

Research article

## Ontogenetic origin of mermithogenic *Myrmica* phenotypes (Hymenoptera, Formicidae)

S. Csósz<sup>1</sup> and G. Majoros<sup>2</sup>

<sup>1</sup> Department of Zoology, Hungarian Natural History Museum, H-1088 Budapest, Baross u. 13., Hungary, e-mail: csosz@zoo.zoo.nhmus.hu

<sup>2</sup> Department of Parasitology and Zoology, Faculty of Veterinary Sciences, Szent István University, H-1078 Budapest, István u. 2., Hungary, e-mail: majoros.gabor@aotk.szie.hu

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**Abstract.** Entomo-pathogen parasites typically induce alternative "parasitogenic" phenotypes in ants and other insects. However, the basis of generated developmental changes is poorly understood. Parasitic mermithid nematodes also cause the formation of three discrete and aberrant morphologies within *Myrmica* ants. These have been called "worker-like" ("mermithergate"), "intermorphic" ("gynaecoid mermithergate") and "gyne-like" ("mermithogyne") and their formation has been attributed to infection of worker- and queen-presumptive larvae, respectively. In order to better understand the developmental mechanisms that lead to the formation of these alternative parasitogenic phenotypes we observed allometric patterns of parasitogenic *Myrmica gallienii* phenotypes in comparison with uninfected workers and gynes from the same nests. It was revealed that the three discrete morphologies of parasitogenic female phenotypes did not differ significantly from each other in their scaling indicating that these were trapped in the same developmental pathway. Infected individuals scaled according to basically gyne-like allometry, however significantly differed from workers in their scaling. Based on the observed scaling patterns we herein raise an alternative explanation according to which both "mermithergate", "gynaecoid mermithergate" and "mermithogyne" *Myrmica* phenotypes develop from the same type of larvae, namely from the queen-presumptive larvae and their formation, therefore, is rendered as a diverging process. According to the mechanism we propose effect of nematodes may turn out to be the determining factor in the formation of alternative parasitogenic morphologies.

**Keywords:** Allometry, castes, intermorphs, development, parasites, *Mermis*.

### Introduction

Mermithid nematode infection resulted in three alternative parasitogenic phenotypes in *Myrmica* species that superficially resembled either workers ("mermithergates") or intermorphs ("gynaecoid mermithergates") or gynes ("mermithogynes"). Relevant papers describe the differences between the infected and uninfected individuals (Wheeler, 1910; Kloft, 1949; Czechowski et al., 2007 etc.). However, very little is known concerning developmental homologies and the ontogenetic origin of infected individuals, as well as the effects of infestation on the developmental process itself.

Mermithid nematodes complete their larval development in the haemocoel of ant larvae and pupae (Czechowski et al., 2007) and attack both sexuals and workers (Passera, 1975, 1976), inducing scrambled caste features on postmetamorphic host individuals. Wheeler (1928, 1937) categorised "parasitogenic anomalies" morphologically, including those that are indicated by mermithid nematodes, and accordingly introduced the following "mermithogenic" categories: **1.** "mermithergates" are infected individuals with worker-like appearance, having extremely reduced thoracic sclerites (Figs 2b-c); **2.** "gynaecoid mermithergate" is a so called "intermorphic" (Heinze, 1998) form, this category was introduced in order to describe individuals that possess both worker-like and gyne-like characteristics, e.g. that are wingless but have easily visible thoracic sclerites (Fig. 2d), well-developed ocelli etc.; **3.** "mermithogynes" are brachypterous or more rarely female individuals with normal wings but slightly reduced in size (this latter parasitogenic category is rather rare within *Myrmica* ants). Wheeler also introduced a "mermithogenic" category for males called "mermithaner".

Wheeler's parasitogenic categories are founded on analogies involving morphological similarities with uninfected individuals from the various castes (workers and gynes) and the explicit assumption that the anomalous individuals developed directly from the corresponding healthy caste by parasitogenic malformation. However, each alternative parasitogenic ("mermithergate", "gynaecoid mermithergate" and "mermithogyne") female phenotype of *Myrmica* ants bears both reduced, worker-like characteristics and gyne-like structures, i.e. relatively exaggerated traits, including the unusually well developed lateral ocelli and particularly the well separated thoracic sclerites (Figs 3b-d).

The very evident anomalies in morphological patterns prompted us to put an alternative explanation to the test that proposes that each nematode-induced female form of *Myrmica gallienii* ants derives from infections of queen-presumptive larvae.

