

## Research Article

# Discrimination of the Social Parasite *Ectatomma parasiticum* by Its Host Sibling Species (*E. tuberculatum*)

Renée Fénéron,<sup>1</sup> Chantal Poteaux,<sup>1</sup> Marie Boilève,<sup>1</sup> Jorge Valenzuela,<sup>2</sup> and Fabrice Savarit<sup>1</sup>

<sup>1</sup>Laboratoire d'Ethologie Expérimentale et Comparée, EA 4443, Université Paris 13, Sorbonne Paris Cité, 99 avenue J.-B. Clément, 93430 Villetaneuse, France

<sup>2</sup>Departamento de Entomología, Instituto de Ecología, Antigua Carretera a Coatepec Km 2.5, A. 63, 91000 Xalapa, Ver, Mexico

Correspondence should be addressed to Renée Fénéron; [feneron@leec.univ-paris13.fr](mailto:feneron@leec.univ-paris13.fr)

Received 6 March 2013; Revised 30 April 2013; Accepted 15 May 2013

Academic Editor: Jean-Paul Lachaud

Copyright © 2013 Renée Fénéron et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Among social parasites, workerless inquilines entirely depend on their host for survival and reproduction. They are usually close phylogenetic relatives of their host, which raises important questions about their evolutionary history and mechanisms of speciation at play. Here we present new findings on *Ectatomma parasiticum*, the only inquiline ant described in the Ectatomminae subfamily. Field data confirmed its rarity and local distribution in a facultative polygynous population of *E. tuberculatum* in Mexico. Genetic analyses demonstrated that the parasite is a sibling species of its host, from which it may have diverged recently. Polygyny is suggested to have favored the evolution of social parasite by sympatric speciation. Nevertheless, host workers from this population were able to discriminate parasites from their conspecifics. They treated the parasitic queens either as individuals of interest or as intruders, depending on their colonial origin, probably because of the peculiar chemical profile of the parasites and/or their reproductive status. We suggest that *E. parasiticum* could have conserved from its host sibling species the queen-specific substances that produce attracting and settling effect on workers, which, in return, would increase the probability to be detected. This hypothesis could explain the imperfect social integration of the parasite into host colonies.

## 1. Introduction

Parasitism is found at all levels of biological organization from genes to societies. Social parasites are specialized in exploiting the social living conditions of one or several species [1]. They have evolved manifold in social Hymenoptera, especially in ants where they occur with a huge diversity [1–3]. Parasitic ants can take advantage of the host-colony resources only during the phase of colony founding (temporary social parasitism) or throughout their life cycle, either by raiding host brood and then enslaving workers (slave-making) or by cohabiting in the nest alongside the host queens (inquilinism) [1–3]. In the most derived form, inquilines have developed a set of adaptations such as the loss of the worker caste and a reduced body size (the “inquiline syndrome” [4]).

Typically, social parasites and their respective hosts are close phylogenetic relatives. This trend has been formalized as Emery's rule and generalized in two versions [5, 6]. In the strict version, the parasite is a sibling species of its host; in the loose version, the parasite and the host are nonsiblings but

belong to the same or a closely related genus. Some empirical studies support the strict version of Emery's rule hypothesis (see, e.g., [7, 8]). This has major evolutionary implications since it may argue for sympatric speciation. Indeed, although still in debate, it has been repeatedly suggested that inquilines may have diverged from their sister host species (or from a common ancestor) through intraspecific parasitism [1, 6, 9, 10]. Reproductive isolation in sympatry has been probably facilitated by the social biology and ecology of the host ant species. In particular, polygyny and later miniaturization of polygynous queens are considered as prerequisites for this scenario, as it is assumed for some *Myrmica* [7, 11, 12] and *Acromyrmex* [8]. It could also be the case for *Ectatomma tuberculatum* [13], but not for all cases of reduced-size queens (see e.g., [14, 15]). Beyond the species model, understanding the evolutionary processes and ecological constraints that could lead to speciation and promote the emergence of social parasitism is thus of a high relevance for evolutionary biologists.

Association between species requires well-matched communication systems. Cuticular hydrocarbons, a blend of surface chemicals, are involved in multiple levels of recognition in ants [16, 17]. They are shared between all colony members thus acting as nestmate recognition cues, and they also provide information on certain individuals inside the colony thus potentially signaling age, caste, or fertility [18, 19]. Inquilines that invade established host colonies to be adopted therein have to overcome the colony-specific barriers [1]. To this end, they can mimic the chemical profiles of their hosts. We refer to “chemical mimicry” following Von Beeren et al. [20] (see also [21]) when social parasites either express no identification cues, produce, or acquire host-specific chemical cues from the host individuals and nest materials [3, 22, 23]. In addition, specific chemicals such as appeasing or propaganda signals can be released by the parasites during host-colony invasion [23]. More generally, chemical strategies can also be combined with behavioral adaptations, for example, to promote colony odor transfer [24, 25].

Workerless inquilines are scarce in ants, and most of them are confined to the Formicinae and Myrmicinae subfamilies. *Ectatomma parasiticum* is the only parasitic species described in the Ectatomminae subfamily [26] and among the rare inquilines from the tropics. It was found to be associated with its host ant, *E. tuberculatum*, in one Mexican population, and to possess several parasitic life-history traits, such as the miniaturization of the queen [13, 27]. However, previous observations have shown that some parasitic queens were attacked by the host workers into their own colony, suggesting a probable failure in their social integration [25]. This could be due to an imperfect chemical mimicry as a result of coevolutionary processes [28].

To get a broader knowledge of the relationship between the parasite and its host, we present here up-to-date field, genetic, and behavioral data in these ants. First, we characterized the population of *E. parasiticum* by compiling data from all our field collection trips in the site of Apazapan. Second, we performed new genetic analyses including data from other Mexican populations (from Chiapas) but presenting neither polygyny nor social parasitism in order to refine phylogenetic relationships of *E. parasiticum* and *E. tuberculatum*. Finally, we conducted discrimination tests to determine the extent to which the host species is able to recognize its social parasite. If chemical mimicry is effective, the parasites should be either undetected by any host, or treated as nestmates by hosts of their own colony and as intruders by hosts of all other colonies. In case of an imperfect chemical mimicry, as suggested in *E. parasiticum* [28], we expected to find some differences from these patterns of responses.

## 2. Material and Methods

**2.1. Studied Sites and Colonies.** A total of 98 colonies of *E. tuberculatum* were collected in the population of Apazapan, Veracruz State, Mexico (19°19′38″ N; 96°43′21″ W, 300 m above sea level) during six field trips between September 1999 and November 2011. They were sampled from three sites

(referred as Apz1, Apz2, and Apz3) about 500 m apart and covering a surface area of about 10 hectares each. These sites are remnants of tropical dry forest [29] and are characterized by a warm and subhumid climate, with heavy rains in early and late summer, sparse rains in winter, and a dry period in the middle of summer [30]. In addition, four colonies were collected in 2007 around Tapachula, Chiapas State, Mexico (14°54′00″ N; 92°15′60″ W), and were used for genetic and behavioral analyses.

