other members of this subcomplex seems weaker than the association between these other subcomplex I subunits. However, Sec3 is indispensable for the assembly of this subcomplex because its silencing significantly reduced the interactions between Sec5 and Sec8 [5]. It is also intriguing that, even though mammalian and yeast Sec3 are predicted to be very similar in structure, both containing an amino-terminal pleckstrin homology (PH) domain, the long coiled-coil CorEx motif and an extended helical rod at the carboxyl terminus, their characteristics appear to be quite different. Mammalian Sec3 is found on the vesicle prior to fusion, whereas in yeast Sec3 is located at the plasma membrane before the arrival of the vesicles [10].

The current study by Ahmed *et al.* [5] describes the dynamics of exocyst assembly in mammalian cells, from which a model of exocyst assembly and disassembly during exocytosis can be proposed (Figure 1). Before being loaded onto the secretory vesicles, the exocyst subunits begin to assemble into their subcomplexes. Then subcomplex I and subcomplex II are independently recruited to the vesicles. Once arriving at the plasma membrane, the two subcomplexes assemble into the holo-exocyst complex to tether the vesicle to the plasma membrane and promote the subsequent membrane fusion. Right before fusion happens, the exocyst complex disassembles to release Sec3. Then the other subunits leave the fusion site 2 seconds after fusion.

This new exocyst movie raises a number of further questions. When are subcomplex I and subcomplex II loaded onto the vesicles? Is there an inhibitory mechanism that prevents the two subcomplexes from assembling into the holo-exocyst complex before the arrival of the secretory vesicles at the plasma membrane? If so, how is the inhibition removed when the vesicles arrive at the sites of secretion? Also, why do the vesicles, the tethering machinery, and some tethering regulators [5,11,12] remain at the plasma membrane for ~12–18 seconds, and what happens during this period of time? Last but not least, how is the assembly of the exocyst regulated in different cellular contexts and under different pathophysiological conditions? No doubt more exocyst sequels will follow.

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Larval Development: Making Ants into Soldiers

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Many ant species have complex caste systems, with reproductive queens and sterile workers, which often play distinct roles in the maintenance and defense of the colony. A new study sheds light on how these worker caste systems evolved and the mechanisms by which totipotent larvae give rise to the alternative adult castes.

The ant genus *Pheidole* contains more than a thousand species [1,2] that produce colonies with two distinct non-reproductive castes: small-bodied workers and large-bodied soldiers, the latter of which often have exceptionally large heads and mandibles. Any larva can develop into either a worker or a soldier, governed largely by the nutritive and pheromonal signals it receives. But soldiers are not just large-bodied workers. When a larva is set on a soldier-bound trajectory, it not only grows to be larger, but the scaling relationship among its

