

Shift from independent to dependent colony foundation and evolution of ‘multi-purpose’ ergatoid queens in *Mystrium* ants (subfamily Amblyoponinae)

MATHIEU MOLET^{1*}, BRIAN L. FISHER², FUMINORI ITO³ and CHRISTIAN PEETERS¹

¹Laboratoire d'Ecologie CNRS UMR 7625, Paris Universit s UPMC, 7 quai Saint Bernard, 75005 Paris, France

²Department of Entomology, California Academy of Sciences, 55 Concourse Drive, San Francisco, CA 94118, USA

³Faculty of Agriculture, Kagawa University, 761-0795 Ikenobe, Miki, Japan

Received 27 November 2008; accepted for publication 5 February 2009

Division of labour improves fitness in animal societies. In ants, queens reproduce, whereas workers perform all other tasks. However, during independent colony founding, queens live as solitary insects and must be totipotent, especially in species where they need to forage. In many ants, solitary founding has been replaced by dependent founding, where queens are continuously helped by nestmate workers. Little is known about the details of this evolutionary transition. *Mystrium rogeri* from Madagascar and *Mystrium camillae* from Southeast Asia (subfamily Amblyoponinae) have winged queens, but three congeneric species from Madagascar reproduce with permanently wingless queens instead. We show that this ‘ergatoid’ caste has distinct body proportions in all three species, expressing a mixture of both queen and worker traits. Ergatoid queens have functional ovaries and spermatheca, and tiny wing rudiments. They can be as numerous as workers within a colony, but only a few mate and reproduce, whereas most behave as sterile helpers. The shape of their mandibles makes them unsuited for hunting and, together with a lack of metabolic reserves (i.e. in the form of wing muscles), this means that ergatoid queens cannot be solitary foundresses. In comparison with winged queens, ergatoid queens are less costly per capita and they experience lower mortality. They remain in their natal colonies where they can either reproduce or function as helpers, making them a ‘multi-purpose’ caste. Within the Amblyoponinae, ergatoid queens replace winged queens in *Onychomyrmex* as well. However, in this genus, ergatoid queens are ‘sole-purpose’, few are produced each year and they reproduce but do not work. Hence, different types of ergatoid queens evolved to replace winged queens in ants.   2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, 98, 198–207.

ADDITIONAL KEYWORDS: allometry – caste – fission – gamergate – intercaste – morphometry – nonclaustral.

INTRODUCTION

Division of labour is a key feature of all animal societies. Individuals that specialize on distinct tasks perform more efficiently; hence, the fitness of the society is increased (e.g. honeybees; Seeley, 1982). Although, in many social species, all individuals retain the ability to switch between tasks during their

life, in ants and some other eusocial insects, selection has shaped adult phenotypes that are dedicated to specific tasks. The most widespread examples are queen and worker castes, which are morphologically specialized for reproductive and nonreproductive tasks, respectively (Oster & Wilson, 1978). Although either caste cannot function alone (e.g. workers usually cannot mate), together, they allow for improved functioning of the colonial unit.

In many social insects, colonies are founded by solitary individuals (independent colony foundation;

*Corresponding author. E-mail: mathieu.molet@snv.jussieu.fr

ICF) and division of labour is impossible during this initial phase. A foundress needs to carry out all tasks, including reproduction, nest building, foraging, and nest defence (e.g. *Polistes* wasps; Reeve, 1991). Accordingly, strong selective pressures act on the phenotype of such foundresses so that they remain totipotent. In many ants, winged queens disperse by flight and found new colonies alone (Peeters & Molet, 2009). In basal lineages, founding queens must even forage outside the nest before their first brood emerges (nonclaustral ICF), although, in the majority of higher ants, queens are provisioned with large metabolic reserves that are sufficient to feed the first brood (claustral ICF). Mortality is high during ICF, and has been selected against in numerous unrelated ant genera. Instead, dependent colony foundation (DCF) has evolved, whereby established colonies divide on a regular basis. This strategy eliminates the solitary founding stage and reproductives cannot succeed without sterile nestmates (Peeters & Ito, 2001). Among social wasps and bees, DCF involves both winged queens and winged workers that fly together to new nests (Ross & Matthews, 1991; Visscher, 2007). In ants, however, workers are always wingless and the queen(s) is forced to disperse on foot. Consequently, the ancestral winged queens have disappeared in many species (Heinze & Tsuji, 1995; Peeters & Ito, 2001). In 200–300 species, they are replaced by sexually reproducing workers ('gamergates'). However, in most ants, workers cannot mate and store sperm; hence, wingless reproductives have evolved that are morphologically distinct from the worker caste. They have a functional spermatheca but their thorax is reduced and resembles that of the workers; hence, they are termed 'ergatoid' queens (Wheeler, 1910; Peeters, 1991).

Ergatoid queens occur in a large number of unrelated ant taxa, but relatively few studies have described their morphological characteristics and the pattern of investment in sexuals. The worldwide subfamily Amblyoponinae is basal among ants (Saux, Fisher & Spicer, 2004; Brady *et al.*, 2006) and exhibits a variety of colonial reproductive strategies and associated queen phenotypes. Thus, it comprises an ideal taxon to assess the consequences of the shift from ICF to DCF. For example, in *Onychomyrmex*, winged queens do not exist and colonies possess a single ergatoid queen that is physogastric and highly fertile (Brown, 1960). This corresponds to the general pattern found in ants with ergatoid queens. However, a recent study in *Mystridium* 'red' revealed that each colony has numerous ergatoid queens that are smaller than the workers (Molet, Peeters & Fisher, 2007a). There are no winged queens, unlike other species in this genus. Whether this is an isolated example is unknown. Accordingly, we compared

colony demography, caste morphometry, and reproductive division of labour in two other species from Madagascar (*Mystridium mysticum* and *Mystridium oberthueri*) as well as in two species with winged queens (*Mystridium camillae* from Southeast Asia and *Mystridium rogeri* from Madagascar). We review the repeated shifts to obligate DCF in the subfamily Amblyoponinae, where the ancestral winged queens have been replaced by different types of wingless reproductives, including gamergates. We find two contrasting patterns (i.e. 'sole-purpose' versus 'multi-purpose' ergatoid queens), indicating that recurrent selection against winged queens can be solved in different ways. We suggest that wingless reproductives can readily evolve in ants because two castes (winged queens and wingless workers) already exist, and larval development can be modified to create intermediate adult phenotypes that are cheaper than winged queens.

