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ORIGINAL PAPER

Preference for high concentrations of plant pyrrolizidine alkaloids in the specialist arctiid moth *Utetheisa ornatrix* depends on previous experience

Adam Hoina · Carlos Henrique Zanini Martins ·
José Roberto Trigo · Rodrigo Cogni

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Abstract Secondary metabolites are one the most pervasive defensive mechanisms in plants. Many specialist herbivores have evolved adaptations to overcome these defensive compounds. Some herbivores can even take advantage of these compounds by sequestering them for protection and/or mate attraction. One of the most studied specialist insects that sequesters secondary metabolites is the arctiid moth *Utetheisa ornatrix*. This species sequesters pyrrolizidine alkaloids (PAs) from its host plant, the legume *Crotalaria* spp. The sequestered PAs are used as a predator repellent and as a mating pheromone. We used this species to test larval preference for different concentrations of PAs. We purified PAs from plant material and added them at different concentrations to an artificial diet. Larvae of *U. ornatrix* previously feeding on low and high PA concentration artificial diets were allowed to choose between two new artificial diets with different PA concentrations. The amount of PAs sequestered and larval preference were dependent on their previous exposure to low or high PA content in the diet. Larvae that were pretreated with a low PA diet significantly consumed more diet with the high PA concentration, while larvae that were pretreated with a high PA diet showed no discrimination between future feeding of different PA concentration diets. We discuss our results using mechanistic and evolutionary approaches. Finally, we discuss how these results have important implications on the evolution of plant herbivore interactions and how specialist herbivores may decrease the levels of chemical defenses on plant populations.

Keywords Coevolution · *Crotalaria* · Chemical defense · Diet choice · Mating choice · Taste receptors

Introduction

Secondary metabolites are one the most pervasive defense mechanisms in plants (Rosenthal and Berenbaum 1992). Due to the long-term evolutionary association between insects and plants, herbivores are considered an important driving force for the production and great diversity of these substances (Ehrlich and Raven 1964). In order to counteract the chemical defense of plants, herbivores have evolved different behavioral and physiologic mechanisms to better exploit their host plants (e.g., feeding specialization, detoxification, sequestration, gall forming) (Rausher 2001; Karban and Agrawal 2002). The effectiveness of these plant defenses and the subsequent herbivore counter responses is dependent on the level of specialization of the interaction. In most cases, specialist insect herbivores are less affected by plant chemical defenses from the hosts they are adapted to than non-specialist herbivores (van Dam et al. 1995; van der Meijden 1996, but see Agrawal and Kurashige 2003 and references therein).

Among the variety of chemical defenses in plants, pyrrolizidine alkaloids (PAs) encompass a group of about

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A. Hoina · R. Cogni (✉)
Department of Ecology and Evolution, Stony Brook University,
650 Life Sciences Building, Stony Brook, NY 11794-5245, USA
e-mail: rcogni@life.bio.sunysb.edu

C. H. Z. Martins · J. R. Trigo
Laboratório de Ecologia Química, Departamento de Biologia
Animal, Instituto de Biologia, Unicamp, Caixa Postal 6109,
Campinas, São Paulo 13083-970, Brazil

R. Cogni
Department of Genetics, University of Cambridge, Downing
Street, Cambridge CB2 3EH, UK

360 chemical structures with restricted occurrence in higher-plant taxa (Hartmann 1999). Pyrrolizidine alkaloids have deterrent and toxic effects on a variety of non-specialist insect herbivores (see reviews in Macel 2011; Trigo 2011). On the other hand, several specialist herbivores are adapted to feed on PA-containing host plants as well as to sequester PAs (e.g., ithomiine and danaine butterflies, arctiid moths, some leaf beetles) (see reviews in Nishida 2002; Trigo 2000, 2008, 2011; Macel 2011). PA sequestration provides advantages in terms of survival and reproductive success for adults and their offspring (Eisner and Meinwald 1995; Brown 1984; Pasteels et al. 2001). What often distinguishes PA-specialist and non-specialist herbivores that specialists are able to sequester these toxic compounds. Plants usually store the PAs in their *N*-oxide non-toxic form (Hartmann and Ober 2008).

