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# Uncoupling Flight and Reproduction in Ants: Evolution of Ergatoid Queens in Two Lineages of *Megalomyrmex* (Hymenoptera: Formicidae)

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## Abstract

*Megalomyrmex* Forel (Myrmicinae: Solenopsidini) consists of 44 species with diverse life history strategies. Most species are predatory and may also tend honeydew-producing insects. A morphologically derived group of species are social parasites that consume the brood and fungus garden within fungus-growing ant nests. The reproductive strategies of *Megalomyrmex* queens are somewhat aligned with these life-style patterns. Predatory species in the *leoninus* species group are large in body size and have ergatoid (i.e., permanently wingless) queens whereas the social parasitic species are smaller and typically have winged queens. We examined two ergatoid phenotypes of *Megalomyrmex foreli* Emery and *Megalomyrmex wallacei* Mann and compared them to winged species, one a social lestobiotic or "thief ant" parasite (*Megalomyrmex mondabora* Brandão) and the other a predator (*Megalomyrmex modestus* Emery). *Megalomyrmex foreli* colonies have a single queen with an enlarged gaster that is morphologically distinct from workers. *Megalomyrmex wallacei* colonies have several queens that are similar in body size to workers. Queens in both species showed a simplification of the thorax, but there was a dramatic difference in the number of ovarioles. *Megalomyrmex foreli* had 60–80 ovarioles compared to eight in *M. wallacei* and *M. mondabora* and *M. modestus* had 22–28. Along with flight loss in queens, there is an obligate shift to dependent colony founding (also called budding or fission) consequently influencing dispersal patterns. These constraints in life history traits may help explain the variation in nesting biology among *Megalomyrmex* species.

**Key words**: ovary, ergatoid, Megalomyrmex foreli, Megalomyrmex wallacei, dispersal

The evolution of strongly dimorphic queens and workers (winged vs. wingless) is a fundamental characteristic of ants. Flying queens follow the basic design of aculeate hymenopterans although the thorax in many species houses hyperdeveloped wing muscles that serve as a metabolic reserve to feed the first generation of workers during independent colony founding. Moreover, a bigger abdomen (gaster) allows enhanced fecundity (more ovarioles enable many eggs to develop simultaneously). This combination of thorax and abdomen modifications contrasts sharply with the phenotypes of workers (Peeters and Molet 2010). The lack of wings and wing muscles causes an extreme reduction in thorax volume, and fusion of sclerites in most species. The diminished thorax of workers is coupled with an abdomen that is generally much smaller than queens', with fewer ovarioles and an almost universal loss of spermathecae.

Compared to various solitary wasps that evolved winglessness, ant workers show extreme simplification in the thorax. The same is also true for the permanently wingless ("ergatoid") queen caste, occurring in at least 55 ant genera (Peeters 2012). Their thorax resembles that of workers, although the abdomen is often bigger with more ovarioles and a spermatheca. Ergatoid queens have evolved convergently across diverse ant lineages therefore their morphology is highly heterogeneous. Similarly, the life history characteristics of their colonies vary immensely, except that dependent colony founding (colony fission or budding) appears to be the rule (Cronin et al. 2013).

The *Megalomyrmex* genus consists of 44 described species (Boudinot et al. 2013), most of which are ground-living ants inhabiting subtropical forests in Central and South America. Species have been separated into four species groups on the basis of morphology and behavior (Brandão 1990). In a recent revision of the Central American species, new species were described and worker morphology indicates that *leoninus* and *modestus* groups, as well as the