MATERIAL AND METHODS

COLONIES

Fifty-nine complete colonies of *Mystridium* were collected in 2003–2005 in primary rainforests in Madagascar, including 16 *M. mysticum* (Forêt de Binara 13°15.8'S; 49°36.2'E), 12 *M. oberthueri* (Parc National de Marojejy 14°26.2'S; 49°56.5'E), 14 *M. 'red'* (Forêt d'Antsahabe 13°13.7'S; 49°33.4'E), and 17 *M. rogeri* (Binara, Marojejy and various southern localities; see <http://www.antweb.org>). Adults and brood were counted in the field, and some cocoons were opened a few days after collection to identify the pupae inside (worker, gyne or male). In addition, ten colonies of *M. camillae* were collected from secondary forests in Java (1995–2003). Specimens from *M. camillae* colonies are in F. Ito's collection; specimens from the four other species are deposited at the California Academy of Sciences and imaged on <http://www.antweb.org>.

MORPHOMETRY

We used a Leica MZ6 microscope to take dorsal and lateral photographs of all adults from one colony per species: 23 ergatoid queens and 61 workers of *M. 'red'*; 22 ergatoid queens and 23 workers of *M. mysticum*; 21 ergatoid queens and 30 workers of *M. oberthueri*; one dealate queen, 19 winged gynes, 24 workers, and one intercaste of *M. rogeri*; and one dealate queen and 24 workers of *M. camillae*. We measured mandible length, maximum head width, thorax volume, and cross-sectional area of the first gaster segment with ImageJ (<http://rsb.info.nih.gov/ij>) *sensu* Molet *et al.* (2007a). To compare mandible shape among workers and ergatoid queens of *M. 'red'*, we measured cross-sectional area at the base, mid-way, and tip in ten

individuals of each caste. In addition, the thorax of various adults was studied by scanning electron microscopy. A few pupae were photographed to study thorax structure.

DISSECTIONS

We dissected a proportion of queens ($N = 681$) and workers ($N = 223$) from 52 colonies to check for ovarian development and spermatheca. Individuals were classified according to mating status (full or empty spermatheca) and the type of oocytes in their ovaries. Yolky oocytes were divided into two categories: 'mature' (i.e. the size of an egg) and 'immature'. Non-yolky oocytes were not counted.

STATISTICAL ANALYSIS

We performed allometry analyses between thorax volume and gaster area in all species and castes. The one exception was *M. camillae*, where only one queen was available for measurement. We used (S)MATR, version 1.0 (Warton & Weber, 2002) (available at: <http://www.bio.mq.edu.au/ecology/SMATR/>) to compute allometry coefficients (i.e. the slope of the regression lines) using standardized major axis regression, and to test for isometry and compare allometries between groups (Molet *et al.*, 2007a). All other statistical analyses were performed with STATISTICA, version 7.1.

RESULTS

ERGATOID QUEENS CONSTITUTE HALF OF COLONIES

We never found winged queens in *M. mysticum* and *M. oberthueri*, either as adults or as pupae. Moreover, winged queens have never been reported in museum

collections or caught in 4000 malaise traps over the last decade in Madagascar. Only workers, ergatoid queens and males (one, two, and six in three colonies of *M. mysticum*) were found, as in *M. 'red'* (Molet *et al.*, 2007a). All three species with ergatoid queens showed similar colony demography (Fig. 1) with 43 ± 33 adults (mean \pm SD) [analysis of variance (ANOVA): $F_{2,39} = 1.92$, $P = 0.16$], including 21 ± 16 ergatoid queens (ANOVA: $F_{2,39} = 0.23$, $P = 0.80$) and 22 ± 20 workers (ANOVA: $F_{2,39} = 4.04$, $P = 0.026$, as a result of a small difference between *M. oberthueri* and *M. 'red'*), and a similar number of pupae (25 ± 23 ; ANOVA: $F_{2,35} = 0.01$, $P = 0.99$). Ergatoid queens thus accounted for $51 \pm 18\%$ of adults (ANOVA: $F_{2,39} = 3.31$, $P = 0.047$), and this percentage was marginally lower in *M. 'red'* ($41 \pm 15\%$) compared to *M. oberthueri* ($58 \pm 18\%$; *t*-test with Bonferroni correction: $P = 0.057$), but no difference was detected compared to *M. mysticum* ($53 \pm 18\%$; $P = 0.20$). Ergatoid queens were present in all 42 colonies of these three species.

By contrast, *M. rogeri* colonies had a single dealate queen (Fig. 2) and 156 ± 178 workers (seven colonies were collected without a queen; Fig. 1). Colonies were larger than in the species with ergatoid queens (*t*-test against all colonies from species with ergatoid queens: $t_{57} = 4.01$, $P < 10^{-6}$), and had more pupae (69 ± 123 ; *t*-test: $t_{53} = 2.16$, $P < 10^{-6}$). Maximum colony size in *M. rogeri* was larger than in *M. mysticum*, *M. oberthueri*, and *M. 'red'* (510 workers versus 134, 62, and 139 adults, respectively). Field-collected cocoons of *M. rogeri* yielded workers, winged gynes (four, 97, and 149 in three colonies) and males (one, 11, 47, and 148 in four colonies). *Mystrium camillae* colonies had similar number of workers and pupae as *M. rogeri* colonies (58 ± 55 workers; *t*-test: $t_{25} = 1.69$, $P = 0.10$; 11 ± 19 pupae; *t*-test: $t_{25} = 1.47$, $P = 0.15$), and one to five dealate queens (Fig. 1).

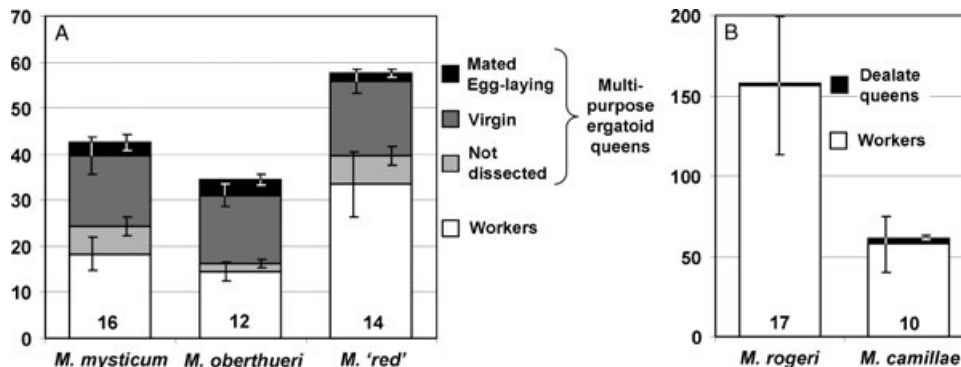


Figure 1. Queen and worker demographics (mean \pm SE) in colonies of three *Mystrium* species with ergatoid queens (A) and two *Mystrium* species with winged queens (B). The number of colonies is indicated at the bottom of each bar. Data regarding brood and sexuals are provided in the text.

