Behavioural Ecology of Bark-digging Ants of the Genus *Melissotarsus*

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ABSTRACT

In *Melissotarsus beccarii* and *M. weissi*, two ant species which damage trees by digging nest galleries in bark, we observed numerous egg-producing physogastric queens situated more than one meter from each other, but within the same colony, making this the first record of oligogyny in this genus. Our analysis of ovarian development and degree of mandible wear reveals that a temporal polyethism exists and that gynes perform the worker duty of digging galleries. This is a new and previously unreported case of worker-like behaviour in gynes. These ants appear to follow a dynamic process wherein newly-inseminated gynes accepted by colonies perform worker-like tasks and do not produce eggs until they have the opportunity to dominate their own section of the colony and become physogastric. Workers that elude the queen's influence and produce chorioned, viable eggs probably play a role in the control of reproduction by non-physogastric gynes. Intraspecific aggressiveness between colonies was low. While spreading beneath the bark, both incipient and mature colonies can merge, forming very large colonies over vast areas of bark. This study demonstrates that *Melissotarus* should be of special concern for the management of forest and tree crop plantations.

INTRODUCTION

Ants of the genus *Melissotarsus*, found from Saudi Arabia to South Africa and Madagascar, threaten tree crop plantations and managed forests. In West Africa they have a major impact on trees of the family Burceraceae such as Okoumé (*Okoumea klaineana* Pierre), whose wood is used in the manufacture of plywood (Ben-Dov 1978; Prins *et al*. 1990; Fisher and Robertson 1999; Mony *et al*. 2002). Colonies live in a network of chambers and tunnels dug by workers in both bark and live wood. Workers never forage outside their nest galleries. These colonies can become extremely large, spreading to the highest branches, so that in South Africa large *Ficus sur* Forssk limbs have had to be removed to prevent their collapse onto public ways (Fisher and Robertson 1999). *Melissotarsus* is uniquely adapted to living in galleries. (1) The workers walk with their middle pair of legs pointing upwards in contact with the ceiling of the galleries, so that they stagger when placed on a flat surface (Delage-Darchen 1972). (2) Their large head glands produce silk that is excreted around the hypostoma, corresponding to the only known case of silk production by adult ants (Fisher and Robertson 1999). The modified protarsi of their forelegs are used to pull and spin the silk to line and plug the galleries. (3) Within the galleries, *Melissotarsus* workers tend phloem-feeding hemiptera of the family Diaspididae. Considered to be the most evolved of the Coccoidea, Diaspididae do not produce honeydew, so that Ben-Dov (1978) deduced that they are eaten by attending ants, something confirmed by our observations. (4) They are one of the few ants specialized for tunneling through live wood. Older workers erode their mandibles tunneling so that the age of individuals can be identified by the degree of wear on their mandibles, in particular by the length and sharpness of their apical tooth (Delage-Darchen 1972).

Despite these unique adaptations and puzzling relation-ship with Diaspididae, we know very little about the behavioural ecology of *Melissotarsus*. In this study, we explore factors related to colony growth and reproduction in *M. beccarii* Emery and *M. weissi* Santschi whose workers also produce silk (pers. observation). Specifically, we investigate how colonies reach colossal sizes that strongly affect host trees (Mony *et al*. 2002). This is of particular interest for managing this genus when it becomes a pest.

This study had three primary goals: (1) to evaluate the density and condition of the ovaries of the gynes, and determine whether or not workers are able to elude the queen's control and lay viable male eggs (a possible explanation for why worker-produced males are noted all year long); (2) obtain information on temporal polyethism by comparing the state of workers' ovaries with the degree of wear on their mandibles; and (3) determine whether well-established colonies are likely to merge with incipient colonies that become established on the same tree, favouring the formation of huge colonies.

MATERIALS AND METHODS

Background information

As with other social Hymenoptera, ants produce both queens and female workers from fertilized, diploid eggs, while non-fertilized eggs produce haploid males. In most species, queens found their own colonies and assume all tasks necessary to rear the first set of workers. Whereas queens founding independent claustral colonies metabolize their wing muscles for the energy to rear their first generation of offspring, the queens of other species must forage during this time (semi-claustral foundation). Later, queens specialize in egg-laying, while workers assume all other tasks (Hölldobler and Wilson 1990). Workers generally have functional ovaries, but are unable to mate and lack spermathecae, leaving them with the potential to produce only male progeny. The queens release

pheromones that inhibit the functioning of worker ovaries. But workers can elude this influence in a number of ways, causing competition over the production of males. Typically, ant colonies have a single queen (monogyny; about 50% of the cases) where workers have a stronger incentive to rear the queen's diploid larvae, as they share ¾ of their genes (coefficient of relatedness r = 0.75) with these offspring (Bourke and Franks 1995). This scheme is more complicated when multiple mating occurs and in colonies with several queens (polygyny).

 Indeed, the colonies of many species may be founded by multiple queens (primary polygyny), but more frequently, polygynous colonies result when new queens are accepted into established colonies (secondary polygyny). Factors favouring polygyny include queens with a lifespan shorter than their colonies and/or a high probability that nests will lose their queens through predation or other events. In both cases, polygyny decreases the risk of colony extinction. Queens in most polygynous colonies compete for reproductive dominance. They release pheromones diminishing the production of the juvenile hormone in other queens, dampening the ovarian activity and egg production of these competitors. As a result, polygyny is correlated with a decrease in individual fecundity (Hölldobler and Wilson 1990; Vargo 1992; Keller 1995; Brent and Vargo 2003). Also, in polygynous colonies the determinants of the reproductive skew (the partitioning of reproduction between queens) varies within and between species and a large part of the sexual progeny (young queens and males) are not the offspring of the extant queens, implying the existence of a turnover of queens during the colony's lifespan (Bourke *et al*. 1997; Fournier *et al*. 2004; Bargum and Sundström 2007; Kümmerli and Keller 2007).

