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Article

Pyrrolizidine Alkaloids in the Pericopine Moth *Scearctia figulina* (Erebidae: Arctiinae): Metabolism and Chemical Defense

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Pyrrolizidine alkaloids (PAs) are defensive compounds present in several plant families. However, some specialist herbivore insects have overcome these toxic compounds and sequester PAs converted to *N*-oxide as a defense against predators and a precursor of male sexual pheromones. In this context, we investigated PA sequestration by the specialist pericopine moth *Scearctia figulina* (Erebidae: Arctiinae), which feeds on leaves of *Heliotropium transalpinum* (Boraginaceae) as larvae. Additionally, we examined the role of PAs against different predators. The PAs sequestered from the host plant were metabolized by larvae and transferred to adults via two main pathways: (*i*) rinderine and its acetyl derivative (7*S*,3'*R*) were epimerized to intermedine (7*R*,3'*R*) and lycopsamine (7*R*,3'*S*), and (*ii*) insect PAs were biosynthesized from necine bases obtained from plant-acquired PAs, with necic acids of insect origin. Both metabolic products may be related to the biosynthesis of 7*R* male pheromone and to chemical defense. Larvae and adults were chemically protected against the spiders *Nephila clavipes* and *Lycosa erythrognatha* and the chick *Gallus gallus*, and this defense may be associated to PAs.

Keywords: callimorphine, *Heliotropium transalpinum*, insect PAs, lycopsamine, predation

Introduction

Pyrrolizidine alkaloids, particularly 1,2-dehydropyrrolizidines (hereafter PAs), are powerful defensive compounds in several plant taxa, such as Eupatorieae and Senecioneae (Asteraceae), Boraginaceae, and Crotalarieae (Leguminosae).[1,2] However, specialized herbivores, such as arctiine moths, danaine and ithomiine butterflies and chrysomeline beetles, among others, are able to cope with pro-toxic free-base PAs from these plants, converting them into non-toxic *N*-oxides.[3,4] These insects sequester PA *N*-oxides, incorporating them into their tissues, and this renders chemical protection against predators and parasitoids.[1,5] Arctiine moths and danaine and ithomiine butterflies also use PAs as precursors of male sexual pheromones, such as the dihydropyrrolizine hydroxydanaidal.[6-8] PAs were also recorded in the grasshopper *Zonocerus variegatus*, the aphid *Aphis jacobaeae*, the bug *Largus rufipennis*, and the coccide *Ceroplastes albolineatus*.[1] PA-containing insects are generally aposematic, i.e., their unpalatability is associated with warning signals alerting predators of this trait; warning signals can be visual, sonorous, or odorant.[9]

Among arctiine moths,[10] PAs were found in the tribes Amerilini and Arctiini (subtribes Callimorphina, Arctiina, Pericopina, Ctenuchina, Euchromiina, and Phaegopterina), but not in Lithosiini and Syntomini.[11] Three PA acquisition strategies were found in these moths: (*i*) PA-obtained as adults, (*ii*) PA-specialist feeding as larvae, and (*iii*) PA-generalist feeding as larvae.[11] In the first syndrome, adults either feeding on nectar containing PAs or display a pharmacophagous behavior, or obtaining the alkaloid visiting withered PA-containing plants.[2,12,13] This syndrome was widely found in Ctenuchina, Euchromiina, and Phaegopterina.[11] In the second, larvae are generally monophagous, feeding on a single PA-host plant genus, from which they sequester the alkaloids; subtribes Callimorphina and Pericopina shared this syndrome.[11,14] The third syndrome comprises polyphagous larvae, feeding both on non-PA and PA-host plants.[5,11] Species from the subtribe Arctiina showed this syndrome.[11] In the two last syndromes, adults can also sequester these alkaloids, even when feeding on PA-plants as larvae.[11]