One of the most studied PA-specialist insects is the arctiid moth *Utetheisa ornatrix*, the larvae of which feed on leaves and unripened seeds of several species of PA-containing plants of the genus *Crotalaria* (Fabaceae: Papilionoideae: Crotalarieae) (Eisner and Meinwald 1995; Ferro et al. 2006; Guimarães et al. 2006; Cogni and Futuyma 2009; Cogni 2010a, b; Cogni et al. 2011). This insect species sequesters PAs from their host plants and uses them as a predator deterrent through their entire development as well as against egg parasitoids (see for reviews Eisner and Meinwald 1995; Trigo 2011). Eisner and Meinwald (1995) reviewed the role of PAs and their hydroxydanaidal-derived pheromone in the mating behavior of *U. ornatrix*. They showed that males with high concentration of these compounds are selected by females since the content of hydroxydanaidal in male coremata allows the females to assess the amount of PAs in the male body that will be transferred to them by mating, thus providing the best defense for themselves and their offspring (Kelly et al. 2012).

The specialist *U. ornatrix* can consume PAs in large quantities and for a prolonged period in laboratory experiments without significant reduction of larval or adult fitness (Cogni 2010a; Cogni et al. 2012). Similarly, in the related species *Estigmene acrea*, consumption of PAs does not negatively affect larval performance (Hartmann et al. 2005). Larvae of *U. ornatrix* and related species have taste receptors specific to PAs that enable the detection of a wide range of concentrations of these compounds (Bernays et al. 2003a, 2004). In *E. acrea*, the gustatory response by these receptors is lost after prolonged feeding in PA-containing plants or injection of PA into the hemolymph (Bernays et al. 2003b). In the wild, females of *U. ornatrix* oviposit on leaves, and the larvae can walk about the plant. The larvae feed generally on unripe seeds and less often on leaves. In one of the main host plants, *Crotalaria pallida*, unripe seeds have an average of 0.24 μg/mg of PAs and leaves have only 0.054 μg/mg (Ferro et al. 2006). Larvae reared exclusively on seeds in the laboratory sequester around 15 times more PAs than larvae reared exclusively on leaves (Ferro et al. 2006). There is also great variation in PA content among other *Crotalaria* species used as hosts (J.R. Trigo, personal communication). Therefore, it is of considerable relevance to understand how this specialist moth behaviorally responds to different concentrations of PAs in the diet and if such response is influenced by the previous level of PAs in its body.

Since all developmental stages of *U. ornatrix* are PA dependent to some extent, and these compounds are sequestered as larvae, we hypothesized that previous feeding on a low PA-content diet will lead larvae to choose a high PA-content diet to supply their PA-deficient tissues. However, larvae feeding previously on a high PA-content diet may not need to make such a choice since PAs in their tissues may be enough for defense and courtship.

In order to test our predictions, we purified PAs from plant material and added them at different concentrations to an artificial diet. Two groups of larvae, one previously feeding on high and the other on low PA concentration artificial diets, were later allowed to choose between two artificial diets with different PA concentrations.

Methods

PA extraction

PAs were extracted from the leaves and flowers of *Senecio brasiliensis* (Asteraceae) as free bases following Trigo et al. (1993). GS-MS and GC-FID analysis confirmed that these PAs consisted of a mixture of senecionine-type PAs: 69 % of integerrimine (relative abundance of ~69 %), retrorsine (27 %), and senecionine (4 %). These are the same type of PAs (senecionine-type) found in the unripe seeds of *C. pallida* (usaramine ca. 85 % and integerrimine ca. 15 %), the most common host of *U. ornatrix* (Ferro et al. 2006; Cogni and Futuyma 2009; Flores et al. 2009; Cogni 2010a, b). Other *Crotalaria* species, such as *C. incana* and *C. micans*, with integerrimine as the main PA (Flores et al. 2009), are also used as the host plant by *U. ornatrix* in the Neotropics (Cogni 2010b, J. R. Trigo personal communication). We used *S. brasiliensis* as the PA source (Trigo et al. 2003) since the yield of these alkaloids is higher than in *C. pallida* seeds (J.R.Trigo personal communication). In addition, *S. brasiliensis* is not a host of *U. ornatrix*, decreasing the chance of carryover of non-PA feeding stimulants. To test how pure the PA extract was, we used a colorimetric method (Trigo et al. 1993) to quantify the amount of PAs in replicated weighted amounts of the extract compared to weighted amounts of the reference pure crystallized monocrotaline. There were no significant difference in colorimetric readings of the extract and the reference substance, indicating that the extract was 100 % pure.

