

# Winged queens replaced by reproductives smaller than workers in *Mystrium* ants

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**Abstract** In ants, winged queens that are specialized for independent colony foundation can be replaced by wingless reproductives better adapted for colony fission. We studied this shift in reproductive strategy by comparing two *Mystrium* species from Madagascar using morphometry, allometry and dissections. *Mystrium rogeri* has a single dealate queen in each colony with a larger thorax than workers and similar mandibles that allow these queens to hunt during non-claustral foundation. In contrast, *Mystrium 'red'* lacks winged queens and half of the female adults belong to a wingless 'intermorph' caste smaller and allometrically distinct from the workers. Intermorphs have functional ovaries and spermatheca while those of workers are degenerate. Intermorphs care for brood and a few mate and reproduce making them an all-purpose caste that takes charge of both work and reproduction. However, their mandibles are reduced and inappropriate for hunting centipedes, unlike the workers' mandibles. This together with their small thorax disallow them to perform independent colony foundation, and colonies reproduce by fission. *M. rogeri* workers have mandibles polymorphic in size and shape, which allow for all tasks from brood care to hunting. In *M. 'red'*, colonial investment in reproduction has shifted from producing expensive winged queens to more numerous helpers. *M. 'red'* intermorphs are the first case of

reproductives smaller than workers in ants and illustrate their potential to diversify their caste system for better colonial economy.

**Keywords** Intermorph · Morphology · Caste · Reproduction · Allometry

## Introduction

Various solitary insects exhibit dispersal polymorphism whereby winged morphs disperse and non-flying morphs remain in their natal area (Harrison 1980; Roff 1990). Social insects exhibiting dispersal polymorphism are particularly interesting because the colonial structure can be dramatically modified in addition to individual morphology. In many termites, winged reproductives found new colonies alone and are replaced upon their death by several wingless reproductives (neotenics: Myles and Nutting 1988). In ants, winged queens that can perform independent foundation is the ancestral condition. They were replaced by wingless reproductives in many species, e.g. intermorphs (Heinze and Buschinger 1989; Ohkawara et al. 1993) or gamergates (mated reproductive workers: Peeters 1997; Ward 1983). These wingless phenotypes function as secondary reproductives or leave the nest with workers to found a new colony (fission). Wingless reproductives are morphologically and behaviourally very different from winged queens and they cannot survive without sterile nestmates. Hence, the colonial pattern of reproductive investment has shifted to an increased production of sterile helpers instead of winged gynes.

Independent colony foundation (ICF) by winged queens is risky in ants. They disperse, mate and start new colonies without the help of workers and this solitary life can last a

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few months. ICF is claustral in many taxa (e.g. Formicinae and Myrmicinae) where founding queens never leave the nest to forage because they have large metabolic reserves (Passera and Keller 1990; Wheeler and Martinez 1995), including the wing muscles that are histolysed to feed the first brood of workers. However, the inferred ancestral condition is non-claustral (semi-claustral) ICF, which is exhibited in many species of ‘basal’ subfamilies e.g. Amblyoponinae and Ponerinae. As a consequence of limited caste dimorphism (another plesiomorphic trait), founding queens lack sufficient reserves and need to hunt until the first generation of workers become adult (reviewed in Peeters 1997). Consequent exposure to predators and parasites likely leads to lower success during non-claustral as opposed to claustral ICF. Non-claustral ICF has also re-evolved in several ‘higher’ ant species when the cost of provisioning queens becomes too high or mortality faced by foundresses gets lower (Brown and Bonhoeffer 2003). Winged queens are the inferred ancestral trait in ants and their complete absence in many unrelated Amblyoponinae and Ponerinae species means that non-claustral foundation and long-range aerial dispersal were repeatedly selected against and replaced by colony fission (budding) and short-range dispersal on the ground (Peeters and Ito 2001).

A crucial characteristic of colony fission is that reproductives totally depend on workers during colony initiation and there is no longer a solitary stage. When fission is obligate, the production of winged queens brings no benefits and consequently in these species reproductives are often wingless (Peeters and Ito 2001; Heinze 1998). The success of colony fission mainly depends on the number of workers that help the wingless reproductives (Pamilo 1991), while in the case of ICF it depends on the number and size of winged queens. Hence, switching from ICF to fission requires different colonial investments and caste specializations. Comparative studies of closely related species that have either winged or wingless reproductives can help to understand evolutionary modifications in colonial dispersal.

