Trends in
Ecology & Evolution

CellPress

Forum

Hourglass Model for Developmental Evolution of Ant Castes

Waring Trible [1,*] and
Daniel J.C. Kronauer[2]

Classic models for the development and evolution of ant castes struggle to explain recent empirical results. Here, we propose an hourglass model compatible with all existing data, providing a formal, falsifiable framework for future study. This illustrates how phenotypic variation can be used to infer underlying developmental and genetic architecture.

The Diversity of Caste Morphology

When a biological network has a diverse array of inputs and outputs, it may seem impossible to attain a simple mechanistic description of the system. For example, the ants in a colony can be divided into multiple sets of distinct adult females, or **morphological castes** (see Glossary), such as workers, soldiers, and queens (Figure 1). Caste morphology is diverse both within and between ant (Formicidae) species, and a range of environmental and genetic factors influence the caste fate of developing larvae [1,3,4].

Existing models account for this complexity by assuming that each caste is induced by an independent developmental pathway that can diverge arbitrarily during evolution (Box 1). We propose that caste diversity arises from a simpler and more constrained developmental-genetic architecture, with a range of inputs feeding into a central integrating mechanism that produces diverse phenotypic outputs (Figure 1A). From this realization follows an explicit framework for the study of caste development and evolution.

Empirical Hallmarks of Hourglass Architecture

Hourglass network architecture is expected to evolve under conditions of phenotype switching, when distinct, partially interchangeable inputs are integrated to produce one of several complex outputs [5]. Under such an architecture, the central integration mechanism is a crucial feature of the network, the identification of which opens avenues for understanding how inputs are converted into outputs. This approach has been successful across a range of systems, including cell type differentiation, sex development, and metabolism [5,6]. Before arguing that caste development in ants has an hourglass architecture, we use sex development to illustrate the patterns of phenotypic variation that these types of network produce.

Males and females are homologous across the insects and differ from each other in many traits, including reproductive apparatus, behavior, and external morphology. The complexity of sexual dimorphism appears incongruent with the fact that male/female development can be induced by distinct sex **determination mechanisms**, including various chromosomal configurations, bacterial infection, temperature, and nutrition [7]. To take an example from butterflies, how is it possible that sex determination mechanisms as distinct as W chromosomes and *Wolbachia* infection induce an identical suite of adult female traits in the same species [7,8]? The answer is that variable determination mechanisms act as upstream switches that modulate the activity of a set of virtually invariant sex **differentiation mechanisms**, including the gene products of *doublesex* and *transformer* [7,8]. These molecules are then responsible for producing the correlated suite of male or female **phenotypic outputs**. When a system has an hourglass-shaped regulatory architecture, evolutionary or developmental perturbations at or upstream of the differentiation factors are capable of shifting all

Glossary

Caste-associated traits: phenotypic traits that distinguish the morphological castes of ants. Workers and queens are distinguished primarily by the growth of the reproductive, visual, and wing tissues. Soldiers typically have enlarged heads or mandibles compared with workers and queens.

Determination mechanism: an intrinsic or extrinsic factor that biases a cell or individual toward a particular state of differentiation. Caste determination mechanisms often produce multimodal distributions of larval body mass.

Differentiation mechanism: a factor downstream of determination that induces the formation of output phenotypes. In an hourglass network, the central differentiation mechanism, or factor, operates at the integration layer, downstream of determination but upstream of tissue-specific morphogenesis.

Hourglass network architecture: one of multiple common network motifs. The width of the hourglass refers to the number of parallel developmental/genetic pathways at the input, integrator, and output layers of the network. Inputs, or determination mechanisms, initially affect caste development partially or entirely independently of each other, but their effect on caste development is coordinated because they all modulate the same downstream integrator, or differentiation mechanism. This integrator then regulates the growth of many cells and tissues, thereby affecting many partially or entirely independent pathways. Hourglass networks are also referred to as bowtie networks [5].

Imaginal discs: clusters of cells in insect larvae that develop into particular adult tissues, such as eyes, legs, and wings, during metamorphosis. These discs proliferate and differentiate into adult tissues during the late larval and prepupal stages. Ant castes vary in the differentiation of imaginal discs, many of which exhibit suppressed growth in small-bodied individuals.

Juvenile hormone: an essential insect hormone. Application of juvenile hormone to late-instar larvae can prolong larval development and increase the mass at pupation in a range of holometabolous insects [1]. In ants, juvenile hormone application can cause worker-destined larvae to develop into soldiers or queens [1,9,10].

Morphological castes: sets of adult ants with distinct morphological traits. Ants ancestrally have a worker and a queen caste, but some species have evolved novel castes, such as soldiers and wingless queens [1,3]. Other species have evolved simplified caste systems, resulting in workerless social parasites and queenless ants [1,12,13].

