previous cases, submissive behaviours were noted only on the part of the *S. saevissima* individuals when they were confronted with *E. burchellii* individuals; the latter were the aggressors in all cases. Note that intraspecific submissive behaviours were also observed for *S. saevissima* (Fig. 3).

3.2. Testing semiochemicals on “stranger” and nestmate *S. saevissima* workers

Dyadic confrontations between “stranger” (non-colony mate) *S. saevissima* workers where one of them was “made-up” with the CCs from *Cr. tenuicula*, *W. auropunctata*, *Ca. melanoticus*, *A. sexdens*, *D. pyramicus* and *P. fallax* resulted in a lower, but non-significant, level of aggressiveness compared to the second control group (where one *S. saevissima* was rinsed; Fig. 4). All previous levels of aggressiveness were significantly lower than those for the first control group (i.e., untreated *S. saevissima* workers) or when one of the workers was “made-up” with extracts from *S. geminata*, *Ca. blandus* or *E. burchellii* (Fig. 4).

The same experiments conducted between *S. saevissima* nestmates produced a significant increase in the level of aggressiveness only for extracts taken from *Ca. blandus* and *E. burchellii* ($P < 0.01$; figure not shown). Here, the aggressiveness values for confrontations between two untreated *S. saevissima* (first control) and

between one untreated *S. saevissima* worker and another rinsed (second control) were identical and equal to 1.

4. Discussion

4.1. DEP, NNE and the biological and ecological traits of the different ant species

Because a similarity in colony odour can be excluded as the confronted workers belong to different species, DEP can only result from habituation or a type of learning of the neighbour's odour leading dominant ants to tolerate workers from certain other species (Peeke, 1984). Nevertheless, the decision to fight or not with a neighbour resides in (1) colony-mate recognition (the role of the CCs), (2) the behaviour of the workers (submissive or not) and (3) ecological characteristics of each species based on their propensity to compete for the same resources and the threat they represent. Indeed, DEP and NNE occur based on the potential costs and benefits related to foraging for food and territorial defence (Langen et al., 2000; Grangier et al., 2007; Abril and Gomez, 2009; Menzel et al., 2010).

One might expect that species eliciting DEP have "appeasing" CCs, while those eliciting NNE have CCs that trigger greater aggressiveness, yet our results did not bear this out (Fig. 4). This is likely due to the interference between the traits we studied here and the biological and ecological characteristics of each species studied, presented below.

Among the eight ant species able to live in the vicinity of *S. saevissima* nests, *S. geminata* and *W. auropunctata* are also supercolonial and their workers are known for their interspecific aggressiveness (Holway et al., 2002); however we noted DEP in the former case and NNE in the latter case. Because they share several traits such as nest site selection, means of defending food resources, high nest density, and the quick colonization of open, disturbed areas, *S. saevissima* and *S. geminata* are in close competition (Perfecto, 1991, 1994; Trager, 1991). This explains the high levels of aggressiveness depicted in Fig. 4. Although small, *W. auropunctata* workers are fierce competitors for food resources and are able to displace most other ants (Holway et al., 2002; Orivel et al., 2009). Yet, they share foraging areas with *S. saevissima* thanks to a high rate of submissive behaviour (Fig. 3), and their CCs do not trigger greater aggressiveness (Fig. 4). Unexpectedly, the same was true for the leaf-cutting ant *A. sexdens* (Figs. 3 and 4) that has populous colonies containing 5–8 million workers (Hölldobler and Wilson, 1990).

Very frequent in the area studied, the diurnal workers from the large, polydomous *Ca. blandus* colonies are dominated at dawn and dusk by the nocturnal *S. saevissima* individuals (Orivel and Dejean, 2002). Accordingly, they very frequently behaved submissively (Fig. 3), while their CCs elicited aggressiveness by *S. saevissima* individuals (Fig. 4).

Ca. melanoticus forms large, polydomous colonies extending over ca. 200 m and the workers, nocturnal, dominate *S. saevissima* individuals in natural conditions (Orivel and Dejean, 2002). Correspondingly, they only rarely displayed submissive behaviours in this study (Fig. 3), so that the low level of aggressiveness noted was likely due to DEP in addition to CCs that do not trigger greater aggressiveness (Fig. 4). The same was true for *D. pyramicus* although the colonies are small and the workers are typically opportunist, discovering food resources quickly but not conducting mass recruitment, and, so, not defending these resources (AD, pers. obs.).

