



Costs and Benefits of Plant Defence Suppression by Tetranychus Evansi Spider Mites
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Summary

Herbivores eat plants, and plants defend their tissues. To overcome these plant defences, herbivores evolved a variety of offensive strategies. Some herbivores use enzymes to detoxify defensive compounds of their hosts, other herbivores cut veins in the leaves of their hosts to prevent exposure to dangerous defensive latex, and yet others sequester the toxic compounds of their hosts as protection against predators. Why has this diversity of herbivore offensive strategies evolved? Understanding the evolution of herbivore offensive strategies requires insight into their costs and benefits, in relation to the environment in which they are employed. In this thesis, I studied costs and benefits of a recently described offensive strategy, defence suppression, in the herbivorous spider mite *Tetranychus evansi*.

Herbivores can suppress plant defences by manipulating a plant's physiological processes, for example by manipulating signalling pathways of plant hormones involved in defence, including jasmonic acid and salicylic acid. This allows herbivores to prevent expression of inducible plant defences, thereby increasing their own performance. How much do *T. evansi* spider mites benefit from suppressing the defence response of their tomato hosts? In CHAPTER 2 I quantitatively review evidence, published and unpublished, of the benefits of defence suppression conferred to *T. evansi* as measured by fecundity. I found that, on average, *T. evansi* increase their oviposition rate by approximately one egg per day on tissues previously infested by conspecifics, corresponding to an increase in fecundity of 9 - 12% each day. However, this benefit varied considerably among studies, ranging from strongly positive to none at all, or even slightly negative. Some of this variation could be explained by the time that spider mite populations had been cultured in laboratories before being used in experiments, such that weaker benefits were found when populations had been maintained in the laboratory for longer time. These results indicate that *T. evansi* adapted to culture conditions, and that the net effect of suppression of plant defence on the performance of spider mites in subsequent infestations is subject to phenotypic change over generations. Consequently, *T. evansi* populations harbour heritable variation for their plant-mediated interactions with other herbivores, and thus have the potential to adapt to plant-mediated ecological interactions.

The meta-analysis in CHAPTER 2 quantifies the average benefit of defence suppression for *T. evansi*, but also confirms previously published assertions regarding potential ecological costs. Specifically, I found that induction of tomato defences by defence-inducing genotypes of the spider mite species *Tetranychus urticae* decreased the fecundity of spider mites in subsequent infestations by approximately one egg per day. This confirms that the presence of *T. urticae* can pose an ecological cost to *T. evansi* through its effects on plant defence. Moreover, I found that *T. urticae* also attained an increase in fecundity of one egg per day from defence suppression by *T. evansi*. This shows that lowered tomato defences are profitable for spider mites in general, and that, on average, defence suppression increases not only the performance of *T. evansi* itself, but also that of its competitors.

The phenotypic change in plant-mediated interactions with other spider mite populations observed in CHAPTER 2 provides indirect evidence for intraspecific variation in defence suppression within *T. evansi* populations. To measure intraspecific variation in defence suppression more directly, in CHAPTER 3 I investigate *T. evansi* populations from several locations around the world. Because the costs of defence

suppression may depend on biotic interactions with competitors and natural enemies, I expected that *T. evansi* from different environments would suppress plant defences to varying levels. By measuring the expression of several marker genes for tomato defence induction, I found significant variation among *T. evansi* populations in the degree to which they suppress jasmonic acid-dependent and salicylic acid-dependent defence pathways. This complements the findings of CHAPTER 2, by showing that variation for defence suppression is not only present within, but also among *T. evansi* populations.

CHAPTERS 2 and 3 demonstrate that *T. evansi* populations harbour intraspecific variation for defence suppression within and among populations. I then hypothesised that manipulating the benefits of defence suppression through experimental evolution could expose its costs. Specifically, in environments where inducible plant defences are either absent or constitutive, I expected *T. evansi* to lower its level of defence suppression through drift or through selection against metabolic costs. Experimental evolution, however, requires a source population with sufficient genetic variation. I hypothesized that a cross between *T. evansi* mites from genetically differentiated lineages would produce such a genetically diverse population. Therefore, in CHAPTER 4, I perform reciprocal crosses between *T. evansi* from two genetically differentiated lineages. First, I confirmed that offspring suffered substantial post-zygotic hybrid breakdown, by showing that only ~5% of hybrid F2 offspring hatched. Then, by genotyping viable and inviable hybrid offspring at eight microsatellite loci, I showed that Bateson-Dobzhansky-Muller incompatibilities underlie hybrid breakdown among these lineages. Moreover, I also found that viable hybrids contained recombined genetic material, and that heterosis contributes to their viability.

After establishing a genetically diverse hybrid *T. evansi* population, in CHAPTER 5 I expose this population to experimental evolution on host plants with an altered inducible jasmonic acid-dependent defence response. I used *defenseless-1* (*def-1*) tomato mutants in which JA-dependent inducible defences are absent, the *35S::prosystemin* (PS) tomato genotype in which these defences are constitutively overexpressed, and *Phaseolus vulgaris* bean as a distantly related host with different defences. I expected that in the absence of inducible defences (*def-1*), when suppression of plant defences is not possible because they are constitutively overexpressed (PS), or because they are regulated differently than in tomato (bean), suppression is not necessary and would erode through drift or be selected against because of metabolic costs. I maintained replicate populations of the hybrid *T. evansi* base population for approximately 60 generations in these three environments and on wildtype controls, and then measured the performance of these mites on wildtype tomato, *def-1*, PS and bean. I found that mite fecundity showed a general response to selection, but that fecundity on wildtype tomato remained high and was similar among all evolved strains. In line with these results, the degree to which wildtype tomato plants infested with these mites accumulated phytohormones and expressed defence gene markers was also similar among these strains. This shows that evolved strains retained their level of tomato defence suppression, and that metabolic costs of defence suppression are likely low.

In conclusion, I found that *T. evansi* can attain a considerable fecundity benefit by suppressing the inducible defences of their tomato hosts. However, competing herbivores can impose considerable costs on this offensive strategy, because they can also benefit from the suppressed defences of a shared host plant, and can subsequently induce these defences to the disadvantage of *T. evansi*. Furthermore,

suppression of plant defences is variable within and among *T. evansi* populations, and metabolic investments required to produce defence-suppressing effectors are likely low. Although these conclusions help to better understand the evolution of defence suppression by herbivores, several open questions remain. In CHAPTER 6, I highlight gaps in our knowledge of defence suppression by *T. evansi*, such as its effect on the recruitment of natural enemies, the range of host plant species in which *T. evansi* can suppress defences, and whether aspects of plant quality other than defence, such as nutrient concentration, are also manipulated. Lastly, I provide three detailed suggestions for further research that could answer some of these open questions.