Ecology/Écologie

An invasive ant species able to counterattack marabunta raids

Une fourmi invasive capable de contrecarrer les raids de marabuntas

Alain Dejean [a,b,*], Frédéric Azémar [b,c], Olivier Roux [d]

[a] CNRS UMR 8172, Écologie des forêts de Guyane, BP 316, 97379 Kourou cedex, France
[b] Université de Toulouse, UPS, INP, laboratoire Écologie fonctionnelle et Environnement (Ecolab), 118, route de Narbonne, 31062 Toulouse, France
[c] CNRS UMR 5245, Ecolab, 31062 Toulouse, France
[d] IRD; MIVEGEC (IRD 224 CNRS 5290–UM1–UM2) Équipe BEES, 911, avenue Agropolis, BP 64501, 34394 Montpellier cedex 5, France

ABSTRACT

In the Neotropics where it was introduced, the invasive ant *Pheidole megacephala* counterattacked raids by the army ants *Eciton burchellii* or *E. hamatum*. The *Eciton* workers that returned to their bivouac were attacked and spread-eagled and most of them killed by their outgoing colony mates. Little by little the zone where returning and outgoing *Eciton* workers encountered one another moved away from the *Pheidole* nest which was no longer attacked, so that most of the colony was spared. Using a water-based technique rounded out by bioassays, we show that *Pheidole* compounds were transferred onto the *Eciton* cuticle during the counterattacks, so that outgoing workers do not recognize returning colony mates, likely perceived as potential prey. Because *P. megacephala* is an introduced African species, this kind of protection, which cannot be the result of coevolutive processes, corresponds to a kind of by-product due to its aggressiveness during colony defence.

© 2014 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

RÉSUMÉ

Quand introduite dans les Néotropiques, la fourmi invasive *Pheidole megacephala* contrattaque les raids des fourmis légionnaires *Eciton burchellii* ou *E. hamatum*. Les *Eciton* qui retournent à leur bivouac après avoir investi le nid de *Pheidole* sont attaquées et généralement tuées par les congénères qui arrivent. La zone de rencontre entre ces deux lots d'*Eciton* s'éloigne du nid de *Pheidole* qui finit par ne plus être attaqué, la plus grande partie de la colonie étant ainsi épargnée. L'utilisation d'une technique utilisant une émulsion d'eau, complétée par des bio-essais, montre que des composés venant des *Pheidole* sont transférés sur la cuticule des *Eciton* lors des contre-attaques. Ainsi, les *Eciton* sortant du bivouac ne reconnaissent pas leurs congénères comme telles. *P. megacephala* étant une espèce africaine introduite, cette défense indirecte ne résulte pas d'un processus coévolutif, mais correspond plutôt à un sous-produit dérivé de son agressivité durant la défense de la colonie.

© 2014 Académie des sciences. Publié par Elsevier Masson SAS. Tous droits réservés.

* Corresponding author at: CNRS UMR 8172, Écologie des forêts de Guyane, BP 316, 97379 Kourou cedex, France.
E-mail address: alain.dejean@wanadoo.fr (A. Dejean).

1. Introduction

Army ants are characterized by large colonies, nomadism and obligate group predation. The colony cycle of *Eciton burchellii* and *E. hamatum*, both epigaeic foraging Neotropical Ecitoninae, is characterized by two alternating phases. During the "statary" phase, the colonies reside in a fixed bivouac from which successive raids radiate away; the queen lays numerous eggs that hatch into larvae at the end of this phase. To feed these larvae, the colonies enter their "nomadic" phase, emigrating and forming new raids daily. As the larvae pupate, a new statary phase begins. Swarm-raiding *E. burchellii* workers form a main column whose front widens, creating a "carpet" of workers that fans out to a width of up to 20 m; the swarm front proceeds at a speed of ca. 15 m/h. The raids last more than 10 hours each day with workers continuously leaving the bivouac to join the front and then returning, sometimes carrying a reward, so that the flow of workers along the trails is bi-directional. The same is true for *E. hamatum*, but it is a column raider whose workers separate into small foraging groups and produce trails that branch out into a tree-like pattern [1–3].

