COMMENTARY

Snapshot of insect–fungus arms race

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The general populace associates the arms race with nations preparing for or attempting to avoid conflicts by developing and divulging their military superiority. Unfortunately, it is not just nations that engage in excessive preparation: so do cities. For example, the City of Davis, California, population 66,000 residents and 40,000 students (University of California, Davis) acquired a Mine Resistant Ambush Protected military vehicle (1), later returned under pressure from this bike-friendly community.

In the insect world, something else drives the arms race: selection as exemplified by host-evolving defenses and pathogens' coevolution of detoxifying mechanisms. Entomologists examine these host–pathogen interactions not only to obtain better insights on the forces driving natural selection, but also to devise novel environmentally safe alternatives to control insect population while reducing our nation's dependence on conventional agricultural chemicals. In PNAS, the multidisciplinary and multinational team of Pedrini et al. unravel a mystery in the insect–fungus arms race (2).

Throughout the world, entomologists use fungi that ultimately kill insects (entomopathogenic fungi) to control human disease vectors, particularly mosquitoes, and agricultural pests (3). One of these widely used pathogens is *Beauveria bassiana*. Typically, the fungus overcomes the first line of insect defense, the cuticle, with enzymes that degrade the cuticle (4), thus allowing penetration into the insect's open circulatory system. Once its immune system is compromised, the insect dies. Intriguingly, the red flour beetle, *Tribolium castaneum*, is resistant to this fungus (Fig. 1). These beetles use "leftover" quinones, which they produce primarily in the process of cross-link proteins (5). The quinones harden the cuticle, render it impenetrable (5), and serve as a chemical-defense weapon (6). These quinones are very toxic (7) and keep fungus at bay. Intrigued that degradation of quinone derivatives by other fungi is very common, Pedrini et al. (2) asked the question: Why doesn't *B. bassiana* use its biochemical machinery to degrade/detoxify quinones and get one step ahead in this race? Surprisingly, the authors found that the fungus is working on it.

In their elegant work, Pedrini et al. (2) first cloned a gene in the genome of the fungus NAD(P)H:1,4-benzoquinone oxidoreductase (*BbbqrA*) encoding for an enzyme of interest, benzoquinone reductase. The authors noticed a hallmark of an inducible gene. They observed that transcript levels of this gene increased up to 40-fold when they exposed cultures of the fungus to benzoquinone. Pedrini et al. surmised that the beetle's secretion is the key to switch on that gene. Next, they engineered the fungus into two different strains, one strain with *BbbqrA* silenced and another one that overexpresses the gene. Then the authors tested the antifungal properties of the beetle secretion against these two strains and the WT *B. bassiana*. The results were remarkable: the beetle defensive compound caused dose-dependent inhibition in the *BbbqrA*-overexpressing strain higher than those observed with the WT, whereas the *BbbqrA*-silent strain showed the lowest level of inhibition.

To unambiguously determine a direct role of the enzyme in the inhibitory effect of growth, Pedrini et al. (2) first measured enzyme activity of extracts from the WT and two engineered strains grown in the presence and absence of benzoquinone. Clearly, enzyme activity was increased in the WT strain when grown in the presence of benzoquinone, and enzymatic activity was low in the silent strain, whereas in the overexpressing strain enzymatic activity was very high both in the presence and absence of benzoquinone. Next, Pedrini et al. expressed the enzyme in a heterologous system to measure the kinetic properties of *BbbqrA* toward reduction of two substrates from the beetle's weaponry: 1-4-benzoquinone and 2-methyl-1-4-benzoquinone.

Having just studied acetylcholinesterase, carbonic anhydrase, and other textbook enzymes, my undergraduate biochemistry students were not overly impressed by the catalytic efficacy of *BbbqrA*. I argue that the fungus does not have to recruit an enzyme with high efficacy, nor is high substrate specificity essential to override the beetle's defensive secretion. Detoxification does not need to be as rapid and specific a process as degradation of a neurotransmitter or formation of carbonic acid in human blood.

Fig. 1. The red flour beetle, *Tribolium castaneum*. Image courtesy of US Department of Agriculture.

The research by Pedrini et al. (2) suggests that they obtained a snapshot in the ongoing arms race. At the moment, the red flour beetle is winning, but the fungus seems to be working on a solution to win. After all, an arms race in the insect world is like running on a treadmill or pedaling a stationary bike: at the end of a long journey one is still at the starting point. The authors envision that exposing the beetle to the silent and overexpressing strains could lead us to speculate past and future insect–fungus interactions. Indeed, the red flour beetle is currently a poor host for the fungus because it kills only about 20% of the beetles. In contrast, the silent and overexpressing strains killed ∼10% and 40%, respectively. In short, at the time of this writing, the beetle is winning, but another snapshot of a different image is likely to appear later in this arms race.

Molecular biologists may argue that a rigorous test for their hypothesis would require knockdown/silencing of the beetle's biochemical machinery involved in benzoquinone formation. This was already done and indeed reducing production of quinones leads to reduced antimicrobial activity (6). However, the picture is not clear, because of the multiple roles of quinones, including proper formation and rigidity of an insect's cuticle.

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Evolutionary biologists would probably inquire whether this could indeed be an arms race with broader implications in natural selection. The main reason for possible skepticism could be that the red flour beetle might be remarkably different from related species in the wild. After all, the red flour beetle lifestyle is a recent event in the face of millions of years of coevolutionary insect–fungus history. Testing this hypothesis with close species from the wild remains an interesting topic for future research. With the tools at their disposal, Pedrini et al. (2) do their best to counter possible arguments raised by evolutionary biologists. They compared the effects of WT and the two engineered-strains of *B. bassiana* on beetles and moths that do not secrete quinones. They found that neither silencing nor overexpressing *BbbqrA* had an effect of virulence against the rice weevil, *Sitophilus orizae*, and the greater wax moth, *Galleria mellonella*.

Paraphrasing insect physiologist and chemical ecologist James L. Nation (University of Florida), no paper is perfect and no paper can tell a complete story. Pedrini et al. (2) admit that the red flour beetles might have additional barriers against *B. bassiana*, given that even with *BbbqrA*, overexpressing strain mortality did not reach more than 50%. However, their novel findings significantly expand our knowledge of insect–fungus interactions and advance the field. Entomologists and chemical ecologists, in particular, should follow these developments with vivid interest. After all, in the insect–entomologist race, exploring new strategies may lead us to improve the human condition in the framework of sustainability.

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2 Perdrini N, et al. (2015) Tenebrionid secretions and a fungal benzoquinone oxidoreductase form competing components of an arms race between a host and pathogen. *Proc Natl Acad Sci USA* 112:E3651–E3660.

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