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Other interesting PA pathways in arctiine moths are the *de novo* biosynthesis of insect ester alkaloids (insect PAs) and the stereochemical inversion of 7*R* configuration to 7*S* pyrrolizidine rings.[14-16] Both biochemical mechanisms are involved in the biosynthesis of dihydropyrrolizine male sexual pheromones.[16-19] Insect PAs are esters biosynthesized from plant-acquired necine bases with necic acids of insect origin, and it has been suggested that they are biosynthesized via transesterification of plant PAs.[19] In the insect PAs of the callimorphine type, necic acids are derived from isoleucine, while in the creatonotine type, they are derived from valine.[16,20] Callimorphine type PAs were found in the Arctiina *Arctia caja*, *Creatonotos transiens*, *Estigmene acrea*, and *Grammia geneura*, in the Callimorphina *Callimorpha dominula* and *Tyria jacobaea*, and in the Pericopina *Gnophaela latipennis* and *Hyalurga syma*.[14-16] PAs of the creatonotine type were found in the Arctiina *Creatonotos transiens*, *Estigmene acrea*, and *Grammia geneura*, and in the Callimorphina *Utetheisa ornatrix*.[16] Insect PAs were not found in the Callimorphina *Nyctemera annulata*.[21] No chemosystematic patterns of insect PAs seem to emerge from different subtribes of Arctiini. Stereochemical inversion must take place in Arctiini, since a general feature of male sexual pheromones seems to be the 7*R* configuration.[6-8,16-19]

Although Arctiini comprises around 11,000 species, only a small fraction was studied regarding PAs.[16,22] For instance, in Pericopina, a single species, *Hyalurga syma*, was studied in relation to PA profile and chemical defense.[14] This gap makes it difficult to search any patterns of PA acquisition syndromes or insect PA production in the arctiine phylogeny, i.e., it is difficult to infer about the evolution of PAs in the Arctiinae subfamily. We have observed the sequestration of PAs from host plants in the pericopine moth *Scearctia figulina*, adding more data to this poorly studied subject. Moreover, we carried out bioassays to test if *S. figulina* was protected against predation, as suggested for PA-containing insects.

Experimental

Studied organisms

The moth *Scearctia figulina* (Butler) (Erebidae: Arctiinae: Pericopina) is a Neotropical species that feeds on leaves of *Heliotropium transalpinum* Vell. (Boraginaceae) as larvae (Figure S1). Adults were found flying around their host plants. The genus *Heliotropium* has a Pantropical distribution with around 200 species, and *H. transalpinum* is a Neotropical species with wide distribution, from Mexico to Argentina, occurring in the open areas of Cerrado savanna and in tropical seasonal forests.[23,24] These species, as well as other Boraginaceae species, show PAs in their tissues.[14,25,26]

Scearctia figulina sampling and rearing

Gregarious moth eggs (87 ± 11 eggs, n = 10, mean ± standard error) were sampled in individuals of *H. transalpinum* in an open area near the Animal Biology Department, Institute of Biology at the State University of Campinas, Campinas, São Paulo, Brazil (22°49'15.38"S, 047°04'8.87"W). After eclosion, gregarious larvae were kept in plastic containers (18 cm high, 11 cm diameter, 10-15 larvae *per* container) until pupation in an incubator at 27 °C, L:D 12:12 photoperiod, with no relative humidity control. The containers were cleaned on a daily basis and old, eaten leaves of *H. transalpinum* were replaced by new, intact ones. Pupae were individualized in small containers (6 cm high, 5 cm diameter) until adult emergence. Eggs, fourth instar larvae, pupae, and adults of both sexes were sampled and immediately frozen at −20 ºC for PA analysis. The fourth instar larvae were sampled immediately after ecdyse to prevent feces in their midguts.

Pyrrolizidine alkaloid analysis

Ten samples of freeze-dried gregarious eggs, fourth instar larvae, pupae, and adults of both sexes reared in laboratory, and five samples of *H. transalpinum* leaves were quantified for PAs by colorimetric assay according to Trigo *et al.*[14] The alkaloids were characterized by gas chromatography-mass spectrometry (GC-MS) using a mass fragmentation pattern and van den Dool & Kratz retention index (see Table S1).[14,15,27-29] We assigned the absolute stereochemistry of PAs based only in retention index of chiral PAs reported in the literature.[20,27]