Diet preparation and rearing system

The diet was made using *Phaseolus* beans (Signoretti et al. 2008), which were cooked and blended with PAs dissolved in soy oil. Diet PA concentrations included 1X (0.024 % dry weight = the same concentration found in seeds of *C. pallida*; Ferro et al. 2006), 5X (0.12 %), and 100X (2.4 %). The diet was cooked and poured into their respective test tubes. The tubes were covered and allowed to sit for 1 day at room temperature to assure the diet was of proper (dense enough) consistency. For the pretreatment, 3 ml of the diet was put in 10-ml test tubes; for the choice tests, 0.6 ml of the diet was put in 2-ml microcentrifuge tubes (these amounts were similar to all tubes and treatments). We used a colorimetric method (Trigo et al. 1993) to quantify the PAs in the diet at time zero (just after diet preparation) and after 1 week ($n = 10$ per concentration per time); there was no significant difference between the expected concentration and the observed concentration in the diet. Also, there was no difference between the concentration of PAs between time zero and after 1 week, indicating that no degradation has taken place in quantitative terms. We also used GC/MS to show that no qualitative degradation has taken place in the diet.

Larvae were bred in the laboratory from *U. ornatrix* adults collected in Campinas, SP, Southeastern Brazil (22°47′S, 47°04′W). One male and one female were confined to paper cages, fed a 5 % honey solution, kept in an incubator at 29 °C, and allowed to mate. Females laid clustered eggs on the paper walls, which were cut out from the cages. The egg clusters were placed in separate containers and allowed to hatch. Newly hatched larvae were randomly assigned to either of two pretreatments: those reared on 100X (high PA concentration) or those on 1X (same PA concentration as the host plant) diet until the experiment commenced.

PA quantification in larvae, feces, and coremata

To test the effects of the 1X diet versus the 100X diet pretreatments, we quantified the amount of PAs sequestered in the larvae, the amount of PAs in larval feces, and the amount of PAs in coremata of the males that have previously fed on each of the two diets. The total PAs in larvae and feces were quantified by a colorimetric method as in Trigo et al. (1993). The amount of hydroxydanaidal in coremata was quantified by GC–MS with the selective ion monitoring (SIM) method, using a Hewlett Packard 6890 gas chromatograph coupled with an HP-5973 mass selective detector equipped with a HP-5 column (30 m × 0.32 mm × 0.25 μm). The GC–MS conditions were as follows: injection temperature 250 °C; temperature program: 70–150 °C, 4 °C/min, 150–300 °C, 30 °C/min; splitless, carrier gas He 1.2 mL/min; monitored ion (m/z): 151. The comparison of PA and hydroxydanaidal amounts between the two diets was carried out using an independent t test. The amount of hydroxydanaidal was given by peak abundance of m/z 151 ion. Sample size per treatment was 10 larvae, 10 larval feces (collected daily from first larval instar until pupation), and four coremata.