In natural populations ant workers and queens often differ dramatically in their scaling relationships for the same trait (Nijhout and Wheeler, 1996; Molet et al., 2007). Observation of their allometric patterns has proven to be the standard tool in understanding the developmental patterns of alternative phenotypes (i.e. the switches in scaling) (Stern and Emlen, 1999). In order to clear up the origin of infected female individuals of *Myrmica*'s we were particularly interested to know whether infected phenotypes differ in their scaling from each other and seemingly anomalous exaggerated traits of infected individuals exhibited either gyne-like or worker-like allometric relationships.

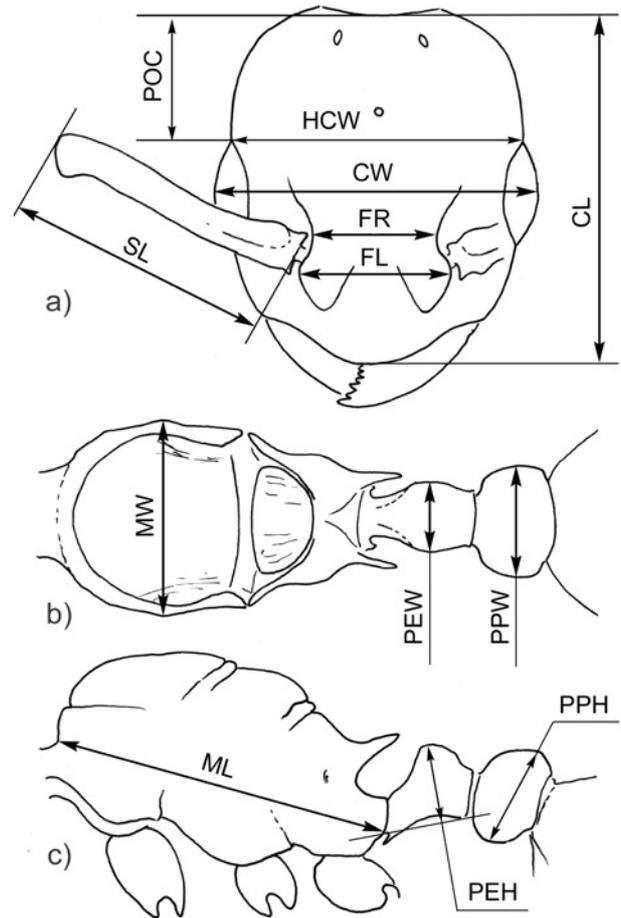
*Myrmica* males do not have morphological caste, hence males always exhibit linear scaling. Comparison of scaling of infected and uninfected males hereafter may serve as an additional test to claim whether or not nematode infection leads to shift in scaling.

This study provides important insight into mermithid parasitism and how it induces mermithogenic individuals in ants. We furthermore suggest that this natural experiment provides an exciting test of recent models for the developmental mechanisms generating trait allometries in insects (Shingleton et al., 2005; 2007), with the very important addition that parasite generated nutritional competition escalates during the pupa stage.

## Materials and methods

Altogether 176 *Myrmica gallienii* females (75 infected female individuals 29 uninfected gynes and 72 uninfected workers), and 42 *M. gallienii* males (12 infected and 30 uninfected males) of four nest samples were compared in statistical analyses.

