PNAS COMMENTARY

Flies co-opt bacterial toxins for use in defense against parasitoids

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Horizontal gene transfer (HGT) is a recurrent driver of innovation in bacteria and archaea, but the broader significance of this phenomenon in animals is less clear (1, 2). Despite barriers impeding the transfer and establishment of foreign DNA, animal genomes frequently encode genetic material derived from other branches of the tree of life. Even if only a small fraction of incoming genetic material is destined to become functional, animals stand to benefit from access to the larger gene reservoir present across microbes. Consistent with this view, studies increasingly show that select HGT events have provided animals with novel functionality (1). In bacteria, HGT has played key roles in supplying virulence factors and resistance to antimicrobials (2). Do similar patterns of HGT occur in animals, arising from shared challenges, in which specific traits are more extensively shared? The answer may be yes. One nearly universal challenge faced by insects is attack by specialized natural enemies called parasitoids (3). Host–parasitoid interactions are typically life or death, leading to strong selection for resistance (4). In PNAS, Verster et al. (5) explore whether toxins of bacterial origin that have been repeatedly transferred into the genomes of insects function in fruit flies to aid in their defense against parasitoids.

Another way that insects access the large gene repertoires of microbes is by harboring maternally transmitted symbionts (6). Many heritable symbionts provide protection against specialized enemies, often through the production of eukaryotic toxins (7, 8). In aphids, the bacterial symbiont *Hamiltonella defensa*, together with its toxin-encoding bacteriophage *Acyrthosiphon pisum* secondary endosymbiont (APSE), confer protection against hymenopteran parasitoids (9, 10). Some APSE variants encode homologs of a genotoxin called cytolethal distending toxin (*cdtB*) and an apoptosis-inducing protein (*aip56*) (11–13), both of which also occur in pathogenic bacteria. Amazingly, Vertser and colleagues (5, 14, 15) have found that homologs of one or both toxins have been horizontally transferred into the genomes of insect species spanning at least five orders. The transferred toxins share numerous features with those occurring in APSE phages, including sequence similarity, gene order, and the retention of only the active subunit of cytolethal distending toxin; the binding units (CdtA/CdtC) that facilitate toxin delivery into target cells have been discarded.

Despite these similarities, *Hamiltonella* is unlikely to be the immediate toxin donor because this symbiont appears restricted to hemipteran insects (16, 17). However, APSE-like phage elements and associated toxins have been found in

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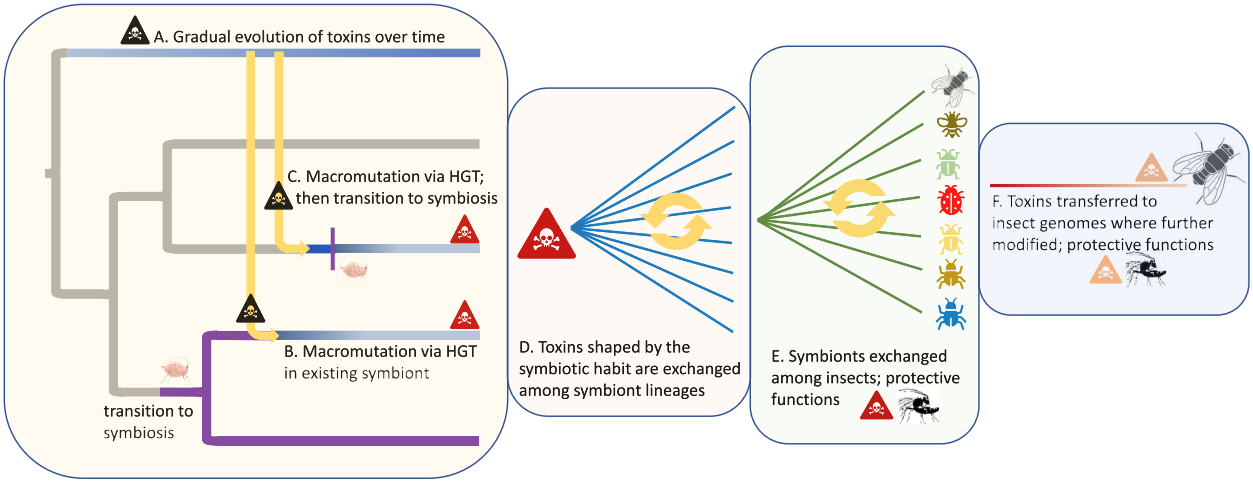

