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Intercontinental chemical variation in the invasive ant *Wasmannia auropunctata* (Roger) (Hymenoptera Formicidae): a key to the invasive success of a tramp species

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Abstract Unicoloniality emerges as a feature that characterizes successful invasive species. Its underlying mechanism is reduced intraspecific aggression while keeping interspecific competitiveness. To that effect, we present here a comparative behavioural and chemical study of the invasive ant *Wasmannia auropunctata* in parts of its native and introduced ranges. We tested the hypothesis that introduced populations (New Caledonia archipelago) have reduced intraspecific aggression relative to native populations (e.g., Ilhéus area, Brazil) and that this correlates with reduced variability in cuticular hydrocarbons (CHCs). As predicted, there was high intraspecific aggression in the Brazilian populations, but no intraspecific aggression among the New Caledonian populations. However, New Caledonian worker *W. auropunctata* remained highly aggressive towards ants of other invasive species. The chemical data corresponded with the behaviour. While CHCs of ants from the regions of Brazil diverged, the profiles of ants from various localities in New Caledonia showed high uniformity. We suggest that

in New Caledonia *W. auropunctata* appears to behave as a single supercolony, whereas in its native range it acts as a multicolonial species. The uniformity of recognition cues in the New Caledonia ants may reflect a process whereby recognition alleles became fixed in the population, but may also be the consequence of a single introduction event and subsequent aggressive invasion of the ecosystem. Chemical uniformity coupled with low intraspecific but high interspecific aggression, lend credence to the latter hypothesis.

Introduction

The success of invasive species can be attributed to the lack of biotic and abiotic constraints that generally regulate the species in its native range (Suarez et al. 2001). Furthermore, changes in key biological traits of the invasive population associated with the introduction event might increase their competitiveness and result in their rapid spread (Tsutsui et al. 2000; Giraud et al. 2002; Tsutsui and Suarez 2003; Le Breton et al. 2004). Social organization appears to be a key attribute responsible for the ecological domination of invasive ants (Passera 1994; Holway et al. 1998; Giraud et al. 2002), among which unicoloniality, i.e., having no colony boundaries within populations, is most remarkable. These species, which generally do not exhibit aggression towards workers within the unicolonial society, generate networks of intercommunicating aggregations of workers, brood and fertile queens. Nestmate recognition in ants relies on non-volatile olfactory cues, mainly cuticular hydrocarbons (CHCs) that constitute a “colony odour”, according to the “gestalt model” (reviewed by Lenoir et al. 1999). Cues comprise of endogenous compounds (genotype) that may be further modulated by exogenous, environmental, sources (Vander Meer and Morel 1998; Liang and Silverman 2000). Elucidating the chemical basis of nestmate recognition may therefore shed light on the mechanisms leading to the formation and maintenance of unicolonial populations of invasive ants (Astruc et al. 2001; Suarez et al. 2002). Among these, the little fire ant *Wasmannia auropunctata* (Myrmicine) is of growing

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concern in areas with tropical climates, especially in the Pacific (Jourdan et al. 2002; Wetterer and Porter 2003). It has spread worldwide from its Neotropical origin by means of man (Jourdan et al. 2002), and is notable for occupying disturbed habitats, leading to subsequent ecological dominance of undisturbed ecosystems within its invasive range (Majer and Delabie 1999; Armbrrecht et al. 2001; Armbrrecht and Ulloa-Chacón 2003). *Wasmannia auropunctata* thus constitutes an excellent model to study questions relevant to successful invasion.

In the present study we examined the intraspecific aggression of this species in both its native (Brazil; J. Delabie, unpublished data) and invasive ranges (New Caledonia) in concordance with CHC profiles. The Brazilian population is not necessarily the origin of the NC population, since *W. auropunctata* is widely spread in the Neotropics both in natural and disturbed environments, and the specifics of its introduction to New Caledonia are unknown. We tested the hypothesis that reduced intraspecific aggression is due to chemical uniformity in CHCs. To estimate the competitive power of invasive *W. auropunctata*, we also investigated the interspecific aggression towards two invasive ant species, *Tetramorium bicarinatum* (Nylander) (Myrmicinae) originating from Southeast Asia with a current range that extends through all the tropical and subtropical areas (Bolton 1977); and *Linepithema humile* (Mayr) (Dolichoderinae), originating from South America and now spreading worldwide (Suarez et al. 2001).