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*pusillus* and *silvestrii* groups, share many features (Boudinot et al. 2013). Consistent with a DNA-based phylogeny (Adams 2008), the *leoninus* and *modestus* groups will likely be merged in the future (Boudinot et al. 2013). Social parasites (thief ants, raiding agro-predator ants, and guest ants; see Boudinot et al. 2013 for details) occur in the *silvestrii* group and the *pusillus* group and have been the focus of recent behavioral (Adams et al. 2013, 2015) and phylogenetic (Adams 2008) studies. The alkaloidal venom (Jones et al. 1991) and ergatoid queen morphology has been investigated in the larger species (Brandão 1987, 1990). Queen winglessness has significant evolutionary consequences because queens disperse on foot together with nestmate workers; hence gene flow is geographically restricted. Studies of natural history and ovarian development are required to firmly establish the reproductive strategy of a species (Heinze 1998), and this has not yet been done in a comparative manner within *Megalomyrmex*.

In this study we investigated queen characteristics in four Central American species of *Megalomyrmex* with distinct natural history characters (Longino 2010, Boudinot et al. 2013). Queens are ergatoid in both *M. foreli* Emery (*leoninus* gr.) and *M. wallacei* Mann (*modestus* gr.) but they differ strikingly in fecundity and morphological dimorphism relative to their respective workers. We review the attributes of winged queens from *M. modestus* Emery (*modestus* gr.) and *M. mondabora* Brandão (*silvestrii* gr.) and compare them with ergatoid queens in *M. foreli* and *M. wallacei*.

## Materials and Methods

Fieldwork was done in Costa Rica, Braulio Carrillo National Park from 21 March 2011 to 30 March 2011. Three colonies of *M. foreli* and three colonies of *M. mondabora* (thief ant parasite of *Cyphomyrmex cornutus* Kempf) were collected from El Ceibo station (10°20′N, 084°05′W, 450–550 m elevation, Waterline trail). Twelve colonies of *M. wallacei* and four colonies of *M. modestus* were collected from La Selva Biological Station (10°24′59″N, 084°01′12″W, 50 m elevation). Worker numbers were either counted or approximated. In *M. foreli*, adults and brood were counted and egg-laying was monitored. Ovaries and spermatheca were dissected in queens and workers of all species. Except for *M. mondabora*, queen and worker specimens were examined by SEM. Species determination was conducted by R.M.M.A. (Longino 2010, Boudinot et al. 2013) and vouchers are at The Ohio State University, Museum of Biological Diversity.

## Results

Three *M. foreli* colonies were excavated from within a large wet log (RMMA110324-03: workers = 226; brood, excluding eggs = 130; zero males; one ergatoid queen), at the base of a *Pourouma* tree (RMMA110323-01: workers = 455; brood, excluding eggs = 219; zero males; one ergatoid queen), and at the base of a small plant (RMMA110323-05: workers = 2,231; brood, excluding eggs = 134; 64 males; one ergatoid queen). Queens were strikingly distinct from workers due to their big gasters (Fig. 1). The *M. wallacei* ergatoid queens were much less conspicuous with a gaster only slightly larger than workers, consistent with other reports (Boudinot et al. 2013). Up to six ergatoid *M. wallacei* queens occurred together with 110 ± 88 workers and brood ($n = 12$). In both species, the queen's thorax was highly reduced, consistent with their ergatoid nature. Distinct ocelli typical of flying queens, were not observed in *M. wallacei* queens and only one faint ocellus was present in the *M. foreli* queens.

**Fig. 1.** Comparison of ergatoid queen (bottom right corner) and workers in *M. foreli*, showing the large difference in gaster size.

*Megalomyrmex mondabora* colonies live within the soil-based nests of *C. cornutus* that hang from vines and tree trunks (Adams and Longino 2007). Two previously censused colonies contained ca. 250 parasite workers (Adams and Longino 2007); our estimations for two colonies examined, ranged from ca. 50–200 and one only had a queen (4 of 27 host colonies or 15% were parasitized, one was queenless). *Megalomyrmex modestus* nests (in logs and soil) were diffuse in structure, with multiple queens and ca. 500–2,000 workers. The queens of both *M. mondabora* and *M. modestus* exhibit morphological characters typical of winged queens. *Megalomyrmex modestus* was used as a reference for the flight thorax of a normal winged queen: segmentation is distinct, the pronotum is very small, and the mesonotum is the biggest sclerite (reflecting size of the wing muscles; Fig. 2A). In contrast, the pronotum in both *M. wallacei* (Fig. 2B) and *M. foreli* (Fig. 2C) queens is comparable in size to *M. foreli* workers (Fig. 2D), while the mesonotum is reduced due to the lack of wing muscles. There is a faint promesonotal suture in ergatoid queens, while all sclerites are fused in workers.