Chemical defense of *Scearctia figulina*: predation bioassays

The chemical defense in moths was investigated against three kinds of predators: the orb-weaving spider *Nephila clavipes* (Nephilidae), the wolf spider *Lycosa erythrognatha* (Lycosidae), and the chick *Gallus gallus* (Phasianidae). The two former are potential predators of *S. fugilina* in nature, and the latter is generally used as a predator model for visually hunting vertebrate predators.[30] The license for research involving wild animals was provided by IBAMA-ICMBio (Ministério do Meio Ambiente, Brazil). The Ethics Committee for Animal Use of the University of Campinas approved all experimental procedures. Chicks were donated to free range farms by the

end of the experiment. Bioassays followed experimental procedures of previous works from our research group.[15,31]

Statistical analysis

We checked if the PAs from the host plant *H. transalpinum* can explain the PAs in lab-reared *S. fugilina*, using a detrended correspondence analysis (DCA).[32] Additionally, we checked if PA concentrations differed among developmental stages of lab-reared moths using an one-way analysis of variance (ANOVA).[33]

We compared predator responses (prey or release) among three predators and larvae, pupae, and between adults of both sexes using two approaches: (*i*) comparing the response of three predators in relation to adults, and (*ii*) comparing the response of *L. erythrognatha* and *G. gallus* in relation to larvae. No bioassays with larvae of *N. clavipes* were carried out because their larvae may not be in contact with this predator in the natural environment. We analyzed the frequency of individuals preyed or released, using a generalized linear model (GLM) with binomial distribution and logit link function, using the package "bbmle" in R 3.1.0 for Windows.[34] In the first approach we performed a pair-pair comparison with Bonferroni correction,[33] using $\alpha = 0.05$ and k number of comparisons = 3; Bonferroni correction decreases the significant threshold to 0.0167.

Results

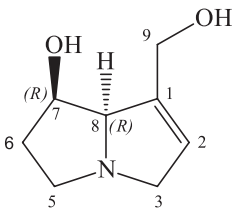
Pyrrolizidine alkaloids

We characterized 16 PAs in the system *H. transalpinum-S. fugilina*, and five were designated as unidentified (Table 1, Figure 1). The leaves of the host plant had predominantly riderine (IX, 7*S*,3'*R*) and 3'-acetylrinderine (XI), which together account for 90% of total PAs; supinine (IV, 3'*R*), 3'-acetylsupinine (VI), and 3'-acetylintermidine (X, 7*R,* 3'*R*) are present in low relative abundances (Table 1). However, moths did not show a similar profile. Only supinine was sequestered unchanged and maintained throughout the moths' life-cycle; traces of 3'-acetylintermedine were found only in larvae. The other host plant PAs were not present in moths. We observed that intermedine (VII, 7*R*, 3'*R*) and lycopsamine (VIII, 7*R*, 3'*S*) were the main alkaloids in the moths, reaching 70% of relative abundance; amabiline (V, 3'*S*), the necine base retronecine (I), and 7- and 9-senecioylretronecine-type PAs (II, III) were found in low amounts. Additionally, we found insect PAs of the callimorphine and creatonotine

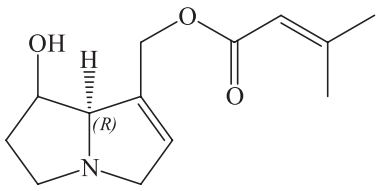
Table 1. Relative abundance of pyrrolizidine alkaloids (mean ± standard error) in developmental stages of *Scearctia figulina* and in the leaves of its host plant, *Heliotropium transalpinum*

