

RESEARCH HIGHLIGHT

Old soldiers never die

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Abstract

An ancestral supersoldier phenotype of *Pheidole* ants can be recovered when selection for supersoldiers re-emerges, indicating that the developmental potential for caste pathways is retained.

There is something truly intriguing about soldiers, individuals that specialize in the defense of the society they are part of. Small societies do not have specialized soldiers, because their enemies are invariably stronger, so keeping a low profile after abandoning camp is likely to be a survival strategy that is superior to fighting. Standing armies tend to arise when states become large and stratified, so that cities and territories are vast and precious enough to justify the expenses incurred. This logic appears to apply also in the advanced insect societies, in which most individuals are sterile workers that have lost the ability to reproduce. Ants are more likely to have a specialized soldier caste when they have large nests in the middle of defendable territories [1]. This so-called central-place foraging closely resembles the classic human city states, which originated as walled fortresses surrounded by their hinterlands [2].

The same logic seems to explain why advanced social wasps and bees have only normal workers and no specialized soldier-morphs. Their workers are powerful defenders by default as they have both wings and stings and they operate in overlapping three-dimensional territories where competitors may dilute food intake but rarely eliminate neighboring colonies. The very first bee species that has a specialized soldier caste has just been discovered [3], an exception that proves the rule because it has an unusually large robber-bee enemy that can exterminate entire colonies. Thus, the costs and benefits of having a standing army seem straightforward, but the set of mechanisms by which individuals commit to a soldier career is poorly understood. Ant soldier caste

differentiation is molded by natural selection at the colony level, but realized by individual development pathways that have rarely been studied in detail. A recent article by Rajakumar *et al.* [4] sheds intriguing light on the molecular mechanisms of caste development, and on the evolutionary persistence of these pathways in lineages that no longer use them.

(Re-)evolving supersoldier ants

Rajakumar *et al.* [4] focus on one of the most speciose genera of ants, *Pheidole*, which has approximately 1,100 described species worldwide and is generally characterized by caste polymorphism. Colonies have minor workers, much larger queens and a moderate number of major workers or soldiers of intermediate size [5]. As the individual ants in a colony are normally close relatives, these highly divergent female phenotypes are mostly shaped by developmental differences, controlled to a large extent by larval nutritional status and juvenile hormone (JH) signaling. High JH titers during a crucial developmental checkpoint will determine whether an individual will become a queen phenotype, whereas low JH will induce a worker phenotype. A second checkpoint determines whether a non-queen larva will develop into a soldier or minor worker [4]. Quite peculiarly, however, eight species of *Pheidole* from desert habitats in the south-western USA and north of Mexico are known to have an extra supersoldier caste (Figure 1). In a wild colony of *Pheidole morrisoni*, a species that does not naturally produce supersoldiers, Rajakumar *et al.* [4] observed oversized soldiers that resembled supersoldiers, prompting these authors to determine whether such individuals shared developmental pathways with the true supersoldiers found in other *Pheidole* species.

Surprisingly, a phylogeny of eleven *Pheidole* species revealed that one of the two species that produces supersoldiers (*Pheidole rhea*) belongs to a basal lineage, whereas the other (*Pheidole obtusospinosa*) belongs to an evolutionarily derived clade. The standard explanation for such a pattern is that supersoldiers evolved twice, independently, but this appeared not to be the case. In a series of elegant experiments, Rajakumar and colleagues showed that extra application of a JH analogue before the checkpoint that differentiates minor workers from soldiers induced a supersoldier-like phenotype in species

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Figure 1. Three worker castes of *Pheidole* ants. Minor and soldier worker castes are found in all *Pheidole* species, but supersoldiers (bottom right) are only found in some species, where they function as defenders or specialize in other distinct tasks such as seed milling, as in *Pheidole tepicana* shown here. Rajakumar *et al.* [4] have shown that supersoldier-like phenotypes can be induced by applications of juvenile hormone in *Pheidole* species that do not normally have this caste, implying that the underlying developmental potential is conserved. Photo © Alexander Wild, used with permission.

that normally do not have supersoldiers. This implies that the genetic pathways needed to express this unusual phenotype have remained intact, even though supersoldiers were apparently not produced for millions of years.

Supersoldiers seem most likely to (re-)evolve in those *Pheidole* species that are under continuous risk of annihilation by raiding army ants [4]. In these situations, supersoldiers are perfect defenders as they block the nest entrance with their big heads. By applying JH to larvae of *P. obtusospinosa* beyond the developmental checkpoint at which minor workers and soldiers are differentiated, Rajakumar *et al.* were also able to show that the developmental switch towards supersoldiers is determined by a third JH-sensitive checkpoint. These results are consistent with the generally accepted model of caste differentiation in ants, which hypothesizes that each caste phenotype has a size checkpoint at some stage of larval growth and an associated developmental program [6].