The big gaster of *M. foreli* queens ($n = 3$) was associated with 60–80 tightly packed ovarioles (Fig. 3A). There was a dense network of tracheoles around the ovaries. All three queens of *M. foreli* were mated and showed different degrees of ovarian activity. In the large colony (2,231 workers), the queen was physogastric (i.e., intersegmental membranes stretched) with many big yolky oocytes and dark "yellow bodies" indicating that many eggs had been previously laid. In the two small colonies (455 and 226 workers), queens lacked dark "yellow bodies" and had fewer yolky oocytes, suggesting they were recently mated. Accordingly, these two colonies may have resulted from a recent fission event.

*Megalomyrmex wallacei* queens had eight ovarioles ($n = 21$) (Fig. 3B). Several queens were inseminated in each colony, but only some had active ovaries with yolky oocytes. Other mated queens had few yolky oocytes and lacked dark "yellow bodies". For comparison, *M. modestus* queens had 22–28 ovarioles ($n = 6$) (Fig. 3C), and queens of *M. mondabora* had eight ovarioles ($n = 3$) (Fig. 3D). Workers in all four species had two ovarioles ($n = 14$).

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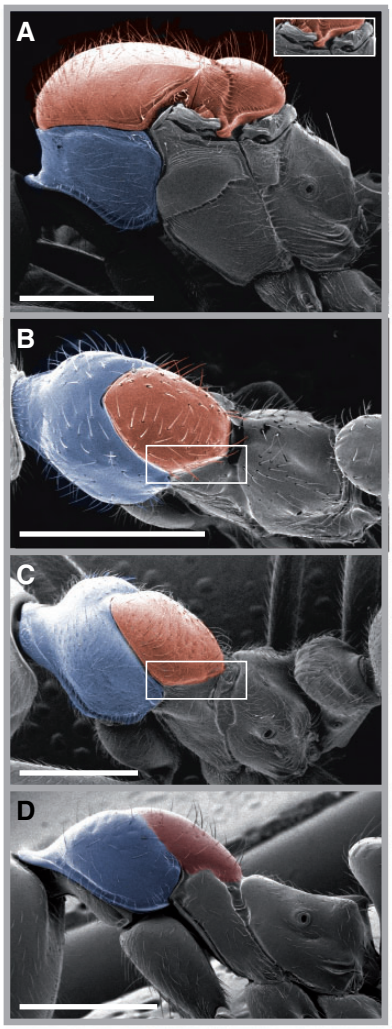


**Fig. 2.** Thoracic segmentation of three *Megalomyrmex* queens, (A) dealate *M. modestus*; (B) *M. wallacei*; (C) *M. foreli*. A worker of *M. foreli* is included for comparison (D). Pronotum (blue) is small in winged queens while the mesonotum (red) is reduced in ergatoid queens. Note the absence of wing bases (insert in A) in the ergatoid queens B and C. Scale bar = 1 mm.