Pyrrolizidine alkaloid[a]	RI[b]	$[M]^+$	Relative abundance / %					
			Egg	Larva	Pupa	Male	Female	Host plant
Retronecine (I)	1484	155	–	1.25 ± 0.12	0.53 ± 0.11	0.46 ± 0.08	0.82 ± 0.09	–
Unidentified PA	1858	–	–	–	–	–	–	0.83 ± 0.03
7-Senecioylretronecine type (III)	1864	237	–	1.18 ± 0.21	0.72 ± 0.07	0.66 ± 0.06	0.57 ± 0.06	–
Isocreatonotine A (XIII)	1877	255	0.69 ± 0.10	0.31 ± 0.08	0.27 ± 0.01	0.14 ± 0.01	0.30 ± 0.07	–
7-Deoxy-1,2-dihydrocallimorphine (XVI)	1883	283	1.59 ± 0.20	1.51 ± 0.09	1.25 ± 0.21	1.23 ± 0.09	0.96 ± 0.06	–
7-Deoxycallimorphine (XV)	1890	281	1.39 ± 0.31	1.99 ± 0.13	0.63 ± 0.07	1.94 ± 0.31	0.80 ± 0.05	–
9-Senecioylretronecine type (II)	1895	237	–	1.43 ± 0.11	0.84 ± 0.10	1.09 ± 0.06	1.24 ± 0.10	–
Creatonotine A (XII)	1938	255	1.50 ± 0.16	1.97 ± 0.16	1.73 ± 0.28	1.20 ± 0.16	1.4 ± 0.12	–
Supinine (IV)	2020	283	3.76 ± 0.27	6.78 ± 0.33	7.13 ± 0.13	7.27 ± 0.44	8.06 ± 0.59	2.39 ± 0.40
Callimorphine (XIV)	2024	297	12.47 ± 0.78	11.83 ± 1.02	13.75 ± 1.79	14.13 ± 1.70	12.58 ± 1.53	–
Amabiline (V)	2027	297	–	2.5 ± 0.12	1.91 ± 0.36	2.71 ± 0.57	2.31 ± 0.34	–
3'-Acetylsupinine (VI)	2108	325	–	–	–	–	–	5.32 ± 0.85
Unidentified PA	2163	–	–	–	–	–	–	1.41 ± 0.29
Intermedine (VII)	2167	299	52.99 ± 1.0	47.26 ± 0.37	48.01 ± 0.52	47.98 ± 2.15	48.41 ± 1.05	–
Lycopsamine (VIII)	2175	299	18.10 ± 0.61	15.92 ± 0.42	17.57 ± 1.04	16.71 ± 0.51	17.04 ± 1.74	–
Rinderine (IX)	2185	299	–	–	–	–	–	9.11 ± 0.55
3'-Acetylintermedine (X)	2220	341	–	1.26 ± 0.08	–	–	–	1.29 ± 0.27
Unidentified PA	2231	–	2.99 ± 0.29	2.05 ± 0.10	1.76± 0.53	1.95 ± 0.49	2.48 ± 0.50	–
3'-Acetylrinderine (XI)	2245	341	–	–	–	–	–	79.65 ± 1.51
Unidentified PA	2543	–	2.42 ± 0.33	2.08 ± 0.22	2.47 ± 0.16	1.40 ± 0.35	1.42 ± 0.41	–
Unidentified PA	2581	–	2.10 ± 0.33	1.23 ± 0.19	1.70 ± 0.21	1.13 ± 0.36	1.61 ± 0.30	–

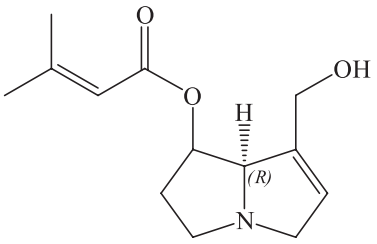
[a]Retronecine (I) was identified by coinjection of pure substances; all other PAs were characterized by comparison with literature retention indices and mass fragmentation patterns (see Table S1); [b]retention indices.



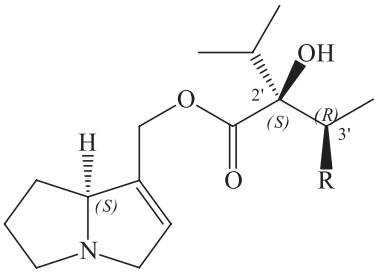
Retronecine (I)



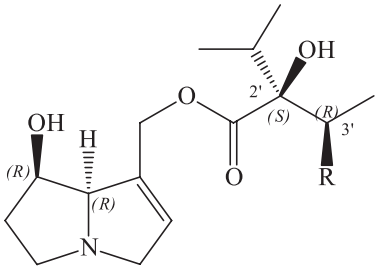
9-Senecioylretronecine (II)



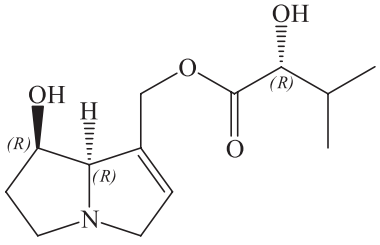
7-Senecioylretronecine (III)