After nest collection, colonies were carried to the laboratory to both check for the presence of the social parasite and count the number of *E. tuberculatum* queens and workers. Queenless colonies having less than 40 workers were excluded from the analysis, as considered to be not entirely collected. Ninety colonies were transported to the LEEC in Paris where they were reared in an experimental room ( $T = 28 \pm 2^\circ\text{C}$ , 60%–80% of relative hygrometry, light-dark cycle = 12 h : 12 h). They were housed in plaster nests each connected to a foraging area where food and water were provided. They were fed on the same diet composed of honey-apple mixture, mealworms, and crickets. Groups of ants were sampled in the field and from the rearing colonies, and they were preserved in 95°C alcohol for further genetic analysis.

**2.2. Genetic Analysis.** Previous sequences of a fragment of the cytochrome b region (cyt b) of the mitochondrial genome were published in Hora et al. [13]: twenty-seven individuals (9 parasites, 5 queens, and 13 workers of *E. tuberculatum*) from seven parasitized colonies of Apazapan were sequenced for a 750-base pair of cyt b (using the set of primers CB1 and tRS designed from *Apis*, according to standard conditions of amplification, [31]). We compared them with the sequences of individuals from two other Mexican nonparasitized populations (5 individuals from Tapachula (GenBank AF452379) and five from Tuxtla (AF452380)) together with 5 individuals from a Brazilian population (Bahia, AF452381). Purified PCR fragments were sequenced using an ABI 370 automated sequencer and a dye terminator cycle sequencing kit. All sequences were unambiguously aligned using the algorithm CLUSTAL W [32], and checked by eye, on the sequence of *Rhytidoponera victoriae* present in GenBank (U75350). Distances between sequences were calculated according to Jukes and Cantor [33]. A neighbor-joining (NJ) tree based on these distances was constructed using MEGA 5.1 [34], and nodes support was assessed by conducting 1000 bootstrap replicates.

### 2.3. Behavioral Experiments

**2.3.1. Description of the Discrimination Test.** These experiments investigated whether *E. tuberculatum* workers distinguish the social parasites from their conspecifics, from either their own colony or another one. For this we performed discrimination tests where a single host worker faced two stimuli-ants in a neutral arena (Figure 1(a)). The test was modified from Fénéron [35] by using only two (instead of four) categories of stimuli-ants and confronting the workers to stimuli-ants issued from the same parasitized colony. This

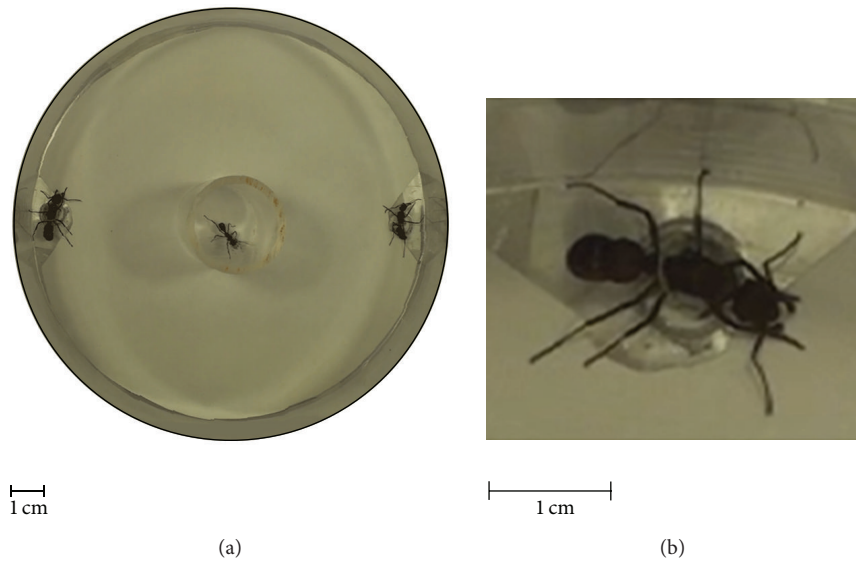


FIGURE 1: The experimental device used for discrimination tests. (a) Overview of the device composed of a round plastic box (11.8 cm diameter) and two fixation systems. The test-worker faced two immobilized stimuli-ants, here a parasite and a conspecific queen. (b) Detailed view of the fixation system on which a queen was immobilized.

allowed us to measure the differential behaviors towards stimuli-ants, while the confounding effects of the stimuli-ants' responses were minimized.

During each test, one parasite and one host were used as stimuli-ants, both from the same colony collected in the Apz1 site. Stimuli-ants were kept alive but immobilized by a thread over the petiole (Figure 1(b)). The test-workers came from different colonial origin as mentioned in the next section. They were sampled from the foraging area by selecting workers that behaved aggressively towards entomological pliers. Foragers are both discriminating and aggressive towards nonnestmate conspecific ants [35], and they are then supposed to be able to reject the parasite. Each test-worker was used only once, but stimuli-ants could be used for several consecutive trials.

After the stimuli-ants have been carefully immobilized, the test-worker was introduced into a glass cylinder in the middle of the arena and was allowed to calm down for about 1 min. The cylinder was then gently removed and the test video-recorded for 5 min (SONY DCR-SR58 camera). After each test the edges of the arena were cleaned with alcohol and the filter paper covering the arena surface was changed to remove any potential chemical marking. The behaviors of the test-workers towards the two stimuli-ants were quantified by scan sampling the video every 5 s (60 scans per individual). Videos were analyzed blindly with respect to the colonial origin of the test-workers.

**2.3.2. Conducted Discrimination Tests.** Two experiments were conducted. In the first one, the *E. tuberculatum* test-workers faced one parasitic queen and one host worker from the same colony of the Apz1 site. Different tests were defined according to the colonial origin of the test-workers. The tests were (1) homocolonial when the test-workers were the nestmates of the stimuli-ants (Apz1H) and allocolonial in all

other cases, (2) nonnestmates from parasitized colonies of the Apz1 site (Apz1P), (3) nonnestmates from nonparasitized colonies of the Apz1 site (Apz1NP), (4) nonnestmates from a different and nonparasitized site (Apz2), and (5) nonnestmates from the nonparasitized population of Tapachula (Tap). A total of 124 tests were performed (22–31 replicates per condition; 8 colonies). Eleven tests were stopped before the 5 min period due to a strong attack against one of the stimuli-ants (i.e., instantaneous and continuous biting over more than 15 s and stinging attempt), and insects were pulled apart. These tests were excluded from the analysis of the behavioral scans.

In the second experiment, we used the same protocol but the test-workers faced one parasitic queen and one host queen from the same colony. In order to prevent *E. tuberculatum* queens from being injured, we carried out only the three types of tests expected to be less aggressive: Apz1H, Apz1P, Apz1NP. A total of 57 tests were performed (12–27 replicates per condition; 4 colonies).