## Discussion

Morphological and biological comparisons of ergatoid queens in two unrelated species of *Megalomyrmex* revealed distinct levels of egg-laying specialization. Their thorax is similarly reduced (Fig. 2B and C) indicating the uncoupling of modifications in the thorax and gaster. The three *M. foreli* colonies examined had a single highly fecund queen with a worker-like thorax Fig. 2C and D and a considerably larger gaster than the workers' (Fig. 1). In contrast, the gaster of *M. wallacei* queens is similar to workers', and the two castes differ slightly in body size. This may explain why Brandão (1990) reported gamergates (i.e., mated, egg-laying workers) as reproductives in *M. wallacei*. Similarly, Longino (2010) noted that *M. wallacei* workers "show considerable size variation, and larger workers have an enlarged mesonotum and no morphologically distinct queen". Our results clearly show that the reproductives do not belong to the worker caste. They have a spermatheca and more ovarioles than workers (Fig. 3B).

The fecundity of ergatoid queens underlies colony size, and this may be correlated with some aspects of the biology of colonies. *Megalomyrmex foreli* builds shallow but large permanent nests in the soil that can be observed year after year (R. M. M. Adams, personal observation). The ants aggressively guard extrafloral nectaries of *Cassia* sp. and *Inga* spp. (Leguminosae) (Jones et al. 1991), riodinid butterflies (Lepidoptera) (DeVries et al. 1992), and build soil structures that cover membracids (Longino 2010, Boudinot et al. 2013). Few foragers can be seen during the day but more can be drawn out with baits (R. M. M. Adams, personal observation). They are apparently more active at night (Jones et al. 1991) therefore estimating colony size is difficult unless the entire colony is excavated. The three colonies that we dug took four researchers, large trash bags, and careful attention to collect the majority of workers and find the queen. Colonies are widely spaced suggesting that foraging territories are established and perhaps their honeydew producing associates help drive this (R. M. M. Adams, personal observation).

In contrast, *M. wallacei* colonies were found in dense populations within deep leaf litter. The population is stable and colonies have been retrieved from the same trails 8 years apart (2003, 2005, 2011) (R. M. M. Adams, personal observation). The nests however are temporary structures found in the litter between dead leaves and at the base of the leaves of short palms (Boudinot et al. 2013). Colonies are small and readily emigrate following the slightest disturbance. High polygyny means a high probability that at least one mated queen will be present following accidental fragmentation, thus leading to autonomous colonies (Cronin et al. 2013).

Unlike flightless insects that live and reproduce alone, flightless reproductives in most ant species have lost the ability to found new colonies independently, and they completely rely on nestmate workers during dependent colony founding (DCF) (Peeters 2012). The multiple occurrences of ergatoid queens in *Megalomyrmex* reveal that DCF (fission or budding) has replaced independent foundation (ICF) repeatedly. However little data on colony founding behavior are available in *Megalomyrmex*. As the two congeneric species studied here differ in queen number, it is evidence against the persistent belief in the literature that DCF species are always polygynous.

The mosaic nature of ergatoid queens in *Megalomyrmex*, (i.e., exhibiting traits specific to both workers and winged queens), suggests that morphological modules became recombined during development (Molet et al. 2012). Ergatoid queens are novel phenotypes that evolved by uncoupling the growth of the thorax and abdomen. Because they evolved convergently across the ants, the phenotypes of ergatoid queens are very heterogeneous. Molet et al. (2009) recognized two categories of ergatoid queens: (1) "single-purpose", few are produced in each colony, they are much bigger than workers and function exclusively for reproduction and (2) "multi-purpose", many are produced in each colony and they are similar in size to workers, only one or few reproduce while others remain virgin and function as labourers. This corresponds to the phenotypes of *M. foreli* and *M. wallacei* queens, respectively, which appear to be alternative solutions to the replacement of winged queens.