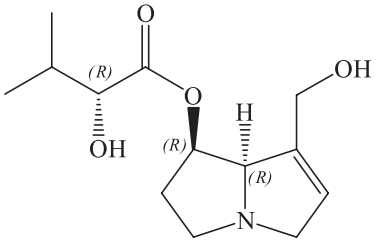
Supinine (IV) - *3′R*, R = OH
Amabiline (V) - *3′S*, R = OH
3'-Acetylsupinine (VI) - *3′R*, R = OAc



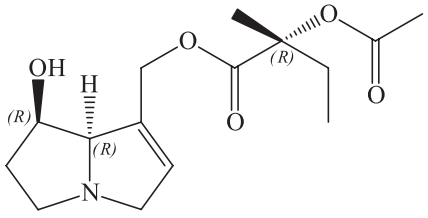
Intermedine (VII) - *7R, 3′R*, R = OH
Lycopsamine (VIII) - *7R, 3′S*, R = OH
Rinderine (IX) - *7S, 3′R*, R = OH
3'-Acetylintermedine (X) - *7R, 3′R*, R = OAc
3'-Acetylrinderine (XI) - *7S, 3′R*, R = OAc



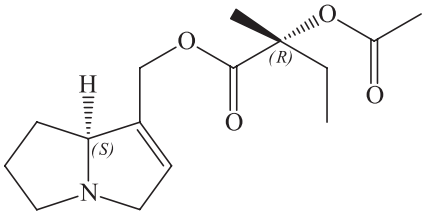
Creatonotine A (XII)



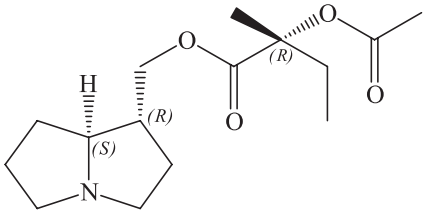
Isocreatonotine A (XIII)



Callimorphine (XIV)



7-Deoxycallimorphine (XV)



7-Deoxy-1,2-dihydrocallimorphine (XVI)

Figure 1. Pyrrolizidine alkaloids found in leaves of *Heliotropium transalpinum* and in eggs, larvae, pupae, and adults of *Scearctia figulina*.

types in all developmental stages (Table 1). The predominant insect PA was callimorphine (XIV); other insect PAs were 7-deoxycallimorphine (XV), 7-deoxy-1,2-dihydrocallimophine (XVI), creatonotine (XII), and isocreatonotine (XIII). Based on the retention index, we assume that all insect PAs have a 7*R* configuration, and show the absolute configuration of necic acid moiety identical to those reported by literature (Figure 1).[20] For the 7-deoxy-1,2-dihydrocallimorphine (XVI), the asymmetric center at C1 has *R* configuration.[20] Insect PAs accounted for

approximately 20% of PAs in *S. figulina*. Three unidentified PAs were found, and they accounted for 8% of relative abundance.

DCA showed that PAs did not cluster host plants and moths; 3'-acetylrinderine (XI) may explain the host plant cluster, while intermedine (VII) explains the moth cluster (Figure 2). The lack of retronecine (I) in eggs (see Table 1) may explain why eggs were clustered apart from the other developmental stages of *S. figulina*.

We did not find any significant differences in PA concentrations in larvae (32.3 ± 2.7 µg of PAs *per* mg of dry weight), pupae (35.5 ± 3.0), males (41.4 ± 1.8), and females (34.0 ± 3.4) of lab-reared *S. figulina* (one-way ANOVA, $F_{3,36} = 1.974$, P = 0.135). Eggs were not quantified since PAs in gregarious eggs did not reach the threshold for the colorimetric analysis. The leaves of *H. transalpinum* had approximately fifty times less PAs than *S. figulina* (0.75 ± 0.05 µg mg^{-1}, n = 5).