Both species prey particularly on wasps and/or ants whose brood represents more than 50% of the *E. burchellii* prey and most of the *E. hamatum* diet. When they successfully raid an ant colony, they generally collect only the brood and callow workers, so that only a relatively minor proportion of older workers are injured or killed; surviving workers and queens, which are not generally injured, later re-establish the colony [1,2]. Workers from species with small or vulnerable colonies panic and carry a part of their brood away from the nest; the workers of some other species are aggressive toward army ants [4]. Among the latter, the African ant, *Pheidole megacephala*, is an invasive species that, after being introduced into the Neotropics, forms huge colonies depleting the prey available for colonies of native ant species and even raiding them [5–9]. Also, *P. megacephala* counterattacks raiding *Eciton* workers; when returning to their bivouac, these *Eciton* workers are spread-eagled and killed by outgoing colony mates [4].

In ants, nestmate recognition is based on chemical cues that are mainly a mixture of low-volatile cuticular hydrocarbons of genetic origin, but other compounds can be acquired from the environment [10]. During self- and allogrooming, trophallaxis and inter-individual contact, the workers continually gather their own chemicals and those of their colony mates in the postpharyngeal gland where they homogenize them into a mixture that is then redistributed. The resulting "colony odour", learned by colony members, represents a neural template, which is compared to the cuticular chemicals of encountered individuals, a mismatch generally resulting in aggressiveness [10].

In this study, we hypothesized that some compounds from *P. megacephala* are passed onto the cuticle of the raiding *Eciton* workers during the combats, so that they are not recognized by their outgoing colony mates. In keeping with the idea that simple, practical research approaches are needed to study the basic biology of social insects [11], we used a water-based technique permitting different compounds, including cuticular hydrocarbons, to be extracted from and transferred onto live ants (Fig. 1). The success of this process was previously demonstrated using gas chromatography-mass spectrometry analysis and bioassays [12].

2. Materials and methods

This study was conducted along the Caribbean coast of Quintana Roo, Mexico, around Puerto Morelos (20° 50′ 52.2″ N, 86° 52′ 41.3″ W) where the *E. burchellii* and *E. hamatum* density (i.e., 0.71 colony per hectare) is relatively high [13].

For those cases where we were able to observe *Eciton* workers leaving the *P. megacephala* galleries being attacked by their outgoing colony mates, we noted the details of the attacks. Each time we arrived early enough to witness the beginning of the *Eciton* raids on the *P. megacephala* colony, we noted the duration of time separating this moment from the first attacks on returning *Eciton* workers by their colony mates, and then between those first attacks and the end of this intracolonial slaughter. In six cases for both *E. burchellii* and *E. hamatum*, we noted the number of returning individuals killed during 10 two-minute-long periods of observation of the zones where the ants encountered one another. We then estimated the total number of individuals killed by their colony mates by extrapolating these results to the duration of the raids on the *P. megacephala* colonies.

We also conducted bioassays where we confronted each time an *E. burchellii* or an *E. hamatum* worker with colony mates from a column after it was soaked in an emulsion made using 30–50 *P. megacephala* workers. These workers were placed in a test tube containing 20 mL of ultrapure water (at ambient temperature or 30–35 °C) and vigorously shaken by hand for 5 min with the aim of emulsifying the cuticular compounds (other chemicals can also be added; see details of this water-based method in [12]). These ants were removed and the *Eciton* workers from the experimental lots were placed directly into the emulsion, shaken for 10 s, and left for 5 min (see A in Fig. 1). The *Eciton* workers from the control lot were placed into test tubes containing 20 mL of ultrapure water, shaken for 10 s, and then left in the water for 5 min (see B in Fig. 1). In both cases, the *Eciton* were then removed and put into a glass container where they dried for 20 min. The behavioural confrontations that followed consisted in placing the 'experimental' *Eciton* workers by the side of one of their colony's trails and noting their reactions, those of their colony mates in the column, and if they were killed or not (see bottom of Fig. 1). We used *E. burchellii* minor, media and major workers (30 cases each; the worker caste is highly polymorphic). The experiments were conducted using workers from six different raids for *E. burchellii* and two raids for *E. hamatum*. Note that this water-based method, previously used for ants and termites [12,14,15], is possible because many tropical ground-nesting ant species are adapted to flooding and do not drown when they are immersed [16].

