

Coexistence between *Cyphomyrmex* ants and dominant populations of *Wasmannia auropunctata*

Julien Grangier*, Julien Le Breton, Alain Dejean, Jérôme Orivel

Laboratoire Evolution et Diversité Biologique, UMR-CNRS 5174, Université Toulouse III, 31062 Toulouse Cedex 9, France

Received 16 September 2005; received in revised form 17 October 2006; accepted 17 October 2006

Abstract

The little fire ant *Wasmannia auropunctata* is able to develop highly dominant populations in disturbed areas of its native range, with a resulting negative impact on ant diversity. We report here on the tolerance of such populations towards several fungus-growing ants of the genus *Cyphomyrmex* (*rimosus* complex) in French Guiana. This tolerance is surprising given the usually high interspecific aggressiveness of *W. auropunctata* when dominant. In order to understand the mechanisms behind such proximity, aggressiveness tests were performed between workers of the different species. These behavioural assays revealed a great passivity in *Cyphomyrmex* workers during confrontations with *W. auropunctata* workers. We also found that the aggressiveness between *W. auropunctata* and two *Cyphomyrmex* species was more intense between distant nests than between adjacent ones. This dear–enemy phenomenon may result from a process of habituation contributing to the ants' ability to coexist over the long term.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Aggressiveness; *Cyphomyrmex*; Dear–enemy phenomenon; Habituation; *Wasmannia auropunctata*

1. Introduction

Native to the Neotropics, the little fire ant *Wasmannia auropunctata* (Roger) (Myrmicinae) is one of the most problematic invasive ants known, with accompanying ecological and economical consequences (Holway et al., 2002). It is most notably present on numerous Pacific Islands, in West Central Africa, and in the southern United States, where it forms huge unicolonial populations that have a negative impact on native faunal diversity, especially ants, thanks to both exploitative and interference competition (Clark et al., 1982; Wetterer and Porter, 2003; Le Breton et al., 2003, 2005).

In its native range, while it is a common but non-dominant species in primary forests, population explosions can occur locally in disturbed areas and affect the overall ant diversity (Majer et al., 1994; Tennant, 1994; Armbrrecht and Ulloa Chacón, 2003; Le Breton, 2003). While working in French Guiana, within the native range of the little fire ant, we often found nests of *Cyphomyrmex* ants (Myrmicinae) in close proximity with such dominant *W. auropunctata* colonies. In the

present study, we evaluate the possible mechanisms behind this coexistence.

2. Materials and methods

2.1. Study sites and sampling

In February and March 2004, we collected ant colonies from disturbed sites in Petit Saut, French Guiana (4°59'N, 53°08'W). We prospected three different sites (i.e., a secondary forest, a forest fragment and roadsides), all dominated for the past several years by large *W. auropunctata* populations, which locally tend to adopt a unicolonial organization (Le Breton, 2003). At each of the three sites, the *W. auropunctata* workers tolerated members of their own population (even those from different nests), but were aggressive towards other ants. We collected six *Cyphomyrmex* nests, each with the nearest *W. auropunctata* nest located at the most several tens of centimetres away (adjacent nests). Finally, we also collected *W. auropunctata* workers from a distant (≈30 km) coffee plantation dominated by this species (distant nests). The latter population also had a unicolonial organization as did the *W. auropunctata* populations originating from the disturbed areas. All of the colonies were then placed into artificial nests.

* Corresponding author. Fax: +33 5 61 55 73 27.
E-mail address: grangier@cict.fr (J. Grangier).

All of the fungus-growing ants belong to the *Cyphomyrmex rimosus* complex. Two major revisions were not enough to completely resolve the taxonomic confusion existing among the different cryptic species of this group (Kempf, 1966; Snelling and Longino, 1992). Nevertheless, the attentive examination of the *Cyphomyrmex* individuals revealed *a posteriori* that we were in the presence of three species: *C. major* (one nest), *C. minutus* (three nests) and *C. nr. minutus* (two nests).

2.2. Aggressiveness tests

In order to quantify aggressiveness between *W. auropunctata* and the fungus-growing ants, we adapted a well-known protocol commonly employed in such studies (Suarez et al., 1999; Tsutsui et al., 2003). One randomly selected worker from each species was placed in a neutral arena (diameter = 1.5 cm, height = 0.7 cm) whose walls were coated with Fluon® to prevent the ants from climbing out. The test began at the first interaction and continued for 5 min during which all interactions were scored with the following indexes—1: short antennation (≤ 1 s); 2: long antennation (≥ 1 s); 3: threatening postures with open mandibles or quick bites; 4: fighting (prolonged biting and eventually stinging). The interactions were timed, and the species initiating the aggressive acts (levels 3 and 4) was also noted (but it was often impossible to know with certainty which species initiated the antennations). For each tested pair, 15 trials were performed using different individuals each time.