Within the *modestus* group, both ergatoid and winged queens have been recorded in separate populations of *Megalomyrmex goeldii* (Brandão 2003). Similarly, *M. wallacei* has winged queens in Brazil (Brandão 2003) but not in Costa Rica (this study). Molecular and/or morphological studies would help determine if Brazilian populations represent a different species or an intraspecific polymorphism. Both winged and ergatoid queens have also been described in the facultative thief ant social parasite, *M. silvestrii* (Boudinot et al. 2013). Although only one ergatoid was examined, it is slightly larger than workers and has a less voluminous thorax compared to the winged queens. The raiding behavior (Adams et al. 2015), nesting

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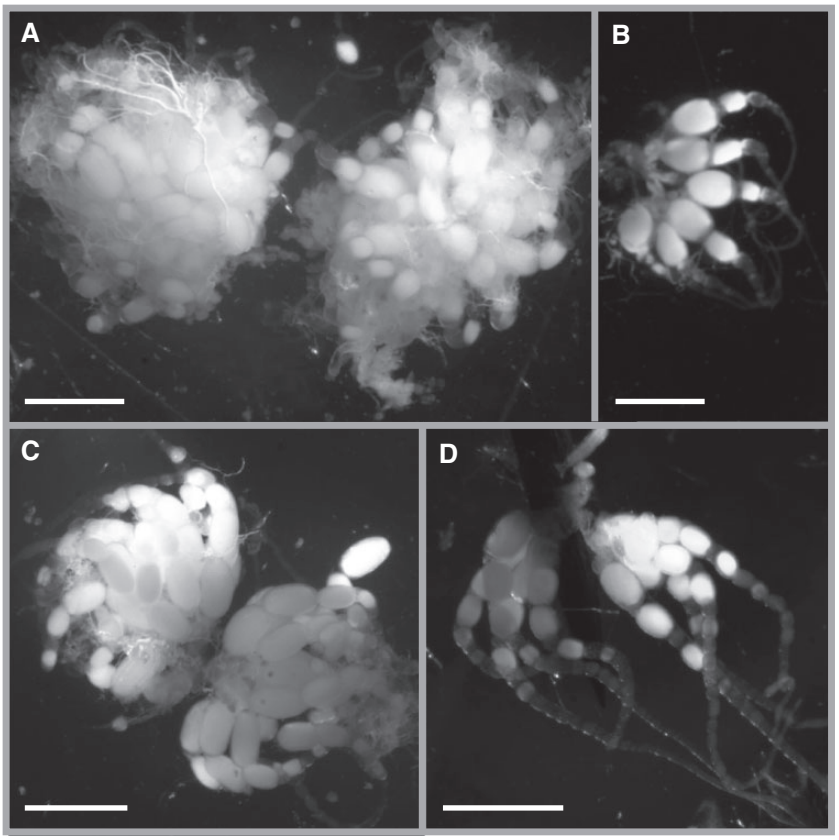


**Fig. 3.** Developed ovaries of ergatoid queens in (A) *M. foreli* and (B) *M. wallacei* compared to winged queens (C) *M. modestus* and (D) *M. mondabora*. The large difference in ovariole numbers indicates different rates of egg-laying. Scale bar = 1 mm.

habits (Boudinot et al. 2013), and morphology suggest that the *M. silvestrii* ergatoids may be the "multi-purpose" type (Molet et al. 2009). Studies of *Megalomyrmex* species that include colony demography, ovarian development, and population genetics will advance our understanding of the evolutionary implications of queen winglessness at the ecological timescale and shed light on the plasticity of independent and dependent colony foundation. Comparative studies conducted on the variety of *Megalomyrmex* species with obligate (e.g., *M. foreli*, *M. leoninus*, and *M. staudingeri*) and facultative ergatoid queens (e.g., *M. wallacei*, *M. silvestrii*, and *M. goeldii*) will elucidate factors that influence the evolution of queen winglessness. Furthermore, future work can draw on the developmental pathways identified in worker winglessness and link these to genomic changes within the obligate ergatoid species. Finally, phylogenetic studies of *Megalomyrmex* and other genera in the tribe Solenopsidini (Ward et al. 2015) will reveal the number of independent evolutionary events of ergatoid queens (e.g., *Monomorium* and *Megalomyrmex*) for broader comparative analyses.

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