

# Selfish responses by clone invaders

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One's first encounter with aphids is likely to have been as a green velvety mat of tiny bodies placidly sucking the sap out of rosebushes with their tiny soda-straw stylets. The impression of gentleness is further enhanced if one knows that aphids are also the cows of the ant world, tended and defended for the honeydew that they excrete nearly constantly as they process enough sap for their protein needs. However, recent research has revealed that a fraction of aphids—perhaps 1% of the over 4,000 species (1)—are less like cows and more like killer bees. Evolution has endowed some of them with bulked-up Schwarzenegger arms (six of them, of course) and turned their soda straws into lethal weapons. These fierce soldiers can repel and even kill predators many times their size. Now, as Abbot *et al.* show in this issue (2), some of these social aphids demand a further revision of their aphid-as-placid-cow reputation. Not only are they fierce, but they are also sneaky and clever. They exhibit a complex and variable suite of responses to their specific social situation and are able to freeload on the protection offered by their unrelated neighbors. As such, they illustrate an important issue in the evolution of complex life.

The discovery of fighting soldiers in aphids by Aoki in 1977 (3) gave Hamilton's kin selection theory support from an unexpected quarter (4). These diminutive warriors aid their clone mates, protecting them from the likes of lacewing and fly larvae (Fig. 1). Although the soldiers themselves often die in defense of the colony, they may save an entire gall full of genetically identical siblings, the offspring of a single female that founded the gall and churned out parthenogenetically identical daughter copies of herself. At first blush, it might seem that clonal species are ideally suited to evolving sociality because of their extraordinarily high relatedness. However, an aphid helping to rear its clonal siblings [relatedness ( $r$ ) = 1] gives up raising its own clonal offspring ( $r$  = 1), and this tradeoff is no different from an individual in a sexual species

raising full siblings ( $r$  = 1/2) instead of offspring ( $r$  = 1/2).

Where clonality does make a difference is for conflicts within the colony. Although the standard, sexual social insects—such as ants, bees, wasps, and termites—can be highly cooperative, they have had to evolve cooperation against a background of potential and actual conflict. Kin selection theory predicts that even if they are relatives, genetically different individuals will often have different optimal ways to pass on their genes.

Therefore, even close relatives can be in conflict. For example, in haplodiploid social insects, males develop from haploid unfertilized eggs, and females develop from diploid fertilized eggs. There-

fore, female workers are more related to their sisters ( $r$  = 3/4) than to their brothers ( $r$  = 1/4). Thus, they gain from raising more sisters, whereas the queen, who is equally related to her daughters and sons ( $r$  = 1/2), does best with equal investment in each (5). So, in many species, we see a waste of colony resources as queens produce far too many males for the workers' taste, and the workers sometimes execute male larvae even when they are nearly mature (6). Other conflicts can arise over who gets to lay the eggs and over whether an individual should develop as a worker or queen (7, 8).

Clones are immune to this kind of disruptive conflict as long as they can maintain their clonal integrity against interlopers. Aphids living in plant galls founded by a single female would seem to be well set up to maintain clonal integrity. But, Abbot *et al.* (2) show that different clones do mix, with interesting consequences. Understanding this case requires an introduction to the unusual biology of gall-forming aphids and, in particular, *Pemphigus obesinymphae*, the species studied by Abbot *et al.* (2).

In *P. obesinymphae*, individual females form galls in the spring at the base of cottonwood leaves (9). This home lasts only until the tree sheds its leaves in autumn, limiting the active season of aphids on this host. The plant tissue

swells into a gall around the female, forming a protected cave for feeding on plant sap and producing hundreds of exact copies of herself. However, like most aphid galls, the gall of *P. obesinymphae* is not completely closed. The aphids require an exit large enough for the winged females to leave in late autumn, and for the removal of the sticky honeydew, without which the aphids could drown in their own waste. Unfortunately for the aphids, it is also large enough for predators to enter. At the slightest disturbance, *P. obesinymphae* soldiers rush out in defense of their gall. If they encounter a predator, such as a lacewing larva, they grab it with their heavy legs and pierce it with their stylets. Although any one aphid may have little impact, the unpleasant effect of many such piercings is evident from the writhing of the predator, and its efficacy is manifest by the dropping of the predator from the plant.

In ants and termites, soldiers represent a terminal stage in development, with specialized and irreversible features for fighting. This is not the case in aphids, in which the soldiers always come from immature stages (1). Developmental arrest as soldiers may be permanent or temporary; the latter is the case for *P. obesinymphae*. All daughters of the gall initiator pass through the tiny, first-instar stage that has defensive modifications. Development is often stalled in this stage as long as the queen is alive and the end of the season has not been reached; but, at the proper time, the soldiers can pass through the additional molts necessary to become a winged reproductive (9, 10). Some galls thus end up with hundreds more first-instar soldiers than can mature in the cramped gall. Some of these soldiers die defending the gall, but many galls still have an excess of soldiers.