Materials and methods

Collection of ants

We collected native populations of *W. auropunctata* from cocoa plantations in the Ilhéus region (Bahia, Brazil; $n=32$ nests), over between-nests ranges of 0.05 km to 123 km. We collected introduced *W. auropunctata* ($n=40$ nests) from eight localities throughout New Caledonia main is-

land over between-nests ranges of 0.02 km to 250 km (list of the sites sampled is available upon request). We collected two colonies of *T. bicarinatum* from Crasto, Santa Luzia do Itanhi (Sergipe, Brazil) and two colonies of introduced populations of *L. humile* from Port-Leucate (Aude, France). All colonies were reared in the laboratory in artificial plaster nests (28×28 cm) and maintained under standard conditions (26°C±2°C, 70% RH, 10:14 h D/L). Each colony consisted of several queens, brood and several hundred workers. They were regularly fed with an equal diet, consisting of honey, sugared water, and artificial food cubes (Keller et al. 1989).

Behavioural assays

We quantified the intraspecific aggression between colonies of *W. auropunctata* using dyadic encounters according to Holway et al. (1998). For each test, one worker was confronted with either a nestmate or a non-nestmate in a neutral arena (Petri dish: 4.5 cm diameter, 1 cm high). Between-worker aggression was scored during 5-min tests on a scale from 1 to 4 (low to high aggression). We conducted ten replicates using different workers for each test to avoid possible effects of familiarization.

Interspecific aggression was assessed using group encounters (Clément 1986) between *W. auropunctata*, and *L. humile* or *T. bicarinatum*. Ten ants of each species were encountered for 30 min in a glass Petri dish (4.5 cm diameter, 1.7 cm high). Before each test, the ants were allowed to calm down by secluding them in a glass tube for 1 min in the test arena. At the end of the test, the number of dead and injured ants of each species was recorded and an aggression index was calculated as $Ag=D+W/2$, where D represents the number of dead ants and W the number of injured ants. For each encounter we scored the aggression index of each of the species involved separately. Each interspecific encounter was replicated ten times, using different individuals. For each species, intraspecific encounters (control) were also conducted between ants from different nests.

Table 1 Aggression of *W. auropunctata* ants in (1) intraspecific dyadic encounters as a function of the intercolonial distances^a; (2) interspecific intercolonial encounters between *W. auropunctata* vs. *L. humile* and vs. *T. bicarinatum*^b

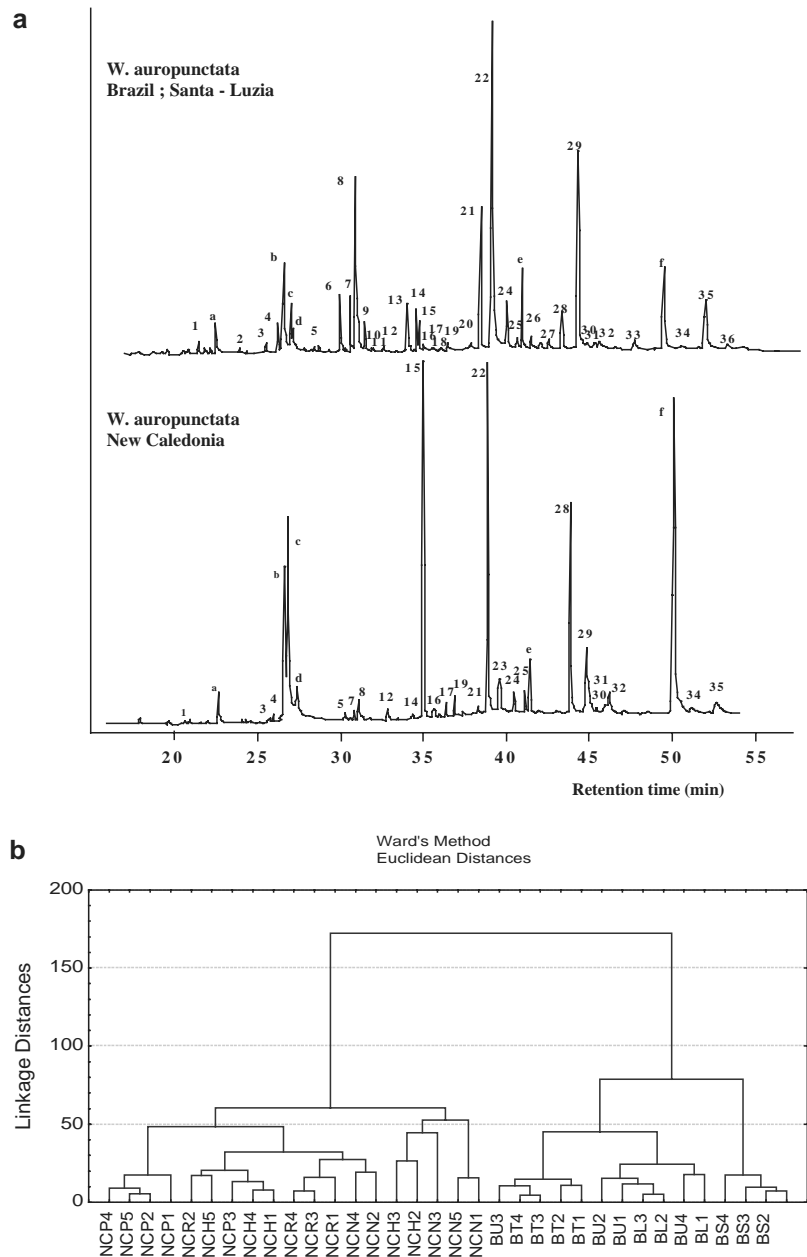
Test species	Encountering ants	Aggressive Index (Holway et al. 1998) (0–4)
<i>Wasmannia</i> – <i>Wasmannia</i>	Brazil	1.04±0.05
	Same population	
<i>Wasmannia</i> – <i>Wasmannia</i>	Brazil	3.12±0.11
	Different populations	
<i>Wasmannia</i> – <i>Wasmannia</i>	New Caledonia	1.22±0.09
	Same population	
<i>Wasmannia</i> – <i>Wasmannia</i>	New Caledonia	1.69±0.15
	Different populations	
		Aggressive Index (Clément 1986) (0–10)
<i>Wasmannia</i> – <i>Wasmannia</i>	New Caledonia Control test	0
<i>Wasmannia</i> – <i>Linepithema</i>	Interspecific test	9.15±0.25
<i>Linepithema</i> – <i>Wasmannia</i>	Interspecific test	3.65±1.13
<i>Wasmannia</i> – <i>Tetramorium</i>	Interspecific test	1.02±0.19
<i>Tetramorium</i> – <i>Wasmannia</i>	Interspecific test	1.1±0.65

