Ecology-relevant bacteria drive the evolution of host antimicrobial peptides in Drosophila

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Introduction

- Antimicrobial peptides (AMPs) fight infection and determine the microbiome of both plants and animals. Using flies lacking AMPs, we recently confirmed this *in vivo.*[1,2,3]
- In *Drosophila*, *Diptericin (Dpt)* genes evolve rapidly, including an S69R polymorphism in DptA that predicts defence against *Providencia rettgeri* bacteria.[4] Follow-up work found this sort of AMP-microbe specificity is common.[5]
- Many studies have shown rapid evolution of AMPs, but the selective pressures driving AMP evolution aren't clear. Likewise, explaining AMP-microbe specificity has been challenging.
- We expected the host microbiome should be important. So we systemically infected our *Drosophila* AMP mutants (Fig. 1) with the common mutualist bacteria *Acetobacter* to screen for AMP(s) relevant to this microbe.

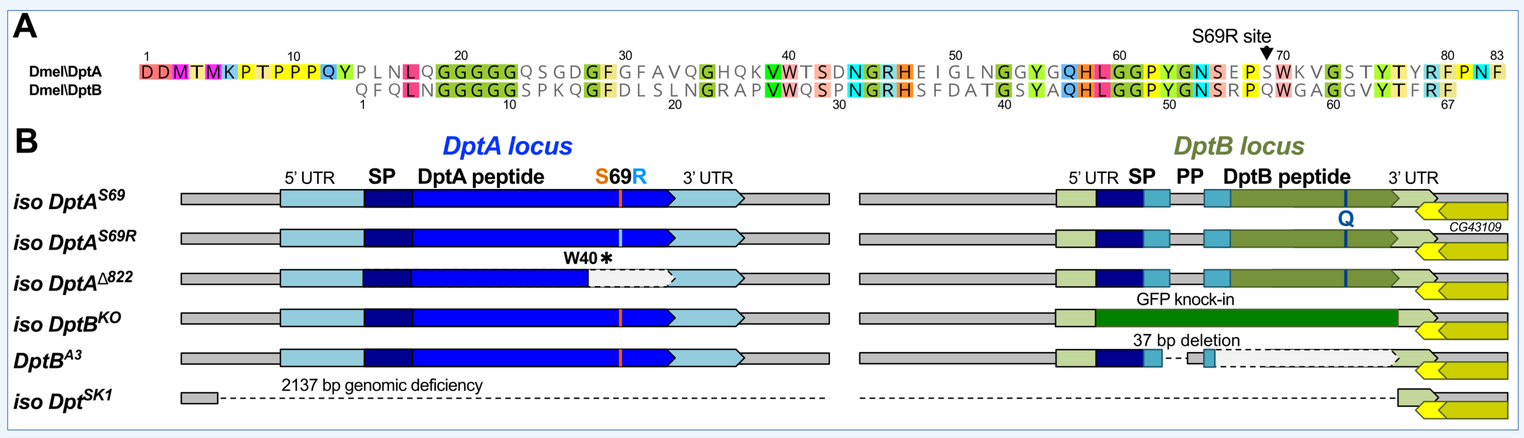

Figure 1: The *Diptericin* toolkit. A) alignment of *D. melanogaster* DptA and DptB mature proteins. B) *Diptericin* loci of key fly stocks used in this study.