All statistics were conducted using Fisher's exact-tests (Past 2.14 software).
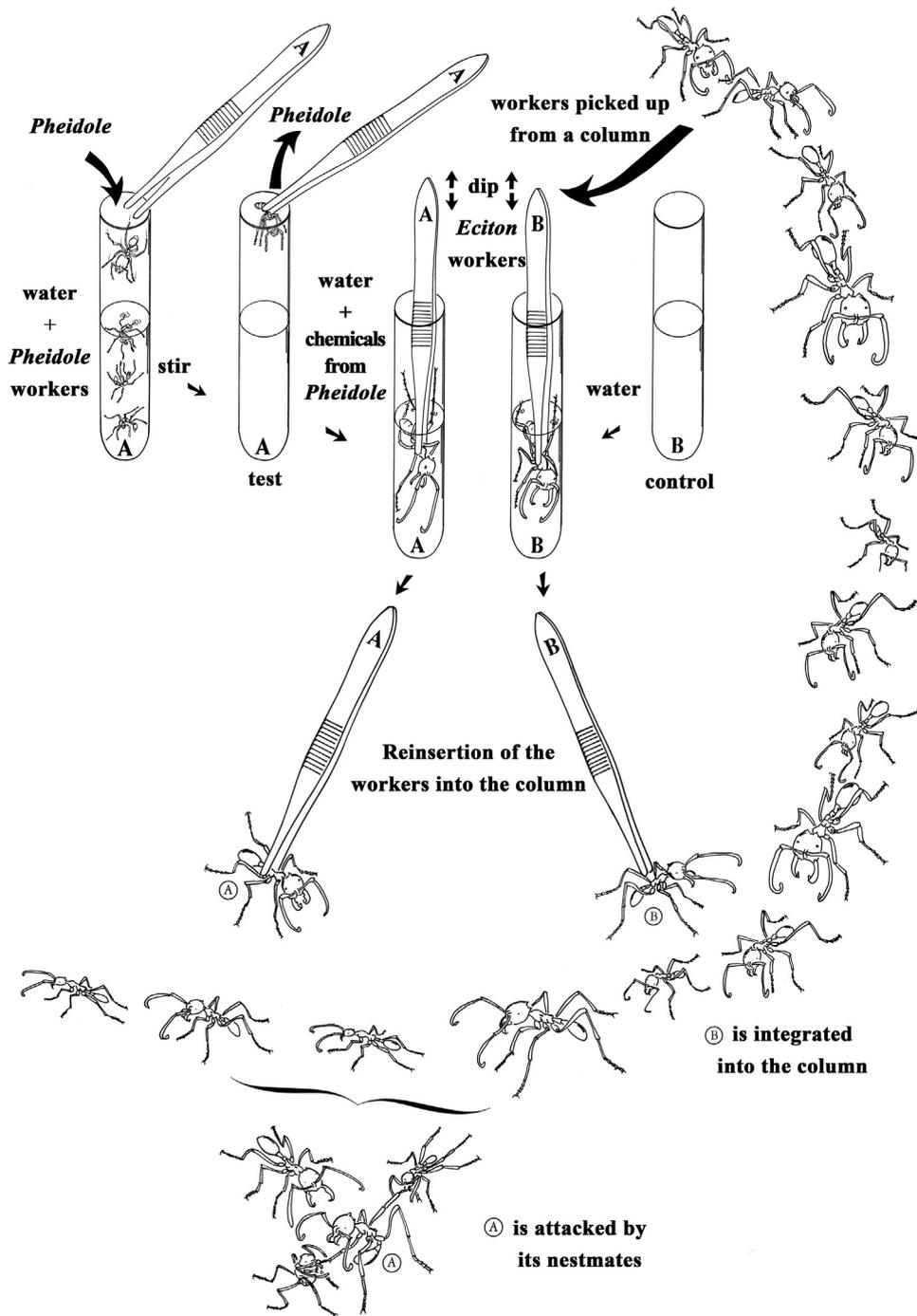
Fig. 1. Representation of the experimental set-up. At top left, the preparation of experimental *Eciton burchellii* workers, which are soaked in a water-based emulsion of the cuticular compounds of *Pheidole megacephala* workers. At top right, the preparation of *E. burchellii* from the control lot which were soaked in ultrapure water. At the bottom of the figure, representation of the release of the manipulated *E. burchellii* workers into their own column; those from the experimental lot were attacked, spread-eagled and killed by their colony mates, while those from the control lot entered the column without being attacked.

