



## Selective interspecific tolerance in tropical *Crematogaster*–*Camponotus* associations

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Associations between ants of the genera *Crematogaster* and *Camponotus* are found in many parts of the world. Associated species use common trails (trail sharing) or even share a common nest (parabiosis). In a tropical lowland forest in Malaysian Borneo, we studied intraspecific and interspecific aggression among the parabiotic species *Crematogaster modiglianii* and *Camponotus rufifemur* using both field and laboratory assays. *Cr. modiglianii* tolerated *Ca. rufifemur* workers from certain foreign colonies but fiercely attacked those of others. In contrast, *Ca. rufifemur* was tolerant even towards attacking allocolonial *Cr. modiglianii* workers but killed other *Crematogaster* species. By analogy, other *Camponotus* species usually attacked and killed *Cr. modiglianii*. Intraspecific confrontations among *Ca. rufifemur* colonies yielded a gradient from allocolonial tolerance to strong aggression. The aggression patterns coincide with those of *Cr. modiglianii* towards *Ca. rufifemur* workers from the same colonies. Our results suggest either that *Ca. rufifemur* is not able to recognize allocolonial *Cr. modiglianii* workers as foreign or that they are recognized but tolerated. The unilateral, species-specific but not colony-specific tolerance of *Ca. rufifemur* towards its partner species contrasts with highly colony-specific tolerance found among neotropical parabioses.

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Most social insects are highly aggressive towards individuals other than their nestmates. Nest entrances are defended especially fiercely against nonnestmates (Breed & Bennett 1987; Roubik 1989; Hölldobler & Wilson 1990). In ants, nonnestmates are often aggressively displaced from trails or food resources (Swain 1980; Fellers 1987; Savolainen & Vepsäläinen 1988; Blüthgen & Fiedler 2004). Discrimination between nestmate and nonnestmate thus plays a central role in encounters between individual ants. Ants recognize their nestmates through colony-specific chemical signals. Located on the body surface, they are perceived by other individuals through contact chemoreception (Vander Meer & Morel 1998) and compared to a cognitive reference template (Errard 1994; Lenoir et al. 1999). Most signalling substances are hydrocarbons (Lenoir et al. 1997; Lahav et al. 1999;

Wagner et al. 2000; Suarez et al. 2002, but see Hernández et al. 2006). Ants continually exchange surface chemicals among nestmates through allogrooming and trophallaxis. Through this process, a mixed colony odor is created and continually redistributed among nestmates (Crozier & Dix 1979; Vander Meer & Morel 1998; Boulay et al. 2000) albeit chemical differences between certain groups, e.g. castes, may be maintained (Endler et al. 2004).

Social parasites (ants and other arthropods) overcome nestmate recognition and manage to get accepted in foreign ant colonies. In many associations, parasite and host possess similar chemical profiles. Thus it is assumed that the hosts do not regard them as alien (chemical mimicry; Howard et al. 1990; Akino et al. 1996, 1999; Howard et al. 2001; Lenoir et al. 2001a; Akino 2002; Elgar & Allan 2004). In contrast, other social parasites possess distinct profiles that do not resemble their hosts. In these cases the host probably recognizes but tolerates the parasite since it has habituated to the parasite's profile (Errard et al. 1996; Liu et al. 2000, 2003).

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However, there are other, presumably nonparasitic, associations between ant species, mostly from different genera, that co-occur without aggression. Two types of association can be observed. The more frequent type of association is that of two ant species sharing a common trail, which has been reported from many parts of the world (Wilson 1965; Baroni Urbani 1969; Davidson 1988). Interspecific tolerance (i.e. absence of aggression) in trail-sharing species is often achieved through submissive avoidance behaviour of one species towards the other (Baroni Urbani 1969; Adams 1990). Similarly, appeasement behaviour also reduced aggression among competing ant species (Mercier & Dejean 1996).

The other type of association is that of two ant species living together in the same nest (parabiosis). The most extensively studied parabioses are associations in neotropical ant gardens. These are inhabited by species of the *Crematogaster limata* complex (see Longino 2003) associated with *Camponotus* (most often *Ca. femoratus*), *Odontomachus*, *Pachycondyla* or *Dolichoderus* (Swain 1980; Davidson 1988; Orivel et al. 1997; Vantaux et al. 2007). The species keep their brood separate but otherwise share major or even all parts of the nest (Orivel et al. 1997). A few other parabiotic or parabiotic-like associations have been studied, including associations of *Brachymyrmex giardii* with *Ca. morosus* and *Ca. chilensis* (Errard et al. 1996) and *Camponotus* nests inside *Iridomyrmex* mounds (Greaves & Hughes 1974; Hölldobler & Wilson 1990, page 467). A putatively parabiotic association between *Ca. morosus* and *Solenopsis gayi* is probably a lestopiosis; that is, *S. gayi* is probably a brood parasite that lives inside the *Ca. morosus* nest (Errard et al. 1996; Lenoir et al. 2001b; Errard et al. 2003).