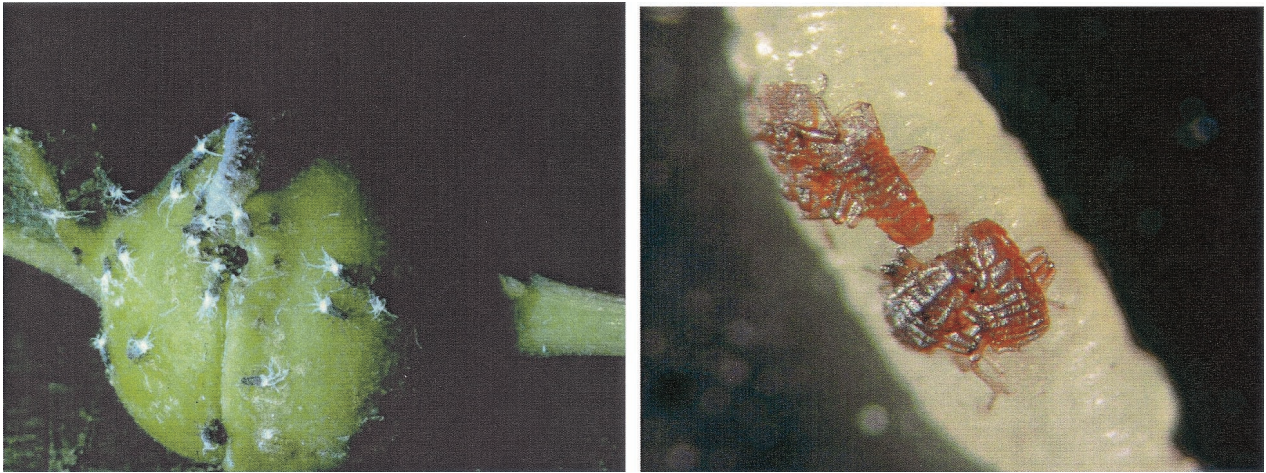
Abbot *et al.* (2) have discovered that appreciable numbers of first-instar soldiers leave their natal gall and infiltrate other galls, where they are unrelated to the mother and her daughters. On average, 41% of young aphids found in galls

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COMMENTARY

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**Fig. 1.** Social behavior in aphids. Aphid soldiers aggressively attack insects much larger than themselves, as shown in *Left*, in which *P. obesinymphae* soldiers (no longer alive in this photo) have attacked a fly larva. (*Right*) The soldiers, trailing whitish wax, have emerged to deter a larval predator attempting to enter the small, round hole in the gall. Inside the gall are both clonemates of the defending soldiers and unrelated intruders from other galls.

in late August were born in other galls. To test defensive reactions in such infiltrated colonies, Abbot *et al.* introduced *Drosophila* larvae into galls. (The reader, having been asked to accept aphids as fierce, is not now being urged to reevaluate *Drosophila* larvae as ferocious predators. *Drosophila* were used as readily available mock predators, which the aphids attack in the same manner as their real enemies.) After a short time, they removed the pincushion larva with its aphid soldier pins, and genotyped the soldiers to ascertain clonal membership. Migrants from other galls turned out to be far less likely to attack these invaders than were the native soldiers. Furthermore, the migrating soldiers do not remain as soldiers for long, but instead, quickly develop into mature reproductive females. Selfish aphids that are loath to attack and keen to develop are just what Hamilton's kin selection theory would predict for individuals faced with a group of nonclone mates. Any clone with the clever trick of developing more individuals faster by putting the defensive burden on other clones could gain a significant advantage. A big question remaining is why this clever strategy is not matched by clever defense. Why are aphids stupid enough to allow aliens into the gall at all? Kin selection would also predict recognition and exclusion of nonclone mates; such recognition systems are common in other clonal organisms (11). Perhaps the reason that soldiers in invaded galls continue to defend and yet do not expel nonclone mates is because the nature of their sociality precludes more advanced or complex social development. No matter how well or poorly aphids maintain their clones, the leaf will fall in autumn, and this social stage will end. It may be particularly hard to pre-

vent invasion shortly before the end of this stage when migrating soldiers are eager to harvest the last fruits of the gall.