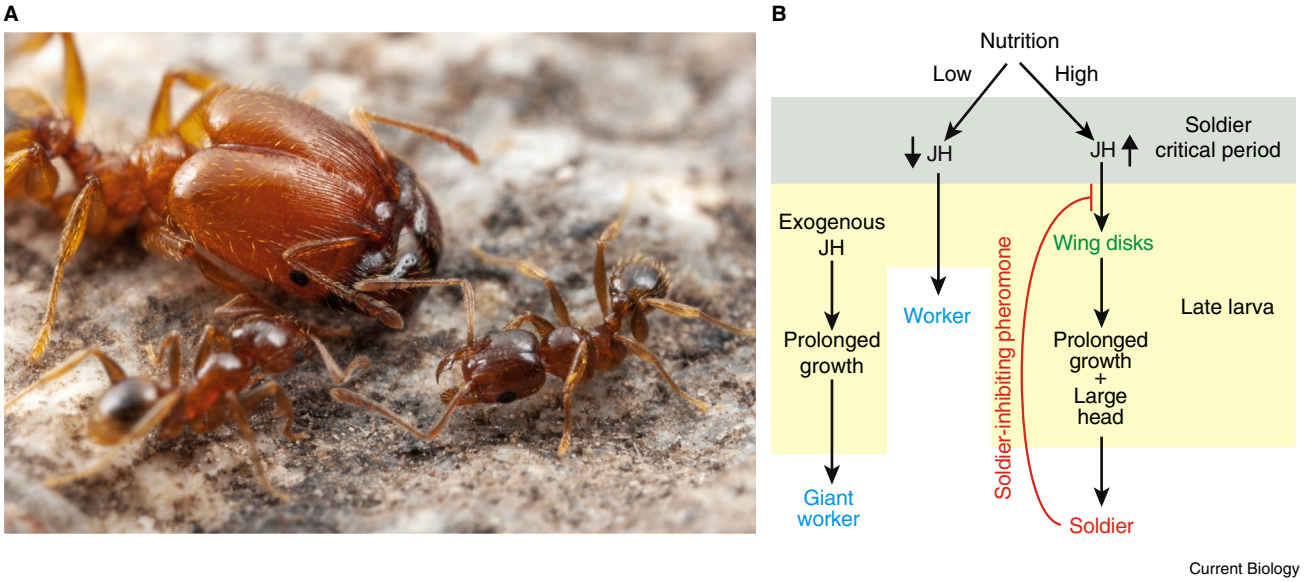

Figure 1. Development of castes in the ants of the genus *Pheidole*.
(A) Soldier and workers of *P. spadonia* from Arizona (photo copyright Alex Wild, used by permission). (B) Model for the role of wing disks in soldier determination in *Pheidole*. Nutrition affects the levels of juvenile hormone (JH) during a critical period of soldier determination. Low JH levels lead to development of a worker. High JH levels lead to development of wing imaginal disks, which control prolonged growth of body and head imaginal disks, leading to the big, large-headed soldier caste. Soldiers produce an inhibitory pheromone that blocks the effect of JH. Exogenous JH applied to a worker larva after the soldier-critical period can extend the growth period and result in giant workers.

body and head also changes: soldiers have disproportionally large heads (Figure 1A). Understanding the mechanisms by which such dramatically different alternative castes arise have challenged biologists for a century and-a-half. A new study by Rajakumar and associates [3] describes the most recent advance in this puzzle with a remarkable discovery that rudimentary wing imaginal disks in soldier-destined larvae play a critical role in the differentiation of the soldier and worker castes.

A curious feature of *Pheidole* soldier-destined larvae is that they develop rudimentary wing imaginal disks, which degenerate later in development [4]. Wings are characteristic of the sexual castes (queens and males), but never develop in the worker/soldier castes. The appearance of wing imaginal disks in soldier larvae has been interpreted as an indication that soldiers develop by retaining or adopting parts of the queen development pathway [5,6]; however, adult soldiers express no queen-like characters. In a recent study in *Nature*, Rajakumar *et al.* [3] set out to investigate a possible functional role for the wing disks in soldier development. Using RNAi to knock down the expression of vestigial (vg), a gene required for normal wing development, the authors found that treated larvae grew to a much smaller size than expected of a soldier and had a head–body allometry that more resembled that of workers. They then microcauterized the wing imaginal disks (using microcautery of leg imaginal disks as sham-operated controls), and those larvae likewise grew to a much smaller size than soldiers (and controls), also with a head–body allometry resembling that of workers. Evidently, the wing disks are required for larvae to achieve both a large body size and the disproportionally large head characteristic of adult soldiers. Suppression of wing-disk development in male larvae (adult males are fully winged) did not alter the male's body size or its head:body ratio, so the role of wing disks in controlling large body size and heads appears to be restricted to the wingless worker caste. Thus, wing disks appear to be specifically required for larvae to grow into soldiers with large bodies and even larger heads. This raises the question as to what developmental signal they are supplying to cause this dramatic change in size and morphology.

It turns out that there are two other factors known to affect differentiation of the soldier caste in *Pheidole*. The first is juvenile hormone (JH), a developmental hormone known to be involved in the control of metamorphosis and reproduction in insects, and also in the development of alternative phenotypes, or morphs [7]. When methoprene (a JH mimic) is applied to larvae during a critical period in the last larval instar, these larvae can grow to a very large size and develop into soldiers [8]. The second is a soldier-inhibiting pheromone, the existence of which was deduced from the finding that when larvae are reared in the presence of soldiers, they become relatively insensitive to soldier induction by JH. The soldier-inhibiting pheromone is a contact pheromone that occurs in the cuticular hydrocarbons of soldiers and functions to limit excessive production of soldiers in an ant colony.