Since parabiotic species need to tolerate heterospecific ants as nestmates, their nestmate recognition system might be adapted accordingly. Similar to social parasites, interspecific tolerance might be achieved either by chemical mimicry or by habituation to the other's profile. The latter seems probable in the neotropical parabiotic ants *Odontomachus mayi* and *Cr. limata*, which possess completely different chemical profiles (Orivel et al. 1997). In artificially mixed colonies of *Formica selysi* and *Manica rubida*, however, both species changed their hydrocarbon profiles towards a higher similarity to the other species when reared together, suggesting a mixed colony odor (Vienne et al. 1995; Errard et al. 2006). Chemical mimicry or habituation can also result in selective interspecific tolerance. In the neotropical parabioses of *Cr. limata* and *O. mayi*, interspecific tolerance was restricted to the respective partner colony, while both species were aggressive towards allocolonial workers of their partner species (Fig. 1a; Orivel et al. 1997). However, individuals of both *S. gayi* and *Ca. morosus*, which can live together in a parasitic (lestopiotic) association, showed allocolonial interspecific tolerance when the involved *Ca. morosus* individuals were part of a lestopiotic association (Fig. 1b; Errard et al. 2003); similar patterns were found in associations between *B. giardii* and *Ca. morosus* or *Ca. chilensis* (Errard et al. 1996).

In the lowland rainforest of Borneo, parabiotic associations between *Cr. modiglianii* and *Ca. rufifemur* are common. The aim of this study was to investigate the extent

of interspecific tolerance as well as the behavioural mechanisms of nestmate recognition and tolerance in these associations. Our research questions were as follows.

(1) Do these parabiotic ant species discriminate between intra- and allocolonial workers of their partner species or between their partner species and other congeneric species?

(2) Do parabiotic species show an unusually high degree of intraspecific tolerance?

(3) Is interspecific tolerance mediated by behavioural mechanisms such as submissive or appeasement behaviour?

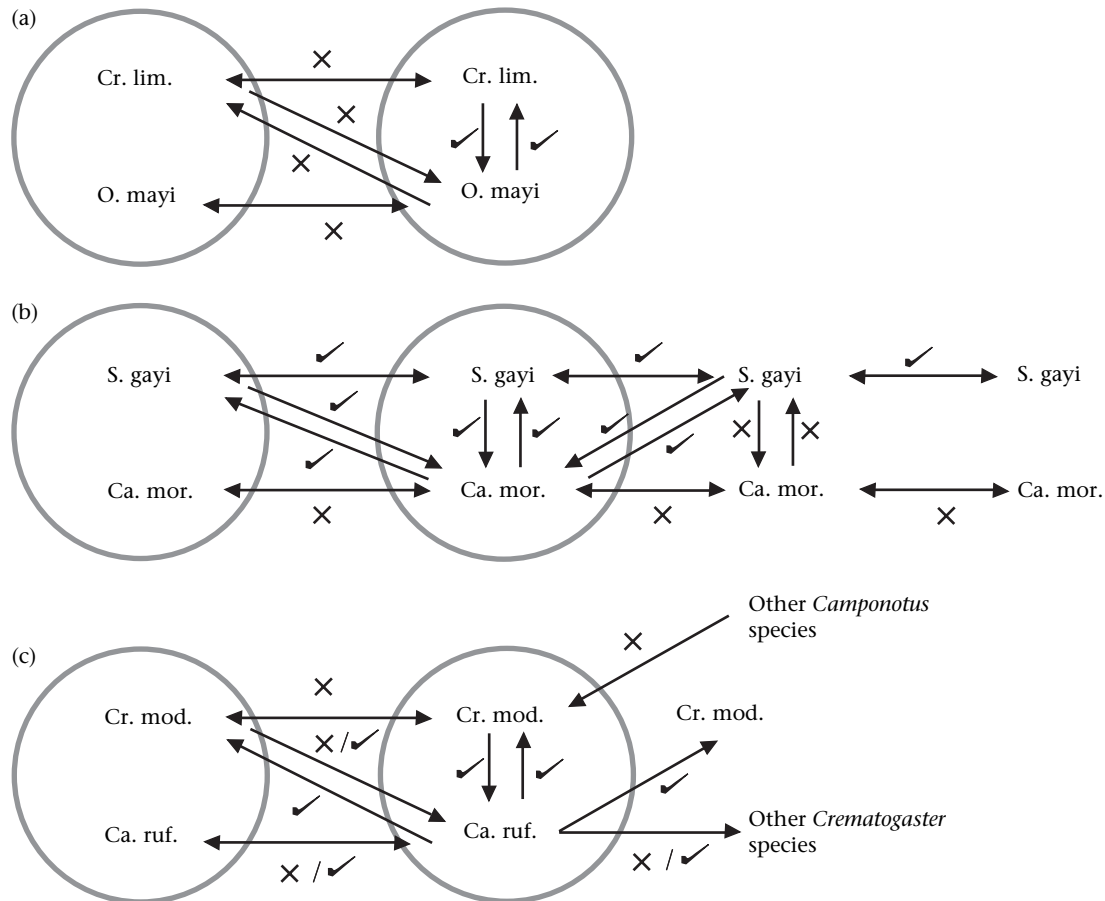
## METHODS

### Study Site

The study was conducted in September–November 2004 and September–November 2006 at Danum Valley Conservation Area. The area is located at 5°N 117°50'E and is approximately 100 m above sea level in Sabah (Malaysian Borneo). It represents one of the major remaining patches of Sabah's primary lowland rainforest. Danum Valley has a typical equatorial rainforest climate, with a mean annual temperature of 26.7°C and a yearly rainfall of 2700 mm. Rainfall is usually minimal during March–April and highest from October to January (Walsh & Newberry 1999).