Parallel evolution: independent evolution of highly similar phenotypic traits in multiple species. Unlike for convergent evolution, these traits hypothetically arise from a similar change to the same ancestral mechanism.

Phenotypic output: an organismal phenotype that results from the activity of the hourglass network. This might include traits such as behavior, metabolism, morphology, and physiology.

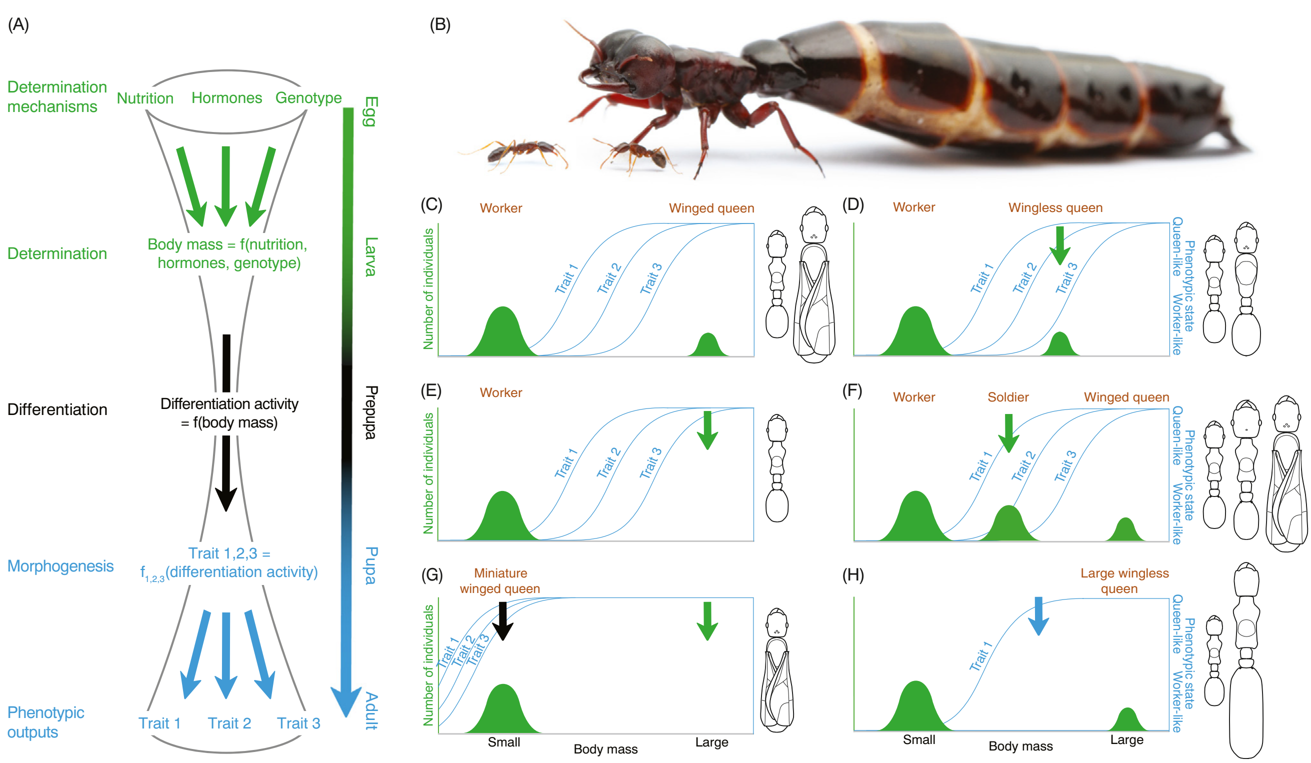
phenotypic outputs in tandem, such as moving individuals along the male–female axis, whereas perturbations downstream of the differentiation factors affect a subset of phenotypic outputs, such as modulating male- or female-limited traits [7].

The Morphological Castes of Ants

A vast set of interconnected variables are known to affect adult body size in insects. These include developmental hormones, temperature, nutrition, wounding, parasite infection, maternal effects, and hybridization. Perturbations that affect overall size also affect **caste-associated traits** in ants in a coordinated and stereotyped manner: as in sex development, distinct perturbations produce indistinguishable output phenotypes [1] (Figure 1A). Therefore, these inputs can be considered as caste determination mechanisms.