Fig. 1. Bacterial toxins widely dispersed across insects through symbioses, with some subsequently transferring into animal genomes. After gradual evolution in bacteria (*A*), HGT moves functional toxins to new bacterial lineages. Toxins can transfer into existing insect symbionts providing novel functions (macromutation) (*B*). Alternatively, the acquisition of new capabilities via HGT may facilitate transitions to symbiosis (*C*). Toxins shaped by the symbiotic habit (e.g., holotoxin loses delivery subunits) are exchanged among symbionts (*D*), and symbionts move among insect lineages broadly disseminating toxins (*E*). Aided by access to the germ line, some toxins move into insect genomes (*F*), where they undergo further modifications, including gene duplication and acquisition of eukaryotic motifs.

other symbionts with broader host ranges (11), and these remain candidate donors. More likely, the repeated exchange of toxin cassettes among insect-associated bacteria over large expanses of time simply renders the donor unknowable. Nonetheless, heritable symbionts provide a parsimonious explanation for how similar toxin homologs have been shuttled around the insect phylogeny. First, they have unusual access to the insect's sequestered germ line as they typically persist intracellularly and are intimately connected to host reproductive tissues (1, 18). Second, while primarily vertically transmitted, heritable symbionts also move laterally into new host species, often displaying exceptionally wide host ranges (16, 19).

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To investigate whether the horizontally transferred toxins actually function in defense against parasitoids, Verster et al. (5) turned to the fruit fly *Drosophila ananassae*. Genomic data supported gene duplication and fusion events following toxin acquisition resulting in a single *cdtB* gene upstream of a pair of hybrid genes each containing *cdtB* and *aip56.* Consistent with a defensive function, *cdtB*:*aip56* fusion genes and their products were highly expressed in larval flies following parasitism by the wasp *Leptopilina boulardi*. To determine whether these toxins influenced the outcome of fly-parasitoid interactions, Crispr-Cas9 mutagenesis was used to create experimental fly lines in which some or all toxin elements were knocked down. In separate bioassays, flies were then parasitized using three *Leptopilina* species varying in specialization and virulence (i.e., ability to complete development) toward this fly. When parasitized by the least virulent wasps, *L. boulardi* and *L. heretotoma*, wild-type flies containing the full set of toxins exhibited higher rates of survival compared to fly lines with disabled toxins. Interestingly, wild-type flies did not benefit following parasitism by the specialist and more virulent wasp, *L. victoriae.* But fewer wasps emerged from wild-type flies, indicating that the toxins negatively impacted parasitoids.

Altogether, this illustrates a quite remarkable path taken by these toxins (Fig. 1). Following gradual evolution over the eons in bacteria, toxins became affiliated with, and exchanged among, insect symbionts. Toxin-containing symbionts then spread to diverse insect lineages often providing host defense. In some cases, toxin genes moved into animal genomes where they were co-opted to function in defense against parasitoids.

Many questions remain for future research, including understanding the precise manner in which these toxins harm parasitoids, how flies avoid harm to their own tissues when producing toxins, and trajectories of toxin evolution after acquisition by animals. Another is how lateral acquisition of antiparasitoid factors subsequently impacts the evolution of the insect immune system? Interestingly, noncanonical encapsulation responses are observed in *D. ananassae* flies and aphids (via symbionts) where CdtB/AIP56 toxins are implicated in harming parasitoids (5, 17). Did the lateral acquisition of novel defenses render components of innate immunity redundant, leading to relaxed selection for their maintenance? Or were the immune systems of these groups already compromised, resulting in stronger selection for retaining laterally acquired toxins?

There is also an interesting question regarding the relative costs and benefits of HGT versus housing protective symbionts for use in defense against parasitoids. Symbionts like *Hamiltonella* can be costly when natural enemies are not present (17), and HGT may reduce protection costs by eliminating the middleman (5). On the other hand, symbionts potentially provide a more dynamic response to parasitism by encoding a more diverse arsenal of toxins capable of targeting specific parasitoid genotypes and species (17). This may be important because wasps rapidly evolve counterresistance to specific toxins, including CdtB (20). If toxins acquired via HGT present a more static target for parasitoids, they may be more effective against generalists. Results from Verster et al. (5) appear consistent with this as the laterally acquired toxins did not improve fly viability following attack by the specialist parasitoid.

Of course, it will also be fascinating to see whether the CdtB and AIP56 toxins function in defense against parasitoids in the other insect systems where HGT events were documented (5, 14, 15). This would support an unexpectedly large role for HGT in insect immunity against parasitoids. Is this the tip of the iceberg? Insect symbionts encode diverse toxins and confer protection to other groups of specialized natural enemies, including nematodes, entomopathogenic fungi, and viruses (7, 8). The ribosome-inactivating proteins of *Spiroplasma* implicated in defending fruit flies against nematodes and parasitoids, for example, have been found in insect genomes (from a different donor) (21). Targeted approaches like those used by Verster et al. (5) are likely to identify additional examples of recurrent HGT, in which specific microbial genes are repeatedly co-opted by insects for use in defense.

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