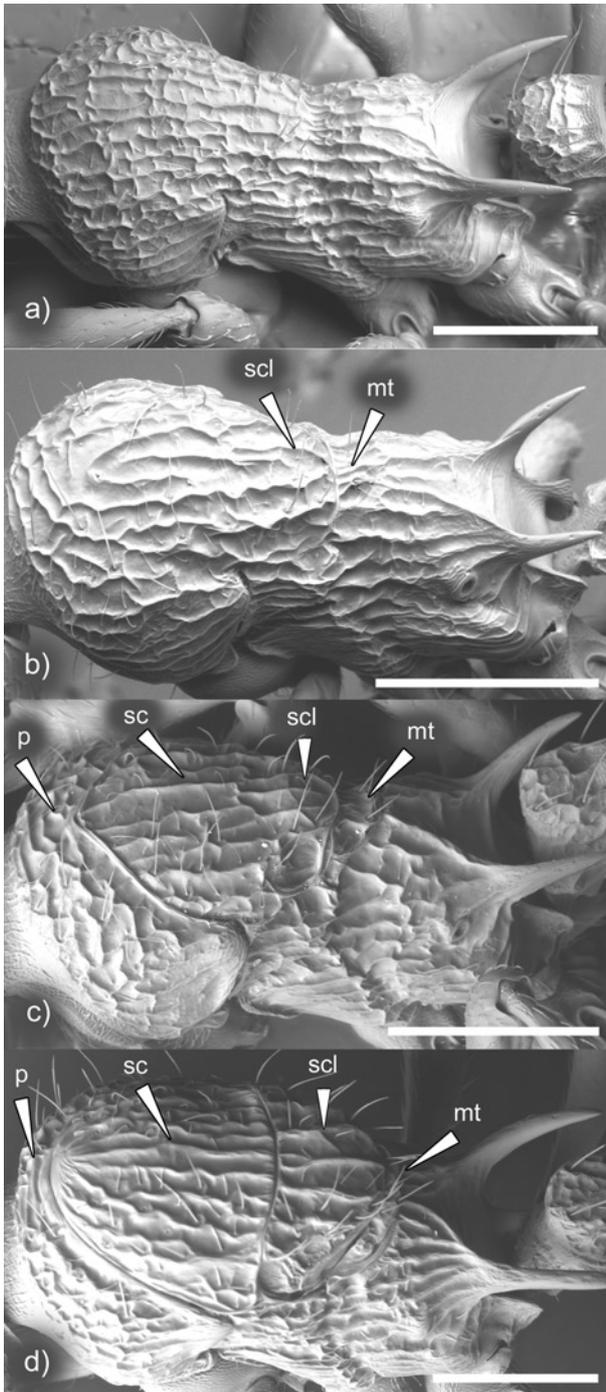
Nest samples were collected from a wet meadow near Kunpeszér (Central Hungary) in August of 2005 (one nest) and June to July of 2008 (three nests). The nests were close to each other: all of them were placed in a circle of 10 m diameter. The infected individuals were easy to recognize in field by their swollen gaster. In order to make sure that our original grouping (i.e. infected vs. uninfected) was correct gaster of each measured individual was dissected.



**Figure 1.** Trait measurements. Morphological characters measured are as follows. Head in full-face view (a). Cephalic length (CL) measured from the anteriormost point of median clypeal margin to the mid-point of the occipital margin. Concavity of occiput reduces CL. Cephalic width (CW) measured including the compound eyes. Head capsule width (HCW) measured just above the compound eyes. Post ocular distance (POC) measured from the reference line fitted just above the compound eyes to the mid-point of the occipital margin. Frons width (FR) measured the minimum distance between the frontal carinae. Frontal lobe width (FL) measured the maximum distance between external borders of the frontal lobes. Scape length (SL) measured from the neck to the distal end of the scape. Mesosoma, petiole and postpetiole in dorsal view (b). Maximum width of mesosoma (MW), petiole (PEW) and postpetiole (PPW) measured in dorsal view. Mesosoma, petiole and postpetiole in lateral view (c). Diagonal length of mesosoma (ML) measured in lateral view from the anteriormost point of the pronotal slope to the posterior (or postero-ventral) margin of the metapleural lobes. Petiole height (PEH) measured the maximum height of the petiole in lateral view. Postpetiole height (PPH) measured the maximum height of the postpetiole in lateral view.

Infected ants carried usually one, rarely 2, 3 or 4 living larvae of an unidentified mermithid species. All the mermithid larvae showed uniform appearance (i.e. their anatomical structures were identical) in each host but their size negatively correlated upon their quantity in a host. No other metazoan parasites were observed in the investigated specimens.

The traditional grouping of "mermithogenic" categories (i.e. the "mermithergates", the "intermorphic" "gynaecoid mermithergates" and the "gyne-like" "mermithogynes") proved to be less repeatable for statistical purpose. Hence we slightly modified the grouping of these "mermithogenic" categories based on clearly notable characteristics,



**Figure 2.** SEM pictures of the mesosoma of parasitogenic forms. Sclerites and sutures on the mesosoma of a worker (a), infected “mermithergate” (b), “gynaecoid mermithergate” (c) and “mermithogyne” (d) individuals of *Myrmica gallienii*. The mesosoma of an uninfected worker (a) is the less differentiated than each other form, where there are no discernable sclerites, articulations or sutures. Mesosoma of the “mermithergate” individual (b) is similar to that of a normal worker: the metanotum (mt) is reduced, but usually separated from the mesoscutellum (scl) and propodeum by weak sutures. The newly introduced *phenotype A* and *B* correspond to this category accordingly the presence or absence of ocelli. Well-developed “gynaecoid mermithergate” (c) and “mermithogyne” (d) correspond to the newly introduced *phenotype C*: the pronotum (p) is well separated by a movable articulation from the scutum (sc). Scutum and scutellum (scl), the scutellum and metanotum (mt) as well as the metanotum and propodeum are almost perfectly separated. Scale 500  $\mu\text{m}$ .