Conflicts between queens and workers or among workers themselves often arise in polygynous colonies where within-nest relatedness is low. This is particularly common in colonies where queens mate more than once. Areas of potential conflict include: (1) Queen care, where workers preferentially rear related female larvae; this has been found only once in polygynous ants (Ratnieks *et al*. 2006). (2) Queen policing, when conflict arises in male care because workers can reproduce. Each worker is more closely related to her own sons (r = 0.50) than to her brothers (r = 0.25), while the queen is more closely related to her sons (r = 0.50) than to the workers' sons (grandsons; r = 0.25). Queen selection is driven by the ability to prevent workers from reproducing via pheromones or other means. (2) Worker policing, where selection favours workers who can prevent each other from reproducing (Ratnieks *et al*. 2006), as each worker is more closely related to her sons (r = 0.50) than to her brothers and nephews (other workers' sons; r < 0.25 for multiply-mated queens and/or polygynous colonies).

In this study we use the following terms for female members of the reproductive caste. Gynes is a broad term that includes both unmated and mated females that are potentially reproductive. Queens are mated gynes that are fertile and capable of egg-laying. Queens are termed physogastric when they exhibit extreme ovarian development and expanded abdomens (see Hölldobler and Wilson 1990).

Study sites and ant collection

This study was conducted in southern Cameroon on ant colonies found on the University of Yaoundé campus (3°53'N; 11°30' E) and in the old secondary forest at Kala (3°50'N; 11°21' E).

We searched for colonies by peeling away bark at the base of nine safoo trees (*Dacryodes edulis* [G. Don] H.L. lam; Burceraceae) to collect *M. beccarii* and 13 mango trees (*Mangifera indica* L.; Anacardiaceae) to collect *M. weissi*. When we realized that incipient colonies prefer the smooth bark of trunks and main branches, we also peeled away bark from young trees. We collected the bark, which included living ants, for further study in the laboratory. We estimated the proportions of gynes and workers by sampling 10 × 10 cm sections of bark taken at random in areas where the bark is rough up to 4 m high: 44 sections on one safoo tree, and 31 and 33 sections on two mango trees, respectively.

Specimens have been deposited at the California Academy of Sciences (CAS) and the Natural History Museum, London (BMNH). Specimens of both species have been imaged and are available on www.antweb.org.

## Attempts to breed parts of *Melissotarsus* colonies

As *Melissotarsus* workers are unable to walk on a flat surface, we tried to breed colonies in the laboratory by sandwiching them between various substrates and a plate of glass positioned at an adequate height so their middle legs reached the ceiling. The device consisted of water absorbent cardboard serving as substrate over which we put a plate of glass held in place by pillars of modelling clay; the adequate height was adjusted by pressing down on the plate of glass (flattening the modelling clay) until the workers were able to move about easily.Our attempts were ultimately unsuccessful because we had trouble feeding the colonies' Diaspidides and controlling the level of humidity. Nevertheless, we were able to keep queenright parts of colonies alive for two weeks or more, permitting us to see whether any workers laid eggs.

State of ovaries related to mandible wear

In a preliminary study we verified the mandible wear on workers and gynes. Using a stereomicroscope equipped with a micrometer, we measured for each left mandible (1) the dorsal length of the apical tooth from its base to the apex "L_1", and (2) the length of the external margin of the entire mandible from the base to the
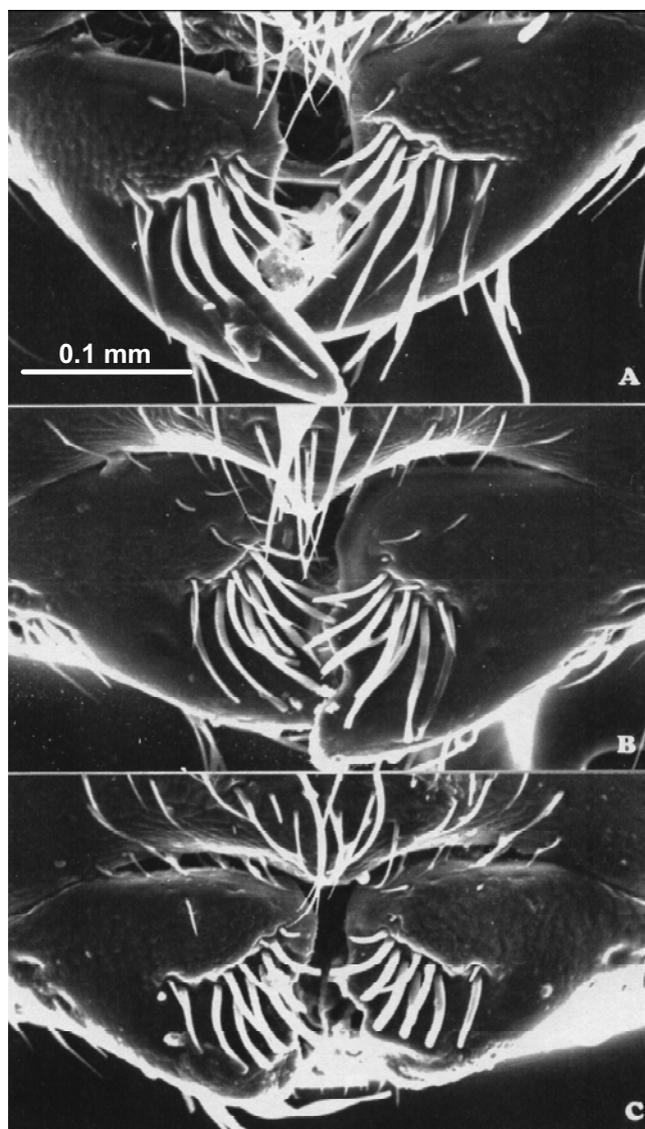
Fig. 1 Three typical states of mandible wear in *Melissotarsus beccarii*. (**A**) Intact, sharp mandibles (≈ 0.3 mm); the bristles at the base of the dorsal face are long and sharp. (**B**) Worn mandibles, or mandibles worn at the edge of the masticatory margin. Note that the apical teeth are no longer sharp and the bristles at the base of the dorsal face are worn at the ends. (**C**) Very worn mandibles (≈ 0.22 mm); the bristles at the base of the dorsal face are also very worn, measuring approximately one-third of their initial length.

