



Ecology/Écologie

Reactions by army ant workers to nestmates having had contact with sympatric ant species

*Réactions de fourmis légionnaires à des congénères ayant été en contact avec des espèces sympatriques*Alain Dejean ^{a,b,*}, Bruno Corbara ^{c,d}^a CNRS, Écologie des forêts de Guyane (UMR-CNRS 8172), 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 6023, Laboratoire Microorganismes, Génome et Environnement, Université Blaise-Pascal, Complexe scientifique des Cèzeaux, 63177 Aubière cedex, France^d Clermont Université, Université Blaise-Pascal, LMGE, BP 10448, 63000 Clermont-Ferrand, France

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ABSTRACT

It was recently shown that *Pheidole megacephala* colonies (an invasive species originating from Africa) counterattack when raided by the army ant, *Eciton burchellii*. The subsequent contact permits *Pheidole* cuticular compounds (that constitute the “colony odour”) to be transferred onto the raiding *Eciton*, which are then not recognised by their colony-mates and killed. Using a simple method for transferring cuticular compounds, we tested if this phenomenon occurs for Neotropical ants. *Eciton* workers rubbed with ants from four sympatric species were released among their colony-mates. Individuals rubbed with *Solenopsis saevissima* or *Camponotus blandus* workers were attacked, but not those rubbed with *Atta sexdens*, *Pheidole fallax* or with colony-mates (control lot). So, the chemicals of certain sympatric ant species, but not others, trigger intra-colonial aggressiveness in *Eciton*. We conclude that prey-ant chemicals might have played a role in the evolution of army ant predatory behaviour, likely influencing prey specialization in certain cases.

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R É S U M É

Il a été montré dernièrement que les colonies de la fourmi invasive *Pheidole megacephala* contrattaquent lors de raids par les fourmis légionnaires *Eciton burchellii*. Les contacts qui en résultent permettent le transfert de composés cuticulaires (ils constituent l'odeur coloniale) des *Pheidole* vers les *Eciton*. Ces dernières ne sont plus reconnues par leurs congénères, qui les tuent. Utilisant une méthode simple pour transférer des composés cuticulaires, nous avons testé si ce phénomène existait pour des fourmis néotropicales. Des *Eciton* frottées avec des ouvrières *Solenopsis saevissima* ou *Camponotus blandus* ont été attaquées par leurs congénères, mais pas celles frottées avec des *Atta sexdens*, des *Pheidole fallax* ou des congénères (lot contrôle). Ainsi, les composés cuticulaires de certaines

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espèces sympatriques déclenchent l'agressivité entre ouvrières *Eciton* s'ils sont transférés, alors que d'autres sont sans effet. Nous concluons que ces composés pourraient avoir joué un rôle dans l'évolution du comportement prédateur chez les fourmis légionnaires.

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1. Introduction

In ants, colony-mate recognition is based on chemical cues, mainly a mixture of low-volatile cuticular hydrocarbons of genetic origin, but other compounds can be acquired from the environment. During self- and allo-grooming and trophallaxy, the workers continually gather their own compounds and those of their colony-mates in the postpharyngeal gland where they homogenize them into a mixture, or “colony odour”, which is then spread onto their cuticle. This colony odour is learned by the colony members, becoming a neural template that they compare to the cuticular compounds (CCs) of encountered individuals; a mismatch generally results in aggressiveness [1].

Eciton burchellii is an epigaeic-foraging, Neotropical army ant characterized by large colonies, nomadism and obligate group predation during raids consisting of a main column whose front widens to up to 20 m. Daily raids last more than 10 hours with workers continuously leaving the bivouac to join the front and then returning, some of them carrying prey, so that the flow of workers along the trails is bi-directional. This species preys mostly on wasp and ant brood, with ant brood representing more than 50% of its diet. When raiding a prey-ant colony, *E. burchellii* workers collect only the brood and callow workers, so that almost all older workers and queens are neither killed nor injured and later re-establish the colony [2,3]. Workers from many prey-ant species run away (likely due to the perception of a propaganda-like substance, as is known in slave-making ant raids; see [4]) and carry a part of their brood away from the nest, so that they are seldom in contact with the raiding *Eciton*. The workers of some other species are aggressive toward army ants; among them, *Pheidole megacephala*, an invasive species of African origin, counterattacks *Eciton* raids [3]. When returning to their bivouac, these *Eciton* workers are killed by outgoing colony-mates. Indeed, whereas the *P. megacephala* workers' defence is primarily based on the fact that their huge colonies permit them to mount efficacious counterattacks, it has also been shown that their CCs are transferred from their cuticle onto that of the *Eciton* raiders through worker-to-worker contact, so that outgoing *Eciton* workers mistake their returning colony-mates for prey and kill them [3,5].