3. Results

3.1. Details of the attacks on the Pheidole megacephala colony and the consequences

Both *E. burchellii* and *E. hamatum* raided *P. megacephala* nests (24 and 11 cases observed, respectively) and, in all cases, they began by successfully plundering a part of the brood. Yet, both around their nest entrances and inside the nests (which we observed by lifting rocks and concrete slabs), the *P. megacephala* workers reacted by counter-attacking and spread-eagling numerous *Eciton* raiders.

When we arrived before the *Eciton* began the raids, we noted that the first returning *Eciton* workers leaving the *P. megacephala* nests were attacked by outgoing colony mates that spread-eagled them, killing most of them (see Table 1). Attacked returning individuals did not defend themselves but rather crouched, their antennae folded backward or adopted a posture where their thorax was vertical and gaster bent forward (bottom of Fig. 1).

The counterattacks by the *P. megacephala* workers kept the *Eciton* raiders inside their nest during a relatively long time as more than 30 min passed before the first individuals left the *Pheidole* nests; then, other *Eciton* individuals continued to leave the *Pheidole* nests during 40–50 min (details in Table 1). Meanwhile, the line of encounter between outgoing and returning *Eciton* workers moved away little by little from the *Pheidole* nest entrances. When the flow of returning *Eciton* workers stopped, the line of encounter with outgoing individuals was more than 10 m further from the *Pheidole* nests, and the column had already begun to move in a new direction as other prey were discovered. The *P. megacephala* nests

were finally abandoned because little by little the recruitment of new *E. burchellii* or *E. hamatum* workers ceased. So, the *P. megacephala* lost a part of their brood, but the core of the colony was spared.

During such raids, *E. burchellii* colonies lost ca. 1800 workers each time and *E. hamatum* ca. 1300 workers, representing between 0.24% and 0.67% of the worker force of the colonies (see details in Table 1).

3.2. Experiment using a water-based transfer of compounds

The *E. burchellii* workers from the control lot, previously soaked in water, easily returned to their columns when released (only one major was attacked but not killed), while all of those from the experimental lot, previously soaked in an emulsion made using *P. megacephala* workers, were spread-eagled. Only two of these workers survived whereas the corpses of the others were retrieved to the bivouac as were the prey; the differences between the control and experimental lots were significant for each sub-caste of workers ($P < 0.0001$; Fig. 2).

When re-introduced into their column, workers from the experimental lot crouched, their antennae folded backward or adopted a posture where their thorax is vertical and gaster bent forward (see again bottom of Fig. 1). Minor workers always crouched, while media and major workers crouched in 63.3% and 40% of the cases, respectively, or adopted a vertical position in the other cases ($n = 30$ for each sub-caste of workers). Then, these workers let themselves be attacked to death by their colony mates.