WI *Melissotarsus beccarii*

WI *Melissotarsus weissi*
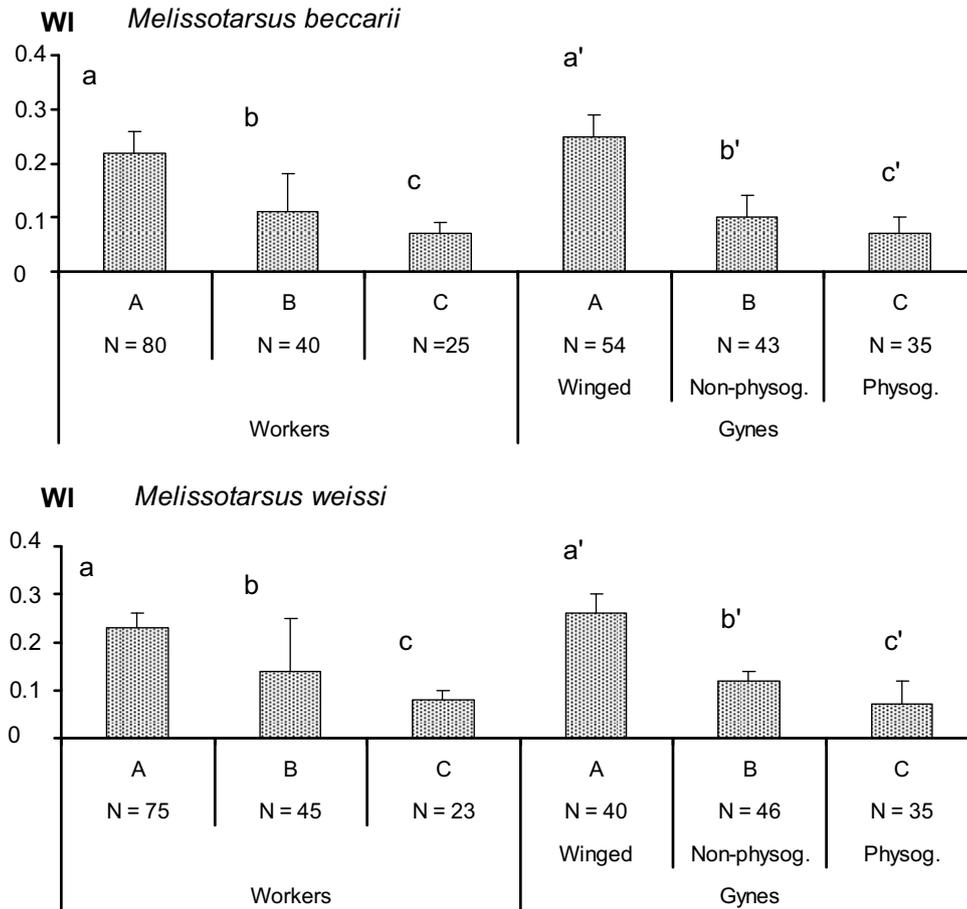
Fig. 2 Calibrating the degree of mandible wear based on the three states presented in Fig. 1. The histograms represent the mean (±SD) values of the mandible wear indices "WI" in series of *Melissotarsus beccarii* and *M. weissi* workers and gynes. Statistical comparisons (ANOVA from square-root transformed data). *Melissotarsus beccarii* workers: $F_{2, 142} = 137.97$; $P < 0.001$; gynes: $F_{2, 129} = 225.29$; $P < 0.001$; *M. weissi* workers: $F_{2, 140} = 67.6$; $P < 0.001$; gynes: $F_{2, 118} = 196.13$; $P < 0.001$. Multiple pairwise comparisons: different letters indicate significantly different means ($P < 0.01$).

apex of the apical tooth "L_2" (see also Delage-Darchen 1972). The state of mandible wear was evaluated using the ratio "$WI = L_1/L_2$," wherein WI indicates the "wear index"; values close to 0 indicate a highly worn state. We distinguished three stages of mandible wear (**Figs. 1, 2**) eliminating the need to isolate the mandibles and conduct time-consuming measurements.