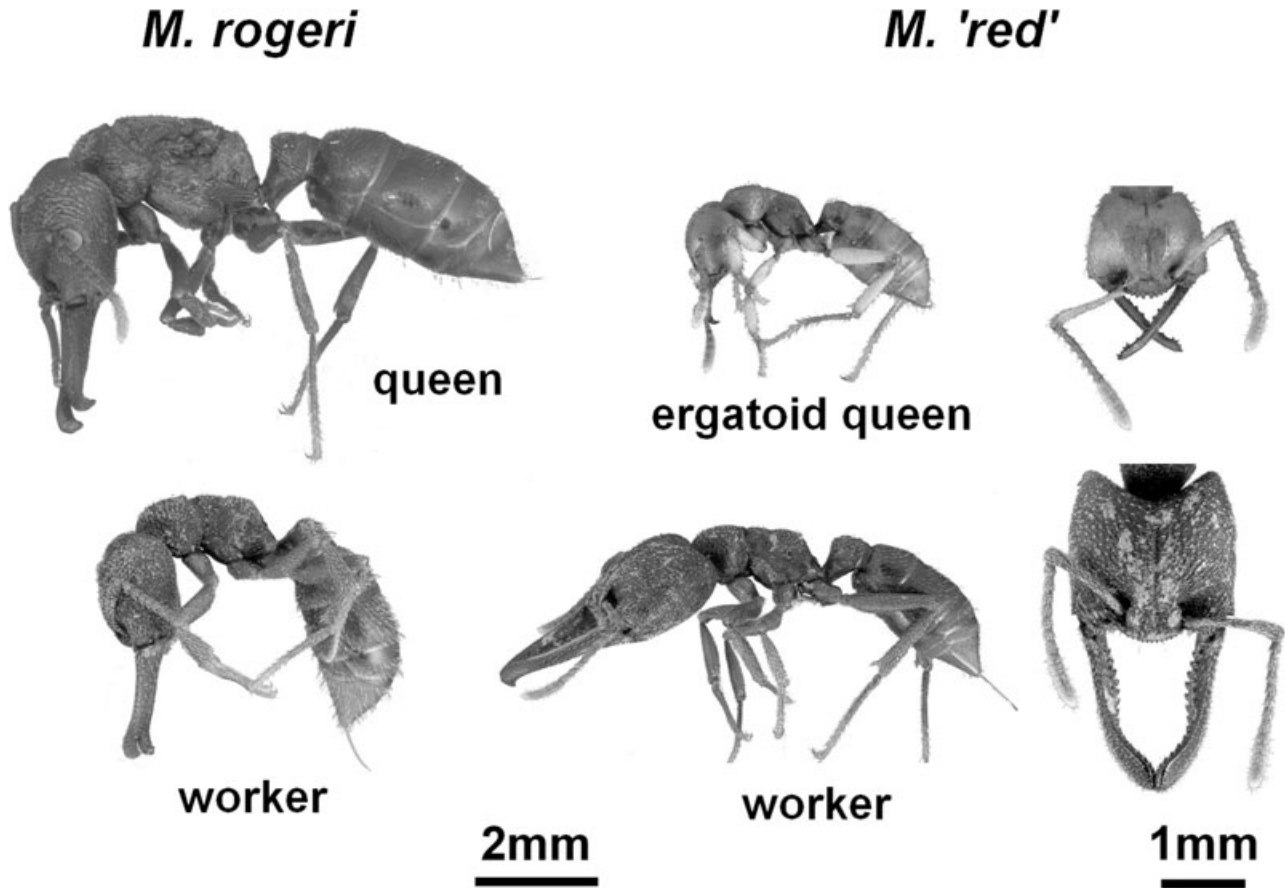


Figure 2. Comparison of the reproductive castes in *Mystridium rogeri* and *Mystridium 'red'*, together with conspecific workers. Details of the heads and mandibles of the *M. 'red'* ergatoid queen and worker are also shown (right; separate scale). Images are reproduced with permission from <http://www.antweb.org>.

ERGATOID QUEENS LACK A FLIGHT THORAX BUT PUPAE GROW WINGS

The thorax of *M. rogeri* and *M. camillae* queens is much bigger than that of conspecific workers (Fig. 3A) and has wings and flight sclerites [i.e. a clearly segmented mesonotum (separated into scutum and scutellum) and a distinct metanotum] (Fig. 4). By contrast, workers from all species exhibit a thorax with fused sclerites [i.e. an unsegmented mesonotum (scutum and scutellum are fused) and an indistinguishable metanotum (fused with the propodeum)].

By contrast, *M. mysticum* and *M. oberthueri* ergatoid queens have a smaller thorax volume than conspecific workers (Fig. 3A), similar to *M. 'red'* (Molet *et al.*, 2007a). The thorax of ergatoid queens has an appearance similar to that of workers because the pronotum is relatively long (Fig. 4). Importantly, however, ergatoid queens retain rudiments of both anterior and posterior wings. Unpigmented pupae

have large wing pouches (Fig. 4) similar to winged queen pupae, but young adult ergatoid queens only retain tiny wing rudiments at the exact location of the wings in *M. rogeri* and *M. camillae* queens. In addition, the pleurite and notum of ergatoid queens, unlike those of workers, are incompletely fused in the region of the wing rudiments (Fig. 4).

We found five 'intercastes' (i.e. erratically produced morphological intermediates between queens and workers; Peeters, 1991) in two *M. rogeri* colonies (BLF12223 and BLF14779). These individuals varied in the degree of fusion of the thoracic sclerites. The scutum and scutellum were reduced but distinct, and the metanotum was recognizable, although partly fused with the propodeum. The volume of the thorax was intermediate between that of winged queens and workers (Fig. 3A). In *M. camillae*, most queens were winged but, of the five queens that emerged from cocoons in one colony, three were brachypterous (i.e. wings were too short to fly).