Very similar results were obtained using *E. hamatum* workers subjected to the same treatment as all 30 workers

Table 1

Duration of *Eciton burchellii* and *E. hamatum* raids on *Pheidole megacephala* nests, and estimation of the number of returning *Eciton* individuals killed by their outgoing colony mates after they left the *Pheidole* nests.

Eciton burchellii: 300,000 to 650,000 workers per colony [4]		
Total number of cases observed	24	
No. of cases analyzed for evaluation	17	
Duration of the raids[c]		
First phase[a]	34.1 ± 2.7 min	ca. 0 h 34′
Second phase[b]	46.7 ± 4.5 min	ca. 0 h 46′
Total	80.8 ± 3.9 min	ca. 1 h 21′
Mean no. of workers killed per series of observation[d]	76.77 ± 2.33	
Number of workers killed during the second phase[e]	From 1571 to 2025	Mean: 1792
% Workers killed based on 300,000 workers	From 0.52% to 0.67%	Mean: 0.60%
% Workers killed based on 650,000 workers	From 0.24% to 0.31%	Mean: 0.27%
Eciton hamatum: up to 250,000 workers per colony [4]		
Total number cases observed	11	
Number of cases analyzed for evaluations	6	
Duration of the raids[c]		
First phase[a]	36.5 ± 4.3 min	ca. 0 h 36′
Second phase[b]	40.7 ± 7.1 min	ca. 0 h 41′
Total	77.2 ± 6.5 min	ca. 1 h 17′
Mean number of workers killed per series of observation[d]	64.7 ± 2.0	
Number of workers killed during the second phase[e]	From 1052 to 1594	Mean: 1315
Percentage of workers killed based on 250,000 workers	From 0.42% to 0.64%	Mean: 0.52%

[a] First phase: from entering the *Pheidole megacephala* nest to the first attack on returning colony mates.
[b] Second phase: from the first attack on returning colony mates to the end of the raid.
[c] Means ± SE calculated from 17 cases for *Eciton burchellii* raids and from 6 cases for 11 *E. hamatum* raids.
[d] Means ± SE calculated from six cases; 10 series of 2-min observations each time.
[e] Evaluation of the number of returning workers killed by their colony mates calculated from the duration of the second phase.
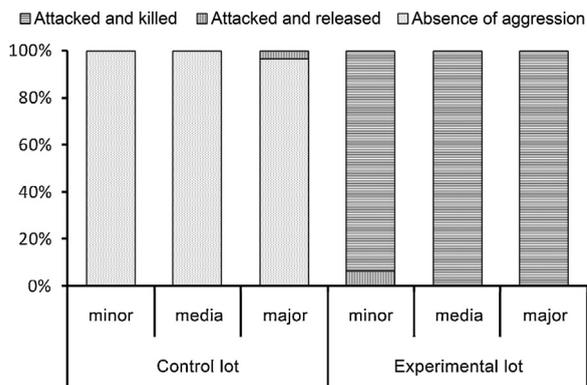
Fig. 2. Results of the fate of *Eciton burchellii* workers from the control (soaked in ultrapure water) and the experimental (soaked in an emulsion of cuticular compounds from *Pheidole megacephala* workers) lots. Each time minor, media and major workers were tested (30 cases each). Statistical comparisons: Fisher's exact-test, $P < 0.0001$ for each sub-caste of workers.

from the control lot returned safely to their columns, while all 30 from the experiment lot were spread-eagled (Fisher's exact-test: $P < 0.0001$).