So, aphid colonies are faced with problems of cheating and conflict, just like colonies of nonclonal social insects. Why do aphid soldiers sacrifice their own offspring production for a class of gallmates who are less related owing to intrusions? The answer must come from the benefits of helping relatives in protected galls, even if these benefits sometimes accrue to nonrelatives. Helping is favored if, despite the intruders, soldiers save more clonal siblings than the clonal offspring they forego. Living and feeding inside galls seems particularly conducive to this trade-off. The gall provides a long-lasting resource that can accommodate a large number of relatives, but it needs to be defended. This kind of food/shelter resource has probably been important in the evolution of all of the parvenu social insects—certain aphids and thrips, as well as a beetle—that have only recently been admitted to the high society of eusociality, largely because their specialized and reclusive lifestyle make them less noticeable (12). This resource was probably also important in the evolution of the termites. These fortress defenders can be contrasted with the ants, bees, and wasps, where predation selects for a life-insurance advantage of sociality (13). Here, the offspring are fed by adults, who must face the hazards of the outside world to procure food. The combination of long offspring dependence and high risk of adult death selects for group care, with the presence of each adult insuring against the loss of others.

The maintenance of clonal purity is not an issue restricted to aphids. It occurs frequently in microorganisms that are asexual or have an asexual phase (14). Bacteria that feed by digestion external to

the cell face issues of cooperation and cheating, because costs borne by individual cells yield products available to anyone sufficiently close by. If neighbors are not clone mates, why not parasitize them? Pathogens may play a similar game whenever they secrete virulence factors that enhance the growth or reproduction of the population of cells but also cost the cells that produce it. Smith (15) argues that this fact may be why toxins such as those produced by the cholera bacterium are carried on plasmids. These toxins are expensive to produce, and are released into the lumen of the host where they benefit all of the cholera bacteria. Any mutant that ceased producing them would out-reproduce those individuals still producing the toxin, and the mutants would still benefit from the toxins as long as mutant cells were rare. But if these genes are on plasmids, then the mutants losing the ability to produce toxins can simply be reinfected, which is a kind of evolutionary control against cheating.

More visually arresting cases come from the cellular slime molds, of which *Dictyostelium discoideum* is best studied. Here, dispersed single cells aggregate to form a fruiting structure, with about 20% of the cells becoming dead stalk cells to support the spores, the functional equivalent of soldier aphids that give their lives for their gallmates. As in aphids, different clones do not exclude each other, and the result is cheating: some natural clones exploit others by devoting less to stalk (16). These exploitative clones are developmentally completely competent on their own, but other exploiters, from both the lab and the field, are known that suffer severe costs, such as stalklessness, without a partner to exploit (17, 18). Parallel issues, and similar cheating mutants, arise in *Myxococcus* bacteria that have convergently evolved a

life cycle and fruiting body quite similar to *Dictyostelium* (19). Clearly, in all these cases, we would like to know much more about micropopulation structure. Are most natural interactions among clone mates?

Such issues might seem less relevant to multicellular organisms such as metazoans, where asexual reproduction is less common. But, in fact, issues of clonal identity have been crucial in the evolution of multicellular forms. Each metazoan begins as a single cell that reproduces asexually. This single-cell bottleneck, coupled with mechanisms that exclude other clones, means that the multicellular organism will be clonal, and thus lack genetic conflicts between somatic and germ-line cells (20, 21). The importance of such defenses can be shown by what happens when they are breached. Self-recognition systems are widespread, particularly in clonal marine invertebrates, and allow not only for discrimination against nonrelatives but also fusion with relatives (11, 22). In the tunicate *Botryllus schlosseri*, sessile colonies can join together, thus forming a

common circulatory system, provided that they share at least one allele at the highly polymorphic histocompatibility locus (23). Of course, sometimes different clones will fuse because they match at this locus, but are not otherwise genetically identical. When this event happens, one clone can dominate the germ line (24, 25).

Clonal purity issues have also had a major impact on organelles. Mitochondria and chloroplasts replicate clonally within their cells, but sexual reproduction of their cells brings two different clones together. The potential for disruptive conflict has been squelched, however, because various mechanisms ensure that only one set of parental organelles survives (26). This result is presumably coordinated by nuclear genes that have no particular stake in the survival of their own mitochondria. Uniparental inheritance of or-

ganelles may be the most fundamental asymmetry underlying the existence of two sexes (27).

Abbot *et al.* (2) have shown that, like other social insects, clonal social insects are vulnerable to conflicts of interest. Their result connects not just to social insects, but to a much wider variety of organisms, as shown by the selfish responses in

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systems as diverse as the social bacteria and amoebae (16–19) and the fusing tunicates and cnidarians (11, 22–25). The issues of cooperation, conflict, and control that have long intrigued social insect biologists are crucial for understanding the evolution of multicellularity, which can be viewed as the most complex and successful form of sociality (28–31).

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