**2.3.3. Behaviors and Data Analysis.** The behaviors displayed towards the stimuli-ants were recorded and categorized as agonistic acts (i.e., escaping, threatening with wide open mandibles, and biting), antennation (i.e., antennal contact on any part of the ant's body), and immobility close to an ant (i.e., standing motionless less than 2 cm away from a stimulus-ant). The latter usually followed antennation and was interpreted as an attracting and settling effect [36].

For each experiment and each type of test, the proportions of tests including aggression, that is, in which at least one agonistic behavior was directed towards the parasite or the conspecific individual, were calculated. They were compared between the types of test for the parasite and the conspecific individual separately using Pearson's exact Chi-Square tests applied to raw data. The behaviors directed

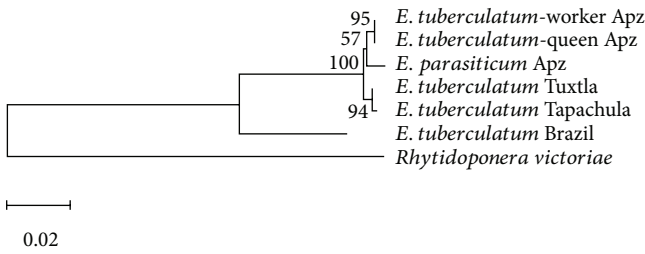


FIGURE 2: Neighbor-joining tree for the different populations. Bootstrap values (1000 replicates) are shown for each node.

toward the parasite and the conspecific individual were quantified as percentages of scans for each test-worker and were compared with Permutation tests for paired samples using the exact method. All statistical analyses were performed using the StatXact-8 software.

### 3. Results

**3.1. Field Study.** Details of the different collections in Apazapan are presented in Table 1. Adult parasites were found only during two out of six field trips, and only in the Apz1 site (but sampling effort was scarce in Apz3). In these cases, alate and dealate parasites were abundant since they were present in 15 out of the 24 collected colonies (63%), and they included a median of 3 alate parasites (range: 0–17) and of 1 dealate parasite (0–5) per colony. In addition, some parasites emerged during March–April 2009 in the laboratory from three colonies collected in January 2009, implying that the parasite was still present in this site at this date.

In the Apazapan population, 26 out of the 98 colonies of *E. tuberculatum* (27%) were polygynous, with a median of two queens (2–8). However, neither the number of host queens (median (and range): 1 (0–3) in the parasitized colonies; 1 (0–8) in nonparasitized colonies, respectively; Permutation tests for independent samples:  $P = 0.48$ ) nor the number of host workers (121 (12–428) in the parasitized colonies; 178 (22–383) in nonparasitized colonies, respectively;  $P = 0.43$ ) was found to differ between parasitized colonies and nonparasitized colonies of the same site (see Supplementary Material available online at <http://dx.doi.org/10.1155/2013/573541>). This showed that host colony size may not limit successful invasion of the parasite and that the parasite did not select specifically populous colonies, or polygynous colonies. The nest distribution of *E. tuberculatum* was patchy, with a distance between nests from 0.6 to 15 m, and we often found several colonies parasitized in the same patch.

**3.2. Genetic Analysis.** Intracolony variation in Apazapan was constituted by two haplotypes, which discriminate *E. parasiticum* from the group composed of host workers and queens from the same colony (Figure 2). There was no haplotype polymorphism between Apazapan colonies, except between the parasite and its host. The two haplotypes diverged by seven variable sites, all of them being transitions, with a nucleotide sequence difference of 0.95%.

Biogeographic variation between *E. tuberculatum* colonies was quite low, with only 6 polymorphic sites discriminating Apazapan from Tapachula (5 transitions and

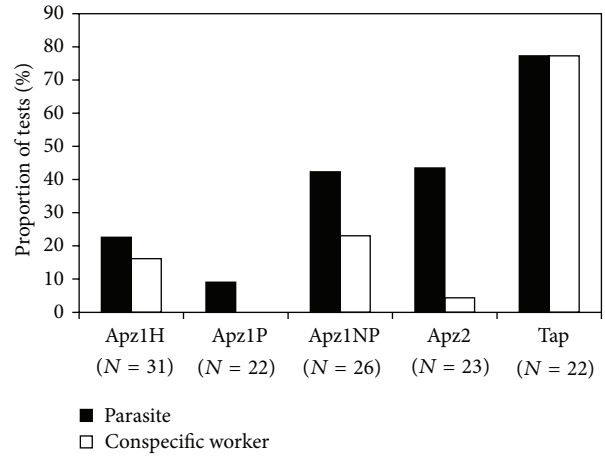


FIGURE 3: Proportions of tests including aggression towards the social parasite or the conspecific worker according to the type of tests. Apz1H = homocolony tests, Apz1P = tests between nonnestmates from parasitized colonies, Apz1NP = tests between nonnestmates from parasitized and nonparasitized colonies of the same site, Apz2 = tests between sites, Tap = tests between populations, and  $N$  = number of tests.

1 transversion, 0.81%) whereas the parasite diverged from Tapachula colonies by 9 variable sites (8 transitions and 1 transversion, 1.08%).

### 3.3. Behavioral Experiments

**3.3.1. Discrimination Tests between a Parasitic Queen and a Conspecific Worker.** The proportions of tests including at least one aggression towards the parasite differed among the type of tests (Figure 3; Pearson's exact Chi-Square test,  $P < 0.001$ ). These proportions were higher in nonparasitized colonies than in parasitized colonies within the Apazapan population ( $P = 0.026$ ), and they reached a maximum level between populations ( $P < 0.001$ ). By contrast, the proportion of tests including aggression against the conspecific workers remained low, except between populations ( $P < 0.001$ ).

Agonistic acts were rare and not specifically directed towards the parasite in homocolony tests (Apz1H) and allocolony tests between parasitized colonies (Apz1P) (Figure 4(a)). By contrast, the tests using nonparasitized colonies showed aggression against the parasite, but the difference was significant only between sites (Apz2). In the two other conditions, the rate of aggression was probably underestimated due to strong attacks which put an end to some tests and excluded them from the statistical analysis. This could explain the absence of significant difference for Apz1NP as 2 out of 26 tests were stopped due to a strong aggression against the parasite, but not for Tap as 9 out of 22 tests were stopped but equally distributed across both species (i.e., 4 against the parasite and 5 against the conspecific).

Antennation was much more frequent towards the parasite than the conspecific worker whatever the tests within the Apazapan population, showing a clear discrimination (Figure 4(b)). This was not the case for the tests between populations where the rate of antennation remained low.