We confronted workers from the six *Cyphomyrmex* nests with those from adjacent *W. auropunctata* nests, then with those from a distant population originating from the coffee plantation. The same tests were set up between *W. auropunctata* workers and those from distant *Crematogaster* sp. and *Pheidole fallax* nests (one nest of each) to serve as a reference. These two Guianian ant species are sympatric with *W. auropunctata*, but their nests are never adjacent to those of the latter species.

To analyse the results, we used an aggressiveness index calculated with the following formula (Errard and Hefetz, 1997):

$$\frac{\sum_{i=1}^n \delta_i t_i}{T}$$

where δ_i and t_i are the interaction index and duration of each act, respectively, and T is the total interaction time defined as the sum of time during which the ants were in physical contact. This index was used in two different ways.

First, we compared the results of the tests between adjacent and distant *W. auropunctata* and *Cyphomyrmex* nests. In this case, all the interactions (levels 1–4) were used to calculate the aggressiveness index. Because most of the interactions were initiated by *W. auropunctata* workers, the overall aggressiveness exhibited during each encounter was a good reflection of *W. auropunctata*'s aggressiveness.

A second type of analysis was necessary to compare the results obtained between *W. auropunctata* workers and those from the distant nests of the three *Cyphomyrmex* species, *Crematogaster* sp. and *P. fallax*, since, the two latter species initiated a quite significant number of aggressive acts. Because, we were

unable to determine which species initiated the antennations, we calculated an aggressiveness index based only on the aggressive acts (levels 3 and 4) initiated by *W. auropunctata*.

For statistical comparisons we used non-parametric tests (GraphPad Prism 4.00 software), in particular Wilcoxon's test to compare the results of confrontations between *W. auropunctata* and *Cyphomyrmex* species. Indeed, although different individuals were confronted between trials, we considered as paired the tests between adjacent and distant nests because *Cyphomyrmex* workers came from the same nest each time, and so were related.

3. Results

Behavioural assays pointed out the great passivity of the *Cyphomyrmex* workers when confronted with *W. auropunctata*. In most cases, the slightest contact led the fungus-growing ants to adopt a submissive attitude, remaining totally motionless with their antennae folded in their scrobes for anywhere from a few seconds to several minutes. Confronted with this behaviour, *W. auropunctata* generally ignored the fungus-growing ants, sometimes after some biting. As a result, nearly all of the aggressive acts (levels 3 and 4) were initiated by *W. auropunctata* during encounters between adjacent nests, as well as between distant ones. Among the 239 aggressive acts recorded during all of the encounters, only two were initiated by *Cyphomyrmex* workers (the three *Cyphomyrmex* species were pooled).

For the confrontations between *W. auropunctata* and *C. minutus* or *C. nr. minutus*, the global aggressiveness index was significantly lower during the tests involving workers originating from adjacent nests than for those from distant nests, but this was not the case for the confrontations with *C. major* (Fig. 1).

Finally, the aggressiveness expressed by *W. auropunctata* was not significantly different during the ant's confrontations with distant *Cyphomyrmex*, *Crematogaster* sp. and *P. fallax*. The proportion of replicate trials during which *W. auropunctata* workers initiated aggressive acts was not different regardless of the species encountered. In addition, when aggressive acts took

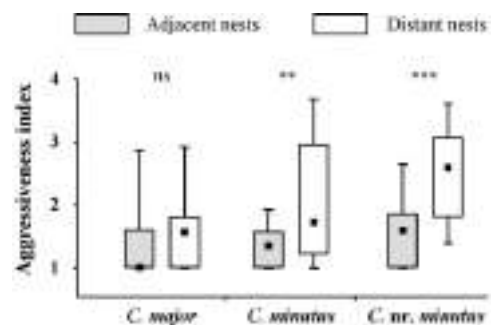


Fig. 1. Aggressiveness index based on all the interactions recorded during confrontations between *Wasmannia auropunctata* and the three *Cyphomyrmex* species. Filled squares represent median values. Error-bars above and below the boxes indicate the 90th and 10th percentiles, and the ends of the boxes indicate the 25th and 75th percentiles. The sample size of "adjacent nests" and "distant nests" tests were each time identical: *C. major* ($n=15$), *C. minutus* ($n=45$) and *C. nr. minutus* ($n=30$). Significant difference at ** $p < 0.01$ and *** $p < 0.001$, respectively (Wilcoxon test). For *C. major*: $W = -16$, $p = 0.52$; for *C. minutus*: $W = -541$, $p = 0.0017$; and for *C. nr. minutus*: $W = -391$, $p < 0.0001$.