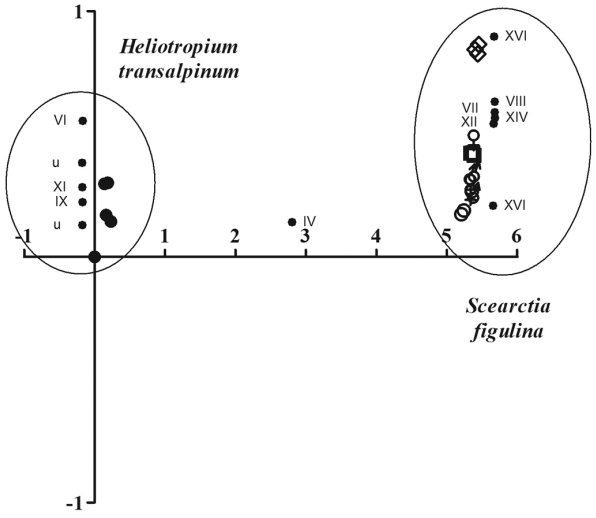


Figure 2. Detrended correspondence analysis (DCA) for pyrrolizidine alkaloids (roman numbers; u: unidentified PA, see Table 1) in leaves of *Heliotropium transalpinum* and eggs, larvae, pupae, males, and females of *Scearctia figulina*.

Chemical defense of *Scearctia figulina* against predators

All three predators consumed all the palatable preys offered. There is a significant difference in the response of three predators regardless of the sex of adult *S. figulina* (GLM, log-likelihood = −57.343, df = 2, $\chi^2 = 22.786$, P = 0.002). The orb-weaving spider *N. clavipes* released all 40 adults bioassayed (100%), while the wolf spider *L. erythrognatha* preyed upon 3 of 40 adults (92.5% release), and the chick *G. gallus* preyed upon 7 out of 40 (82.5% release). A *post hoc* pair-pair comparison showed that *N. clavipes* preyed on lesser *S. figulina* than *L. erythrognatha* (log-likelihood = −38.666, df = 1, $\chi^2 = 15.518$, P < 0.001) and *G. gallus* (log-likelihood = −38.000, df = 1, $\chi^2 = 22.747$, P < 0.001), but no differences occurred between the response of *G. gallus* and *L. erythrognatha* (log-likelihood = −38.229, df = 1, $\chi^2 = 3.392$, P = 0.065). No significant differences were found between sexes (log-likelihood = −30.617, df = 1, $\chi^2 = 0.109$, P = 0.741), and there was no interaction between predator response and sex (log-likelihood = −0.488, df = 2, $\chi^2 = 0.258$, P = 0.879). No significant difference was found between *L. erythrognatha* and *G. gallus* in relation to larvae of *S. figulina* (log-likelihood = −14.50, df = 1, $\chi^2 = 1.412$, P = 0.235). The wolf spider preyed upon one larva out of 13 bioassayed (92.3% release) and the chick preyed upon 4 out of 18 (77.8% release).

Discussion

The PA profile of *H. transalpinum* has already been described by Trigo *et al*.[14] We did not find the PAs transalpinecine, subulacine, and their stereoisomers, as given by Medina *et al*.[25] As other arctiines that feed on PA plants as larvae,[12-14] our results showed that *S. figulina* also uptakes these defensive compounds from its host plant. Although no analyses were performed to check if PAs are present only in the *N*-oxide form, we have made this assumption, since it has occurred in other arctiines studied.[3,4,15,16] The uptake of PA from plants may only occur in larvae of *S. figulina*, since we did not observe adults in PA sources.

Regarding PA patterns, the alkaloid profile in moths might be expected to be a fingerprint of the host plant profile. However, this does not occur in *S. figulina*, as well as in other PA specialist lepidopterans. When these insects feed on plants with 7*S*-configured PAs, they invert this chiral center to the 7*R* configuration.[35] The *raison-d'etre* for this is male sexual pheromone. In danaine and ithomiine butterflies and arctiine moths, all dihydropyrrolizine male pheromones identified have 7*R* configuration.[6-8] As the clades danaine/ithomiine and arctiine are not monophyletic,[36] the 7*R* trait may have evolved twice, independently. Trigo *et al*.[35] suggested that ancestral PA-plants with 7*R* alkaloids may have molded this trait. In PA specialist leaf beetles, such as *Longitarsus* and *Platyphora* species, dihydropyrrolizines are not present, and these beetles show 7*S* PAs sequestered from their host plants, even if some epimerization to 7*R* does occur.[37,38] However, in *S. figulina* we did not find an androconial organ that produces pheromones. It is suggesting that epimerization in *S. figulina* might be due to a phylogenetic constraint, since the more basal species *Amerila* spp.[11] have androconial organs and might produce PA-derived pheromones.[39]