A few recent studies highlight the input end of the caste hourglass. One species of desert ant, *Cataglyphis mauritanica*, has a strong genetic bias to caste development such that, under normal circumstances, certain genotypes always develop into large queens, while others always develop into small workers. However, **juvenile hormone** treatment causes worker-destined genotypes to develop into large queens, and queen-destined genotypes develop into small workers if reared in small colonies, where the larvae are likely starved [9]. These findings imply that the

Reaction norm: depicts how environmental variation affects a phenotypic measure in a particular genotype. The caste reaction norm depicts the relationship between body mass and caste-associated traits in ants [1,4].
Social parasites: in ants, these are species that exploit the colonies of other ant species as an obligate component of their life cycle. Many socially parasitic ants, especially workerless social parasites, have queens that are less massive than those of their closest free-living relatives. Small queens arise via mutations that lower the body mass threshold for the attainment of queen-like morphological traits [1,12].



Trends in Ecology & Evolution

Figure 1. Caste Evolution. (A) Hourglass model of caste development. Caste determination operates during larval development to produce variation, often multimodal, in body mass and associated factors (green). At the onset of metamorphosis, individuals of varying mass have varying levels of caste differentiation activity (black). Each tissue responds with a particular degree of worker-like or queen-like morphogenesis (blue). (B) Queen and two small workers of the army ant *Dorylus molestus*. Unlike most ant queens, army ant queens lack wings and eyes, but have extraordinarily large ovaries. (C) Caste reaction norm and cartoons of adult phenotypes in the ancestral worker/queen system. (D–H) Five types of derived caste system. Arrow positions indicate changes relative to the ancestral state in (C); green, black, and blue arrows denote changes that affect determination, differentiation, and morphogenesis, respectively. (D) Worker/wingless queen. (E) Worker only. (F) Worker/soldier/winged queen. (G) Miniature winged queen only (workerless social parasite). (H) Worker/large wingless queen; this caste system is represented by the army ants in (B). Here, traits 1, 2, and 3 refer to the reproductive, visual, and flight system, respectively, measured relative to the state in the queen caste [1]. In principle, however, this model can be applied to any phenotypic trait that varies along the worker-queen axis. Images from [2].

Box 1. The New Hourglass Model versus the Bifurcating Developmental Program Model

In classic models of caste development, each caste originates from partially or entirely independent developmental programs. For instance, caste development in *Pheidole* is depicted as a series of bifurcations: early determination events split larvae into worker-, soldier- and queen-destined populations [10]. Soldier determination was recently proposed to induce a novel rudimentary wing disc, which then induces soldier-like head growth and body size [10]. Under this model, soldier evolution is not constrained: if the rudimentary wing disc induces a unique program of soldier differentiation, it could evolve to induce many combinations of traits without interference or trade-offs between castes [3,10]. Unconstrained caste-specific differentiation programs are central to many prior models of caste development and evolution [3,10–12].

In our hourglass model, multiple determination mechanisms converge on a 1D axis of larval body mass, which, directly or indirectly, results in a 1D axis of caste differentiation at the onset of metamorphosis [1] (Figure 1A). Thus, soldiers are not derived from a novel soldier-specific developmental program, but are instead modified worker–queen intermediates [3]. This differs from bifurcating developmental program models, which predict that castes will not exhibit strict trade-offs but instead have independent dimensions of developmental and, therefore, evolutionary variation.

genetic caste bias affects caste determination and does not entail loss or modification of the downstream caste differentiation mechanisms (Figure 1A). Another study found that removal of the larval wing **imaginal discs** produced worker–soldier intermediates in *Pheidole hyatti* [10]. Such intermediates do not typically occur in *Pheidole*, but resemble worker–soldier intermediates in other species of ant. These results support the growing consensus that changes to caste determination can fill in unoccupied regions of the caste **reaction norm** and might underpin the **parallel evolution** of modified caste systems [10,11] (Figure 1D–F). In both cases, the applied experimental perturbations were not found to alter the relationship between body size and caste morphology.

Caste differentiation operates at the integration layer of the caste hourglass and is downstream of caste determination (Figure 1A; Box 1). Put differently, caste differentiation creates a reaction norm of body mass and phenotypic outputs. Unlike caste determination, no experimental perturbations affecting caste differentiation have been reported. However, it is clear that differentiation is distinct from determination, because genetic variation between populations or species can affect the differentiation process without influencing determination [11]. Clear examples come from ant species, often **social parasites** that exploit other ants, that express queen-like morphological traits at worker or soldier-like body sizes [12] (Figure 1G). For instance, species of the parasitic *Pheidole lucida* group exploit the colonies of other *Pheidole* spp. [13]. In most *P. lucida* group species, the soldier caste has been lost and replaced with miniature, soldier-sized queens. Thus, a reduction in the size at which larvae undergo queen-like differentiation reveals a trade-off between queen size and soldier size [11–13]. As further support for the hourglass model, one undescribed species in this group, *P.* gf010, has queens the size of regular workers and has lost the worker caste entirely [13] (Figure 1G).