Rajakumar *et al.* [3] investigated the possible interactions among wing imaginal disks, JH signaling, and the soldier-inhibiting pheromone. They found that rearing soldier-destined larvae in the presence of soldiers reduced the size of their wing imaginal disks, and those larvae developed into smaller ants with a significantly reduced head:body ratio, approaching that of workers. Similarly, simply treating soldier-destined larvae with the cuticular hydrocarbon extract

that contains the soldier-inhibiting pheromone likewise inhibited wing-disk growth in soldier-destined larvae and resulted in ant sizes and morphologies approaching those of workers.

Treatment with JH resulted in two kinds of animals: in some, wing disks were induced and those developed into soldiers, but in others no wing disk appeared, and these grew into very large, soldier-sized workers, with worker-like head-body scaling. The cause of the difference between these two responses is not reported, but it is likely that giant workers developed in cases where JH was applied outside the critical sensitive window for soldier (and wing imaginal disk) induction [8,9].

Thus, we are faced with two questions: how are the rudimentary wings induced, and how do those wing disks, in turn, induce soldier development? Some of the answers to these questions can be deduced from the results of previous studies on ants and other holometabolous insects. It is well-known that JH is sufficient to induce soldier development, so long as it is applied during a critical period [9,10]. At other times in the last larval instar, JH is known to inhibit the secretion of the molting hormone ecdysone [11,12]. This inhibitory effect is thought to be a safety mechanism to ensure that the metamorphic molt does not occur until all JH has disappeared. One consequence of this mechanism is that if extra JH is supplied during the last larval instar, after the soldier-critical period, the larva will continue to grow, often to an abnormally large size, until all of the JH is metabolized. This is probably why JH can produce large soldier-sized worker larvae. The level of JH reflects a larva's nutritional status. Well-nourished larvae have higher JH levels and are more likely to develop into soldiers, whereas poorly nourished ones seldom, if ever, do. This is where the soldier-inhibiting pheromone comes in. In a healthy well-nourished colony there is a danger that too many larvae will develop into soldiers. The percentage of soldiers is, however, carefully regulated [13,14]. The soldier-inhibiting pheromone raises the threshold of sensitivity to JH, so that even with high JH levels soldier induction does not proceed. The idea here is that the percentage of soldiers in a colony is homeostatically maintained by the counteracting forces of induction by JH and inhibition by the pheromone. Both of these forces are system properties of the ant colony as a society.

The work of Rajakumar *et al.* [3] shows that the effect of JH on the developmental switch to the soldier pathway is mediated by the rudimentary wing imaginal disks (Figure 1B). Two things are involved in this developmental switch: a revision of the size at which larval growth will stop, and a reprogramming of the head imaginal disks to grow disproportionally large. The body size effect is most likely mediated by the critical weight at which secretion of JH stops. As noted above, JH inhibits ecdysone secretion, so if JH secretion, or its persistence, is prolonged, then so is the growth period, resulting in a larger body size. Thus, one of the roles of the wing disks could be to alter the body size at which JH secretion stops.

A plausible mechanism by which wing disks could have this effect comes from the finding that in *Drosophila*, wing imaginal disks secrete an insulin-like protein (DILP-8) that inhibits metamorphosis while the disks are growing [15]. It does so by inhibiting the biosynthesis of ecdysone [15,16]. A similar effect could exist in *Pheidole* and explain the prolonged larval growth while the rudimentary wing disks are growing. The excessive growth of the head imaginal disks in soldier-bound larvae could also be explained by the prolonged action of an insulin-like growth factor, because the growth of imaginal disks is known to require insulin signaling [17,18].

Much still remains to be discovered about how ants regulate their castes, but the work of Rajakumar *et al.* [3] has revealed a heretofore unimagined mechanism that captures a feature thought to be evolutionarily lost, and redeploys it in a novel and unexpected way. The mechanism they uncovered may be widespread in ants, since rudimentary wing disks also occur in the soldier caste of the genera *Camponotus* (carpenter ants) and *Solenopsis* (fire ants) [3], but there is not sufficient information at present to say whether they function as they do in *Pheidole*.

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