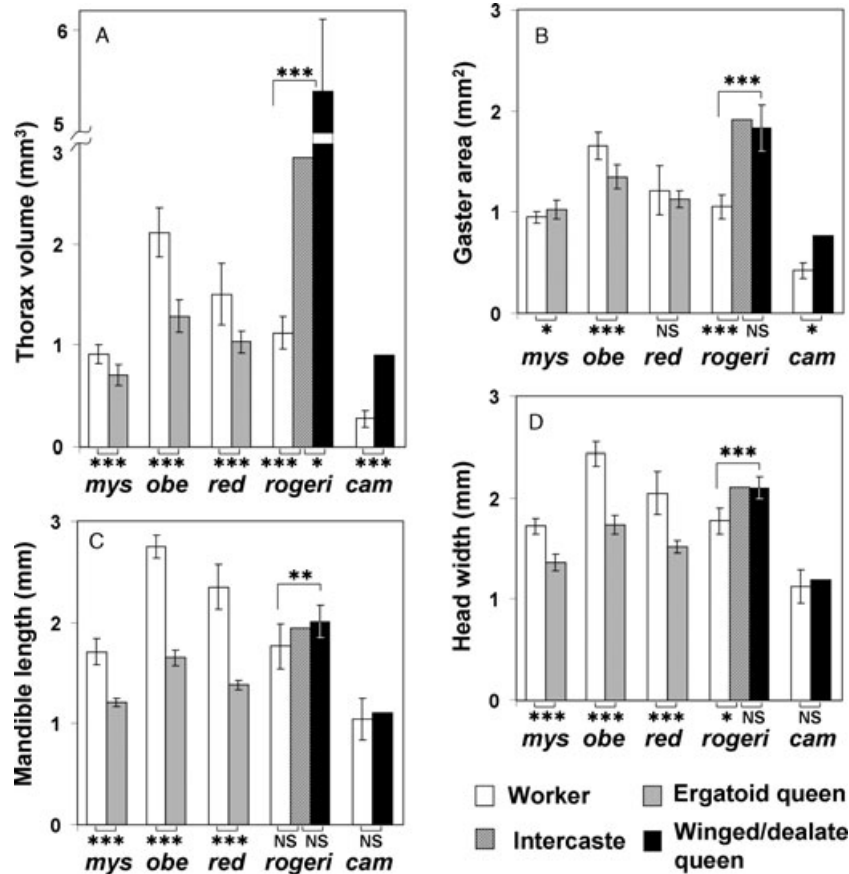


Figure 3. Size of body parts (A, thorax volume; B, gaster area; C, mandible length; D, head width) (mean \pm SE) in *Mystridium mysticum* ('mys'), *Mystridium oberthueri* ('obe'), *Mystridium* 'red' ('red'), *Mystridium rogeri* ('rogeri'), and *Mystridium camillae* ('cam') (one colony each). The significance of the *t*-tests are given at the bottom of the bars. *** $P \leq 10^{-6}$; ** $P \leq 10^{-4}$; * $P \leq 0.05$; NS ($P > 0.05$).

ERGATOID QUEENS HAVE FUNCTIONAL OVARIES AND SPERMATHECA

Among the 668 ergatoid queens dissected (Fig. 1), 93 were mated reproductives (0–23 per colony in *M. mysticum*; 0–10 in *M. oberthueri*; and 0–8 in *M. 'red'*). The most fecund were *M. 'red'*, followed by *M. mysticum*, and finally *M. oberthueri* (mature oocytes: 2.5 ± 1.3 , 2.2 ± 0.9 , and 0.6 ± 0.8 per ovary, respectively; ANOVA: $F_{2,82} = 32.46$, $P < 10^{-6}$; immature oocytes: 4.5 ± 1.2 , 1.3 ± 1.1 , and 0.8 ± 0.9 per ovary, respectively; ANOVA: $F_{2,82} = 57.03$, $P < 10^{-6}$). The other 575 ergatoid queens were virgin, and only 3% had active ovaries and yellow bodies (suggesting that they lay haploid male eggs). Thus, $87 \pm 20\%$ of ergatoid queens in each colony were not reproductives, a proportion similar in all three species (ANOVA: $F_{2,34} = 1.69$, $P = 0.2$). All 183 workers dissected had eight ovarioles, similar to the ergatoid queens, but these were extremely short [less than 1 mm (i.e. less than the size of a mature oocyte); Molet *et al.* (2007a)] and lacked growing oocytes. We consider that such

highly reduced ovaries cannot produce eggs. A tiny spermatheca-like structure was seen but was always empty. *Mystridium mysticum* and *M. oberthueri* ergatoid queens have a high gaster/thorax ratio relative to workers ($1.44 \pm 0.17 \text{ mm}^{-1}$ versus $1.04 \pm 0.11 \text{ mm}^{-1}$, *t*-test: $t_{43} = 9.80$, $P < 10^{-6}$ in *M. mysticum* and $1.04 \pm 0.07 \text{ mm}^{-1}$ versus $0.79 \pm 0.08 \text{ mm}^{-1}$, $t_{49} = 11.90$, $P < 10^{-6}$ in *M. oberthueri*; Fig. 3A, B), as found in *M. 'red'* (Molet *et al.*, 2007a). In ergatoid queens, the growth of the gaster versus the thorax is isometric (test for isometry: $P = 0.82$ in *M. mysticum*; $P = 0.36$ in *M. oberthueri*; and $P = 0.82$ in *M. 'red'*) and similar among the species ($P > 0.7$). No clear pattern was found in workers (isometry in *M. mysticum* and *M. oberthueri*; allometry in *M. 'red'*).

In *M. camillae* and *M. rogeri*, both winged queens (based on the dissection of $N = 13$ and $N = 3$ queens, respectively) and workers ($N = 40$ and $N = 105$) have ovaries with eight ovarioles (except in one colony of *M. rogeri*; see below). However, these queens have a much larger gaster than conspecific workers (Fig. 3B). Active