We then dissected the ants to determine their ovary activity. For each *Melissotarsus* species we dissected 300 workers and 150 winged females (a detailed examination of the ovaries was conducted on only 70 *M. beccarii* and 104 *M. weissi* individuals), 130 non-physogastric and 35 physogastric gynes for which we also noted the state of the mandibles. We noted the presence of physogastric gynes. In these individuals, the normally overlapping sclerites separate to accommodate the enlarged ovaries, and the intersegmental membranes of the gaster stretch (**Fig. 3**). We also dissected 17 *M. beccarii* and 15 *M. weissi* founding queens which are non-physogastric. Finally, the spermathecae were isolated from the gynes and examined under a microscope for the presence of spermatozoa.

Task Distribution (polyethism)

Except during the alate dispersal period, the life cycle of *Melissotarsus* takes place entirely within cavities and galleries inside tree bark. Because these ants spend much of their time hidden from sight, we were obliged to study task distribution by deduction rather than direct observation and experimentation.

To that end, we selectively harvested workers performing four specialized tasks while we gathered colonies: (1) workers on the brood masses that remained immobile when we opened the galleries; (2) workers that grouped themselves around the physogastric gynes; (3) workers that plugged the opened galleries with wads of silk and wood particles; and (4) workers found around the Diaspididae. We selected 120 workers performing each of these tasks in each species and recorded their level of pigmentation, degree of mandible wear, and ovarian development. These 120 individuals selected for the study on task distribution were different from the 300 workers dissected in the study on ovaries and mandible wear. Temporal polyethism was analyzed by grouping ants into five distinct classes based on worker pigmentation, degree of mandible wear, and ovarian development (see **Fig. 4**).

For statistical comparisons we used the Student's *t*-test and an analysis of variance (square-root transformed data) using Sigma Stat 2.03. For multiple comparisons we performed a *post-hoc* test for pairwise mean differences with a Bonferroni adjustment; values are given in terms of means ± SD.

Fig. 3 Two physogastric gynes. The sclerites are separated and the intersegmental membranes of the gaster are stretched to accommodate the enlarged ovaries. These individuals were significantly longer than the other gynes (3.63 ± 0.12 mm; N = 15 *versus* 2.69 ± 0.04 mm; N = 35; t = 9.75; 48 df.; P < 0.0001). When placed on a flat surface, the workers clearly stagger.
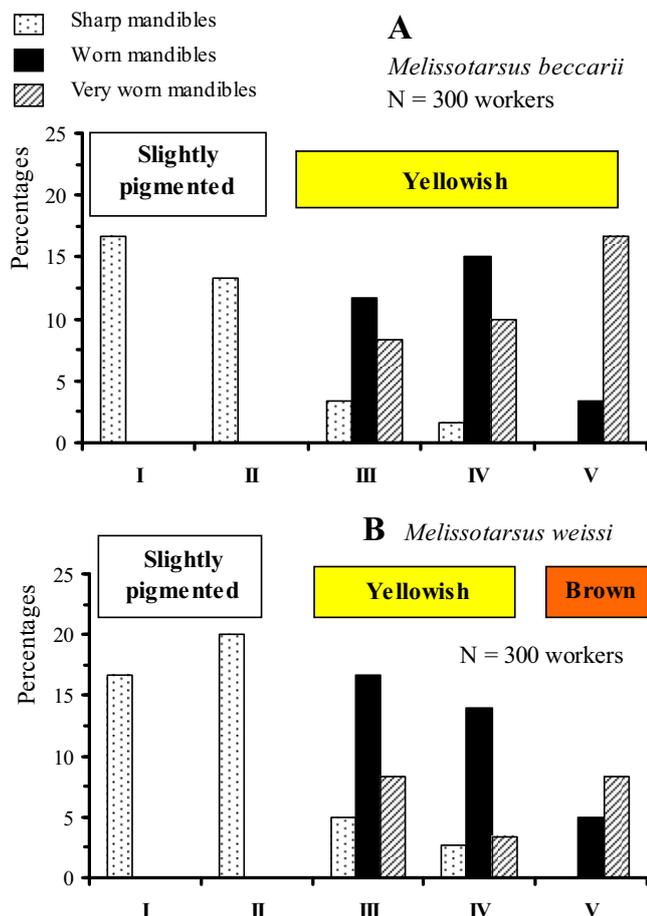
Fig. 4 Developmental stages of the ovaries in *Melissotarsus beccarii* (A) and *M. weissi* (B) workers based on their pigmentation and degree of mandible wear. I: ovaries with undifferentiated tubes; II: ovaries with a few yellow bodies; III: developed ovaries with a few mature basal oocytes having a distinct chorion and the presence of yellow bodies; IV: ovaries with several yellow bodies at the base of degenerating oocytes; V: highly degenerated ovaries with several yellow bodies at the base of transparent ovarioles.

Fusion of colonies and one-on-one confrontation tests

We found that the roughened bark of mango trees tended to indicate areas inhabited by *Melissotarsus* colonies with the zones between these areas devoid of galleries (Mony *et al*. 2002). This observation permitted us to determine both the presence of colonies and their boundaries. Incipient colonies inhabit areas of trees where the bark is not yet rough, including the trunks of relatively young trees and large branches on old trees. The presence of incipient colonies was recognizable by circular zones that have a different structure and colour than the rest of the bark (Mony *et al*. 2002).

We used pushpins with different-coloured heads to delineate the territorial boundaries of main colonies and incipient colonies (diameter between 10 and 22 cm in this study) on five mango trees at the beginning of the experiment. By updating this assessment every six months over the next two years, we were able to follow the spread of colonies beneath the bark. This permitted us to verify whether colonies are able to fuse. At the end of the experiment we peeled away the bark above the original location of each colony to determine whether physogastric females were present (see Mony *et al*. 2002).