In principle, mutations acting downstream of caste differentiation could affect some caste-associated traits but not others. Such mutations allow one caste to 'borrow' traits from another, analogous to the gain, loss, and exaggeration observed in the evolution of sex-limited traits [7]. For example, the unusual queens of army ants (Figure 1B) may have originated in part via mutations downstream of caste differentiation that cause large individuals to lack wings and eyes, but nevertheless develop an exaggerated reproductive system (Figure 1H).

Empirical Approaches to Test and Elaborate the Hourglass Model

The hourglass model predicts that caste morphology in each species can be described along a 1D worker–queen axis. This can help explain the extreme parallel evolution of ant castes. Novel intermediates, such as soldiers, super-soldiers, and wingless queens, do not reflect a derived genus- or species-specific developmental program (Box 1), but an elaboration of intercaste morphology that can arise in any lineage [1,3,4]. Such worker–queen intermediates should on average always be lighter in body mass than queens at the onset of caste differentiation, even if they exceed queens in some traits, such as head width.

Determination mechanisms should carry no information for caste development other than their effect on differentiation. Therefore, within the same genotype, manipulations of nutrition, hormones, or generally the larval rearing environment should all reproduce the same caste reaction norm. Another implication is that, when a shift to a new position along the reaction norm confers an adaptive trait, such as an enlarged soldier head, non-adaptive traits, such as wing buds or eye development, might phenotypically hitchhike. This constitutes an alternative to previous interpretations [10].

It should also be possible to map genetic elements that affect each layer of the hourglass. To understand determination, one could compare species or genotypes with small wingless queens to closely related species or genotypes with winged queens. Looking for caste-biasing factors that operate on embryos, including maternal effect factors or genetic elements that bias body size (genetic caste determination), would be another approach.

Differentiation can be understood by comparing species or genotypes that vary in the body mass at which caste-associated traits are attained. We have discussed workerless social parasites, but this approach could also be used to identify quantitative loci that mediate variation in caste thresholds [11,12]. Studying development and looking for a receptor or pathway enriched in cells that actively differentiate in a worker- or queen-like manner is another promising avenue.

[1]John Harvard Distinguished Science Fellowship Program, Harvard University, Cambridge, MA, USA
[2]Laboratory of Social Evolution and Behavior, The Rockefeller University, New York, NY, USA

*Correspondence:
bucktrible@fas.harvard.edu (W. Trible).

https://doi.org/10.1016/j.tree.2020.11.010

References

1. Trible, W. and Kronauer, D.J.C. (2017) Caste development and evolution in ants: it's all about size. *J. Exp. Biol.* 220, 53–62
2. Kronauer, D.J.C. (2020) *Army Ants: Nature's Ultimate Social Hunters*, Harvard University Press
3. Molet, M. *et al.* (2012) Evolution of novel mosaic castes in ants: modularity, phenotypic plasticity, and colonial buffering. *Am. Nat.* 180, 328–341
4. Londe, S. *et al.* (2015) Phenotypic plasticity and modularity allow for the production of novel mosaic phenotypes in ants. *Evodevo* 6, 36
5. Friedlander, T. *et al.* (2015) Evolution of bow-tie architectures in biology. *PLoS Comput. Biol.* 11, 1–19
6. Wagner, G.P. (2014) *Homology, Genes, and Evolutionary Innovation*, Princeton University Press
7. Verhulst, E.C. and Van de Zande, L. (2015) Double nexus-*Doublesex* is the connecting element in sex determination. *Brief. Funct. Genomics* 14, 396–406
8. Traut, W. *et al.* (2008) Sex chromosomes and sex determination in Lepidoptera. *Sex. Dev.* 1, 332–346
9. Kuhn, A. *et al.* (2018) Phenotypic plasticity in an ant with strong caste – genotype association. *Biol. Lett.* 14, 20170705
10. Rajakumar, R. *et al.* (2018) Social regulation of a rudimentary organ generates complex worker-caste systems in ants. *Nature* 562, 574–577
11. McGlynn, T.P. *et al.* (2012) Tradeoffs in the evolution of caste and body size in the hyperdiverse ant genus *Pheidole*. *PLoS ONE* 7, e48202
12. Nonacs, P. and Tobin, J.E. (1992) Selfish larvae: development and the evolution of parasitic behavior in the Hymenoptera. *Evolution* 46, 1605–1620
13. Fischer, G. *et al.* (2020) Socially parasitic ants evolve a mosaic of host-matching and parasitic morphological traits. *Curr. Biol.* 30, 3639–3646