^a(Aggression Index, Holway et al. 1998); ^b(Aggression Index, Clément 1986); The aggression index refers to the first species in each couple (mean ± SEM)

Chemical analyses

Total body washes of 50-pooled workers of *W. auropunctata* from each locality were used for identification of CHCs by GC/MS. The extracts were run on a DB5 capillary column, temperature programmed from 120°C to 300°C at 5°C/min. The compounds were identified by their mass fragmentation pattern and retention time comparison. Quantitative analyses were performed using pools of ten workers by GC equipped with a CPSIL 5 capillary column, temperature programmed from 80°C to 180°C at 10°C per minute, and from 180°C to 280°C at 5°C per minute, then kept for 10 min at 280°C. The relative amount of each peak was quantified by peak integration and the various profiles compared thereafter by hierarchical cluster analyses.

Fig. 1 **a** Gas Chromatograms of total body wash of 50 workers of *Wasmannia auropunctata* from Santa Luzia Brazil (top: putative native population) and New Caledonia (bottom: invasive population). Peak numbers correspond to the list in Table 2. Peaks denoted from a–f are of non-hydrocarbons as follows: a palmitic acid; b linoleic acid; c oleic acid; d stearic acid; e squalene; f cholesterol. **b** Similarity between chemical profiles of the different colonies was assessed by using 54 peaks and comparing 34 extracts (one extract per nest), 16 from Brazil (4 from Santa Luzia: BS1-4, 4 from Uruçuca: BU1-4, 4 from Itajuípe: BT1-4 and 3 from Ilhéus: BL1-3) and 19 from New Caledonia (5 from Pouembout: NCT1-5, 5 from Ponérihouen: NCH1-5, 5 from Nouméa: NCN1-5 and 4 from Rivière Bleue: NCR1-4)



Results

In the intraspecific aggression tests, workers of *W. auropunctata* from native habitats in Brazil showed little aggression between nests within populations, but exhibited high aggression between nests from different populations (Table 1). Among the New Caledonian ants there was no intercolonial aggression irrespective of the population of origin. In contrast, the interspecific encounters involving *W. auropunctata* from New Caledonia culminated in high aggression. Although we can not tell which species initiated the aggressive acts, the resulting indices indicated that *W. auropunctata* were highly aggressive towards nests of *L. humile* (Kruskal-Wallis: $H=16.47$; $p<0.001$), considerably more than reciprocally. Towards *T. bicarinatum* they

showed only little aggression (Kruskal-Wallis: $H=13.95$; $p<0.001$) that was nevertheless significantly higher than the control (Kruskal-Wallis: $H=25.96$; $p<0.001$). This confirms that *W. auropunctata* forms a unicolonial system in New Caledonia, as suggested by Le Breton et al. (2004).