Because we hypothesized that colonies do fuse, we conducted confrontation tests to see whether workers from different colonies are aggressive towards one another. We adapted a standard behavioural assay commonly employed in studies to test whether different individuals belong to the same colony (Holway *et al*. 1998; Le Breton *et al*. 2004). First, we placed the individuals to be tested on a substrate made of slightly rough carton. We then created neutral, transparent arenas by covering the edges of a microscope slide with modelling clay, and pressed this over a neutral clay substrate. The ants were still able to place their middle pair of legs in contact with the microscope slide and move about without staggering. We were able to lift the slide easily to introduce or remove pairs of workers being tested.

We tested workers with slightly worn mandibles originating (1) from the same colony/trees, (2) two different colonies of the same species from the bark of two trees separated from each other by 5 to 10 km, and (3) interspecific confrontations. Workers originated from a total of 22 trees. We tested each pair of workers for five minutes, scoring their interactions on a scale from 1 to 3: 1 = touch (antennation and physical contact, but no aggressive response), 2 = low-level aggressiveness (brief physical attack with biting by one or both of the workers), and 3 = fighting (prolonged biting and pulling, gaster flexion). The highest value obtained in each series of five minutes was retained for comparison. For each type of confrontation we conducted 50 replications and used each worker only once. Levels of aggressiveness between colony pairs were compared using the Kruskal-Wallis test. A *post-hoc* test (Dunn's method) was then performed to isolate any groups that differed from the others (Graph Pad Prism 4 software).

RESULTS

Workers' ovarian activity and temporal polyethism

Only one ovariole per ovary was present for all of the *M. beccarii* and 98.7% of the *M. weissi* workers; the remaining 1.3 % had 2 to 4 ovarioles per ovary (N = 300 workers per species; none had spermathecae). The length of the basal oocytes was 0.23 ± 0.01 mm (N = 132) and 0.25 ± 0.01 mm (N = 147) for *M. beccarii* and *M. weissi* workers, respectively. Just after parts of colonies were set up in the laboratory, workers with worn to very worn mandibles laid eggs that appeared to be chorioned (N = 16 cases).

Temporal polyethism was analyzed by studying worker pigmentation, degree of mandible wear, and ovarian development. We separated these ants into five distinct classes (**Fig. 4**). In both *Melissotarsus* species, we found that young, slightly pigmented workers with sharp mandibles were not yet able to lay eggs due to the immature state of their ovaries: Class I had undifferentiated tubes, and class II had only a few yellow bodies. In each species, all 120 workers grouped on the brood were slightly pigmented with sharp mandibles and class I ovaries. All the workers grouped on physogastric gynes appeared very similar but had class II ovaries. Workers engaged in plugging the galleries with wads of silk also had class II ovaries, were slightly pigmented and always had sharp, or only slightly worn, mandibles.
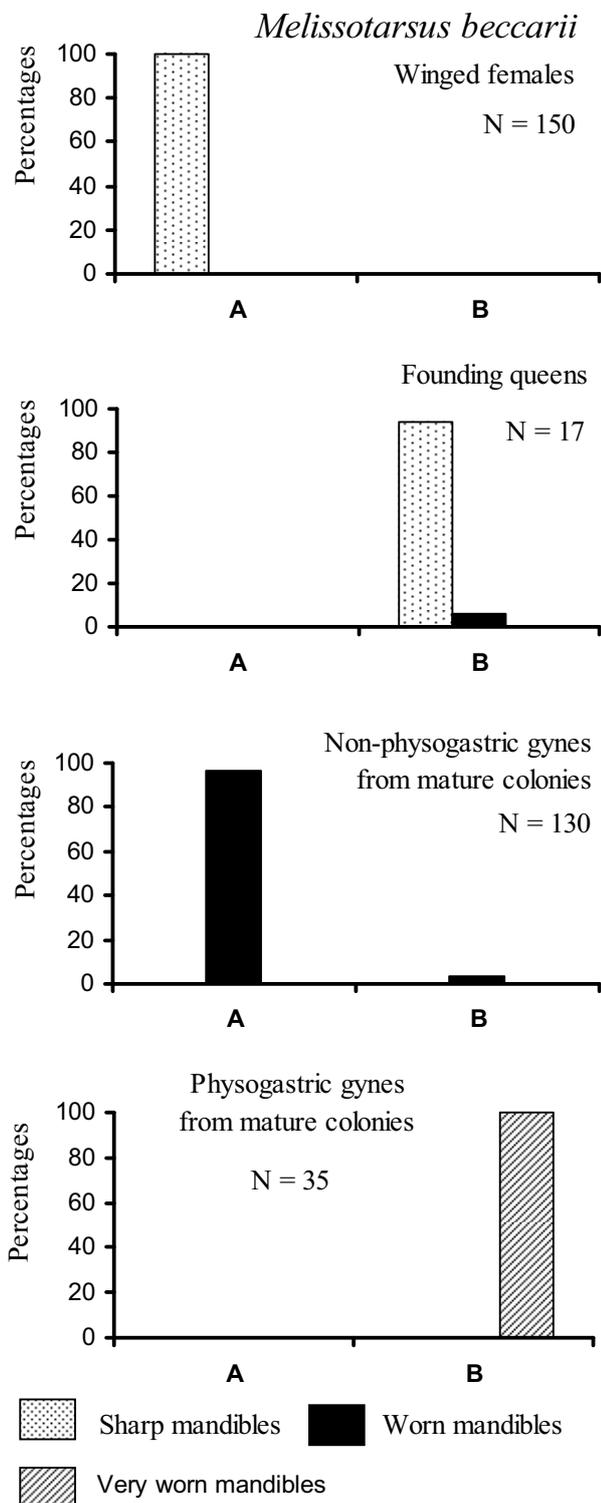
Mature workers, which had a yellowish cuticle, had active, developed ovaries: Class III had a few mature basal oocytes and a distinct chorion, plus the presence of yellow bodies; and class IV had several yellow bodies at the base of degenerating oocytes. They probably produce eggs. Most also had worn to very worn mandibles, indicating they dig galleries.