In *Myrmium* (subfamily Amblyoponinae), several species have typical winged queens, including *Myrmium rogeri* from Madagascar. However, *Myrmium ‘red’* from Madagascar lacks winged queens and colonies have two wingless female phenotypes: black adults, which are similar to the workers of *M. rogeri*, and smaller red adults (Fig. 1). Our aim was to establish their respective function, especially for reproduction. Therefore, we compared the morphology, ovarian activity and mating status of these two phenotypes with one another and also with queens and workers of *M. rogeri*. We performed allometry analyses to establish whether they constitute distinct castes. A phylogeny based on five genes indicates that species with winged queens are basal in *Myrmium* (B. Noonan and B. Fisher, unpublished



**Fig. 1** Intermorphs of *M. ‘red’* (small and reddish) have larger ovaries and several mate and lay eggs. In contrast, workers (large and black) cannot lay eggs and have larger mandibles for hunting (© Alex Wild)

data) and we discuss the evolutionary shift in colonial reproductive strategy that occurred in *M. ‘red’*.

## Materials and methods

### Colonies

From 1992 to 2005, 14 colonies of *M. ‘red’* and 16 colonies of *M. rogeri* were collected in primary rainforests in Madagascar. Voucher specimens for each colony are deposited in the California Academy of Sciences and collection data on <http://www.antweb.org>. Cocoons were opened and differentiated pupae identified as black adults, red adults, winged gynes (infertile young queens) or males. Colonies were housed in plaster nests with a glass roof allowing behavioural observations.

### Dissections

Individuals from 14 *M. ‘red’* and 3 *M. rogeri* colonies collected in 2003 and 2004 were dissected. Ovarian development and spermatheca were checked under a Leica stereomicroscope. Yolky oocytes can be distinguished visually from smaller non-yolky oocytes because they are opaque and white instead of translucent; the former were counted and classified as ‘mature’ (size of an egg) or ‘immature’ (smaller than an egg). The presence of yellow bodies gives evidence of past egg-laying activity (Billen 1982). The length of ovaries was measured in one queen of *M. rogeri* and in one black adult and one red adult of *M. ‘red’*.

### Morphometry

To document intra-colonial variability in size and morphology, we measured all individuals from colonies BLF10859

(61 black adults and 23 red adults of *M. 'red'*), BLF10950 (34 workers and one dealate queen of *M. rogeri*) and some individuals from colonies BLF10952 (1 dealate queen) and BLF02648 (19 winged gynes). We also measured a few random individuals from all colonies to check if our sample was representative of the species (12 black adults and 12 red adults from 4 different *M. 'red'* colonies; 12 workers from 4 different *M. rogeri* colonies; data not shown). Dorsal and lateral photographs were taken with a Sony digital video camera. We then measured (ImageJ <http://rsb.info.nih.gov/ij>) mandible length, maximum head width, thorax (mesosoma) volume and cross-sectional area of the first gaster segment (abdominal segment III). These characteristics are biologically meaningful in that the mandibles and associated muscles in the head are important for brood care or hunting, the gaster volume is linked to fecundity and the thorax is a good measure of overall body size. An ant thorax has a complex shape and we approximated its volume as the mean of two volumes: dorsal width times lateral area and lateral height times dorsal area. Since the gaster can be contracted, its length is highly variable even in one individual. Thus, the gaster volume was estimated by the area of the first segment of the gaster for which the tergite and sternite are fused in *Mystrium*. This area was assumed to be an ellipse, the diameters of which are lateral height and dorsal width.

#### Data analysis

To establish whether red and black adults represent different castes and to assess inter-specific differences, we performed linear regressions and allometry analyses between our morphometric variables. Because measurements are subject to errors, we could not use the model I regression that hypothesises that the independent variable is error-free. Instead, we used the standardised major axis regression (Fairbairn 1997). We also tested for isometry and compared allometry coefficients between groups (both castes and species). These three statistical analyses were performed with the software (S)MATR 1.0 (Warton and Weber 2002) available at <http://www.bio.mq.edu.au/ecology/SMATR/>. Details on how the test statistic and *P* value are computed are available on this website. Other statistical analyses were performed with Statistica 5.1.

