



## Patterns in parasitism frequency explained by diet and immunity

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**We sought to explain patterns in parasitism frequency of two specialist herbivores (Geometridae) by investigating the influence of plant diet as a source of variation in immune response variables important for defense against parasitism. Field collected caterpillars (*Eois apyraria* and *Eois nympha*) were assigned to one of two species in the plant genus *Piper* (Piperaceae): 1) a host species with high diversity of defensive chemistry, *P. cenocladum* C.DC., or 2) a host species with lower investment in chemical defense, *P. imperiale* C.DC. Hemolymph was extracted from fifth instar larvae, and immune strength measured unidimensionally using a phenoloxidase (PO) enzyme assay. Parasitism data came from 19 yr of accumulated host plant–caterpillar–parasitoid associations from a long-term rearing project at La Selva Biological Station in Costa Rica, where the experiment took place. We found that immunity was significantly weakened when caterpillars were reared on the host plant with higher phytochemical diversity (*P. cenocladum*). Moreover, host plants inducing a weak immune response hosted caterpillars with higher parasitism rates. We conclude that patterns in parasitism frequency can be partially explained by cascading effects of host plant traits.**

Variation in the immune response of insects has been linked to host plant chemistry (Smilanich et al. 2009a, Lampert and Bowers 2015). Since the strength of the immune response is an important predictor of parasitoid success (Smilanich et al. 2009b), we hypothesized that patterns in parasitism rates between herbivore species can be partially explained by host plant derived variation in the immune response. To link variation in host plant chemistry and immunity to higher trophic levels, we used a long-term lepidopteran rearing dataset which includes parasitism frequency for individual caterpillar species to estimate differential parasitism rates in two *Piper* (Piperaceae) specialist caterpillars (*Eois apyraria* and *Eois nympha*) (Dyer et al. 2012, Supplementary material Appendix 1). These two caterpillars were then reared on two species of *Piper* that have distinct phytochemistry, differing in both concentration and composition (*Piper imperiale* and *Piper cenocladum*) (Richards et al. 2015, Supplementary

material Appendix 1), and measured the activity of the enzyme, phenoloxidase, a key enzyme in the immune response of the melanization cascade (Beckage 2008, Gonzalez-Santoyo and Cordoba-Aguilar 2012). Prior work with these two species of caterpillars showed that amount of melanization on a bead injected into the hemocoel was a strong predictor of parasitism success (Smilanich et al. 2009b). If parasitoid success can be linked to variation in the immune response as measured by PO enzyme, which in turn can be linked to differences in host plant traits, such as phytochemical diversity (Richards et al. 2015), then plants, herbivores, and parasitoids are linked in a tri-trophic relationship where variation in the defensive chemistry of plants act as bottom-up determinants of biodiversity by modifying herbivore responses and affecting parasitoid abundance and richness (Bukovinszky et al. 2009).

For the caterpillar species studied here, (*Eois apyraria* and *Eois nympha*, Geometridae: Larentiinae), the PO activity was significantly stronger when fed *Piper imperiale* foliage compared to *P. cenocladum* foliage ( $F_{1,95} = 10.31$ ,  $n = 98$ ,  $n = 0.001$ , Fig. 1A), and the interaction between *Piper* species and the *Eois* caterpillars was non-significant ( $F_{1,91} = 2.39$ ,  $n = 98$ ,  $p = 0.125$ ), although the raw effect size of host on *E. nympha* is greater than that on *E. apyraria* (Fig. 1A). This difference represented a 46% decrease in immunity for caterpillars on the poorer host. Concomitant with the strength of the immune response, we found that parasitism frequency (from the long-term rearing dataset) was higher on *P. cenocladum* (12%) compared to *P. imperiale* (1.7%) (parasitism by *Piper* species association,  $X^2 = 30.58$ ,  $DF = 1$ ,  $p < 0.001$ ; Fig. 1B), supporting our hypothesis that the differences in parasitism rates are associated with variation in the herbivore immune response. This raw effect size is considered a very strong decline in parasitism compared to other studies that report ecological effects on parasitism rates for macrolepidopterans (Stireman et al. 2005), and these studies indicate that such changes in parasitism result in substantial ecological consequences (Stireman et al. 2005). Although we do not know the exact mechanism by which the efficacy of the immune response is altered, the variation in the immune response is possibly due to the difference in