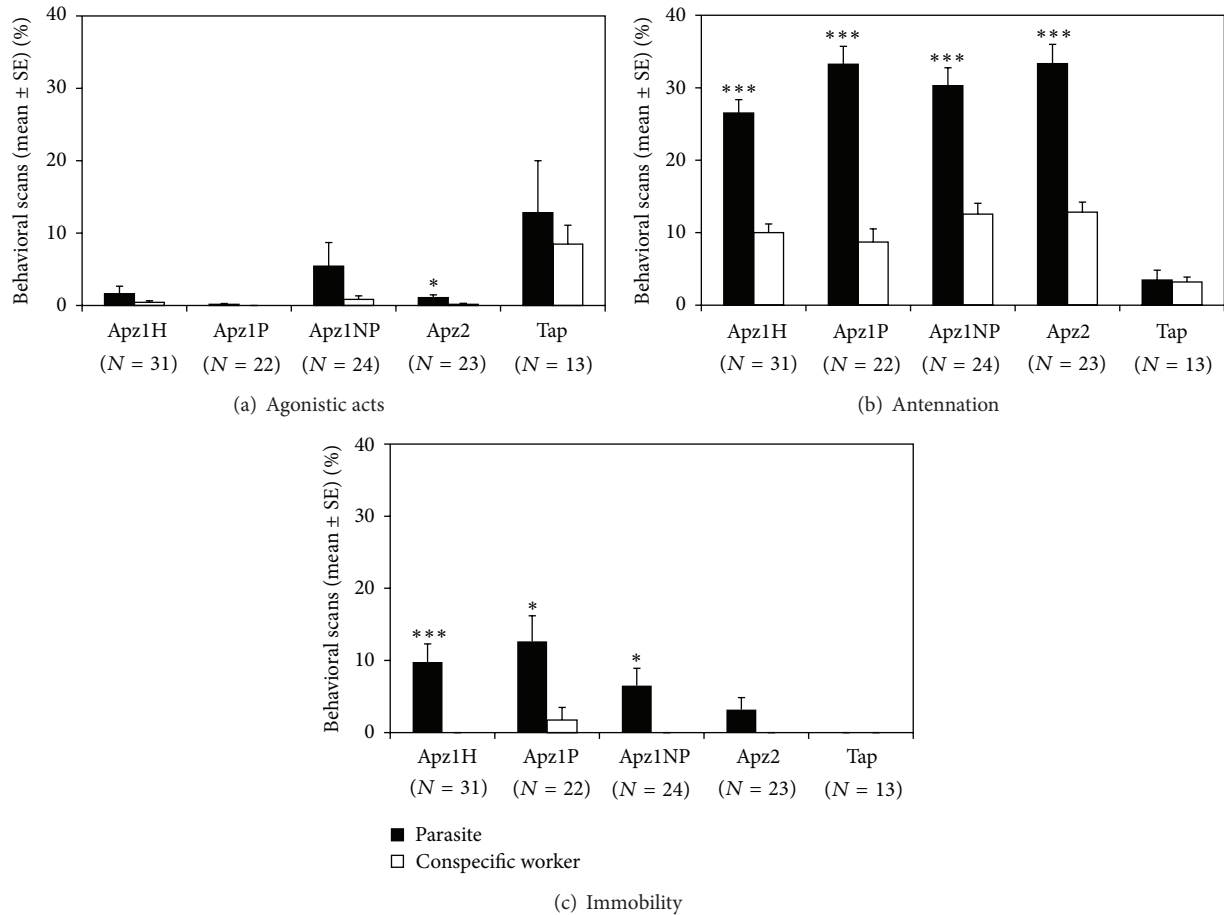


FIGURE 4: Comparison of the behavioral reactions towards the social parasite (black bars) and the conspecific worker (white bars) in the different types of tests (see Figure 3 for the abbreviations). Pairwise comparisons were made with Permutation tests: \* $P < 0.05$ , \*\*\* $P < 0.001$ .  $N$  = number of tests.

Similarly, although at a lesser rate, workers stayed more often motionless near a parasite than a conspecific worker, but the difference was not significant in the tests between sites and never occurred with the *Tapachula* population (Figure 4(c)).

**3.3.2. Discrimination Tests between a Parasitic Queen and a Conspecific Queen.** In this experimental condition, only a few tests included at least one aggression (Figure 5), and no difference between the types of tests was found for the parasitic queen (Pearson's exact Chi-Square test,  $P = 0.21$ ) and the conspecific queen ( $P = 0.66$ ). When occurring, the rate of aggression was low and similar towards both queens (Figure 6(a)). However, the parasite was discriminated through a lesser rate of antennation and immobility compared with the conspecific queen (Figures 6(b) and 6(c)). All differences were statistically significant, except for antennation between nestmates.

## 4. Discussion

**4.1. Field Study.** Field data confirmed and strengthened our previous reports [13, 27] that, unlike the host species [37], the social parasite *E. parasiticum* is rare and very local in

occurrence. Along with its patchy distribution, this suggests a short-range dispersal of the species. Moreover, we showed a change in abundance of the parasite over the time. This could be due to not only its rarity, but also its vulnerability to environmental conditions. Unfortunately climatic data were not available for the whole period, but it seems that the successful collections of the parasites in 1999 and 2000 were preceded by rainy periods, and the unsuccessful one in 2002 was characterized by a long dry period.

Furthermore, environmental constraints, along with genetic factors, are known to explain variation in reproductive strategies [39, 40]. Our data confirm that the colonies of *E. tuberculatum* exhibit a facultative polygyny in the Apazapan population with queens being functionally reproductive [13, 25]. By comparison, in the whole Soconusco region including Tapachula, only three out of 253 colonies collected (1%) were polygynous, including only two queens, and the parasite was never found [38]. A polygynous social organization, by re-adoption of daughter queens, seems to be the rule in *E. tuberculatum* in Brazil, where 49% of the nests exhibited at least two reproductive queens (2–14 queens per nest,  $n = 165$ , recalculated from Hora et al. [13] and Zinck et al. [41]). The social organization

TABLE 1: Composition of the *Ectatomma tuberculatum* colonies sampled in three sites of Apazapan between September 1999 and November 2011.

Date	Site Apz1			Site Apz2			Site Apz3		
	Collected	N of colonies	N of workers Median (range)	Collected	N of colonies	N of workers Median (range)	Collected	N of colonies	N of workers Median (range)
September 1999	3	3	51 (12–120)	—	—	—	—	—	—
June-July 2000	21	12	118 (15–428)	—	—	—	—	—	—
March-April 2002	19	0	178 (64–358)	7	0	146 (55–243)	6	0	139 (51–225)
January 2009	8	0 (3*)	263 (163–341)	15	0	198 (46–287)	—	—	—
September 2010**	—	—	—	8	0	68 (47–150)	—	—	—
November 2011	4	0	162 (95–383)	4	0	242 (74–254)	3	0	94 (57–148)
Total	55	18	157 (12–428)	34	0	146.5 (46–287)	9	0	99 (51–225)

—: no sampled data; \* emergence of parasites from larvae collected in field; \*\* data from Pérez-Lachaud et al. [38].