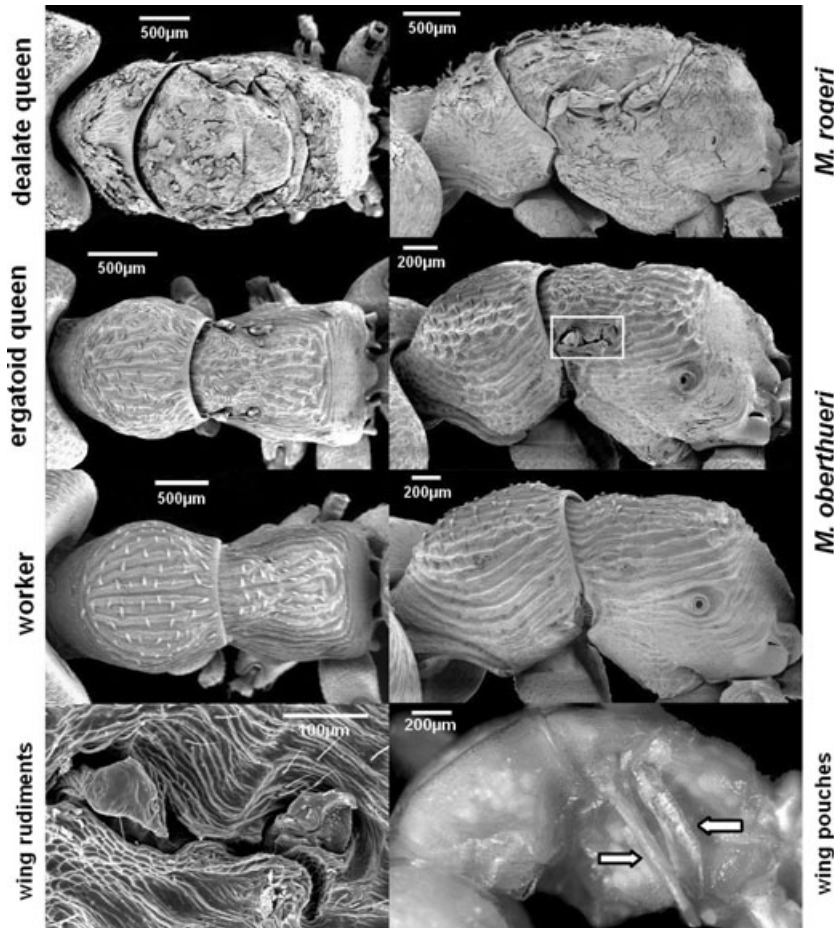


Figure 4. Dorsal (left) and lateral (right) scanning electron microscopy images of the thorax of a *Mystrium rogeri* queen (top), a *Mystrium oberthueri* ergatoid queen (middle), and a *Mystrium oberthueri* worker (bottom). The thorax of ergatoid queens bears wing rudiments (zoom in bottom left corner) corresponding to the wing pouches in pupae (arrows, bottom right corner).

ovaries in *M. rogeri* queens are longer than in fertile ergatoid queens (Molet *et al.*, 2007a). However, in contrast with workers from ergatoid species, *M. rogeri* workers have functional ovaries and can lay eggs following the death of the queen (after 3 months in the laboratory; M. Molet & C. Peeters, unpubl. data). One of the *M. rogeri* intercastes had the same gaster area as queens, but the other four had smaller gasters. These four intercastes were dissected and had ten, 11, eight, and eight ovarioles, whereas the queen of the same colony had 13. *Mystrium rogeri* colony BLF10994 lacked a queen, and workers had more ovarioles (i.e. 8–16) than in other colonies, with 14 workers having mature oocytes.

ERGATOID QUEENS HAVE SMALL MANDIBLES

Mystrium mysticum and *M. oberthueri* workers have longer mandibles and a wider head than ergatoid

queens (Figs 2, 3C, D), as also found in *M. 'red'* (Molet *et al.*, 2007a). Furthermore, workers have a higher mandible/head ratio than ergatoid queens (1.00 ± 0.09 versus 0.89 ± 0.05 , *t*-test: $t_{35} = 4.47$, $P < 10^{-4}$ in *M. mysticum*; 1.14 ± 0.04 versus 0.95 ± 0.04 , $t_{30} = 14.60$, $P < 10^{-6}$ in *M. oberthueri*; and 1.17 ± 0.06 versus 0.91 ± 0.04 , $t_{61} = 16.52$, $P < 10^{-6}$ in *M. 'red'*). Finally, mandibles are wider in workers than in ergatoid queens at the base, middle, and tip (*t*-tests: $P < 10^{-6}$; Fig. 2). From the tip of the mandibles to mid-way, the cross-sectional area increases at the same rate in both workers and ergatoid queens (ANOVA: no interaction $F_{1,36} = 1.26$, $P = 0.27$), but increases much faster from mid-way to the base among workers (ANOVA: interaction $F_{1,36} = 16.12$, $P < 10^{-3}$); thus, the base of mandibles is disproportionately enlarged in workers relative to ergatoid queens.

Mystrium rogeri queens have longer mandibles and a wider head than *M. rogeri* workers (Fig. 3C, D).

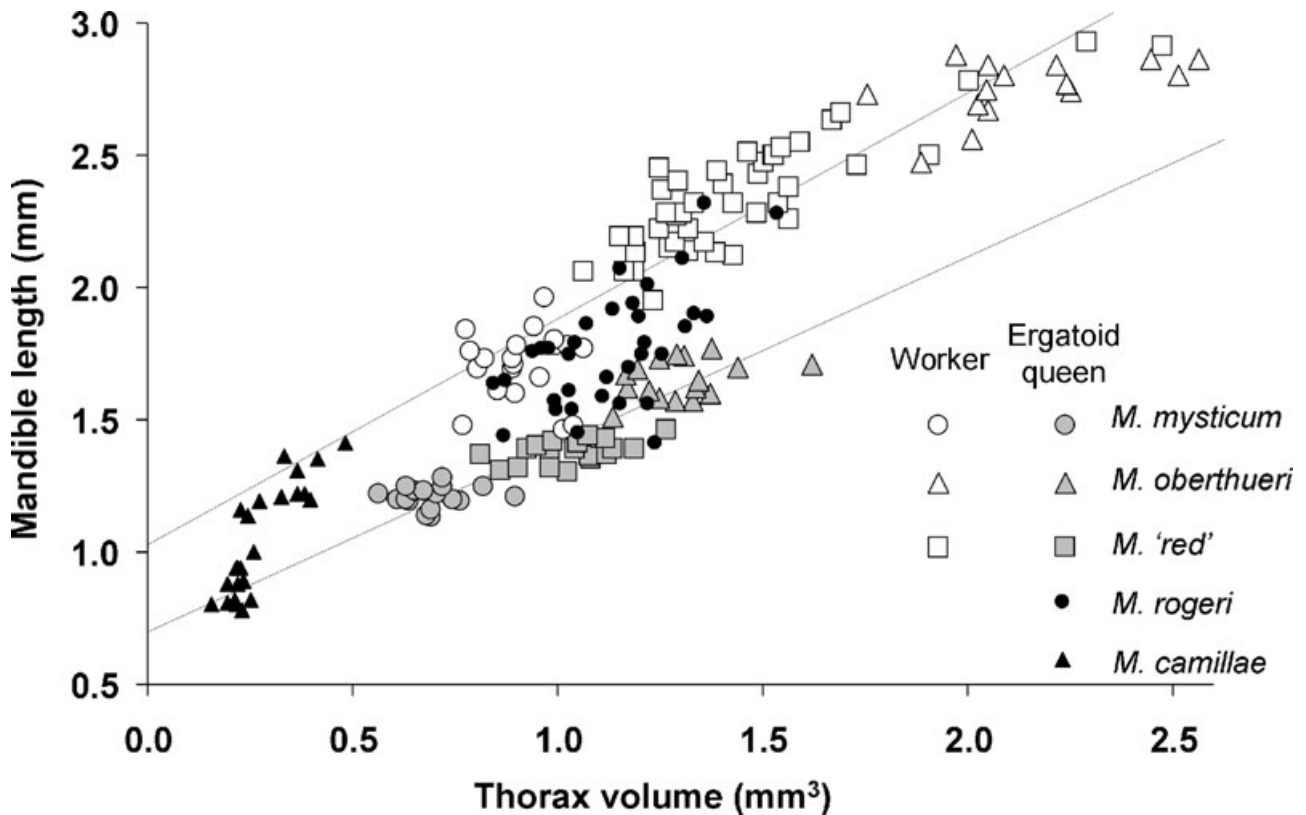


Figure 5. Proportions between body parts in queens and workers of the three species with ergatoid queens compared to workers of the two species with winged queens. Workers of the latter two species have shapes that overlap those of ergatoid queens and conspecific workers. Each point corresponds to one individual.