### In Situ Assays

We studied four parabiotic colonies (A–D) of *Ca. (Myrmotarsus) rufifemur* and *Cr. (Paracrema) modiglianii*. These parabioses occur in hollow, living trunks of various tree species, especially often in *Eugenia chrysantha* trunks. For the colonies A, B and C, we separately tested the behaviour of both parabiotic species towards an introduced individual. The introduced individuals were *Ca. rufifemur* workers (majors or minors) and *Cr. modiglianii* workers from the other two colonies or control individuals from the same colony that had been captured several hours earlier and kept in a small plastic container. In the following, these colony combinations are given with two letters, the first referring to the resident colony and the second to the introduced individual. Intraspecific recognition within *Ca. rufifemur* was additionally tested with the colony combinations (A–D) and (D–A). The colonies A–C were located at least 500 m from each other and separated by rivers; colony D was ca. 10 m from colony B. For each combination, nine to 13 replicates were performed. The in situ assays were conducted in plastic arenas that were attached to the nest trunk. They consisted of a plastic platform covered with tissue paper. This platform carried the arena, a plastic ring (diameter 11.5 cm, height 5 cm) coated with paraffin oil so that the ants could not walk on it. The arena was connected to the nest tree with a small twig to allow the ants to walk into the arena, and a tuna bait was placed into the arena to attract ants. At 30–60 min after placing the bait, the bridge was carefully removed so that the ants (at least 10 individuals) were



**Figure 1.** Tolerance patterns between colonies in different associations. The arrows indicate mutual tolerance (✓) or mutual aggression (×) between workers. Each circle represents a nest association; names outside circles represent nonassociated colonies. (a) Parabiotic association of *Crematogaster limata* and *Odontomachus mayi* (Orivel et al. 1997); (b) lestobiotic associations of *Solenopsis gayi* and *Camponotus morosus* (Errard et al. 2003); (c) parabiotic associations of *Crematogaster modiglianii* and *Camponotus rufifemur* (present study).

captured in the arena with a minimum of disturbance. Since *Ca. rufifemur* is a nocturnal species, we brought the baits out shortly before dusk and then conducted the experiments at night under red light. Some of the tests with *Cr. modiglianii*, however, were performed during the day because we did not find a difference in nocturnal and diurnal activity or behaviour in this species. The behaviour of the two species was recorded in separate assays although both species were present in the arena in 22% of the cases ( $N = 391$ ).

All interactions of any resident individual towards the intruder were counted for 3 min. For each interaction, the behaviour was recorded separately and categorized into amicable (antennating or trophallaxis), weakly aggressive (antennating with open mandibles) or aggressive (biting or locked mandibles) for further analysis. An interaction was regarded finished when the resident ant moved away from the intruder and counted as a new interaction if it returned later. Temporal effects in the recognition process were recorded by dividing the 3 min into 18 steps of 10 s. For each interaction, we recorded the time step during which it was observed. If interactions lasted longer than 10 s ('continued interactions'), they were recorded again in the following time step.

## Arena Confrontations

These confrontations were performed to estimate the reciprocal aggressiveness of *Camponotus* and *Crematogaster* colonies, measured by counting the numbers of workers killed. Of either colony, three individuals were placed into an arena with forceps almost simultaneously. The arena consisted of a fluton-covered plastic cylinder (diameter 7.5 cm, height 5 cm) on top of a paper sheet that was replaced after each experiment. The number of dead individuals was counted after 60 min, with seriously injured individuals being regarded as half dead (i.e. a value of 0.5, which rarely occurred). Arena confrontations were performed for all pairwise combinations of a set of six *Crematogaster* colonies (*Cr. (Paracrema) modiglianii*: four parabiotic colonies; *Cr. (Paracrema) coriaria* and *Cr. (Physocrema) inflata*: one colony each) and seven *Camponotus* colonies (*Ca. (Myrmotarsus) rufifemur*: four parabiotic colonies; *Ca. (Colobopsis) saundersi*, *Ca. (Colobopsis) sp. 1*, and *Ca. (Tanaemyrmex) arrogans*: one colony each) with six to eight replicates per combination. All studied ant colonies were scattered along the forest trails within 2 km distance to the Danum Valley Field Center. Voucher specimens of all colonies will be deposited

both at the Department of Zoology III, University of Würzburg and the Forest Research Center in Sepilok, Sabah (Malaysia).