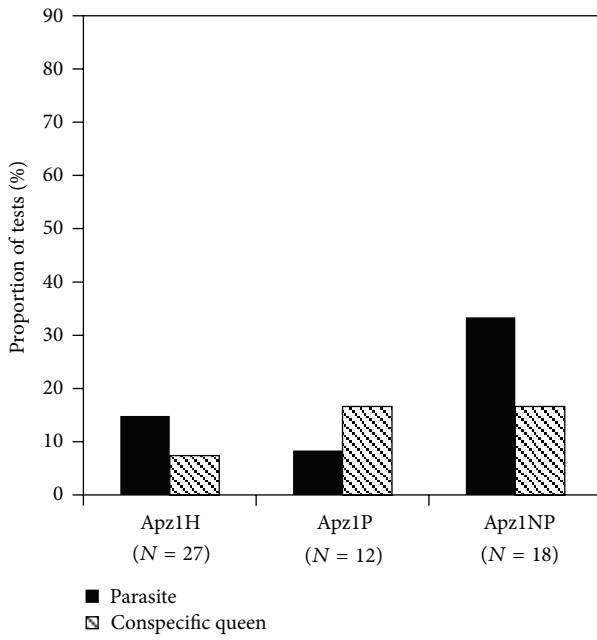


FIGURE 5: Proportions of tests including aggression towards the social parasite or the conspecific queen according to the type of tests (see Figure 3 for the abbreviations).  $N$  = number of tests.

in Brazil is characterized by a polydomous structure and reproduction by nest budding, both characteristics increasing the size of *E. tuberculatum* colonies territory, and therefore insuring the ecological dominance of the species [42, 43]. In the case of Apazapan in Mexico, nest distribution of *E. tuberculatum* is also patchy, but the soil is highly rocky, the stone often forming a horizontal homogeneous layer that limits abilities for queens to found new nests. The nest site limitation, plus other ecological factors yet unidentified, might have constrained polygyny, as already suggested for the *E. tuberculatum* population of Apazapan [38] and shown in other ant species [44]. In a second step, polygyny might have favored the selection of selfish reproductive strategies and then the evolution of social parasite by sympatric speciation [1, 6, 10].

**4.2. Genetic Analysis.** The node built from cytochrome b sequences was poorly supported between *E. tuberculatum* and *E. parasiticum*. Investigation in both other genes sequencing and more populations are needed to resolve this divergence. However, the low levels of divergence between *E. parasiticum* and its host combined with the observed geographic variation are consistent with the strict acceptance of Emery's rule [5] and support the hypothesis of a recent divergence between *E. tuberculatum* and its parasite. *Ectatomma parasiticum* might have evolved by sympatric speciation from its host species in Apazapan, due to a previous evolution of *E. tuberculatum* to polygyny (polygyny syndrome [45]) and environmental conditions. Miniaturization of queens was linked to social parasitism in several ant species (see [46, 47], and also see, e.g., [14, 15]). Convergent arguments from field studies and laboratory experiments suggest that assortative mating through direct mate choice, or through choice of

different mating habitat between miniaturized and large queens, led divergent selection up to sympatric speciation [7].

**4.3. Discrimination Ability and Social Tolerance.** Our results show that *E. tuberculatum* host workers were able to distinguish the social parasites *E. parasiticum* from their conspecifics. Such discrimination occurred only within the parasitized population (Apazapan) and was inferred from differential responses in antennation and immobility, and in some cases in aggression. By contrast, workers from the nonparasitized, monogynous, and geographically distant population (Tapachula) attacked vigorously both parasitic and conspecific ants, considering both as intruders.

When confronted to *E. tuberculatum* workers from its own colony, the parasitic queen was more antennated and more attractive than a nestmate worker, but less attractive than a nestmate queen. The parasite was thus perceived as a distinct entity, even by the members of its own host colony. This is unusual because inquiline species are expected either to avoid any detection or to be treated as a nestmate, depending on the chemical strategy (see e.g., [24, 48] in ants, [49], in bumblebees, and [50] in wasps). Because our test was independent of the stimuli-ants' behaviors, such discrimination was supposed to be primarily based on chemicals, even if differences in size could also be detected. This is congruent with recent chemical analyses showing that *E. parasiticum* was chemically distinct from its host species [28]. In particular, the parasite had reduced amounts of cuticular hydrocarbons, and it differed from its host in the relative composition of some of these compounds. This is also consistent with behavioral observations in a more natural context, as some parasites were specifically antennated or attacked by the host workers within their colony [25].

Allocolonial tests within the Apazapan population showed that workers responded differentially towards parasites and conspecific nonnestmates, either workers or queens. Both parasitic and conspecific queens from another colony were considered as individuals of interest, as they elicited intense antennal inspection. It could be a result of novelty due to the detection of unfamiliar odors. These odors, however, could not be exclusively colony specific as nonnestmate workers of *E. tuberculatum* were treated differently from conspecific nonnestmate queens. Because antennation and immobility were mostly associated with the presence of *E. tuberculatum* queens, we supposed that workers were attracted to queen-specific substances. Queen pheromones are known to produce an attracting and settling effect on workers and cause the retinue behavior in ants [36, 51] and honeybees [52]. In ants, this effect can be elicited by surface molecules probably linked to fertility signals and esters from Dufour's gland secretion [2, 18, 53]. The hydrocarbon cuticular profile of *E. tuberculatum* queens differed from that of workers [28, 54] and virgin queens [55]. Some alkanes have been proposed as fertility signals in this species [55], but we also found esters on the queens' cuticle that could be involved as well [28]. The lesser amount of these compounds on the parasite's cuticle compared with conspecific queen could explain the lower effect of attractiveness on *E. tuberculatum* workers.