the presence/absence of ocelli and the discrete mesoscutum separated by well visible sutures and hereafter we call them *parasitogenic phenotypes (A,B,C)*. Ocelli are sometimes hardly discoverable, however using transmitting light these become well visible. The introduced parasitogenic phenotypes are as follows:

“*phenotype A*”: infected individuals with no ocelli and no discrete mesoscutum either (n = 29) (Fig. 2b);

“*phenotype B*”: infected individuals with ocelli, but without discrete mesoscutum (n = 31) (Fig. 2b);

“*phenotype C*”: individuals with both, ocelli and discrete mesoscutum (n = 15) (Figs 2c–d), this class comprised two completely winged individuals.

These *parasitogenic phenotypes* more or less correspond to Wheeler’s mermithogenic categories, the “mermithergates”, the “gynaecoid mermithergates” and the “mermithogyne” respectively. We also investigated infected *M. gallienii* males called “mermithaner” (n = 12). For purposes of comparison to uninfected phenotypes we measured random samples of uninfected *M. gallienii* workers (n = 72), uninfected gynes (n = 29) and uninfected males (n = 30) from the same pool of four nests.

### Morphometrics

All measurements were made with an ocular micrometer using an Olympus SZX9 stereomicroscope at a magnification of  $\times 150$ . Data are given in  $\mu\text{m}$ , estimated precision was  $\pm 2 \mu\text{m}$ . We tested the repeatability of measurements; all variables were measured twice for 14 randomly chosen ant individuals, the average measure of intraclass correlation coefficient (R) were calculated (Lessells and Boag, 1987). Each variable was highly repeatable (ranges:  $r = 0.953, 0.999$ ;  $F_{1, 13} = 21.385, 963.231$ , each  $P < 0.001$ ). Morphometric measurements (Fig. 1) were used as follows:

- Cephalic length (CL) measured from the anteriormost point of clypeal margin to the mid-point of the occipital margin, in full-face view. Concavity of occiput reduces CL;
- Cephalic with (CW) measured in full-face view, including compound eyes;
- Cephalic size (CS) calculated from the arithmetic mean of head length (CL) and head with (CW);
- Frons width (FR) measured the minimum distance between the frontal carinae;
- Frontal lobe width (FL) measured the maximum distance between external borders of the frontal lobes. This characteristic was not measurable on males;
- Head capsule width (HCW) measured maximum width of head capsule in full-face view just above compound eyes;
- Mesosoma length (ML) measured along a diagonal line in lateral view from the anteriormost point of the pronotal slope to the posterior (or postero-ventral) margin of the metapleural lobes;
- Mesosoma width (MW) measured in dorsal view;
- Petiole height (PEH) measured the maximum height of the petiole in lateral view;
- Petiole width (PEW) measured the maximum width of the petiole in dorsal view;
- Post ocular distance (POC) measured from the reference line fitted just above the compound eyes to the mid-point of the occipital margin.
- Postpetiole height (PPH) measured the maximum height of the postpetiole in lateral view;
- Postpetiole width (PPW) measured the maximum width of the postpetiole in dorsal view;
- Scape length (SL) measured from the neck to the distal end of the scape;
- Size of compound eyes (EYE) arithmetic mean of the minimum and the maximum diameter of the compound eyes.

### Regression analysis

Regression of each available character of parasitogenic phenotypes and uninfected castes were compared using Linear Model (lm) with R version 2.7.0. Twelve characters for female individuals and eleven characters for males were analyzed. Cephalic size (CS) is considered to be a fair indicator of overall body size in ants (Seifert, 2002; Csősz et al., 2007), hence (CS) was used as covariate. Parasitogenic phenotypes and uninfected individuals of females and males were compared in two independent analyses.

## Results

### Trait scaling of infected and uninfected males

Seven of eleven characteristics of infected males did not differ significantly from those of uninfected males in their effect (Table 1) and scaled according to the allometry of the latter. However, four characteristics, the mesosoma width (MW,  $t = 2.90$ ,  $p = 0.006$ ), the postpetiole width (PPW,  $t = -6.59$ ,  $p < 0.001$ ), the postpetiole height (PPH,  $t = -4.32$ ,  $p < 0.001$ ) and compound eyes (EYE,  $t = -4.34$ ,  $p < 0.001$ ) of infected males exhibited effects significantly different from the corresponding trait of uninfected males.

### Trait scaling of parasitogenic female phenotypes and uninfected females

Comparison of parasitogenic female phenotypes (*phenotypes A, B and C*).