Lastly, old workers, yellowish to brown (*M. weissi*) with worn to very worn mandibles, no longer laid eggs. Indeed, their class V, highly degenerated ovaries had several yellow bodies at the base of transparent ovarioles.

The workers found around the Diaspididae were mature and/or old workers: they were well pigmented with worn to very worn mandibles and either had well-developed ovaries or no longer laid eggs.

The gynes

We collected 32 incipient colonies that contained brood, up to 10 workers and diaspidides. All 17 *M. beccarii* were monogynous, while in *M. weissi*, two of the 15 had two queens. None of the founding queens were physogastric. All founding queens were inseminated and had both oocytes in the process of maturation and mature oocytes, suggesting they were actively laying eggs before capture. One gyne of

Melissotarsus beccarii

Winged females

N = 150

Founding queens

N = 17

Non-physogastric gynes
from mature colonies

N = 130

Physogastric gynes
from mature colonies

N = 35

Sharp mandibles   Worn mandibles

Very worn mandibles

Fig. 5 Developmental stages of the ovaries in *Melissotarsus beccarii* gynes in relation to the worn state of the gynes' mandibles. (A) ovaries with undifferentiated tubes; (B) developed ovaries with mature basal oocytes having a distinct chorion and the presence of yellow bodies. Degenerated ovaries were never noted. Differences in *M. weissi* concerned only non-physogastric gynes from mature colonies, with 6.7% having class B ovaries and worn mandibles (*versus* 3.3% for *M. beccarii*); we noted one founding queen of 15 having worn mandibles *versus* one of 17 for *M. beccarii*.

each species had worn mandibles (**Fig. 5**).

We noted the presence of numerous gynes in all mature colonies of both *M. beccarii* and *M. weissi*. Among them, the physogastric individuals were always positioned more than 1 m from each other and covered by a retinue of lightly pigmented workers (see above). While evaluating the proportion of the different sub-castes, we counted 10,512 *M.*

beccarii workers per 149 gynes (one gyne per 70 workers; 4,400 cm^2 of bark); only one gyne was physogastric. In *M. weissi* we noted 11,890 workers per 143 gynes (one gyne per 83 workers; 6,400 cm^2 of bark); two gynes were physogastric (one physogastric queen per 5,945 workers).

When gynes were pooled by morphology (i.e., winged, non-physogastric, and physogastric), we found that each group showed a different degree of mandible wear (**Fig. 5**). All winged gynes had spermathecae devoid of spermatozoa, undifferentiated tubes characterizing class I ovaries, each with 2 to 7 ovarioles, and sharp mandibles (**Figs. 2, 5**). A significant difference was noted between species: 4.5 ± 0.31 ovarioles, N = 70 for *M. beccarii versus* 5.3 ± 0.19 ovarioles, N = 104 for *M. weissi*; t = 3.22; df = 172; P < 0.01. All 130 non-physogastric gynes dissected from each species were inseminated. Among them, only one *M. beccarii* and two *M. weissi* gynes had oocytes in the process of maturation and thus were likely to lay eggs. All but two *M. beccarii* and six *M. weissi* gynes had worn to very worn mandibles (**Figs. 2, 5**). Finally, all 35 dissected physogastric individuals from each species had mature, egg-producing ovaries and very worn mandibles (**Figs. 2, 5**). Indeed, their ovaries contained large numbers of oocytes in the process of maturation, plus mature oocytes, and each laid numerous eggs (mean length of: 0.49 ± 0.01 mm; N = 50 eggs for each species) much larger than those of the workers, whose basal oocyte size was comparable.

Fusion of colonies and one-on-one confrontation tests

One-on-one confrontations between workers from different colonies (different trees) did not result in significant differences, thus illustrating a low level of intraspecific aggressiveness in both *Melissotarsus* species (**Table 1**). A significant difference was noted during interspecific confrontations, demonstrating that workers do have the capacity for aggression.

The edges of the territories of incipient colonies were visibly concentric areas whose maximum radius increased by 21 ± 1.9 cm over two years (N = 20). Monitoring main and incipient *M. weissi* colonies on five mango trees permitted us to record cases of fusion between large "main" colonies, main and incipient colonies, and a combination of both (**Table 2**). By peeling away the bark over zones where incipient colonies were previously identified at the beginning of the experiment, we found nine physogastric queens (out of 20 cases).

DISCUSSION

Workers' ovarian activity

The workers of several polygynous ant species are able to elude the influence of the queen and produce eggs over their lifetimes. Depending on the species, they produce unfertilized trophic eggs with a thin or an incomplete chorion, or viable eggs that develop into males (arrhenotoky) if not eaten (Hölldobler and Wilson 1990). In this study, the basal oocytes had a distinct chorion and were associated with the presence of yellow bodies at the base of the ovarioles. Yellow bodies that originate from degenerating nurse cells point to active oviposition (but see Billen 1985). Therefore, it is likely that many (if not all) *Melissotarsus* workers with worn mandibles regularly produce viable eggs. This result is corroborated by the observation of worker eggs produced in lab part of colonies.