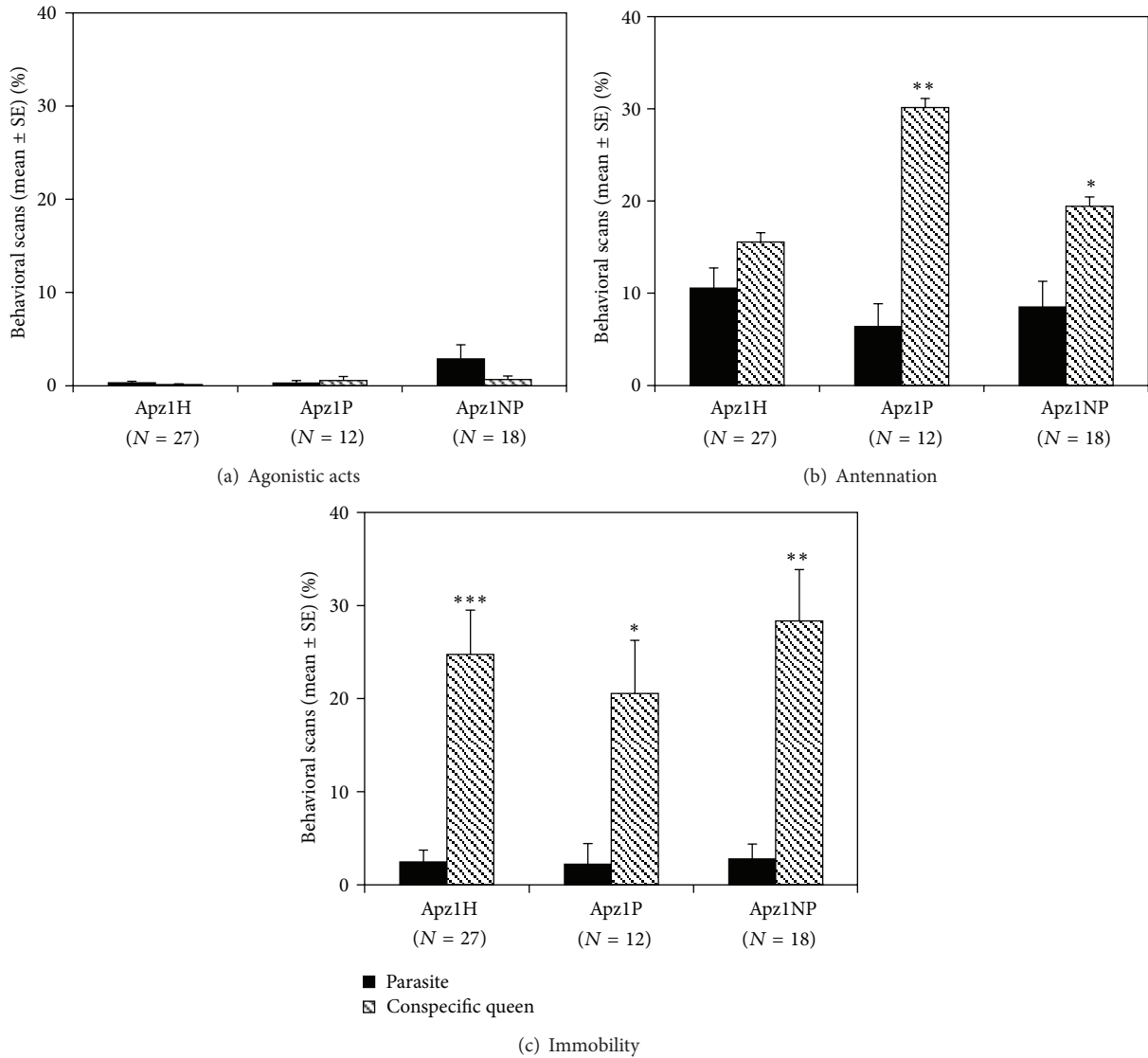


FIGURE 6: Comparison of the behavioral reactions towards the social parasite (black bars) and the conspecific queen (dashed bars) in the different types of tests (see Figure 3 for the abbreviations). Pairwise comparisons were made with Permutation tests: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .  $N$  = number of tests.

However, workers from nonparasitized and distant colonies in the Apazapan population were less attracted by the parasite and some of them attacked it, thus considering it as an intruder. Aggression means a possible rejection that could explain why some colonies were parasitized and the others not. The level of aggression, however, remained low. This could be partly due to the experimental device, as the neutral arena and the immobilization of stimuli-ants are known to limit aggressive reactions [35]. But more likely, because strong aggression between nonnestmate workers of *E. tuberculatum* from the monogynous population of Tapachula was observed using the same discrimination test ([56] and this paper), it could be associated to life-history traits specific to individuals from the Apazapan population. Polygyny by mixing odors from individuals of different genetic lineages (Gestalt model [57]) may affect recognition

systems. It is likely to increase the tolerance threshold of the workers within colonies and to reduce the variation in chemical cues between colonies, resulting in a lower level of aggression between nonnestmates at a population level ([39, 58], but see [59]). Both of these features may have facilitated the exploitation of the host by a social parasite [3].

## 5. Conclusions

*Ectatomma parasiticum* shared several life-history traits with other workerless inquiline ants [1, 3]: rarity, local distribution, variation in abundance, limited dispersal, intracolony mating, queen miniaturization, morphological similarity with its host, and quasiexclusive production of sexuals ([13, 28] and this paper). Some of these parasitic traits, the polygynous population of the host, and the association between sibling



species are arguments which may support the hypothesis of sympatric speciation. Despite a possible recent divergence of the social parasite from its host, we showed that *E. parasiticum* could be discriminated by its host, and then potentially rejected. Nevertheless, most parasites elicited interest and attractiveness from the host, probably because of their peculiar chemical profile (a weak chemical signature) and/or their reproductive status. We suggest that *E. parasiticum* could have conserved from its host sibling species the queen-specific substances that produce attracting and settling effect on workers, then making the exploitation of the host easier. However, recognition in ants is a multi-component system which encodes different types of information [17, 18], but not independently of one another. For example, it has been recently suggested that fertility signal interferes with the production or the perception of colony-specific cues in *Camponotus floridanus* [60]. In case of *E. parasiticum*, host worker attractiveness due to the queen-specific substance could, in return, increase the probability to be detected as carrying distinct recognition cues, and then to be attacked by the most discriminating host workers. This hypothesis would explain why the social integration of the parasite into host colonies is imperfect [25]. Which peculiar compounds or class of compounds are involved in each recognition level remains to be clarified. Further experiments by manipulating queen odors are needed that should also enlighten the function of queen chemicals in social insects, in general.

## Acknowledgments

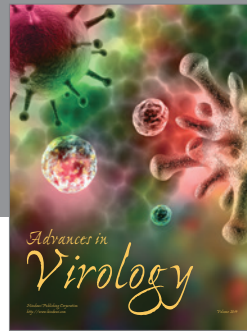
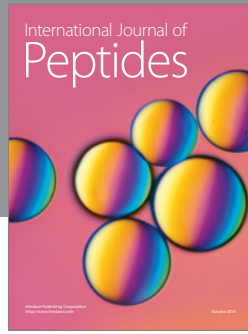
The authors would like to thank Nicolas Châline, Stéphane Chameron, Jean-Paul Lachaud, and three anonymous reviewers for providing critical comments on the manuscript and improving English. The authors are particularly grateful to Mario Favila, the INECOL staff, and students for helping during the fieldwork in Mexico, and also grateful to Dominique Fresneau and Riviane R. da Hora who collected the colonies from 1999 to 2002. The authors are grateful to Marie-Claire Malherbe and Marjorie Labédan as well for rearing the colonies in the laboratory. This work was partly financed by the CREI of the Université Paris 13 (no. 900-RELI-247) and by the ANR (no. ANR-09JCJC-0031).