Table 1

Aggressiveness expressed by *Wasmannia auropunctata* towards distant *Cyphomyrmex*, *Crematogaster* sp. and *P. fallax* workers

	Species confronted with <i>W. auropunctata</i> (all distant nests)					Statistical and <i>p</i> -value
	<i>C. major</i>	<i>C. minutus</i>	<i>C. nr. minutus</i>	<i>Crematogaster</i> sp.	<i>P. fallax</i>	
Frequency	40% (6/9)	55.6% (25/20)	76.7% (23/7)	60% (9/6)	53.3% (8/7)	$\chi^2 = 6.542, p = 0.16$
Index	3 (3–3.64)	3.46 (3–3.82)	3.44 (3–3.76)	3.67 (3.07–4)	3.18 (3.02–3.65)	$H = 3.586, p = 0.47$

The two compared parameters are—(1) “frequency”: proportion of encounters during which *W. auropunctata* initiated at least one aggressive act. Values are given as follows: percentage (number of encounters with/without aggressive acts). Statistical and *p*-values for the χ^2 -test are given and (2) “index”: aggressiveness index based on the aggressive acts (levels 3 and 4) initiated by *W. auropunctata*. Values are given as follows: median (Q25–Q75) (the sample size is the number of encounters with aggressive acts indicated for the preceding parameter). Statistical and *p*-values for the Kruskal–Wallis test are given.

place, they did not differ significantly as shown by *W. auropunctata*'s aggressiveness index (Table 1). Note that contrary to *Cyphomyrmex* workers, *Crematogaster* sp. and *P. fallax* workers initiated a significant part of the overall number of aggressive acts (22.6 and 9.5%, respectively).

4. Discussion

This study confirms the tolerance exhibited between neighbouring *Cyphomyrmex* and *W. auropunctata* nests, a fact that was pointed out about two nests in Mexico by Weber (1947), who specified that “they were probably not hostile”. In our case, the proximity between the nests of the two species is noteworthy because the phenomenon takes place in areas highly dominated by *W. auropunctata* (where we have never found the same high level of proximity with any other ant species), and because of the highly aggressive nature of *W. auropunctata* when it invades disturbed areas of its native range (Le Breton, 2003).

A first possible hypothesis explaining this situation concerns the passive behaviour of the *Cyphomyrmex* workers, which is illustrated by the very few acts of aggressiveness they initiated and the submissive posture they frequently adopted. In Florida, Morrison and Porter (2003) showed that *C. rimosus* is one of the most abundant ants in some of the areas invaded by *Solenopsis invicta*. Such discreet behaviour could be part of a general strategy allowing the *Cyphomyrmex* species to remain in areas dominated by highly competitive ants, as has been observed between dominant and subordinate ants in arboreal ant mosaics (see Mercier, 1999, and references cited therein). In fact, it appears that encounters with distant *Cyphomyrmex* nests triggered in *W. auropunctata* the same level of aggressiveness as did encounters with very competitive species like *Crematogaster* sp. and *P. fallax*. Workers of both latter species, that react or attack in turn, probably trigger group attacks by *W. auropunctata* under natural conditions. On the contrary, the passivity of the attacked *Cyphomyrmex* workers incites their aggressors to abandon them and possibly not recruit nestmates, partially explaining the presence of *Cyphomyrmex* colonies in areas invaded by *W. auropunctata*.

Our study provides another possible explanation for two of the three *Cyphomyrmex* species: contact with workers from the same *C. minutus* or *C. nr. minutus* nest clearly leads to a more intense aggressiveness in *W. auropunctata* workers from distant nests than from adjacent ones. This suggests that a dear–enemy phenomenon facilitates the cohabitation between these fungus-

growing ants and *W. auropunctata*. In this phenomenon, which has been described in a wide variety of animal species, individuals express a more tolerant behaviour towards neighbours than towards aliens (Temeles, 1994). Habitually, this phenomenon is used to explain interactions between conspecific or sometimes congeneric individuals, which thereby have very similar or identical needs (in ants, see Jutsum et al., 1979; Heinze et al., 1996; Langen et al., 2000). However, in our case, the cohabitation of heterogeneric species takes place in a particular context which makes this concept appropriate. Indeed, it has been shown that large populations of *W. auropunctata* can monopolize virtually all resources, which inevitably places every alien ant species into the category of “enemy” (Armbrecht and Ulloa Chacón, 2003; Le Breton et al., 2005). In our study, this is true for the *Cyphomyrmex* species, which represent a potential competitor for at least one resource: the nesting site.