## Results

### Colony composition

*M. 'red'* nested either in rotten logs or in the surface soil under stones, while *M. rogeri* was only found in rotten logs (Table 1). *M. 'red'* colonies contained 26 black adults

**Table 1** Composition and dissection data of 14 *M. 'red'* and 16 *M. rogeri* colonies collected in Madagascar

Month/year of collection	Colony code <sup>a</sup>	Nesting site	Workers (no mature oocytes, virgin+mature oocytes)	Intermorphs (no mature oocytes, virgin+mature oocytes, mated+mature oocytes)
<i>M. 'red'</i>				
12/2003	10173	Log	31 (18, 0)	26 (22, 2, 2)
12/2003	10341	Log	11 (9, 0)	21 (10 <sup>c</sup> , 0, 5)
12/2003	10138	Log	46 (16, 0)	31 (16, 3, 0)
11/2004	10449	Log	16 (14, 0)	18 (10, 4, 1)
11/2004	10480	Stone	33	15 (7, 5, 0)
11/2004	10482	Stone	7 (2, 0)	18 (9, 7, 0)
11/2004	10499	Stone	40 (14, 0)	37 (11 <sup>c</sup> , 10, 6)
11/2004	10859	Stone	62 (61, 0)	25 (17, 6, 0)
11/2004	10860	Log	7 (2, 0)	2 (1, 1, 0)
11/2004	10861	Stone	10 (1, 0)	8 (6, 0, 0)
11/2004	10864	Stone	86 (1, 0)	53 (17, 16, 8)
11/2004	10865	Stone	16	7
11/2004	10866	Stone	21 (1, 0)	5 (5, 0, 0)
11/2004	10867	Stone	82 (1, 0)	54 (24, 2, 0)
<i>M. rogeri</i>				
11/1992	00519 <sup>b</sup>	Log	510	0
01/2001	02642 <sup>b</sup>	Log	47	0
11/2001	04466	Log	507	0
12/2001	04770	Log	496	0
01/2002	05154	Log	16	0
03/2002	06144	Log	187	0
12/2003	09731	Log	128	0
11/2004	10907 <sup>b</sup>	Log	59 (54, 0)	0
11/2004	10919	Log	19 (17, 0)	0
11/2004	10950	Log	33 (33, 1)	0
11/2004	10952	Log	142	0
11/2004	10994 <sup>b</sup>	Log	228	0
06/2005	12213 <sup>b</sup>	Log	140	0
06/2005	12216	Log	33	0
06/2005	12223 <sup>b</sup>	Log	60	0
06/2005	12274	Log	31	0

<sup>a</sup> Colony codes 10#### refer to BLF10#### on <http://www.antweb.org>.

<sup>b</sup> No queen was found.

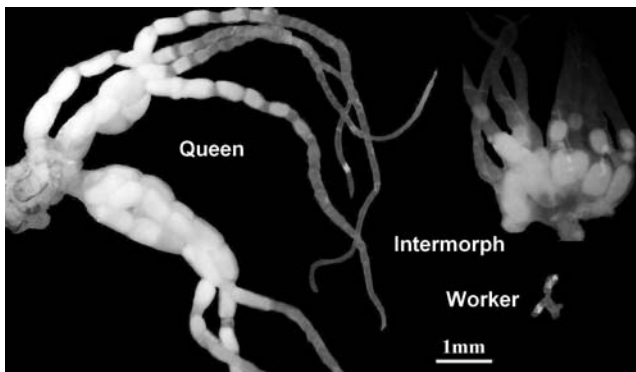
<sup>c</sup> Including 8 mated in colony 10341 and 1 in colony 10499.