## References

- [1] A. Buschinger, "Social parasitism among ants: a review (Hymenoptera: Formicidae)," *Myrmecological News*, vol. 12, pp. 219–235, 2009.
- [2] B. Hölldobler and E. O. Wilson, *The Ants*, Springer, Berlin, Germany, 1990.
- [3] D. R. Nash and J. J. Boomsma, "Communication between hosts and social parasites," in *Sociobiology of Communication: An Interdisciplinary Perspective*, P. d'Ettorre and D. Hughes, Eds., pp. 55–79, Oxford University Press, Oxford, UK, 2008.
- [4] E. O. Wilson, *The Insect Societies*, Belknap Press of Harvard University Press, Cambridge, Mass, USA, 1971.
- [5] G. Le Masne, "Recherches sur les fourmis parasites. *Plagiolepis grassei* et l'évolution des *Plagiolepis* parasites," *Comptes rendus de l'Académie des Sciences*, vol. 243, pp. 673–675, 1956.
- [6] A. F. G. Bourke and N. R. Franks, "Alternative adaptations, sympatric speciation and the evolution of parasitic, inquiline ants," *Biological Journal of the Linnean Society*, vol. 43, no. 3, pp. 157–178, 1991.
- [7] R. Savolainen and K. Vepsäläinen, "Sympatric speciation through intraspecific social parasitism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 12, pp. 7169–7174, 2003.
- [8] S. Sumner, D. K. Aanen, J. Delabie, and J. J. Boomsma, "The evolution of social parasitism in *Acromyrmex* leaf-cutting ants: a test of Emery's rule," *Insectes Sociaux*, vol. 51, no. 1, pp. 37–42, 2004.
- [9] J. A. Smith, S. M. Tierney, Y. C. Park, S. Fuller, and M. P. Schwarz, "Origins of social parasitism: the importance of divergence ages in phylogenetic studies," *Molecular Phylogenetics and Evolution*, vol. 43, no. 3, pp. 1131–1137, 2007.
- [10] A. Buschinger, "Sympatric speciation and radiative evolution of socially parasitic ants—heretic hypotheses and their factual background," *Zeitschrift für Zoologische Systematik und Evolutionsforschung*, vol. 28, no. 4, pp. 241–260, 1990.
- [11] K. Vepsäläinen, J. R. Ebsen, R. Savolainen, and J. J. Boomsma, "Genetic differentiation between the ant *Myrmica rubra* and its microgynous social parasite," *Insectes Sociaux*, vol. 56, no. 4, pp. 425–437, 2009.
- [12] G. Jansen, R. Savolainen, and K. Vepsäläinen, "Phylogeny, divergence-time estimation, biogeography and social parasite-host relationships of the Holarctic ant genus *Myrmica* (Hymenoptera: Formicidae)," *Molecular Phylogenetics and Evolution*, vol. 56, no. 1, pp. 294–304, 2010.
- [13] R. R. Hora, C. Doums, C. Poteaux et al., "Small queens in the ant *Ectatomma tuberculatum*: a new case of social parasitism," *Behavioral Ecology and Sociobiology*, vol. 59, no. 2, pp. 285–292, 2005.
- [14] J. P. Lachaud, A. Cadena, B. Schatz, G. Pérez-Lachaud, and G. Ibarra-Núñez, "Queen dimorphism and reproductive capacity in the ponerine ant, *Ectatomma ruidum* Roger," *Oecologia*, vol. 120, no. 4, pp. 515–523, 1999.
- [15] J. C. Lenoir, J. P. Lachaud, A. Nettel, D. Fresneau, and C. Poteaux, "The role of microgynes in the reproductive strategy of the neotropical ant *Ectatomma ruidum*," *Naturwissenschaften*, vol. 98, no. 4, pp. 347–356, 2011.
- [16] R. W. Howard and G. J. Blomquist, "Ecological, behavioral, and biochemical aspects of insect hydrocarbons," *Annual Review of Entomology*, vol. 50, pp. 371–393, 2005.
- [17] P. D'Ettorre, "Multiple levels of recognition in ants: a feature of complex societies," *Biological Theory*, vol. 3, no. 2, pp. 108–113, 2008.
- [18] A. Hefetz, "The evolution of hydrocarbon pheromone parsimony in ants (Hymenoptera: Formicidae)—interplay of colony odor uniformity and odor idiosyncrasy. A review," *Myrmecological News*, vol. 10, pp. 59–68, 2007.
- [19] P. D'Ettorre and A. Lenoir, "Nestmate recognition," in *Ant Ecology*, L. Lach, C. L. Parr, and K. L. Abbott, Eds., pp. 194–209, Oxford University Press, Oxford, UK, 2010.
- [20] C. Von Beeren, S. Pohl, and V. Witte, "On the use of adaptive resemblance terms in chemical ecology," *Psyche*, vol. 2012, Article ID 635761, 7 pages, 2012.
- [21] K. Dettner and C. Liepert, "Chemical mimicry and camouflage," *Annual Review of Entomology*, vol. 39, pp. 129–154, 1994.
- [22] A. Lenoir, P. D'Ettorre, C. Errard, and A. Hefetz, "Chemical ecology and social parasitism in ants," *Annual Review of Entomology*, vol. 46, pp. 573–599, 2001.