Nine of twelve characteristics of parasitogenic phenotypes did not differ significantly from those of each other in their effect (Table 2). Two characteristics, the postpetiole width (PPW,  $t = -2.15$ ,  $p = 0.011$ ) and mesosoma length (ML,  $t = 2.07$ ,  $p = 0.04$ ) of *phenotype A* and postpetiole height (PPH,  $t = 2.88$ ,  $p = 0.045$ ) of *phenotype C* differed significantly from corresponding traits of the other parasitogenic female phenotypes in their effect.

### Comparison of parasitogenic female phenotypes and uninfected castes (workers and gynes).

Seven of twelve characteristics of parasitogenic phenotypes did not differ significantly from those of uninfected gynes in their effect (Table 2) and scaled according to gyne-like allometry. The remaining five characteristics, the scape length (SL,  $t = -7.03$ ,  $p < 0.001$ ), mesosoma width (MW,  $t = 19.86$ ,  $p < 0.001$ ), petiole width (PEW,  $t = 5.85$ ,  $p < 0.001$ ), mesosoma length (ML,  $t = 22.29$ ,  $p < 0.001$ ) and compound eyes (EYE,  $t = 9.24$ ,  $p < 0.001$ ) of parasitogenic phenotypes exhibited effects significantly different from the corresponding traits of uninfected gynes.

**Table 1.** Effect of parasitism on trait scaling of males. Intercepts of infected and uninfected males were compared using linear model. Upper row: effects and estimated standard errors of the scaling factors are shown in parentheses. Lower row:  $t$  and  $p$  values. Effect of uninfected individuals indicates the difference of their intercept from that of the infected individuals (left column),  $t$  and  $p$  values of infected individuals were redundant. Gray cells mark the effects significantly different from that of infected males.

| characters | Infected males   | Uninfected males                            |
|------------|------------------|---|
|            | (n = 12)         | (n = 30)                                    |
|            | Intercept        | Effect                                      |
| HCW        | -117.54 (50.29)  | -0.96 (7.06)<br>$t = -0.14$ , $p = 0.892$   |
| POC        | 8.81 (26.08)     | 2.87 (3.66)<br>$t = 0.79$ , $p = 0.437$     |
| FR         | -87.09 (31.71)   | 2.39 (4.37)<br>$t = 0.55$ , $p = 0.587$     |
| SL         | -152.82 (50.00)  | -5.21, (6.86)<br>$t = -0.76$ , $p = 0.452$  |
| MW         | -643.68 (181.21) | 72.50, (24.96)<br>$t = 2.90$ , $p = 0.006$  |
| PEW        | 55.36 (40.54)    | 2.12 (5.58)<br>$t = 0.38$ , $p = 0.706$     |
| PPW        | 96.50 (54.52)    | -49.51, (7.51)<br>$t = -6.59$ , $p < 0.001$ |
| ML         | 74.97 (190.64)   | 47.18 (26.26)<br>$t = 1.80$ , $p = 0.080$   |
| PEH        | 87.02 (49.04)    | -19.30 (6.76)<br>$t = -2.86$ , $p = 0.068$  |
| PPH        | 156.30 (78.00)   | -46.42 (10.75)<br>$t = -4.32$ , $p < 0.001$ |
| EYE        | 50.26 (27.68)    | -16.57 (3.81)<br>$t = -4.34$ , $p < 0.001$  |

However, ten of twelve characteristics of parasitogenic phenotypes differed significantly from those of uninfected workers in their effect (Table 2), only mesosoma width (MW,  $t = -1.90$ ,  $p = 0.059$ ) and mesosoma length (ML,  $t = -0.76$ ,  $p = 0.449$ ) of parasitogenic phenotypes scaled according to worker-like allometry.

## Discussion

In natural populations workers and gynes differ in their scaling relationships, made up of two discontinuous and discrete lines (Fig. 3c), indicating that workers and gynes exhibited different developmental pathways, hence observation of allometric relationships of different phenotypes has proven to be an appropriate tool in understanding their developmental patterns (Nijhout and Wheeler, 1996; Molet et al., 2007). *Myrmica* males do not have morphological caste, develop from the same type of larvae, hence males always exhibit linear scaling (Fig. 3a). Unsurprisingly, comparison of scaling of infected and uninfected males revealed that nematode infection did not lead to significant shift in scaling; infected

**Table 2.** Effect of parasitism on trait scaling of female classes. Intercepts of three parasitogenic phenotypes, uninfected workers and gynes were compared using linear model. Upper row: effects and estimated standard errors of the scaling factors are shown in parentheses. Lower row:  $t$  and  $p$  values. Effect of parasitogenic phenotypes and uninfected castes indicates the difference of their intercept from that of the *phenotype B* (center column),  $t$  and  $p$  values of *phenotype B* were redundant. Gray cells mark the effects significantly different from that of *phenotype B*.

