

## Letter

## Ant caste evo-devo: size predicts caste (almost) perfectly

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Under the standard model of ant caste development, a series of switch-like determination mechanisms cause larvae to follow distinct developmental trajectories. These mechanisms are believed to induce independent developmental programs in different larval populations, allowing each caste to evolve phenotypic adaptations that are not correlated with or constrained by other castes (Figure 1A). We proposed an alternative hourglass model, in which caste determination mechanisms induce multimodal distributions of late larval body size/mass. Caste differentiation, the neck of the hourglass, then coordinates the formation of adult phenotypes as a function of size or factor(s) tightly correlated with size. Therefore, differentiation is not independent in each caste, and castes can be arranged along a size axis with minimal overlap [1–3].

Abouheif argues, in contrast to the hourglass model, that castes are developmentally and evolutionarily independent [3]. Here we clarify why the hourglass model is compatible with available evidence and represents a testable alternative to the standard model. New data explicitly collected to distinguish the two models will inform this ongoing discussion.

**Response 1: thresholds in the hourglass and standard models**

Abouheif states that developmental thresholds for body size and tissue growth are incompatible with the hourglass model, but both types of thresholds can be depicted using this model. Regarding body size, early developmental influences that affect larval mass are defined as

caste determination mechanisms (green function in Figure 1A of [2]). Caste determination culminates in discontinuous (or multimodal) outputs that are robust to environmental variation. For example, larger colonies of many species produce larger representatives of each caste without producing caste size overlaps or inter-castes (Figure 1B) [4]. This outcome might result from step-like hormonal responses to environmental inputs and is compatible with both models.

Thresholds that regulate tissue growth are also consistent with the hourglass model. These are depicted as tissue-specific responses to caste differentiation (blue function in Figure 1A of [2]). We agree with Abouheif that the association of tissue growth and body size can be step-like and even nonmonotonic (see soldier head size in Figure 1C). For noisy continuous traits, *y*-axis values on the caste reaction norm represent the mean trait value within a narrow size range, but for step-like (or discrete) traits, they could instead depict the probability of expressing either state of a binary trait.

**Response 2: soldier development and evolution**

Abouheif provides examples of size overlap between workers and soldiers and argues that size and morphology are decoupled (Figure 1D,E; Figure 1C,E in [3]). We agree that the correlation between size and caste differentiation is not perfect. We hypothesize that caste morphology is coordinated by an uncharacterized differentiation factor, such as a developmental hormone, whose activity varies with size. For example, caste differentiation could result from a circulating growth inhibitor that has a higher concentration in smaller larvae and suppresses ovary, eye, and wing morphogenesis in a dose-dependent manner [1,2].

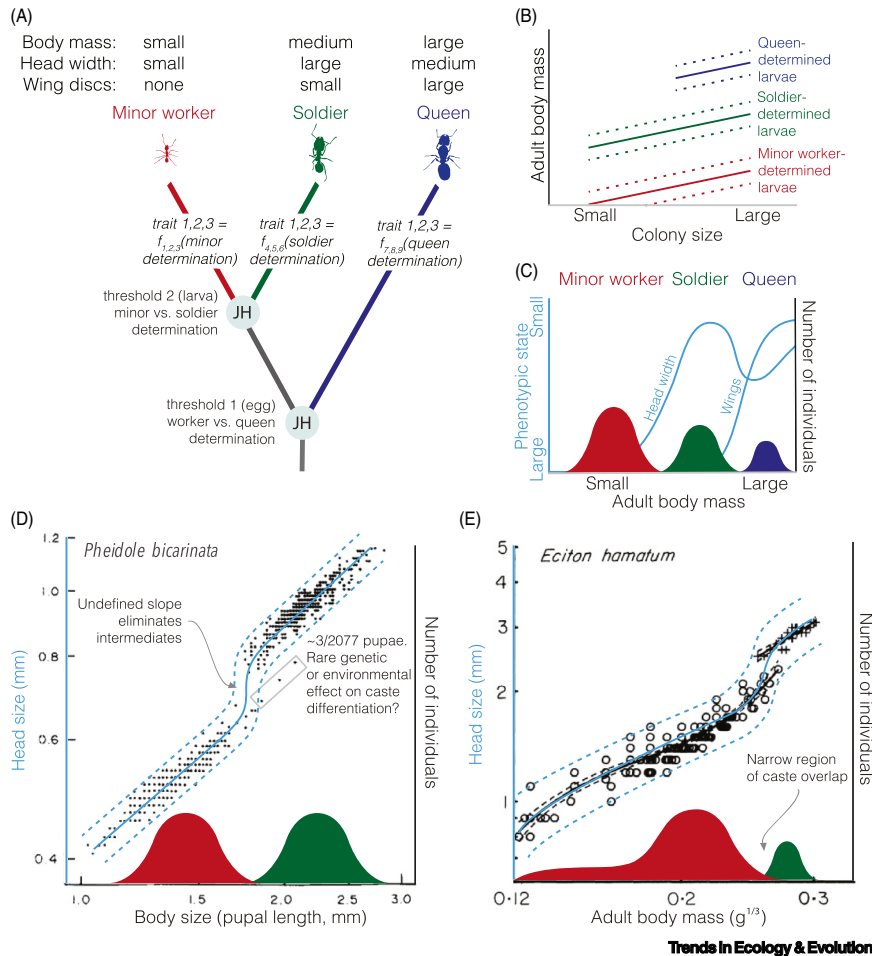
This can be tested by combining mathematical modeling with morphometric data. If caste differentiation is a function