In ants, discrimination between nestmates and intruders is essentially based on the perception of cuticular compounds during antennations (Vander Meer and Morel, 1998; Lahav et al., 1999). That is why, to explain the dear–enemy phenomenon, one could hypothesize that environmentally influenced odours homogenize the recognition cues in adjacent nests. However, a gas chromatography analysis of cuticular extracts from *W. auropunctata* and *Cyphomyrmex* spp. workers revealed markedly different cuticular compounds (unpublished data), making this hypothesis less likely. Each species possesses its own cuticular profile, without any obvious similarities to the cuticular profile of the other species living in adjacent nests. Thus, a more likely mechanism able to explain our results is a phenomenon of habituation. Habituation is a simple form of learning, widespread in animals, and consists of a lack of response to a repetitively presented stimulus. In the present case, *W. auropunctata* workers could learn to recognize the exact cuticular profile of the *Cyphomyrmex* workers living close to their nest, whereas they could consider one of the *Cyphomyrmex* workers living in a distant nest as a new and potentially hostile component of their usual chemical environment.

Habituation has also been advanced to explain interspecific cohabitation in ant gardens, where two or three species belonging to different genera live in the same nest (Orivel et al., 1997). However, in that situation, different species foster mutualistic relationships, while in the present case nothing leads us to believe that the presence of one of the species is beneficial to the other. Colonies of each species live in separate nests, and we have never observed workers of either species moving freely

into the nest of the other, whether in the field or in the laboratory with two nests connected to the same foraging arena (pers. obs.). That excludes, for example, the use by *W. auropunctata* of the fungus cultivated by *C. rimosus*.

Finally, regarding *C. major*, for which there is no evidence for the dear–enemy phenomenon, we assume that some specific traits lead this species not to be identified as a threatening intruder by any *W. auropunctata* worker. For example, passivity may play a more important role than in the two other *Cyphomyrmex* species, but a more in-depth comparative analysis of their behaviour using a larger sample of *C. major* would be necessary to verify this. It is also possible that the odour of *C. major* workers is simply not perceived as a threatening one, whether the *W. auropunctata* workers have previously encountered it or not.

In conclusion, this study highlights an original example of interactions between *W. auropunctata* and the entomofauna of its native range. The *Cyphomyrmex* species belong to the very few ants able to survive in areas highly dominated by *W. auropunctata*. The food-specialization of the fungus-growing ants may represent a shift compared to the ecological niche of the little fire ant, but such a shift is limited since these ants are potential competitors for nesting sites. The discretion of the *Cyphomyrmex* workers when faced with *W. auropunctata* could also play a part in this coexistence, but we show here that this is not enough to entirely explain it. At least for some species, it appears that a phenomenon of habituation may be the main factor permitting such a situation.

Acknowledgements

Financial support was provided by the Programme Ecosystèmes Tropicaux of the French Ministère de l'Écologie et du Développement Durable (project agreement 02-E/2002). We are grateful to Dr. Jacques H.C. Delabie (CEPEC-CEPLAC, Brazil) for the identification of the ants, to the “Laboratoire Environnement de Petit Saut, Hydreco, EDF-CNEH” for logistical support at the field site, to the CIRAD-Guyane who allowed us to work in their coffee plantations, and to Andrea Dejean for proofreading the manuscript. We would also like to thank two anonymous reviewers for useful comments on the manuscript, and Ariane Dor who provided field assistance.

References

Armbrecht, I., Ulloa Chacón, P., 2003. The little fire ant *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae) as a diversity indicator of ants in tropical dry forest fragments of Colombia. *Environ. Entomol.* 32, 542–547.

Clark, D.B., Guayasamín, C., Pazmiño, O., Donoso, C., Páez de Villacís, Y., 1982. The tramp ant *Wasmannia auropunctata*: autecology and effects on ant diversity and distribution on Santa Cruz Island, Galapagos. *Biotropica* 14, 196–207.

Errard, C., Hefetz, A., 1997. Label familiarity and discriminatory ability of ants reared in mixed groups. *Insectes Soc.* 44, 189–198.