## Statistical Analysis

We used generalized linear models (GLMs) to obtain mathematical descriptions of the different variables affecting aggression. For each replicate of the in situ assays, we calculated the sum of aggressive interactions (all time steps pooled, including weak aggression and both starting and continued interactions) versus the sum of all nonaggressive interactions. We used a quasibinomial error distribution with a logit link function since our response variable consisted of two respective proportions (of the total sum of interactions) and was overdispersed. The explanatory variables were 'species combination', 'IN–AL' (intracolony versus allocolony), 'colony combination' and the respective interactions. Species combination refers to the respective species of the observed and the introduced ants and contains four categories. Colony combination refers to the respective parabiotic nests of the observed and the test ants and is nested within IN–AL. The numbers of present *Cr. modiglianii* and *Ca. rufifemur* workers were included as covariates. The impact of each variable was determined by likelihood ratio tests ( $F$  test). All computations were performed in R Version 2.4 (R Development Core Team 2007). We constructed a comprehensive model for all species combinations, excluding the test series A–D and D–A to achieve a balanced experimental design, and analysed separate models for each species combination, whereby the data set for species combination *Ca. rufifemur*–*Ca. rufifemur* also included the colony combinations A–D and D–A.

In addition, temporal aspects of the intraspecific recognition process were analysed using three derived variables. The 'average occurrence time' provided information at which time step aggression (or amicable behaviour, respectively) was observed on average. For each replicate, the scalar product between 'time step number'  $t$  and 'number of aggressive interactions in this time step'  $a_t$  was divided by the total number of aggressive interactions in this replicate, viz.:  $\sum_{t=1}^{18} ta_t / \sum_{t=1}^{18} a_t$ . In addition, aggression latency was determined as the first time step when strong aggression was observed. Aggression latency as well as proportion of continued antennation were then analysed using Kruskal–Wallis  $H$  test followed by Nemenyi's test (for allocolony confrontations only).

The arena confrontations were analysed using generalized linear models with binomial error distribution and a logit link function. The response variable was the number of killed versus living *Crematogaster* workers in each trial. We used two different models to test the following effects: (1) differential aggression of *Ca. rufifemur* towards different *Crematogaster* species and towards intra- and allocolony *Cr. modiglianii* and (2) differential aggression of different *Camponotus* species towards *Cr. modiglianii*.

## RESULTS

### In Situ Assays: Interspecific Recognition

The in situ assays revealed high *Cr. modiglianii* aggression towards allocolony *Ca. rufifemur* but low *Ca. rufifemur* aggression towards both intracolony and allocolony *Cr. modiglianii* (for an overview, see Fig. 1c). The comprehensive model explained 84.6% of the original deviance (Table 1), while the separate models for each of the four species combinations explained 69.1–87.0% of the original deviance, except for the behaviour of *Ca. rufifemur* towards *Cr. modiglianii* (15.5% explained deviance, Tables 2 and 3).

*Cr. modiglianii* discriminated between intracolony and allocolony *Ca. rufifemur*. However, aggression in different allocolony colony combinations differed significantly (Table 2). *Cr. modiglianii* from colonies A and B showed low aggression towards both *Ca. rufifemur* A and B but attacked *Ca. rufifemur* C whereas *Cr. modiglianii* C workers attacked *Ca. rufifemur* from both colonies A and B (Fig. 2a, c). The aggressive reaction of *Cr. modiglianii* did not depend on the number of *Ca. rufifemur* nestmates in the arena (Table 2); altogether, *Ca. rufifemur* was present in only 12% of the *Cr. modiglianii*–*Ca. rufifemur* assays. In turn, *Ca. rufifemur* showed a weak, but significant discrimination between intracolony and allocolony *Cr. modiglianii*, albeit most of the interactions even towards allocolony workers were amicable (Fig. 2c, Table 3). We often observed that, although *Crematogaster* workers heavily attacked the allocolony *Camponotus* worker by locking mandibles into its legs, the latter did not bite *Crematogaster* to defend itself. Attacked *Camponotus* workers ignored the attacking *Crematogaster* or antennated them so that they ceased biting. Generally, *Camponotus* sometimes antennated *Crematogaster* workers very intensely and moved their mouthparts towards them. This behaviour, which was regarded as amicable, was observed in both intracolony and allocolony confrontations.

### In Situ Assays: Intraspecific Recognition

*Crematogaster modiglianii* heavily attacked allocolony conspecifics. We did not detect differences between

**Table 1.** Generalized linear model for aggression in the in situ assays with all species combinations included

Parameter	Deviance	df	$F$	$P$
Species combination	1788.5	3	33.61	<0.0001
IN–AL	3389.7	1	258.76	<0.0001
Colony combination (nested in IN–AL)	1785.8	8	22.36	<0.0001
Number <i>Cr. modiglianii</i>	2.5	1	0.54	0.46
Number <i>Ca. rufifemur</i>	16.5	1	3.60	0.06
Species combination: IN–AL	190.1	3	6.91	0.0002
Species combination: colony combination	524.8	20	5.77	<0.0001
Residual error	1401.0	333		
Total	9098.8	370		