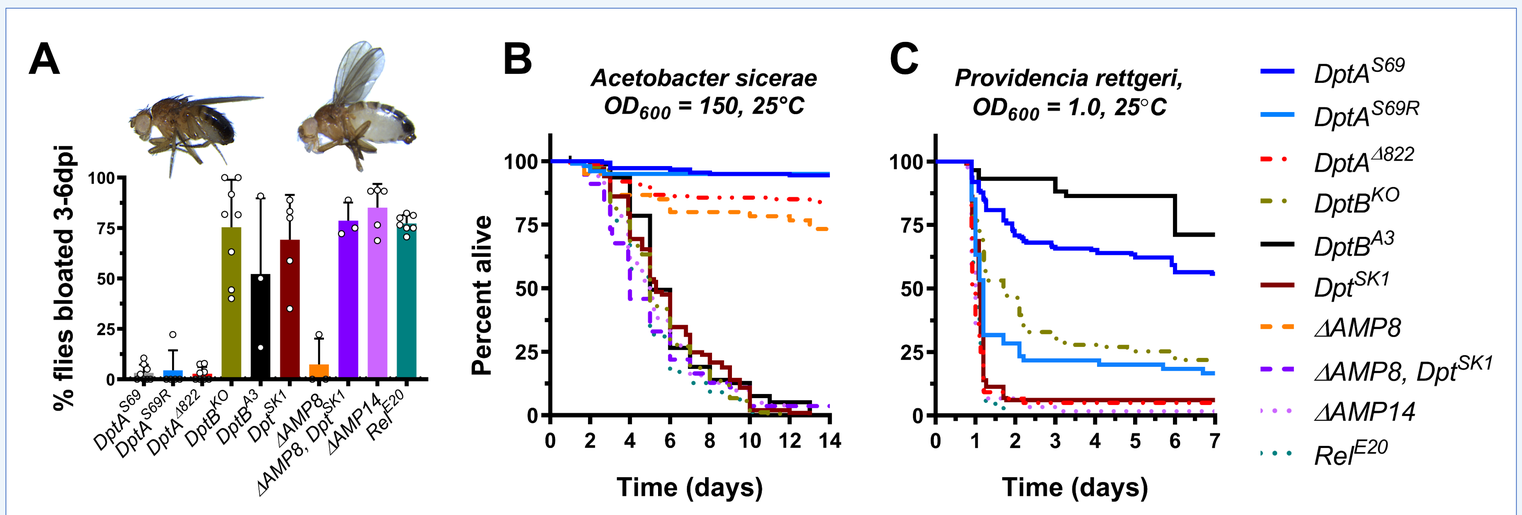

Figure 2: *DptA* and *DptB* are highly important and specific for control of unique microbes. A) Flies lacking *DptB* become bloated after *A. sicerae* infection. B) *Acetobacter sicerae* kills flies lacking *DptB*, but not flies affected in *DptA*, or flies lacking other AMPs *(ΔAMP8),* paralleling specificity of *DptA against P. rettgeri* shown previously.[1] C) $DptB^{A3}$ flies confirm *DptB* does not contribute to defence against *P. rettgeri*, which was not tested previously.[1,4] $DptB^{KO}$ flies have lower induction of their *DptA* gene (not shown), explaining their greater susceptibility.

Ecology:				**Independent correlations**
Providencia	Yes	Yes	No	-
Acetobacter	Yes	No	No	-
DptA-like	Yes	Yes	No	*at least 3*
DptB-like	Yes	No	No	*at least 6*

Figure 3: Correlations aplenty! Ecology predicts presence of microbiome members, and microbiome members predict *Diptericin* evolution.

A much more detailed summary is shown in Fig. 4 of the manuscript, available at the (Open Access link) QR code above.