Their mandible/head ratio (0.95 ± 0.04) is higher than that of ergatoid queens (t -test: $t_{35} = 4.33$, $P < 10^{-3}$ with *M. mysticum*; $t_{35} = 0.34$, $P = 0.73$ with *M. oberthueri*; and $t_{38} = 3.28$, $P < 10^{-2}$ with *M. 'red'*) but lower than that of conspecific workers ($t_{52} = 2.14$, $P < 10^{-2}$). The mandible lengths and head widths of the five *M. rogeri* intercastes were similar to those of *M. rogeri* queens. *Myrmicium rogeri* and *M. camillae* queens have three ocelli, whereas the *M. rogeri* intercastes only have one, and the ergatoid queens of the other species have none. Workers of all species lack ocelli.

Myrmicium rogeri and *M. camillae* workers show a higher variability in their body proportions than workers of ergatoid species (Fig. 5). For example, their mandible coefficient of variation (CV) is much higher than that of *M. mysticum*, *M. oberthueri*, and *M. 'red'* workers (12.5% and 20.1%, respectively, versus 7.6%, 4.2%, and 10.0%). Head and mandible sizes of *M. rogeri* and *M. camillae* workers range from being similar to ergatoid queens to being similar to workers of ergatoid queen species (Fig. 5). Hence the workers in species with ergatoid queens exhibit very little size polymorphism compared to workers of queenright species.

All ergatoid queens of *M. 'red'* have a soft reddish cuticle throughout their adult life, unlike the black ergatoid queens in *M. mysticum* and *M. oberthueri*. Having an unsclerotized cuticle is possible in ergatoid queens because they do not forage outside the nest and thus are less subject to dehydration. The ergatoid queens of *M. 'red'* are also less numerous in each colony, and individually more fecund.

DISCUSSION

ERGATOID QUEENS ARE A CASTE DISTINCT FROM WORKERS

In all three *Myrmicium* species lacking winged queens, reproduction is carried out by ergatoid queens that have distinct morphology and body proportions relative to conspecific workers. They are smaller than workers and, given their tiny mandibles (Fig. 2), they are unlikely to be hunters (confirmed in the laboratory for *M. oberthueri*; Molet *et al.*, 2007b). Together with a lack of wing muscles serving as metabolic reserves, this makes ergatoid queens unfit to be solitary foundresses. This is in

contrast to the winged queens of *M. rogeri* that are larger than workers and have strong mandibles (Fig. 2) and, thus, are able to hunt during ICF. We can conclude that these ergatoid queens are not totipotent, and DCF is obligate (i.e. there is uninterrupted cooperation between queens and nestmate workers). These three species with ergatoid queens are closely related, and are derived relative to *M. rogeri* and *M. camillae* (DNA sequence data from seven nuclear genes, B. Noonan and B. Fisher, unpubl. data). Because *Mystrium* workers apparently lack a functional spermatheca, gamergates do not exist in this genus. Accordingly, the only possible response to selection against winged queens in *Mystrium* was to evolve a new wingless caste that is capable of sexual reproduction: the ergatoid queen caste [termed the 'intermorph caste' in Molet *et al.* (2007a) but corresponding fully to the ergatoid queens found in other ant taxa].

Ergatoid queens were found in large numbers in all colonies of *M. oberthueri*, *M. mysticum* and *M. 'red'*. However, only a few mated and reproduced, whereas most remained virgin and infertile, and the latter performed brood care (e.g. *M. oberthueri*; Molet *et al.*, 2007b). The distinctive mandibles of *Mystrium* workers (Fig. 2) allow specialized predation on large centipedes, as also observed in three species of *Amblyopone* (Gotwald & Léviéux, 1972; Ito, 1993b; Masuko, 1993). In *M. rogeri*, workers are continuously polymorphic (Fig. 5) and their highly variable mandibles are suitable for both brood care and hunting (Molet *et al.*, 2007b). In *M. camillae*, only large individuals were seen in the foraging area of laboratory nests (F. Ito, unpubl. data). In *Mystrium* species with ergatoid queens, the workers have large mandibles suited for hunting but not for brood care. By contrast, ergatoid queens have reduced mandibles and are unfit to hunt, but they can care for the brood. Such atypical division of labour may explain the reduction in size polymorphism of workers relative to *M. rogeri* and *M. camillae*; all but the larger workers have been eliminated in species with ergatoid queens, and ergatoid queens have taken over the tasks of small workers.

The lack of mated ergatoid queens in some colonies may be the result of field collection occurring just before the mating season, so mated queens may have died during the year or left the nest during a colony-founding event. Some virgin ergatoid queens could have mated a few days later (e.g. we observed males flying towards the colonies during collection, and in the laboratory virgin ergatoid queens of *M. oberthueri* performed sexual calling and mated with foreign males). The absence of dealate queens in some *M. rogeri* colonies is due to either natural mortality or escape during collection.

EVOLUTION OF 'MULTI-PURPOSE' ERGATOID QUEENS

Mystrium ergatoid queens do not disperse individually from their natal colony, and they presumably mate near the entrance. They can be as numerous as the workers in a colony, although it is not known whether they are produced year-round. The majority of ergatoid queens are infertile and care for the brood. They contribute to the success of DCF, an endeavour that relies on a large number of nonreproductive helpers (Macevicz, 1979). Such 'multi-purpose' ergatoid queens are also found in other subfamilies; for example, Myrmicinae (all species of *Ocymyrmex*: Bolton & Marsh, 1989; Forder & Marsh, 1989; *Eutetramorium mocquersyi*: Heinze, Hölldobler & Alpert, 1999; *Myrmecina nipponica*: Ohkawara, Ito & Higashi, 1993) and Ponerinae (*Leptogenys diminuta*: Ito & Ohkawara, 2000), and they correspond to the 'reproductive intercastes' listed by Peeters (1991). In *Mystrium*, interspecific comparisons reveal that ergatoid queens are considerably cheaper per capita relative to winged queens (fresh weights of 5.95 mg for *M. 'red'* ergatoid queens versus 14.51 mg for *M. rogeri* queens; Molet *et al.*, 2007a). Moreover, winged queens are generally reared in large numbers once a year (i.e. more than 100 winged gynes in two colonies of *M. rogeri*), although only few are likely to succeed in establishing a new colony. Hence, this large reproductive investment may be dramatically reduced by producing multi-purpose ergatoid queens, where individuals that do not reproduce remain in the colony as labourers.