Rajakumar *et al.* [4] suggest that the *P. obtusospinosa* supersoldier is an example of genetic accommodation: the induction of a novel phenotype from existing genetic variation that was subsequently made permanent by selection at regulating genes. With pathway dormancy going back to a remote ancestor, the development of the new phenotype could even be an atavism, defined as the relatively rapid retrieval of a complex trait. Skeptics would argue, however, that as long as the expression of the smallest (minor worker) and largest (queen) phenotypes are maintained throughout an ant clade, the pathways enabling the existence of a large spectrum of

alternative intermediate phenotypes through pleiotropic effects will necessarily have to be maintained. Comparative studies across ant species have confirmed this notion by showing that size dimorphism between the largest (queen) caste and the smallest worker caste is a good predictor of the likelihood of intermediate castes to arise [1].

To make an atavistic explanation fully credible, evidence for the recurrent emergence of wider extremes (superqueens or mini-minor workers) would be required. Such pathway extensions would be much more likely to become irreversibly eroded when no longer in use, but they do not seem to have happened. Still, Rajakumar *et al.* present an important example of how the interplay between conserved genetic networks and environmental pressure can recurrently produce phenotypic variation that natural selection can work on.

Future perspectives

As incisive studies can be expected to do, the work of Rajakumar *et al.* [4] raises a series of novel questions that are deserving of further research. First, it would be interesting to see these analyses in a greater diversity of *Pheidole* species; this would improve phylogenetic taxon sampling and provide a better understanding of the variation in checkpoint developmental thresholds, which do not seem to be simple qualitative on/off switches [4]. Second, more precise data on the task spectrum of soldiers and supersoldiers across *Pheidole* species would help us to understand how to rank their functional significance as defenders and seed grinders. It seems possible that some soldiers from dimorphic *Pheidole* species are developmentally closer to the supersoldiers than the soldiers of trimorphic species. A more comprehensive molecular phylogeny than that achieved by Rajakumar *et al.* might even show that the supersoldier phenotype evolved before the soldier phenotype [7]. Third, it would be intriguing to know how much genetic variation occurs within these caste-development switches among colonies or populations of the same species, and how this variation affects growth rates when it interacts with larval JH titers. Such variation has been linked to the emergence of socially parasitic caste phenotypes in *Acromyrmex*, a genus of leaf-cutting ants that belongs to the same ant Subfamily as *Pheidole* [8].

Now that reference genomes of non-model eusocial organisms such as ants, bees, wasps and termites are becoming available, it will be increasingly feasible to study caste development at an -omics scale. For example, a recent study by Toth *et al.* [9] showed that gene expression patterns in the brains of worker and breeder wasps differ in ways that indicate that worker behavior is derived from maternal care. Similar high-throughput approaches also offer new and exciting avenues for

studying how castes have become phenotypically distinct, and extensive morphological caste differentiation makes ants ideal model systems for such work. The technology now available might soon make it possible to address meta-questions such as whether an unusually persistent but malleable set of developmental pathways could have helped a genus such as *Pheidole* to become extremely species-rich. Indeed, seven ant genomes have already been published, and the first comparative analyses are underway [10]. The increasing availability of such genomic reference data will reinforce research addressing evolutionary questions similar to those explored in the pioneering study of Rajakumar *et al.* [4].

Concluding remarks

The re-emergence of supersoldiers underscores the interesting evolutionary complexities of phenotypic plasticity in general and of caste-determination in particular. The finding that this trait can also be hormonally induced elegantly shows that alternative pathways of development are likely to be maintained by a set of switches and JH titers that may well apply generally, throughout the ants and beyond, consistent with earlier hypotheses and empirical studies (such as [6]). A more encompassing understanding is needed to address variation in the developmental checkpoints that determine caste, to link this variation to structural and regulatory genes, and to interpret the likely selection forces that lead to the expression of specialized phenotypes.

Even over evolutionary time, it is apparently difficult to make developmental pathways disappear when slightly different versions remain of vital importance. As General Douglas MacArthur remarked in his 1951 farewell address to the US Congress, after being dismissed for

supersoldier-like tendencies: 'Old soldiers never die, they just fade away.'

Abbreviation

JH, juvenile hormone.

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