(median; quartiles: 12.25 and 44.50; *N*=14, Fig. 1) and 19.5 red adults (quartiles: 9.75–19.75), i.e. red adults represent 40% of total adults (quartiles: 31 and 47). *M. rogeri* colonies (*N*=16) contained 94 workers (median; quartiles: 33 and 197.25), which is more than the total number of female adults in *M. 'red'* (Mann–Whitney test: *U*=43.5, *P*=0.0044). Moreover, the largest *M. rogeri* colony consisted of 510 adults, while the largest *M. 'red'* colony had 139 adults. More cocoons (Mann–Whitney test: *U*=2, *P*=0.0018) and as many larvae (Mann–Whitney test: *U*=78, *P*=0.622) were present in *M. rogeri* colonies. Ten of the 16 *M. rogeri* colonies had a single dealate queen; in the others she either escaped or was naturally absent.

## Morphology of the ovaries and oogenesis

Among the 233 red adults dissected, 22 were mated reproductives with a spermatheca full of sperm, active ovaries ( $2.5 \pm 1.3$  mature and  $4.5 \pm 1.2$  immature oocytes per ovary) and large dark yellow bodies (Table 1). Fifty-six red adults had an empty spermatheca but slightly developed ovaries ( $0.6 \pm 0.6$  mature and  $0.8 \pm 0.8$  immature oocytes per ovary) and very few yellow bodies. Mated reproductives had at least four times more mature and immature oocytes per ovary than these virgins ( $t$  test:  $t_{130} = 11.35$ ,  $P < 0.001$ ;  $t_{130} = 19.64$ ,  $P < 0.001$ ). The remaining 155 red adults were virgin and had inactive ovaries or immature oocytes only ( $1.1 \pm 0.9$  immature oocytes per ovary) without yellow bodies. Only 5 out of 14 colonies had mated reproductives (Table 1). In these 5 colonies, 7% to 33% of red adults reproduced sexually and 0% to 39% may have laid unfertilized eggs. In the remaining 9 colonies, 0% to 50% of the red adults may have laid unfertilized eggs. Both red and black adults had four ovarioles per ovary. All of the 140 black adults dissected had much shorter and thinner ovarioles than those of infertile red adults where no oocytes could be seen inside (Fig. 2). Their spermatheca was always empty and much smaller than that of red adults. They never laid eggs even when isolated from red adults for several weeks (unpublished data). Thus, black adults are sterile while red adults can reproduce. Eggs laid by red adults yielded red adults, black adults and males (unpublished data), which excludes the possibility that they are social parasites.

Three *M. rogeri* queens dissected had four ovarioles per ovary and a full spermatheca. They had much longer ovarioles and could have more oocytes (1 to 15 mature and 0 to 14 immature oocytes per ovary) than *M. 'red'* reproductives (Fig. 2). In three *M. rogeri* colonies (105 workers dissected), the workers had 4 ovarioles per ovary. Their ovarioles were longer than the developed ovarioles of red adults in *M. 'red'*, although they almost always lacked



**Fig. 2** Pair of ovaries of a *M. rogeri* queen, *M. 'red'* reproductive intermorph and *M. 'red'* worker. Ovarioles were 9.3, 4.3 and 0.6 mm long, respectively. Both queen and intermorph were inseminated

folly oocytes. Two colonies had 2 and 3 workers with one immature oocyte per ovary, and one colony had one worker with 2.5 mature and 4.5 immature oocytes per ovary (Table 1). Thus, the workers of *M. rogeri* are able to lay eggs, but they were always virgin and their spermatheca was very small relative to queens.