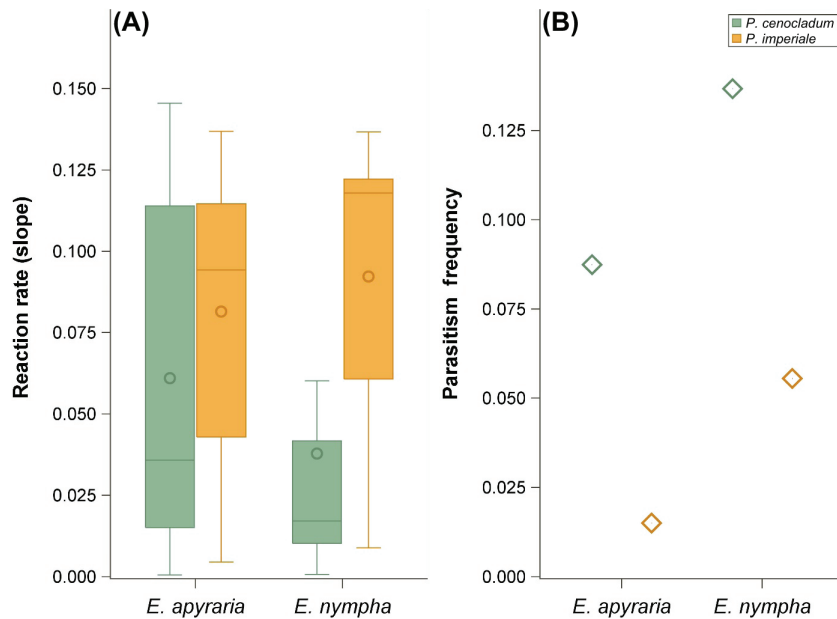


Figure 1. (A) Differences in the immune response of *Eois* caterpillars feeding on the two *Piper* host plants as measured by the reaction rate (enzyme activity) of the phenoloxidase enzyme assay. *Piper cenocladum* has a higher diversity of phytochemistry compared to *P. imperiale*. Means are represented by circles within the box plots and medians are the straight lines. (B) Parasitism frequency of the two caterpillar species corresponded to the immune data from the host plants, such that host plants that induced a poor immune response had higher parasitism frequencies. Parasitism data is from a 19-yr data set of accumulated interactions between host plant, caterpillar, and natural enemies at La Selva Biological Reserve.

phytochemistry between the *Piper* species (Dodson et al. 2000, Fincher et al. 2008, Richards et al. 2015). Richards et al. (2015) measured phytochemical diversity using a metabolomics approach and found that *P. cenocladum* had higher phytochemical diversity (Simpson diversity index = 0.92) compared to *P. imperiale* (Simpson diversity index = 0.84). Another important phytochemical difference between these two *Piper* species is the dramatic concentration difference at which specific secondary metabolites (amide and imide compounds) are present (*P. cenocladum* = 1.068% versus *P. imperiale* = 0.029%; 189% difference). Prior data with *P. cenocladum* showed that parasitism frequency was higher when *Eois* caterpillars were fed experimental diets that had a mixture of *P. cenocladum* secondary metabolites added to the leaf surface compared to control diets without secondary metabolites added (Richards et al. 2010). In that experiment, the strength of the immune response was untested, but we can now hypothesize that the differences in parasitism rates were a result of phytochemical effects on the performance of the immune response.

*Eois nympha* is usually found on *P. cenocladum* and rarely found on *P. imperiale*, while *E. apyraria* can be found equally on both hosts. Overall, *E. apyraria*, the species with a broader breadth of *Piper* hosts, had a significantly higher PO activity than *E. nympha* ( $F_{1,95} = 8.85$ ,  $n = 98$ ,  $p = 0.003$ , Fig. 1A). As with host plant effects, the parasitism frequency corresponded to the strength of the immune response, with *E. apyraria* having a lower frequency (4.0%) compared to *E. nympha* (13.2%) (parasitism by *Eois* species association,  $X^2 = 24.96$ ,  $DF = 1$ ,  $p < 0.001$ ; Fig. 1B). This may be the result of more generalized *Piper* herbivores encountering

a higher diversity of secondary metabolites since they feed on more plant species, and thus able to efficiently detoxify compared to monophagous herbivores whose constrained diet exposes them to a narrow range of phytochemistry (Smilanich et al. 2011).

In conclusion, we uncovered patterns consistent with the hypothesis that host plant effects can cascade to the third trophic level via the insect immune response. Whether this was a direct result of phytochemistry has yet to be elucidated. Nonetheless, these results are important for understanding patterns in parasitism rates in herbivore populations through the interactive effects of plants and predators.

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.98b31>> (Hansen et al. 2016).

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Supplementary material (Appendix ECOG-02498 at <[www.ecography.org/appendix/ecog-02498](http://www.ecography.org/appendix/ecog-02498)>). Appendix 1.