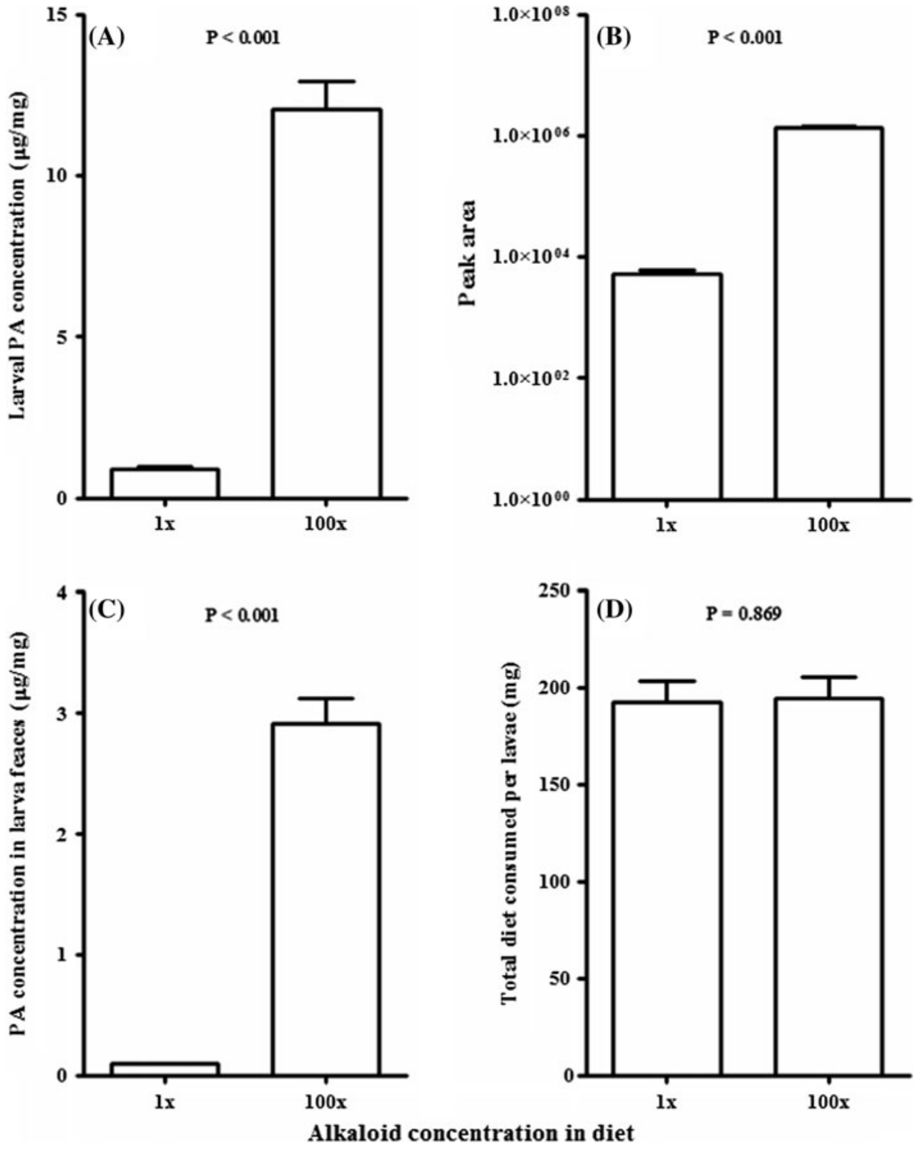
Choice experiment

Larvae in their fourth instar (average dry weight = 33 mg) were subjected to choice tests. The larvae were individually placed in 2-ml microcentrifuge pre-weighted tubes containing two different alkaloid concentration diets. The concentrations tested were 5X versus 1X, 100X versus 1X, and 100X versus. 5X. Each choice test was performed with larvae previously fed on the 1X and 100X diets. Sample sizes varied from 52 to 55 individuals per choice test per pretreatment group. The larvae were placed within the tubes and the open ends of the tubes were bound together by tape so that the larvae were able to choose between the two diets. It was indeed noted that larvae sampled both diets during the experiment. After 2 days, the larvae were removed from the microcentrifuge tubes and the tubes were reweighed to access the amount of diet consumed of each concentration. The initial amount of the diet was large enough to avoid that the larvae finish the diet during the 2-day period. Under the test conditions, water evaporation during the 2-day period caused variation in diet weight of <1 %; the evaporation was similar for the different PA concentrations. For each choice test, the average amount of diet consumed (in mg) for each concentration was compared with a paired t test. Data were $\ln x + 1$ transformed to achieve a normal distribution.