## Morphometry: black vs red adults in *M. 'red'*

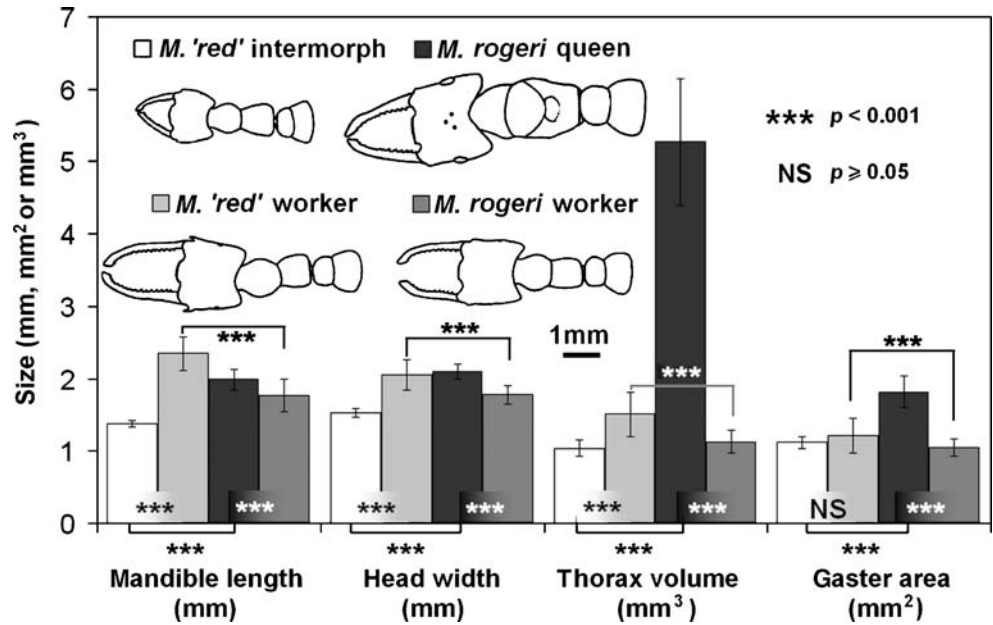
*M. 'red'* black adults have a larger thorax ( $t$  test:  $t_{82} = 7.21$ ,  $P < 0.001$ ; Fig. 3) and are heavier than red adults (fresh weight  $8.84 \pm 1.00$  mg,  $N = 34$  vs  $5.95 \pm 0.61$  mg,  $N = 19$ ,  $t$  test:  $t_{51} = 11.22$ ,  $P < 0.001$ ). They also have longer mandibles and wider heads ( $t_{61} = 19.30$ ,  $P < 0.001$ ;  $t_{81} = 11.78$ ,  $P < 0.001$ ; Fig. 3). There is no continuum between black and red adults (Fig. 4). The growth of mandible length with thorax volume<sup>1/3</sup> is allometric in black adults but isometric in red adults (isometry test:  $F_{1,41} = 31.51$ ,  $P < 0.001$ ;  $F_{1,18} = 0.068$ ,  $P = 0.797$ ; Fig. 4), these allometries differ significantly ( $P = 0.044$ ). The same is true for head width (isometry test:  $F_{1,58} = 43.83$ ,  $P < 0.001$ ;  $F_{1,21} = 0.569$ ,  $P = 0.459$ ), except the allometries do not differ ( $P = 0.173$ ). We thus conclude that the growth patterns of black and red adults are distinct. Moreover, the mandible/head ratio is higher in black adults than in red adults ( $1.17 \pm 0.06$  vs  $0.91 \pm 0.05$ ,  $t$  test:  $t_{61} = 16.52$ ,  $P < 0.001$ ), but the gaster/thorax ratio is lower ( $0.81 \pm 0.09$  mm<sup>-1</sup> vs  $1.09 \pm 0.09$  mm<sup>-1</sup>,  $t$  test:  $t_{82} = 12.64$ ,  $P < 0.001$ ). Hence, black adults have large mandibles and heads while red adults have large gasters. Thus, there are two distinct castes in *M. 'red'*. Black adults are workers and we argue that red adults are an 'intermorph' caste (M. Molet, B.L. Fisher, F. Ito and C. Peeters, unpublished data), i.e. exhibiting a fixed combination of worker and queen traits (wingless and smaller than winged queens, but having functional ovaries and spermatheca).

## Morphometry: workers vs queens in *M. rogeri*

We compared winged queens with workers to assess the degree of caste dimorphism. *M. rogeri* workers are considerably smaller and lighter than queens and this is essentially based on the differences in the thorax volume ( $t$  test:  $t_{52} = 26.77$ ,  $P < 0.001$ ; Fig. 3; fresh weight  $8.33 \pm 1.20$  mg,  $N = 24$  workers vs  $14.51 \pm 1.14$  mg,  $N = 8$  gynes i.e. virgin young queens;  $t$  test:  $t_{30} = 5.82$ ,  $P < 0.001$ ). This supports the idea that *M. rogeri* queens have large metabolic reserves in the form of wing muscles. Queens had longer mandibles and wider heads relative to workers ( $t_{52} = 4.01$ ,  $P < 0.001$ ;  $t_{52} = 9.54$ ,  $P < 0.001$ ; Fig. 3) and mandible/head ratio of *M. rogeri* queens is only slightly lower than that of workers ( $0.95 \pm 0.03$ ,  $N = 20$  vs  $0.99 \pm 0.08$ ,  $N = 34$ ;  $t$  test:  $t_{52} = 2.56$ ,  $P = 0.013$ ), which suggests that single founding queens can hunt the same prey as single