4. Discussion

Here, we show that the aggressive reactions of the workers indirectly protect the *P. megacephala* colonies from raids by *E. burchellii* and *E. hamatum*, two of the main ant predators in the Neotropics. This aptitude stems from two of the most important characteristics known for invasive ant species in their introduced range: the ability to form huge colonies and a high level of aggressiveness towards native ants that are displaced or eliminated [5]. Indeed, due to its ability to develop huge colonies in areas where it has been introduced and its high predatory aptitude, *P. megacephala* can even raid native ant species [6,8]. Subsequently, when raiding *P. megacephala* nests, *Eciton* workers were kept inside these nests during a long time due to the combativeness of the counterattacking *P. megacephala* workers. This likely permits compounds to be transferred onto the cuticle of the raiding *Eciton* workers. So, it is because returning *Eciton* workers were attacked and killed by outgoing colony mates that the *P. megacephala* colony was spared. This corresponds to a kind of by-product benefit for *P. megacephala* due to the different characteristics of the colonies in their introduced range as no coevolutive processes are involved in this case (see also [17]). Yet, one can note that, in their native African range, *P. megacephala* workers react very aggressively to *Dorylus* driver ant raids (the Old World equivalent of army ants), but, here, the reaction is related to predatory behaviour. Indeed, the *Dorylus* workers are raided, spread-eagled and retrieved to be eaten (A.D. pers. obs.; [8]), the same has been noted for other African ant species [18,19].

Our simple, water-based experiment permits us to argue that some *P. megacephala* chemicals are indeed transferred onto the *Eciton* workers' cuticle during the counterattacks occurring during raids. These transferred compounds disturb the recognition system of the *Eciton*

workers to the point that they mistakenly attack and kill colony mates having experimentally acquired *P. megacephala* compounds. This transfer of chemicals is reminiscent of the case of slave-making *Polyergus breviceps* queens, which kill the queens of their *Formica* host colony. Then, they are adopted by the host workers because the chemical signatures of the *Formica* host queens are transferred onto the parasite queens during their aggressive interaction [20]. Note that colony mate recognition is sensitive to only certain modifications of the cuticular signature. For instance, in the Argentine ant, adding one synthetic methylated alkane to an individual confronted with colony mates elicits their aggressiveness if this compound has a different branch position, but not if it has the same branch position, even if the chain length is different [21].

One can note that, in addition to cuticular compounds, defensive chemicals might be transferred onto the *Eciton* cuticle when raiding *P. megacephala* nests. For instance, traces of *Solenopsis saevissima* venom in *Camponotus blandus* cuticular extracts were noted during a water-based experiment [12]. Because they have atrophied stingers, it has been suggested that *P. megacephala* workers release offensive secretions other than venom [6–8]. The pygidial gland is a good candidate for producing such compounds because in *P. biconstricta* it produces repellent and irritant components [22,23].

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References

[1] S. Powell, N.R. Franks, Ecology and the evolution of worker morphological diversity: a comparative analysis with *Eciton* army ants, Funct. Ecol. 20 (2006) 1105–1114.

[2] D.J.C. Kronauer, Recent advances in army ant biology (Hymenoptera: Formicidae), Myrmecol. News 12 (2009) 51–65.

[3] S.K. Willson, R. Sharp, I.P. Ramler, A. Sen, Spatial movement optimization in Amazonian *Eciton burchellii* army ants, Insect. Soc. 58 (2011) 325–334.

[4] A. Dejean, B. Corbara, O. Roux, J. Orivel, The antipredatory behaviours of Neotropical ants towards army ant raids (Hymenoptera: Formicidae), Myrmecol. News 19 (2013) 17–24.

[5] D. Holway, L. Lach, A.V. Suarez, N.D. Tsutsui, T.J. Case, The causes and consequences of ant invasions, Annu. Rev. Ecol. Syst. 33 (2002) 181–233.

[6] A. Dejean, M. Kenne, C.S. Moreau, How predatory abilities favor the success of the invasive ant *Pheidole megacephala* in an introduced area, J. Appl. Entomol. 131 (2007) 625–629.

[7] A. Dejean, C.S. Moreau, P. Uzac, J. Le Breton, M. Kenne, The predatory behavior of *Pheidole megacephala*, C.R. Biologies 330 (2007) 701–709.