**Table 2.** Generalized linear models for *Cr. modiglianii* aggression in the in situ assays

Parameter	<i>Cr. modiglianii</i> towards <i>Ca. rufifemur</i>				<i>Cr. modiglianii</i> towards <i>Cr. modiglianii</i>			
	Deviance	df	F	P	Deviance	df	F	P
IN–AL	353.0	1	46.55	<0.0001	2199.7	1	436.28	<0.0001
Colony combination (nested in IN–AL)	419.1	7	13.30	<0.0001	16.8	7	0.44	0.88
Number <i>Cr. modiglianii</i>	2.4	1	0.53	0.47	6.8	1	1.22	0.28
Number <i>Ca. rufifemur</i>	1.2	1	0.25	0.62	22.2	1	3.84	0.054
Error	346.0	82			335.8	73		
Total	1121.5	92			2581.3	83		

colony combinations (Fig. 2b, Table 2). *Camponotus rufifemur* also clearly discriminated between intra- and allocolonial conspecifics. However, in contrast to *Cr. modiglianii*, there was a highly significant influence of colony combination on intraspecific aggression in this species; several allocolonial *Ca. rufifemur* confrontations yielded only low aggression levels (Fig. 2d, Table 3). Intraspecific *Camponotus* confrontations triggered two different types of aggression: 'biting', which was a short interaction (<1 s) whereby the intruder was quickly dismembered, and 'locked mandibles', which could last for several minutes but rarely resulted in cut limbs. While biting was strictly confined to combinations of *Ca. rufifemur* C with A or B (or vice versa), locked mandibles occurred in other combinations also. The aggressive response towards introduced ants was not always symmetrical. Colony D intruders were tolerated in colony A, but A individuals were heavily attacked in colony D (Mann–Whitney *U* test:  $U = 6$ ,  $N_1 = N_2 = 10$ ,  $P = 0.0009$ ). In contrast to other aggressive colony combinations, D workers showed locked mandibles but never biting behaviour towards A intruders. A similar, albeit weaker, asymmetry was found between the colonies A and B ( $U = 23$ ,  $N_1 = N_2 = 10$ ,  $P = 0.04$ ).

In most allocolonial confrontations between *Ca. rufifemur* individuals, aggression occurred during the first 10 s. Aggression latency significantly differed between allocolonial colony combinations (Kruskal–Wallis test:  $H_7 = 18.52$ ,  $P = 0.0098$ ); it was highest in the less aggressive confrontation A–B, where first aggression occurred on average after  $68.3 \pm 37.5$  s, compared to the confrontations A–D ( $40.0 \pm 15.2$  s), B–A ( $23.8 \pm 12.2$  s) and the remaining allocolonial confrontations ( $\leq 13.0$  s). These three

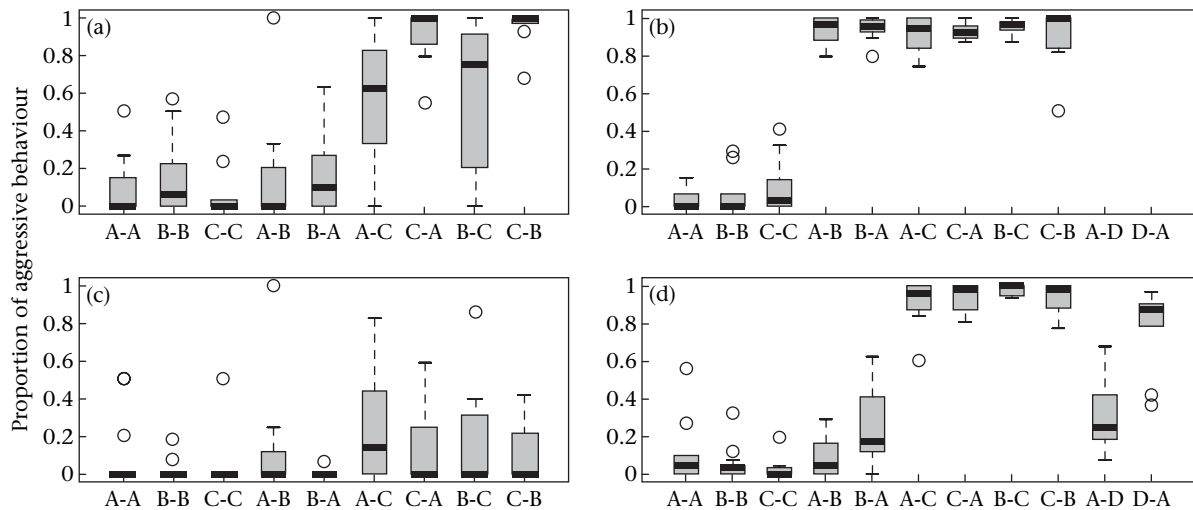
combinations also had high proportions of continued antennation, which were similar to those in intracolonial confrontations but differed significantly from all other allocolonial confrontations except D–A ( $H_7 = 45.68$ ,  $P < 0.0001$ ; Fig. 3e). Intracolonial test ants elicited aggressive behaviour upon reintroduction also. This aggression, however, ceased soon whereas allocolonial intruders received constant or increasing aggression during the course of the observation period. Average occurrence time of aggression differed significantly in both intraspecific *Crematogaster* (Student's *t* test:  $t_{90} = 5.56$ ,  $P < 0.0001$ ; Fig. 3a) and intraspecific *Camponotus* ( $t_{10.84} = 5.42$ ,  $P = 0.0002$ ; Fig. 3b) confrontations. In contrast, the average occurrence time of amicable behaviour did not differ between intra- and allocolonial confrontations in both species ( $t_{49.5} = 1.1$ ,  $P = 0.27$  and  $t_{89.9} = 1.8$ ,  $P = 0.07$ ; Fig. 3c, d).