**Fig. 3** Size of body parts in one *M. 'red'* and one *M. rogeri* colonies. Drawings are based on individuals of mean size (gaster segment 1 was measured, segments 2–5 are not shown). Significance of the *t* tests are between the bars



workers. Furthermore, *M. rogeri* queens have a larger gaster than workers and the growth of the gaster area with thorax volume<sup>1/3</sup> is allometric in queens but isometric in workers (isometry test:  $F_{1,18}=16.96$ ,  $P<0.001$ ;  $F_{1,32}=3.08$ ,  $P=0.089$ ; Fig. 4). Hence, *M. rogeri* queens are morphologically adapted for both non-claustral ICF and higher fecundity.

**Morphometry: *M. 'red'* workers vs *M. rogeri* workers**

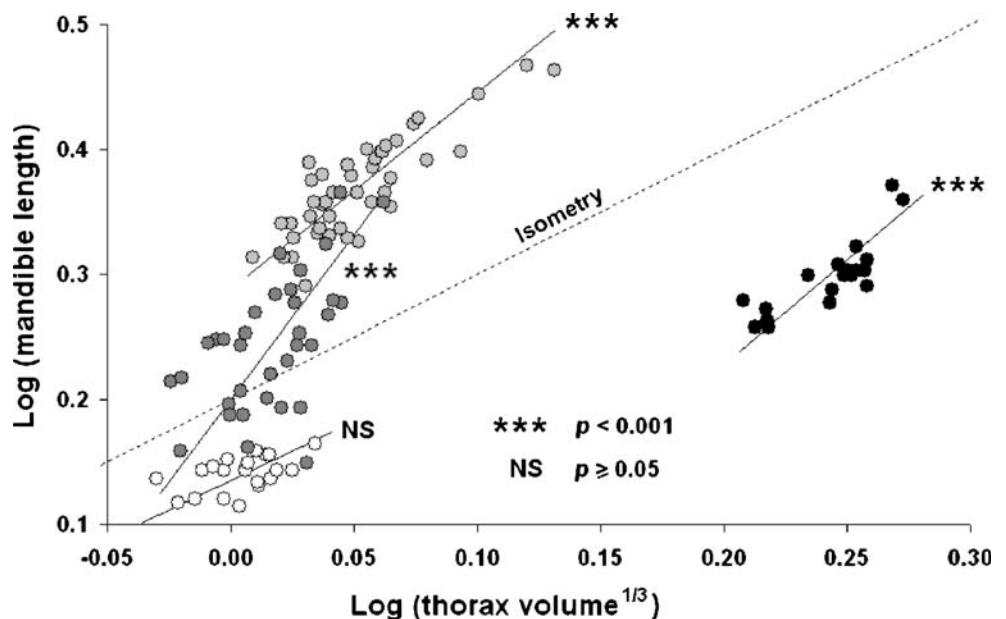
*M. 'red'* workers have longer mandibles, wider head, larger thorax and larger gaster than *M. rogeri* workers (*t* test:  $t_{75}=11.48$ ;  $t_{92}=6.96$ ;  $t_{93}=6.75$ ;  $t_{93}=3.73$ , respectively; all tests  $P<0.001$ ; Fig. 3). The coefficient of variation in mandible

length is lower in *M. 'red'* workers than in *M. rogeri* workers (CV: 9.31 against 12.25) and so is the allometry between mandible length and thorax volume<sup>1/3</sup> ( $P=0.006$ ; Fig. 4). Thus, relative to *M. rogeri* workers, *M. 'red'* workers are larger and less variable in size.