Because workers sometimes control the fertility of other workers (see Ratnieks *et al*. 2006) we cannot assert that the numerous males noted nearly all year long in the colonies (Mony *et al*. 2002) originated partially or completely from the workers. Conversely, we cannot be certain that all of the eggs produced by workers were fed to the queens and larvae. Indeed, worker reproduction is known to be costly to colonies because reproductive individuals do not perform

Table 1 Levels of aggressiveness (scored from one to three) during one-on-one confrontations between *Melissotarsus* workers. A = *M. beccarii* workers from the same colony/tree; B = *M. beccarii* workers from two different colonies (gathered from different trees); C= interspecific confrontations between *M. beccarii* and *M. weissi* workers; A′ and B′: same as A and B, but for *M. weissi* workers. Statistical comparisons (Kruskall-Wallis test): H = 16.310; df = 5; P = 0.0026; *Post-hoc* comparisons (Dunn's method): only A vs. C and A' vs. C resulted in significant differences (P < 0.05 and P < 0.01, respectively).

Levels of aggressiveness	*Melissotarsus beccarii*			*Melissotarsus weissi*	
	A- Intracolonial confrontation	B- Intercolonial confrontation	C- Interspecific confrontations	A′- Intracolonial confrontation	B′- Intercolonial confrontation
1	24	17	11	26	19
2	20	21	21	18	18
3	6	12	18	6	13
Total	50	50	50	50	50

Table 2 Monitoring the spread of main and incipient *Melissotarsus weissi* colonies on five mango trees and verification of their fusion. Large main colony: the area of bark that appeared modified was greater than 1 m². Incipient colony: the diameter of the roughly circular zones of modified looking bark was 10 to 30 cm; the shortest distance between the borders of these colonies and the main colonies was 20 to 50 cm.

Mango Trees	Number of large colonies	Number of incipient colonies	Main colony fusion	Fusions between main and incipient colonies	Fusions between incipient colonies
N° 1	1	3	-	All three	Two out of three
N° 2	1	6	-	All six	Two (twice)
N° 3	1	4	-	Three out of four	Two
N° 4	1	2	-	Both	-
N° 5	2	5	yes	Four out of five	Two (twice)

tasks such as foraging which are typically reserved for their caste, resulting in lower total colony productivity (Cole 1986; Wenseleers *et al*. 2003; Wenseleers and Ratnieks 2004). Nevertheless, because *Melissotarsus* workers spend their entire life in galleries that even the gynes help to dig, foraging has different implications for these species.

Gynes' ovarian activity

In the large, polygynous mature colonies of both *Melissotarsus* species studied, physogastric queens were the only gynes to produce eggs. In addition, young workers only grouped on and around physogastric queens, and were not attracted to other gynes. Physogastry, correlated with high egg productivity, is mostly known in parasitic and nomadic ant species (Hölldobler and Wilson 1990) and can explain the large size of *Melissotarsus* colonies. As the same colony can contain several physogastric queens that live in within bark more than 1 m from each other, this situation is similar to oligogyny (i.e., multiple queens cannot co-exist in the immediate vicinity within a single colony due to antagonism between them, but are tolerated by workers; Hölldobler and Wilson 1990).

Physogastry is not a necessary condition for laying eggs in *Melissotarsus*, as all founding queens as well as three non-physogastric gynes in mature colonies in this study produced eggs. These traits are reminiscent of a polygynous *Leptothorax* species in which egg laying is monopolized by one "alpha-female" that controls the other gynes using a combination of physical dominance and chemical cues (Ortius and Heinze 1999). When *Leptothorax* gynes were experimentally separated in different parts of the same nest, they began to lay eggs, and workers preferred to group around gynes with high fecundity rather than those of high social status before separation (Ortius and Heinze 1999). In *Melissotarsus*, physical dominance is improbable due to the physogastry of queens that cannot move around in the galleries. Apparently, queens (old individuals with very worn mandibles) only control the fertility of gynes over a limited area, resulting in a mosaic of zones within each colony, each under the influence of its own physogastric queen. The fact that workers are able to lay viable eggs suggests low within-colony relatedness and that workers could indeed be key players in controlling gynes' fertility by favouring the most fertile, related individuals from each area of the mosaic (cuticular hydrocarbons encode reliable information about fertility; Tsuji *et al*. 1999; Dietemann *et al*. 2003). This influence is strengthened when most workers in an

area are related to the corresponding physogastric queen whose means of control is more likely to be fertility rather than an inhibitory signal (Keller and Nonacs 1993).

Because physogastric females in mature colonies are older reproductive individuals with very worn mandibles, frequent renewal of queens may be necessary. All inseminated gynes may have the potential to become physogastric egg-layers in turn. This system would result in greater colony longevity if newly inseminated gynes are adopted. Non-physogastric gynes with partially developed ovaries could be prospective queens (relatively similar to founding, non-physogastric queens) ready to replace the physogastric individual; hence, they can compete to attain high levels of fertility. This feature can be compared to polygynous *Formica fusca* colonies where old queens can produce gynes and young queens produce workers while queuing for the opportunity to produce gynes in turn (Bargum and Sund-ström 2007).