DIFFERENT TYPES OF WINGLESS REPRODUCTIVES IN AMBLYOPONINAE

The worldwide subfamily Amblyoponinae comprises ten genera with distinctive morphology and specialized predatory behaviour. This subfamily is placed in the poneroid group, which comprises a heterogeneous assemblage of taxa at the base of the ant tree (Brady *et al.*, 2006). In *Amblyopone* (Gotwald & Léviéux, 1972), *Apomyrma stygia* (Brown, Gotwald & Léviéux, 1970), and *Prionopelta* (Hölldobler & Wilson, 1986; Ito & Billen, 1998), the degree of dimorphism between winged queens and workers is rather limited, meaning that a lone foundress needs to raise workers that are almost as big as herself. Accordingly foundresses must hunt (i.e. ICF is nonclaustral, as documented in *Amblyopone australis*; Haskins & Haskins, 1951). Species that have a poor success rate during nonclaustral foundation have two evolutionary choices: either rear more expensive queens (i.e. larger reserves to reduce the frequency of foraging trips) or shift to DCF. The first scenario is seen in *Myopopone castanea*, where winged queens are much bigger than workers and have more ovarioles (32 in queen, 6–22

in workers: Ito & Ohkawara, 1994, F. Ito unpubl. data); the details of ICF remain unknown. The second scenario is more widespread. In species of the *Amblyopone reclinata* group, winged queens have been replaced by gamergates (Ito, 1993a). In *Adetomyrma*, both winged and ergatoid queens have been found in undescribed species from Madagascar (B. L. Fisher, unpubl. data). In all species of *Onychomyrmex*, winged queens have been replaced by ergatoid queens (Brown, 1960). The gaster of these queens is much larger than that of workers and they are often physogastric (Wheeler, 1916). Very few ($N = 1-6$) ergatoid gynes are reared annually, virgin infertile ergatoids are not present year-round in colonies, and colonies are strictly monogynous (e.g. *Onychomyrmex hedleyi*; Miyata *et al.*, 2003). The ergatoid queens in *Onychomyrmex* can be described as 'sole-purpose' because reproduction is their exclusive function. This syndrome is true in the majority of ants studied [i.e. only one ergatoid queen (also called 'dichthadiiform' in species where they have a huge gaster) occurs in each colony] (Peeters & Ito, 2001). This is in sharp contrast to species with multi-purpose ergatoid queens, where many are produced per colony and can either reproduce or function as labourers, as described in the present study for *Mystrium*. Sole-purpose and multi-purpose ergatoid queens can be found in various other ant taxa, where they convergently evolved to replace winged queens.

DEVELOPMENT OF NOVEL REPRODUCTIVE PHENOTYPES IN ANTS

Ergatoid queens are novel phenotypes that express a mixture of queen and worker traits. Evidence for this is provided by the occurrence of tiny wing rudiments in the ergatoid queens of *M. mysticum*, *M. oberthueri*, and *M. 'red'*, in addition to functional ovaries and spermatheca. Although wings sometimes start developing from imaginal wing discs in ant workers, they only occur in pupae and have disappeared at the time of emergence (Sameshima, Miura & Matsumoto, 2004). In the ergatoid queens of *Mystrium*, wing rudiments persist in adults. These structures have no function, although their persistence suggests a recent origin of winglessness in the three species with ergatoid queens. Wing pouches in pupae may be related to the hormonal mechanisms that activate the development of queen-like ovaries.

We found a few intercastes in *M. rogeri*, which are erratically-produced queen-worker intermediates (e.g. in *Temnothorax nylanderi*; Plateaux, 1970). Importantly, they were first to start laying eggs upon the death of the founding queen in colony BLF 14779 (M. Molet & C. Peeters, unpubl. data). These intercastes varied in thorax morphology, and were all

larger than conspecific workers. They are thus unlike the regularly produced ergatoid queens described in the present study, but provide insights into the evolutionary origins of the latter. In environments where DCF is a better reproductive strategy than ICF, the regular production of cheaper intercastes that can mate and reproduce (e.g. as found in *Pachycondyla obscuricornis*; Düssmann, Peeters & Hölldobler, 1996) could be selected for, resulting in the evolution of an ergatoid queen caste with fixed body proportions (e.g. *Myrmecina nipponica*; Miyazaki *et al.*, 2006). In the ants, the shift from ICF to DCF leads to modifications at the level of both the colony (i.e. reproductive investment and division of tasks) and the individual (i.e. novel phenotypes), resulting in better colonial economy.

ACKNOWLEDGEMENTS

The fieldwork conducted in the present study could not have been completed without the help of the Malagasy Ant Team: Jean-Jacques Rafanomezantsoa, Chrislain Ranaivo, Tantely Nirina Randriambololona, and Clavier Randrianandrasana. We thank G. Mascarell and A. Couté for their help with the scanning electron microscopy conducted at the Muséum National d'Histoire Naturelle, Paris. Roberto Keller and Mary Jane West-Eberhard made constructive comments on the manuscript, as did the anonymous referees. This work was supported by ANR-06-BLAN-0268, CNRS UMR 7625, National Science Foundation (grant DEB-0344731 to B.L.F. and P.S.W.), National Geographic Society (grant no. 7617-04), and JSPS (grants nos. 11691130 and 14405036).

REFERENCES

- Bolton B, Marsh AC. 1989.** The Afrotropical thermophilic ant genus *Ocymyrmex* (Hymenoptera: Formicidae). *Journal of Natural History* **23**: 1267–1308.
- Brady SG, Schultz TR, Fisher BL, Ward PS. 2006.** Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 18172–18177.
- Brown WL. 1960.** Contributions toward a reclassification of the Formicidae. III. Tribe Amblyoponini (Hymenoptera). *Bulletin of the Museum of Comparative Zoology* **122**: 145–230.
- Brown WL, Gotwald WH, Lévioux J. 1970.** A new genus of ponerine ants from West Africa (Hymenoptera: Formicidae) with ecological notes. *Psyche* **77**: 259–275.
- Düssmann O, Peeters C, Hölldobler B. 1996.** Morphology and reproductive behaviour of intercastes in the ponerine ant *Pachycondyla obscuricornis*. *Insectes Sociaux* **43**: 421–425.