of size, then it should be possible to describe minor workers, soldiers, and queens using distinct intervals of a single dimension, and rare or limited caste overlap would represent noise or genetic variation in caste thresholds (Figure 1C–E; Figure 1F in [2]). If caste differentiation and size are independent functions of caste determination, then the data will be best explained using one morphological dimension for each branching point (Figure 1A).

Abouheif argues that soldiers evolved from workers, citing evidence that soldier determination happens after worker determination [3]. We agree that soldier evolution involved an elaboration of worker determination mechanisms, but this poses a challenge to the standard model: if queen-like traits are a function of early queen determination mechanisms rather than late larval mass (Figure 1A), then why would soldiers have partially queen-like wing development [5]? Within the soldier caste, why would larger individuals possess ocelli that smaller individuals lack [6]? In the hourglass model, an increase in size can induce partially queen-like differentiation in soldiers, even if they arise from worker-determined larvae (Figure 1C; Figure 1F in [2]). A decrease in size of queen-determined larvae can explain the wingless queens of many species (Figure 1D in [2]). These and other observations motivated our hypothesis that caste differentiation does not arise directly from previously described caste determination mechanisms like juvenile hormone thresholds (Figure 1A) [7]. Instead, the intermediate traits of intermediate-sized adults raise the testable prediction that adult morphology is more strongly correlated with late larval mass (and its molecular correlates) than earlier developmental events [8].

**Response 3: size overlaps between workers and queens**

Abouheif argues that queens can be smaller than workers and cites data from



**Figure 1.** (A) Standard model for caste development in *Pheidole*. Here, traits 1, 2, and 3 correspond to adult body mass, adult head width, and larval wing disc size, respectively. Under the standard model, determination of each caste regulates both body size and caste morphology independently. Each caste determination event therefore produces a new dimension of adult morphological variation. In the hourglass model (Figure 1A in [2]), caste determination mechanisms have an identical effect on late-larval body mass. However, adult morphology is a function of body mass, not early caste determination mechanisms directly. (B) Schematic relationship of adult body mass and colony size in *Pheidole*. Following early caste determination mechanisms, larvae on minor worker-, soldier-, and queen-developmental trajectories respond to the same social environment by becoming differently sized adults. Larger colonies produce larger minor workers and soldiers (e.g., [4]), likely due to increasing colony nutrition, and this likely also applies to queens, which are not produced by small colonies. Solid and broken lines give hypothetical mean and range, respectively. (C) Caste reaction norm for data in A and B. Head width is not a monotonic function of body mass, causing large soldiers to have bigger heads than small queens [5]. Such a relationship could arise from trade-offs in resource allocation, in this case between head and wing growth [1]. (D) Annotated *Pheidole bicarinata* data [7]. Multimodal size distributions with step-like trait allometry can generate discrete castes without invoking independent developmental programs. This plot shows 2077 individuals pooled across multiple experimental treatments [7], and the specific origins of rare soldier-sized minor workers (gray box) were not reported. Such perturbations, if replicated, could be used to identify molecular caste differentiation mechanisms. (E) Annotated *Eciton hamatum* data [12]. Worker polymorphism is more complex in *Eciton* than in *Pheidole*, but still compatible with the hourglass model. In (A) JH refers to juvenile hormone. In D and E, the left y-axis refers to the original data plot, and the right y-axis refers to the size distribution cartoon. Solid and broken lines give approximate mean and range, respectively.

*Mystrium* and *Brachyponera luteipes*. Like *Mystrium* reproductives are not queen- like in the morphological sense [1,9]. Whether *B. luteipes* winged queens are smaller than workers in late larval mass,

or even adult body mass, has not been reported, to our knowledge [10]. Many ants appear to have experienced selection for reduced worker–queen dimorphism. We hypothesized previously that in most cases selection allows winged queens to become as small as the largest workers, on average, but not smaller [1].

However, it is also possible that some ants have evolved secondary changes to caste differentiation and no longer follow the caste reaction norm. Based on existing evidence, this appears to be rare, but it could have occurred in some clades. For instance, winged queens of the *Formica difficilis* group are unambiguously smaller than workers [11]. One exciting possibility is that they exhibit ‘genetic caste differentiation.’ Regardless of whether they have a plastic or genetic origin, these miniscule queens provide a promising opportunity to study caste differentiation mechanisms in a comparative framework.

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