One may ask if this transfer of CCs and possibly other chemicals triggering intra-colonial aggressiveness in *Eciton* exists only for *P. megacephala* (which is an introduced, invasive species), or if such transfer also exists during raids on Neotropical ants. Indeed, in French Guiana, we noted that some *E. burchellii* workers were attacked by their colony-mates after raiding mounds of the native ant species, *Solenopsis saevissima*. So, we hypothesized that

E. burchellii workers may be sensitive to alterations in the cuticular cues due to contact with workers from certain prey species. These alterations lead to mismatches in colony-mate recognition and may exert a selective pressure on army ants.

2. Materials and methods

This study was conducted at the field station at *Petit Saut*, Sinnamary, French Guiana (5°03'39" N; 53°02'36" W) in July 2013.

Prior to the experiments, we gathered parts or entire colonies of four ground-nesting ant species: *Atta sexdens*, *Pheidole fallax*, *S. saevissima* and *Camponotus blandus*. They were selected because they are commonly found along forest edges where *E. burchellii* raids are frequent and because the size of their workers permits easy manipulation if compared with the much smaller workers of *Crematogaster tenuicula* and *Wasmannia auropuncta*, two other species frequent in this habitat [6,7]. With colonies of 5–8 million workers, *A. sexdens* is a leaf-cutting, fungus-growing ant, while the three other species are generalist foragers [6–8]. *S. saevissima*, whose workers forage mostly nocturnally, forms supercolonies extending over up to 54 km in its native range in French Guiana [6–9]. In *C. blandus* colonies, consisting of ca. 6000 individuals, the workers forage during the hottest hours of the day, whereas *P. fallax* colonies, containing fewer than 1000 workers, are active all around the clock [6–8].

We placed these ants in large plastic boxes with earth from their nests and transported everything to the laboratory. There, we opened the boxes (whose sides were covered with Fluon[®] to prevent the workers from escaping), furnished these ants with pieces of cotton imbibed with water and with diluted honey and allow them to calm down during one night (they rearranged the earth in the boxes, grouped the brood and proceeded with numerous acts of self- and allo-grooming). The next day, we delicately placed the boxes in a freezer where the workers progressively became numb. We took these precautions to limit the possibility that defensive compounds remained on the workers' cuticle (some may have been released while we were gathering them). We then looked for an *E. burchellii* bivouac from which we gathered several hundred workers plus leaf-litter. We placed these ants and the leaf-litter in a large, smooth-walled plastic box, transported the box to the laboratory, and waited until the workers had calmed down.

In keeping with the idea that simple, practical research approaches are needed to study the basic biology of social insects [10], we used the simplest possible technique to transfer cuticular compounds onto live ants, which

consisted of rubbing a given *Eciton* individual with the thorax from a different worker ant [11,12]. After trying this method during a preliminary study conducted in the field on three *E. burchellii* raids, we adapted it for use in laboratory conditions. To do so, we picked up an *E. burchellii* worker using a pair of supple forceps without perturbing its colony-mates. Then, using a second pair of forceps, we picked up a heterospecific worker (which we had allowed to thaw) and rubbed its thorax against the *E. burchellii* worker during 2 min (for small *Pheidole* and *Solenopsis* workers, two successive individuals were used). The *E. burchellii* workers were then released among their colony-mates and we noted if they were admitted, antennated (i.e., successive touching with antennae as a sensory probe [8]), pursued or bitten. The same process was undertaken for each sympatric ant species tested and for a control lot, for which we used an *E. burchellii* colony-mate ($n = 30$ in all cases).

Statistics were conducted using Fisher's exact-tests (GraphPad Prism software) and the 'false discovery rate' adjustment (FDR) (BY correction; [13]) for simultaneous comparisons.

3. Results and discussion

When we reintroduced 'manipulated' workers among their colony-mates, those from the control lot were never attacked. The difference with the control lot was not significant for *E. burchellii* workers rubbed with *A. sexdens* or *P. fallax* individuals, while we noted a significantly greater level of aggressiveness toward those rubbed with *S. saevissima* and *C. blandus* individuals (Fig. 1).

The clear differences in the reactions toward experimented colony-mates show that our very simple technique (having rubbed the ants with the thoraxes of colony-mates

or of workers from different species) is sufficient for such studies (on the condition that basic precautions are taken). This indeed suggests the transfer of some chemicals (likely, mostly CCs) from potential prey-ants to *E. burchellii* individuals. The effects of such a transfer have been discussed in the literature. For instance, adding a dimethylated alkane to the *Camponotus herculeanus* cuticle elicits aggressiveness from nestmates, but not a mono-methylated nor a linear alkane [14]; in the Argentine ant, *Linepithema humile*, adding a methylated alkane to an individual's cuticle elicits its nestmates' 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 [15].