### Arena Confrontations

The GLM for confrontations of *Ca. rufifemur* with workers from *Cr. modiglianii* and other *Crematogaster* species explained 65.0% of the original deviance. *Camponotus rufifemur* workers did not significantly differentiate between intracolonial and allocolonial *Cr. modiglianii* workers (Table 4). Although *Cr. modiglianii* frequently interacted with intra- and allocolonial *Ca. rufifemur* individuals, only 13 of 288 *Cr. modiglianii* workers were killed. In a separate experiment, *Ca. rufifemur* workers also tolerated workers from a nonparabiotic *Cr. modiglianii* colony despite frequent interactions and killed no nonparabiotic

**Table 3.** Generalized linear models for *Ca. rufifemur* aggression in the in situ assays

Parameter	<i>Ca. rufifemur</i> towards <i>Cr. modiglianii</i>				<i>Ca. rufifemur</i> towards <i>Ca. rufifemur</i>			
	Deviance	df	F	P	Deviance	df	F	P
IN–AL	20.7	1	5.77	0.02	1306.3	1	61.00	<0.0001
Colony combination (nested in IN–AL)	42.5	7	1.93	0.07	2064.6	9	32.51	<0.0001
Number <i>Cr. modiglianii</i>	5.2	1	1.71	0.19	22.7	1	3.75	0.06
Number <i>Ca. rufifemur</i>	0.0	1	0.001	0.98	85.1	1	13.14	0.0005
Error	260.5	89			573.1	101		
Total	308.2	98			4052.0	113		

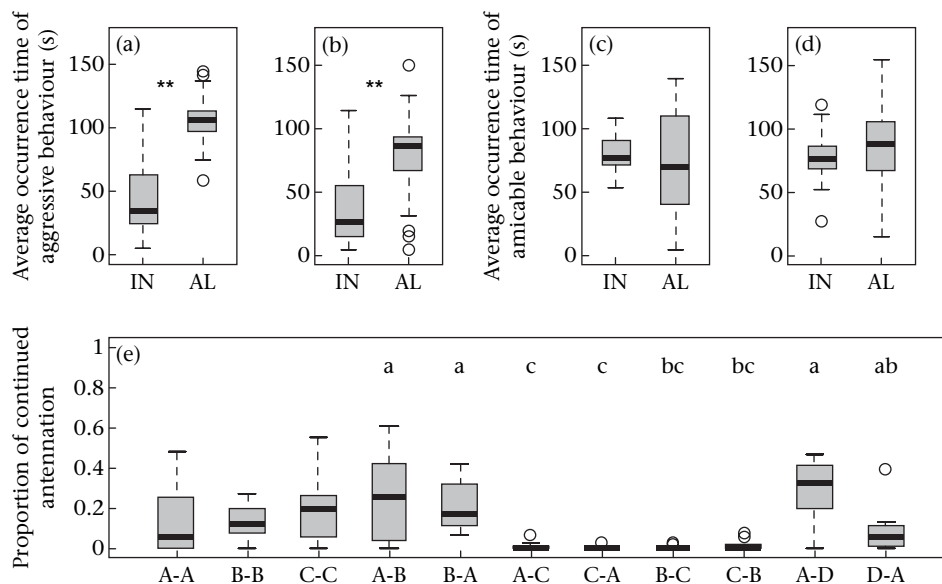


**Figure 2.** Aggression (proportion of aggressive interactions of all interactions) among different colony combinations for in situ assays. Median, 1st and 3rd quartiles and range are shown. The first letter refers to the resident colony; the second letter refers to the intruder. (a) *Crematogaster modiglianii* aggression towards *Camponotus rufifemur*; (b) *Cr. modiglianii* towards *Cr. modiglianii*; (c) *Ca. rufifemur* towards *Cr. modiglianii*; (d) *Ca. rufifemur* towards *Ca. rufifemur*.

*Cr. modiglianii* in four arena confrontations. *Camponotus rufifemur*, however, significantly discriminated between different *Crematogaster* species, tolerating only *Cr. modiglianii* but usually killing *Cr. coriaria* and *Cr. inflata* (Fig. 4a, Table 4). The *Ca. rufifemur* colonies strongly varied in their aggression towards the latter two *Crematogaster* species. Two *Ca. rufifemur* colonies killed on average 79% of the *Cr. coriaria* and 96% of the *Cr. inflata* workers while the other two colonies killed 12 and 15%, respectively. The significant interaction between *Ca. rufifemur* colony and *Crematogaster* species is due to these differences but

equal tolerance of *Cr. modiglianii* by all *Ca. rufifemur* colonies.