- [23] T. Akino, "Chemical strategies to deal with ants: a review of mimicry, camouflage, propaganda, and phytomimesis by ants (Hymenoptera: Formicidae) and other arthropods," *Myrmecological News*, vol. 11, pp. 173–181, 2008.
- [24] N. Franks, M. Blum, R. K. Smith, and A. B. Allies, "Behavior and chemical disguise of cuckoo ant *Leptothorax kutteri* in relation to its host *Leptothorax acervorum*," *Journal of Chemical Ecology*, vol. 16, no. 5, pp. 1431–1444, 1990.
- [25] R. R. Hora, R. Blatrix, D. Fresneau, and R. Fénéron, "Social interactions between an inquiline ant, *Ectatomma parasiticum*, and its host, *Ectatomma tuberculatum* (Formicidae, Ectatomminae)," *Journal of Ethology*, vol. 27, no. 2, pp. 285–288, 2009.
- [26] R. M. Feitosa, R. R. Hora, J. H. C. Delabie, J. Valenzuela, and D. Fresneau, "A new social parasite in the ant genus *Ectatomma* F. Smith (Hymenoptera, Formicidae, Ectatomminae)," *Zootaxa*, no. 1713, pp. 47–52, 2008.
- [27] R. R. Da Hora, R. Fénéron, J. Valenzuela, M. E. Favila, and D. Fresneau, "Queen-size dimorphism in the ant *Ectatomma tuberculatum* (Hymenoptera: Formicidae: Ponerinae)," *Sociobiology*, vol. 38, no. 3, pp. 407–420, 2001.
- [28] F. Savarit and R. Fénéron, "Imperfect chemical mimicry explains the imperfect social integration of the inquiline ant, *Ectatomma parasiticum*," in preparation.
- [29] C. G. Castillo, "Ecología del paisaje del municipio de jalcomulco," in *Facultad de Ciencias*, UNAM, Veracruz MSc, 1995.
- [30] E. Garcia, "Modificaciones al sistema de clasificación climática de Köppen," in *Instituto de Geografía*, Universidad Nacional Autónoma de México, México D.F., 1973.
- [31] R. H. Crozier and Y. C. Crozier, "The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization," *Genetics*, vol. 133, no. 1, pp. 97–117, 1993.
- [32] J. D. Thompson, D. G. Higgins, and T. J. Gibson, "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice," *Nucleic Acids Research*, vol. 22, no. 22, pp. 4673–4680, 1994.
- [33] T. H. Jukes and C. R. Cantor, "Evolution of protein molecules," in *Mammalian Protein Metabolism*, H. N. Munro, Ed., pp. 21–132, Academic Press, New York, NY, USA, 1969.
- [34] K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar, "Mega5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods," *Molecular Biology and Evolution*, vol. 28, no. 10, pp. 2731–2739, 2011.
- [35] R. Fénéron, "A new ethological test to study nestmate recognition in adult ants," *Insectes Sociaux*, vol. 43, no. 2, pp. 131–135, 1996.
- [36] B. Hölldobler, C. Peeters, and M. Obermayer, "Exocrine glands and the attractiveness of the ergatoid queen in the ponerine ant *Megaponera foetens*," *Insectes Sociaux*, vol. 41, no. 1, pp. 63–72, 1994.
- [37] N. A. Weber, "Two common ponerine ants of possible economic significance, *Ectatomma tuberculatum* (Olivier) and *E. ruidum* Roger," *Proceedings of the Entomological Society of Washington*, vol. 48, no. 1, pp. 1–16, 1946.
- [38] G. Pérez-Lachaud, J. E. Valenzuela, and J.-P. Lachaud, "Is increased resistance to parasitism at the origin of polygyny in a mexican population of the ant *Ectatomma tuberculatum* (Hymenoptera: Formicidae)?" *The Florida Entomologist*, vol. 94, no. 3, pp. 677–684, 2011.
- [39] L. Keller, Ed., *Queen Number and Sociality in Insects*, Oxford University Press, Oxford, UK, 1993.
- [40] J. Heinze, "Social plasticity: ecology, genetics and the structure of ant societies," in *Ecology of Social Evolution*, J. Korb and J. Heinze, Eds., pp. 129–150, Springer, Berlin, Germany, 2008.
- [41] L. Zinck, P. Jaisson, R. R. Hora, D. Denis, C. Poteaux, and C. Doums, "The role of breeding system on ant ecological dominance: genetic analysis of *Ectatomma tuberculatum*," *Behavioral Ecology*, vol. 18, no. 4, pp. 701–708, 2007.
- [42] D. Leston, "A neotropical ant mosaic," *Annals of the Entomological Society of America*, vol. 71, no. 4, pp. 649–653, 1978.
- [43] J. D. Majer, J. H. C. Delabie, and M. R. B. Smith, "Arboreal ant community patterns in Brazilian cocoa farms," *Biotropica*, vol. 26, no. 1, pp. 73–83, 1994.
- [44] T. McGlynn, "Serial monodomy in the gypsy ant, *Aphaenogaster araneoides*: does nest odor reduction influence colony relocation?" *Journal of Insect Science*, vol. 10, article 195, 2010.
- [45] L. Keller, "Social life: the paradox of multiple-queen colonies," *Trends in Ecology and Evolution*, vol. 10, no. 9, pp. 355–360, 1995.
- [46] P. Nonacs and J. E. Tobin, "Selfish larvae: development and the evolution of parasitic behavior in the Hymenoptera," *Evolution*, vol. 46, no. 6, pp. 1605–1620, 1992.
- [47] S. Aron, L. Passera, and L. Keller, "Evolution of social parasitism in ants: size of sexuals, sex ratio and mechanisms of caste determination," *Proceedings of the Royal Society B*, vol. 266, no. 1415, pp. 173–177, 1999.
- [48] D. Lambardi, F. R. Dani, S. Turillazzi, and J. J. Boomsma, "Chemical mimicry in an incipient leaf-cutting ant social parasite," *Behavioral Ecology and Sociobiology*, vol. 61, no. 6, pp. 843–851, 2007.
- [49] S. Dronnet, X. Simon, J. C. Verhaeghe, P. Rasmont, and C. Errard, "Bumblebee inquilinism in *Bombus (Fernaldaep-sithyrus) sylvestris* (Hymenoptera, Apidae): behavioural and chemical analyses of host-parasite interactions," *Apidologie*, vol. 36, no. 1, pp. 59–70, 2005.
- [50] M. F. Sledge, F. R. Dani, R. Cervo, L. Dapporto, and S. Turillazzi, "Recognition of social parasites as nest-mates: adoption of colony-specific host cuticular odours by the paper wasp parasite *Polistes sulcifer*," *Proceedings of the Royal Society B*, vol. 268, no. 1482, pp. 2253–2260, 2001.
- [51] C. W. Rettenmeyer, H. Topoff, and J. Mirenda, "Queen retinues of army ants (Hymenoptera, Formicidae, Ectoninae)," *Annals of the Entomological Society of America*, vol. 71, no. 4, pp. 519–528, 1978.
- [52] K. N. Slessor, M. L. Winston, and Y. Le Conte, "Pheromone communication in the honeybee (*Apis mellifera* L.)," *Journal of Chemical Ecology*, vol. 31, no. 11, pp. 2731–2745, 2005.
- [53] T. Monnin, "Chemical recognition of reproductive status in social insects," *Annales Zoologici Fennici*, vol. 43, no. 5-6, pp. 515–530, 2006.
- [54] L. Zinck, D. Denis, R. R. Hora et al., "Behavioral and chemical correlates of long-term queen adoption in the facultative polygynous ant *Ectatomma tuberculatum*," *Journal of Insect Behavior*, vol. 22, no. 5, pp. 362–374, 2009.
- [55] R. R. Hora, A. Ionescu-Hirsh, T. Simon et al., "Postmating changes in cuticular chemistry and visual appearance in *Ectatomma tuberculatum* queens (Formicidae: Ectatomminae)," *Naturwissenschaften*, vol. 95, no. 1, pp. 55–60, 2008.
- [56] R. Fénéron, E. Nowbahari, and F. Dutrou, "Reconnaissance intercoloniale et niveau d'agression chez la fourmi Ponerine, *Ectatomma tuberculatum*," *Actes Colloques Insectes Sociaux*, vol. 12, pp. 33–36, 1999.

- [57] R. H. Crozier and M. W. Dix, "Analysis of two genetic models for the innate components of colony odor in social Hymenoptera," *Behavioral Ecology and Sociobiology*, vol. 4, no. 3, pp. 217–224, 1979.
- [58] L. Sundström, "Queen acceptance and nestmate recognition in monogyne and polygyne colonies of the ant *Formica truncorum*," *Animal Behaviour*, vol. 53, no. 3, pp. 499–510, 1997.
- [59] S. J. Martin, H. Helanterä, K. Kiss, Y. R. Lee, and F. P. Drijfhout, "Polygyny reduces rather than increases nestmate discrimination cue diversity in *Formica exsecta* ants," *Insectes Sociaux*, vol. 56, no. 4, pp. 375–383, 2009.
- [60] D. Moore and J. Liebig, "Mixed messages: fertility signaling interferes with nestmate recognition in the monogynous ant *Camponotus floridanus*," *Behavioral Ecology and Sociobiology*, vol. 64, no. 6, pp. 1011–1018, 2010.



**Hindawi**

Submit your manuscripts at  
<http://www.hindawi.com>