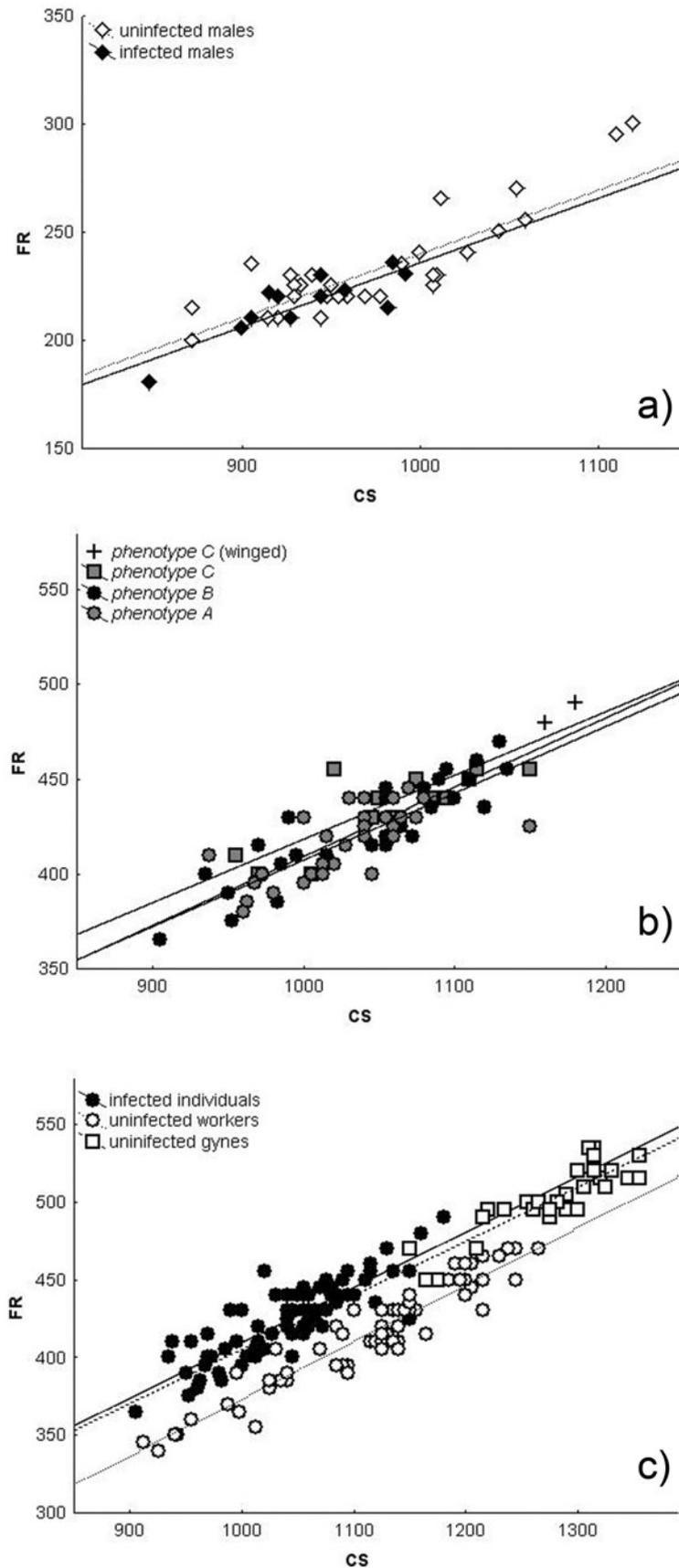
| characters | Uninfected workers                       | Classes of parasitogenic phenotypes     |                      |   | Uninfected gynes                         |
|------------|--|---|----------------------|---|--|
|            | (n = 72)                                 | phenotype A (n = 29)                    | phenotype B (n = 31) | phenotype C (n = 15)                    | (n = 29)                                 |
|            | Effect                                   | Effect                                  | Intercept            | Effect                                  | Effect                                   |
| HCW        | 16.20 (4.85)<br>$t = 3.34, p = 0.001$    | -4.90 (4.49)<br>$t = -1.09, p = 0.277$  | -55.52 (24.78)       | -7.75 (5.54)<br>$t = -1.40, p = 0.164$  | -3.90 (7.67)<br>$t = -0.51, p = 0.612$   |
| POC        | 16.71 (3.33)<br>$t = 5.03, p < 0.001$    | 1.54 (2.99)<br>$t = 0.52, p = 0.608$    | 19.59 (16.78)        | -7.10 (3.93)<br>$t = -1.81, p = 0.073$  | 1.51 (4.48)<br>$t = 0.34, p = 0.736$     |
| FR         | -31.61 (2.57)<br>$t = -10.92, p < 0.001$ | -2.95 (3.30)<br>$t = -0.89, p = 0.373$  | 73.73 (14.53)        | 5.95 (4.21)<br>$t = 1.41, p = 0.159$    | -2.09 (4.57)<br>$t = -0.46, p = 0.648$   |
| FL         | -21.32 (3.18)<br>$t = -6.71, p < 0.001$  | -5.14 (3.62)<br>$t = -1.42, p = 0.157$  | 65.31 (15.95)        | -4.07 (4.62)<br>$t = -0.88, p = 0.379$  | -3.58 (5.02)<br>$t = -0.71, p = 0.477$   |