Results

The amount of PA sequestered by larvae differed significantly between larvae that were previously exposed to low and high PA-content diets (Fig. 1a; $t = 12.764$, $df = 18$, $P < 0.001$); larvae previously reared on the 100X PA diet sequestered 13 times more PAs than larvae reared on the 1X PA diet. There was also a difference in the PA content of the coremata of males from the two pretreatments (Fig. 1b; $t = 16.082$, $df = 2$, $P < 0.001$); males from the 100X diet have on average 260 times more PAs in the

Fig. 1 Differences between larvae fed on a diet with 1X or 100X PA concentrations. **a** Concentration of sequestered PAs in larvae. **b** Amount of PAs in male coremata given by peak area of ion (m/z) 151. The *Y* axis is plotted in log scale. **c** Concentration of PAs in larval feces. **d** Total amount of diet consumed per larva in choice experiments. *Bars* show the average ± SE



coremata than males from the 1X diet. Not all PAs consumed in the diet were sequestered by larvae. Feces from larvae eating the 1X PA diet have an average PA concentration of 0.096 µg/g, while feces from larvae eating the 100X diet have an average of 2.918 µg/g (Fig. 1c; $t = 13.55$, $df = 18$, $P < 0.001$). Larvae that were previously exposed to low and high PA-content diets have similar appetites; the average total amount of diet consumed per larva did not differ between the 1X and 100X PA pretreatments (Fig. 1d; $t = 0.166$, $df = 318$, $P = 0.869$).

Larval dietary preference was dependent on their previous exposure to low or high PA-content in the diet. Larvae pretreated with a low PA diet (1X) consumed more diet with the high PA concentration compared to the low concentration. Larvae significantly preferred the 5X diet over the 1X (Fig. 2a; $t = 2.193$, $df = 52$, $P = 0.033$), and the 100X over the 1X (Fig. 2b; $t = 3.200$, $df = 54$, $P = 0.002$). The preference for the 100X over the 5X was marginally significant (Fig. 2c; $t = 1.989$, $df = 52$, $P = 0.052$). On the other hand, larvae that were pretreated with 100x PA concentration showed no discrimination between the different PA concentrations. The larvae consumed similar amounts of the 5X and 1X diet (Fig. 2d; $t = 0.866$, $df = 52$, $P = 0.390$), the 100X and 1X diet (Fig. 2e; $t = 0.872$, $df = 51$, $P = 0.387$), and the 100X and 5X diet (Fig. 2f; $t = 1.334$, $df = 53$, $P = 0.189$). During the choice tests, we observed the larvae walking between the two tubes and sampling both diets; in almost all cases, the larvae consumed measurable amounts of both diets.