Arctiine moths also biosynthesize their own PA metabolites; the insect PAs.[16,19] These alkaloids are biosynthesized by the esterification of a necine base of plant origin (generally the 7*R* retronecine) with a necic acid of insect amino acid origin.[16,19,40] Only two types of insect PAs were found: PAs of the callimorphine type, whose necic acid is derived from the isoleucine, and of the creatonotine type, whose necic acid is a valine derivative.[16,20,40] Larvae, pupae, and adults of both sexes of *S. figulina* showed both insect PA types, suggesting that biosynthesis occurs in larvae, which is similar to other arctiines that obtain PAs from larval host plants.[16] The function of insect PAs was first associated to the biosynthesis of dihydropyrrolizines, male sexual pheromones, via intramolecular transesterification of insect PA O^9-ester to insect-PAO^7-ester,[19] although this kind of PA is present in both males and females. Our research group has already shown that insect PAs are also defensive compounds against predators.[15,41] When arctiine moths feed on plants containing retronecine, which is innocuous and ineffective against predation,[41] the biosynthesis of insect PAs would be a mechanism to maximize insect chemical defense. However, it is difficult to determine which would be the first function of insect PAs, precursor of sexual pheromones or defensive compounds. Interestingly enough, no free retronecine was found in host plant leaves. Therefore, the moth may first epimerize 7*S* to 7*R* PAs, and then, hydrolyze them to proceed to a further esterification into insect PAs. Additionally, the moth also deacetylates the PAs from the host plant. We can speculate that an O^7-acetyl moiety would prevent the transesterification and the further biosynthesis of insect PAs.

Another unanswered question on insect PAs is: why do some arctiini species biosynthesize a single type of these alkaloids, while others biosynthesize both types? This is more noticeable when we compare the PA sequestration by the pericopines *S. figulina* and *H. syma*. They have a very similar life-style, feeding on the same host plant. The PA epimerization and deacetylation is similar in both species, but insect PA biosynthesis is quite different.[14] *Searctia figulina* biosynthesizes five insect PAs, of the callimorphine and creatonotine types, while *H. syma* biosynthesizes just one callimorphine type PA.[14] A comparative study of insect PA biosynthesis may shed light on the evolutionary mechanisms underlying these findings.

The defensive role of PAs against predators is well known and has been extensively exploited in literature. A further discussion on this function in *S. figulina* would be rhetoric. However, assuming that PAs are responsible for *S. figulina* defenses, our results showed that different predators have different PA release thresholds. The orb-weaving spider *N. clavipes* was the more sensitive predator, releasing all bioassayed individuals. The wolf-spider was less responsive, followed by chicks. We can suggest that a possible high encounter rate of *N. clavipes* with PA-defended insects would account for the high responsiveness. As the orb-weaving spiders build their nets in forest corridors and patches,[42] they may size and release many flying PA insects. The encounter rate of *L. erythrognatha* with PA insects would be lower, since they wander on floors of several environments,[43] where PA-sequestering insects are not so common. Finally, chicks are a model of visually oriented predadors, such as birds. To what extent they share an evolutionary history with PA insects is unkown. In addition, like the wolf spider, chicks show a wandering habit, which decreases the probability of encountering PA insects.

Another point that deserves attention in relation to the defensive role of PAs in adults of *S. figulina* is a possible mimicry pattern with other moths once this species, the arctiine *Episcea extravagans* (Arctiini: Pericopina) and several josiini moths (Notodontidae: Dioptidae) share the same wing color pattern.[44,45] We can hypothesize that *E. extravagans*, as *S. figulina* and other arctiines, is a PA-adapted insect using these compounds for defenses against predators. It is known that Josiini moths use *Passiflora* as larval host plants,[45] which have cyanogenic glucosides in their leaves[46] and they may use these compounds for defense against predators in the same way as other cyanide-feeder insects such as unpalatable *Heliconius* butterflies.[46] If so, Müllerian mimicry can explain the similarities of the wing patterns of these insects.

Conclusions

Although our data on *S. figulina* have shed more light on PAs in arctiine moths, an extensive survey on PA acquisition syndromes and insect PA types from this subfamily should be carried out to suggest any evolutionary trends.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as PDF file.

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