**Investment in reproductives**

*M. 'red'* colonies produced workers, males and intermorphs but never gynes (193 cocoons opened). In contrast *M. rogeri* colonies produced workers, males and winged gynes only (910 cocoons). The product of the number of pupae of workers, intermorphs and gynes times the individual fresh weights gives an idea of the proportion of resources (in mg)

**Fig. 4** Allometry between thorax volume and mandible length in *M. 'red'* intermorphs (white) and workers (light grey) and in *M. rogeri* workers (dark grey) and winged queens (black). The allometry coefficients correspond to the slopes of the regression lines. The dotted line has a slope of 1 (isometry). Significance of the allometry vs isometry tests are at the end of each line



allocated to each female caste for colonial reproduction. *M. 'red'* and *M. rogeri* colonies invest proportionally as much in workers ( $85\pm 23\%$  and  $72\pm 30\%$  of the total investment in females, Mann–Whitney test:  $U=13$ ,  $P=0.306$ ) and in either intermorphs or gynes ( $11\pm 14\%$  of the total investment in females for *M. 'red'* intermorphs and  $2\pm 4\%$  for *M. rogeri* gynes, Mann–Whitney test:  $U=11$ ,  $P=0.188$ ). Males represent  $17\pm 31\%$  and  $13\pm 23\%$  of the total investment in new adults, respectively, which is not different (Mann–Whitney test:  $U=19.5$ ,  $P=0.942$ ).

## Discussion

*Mystrium* intermorphs can both reproduce and care for brood

*M. 'red'* lacks winged queens, but instead a few of the numerous red intermorphs reproduce in each colony. Intermorphs are smaller than workers (Fig. 3) and have short thin mandibles with blunt teeth. In the closely related queenless *M. oberthueri*, intermorphs rear the brood but, unlike the workers, they do not forage outside the nests (M. Molet, C. Peeters, I. Follin and B.L. Fisher, unpublished data). Non-quantitative observations in *M. 'red'* suggest the same pattern of polyethism. Workers of *M. 'red'* have strong mandibles with sharp teeth and their large head grows allometrically with thorax volume, unlike intermorphs, which exhibit isometric growth. The head capsule provides attachment for mandible muscles, thus workers with enlarged heads have more powerful mandibles (Gronenberg et al. 1998) appropriate for catching large centipedes. Indeed, *Mystrium* specialises on this prey, similarly to some species of *Amblyopone* (e.g. Léviex 1972). The gaster exhibits an opposite pattern to the mandibles because it grows proportionally larger in intermorphs than in workers and can thus accommodate more developing oocytes in fertile individuals. In contrast, *M. 'red'* workers have degenerate ovaries and cannot lay eggs. Although their spermatheca is visually distinct, we lack histological data about the epithelium characteristics (see Gobin et al. 2006). Since the spermatheca is much smaller than in intermorphs, it may be non-functional. Accordingly, workers cannot become gamergates. Hence, the intermorphs are not morphologically competent to hunt while the workers are no longer able to reproduce, as also found in two other queenless species of *Mystrium* (M. Molet, B.L. Fisher, F. Ito and C. Peeters, unpublished data).

*M. rogeri* has winged queens but lacks intermorphs. Colonies are monogynous and queens have very active ovaries (Fig. 2) so they can reach a high egg-laying rate and generate large colonies. *M. rogeri* workers have functional ovaries but never mate. After the queen's death, they can lay

unfertilized eggs that develop into males. The mandibles of workers are polymorphic in length, which allows both brood-caring and hunting, unlike *M. 'red'* in which all workers have larger mandibles (Fig. 4). We show elsewhere (M. Molet, C. Peeters, I. Follin and B.L. Fisher, unpublished data) that in the absence of intermorphs, workers in *M. oberthueri* have the ability to raise the brood to adulthood.

Male activity seems seasonal in *M. 'red'* (unpublished data), so most intermorphs cannot mate because they emerge at the wrong time of the year. Inseminated reproductive intermorphs were found in only 5 of the 14 *M. 'red'* colonies collected in November–December. Some mated individuals may have escaped during colony collection. Various data suggest that the dispersal of *M. 'red'* males starts in January, and the number of mated reproductives reaches its lowest level before male dispersal. This may account for the many virgin intermorphs with developed ovaries found in our sample of colonies (Table 1). We do not know whether thelytokous parthenogenesis (e.g. *Platythyrea punctata*, Heinze and Hölldobler 1995) is an option for virgin intermorphs.