[8] A. Dejean, C.S. Moreau, M. Kenne, M. Leponce, The raiding success of *Pheidole megacephala* on other ants in both its native and introduced ranges, C.R. Biologies 331 (2008) 631–635.

[9] J.K. Wetterer, Worldwide spread of the African big-headed ant, *Pheidole megacephala* (Hymenoptera: Formicidae), Myrmecol. News 17 (2012) 51–62.

[10] P. d'Ettorre, A. Lenoir, Nestmate recognition, in: L. Lach, C.L. Parr, K.L. Abbott (Eds.), Ant Ecology, Oxford University Press, Oxford, 2010, pp. 194–209.

[11] W.R. Tschinkel, Back to basics: sociometry and sociogenesis of ant societies (Hymenoptera: Formicidae), Myrmecol. News 14 (2011) 49–54.

[12] O. Roux, J.-M. Martin, N. Tene Ghomsi, A. Dejean, Non-lethal water-based removal-reapplication technique for the cuticular compounds of ants, J. Chem. Ecol. 35 (2009) 904–912.

[13] S. Durou, A. Dejean, I. Olmsted, R.R. Snelling, Ant diversity in coastal zones of Quintana Roo, Mexico, with special reference to army ants, Sociobiology 40 (2002) 385–402.

[14] A. Dejean, B. Corbara, J. Oliva-Rivera, Mise en évidence d'une forme d'apprentissage dans le comportement de capture des proies chez *Pachycondyla (= Neoponera) villosa*, (Formicidae, Ponerinae), Behaviour 115 (1990) 175–187.

[15] O. Roux, V. Rossi, R. Céréghino, A. Compin, J.-M. Martin, A. Dejean, How to coexist with fire ants: the roles of behaviour and cuticular compounds, Behav. Proc. 98 (2013) 51–57.

[16] N.J. Mlot, C.A. Tovey, D.L. Hu, Fire ants self-assemble into waterproof rafts to survive floods, Proc. Natl. Acad. Sci. USA 108 (2011) 7669–7673.

[17] J.F. Bruno, J.D. Fridley, K.D. Bromberg, M.D. Bertness, Insights into biotic interactions from studies of species invasions, in: D.F. Sax, J.J. Stacho-wicz, S.D. Gaines (Eds.), Species Invasions: Insights into Ecology, Evolution and Biogeography, Sinauer Associates, Sunderland, 2005, pp. 9–40.

[18] A. Dejean, B. Corbara, O. Orivel, M. Leponce, Rainforest canopy ants: the implications of territoriality and predatory behaviour, Funct. Ecosyst. Comm. 1 (2007) 105–120.

[19] N. Adgaba, A.G. Shenkute, A.A. Al-Ghamdi, A. Assefa, M.J. Ansari, B. Taylor, S. Radloff, *Crematogaster chiarinii* ants as a potential biological control agent for protecting honeybee colonies from attack by *Dorylus* quadrates driver ants in Ethiopia (Hymenoptera: Formicidae), Agric. Forest Entomol. (2014), http://dx.doi.org/10.1111/afe.12060.

[20] C.A. Johnson, R.K. Vander Meer, B. Lavine, Changes in the cuticular hydrocarbon profile of the slave-maker ant queen, *Polyergus breviceps* emery, after killing a *Formica* host queen (Hymenoptera: Formicidae), J. Chem. Ecol. 27 (2001) 1787–1804.

[21] E. van Wilgenburg, R. Sulc, K.J. Shea, N.D. Tsutsui, Deciphering the chemical basis of nestmate recognition, J. Chem. Ecol. 36 (2010) 751–758.

[22] C. Kugler, Alarm and defense: a function for the pygidial gland of the myrmicine ant, *Pheidole biconstricta*, Ann. Entomol. Soc. Am. 72 (1979) 532–536.

[23] D.W. Davidson, D.A. Clark, T.H. Jones, Gastral exocrine products of a myrmicine ant strongly overlap pygidial gland products of Dolicho-derinae, Insect. Soc. 52 (2005) 305–308.