All three studied nonparasitoid *Camponotus* species were highly aggressive against *Cr. modiglianii* (Fig. 4b–d, Table 5). Both *Ca. (Colobopsis)* sp. 1 and *Ca. (Colobopsis) saundersi* often killed *Cr. modiglianii* but usually tolerated *Cr. coriaria* and *Cr. inflata* workers while *Ca. arrogans* was aggressive against all three *Crematogaster* species. The GLM, which explained 68.0% of the original deviance (number of killed *Cr. modiglianii*), also revealed highly significant differences between *Cr. modiglianii* colonies and a strong



**Figure 3.** Temporal aspects of the intraspecific recognition process in the in situ assays. Median, 1st and 3rd quartiles and range are shown. The first letter refers to the resident colony; the second letter refers to the intruder. (a), (b) average occurrence time of aggressive behaviour in (a) intraspecific *Cr. modiglianii* and (b) intraspecific *Ca. rufifemur* assays; (c), (d) average occurrence time of amicable behaviour in (c) intraspecific *Cr. modiglianii* and (d) intraspecific *Ca. rufifemur* assays; (e) proportion of continued antennation in intraspecific *Ca. rufifemur* assays. \*\*Significant at  $P = 0.001$ . Bars with the same letters are not significantly different according to Nemenyi's test (allocolonial confrontations only).

**Table 4.** Generalized linear model for the number of *Crematogaster* workers killed by workers from four *Ca. rufifemur* colonies in the arena confrontations

Parameter	Deviance	df	F	P
IN–AL (nested within <i>Crematogaster</i> species: <i>modiglianii</i> )	2.8	1	2.84	0.09
<i>Crematogaster</i> species	159.0	2	79.49	<0.0001
<i>Ca. rufifemur</i> colony	93.6	3	31.20	<0.0001
IN–AL: <i>Ca. rufifemur</i> colony	1.9	3	0.62	0.61
<i>Ca. rufifemur</i> colony: <i>Crematogaster</i> species	23.5	6	3.92	0.0006
Residual error	151.3	156		
Total	432.1	171		

interaction term between *Cr. modiglianii* colony and *Camponotus* species. Possible effects of intracolony versus allocolony were not considered here because they had been shown to be not significant in the first GLM (Table 4). In all arena confrontations, *Camponotus* workers usually survived the arena confrontations; in 309 tests with 927 *Camponotus* individuals, only seven *Ca. (Colobopsis)* sp. 1 and eight *Ca. (Colobopsis) saundersi* individuals were killed.

## DISCUSSION

### Interspecific Recognition and Tolerance in Parabiotic Species

Both *Cr. modiglianii* and *Ca. rufifemur* tolerated their respective parabiotic partners. *Cr. modiglianii* also tolerated *Ca. rufifemur* of certain foreign colonies but strongly attacked *Ca. rufifemur* workers of other colonies. These aggression patterns correspond to intraspecific aggression among the same *Ca. rufifemur* colonies (Fig. 2), suggesting that both species use similar recognition cues. Aggression by *Cr. modiglianii* also occurred when *Ca. rufifemur* nestmates were absent and thus did not depend on putative

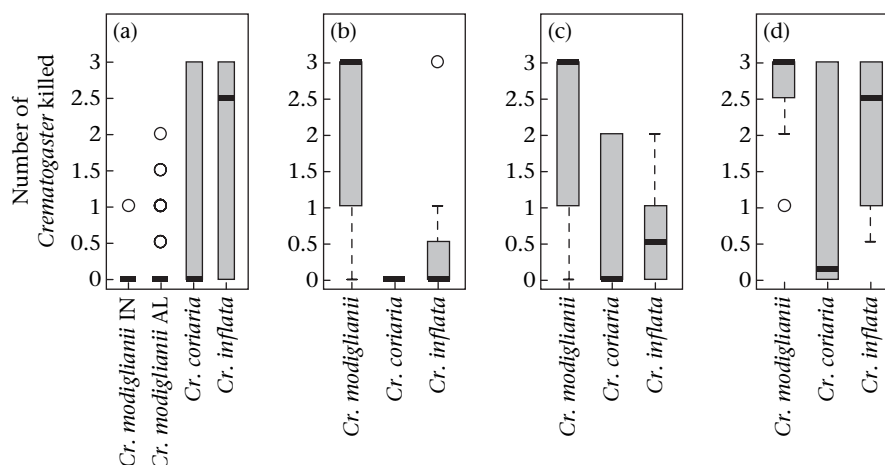
**Table 5.** Generalized linear model for the number of *Cr. modiglianii* workers from four colonies killed by different *Camponotus* species in the arena confrontations

Parameter	Deviance	df	F	P
<i>Camponotus</i> species	382.5	3	127.49	<0.0001
<i>Cr. modiglianii</i> colony	17.2	3	5.73	0.0006
<i>Camponotus</i> species: <i>Cr. modiglianii</i> colony	28.8	9	3.19	0.0007
Residual error	201.5	184		
Total	629.9	199		

nestmate-*Camponotus* signals. *Cr. modiglianii* even differentiated between dead *Ca. rufifemur* workers, biting those from certain foreign colonies but only antennating those from others or their own colony (F. Menzel, unpublished data).