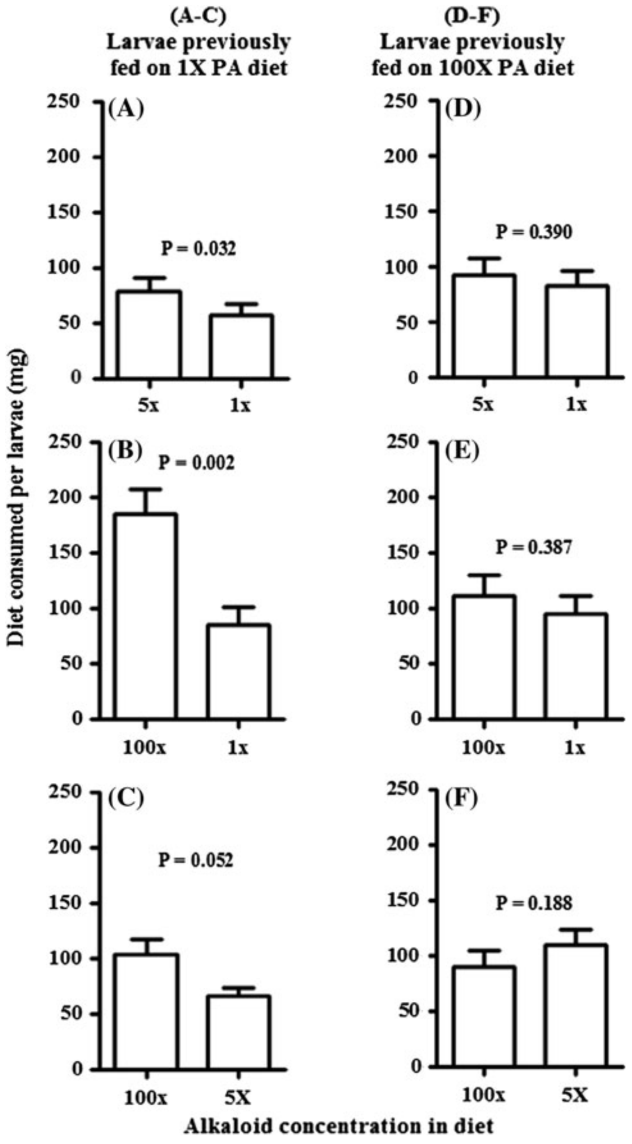


Fig. 2 Larval consumption of diets with different pyrrolizidine alkaloid concentrations in choice tests. *Bars* show the average amount of each diet consumed per larva ± SE. **a–c** Larvae were previously fed on a diet with 1X PA concentration. **d–f** Larvae were previously fed on a diet with 100X PA concentration

Discussion

Larvae that previously fed on a diet with low PA concentration presented low amounts of sequestered PAs in the tissue and showed a strong preference for the diet with the highest PA content in the choice experiments, while larvae that previously fed on a diet with high PA concentration presented high amounts of sequestered PAs in the tissue and showed no dietary preference. These findings agree with our predictions stated above. We will discuss such results using both mechanistic and evolutionary approaches, which are not mutually exclusive.

In terms of physiology, the preference results can be explained by the presence of a taste receptor neuron in each of the lateral and medial galeal styloconic sensilla in *U. ornatrix* larvae (Bernays et al. 2003a). These receptors exhibit a dose-dependent response to pyrollizidine alkaloids that enable the detection of PAs over a wide range of concentrations (Bernays et al. 2003a). The preference for higher PA concentrations seems to be adaptive because higher levels of sequestration provide higher protection against predators and higher mating success in males (Eisner and Meinwald 1995; Ferro et al. 2006; Kelly et al. 2012). In addition, sequestration in this species does not incur a fitness cost. In an experiment in which larvae fed during the entire development on diets with different PAs concentrations (including a diet with 100 times higher concentration), the larvae sequestered amounts of PAs proportional to the diet concentration previously consumed without any important negative effect on larval or adult fitness (Cogni 2010a, Cogni et al. 2012). It is interesting, however, the extent to which larvae prefer diets with PA concentrations well above that found in its main host plant, *C. pallida* (Ferro et al. 2006). PAs in unripe seeds of *C. pallida* can vary up to 10 times among individuals and populations (Cogni 2010b, J.R. Trigo personal communication), a range of variation comparable to our 1X to 5X treatments. However, in some localities, *U. ornatrix* can use alternate hosts with a higher PA concentration; for example, unripe seeds of *C. spectabilis* have an average of 4.6 % (SE = 0.4; $N = 9$) (J.R. Trigo & R. Cogni unpublished results).

When larvae were pretreated with the 100X diet there was no preference for either the high or low alkaloid diet, presumably because they were not able to distinguish between concentrations. How can this lack of larval preference in the high PA pretreatment be explained physiologically? Experiments on the highly polyphagous larvae of *E. acrea* have shown that extensive feeding on PAs or injection of PAs into the hemolymph can cause a loss in response of the PA-sensitive cells. This leads to a behavioral response causing reduced feeding on PA-containing foods for a few hours, possibly to allow physiologic handling mechanisms time to deal with the PAs (Bernays et al. 2003b). Our behavioral results suggest that a similar mechanism may occur in *U. ornatrix*, and we propose that future studies may address this hypothesis by electrophysiologic recordings of the receptors signal as in Bernays et al. (2003b). The suggested decrease in the neural responsiveness to high concentrations of PAs is unlikely to be under negative or positive selection in the specialist *U. ornatrix*. As posed above ingestion of high amounts of PAs are not costly, and larvae that were previously fed high PA concentrations may have enough PAs in their tissues for defense and mating. This response may be more likely explained as an ancestral trait selected in species that may be negatively affected by prolonged ingestions of PAs.