Heinze, J., Foitzik, S., Hippert, A., Hölldobler, B., 1996. Apparent dear–enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderii*. *Ethology* 102, 510–522.

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

Jutsum, A.R., Saunders, T.S., Cherrett, J.M., 1979. Intraspecific aggression in the leaf-cutting ant *Acromyrmex octospinosus*. *Anim. Behav.* 27, 839–844.

Kempf, W.W., 1966. A revision of the Neotropical fungus-growing ants of the genus *Cyphomyrmex* Mayr. Part II. Group of *rimosus* (Spinola) (Hym., Formicidae). *Stud. Entomol.* 8, 161–200.

Lahav, S., Soroker, V., Hefetz, A., Vander Meer, R.K., 1999. Direct behavioral evidence for hydrocarbons as ant recognition discriminators. *Naturwissenschaften* 86, 246–249.

Langen, T.A., Tripet, F., Nonacs, P., 2000. The red and the black: habituation and the dear–enemy phenomenon in two desert *Pheidole* ants. *Behav. Ecol. Sociobiol.* 48, 285–292.

Le Breton, J., 2003. Etude des interactions entre la fourmi *Wasmannia auropunctata* et la myrmécophage. Comparaison d'une situation en zone d'introduction: la Nouvelle-Calédonie et d'une situation en zone d'origine: la Guyane Française. Thèse de Doctorat. Université Toulouse III, p. 230.

Le Breton, J., Chazeau, J., Jourdan, H., 2003. Immediate impacts of invasion by *Wasmannia auropunctata* (Hymenoptera: Formicidae) on native litter ant fauna in a New Caledonian rainforest. *Aust. Ecol.* 28, 204–209.

Le Breton, J., Jourdan, H., Chazeau, J., Orivel, J., Dejean, A., 2005. Niche opportunity and ant invasion: the case of *Wasmannia auropunctata* in a New Caledonian rain forest. *J. Trop. Ecol.* 21, 99–102.

Majer, J.D., Delabie, J.H.C., Smith, M.R.B., 1994. Arboreal ant community patterns in Brazilian cocoa farms. *Biotropica* 26, 73–83.

Mercier, J.L., 1999. Territorialité et agressivité intra- et interspécifique dans les mosaïques de fourmis arboricoles. *Ann. Biol.* 38, 131–148.

Morrison, L.W., Porter, S.D., 2003. Positive association between densities of the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), and generalized ant and arthropod diversity. *Environ. Entomol.* 32, 548–554.

Orivel, J., Errard, C., Dejean, A., 1997. Ant gardens: interspecific recognition in parabiocotic ant species. *Behav. Ecol. Sociobiol.* 40, 87–93.

Snelling, R.R., Longino, J.T., 1992. Revisionary notes on the fungus-growing ants of the genus *Cyphomyrmex*, *rimosus* group (Hymenoptera: Formicidae: Attini). In: Quintero, D., Aiello, A. (Eds.), *Insects of Panama and Mesoamerica: Selected Studies*. Oxford University Press, Oxford, pp. 479–494.

Suarez, A.V., Tsutsui, N.D., Holway, D.A., Case, T.J., 1999. Behavioral and genetic differentiation between native and introduced populations of the Argentine ant. *Biol. Invas.* 1, 43–53.

Temeles, E.J., 1994. The role of neighbours in territorial systems: when are they “dear–enemies”? *Anim. Behav.* 47, 339–350.

Tennant, L.E., 1994. The ecology of *Wasmannia auropunctata* in primary tropical rainforest in Costa Rica and Panama. In: Williams, D.F. (Ed.), *Exotic Ants: Biology, Impact, and Control of Introduced Species*. Westview Press, Boulder, CO, pp. 80–90.

Tsutsui, N.D., Suarez, A.V., Grosberg, R.K., 2003. Genetic diversity, asymmetrical aggression, and recognition in a widespread invasive species. *Proc. Natl. Acad. Sci. U.S.A.* 100, 1078–1083.

Vander Meer, R.K., Morel, L., 1998. Nestmate recognition in ants. In: Vander Meer, R.K., Breed, M., Winston, M., Espelie, K.E. (Eds.), *Pheromone Communication in Social Insects*. Westview Press, Boulder, CO, pp. 79–103.

Weber, N.A., 1947. Lower Orinoco river fungus-growing ants (Hymenoptera: Formicidae, Attini). *Bol. Entomol. Venez.* 6, 143–161.

Wetterer, J.K., Porter, S.D., 2003. The little fire ant, *Wasmannia auropunctata*: distribution, impact, and control. *Sociobiology* 42, 1–41.