Temporal polyethism

In ant colonies, the duties of workers shift with age. When young, workers are responsible for internal activities. As they grow older, they become progressively specialized in defence and foraging. This phenomenon, known as "age polyethism" or "temporal polyethism," permits the deployment in the field of old workers with atrophied ovaries who are generally experienced and efficient (Hölldobler and Wilson 1990; Robinson *et al*. 1994). Although such an "external service" does not exist in *Melissotarsus* because workers never leave the bark of their host trees, we demonstrate here age polyethism in both species studied. After assuming the care of the brood and then that of the physogastric gynes, workers dig galleries (as evidenced by their worn mandibles) and can even lay unfertilized eggs. Later they plug the galleries, and then tend Diaspididae when they have very worn mandibles and no longer produce eggs.

Unexpectedly, we discovered that gynes dig galleries, a worker-like behaviour. They probably begin to dig galleries when founding their colonies, as 6% of the incipient *M. beccarii* and 6.7% of the incipient *M. weissi* colonies had gynes with worn mandibles (founding gynes have sharp mandibles when they first enter tree bark through galleries bored by xylophagous coleopterans; Dejean, pers. obs.). We deduce that gynes continue to dig galleries in mature colonies because all of the physogastric queens had very worn mandibles whereas non-physogastric ones presented different degrees of mandible wear.

In ants, worker-like behaviour in gynes is observed during colony foundation as they dig their first shelter, care for their first brood and, in cases of semi-claustral foundations, even forage (Hölldobler and Wilson 1990). In mature colonies, worker-like behaviour has been reported exceptionally for non-mated or even mated gynes that forage or share tasks with workers (Newell and Barber 1913; Bushinger 1968; Ito *et al*. 1996; Brown 1999). Also, the queens of young *Pheidole fallax* Mayr or *Solenopsis saevissima* (F. Smith) colonies deprived of workers resume brood care (Hölldobler and Wilson 1990). The queens of certain species block nest entrances or the entrances to brood chambers with their modified head (*Cephalotes*) or head and thorax (*Blepharidata*), a task generally reserved for specialized workers (Brandão *et al*. 2001).

Colony fusion and one-on-one confrontation tests

This study shows that very large *Melissotarsus* colonies can result from their own expansion plus the fusion of several colonies (note that different colonies on the same tree are not interconnected by galleries; Mony *et al*. 2002). Indeed, workers have a low level of intraspecific aggressiveness (i.e., belonging to different trees); the same is true for different colonies from the same tree (pers. observation), but in this case it is likely that they are related. Colony boundary monitoring revealed that many types of colony fusion are likely (between already large colonies, between incipient colonies and between large and incipient colonies). From the very start of observations, we found that incipient colonies continually increased the area of bark they occupied, and merged with neighbouring large colonies; 9 out of 20 even acquired a physogastric gyne. This result confirms the presence of a mosaic of zones, each under the influence of a physogastric queen, with new zones resulting from mergers with other colonies. Nevertheless, although this is not in complete conformity with the presence of queens in the centre of areas previously occupied by incipient colonies, it remains possible that a takeover by large colonies occurred when their galleries came into contact with those of small colonies. The large colonies in this case may spread into the galleries of the smaller ones. A specific study on the relatedness between the different queens and between them and the workers surrounding them would be useful.

The large size of *Melissotarsus* colonies is due to a form of oligogyny with several physogastric queens, which are capable of producing very large numbers of eggs (Hölldobler and Wilson 1990). This phenomenon is amplified by low intraspecific colony aggressiveness and colony mergers where new, incipient colonies can fuse with each other or with the main colonies. In addition to these traits, the gallery-nesting and Diaspididae-tending habits of both *Melissotarsus* species studied favour several other novel aspects of social organization. Newly-inseminated gynes seem to undergo a dynamic process where they are first accepted by colonies to perform worker-like tasks but do not produce eggs prior to becoming physogastric. Workers that elude the queen's influence and produce chorioned, viable eggs, also probably play a role in the control of reproduction by non-physogastric gynes. Further investigations using microsatellite loci could provide insights into the following questions (e.g., Kümmerli and Keller 2007). (1) Do workers' chorioned eggs result in males? (2) Are the areas under the influence of a physogastric queen mostly or entirely occupied by her daughters (a concern for both workers and gynes)? (3) Are the gynes from incipient colonies that later fuse with each other or with the main colonies related?

In conclusion, *Melissotarsus* exhibits many characteristics found in the most notoriously invasive ant species. First, invasive ants possess multiple queens and show low intraspecific colony aggressiveness permitting them to achieve unicoloniality (i.e., maintain no colony boundaries within populations) (Hölldobler and Wilson 1990; Tsuji and Yamauchi 1994; Holway *et al*. 1998, 2002). In the same way,

within a single tree, *Melissotarsus* absorbs satellite colonies to form one large colony. Second, according to the "enemy release hypothesis," separation from coevolved parasites, predators, and competitors may allow populations to flourish virtually unchecked (Holway *et al*. 2002). *Melissotarsus* may have escaped predators by living entirely within the galleries of living wood. Third, a mutualistic association with hemipterans with which invaders share no co-evolutionary history can permit outbreaks in the densities of both partners (Holway *et al*. 2002; Le Breton *et al*. 2005). It appears that *Melissotarus* individuals feed exclusively on associated Diaspididae, and since they forage only within host trees the threat from predators is limited compared to free living ant invaders. Nonetheless, the presence of *Melissotarsus* should be monitored in plantations in Africa and special care must be taken to prevent both the spread of this genus and its associated Diaspididae.

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