Even though we showed that previous ingestion of extremely high PA concentrations can result in a lack of preference, under the average PA concentrations found on the most common host plant (*C. pallida*), the larvae showed a strong preference for the highest PA concentration. This finding has important implications for the evolution of interactions among herbivores and their host plants. A strong preference for higher concentrations of secondary metabolites has important ramifications on how specialist herbivores act as agents of natural selection on the level of defenses in their host plants. Since there is an advantage to acquiring higher amounts of plant chemicals without costs (Cogni 2010a; Cogni et al. 2012), it is favorable for the herbivore to eat plants with the highest PA concentrations. Indeed, the present study shows that larvae of *U. ornatrix* present a strong preference for higher concentrations of PAs. From the host plant perspective, specialist herbivores act as agents of natural selection that lowers the levels of defensive chemicals in host plant populations (see van der Meijden 1996 for PAs, and Lankau 2007 for glucosinolates). This concept is seen in introduced populations of the weed *Senecio jacobaea* which lacked a specialist herbivore; these populations evolved increased levels of PAs and as a consequence increased their resistance to non-specialist herbivores (Joshi and Vrieling 2005). In another example, Lankau (2007) reported that non-specialist damage was negatively correlated to glucosinolate sinigrin concentrations in leaves of *Brassica nigra* (Brassicaceae), but specialist damage was positively correlated. The increase of sinigrin concentration was favored when specialists were removed, disfavored when non-specialists were removed, and selectively neutral when plants faced both generalists and specialists (Lankau 2007). These results strongly support the idea that specialist and non-specialist herbivores can exert opposing selection pressures on chemical defenses (van der Meijden 1996; Lankau 2007). Therefore, the balance of selective pressures from specialist and non-specialist herbivores must be considered an important factor in maintaining genetic variation of chemical defenses in natural plant populations.

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References

Agrawal AA, Kurashige NS (2003) A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae*. J Chem Ecol 29:1403–1415

Bernays EA, Chapman RF, Lamunyon CW, Hartmann T (2003a) Taste receptors for pyrrolizidine alkaloids in a monophagous caterpillar. J Chem Ecol 29:1709–1722

Bernays EA, Rodrigues D, Chapman RF, Singer MS, Hartmann T (2003b) Loss of gustatory responses to pyrrolizidine alkaloids after their extensive ingestion in the polyphagous caterpillar *Estigmene acrea*. J Exp Biol 206:4487–4496

Bernays EA, Hartmann T, Chapman RF (2004) Gustatory responsiveness to pyrrolizidine alkaloids in *Senecio* specialist, *Tyria jacobeae* (Lepidoptera: Arctiidae). Physiol Entomol 29:67–72

Brown KS (1984) Adult-obtained pyrrolizidine alkaloids defend ithomiine butterflies against a spider predator. Nature 309:707–709

Cogni R (2010a) Coevolution at the population level: empirical studies in an insect-plant interaction, Ph.D. dissertation. Stony Brook University, Stony Brook, NY

Cogni R (2010b) Resistance to plant invasion? A native specialist herbivore shows preference for and higher fitness on an introduced host. Biotropica 42:188–193

Cogni R, Futuyma DJ (2009) Local adaptation in a plant herbivore interaction depends on the spatial scale. Biol J Linn Soc 97:494–502

Cogni R, Trigo JR, Futuyma DJ (2011) Varying herbivore population structure correlates with lack of local adaptation in a geographic variable plant-herbivore interaction. PLoS One 6:e29220

Cogni R, Trigo JR, Futuyma DJ (2012) A free lunch? No cost for acquiring defensive plant pyrrolizidine alkaloids in a specialist arctiid moth (*Utetheisa ornatrix*). Mol Ecol. doi:10.1111/mec.12086

Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in coevolution. Evolution 18:586–608

Eisner T, Meinwald J (1995) The chemistry of sexual selection. Proc Natl Acad Sci USA 92:50–55

Ferro VG, Guimarães PR, Trigo JR (2006) Why do larvae of *Utetheisa ornatrix* penetrate and feed in pods of *Crotalaria* species? Larval performance vs. Chemical and physical constraints. Entomol Exp Appl 121:23–29