When *E. burchellii* raid ant species with small colonies (the case for *P. fallax* and *C. blandus* [6–8]), the only effective response for the prey-ant is to escape: they lose most of their brood and callow workers, but the society is safe and able to recover. A counterattack at the colony level would be "suicidal" and therefore such a strategy could not have exerted selective pressure. Therefore, even when transferred CCs trigger intra-colonial aggressiveness in *E. burchellii* (as experimentally shown in the case of *C. blandus*), such an event is rare in nature (observed in Mexico during raids on *Camponotus* spp. colonies; AD, pers. obs.). Indeed, in natural conditions, the raided workers evacuate their nests and *E. burchellii* workers gather only the brood and callow workers that have not yet acquired the CCs responsible for the colony odour [16]; contact with "old" raided workers is extremely rare. Moreover, for species with small colonies, the number of raiding army ant workers is so great that the CC transfer is "diluted" and thus cannot become a defence mechanism.

When army ants raid ant species with large colonies, the two species being evenly matched, a counterattack by the raided ants may be an effective strategy as observed for the invasive ant, *P. megacephala*, whose workers react aggressively to the presence of *E. burchellii*. This promotes physical contact between workers that facilitates the transfer of CCs from the raided workers to *E. burchellii* raiders and consequently triggers intra-colonial aggressiveness among the raiding army ants. Those previously in contact with *P. megacephala* workers are mistakenly attacked by their outgoing colony-mates when they return to their bivouac; they are then spread-eagled and killed [5]. This corresponds to a kind of by-product benefit for *P. megacephala* because this is not the result of a coevolutionary process. Among Neotropical ants with large colonies, some instances of intra-colonial aggressiveness among *E. burchellii* colony-mates have been observed after contact with *S. saevissima* individuals, but the core of the individuals from the attacked mound move (polydomous colonies; AD, pers. obs.; this study). This corroborates the fact that *S. saevissima* workers react aggressively to *E. burchellii* CCs [6]. Yet, because most individuals evacuate their nest during *E. burchellii* raids, the situation is relatively similar to that of most ant species having small colonies. Similarly, when raided by *Neivamyrmex compressinodis*, the huge *W. auropunctata* colonies evacuate their nests due to a propaganda effect [17].

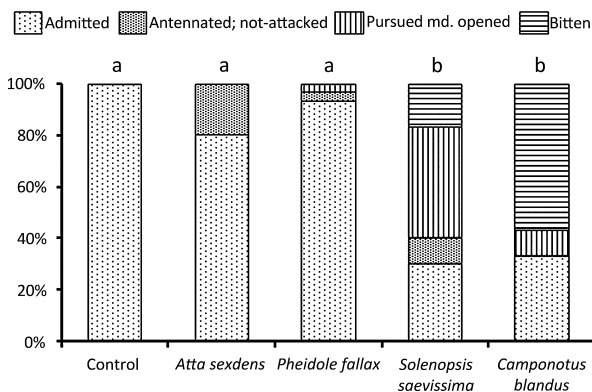


Fig. 1. The fate of *Eciton burchellii* workers from the control (rubbed with the thoraxes of colony-mates for ~2 min) and the experimental lots (rubbed with the thoraxes of heterospecific workers during ~2 min) when introduced into the bivouac of colony-mates ($n = 30$ in all cases). Statistical comparison: Fisher's exact-tests and false discovery rate' adjustment (BY correction); different letters indicate significant differences at $P < 0.001$. Pursued md. opened: the experimented *E. burchellii* worker, which had received cuticular compounds from alien ant species, is itself perceived as an alien by its colony-mates which pursued it their mandibles wide open, ready to bite (this is a level of aggressiveness lower than biting [6,8]).

When the transfer of CCs does not trigger intra-colonial aggressiveness among raiding *E. burchellii*, as for *A. sexdens*, there is no selection pressure to avoid contact with these prey-ants. Yet, these ants are not preyed upon by *Eciton* spp., all generalist predators of ant brood, due to an unknown reason (likely related to CCs). Nevertheless, the army ant, *Nomamyrmex esenbeckii*, is specialized in *Atta* spp. predation although the colonies of the latter contain several million workers, many of them counterattacking and so generating numerous physical contacts with the raiders. It is likely that the transfer of *Atta* chemicals does not trigger any intra-colonial aggressiveness between *N. esenbeckii* individuals (as in the present study) because this fact has never been reported by those having witnessed these raids [18–22].

In conclusion, we have shown differences between potential prey-ants of *E. burchellii* relative to the transfer of chemicals. Because both a transfer of chemicals from prey-ants and the aggressiveness between colony-mates may occur in nature during army ant raids, chemicals from prey-ants likely had a role in the evolution of army ant predatory behaviour. Further research is needed to confirm this hypothesis and to verify what compounds are transferred.

Disclosure of interest

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

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