- Forder JC, Marsh AC. 1989.** Social organization and reproduction in *Ocymyrmex foreli* (Formicidae: Myrmicinae). *Insectes Sociaux* **36**: 106–115.
- Gotwald WH, Léviéux J. 1972.** Comportement d'alimentation et relations entre les individus chez une fourmi primitive, *Amblyopone pluto* Gotwald et Léviéux. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, Série D Sciences Naturelles* **275**: 483–485.
- Haskins CP, Haskins EF. 1951.** Note on the method of colony foundation of the ponerine ant *Amblyopone australis* Erichson. *American Midland Naturalist* **45**: 432–445.
- Heinze J, Hölldobler B, Alpert G. 1999.** Reproductive conflict and division of labor in *Eutetramorium mocquerysi*, a myrmicine ant without morphologically distinct female reproductives. *Ethology* **105**: 701–717.
- Heinze J, Tsuji K. 1995.** Ant reproductive strategies. *Researches on Population Ecology* **37**: 135–149.
- Hölldobler B, Wilson EO. 1986.** Ecology and behavior of the primitive cryptobiotic ant *Prionopelta amabilis* (Hymenoptera: Formicidae). *Insectes Sociaux* **33**: 45–58.
- Ito F. 1993a.** Social organization in a primitive ponerine ant-queenless reproduction, dominance hierarchy and functional polygyny in *Amblyopone* sp. (*reclinata* group) (Hymenoptera: Formicidae: Ponerinae). *Journal of Natural History* **27**: 1315–1324.
- Ito F. 1993b.** Observation of group recruitment to prey in a primitive ponerine ant, *Amblyopone* sp. (*reclinata* group) (Hymenoptera: Formicidae). *Insectes Sociaux* **40**: 163–167.
- Ito F, Billen J. 1998.** Larval hemolymph feeding and oophagy: behavior of queen and workers in the primitive ponerine ant *Prionopelta kraepelini* (Hymenoptera, Formicidae). *Belgian Journal of Zoology* **128**: 201–209.
- Ito F, Ohkawara K. 1994.** Spermatheca size differentiation between queens and workers in primitive ants: relationship with reproductive structure of colonies. *Naturwissenschaften* **81**: 138–140.
- Ito F, Ohkawara K. 2000.** Production and behavior of ergatoid queens in two species of the Indonesian ponerine ant genus *Leptogenys* (*diminuta*-group) (Hymenoptera: Formicidae). *Annals of the Entomological Society of America* **93**: 869–873.
- Macevicz S. 1979.** Some consequences of Fisher's sex ratio principle for social Hymenoptera that reproduce by colony fission. *American Naturalist* **113**: 363–371.
- Masuko K. 1993.** Predation of centipedes by the primitive ant *Amblyopone silvestrii*. *Bulletin of the Association of Natural Science, Shushu University* **24**: 35–44.
- Miyata H, Shimamura T, Hirosawa H, Higashi S. 2003.** Morphology and phenology of the primitive ponerine army ant *Onychomyrmex hedleyi* (Hymenoptera: Formicidae: Ponerinae) in a highland rainforest of Australia. *Journal of Natural History* **37**: 115–125.
- Miyazaki S, Murakami T, Azuma N, Higashi S, Miura T. 2006.** The postembryonic developmental regulation specific to intermorphic queens in *Myrmecina nipponica* (Hymenoptera; Myrmicinae). *Proceedings of the XV Congress IUSSI*, Washington, DC.
- Molet M, Peeters C, Fisher BL. 2007a.** Winged queens replaced by reproductives smaller than workers in *Myrmium* ants. *Naturwissenschaften* **94**: 280–287.
- Molet M, Peeters C, Follin I, Fisher BL. 2007b.** Reproductive caste performs intranidal tasks instead of workers in the ant *Myrmium oberthueri*. *Ethology* **113**: 721–729.
- Ohkawara K, Ito F, Higashi S. 1993.** Production and reproductive function of intercastes in *Myrmecina graminicola nipponica* colonies (Hymenoptera: Formicidae). *Insectes Sociaux* **40**: 1–10.
- Oster GF, Wilson EO. 1978.** *Caste and ecology in the social insects*. Princeton, NJ: Princeton University Press.
- Peeters C. 1991.** Ergatoid queens and intercastes in ants: two distinct adult forms which look morphologically intermediate between workers and winged queens. *Insectes Sociaux* **38**: 1–15.
- Peeters C, Ito F. 2001.** Colony dispersal and the evolution of queen morphology in social Hymenoptera. *Annual Review of Entomology* **46**: 601–630.
- Peeters CP, Molet M. 2009.** Chapter 9: colonial reproduction and life histories. In: Lach L, Parr C, Abbott K, eds. *Ant ecology*. Oxford: Oxford University Press.
- Plateaux L. 1970.** Sur le polymorphisme social de la fourmi *Leptothorax nylanderii* (Förster). I. Morphologie et biologie comparées des castes. *Annales des Sciences Naturelles, Zoologie et Biologie Animale* **12**: 373–478.
- Reeve HK. 1991.** Polistes. In: Ross KG, Matthews RW, eds. *The social biology of wasps*. New York, NY: Cornell University Press, 99–148.
- Ross KG, Matthews RW. 1991.** *The social biology of wasps*. Ithaca, NY: Cornell University Press.
- Sameshima S, Miura T, Matsumoto T. 2004.** Wing disc development during caste differentiation in the ant *Pheidole megacephala* (Hymenoptera: Formicidae). *Evolution & Development* **6**: 336–341.
- Saux C, Fisher BL, Spicer GS. 2004.** Dracula ant phylogeny as inferred by nuclear 28S rDNA sequences and implications for ant systematics (Hymenoptera: Formicidae: Amblyoponinae). *Molecular Phylogenetics and Evolution* **33**: 457–468.
- Seeley TD. 1982.** Adaptive significance of the age polyethism schedule in honeybee colonies. *Behavioral Ecology and Sociobiology* **11**: 287–293.
- Visscher PK. 2007.** Group decision making in nest-site selection among social insects. *Annual Review of Entomology* **52**: 255–275.
- Warton DI, Weber NC. 2002.** Common slope tests for bivariate errors-in-variables models. *Biometrical Journal* **44**: 161–174.
- Wheeler WM. 1910.** *Ants: their structure, development and behavior*. New York, NY: Columbia University Press.
- Wheeler WM. 1916.** The Australian ants of the genus *Onychomyrmex*. *Bulletin of the Museum of Comparative Zoology* **60**: 45–54.