Evolutionary switch from ICF to fission

*M. rogeri* queens have a large flight thorax and caste dimorphism is very pronounced (queen/worker thorax volume ratio=5.7, Fig. 3) for an ant belonging to 'basal' subfamilies. For comparison, queen/worker thorax ratio is 3.9 in *Amblyopone australis* and 1.8 in *Harpegnathos saltator* (unpublished data). Foundresses in Amblyoponinae and Ponerinae species need to hunt to feed the first brood of workers, e.g. *A. australis* and *H. saltator* (Haskins and Haskins 1951; Peeters et al. 2000) and the large metabolic reserves (in the form of wing muscles) in *M. rogeri* probably help to reduce the number of exits from the nest. The mandible/head ratio of *M. rogeri* queens indicates that they are specialized hunters in addition to being specialized breeders. Such totipotent winged queens are expensive and their annual production is costly at the colony level. The low success rate likely to be associated with non-claustral ICF (Peeters 1997) means that they need to be produced in large numbers. The two *Mystrium* species outside Madagascar, *M. camillae* and *M. silvestrii*, have winged queens and a reproductive strategy that appears similar to *M. rogeri* (F. Ito, personal communication; B. Fisher, unpublished data).

In sharp contrast, *M. 'red'* has evolved intermorphs that are much smaller and cheaper than *M. rogeri* queens (queen/intermorph thorax volume ratio=5.23; other body parts in Fig. 3). Colonies with intermorphs are polygynous, which compensates for their lower individual fecundity. It is surprising to note that unlike the wingless reproductives described in other ants (Heinze 1998; Peeters 1991), the intermorphs of *M. 'red'* are even smaller than conspecific

workers (intermorph/worker thorax volume ratio=0.69). Winged queens have disappeared in *M. 'red'* (based on our 14 colonies, 292 additional records of *M. 'red'*, 4,000 Malaise traps throughout Madagascar and museum collections). The inability of intermorphs to hunt confirms that ICF is impossible. Although colony fission was not empirically documented in *M. 'red'*, it is the only alternative mechanism of colony multiplication known in ants. In species where a switch from ICF to fission is selected for and where gamergates are impossible, producing intermorphs is a strategy to acquire cheap wingless reproductives. Little is known about the ecological differences between queenright and queenless species of *Mystrium*. The smaller colonies of *M. 'red'* may exploit a different ecological niche relative to *M. rogeri*, e.g. nesting site under stones.

Since the investment in males is similar in both species, we focus on the investment in females. In *M. rogeri*, the colonial investment in labor and colony reproduction is directly equivalent to the respective biomass of workers and gynes produced each year. In contrast, labor and colony reproduction in *M. 'red'* are not clearly separated between workers and intermorphs. Indeed, intermorphs not only reproduce but also perform all brood care and are thus part of the labor force. Moreover, they share the task of colony fission with workers and both castes are distinct components of the reproductive investment (Pamilo 1991). Intermorphs that remain infertile contribute to colony fitness, whereas gynes of *M. rogeri* that fail to establish a new colony die.

The ancient insular nature of Madagascar (120 million years) and its distance of more than 300 km from the African coast account for the absence of some of the ecologically crucial African genera, like the Formicinae *Oecophylla* (weaver ants) that dominate the forest canopy and the Dorylinae (army ants) that dominate the forest ground (Wheeler 1922). Since both are major predators of ants (Hölldobler and Wilson 1990; Kaspari and O'Donnell 2003; Gotwald 1995), their absence from Madagascar has allowed the radiation of taxa that are relic elsewhere (Fisher 1997), together with the frequent evolution of fission-based colonial strategies, e.g. *Odontomachus coquereli* (M. Molet, C. Peeters and B.L. Fisher, unpublished data). The permanent switch from winged queens to dwarf wingless intermorphs in *Mystrium* illustrates the potential of ants to modify their caste system as an adaptation to new needs in colonial reproduction.

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