We noted neither DEP nor NNE for *P. fallax* and *Cr. tenuicula* (Fig. 4), both having small colonies (Itzkowitz and Haley, 1983; de Oliveira et al., 2009). Active all around the clock, *P. fallax* workers are generalist foragers that use mass recruitment (Itzkowitz and Haley, 1983). They likely attenuated *S. saevissima* aggressiveness

through their high rate of submissive behaviour aided by their CCs that do not trigger greater aggressiveness (Figs. 3 and 4), whereas *Cr. tenuicula* workers were likely tolerated by *S. saevissima* thanks to their CCs as they were very rarely submissive (Figs. 3 and 4).

Colonies of the army ant *E. burchellii* are quite large with up to 2 million polymorphic workers that capture a wide range of prey, including the brood of other ants (Gotwald, 1995). This time it was the *S. saevissima* workers that behaved submissively (Fig. 3), explaining, at least in part, how they survive army ant raids even though the latter plunder larvae, pupae and some callow workers (AD, pers. obs.; see similar cases in Gotwald, 1995).

4.2. CCs affecting the level of aggressiveness of *S. saevissima* workers

Ant cuticular hydrocarbon profiles are composed of more than 100 linear alkanes and methyl-branched alkanes and alkenes whose chains range from 20 carbons to 40 carbons (Guerrieri et al., 2009; Martin and Drijfhout, 2009; van Wilgenburg et al., 2012). It is generally assumed that workers discriminate their nestmates from strangers by qualitatively and quantitatively comparing their own CCs (i.e., their "colony odour" that they learned and that represents a neural template) with the CCs of encountered individuals. A mismatch generally results in aggressiveness (d'Ettorre and Lenoir, 2010). Yet, it appears that overall only one subset of cuticular hydrocarbons are likely used as recognition cues, while the discrimination of a stranger does not obligatorily elicit aggressiveness (Guerrieri et al., 2009; d'Ettorre and Lenoir, 2010; van Wilgenburg et al., 2010, 2012).

S. saevissima workers "made-up" with the CCs from *Ca. blandus*, *S. geminata* and *E. burchellii* triggered greater aggressiveness (Fig. 4 and experiments involving *S. saevissima* nestmates). This means that individuals from these three species were likely recognized as potential threats by *S. saevissima* workers based on their CCs (or a part of their CCs). This is reminiscent of experiments where hydrocarbons were added to natural profiles where only some of them elicited aggressiveness in *Ca. herculeanus* and the Argentine ant (Guerrieri et al., 2009; van Wilgenburg et al., 2010). Note that only *Ca. blandus* shares foraging areas with *S. saevissima*, but with different rhythms of activity, whereas *S. geminata* rather competes with *S. saevissima* for territories and *E. burchellii* preys on all of them.

In the present study, rinsing one individual before dyadic confrontation tests between stranger *S. saevissima* workers (second control) resulted in a significantly lower level of aggressiveness than during confrontations between untreated individuals (first control) (Fig. 4). This is likely because the rinsed individual loses a part of its CCs (a threshold is not reached that discriminates it as a "true" stranger), while the modification of its "template" lowers its own reactions. The addition of the CCs from *Cr. tenuicula*, *W. auropunctata*, *Ca. melanoticus*, *A. sexdens*, *D. pyramicus* and *P. fallax* had no effect on the level of aggressiveness by *S. saevissima* workers (values not significantly different than those from the second control group; Fig. 4). It is as if these tested *S. saevissima* workers were unable to recognize that they are "true" strangers due to the rinsing (second control) or due to the rinsing plus the addition of the CCs from these six ant species. At least in part, this permits the workers of these ant species to share territories with *S. saevissima* individuals without triggering aggressiveness (Fig. 4), while explaining why in nature interspecific encounters between ants result mostly in reciprocal avoidance (the other is recognized but not attacked).

5. Conclusion

In addition to DEP or NNE, ant species able to live in the vicinity of *S. saevissima* lessened its workers' aggressiveness either

through submissive behaviours (essential when species' CCs trigger a greater level of *S. saevissima* aggressiveness) and/or, for certain species, the properties of their CCs that do not trigger aggressiveness by the *S. saevissima* workers. Also, our results bring new insights to the patterns related to colony-mate recognition in *S. saevissima*, something that may also be true for invasive fire ants.

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