

Ecography

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Supplementary material

1 **Appendix 1**

2 **Materials and Methods**

3 *Study System*

4 *Piper*—The plant genus *Piper* (Piperaceae) contains over 2,000 species pantropically, with 1,500
5 species in the Neotropics and 50 described species at our study site in Costa Rica, La Selva
6 Biological Station (Jaramillo et al. 2008). The *Piper* species in this study, *Piper imperiale* C.DC.
7 and *Piper cenocladum* C.DC., grow in the understory of this tropical wet forest as small or mid-
8 canopy shrubs. The phytochemistry of both species has been carefully investigated. *Piper*
9 *imperiale* contains two imides: 5'-desmethoxydihydropiplartine, piplaroxide, an alkene, and at
10 least five different sesquiterpenes (Fincher et al. 2008). *Piper cenocladum* contains the following
11 amides: piplartine, 4'-desmethylpiplartine, and cenocladamide (Dodson et al. 2000). In addition
12 to differences in the chemical profiles, the total amide/imide concentrations differ, with *P.*
13 *cenocladum* containing concentrations as much as 10x higher than *P. imperiale* (0.029% vs.
14 1.068%) (Dodson et al. 2000, Fincher et al. 2008). A recent analysis using NMR data of forty-
15 three *Piper* species from La Selva found that *P. cenocladum* had higher phytochemical diversity
16 compared to *P. imperiale* (Richards et al. 2015).

17 *Eois*—*Eois* (Geometridae) caterpillars are specialists on *Piper* hosts (Dyer and Palmer 2004).
18 The species used here, *Eois apyraria* Guenee and *Eois nympa* Schaus, are both specialists on *P.*
19 *imperiale* and *P. cenocladum*. There is a difference in the degree of specialization for these
20 caterpillars, with 62% (612 individuals) of *E. apyraria* rearings on *P. imperiale*, and 38% (332
21 individuals) on *P. cenocladum*, while *E. nympa* is found mostly on *P. cenocladum* (96%; 884
22 individuals) and rarely on *P. imperiale* (4%; 32 individuals) (Dyer et al. 2012).

23 *Long-term rearing dataset*

24 The rearing dataset at La Selva spans a 19 year time period (1996-2015) and includes the
25 associations between host plant, caterpillar, and parasitoid (Dyer et al. 2012). Caterpillars are
26 field collected then reared in plastic bags or cups with host plant material added ad libitum. The
27 caterpillars are kept in an outdoor open air laboratory and checked daily for events of parasitism,
28 pupation, eclosion to adult, or death due to unknown causes. Parasitism frequency is calculated
29 as the number of reared parasitoids divided by the total number of reared adults plus parasitoids
30 (parasitoids/adults + parasitoids). Only the results of adult moth and adult parasitoid or parasitoid
31 pupa are included in the calculation of parasitism frequency. Caterpillars were determined to be
32 parasitized when either parasitoid larvae, cocoons, pupae, or adults were found in the bag for
33 cup, and the caterpillar was dead. For the *Eois* species used here, all the reared parasitoids
34 emerged from the larvae at fourth or fifth instar (we used fifth instar larvae to measure PO
35 activity). There were no incidences of egg or pupal parasitoids. For the analyses in this paper,
36 parasitism and adulthood data for *E. apyraria* and *E. nympa* were used. For *E. apyraria*, we
37 have records of a total of 550 individuals reared to adulthood or parasitized; 528 were reared to
38 adulthood and 22 were parasitized. Of these, we reared 167 to adulthood on *P. cenocladum* and
39 16 were parasitized on *P. cenocladum*. On *P. imperiale*, there were 327 adults, and 5 parasitism
40 events. For *E. nympa*, we have a total of 397 individuals reared to adulthood or parasitized; 339
41 were reared to adulthood and 58 were parasitized. Of these, we reared 303 to adulthood on *P.*
42 *cenocladum* and 48 were parasitized. On *P. imperiale*, there were 17 adults and 1 parasitism
43 event.

44

45 *Experimental Design*

46 The experiment took place during the wet season June through August 2013 at La Selva
47 Biological Station. Field-collected *E. apyraria* and *E. nympa* were reared on their natal hosts or
48 in some cases assigned to the alternate host to balance sample size. The caterpillars were placed
49 in small cups with a cutting of their assigned *Piper* treatment. The caterpillars were kept at an
50 ambient temperature, under a mesh sheet to simulate their natural understory environment.
51 Individuals were collected at all instars and reared in ambient lab conditions until 5th instar
52 (captivity before bleeding ranged from 2 days to 10 days). All individuals were bled within 24hrs
53 after molting into 5th instar. From *Piper cenocladum* we collected 37 *Eois nympa* and 15 *E.*
54 *apyraria*. From *P. imperiale*, we collected 9 *E. nympa*, and 37 *E. apyraria*. In the majority of
55 cases, caterpillars were reared on their natal host plant they were collected from in the field
56 (N=62/98). However, in order to balance the sample size for each *Eois* species on each *Piper*
57 host plant, some individuals were transferred to the alternative host (N=36/98). Of the
58 individuals that were transferred to a new host 29 were *E. apyraria* and 7 were *E. nympa*.

59 For spectroscopy analysis of phenoloxidase, one μL of hemolymph was obtained by
60 bleeding each caterpillar at its fifth instar. This hemolymph was stored in 25 μL of a phosphate-
61 buffered saline solution and frozen until analysis. ProPO was converted into the PO form by
62 adding 7 μL of α -chymotrypsin and incubating at room temperature, 25 °C, for 20 minutes. This
63 solution was then added to 0.9 mL of L-Dopa, 15 mM, and analyzed at 490nm on a Helios Beta
64 Spectronic Unicam spectrophotometer. Each assay run for PO contained approximately equal
65 number of samples across treatment groups. Absorbance readings were taken every two minutes
66 over a 40 minute period. The reaction rate was linear for all samples between 0 and 8 minutes.
67 Thus, we used reaction rate (slope) from the first 8 minutes of the reaction as the response
68 variable for all statistical analyses.

69

70 **Statistics.** The residuals were normally distributed, thus we utilized standard Analysis of
71 Variance (ANOVA). First, we tested a saturated model which included *Eois*, *Piper*, host
72 switching, and their interactions along with associated parameters and AIC values – but due to
73 insufficient degrees of freedom the 3-way interaction was not included in this model. All
74 interactions were non-significant and were removed from the model to yield a single, simple
75 model that best represented our original hypotheses. The results of that model (which also had
76 the lowest AIC value) are reported here. We found that switching hosts did not significantly
77 affect the PO activity ($F_{1,91} = 0.07$, $N = 98$, $P = 0.792$) nor were there significant interactions
78 between the main effects, *Eois* and *Piper* ($F_{1,91} = 2.39$, $N = 98$, $P = 0.125$) or between switching
79 host plants and the other independent variables (*Eois* × host switch: $F_{1,91} = 1.01$, $N = 98$, $P =$
80 0.317 ; *Piper* × host switch: ($F_{1,91} = 0.65$, $N = 98$, $P = 0.422$), so these terms were not included in
81 the ANOVA model reported in the paper. This parsimonious model had the lowest AIC
82 compared to the model with all 2-way interactions (delta AIC = 1), as well as the model with the
83 3-way interaction (delta AIC = 15.1), and since our intent was to focus on main effects of host
84 and caterpillar species, we report results from the more parsimonious model. We used chi-square
85 contingency tables to examine associations between parasitism frequency, caterpillars and hosts.

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