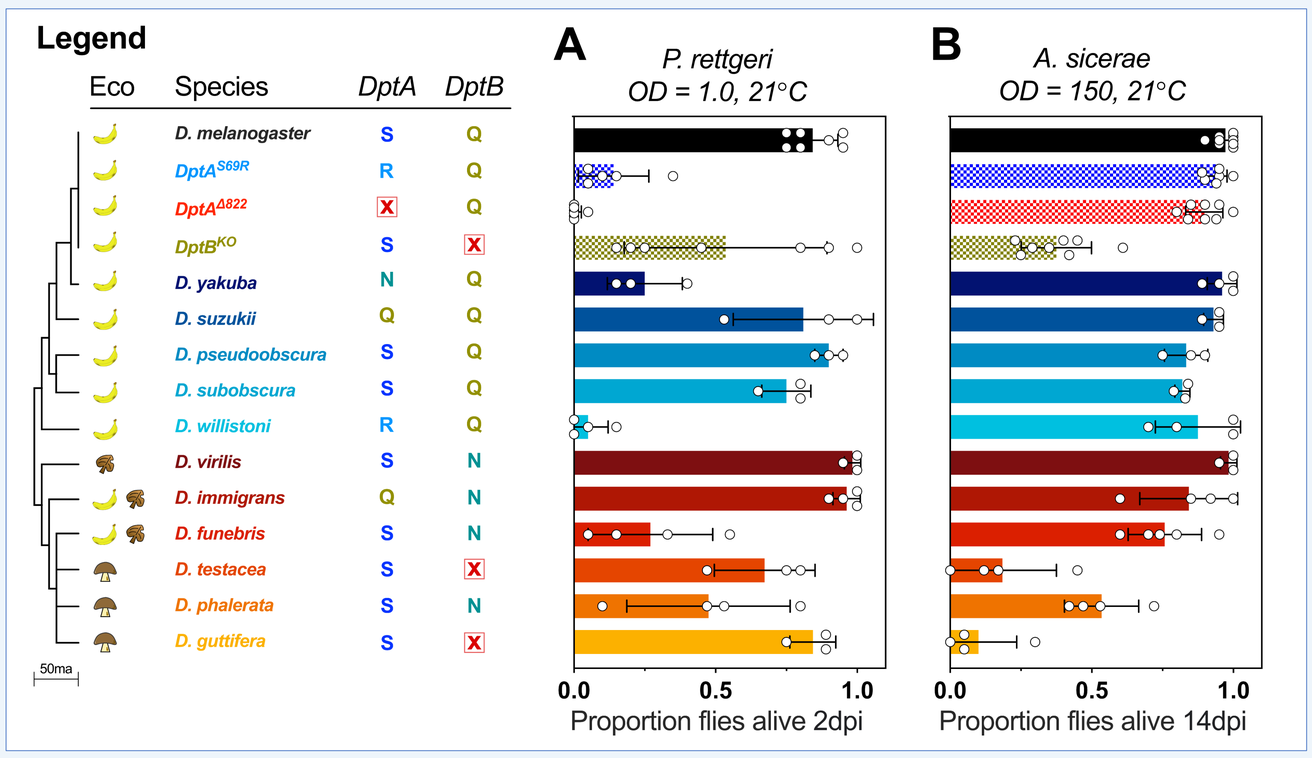

Figure 4: The defence offered by *DptA* and *DptB* against relevant microbes is true across flies separated by 50 million years of evolution. A) *DptA* complement predicts survival against *P. rettgeri* (R^2 = 0.74). B) DptB complement predicts survival against *A. sicerae,* both in *D. melanogaster* and mushroom-feeding flies (R^2 = 0.87).

Artwork by Diego Galagovsky (sci-flies.com)

"Time flies like an arrow, fruit flies like a banana." - AG Oettinger.[11] This dietary preference of the *Drosophila* ancestor exposed it to a new suite of microbes. Among these microbes was *Acetobacter*. As a result, the host immune system evolved a unique immune effector, *DptB*, to prevent *Acetobacter* infection. Similar dynamics can explain the evolution of *DptA* and its role in fighting *P. rettgeri*. Our findings offer an evolutionary logic underpinning recent observations of AMP-microbe specificity.

Conclusions

- AMPs specifically important against ecological microbes are derived given host-microbe association over evolutionary timescales. This finding helps explain the rapid evolution that is so common among animal AMP genes.
- The alternate specificity of DptA and DptB will allow future work using both host and microbe genetics to reveal mechanisms of specificity.
- Here we describe a one-sided evolutionary dynamic: hosts will adapt to the ubiquitous presence of an environmental microbe. The microbe, however, faces selection from many hosts, and is not expected to evolve resistance to any specific host's unique immune mechanism.
- Given recent studies showing specific AMP importance against unique microbes across animals,[9,10] we expect this finding will be highly applicable to understanding the logic of immune evolution in general.

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