Flores AS, Tozzi AMGA, Trigo JR (2009) Pyrrolizidine alkaloid profiles in *Crotalaria* species from Brazil: chemotaxonomic significance. Biochem Syst Ecol 37:459–469

Guimarães PR, Raimundo RLG, Bottcher C, Silva RR, Trigo JR (2006) Extrafloral nectaries as a deterrent mechanism against seed predators in the chemically defended weed *Crotalaria pallida* (Leguminosae). Austral Ecol 31:776–782

Hartmann T (1999) Chemical ecology of pyrrolizidine alkaloids. Planta 207:483–495

Hartmann T, Ober D (2008) Defense by pyrrolizidine alkaloids: developed by plants and recruited by insects. In: Schaller A (ed) Induced plant resistance to herbivory. Springer, New York

Hartmann T, Theuring C, Beuerle T, Klewer N, Schulz S, Singer MS, Bernays EA (2005) Specific recognition, detoxification and metabolism of pyrrolizidine alkaloids by the polyphagous arctiid *Estigmene acrea*. Insect Biochem Mol Biol 35:391–411

Joshi J, Vrieling K (2005) The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores. Ecol Lett 8:704–714

Karban R, Agrawal AA (2002) Herbivore offense. Ann Rev Ecol Syst 33:641–664

Kelly CA, Norbutus AJ, Lagalante AF, Iyengar VK (2012) Male courtship pheromones as indicators of genetic quality in an arctiid moth (*Utetheisa ornatrix*). Behav Ecol 23:1009–1014

Lankau RA (2007) Specialist and generalist herbivores exert opposing selection on a chemical defense. New Phytol 175:176–184

Macel M (2011) Attract and deter: a dual role for pyrrolizidine alkaloids in plant-insect interactions. Phytochem Rev 10:75–82

Nishida R (2002) Sequestration of defensive substances from plants by Lepidoptera. Ann Rev Entomol 47:57–92

Pasteels JM, Termonia A, Windsor DM, Witte L, Theuring C, Hartmann T (2001) Pyrrolizidine alkaloids and pentacyclic triterpene saponins in the defensive secretions of *Platyphora* leaf beetles. Chemoecology 11:113–120

Rausher MD (2001) Co-evolution and plant resistance to natural enemies. Nature 411:857–864

Rosenthal GA, Berenbaum MR (1992) Herbivores: their interactions with secondary plant metabolites, 2nd edn. Academic Press, San Diego

Signoretti AGC, Nava DE, Bento JMS, Parra JRP (2008) Biology and thermal requirements of *Utetheisa ornatrix* (L.) (Lepidoptera: Arctiidae) reared on artificial diet. Braz Arch Biol Technol 51:647–653

Trigo JR (2000) The chemistry of antipredator defense by secondary compounds in neotropical Lepidoptera: facts, perspectives and caveats. J Braz Chem Soc 11:551–561

Trigo JR (2008) Chemical ecology of ithomiine butterflies. In: Epifano F (ed) Current trends in phytochemistry. Research Signpost, Kerala, pp 141–165

Trigo JR (2011) Effects of pyrrolizidine alkaloids through different trophic levels. Phytochem Rev 10:83–98

Trigo JR, Witte L, Brown KS, Hartmann T, Barata LES (1993) Pyrrolizidine alkaloids in the arctiid moth *Hyalurga syma*. J Chem Ecol 19:669–679

Trigo JR, Leal IR, Matzenbacher NI, Lewinsohn TM (2003) Chemotaxonomic value of pyrrolizidine alkaloids in southern Brazil *Senecio* (Senecioneae: Asteraceae). Biochem Syst Ecol 31:1011–1022

van Dam NM, Vuister LWM, Bergshoeff C, Vos H, van der Meijden E (1995) The “raison d’être” of pyrrolizidine alkaloids in *Cynoglossum officinale*: deterrent effects against generalist herbivores. J Chem Ecol 21:507–523

van der Meijden E (1996) Plant defense, an evolutionary dilemma: contrasting effects of (specialist and generalist) herbivores and natural enemies. Entomol Exp Appl 80:307–310