The attacked *Ca. rufifemur* individuals ignored the biting *Cr. modiglianii* ants or antennated them so that they ceased biting. This surprising unilateral tolerance accounts for the low number of killed allocolony *Cr. modiglianii* in the arena confrontations. That *Ca. rufifemur* tolerated allocolony *Cr. modiglianii* workers but still showed slightly more aggression than towards intracolony workers suggests either that *Ca. rufifemur* received few nestmate recognition cues from *Cr. modiglianii* ('chemical insignificance' sensu Lenoir et al. 2001b) and was not able to recognize allocolony *Cr. modiglianii* with certainty or that allocolony *Cr. modiglianii* were recognized as foreign but were nevertheless tolerated. Notably, none of the amicable interactions between the two species involved any appeasement or otherwise submissive behaviour. However, the fact that attacking *Cr. modiglianii* ceased biting upon being antennated by *Ca. rufifemur* might be regarded evidence of appeasement behaviour of *Ca. rufifemur* during aggressive interactions.

The tolerance of *Ca. rufifemur* towards *Cr. modiglianii* was species specific and did not extend to other *Crematogaster* species. By analogy, *Cr. modiglianii* was tolerated only by its partner species and not by other *Camponotus*

**Figure 4.** Median number of killed *Crematogaster* individuals (with quartiles and range) in the arena confrontations. (a) *Ca. rufifemur* (intra- (IN) and allocolony (AL) *Cr. modiglianii* are shown); (b) *Ca. (Colobopsis)* sp. 1; (c) *Ca. (Colobopsis) saundersi*; (d) *Ca. arrogans*.

species. This species-specific high tolerance is in strong contrast to findings from previous studies on other associated species. In parabioses of the neotropical *Cr. limata* and *O. mayi*, both species tolerated their partner but were aggressive against allocolonial workers of the respective partner species in laboratory assays (Fig. 1a; Orivel et al. 1997). The tolerance patterns of the Southeast Asian parabioses rather resemble those of lestopibiotic associations between *S. gayi* and *Ca. morosus* (Fig. 1b, c; Errard et al. 2003).

### Intraspecific Recognition and Aggressiveness in Parabioc Species

In contrast to generally high aggression levels between *Cr. modiglianii* colonies, intraspecific allocolonial confrontations in *Ca. rufifemur* yielded a gradient from complete tolerance to high aggression. Since mutual tolerance between distant colonies separated by rivers also occurred, they were unlikely to be part of a polydomous colony. In certain colony combinations, continued antennating and high aggression latencies provided evidence of recognition uncertainty. Recognition uncertainty has been found in previous studies in *Aphaenogaster senilis* (Lenoir et al. 2001a) and is probably caused by high chemical similarity between intruder and resident (Breed 2003). The low, but often observed, aggression against intracolony intruders may have been induced by keeping the test ants separate for several hours prior to the test, thereby altering the surface recognition cues. However, as evidenced by the average aggression times, this aggression quickly ceased whereas aggression against allocolonial intruders remained constant or increased over time. *Ca. rufifemur* colonies also differed in their overall aggressiveness, as was evident through both asymmetric intraspecific aggression and different aggression towards nonparabioc *Crematogaster* species. The causes for mutual tolerance and for differences in overall aggressiveness are unknown. They might relate to different surface chemistry, different levels of intracolony genetic diversity (Tsutsui et al. 2003) or different stages of the parabioc association (e.g. colony size; Balas & Adams 1996).

### Experimental Setup of Nestmate Recognition Assays

The fundamental problem in detecting nestmate recognition is that recognition is a cognitive process and cannot be observed directly. Whether an ant fails to recognize its counterpart or tolerates it upon recognition cannot be distinguished based merely on behavioural observations. In interspecific associations, another complication is the lack of a plausible null hypothesis; that is, the expected behaviour given recognition failure is unknown. Many studies inferred a recognition failure due to chemical mimicry when two associated species possessed similar chemical profiles (Bagnères et al. 1991; Lenoir et al. 1997, 2001b), but assumed that the foreign profile was learned as an additional template when they differed (Errard 1994, Errard et al. 2003). Although this explanation

is plausible, it is important to keep in mind that recognition failure cannot be shown in principle.

Laboratory assays are a popular method to estimate recognition since they are weather independent and allow an exact experimental design as well as easy replication. Their reliability, however, remains questionable since aggression is often highly context specific (Starks et al. 1998; Breed 2003; Velasquez et al. 2006); in addition, laboratory-reared colonies often display lower intraspecific aggression than wild colonies (Buczkowski & Silverman 2005; Buczkowski et al. 2005) and may not show differential behaviour towards different test ants. As mentioned, lack of differentiation cannot be assigned to lack of recognition. However, significant differentiations between test individuals observed in the laboratory are likely to have biological importance. Although rather coarse and conservative, carefully conducted laboratory assays can be meaningful tools to estimate intercolonial recognition and tolerance.

With the in situ assays we tried to mimic the natural conditions as much as possible, completely avoiding manipulating the resident ants or immobilizing the test ants during the experiment. However, we still did not detect significantly differential behaviour of *Ca. rufifemur* towards different *Cr. modiglianii*. Other ant associations in contrast showed strong differentiation between intra- and allocolonial allospecifics even in laboratory assays (Orivel et al. 1997). Especially given the high aggression detected in the other species combinations of our study, the lack of differentiation of *Ca. rufifemur* towards *Cr. modiglianii* is therefore unlikely to be an effect of experimental design or low sample size.

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