| SL         | -12.11 (5.12)<br>$t = -2.37, p = 0.019$  | 6.69 (5.82)<br>$t = 1.15, p = 0.252$    | 180.89 (25.67)       | -13.07 (7.43)<br>$t = -1.76, p = 0.081$ | -56.75 (8.08)<br>$t = -7.03, p < 0.001$  |
| MW         | -10.03 (5.27)<br>$t = -1.90, p = 0.059$  | 0.44 (6.00)<br>$t = 0.07, p = 0.942$    | 6.90 (26.43)         | 5.49 (7.65)<br>$t = 0.72, p = 0.474$    | 165.14 (8.32)<br>$t = 19.86, p < 0.001$  |
| PEW        | -19.03 (3.50)<br>$t = -5.43, p < 0.001$  | -5.75 (3.99)<br>$t = -1.44, p = 0.151$  | 59.30 (17.57)        | 1.66 (5.09)<br>$t = 0.33, p = 0.745$    | 32.33 (5.53)<br>$t = 5.85, p < 0.001$    |
| PPW        | -51.23 (4.29)<br>$t = -9.86, p = 0.009$  | -12.69 (5.92)<br>$t = -2.15, p = 0.011$ | 56.28 (26.08)        | 13.26 (7.55)<br>$t = 1.76, p = 0.081$   | 21.31 (7.71)<br>$t = 2.83, p = 0.062$    |
| ML         | -5.49 (7.23)<br>$t = -0.76, p = 0.449$   | 17.04 (8.23)<br>$t = 2.07, p = 0.040$   | -65.04 (36.29)       | -3.72 (10.51)<br>$t = -0.35, p = 0.724$ | 254.51 (11.42)<br>$t = 22.29, p < 0.001$ |
| PEH        | -91.86 (35.42)<br>$t = -2.59, p < 0.001$ | 0.10 (3.49)<br>$t = 0.03, p = 0.977$    | 57.67 (15.39)        | 4.01 (4.46)<br>$t = 0.90, p = 0.370$    | 45.44 (4.84)<br>$t = 1.66, p = 0.098$    |
| PPH        | -56.07 (6.24)<br>$t = -8.99, p < 0.001$  | -6.64 (7.11)<br>$t = -0.94, p = 0.351$  | 89.79 (31.32)        | 26.09 (9.07)<br>$t = 2.88, p = 0.045$   | 26.44 (9.85)<br>$t = 2.68, p = 0.060$    |
| EYE        | -6.48 (2.29)<br>$t = -2.83, p = 0.005$   | -4.11 (2.26)<br>$t = -1.82, p = 0.072$  | 21.59 (12.38)        | -3.48 (2.89)<br>$t = -1.21, p = 0.230$  | 33.09 (3.58)<br>$t = 9.24, p < 0.001$    |