The chemical analyses revealed clear differences in CHC patterns between New Caledonia (invasive range) and Brazil (native range) *W. auropunctata* (Fig. 1a, Table 2).

Table 2 Comparative chemical analyses of cuticular hydrocarbons on workers *W. auropunctata* collected from its putative native (Brazil) and invasive (New Caledonia) ranges^a

Compound	New Caledonia	Brazil – Santa Luzia
1 Nonadecane	t	+
2 Eicosane		t
3 Heneicosene	t	t
4 Heneicosane	t	+
5 Docosane	t	t
6 Tricosadiene	t	+
7 Tricosene	t	+
8 Tricosane	+	+++
9 3-Methyltricosane		+
10 Tetracosadiene		t
11 Tetracosene		t
12 Tetracosane	+	t
13 Pentacosadiene		+
14 Pentacosene	t	+
15 Pentacosane	++++	+
16 9-,+11-+13-Methylpentacosane	t	t
17 3-Methylpentacosane	+	t
18 Hexacosadiene		t
19 Hexacosane	+	t
20 Heptacosadiene		t
21 Heptacosene	+	++
22 Heptacosane	++++	++++
23 9-,+11-+13-Methylheptacosane	+	
24 3-Methylheptacosane	+	+
25 Octacosane	+	t
26 4-Methyloctacosane		t
27 Nonacosene		t
28 Nonacosane	++++	+
29 11-+13-+15-Methylnonacosane	+	+++
30 7-Methylnonacosane	t	t
31 5-Methylnonacosane	+	t
32 3-Methylnonacosane	+	t
33 Triacontane		+
34 Hentriacontane	t	t
35 9-+11-+13-+15-Methylhentriacontane	+	+
36 9,19+11,19-Dimethylhentriacontane		t

^at: <1%; (+): 1%-5%; (++): 5%-10%; (+++): 10%-15%; (++++): >15%

Ants from Brazil exhibited greater chemical complexity than those from New Caledonia, mainly in having larger amounts of branched hydrocarbons. A hierarchical cluster analysis based on GC analyses of 34 extracts (15 and 19 nests respectively from native and invasive ranges; Fig. 1b) revealed a significant divergence between the different locations, according to Euclidean distance ($F_{13,225} = 22.35$; $p<0.001$). The first node (172.20) separated Brazilian from New Caledonia populations, while the second node (78.92) divided the Urucuca, Itajuipé and Ilheus Brazilian colonies (BU1-4, BT1-4, BL1-3) from the other Brazilian colonies of Santa Luzia (BS1-4). The third node (60.38) separated the New Caledonian *W. auropunctata* irrespective of locality.

Discussion

The tendency of invasive species to create large supercolonies, often over the entire invasive range, was confirmed here for *W. auropunctata*. In New Caledonia, *W. auropunctata* workers showed no intraspecific aggression, even between nests several hundred kilometers apart, indicating that they behave as a single supercolony, as suggested by Le Breton et al. (2004). Similar supercolonies over wide geographic scales have been described for the Argentine ant in southern California (Tsutsui et al. 2000) and in southern Europe (Giraud et al. 2002). In contrast, in Brazil, as expected from native populations, *W. auropunctata* expressed a normal multicolonial system, as attested by the high intercolonial aggression exhibited by the ants. These results are similar to those obtained in studies of South American populations of *L. humile*. This is consistent with the hypothesis that unicoloniality is associated with invasion of new habitats.

The changes in aggressive behaviour were also correlated with CHC profiles exhibited by workers *W. auropunctata*. These are more diverse in workers coming from the native range as compared to the introduced range, including qualitative differences in CHC composition. A similar negative relationship between CHC-profile similarity and intercolonial aggression was also reported for Argentine ant (Suarez et al. 2002).

If bypassing the nestmate recognition process is a key factor in the success of invasive ant species, *W. auropunctata* in New Caledonia provides a new compelling case. Its loss of intraspecific but not interspecific aggression contributes to its successful displacement of other ant species. A similar pattern was also described for the Argentine ant, whose aggressive behaviour certainly contributes to its competitive success against several native ants species (Human and Gordon 1999; Holway et al. 1998). Whether this loss of aggression is due to the loss of recognition ability remains elusive. The fact that in New Caledonia, but not in Brazil, the ants exhibited similar CHC profiles, lends credence to the loss of recognition ability. However, it is possible that this CHC similarity reflects a single-nest introduction-event of *W. auropunctata*, which, due to high

competitiveness, has grown to become a large unicolonial species in its new habitat.

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