males scaled according to the basic allometry of uninfected males (Fig. 3a).

Parasitogenic female phenotypes (*phenotypes A, B* and *C*) of *Myrmica gallienii* did not differ from each other in their scaling. This finding led us to the conclusion that in fact each parasitogenic female individual developed from the same type of larvae. If each parasitogenic female phenotype develops from the same category of larvae, then what type of larvae is that?

Our results show that parasitogenic female phenotypes scaled according to gyne-like basic relationships. However, parasitogenic female phenotypes differed significantly from the corresponding traits of workers in their scaling, only two of twelve traits of parasitogenic individuals scaled according to worker-like allometry (Table 2, Fig. 3c). Based on the very evident patterns of trait allometries we raise an alternative hypothesis according to which each parasitogenic female phenotype of *Myrmica* ants develop from the same (queen-presumptive) category of larvae. The gyne-like scaling of the infected individuals and uninfected gynes indicate that parasitogenic phenotypes were trapped in gyne-like developmental pathway, but environmental factors might play a role in the formation of these parasitogenic

phenotypes. The most significant environmental factor in this case is the presence or absence of the mermithid parasite, which leads to the smaller individuals with often strikingly worker-like appearance. This new hypothesis (i.e. the solely queen-presumptive origin of parasitogenic female phenotypes) corresponds to the facts known the about life cycle of *Myrmica* ants. Life cycle of the mermithid nematodes and the host ant larvae is well synchronized (Kaiser, 1986). According to the collected material infected individuals appear together with the sexuals (June to August). *Myrmica* workers are continually produced throughout spring and summer, but late season (autumn) larvae may enter winter diapause (Bono and Herbers, 2003). These large larvae that overwinter maintain the capacity to develop into workers or gynes depending on environmental rearing conditions in early spring (Elmes, 1991). Under satisfactory nutritional status these overwintering female larvae are the queen-presumptive larvae. Male larvae develop simultaneously with queen-presumptive larvae. Since mermithid worms lay eggs in autumn these young queen-presumptive larvae and simultaneously developing male larvae have the chance to acquire the infective stages of the mermithids in late autumn or early spring.



**Figure 3.** Trait scaling of infected and uninfected individuals. **a.** Trait allometries of infected (black diamonds) and uninfected males (open diamonds) illustrated on frons width (FR), the head size (CS) as covariate. Infected males (solid regression line) and uninfected males (dotted regression line) scaled according to same slope and intercept ( $t = 0.55$ ,  $p = 0.587$ ). The similar scaling of the infected and uninfected males indicates that nematode infection did not lead to significant allometric shift. **b.** Trait allometries of three parasitogenic phenotypes, phenotype A (gray circles), phenotype B (black circles) phenotype C (gray rectangles) and winged individuals of phenotype C (black crosses) illustrated on frons width (FR), the head size (CS) as covariate. Each phenotype (solid regression lines) scaled according to same slope and intercept ( $t = -0.89$ ,  $p = 0.373$  and  $t = 1.41$ ,  $p = 0.159$ ). The similar scaling indicates that each parasitogenic phenotype was developed from the same type of larvae. **c.** Trait allometries of pooled sample of parasitogenic phenotypes (black circles), uninfected workers (open circles) and gynes (open rectangles) illustrated on frons width (FR), the head size (CS) as covariate. Solid regression line: infected individuals, dashed line: gynes, dotted line: workers. Intercept of infected individuals did not differ significantly from that of gynes ( $t = -0.46$ ,  $p = 0.648$ ), but differed significantly from that of workers ( $t = -10.92$ ,  $p < 0.001$ ).

The gyne-like slope and intercept of parasitogenic phenotypes indicate that infected individuals were trapped in gyne-like developmental pathway, but mermithid parasitism as an environmental factor played a role in the formation of these phenotypes.

This is the first study that raises the hypothesis that each parasitogenic female phenotype of *Myrmica* ants develop from the same (queen-presumptive) category of larvae. This new hypothesis can further explain the formation of anomalous scrambled traits, namely, that each alternative parasitogenic phenotype bears the combination of both worker-like and gyne-like structures (Figs 2b-d). These structures, which were to exhibit the gyne-like developmental pathway, did not degenerate at the reprogramming phase (Sameshima et al., 2004; Emlen and Allen, 2003), but unfortunate conditions that arose after initiating nematode development reduced their final size dramatically. This can be a reason why parasitogenic morphs often bear weak but nevertheless obviously existing gyne-like thoracic sclerites (Figs 2b-d), while workers never do (Fig. 2a).

Our results are relevant to prior works show, that environmentally induced developmental changes in the larval period cause correlated morphological changes in postmetamorphic individuals of ants (Peeters, 1991) and of other insects (Stern and Emlen, 1999; West-Eberhard, 2005; Gomez-Mestre and Buchholz, 2006). Nematodes may alter ant development in ways similar to late larval food shortage, which is also a key role in caste determination of *Myrmica* ants (Bono and Herbers, 2003; McGlynn and Owen, 2002; Wheeler, 1986) and in the expression of alternative morphologies in response to the feeding conditions of other insects (Moczek and Nijhout, 2002). Due to the novelty of the statement of this paper – thought it is supported by weight arguments and physiologically well explained – it might open a discussion whether or not only the nematode caused malnutrition effect, but other processes also play a key role in the expression of parasitogenic forms of *Myrmica gallienii*.

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