Reproductive benefits derived from defensive plant alkaloid possession in an arctiid moth (Utetheisa ornatrix)

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The moth Utetheisa ornatrix (family Arctiidae) depends on pyrrolizidine alkaloids (PAs) for defense. It sequesters the toxins as a larva from its food plants (Crotalaria species: family Fabaceae) and retains them through metamorphosis. We report here that PA-possession in the adult female U. ornatrix has a life-shortening effect, suggesting that, by putting the compounds to use, the moth may be incurring a cost. However, PA-possession also induces the female to oviposit at an accelerated rate, so that she does not, by dying earlier, incur a loss in fecundity. We argue that by “compressing” their adult existence into a shorter period, female U. ornatrix may actually accrue benefits.

Any insects sequester toxic secondary metabolites from their food plants. They absorb the compounds from the gut and incorporate them systemically, with the result that they are protected against predation (1, 2). A question of interest is whether insects that put phytotoxins to defensive use incur a cost by doing so. Are the insects themselves affected negatively by possession of the toxins, and are the negative effects discernable by doing so. We did not think so, but noted that able negative effects on PA possession? We showed earlier that moths raised -containing diet (12). The moth is therefore ideally suited for pressing their adult existence into a shorter period, female dying earlier, incur a loss in fecundity. We argue that by “com-pressing” their adult existence into a shorter period, female U. ornatrix may actually accrue benefits.

Materials and Methods

Basic Protocol. Our experimental procedure involved raising Utetheisa of two kinds: PA-containing, reared on PA-containing diet, and henceforth referred to as (+)moths; and PA-free, reared on PA-free diet, and henceforth referred to as (-)moths. Individuals of both sexes were picked at random as pupae from their respective dietary cultures, and upon adult emergence were either kept unmated, or paired overnight on the third day of adulthood with (+) or (-) mating partners. Six categories of experimental moths were thus established, two for unmated moths, the other four for mated partners, as follows: (i) virgins, (+) and (-)males; (ii) virgins, (+) and (-) females; (iii) mated partners, (-) females, (-) males; (iv) mated partners, (-) females, (+) males; (v) mated partners, (+) females, (-) males; and (vi) mated partners, (+) females, (+) males.

Individuals were confined singly for their entire adult existence (except, in the case of mated individuals, for the night of mating). The following parameters were recorded: male and female longevity (adult lifespan in days); fecundity (lifetime female egg output); oviposition rate (daily female egg output); and egg viability (percentage eggs that hatched). A measure was also obtained of the body mass of the individuals, recorded by weighing them as pupae.

Larval Diet. We had long known (12) that Utetheisa can be reared on a diet based on pinto beans, totally devoid of PAs. Such diet, which we designate herein as (-) diet, has no obviously discernable negative effects on Utetheisa, which when reared on it are totally normal in appearance.

We also knew that when the (-) diet is supplemented with either Crotalaria seeds or pure PA (e.g., monocrotaline), the Utetheisa reared on it acquire PA in quantities (0.6 μg, on average) commensurate with the amounts (0.7 μg, on average) they incorporate when reared on their food plants (13, 14). Therefore, we chose to rear our (+) moths on a diet consisting of the (-) diet, supplemented with 0.5% (dry weight) of monocrotaline, the primary PA in Crotalaria spectabilis, one of Utetheisa’s principal food plants (14). That diet is designated herein as the (+) diet.

Pupal Mass. We knew from previous studies that pupal mass at day 7 after pupation provides a reliable correlate of adult mass (15). Therefore, individuals were all weighed as pupae on that day.

Abbreviation: PA, pyrrolizidine alkaloid.

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Matings. Matings were effected, as described (15), by confining designated pairs overnight in cylindrical containers (0.35 liter). Mating in *Utetheisa* lasts 6–12 h. To ensure that mating took place, the containers were checked visually at intervals of 4 h or less. Females were also dissected after they died to check on presence of the spermatophore remnant (colla) in the bursa.

Adult Maintenance. Adult males and females were maintained in small individual chambers under identical ambient conditions (22°C; 80% relative humidity; 16-h light/8-h dark photoperiod). They were provided with no sustenance except water, offered by way of soaked wicks.

Longevity. Adults were checked twice daily for deaths incurred.

Fecundity and Oviposition Rate. The containers within which the females were maintained were lined with wax paper, upon which the moths oviposited. The papers were removed daily, checked for numbers of attached eggs, and replaced. Mean daily egg output (oviposition rate), as well as total lifetime egg output (fecundity), were then calculated for each female, virgins included. We knew from experience that female *Utetheisa*, like many other moths (16), lay eggs (albeit nonviable ones) when virginal.

Egg Viability. Egg viability was determined for eggs laid by mated females (categories iii–vi) on the first day after mating, when egg output was at a peak. A count was taken of the fraction of eggs from that day’s output that did not hatch, providing the basis for calculation of egg viability (percentage of eggs that hatched) per female.

Statistical Analyses. Standard multiple linear regression analyses were performed to check into the effect of larval diet and male longevity and on female fecundity. Analyses were also carried out to test for the effect of body mass and mating status on these variables. Data were normally distributed in all categories subjected to these analyses.

Repeated measure analyses were performed to check into the effect of larval diet on male and female longevity and on female fecundity. Analyses were also carried out to test for the effect of body mass and mating status on these variables. Data were normally distributed in all categories subjected to these analyses.

Results

Adult Longevity. As is evident from Fig. 1 and Table 1 ( regressions a–d), diet and mating status each had a life-shortening effect on the adult female, but not on the adult male. Longevity also correlated, albeit positively, with pupal mass in the female. Therefore, among females, large, unmated, PA-free individuals tended to live longer than small, mated, PA-laden individuals.

Among males, there was also an effect of pupal mass on

Table 1. Multiple regression analyses performed on data pertaining to adult longevity, female fecundity, and egg viability

<table>
<thead>
<tr>
<th>Regression</th>
<th>Dependent variable</th>
<th>n</th>
<th>Independent variable</th>
<th>Interactions</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Longevity of mated females (categories iii–vi)</td>
<td>110</td>
<td>Female diet &lt;0.001, Male mass &gt;0.05,</td>
<td>Mating status &gt;0.05</td>
<td>0.319</td>
</tr>
<tr>
<td>b</td>
<td>Longevity of females (categories ii–iv)</td>
<td>170</td>
<td>— —</td>
<td>—</td>
<td>0.434</td>
</tr>
<tr>
<td>c</td>
<td>Longevity of mated males (categories iii–vi)</td>
<td>111</td>
<td>Male mass &gt;0.05,</td>
<td>Mating status &gt;0.05</td>
<td>0.125</td>
</tr>
<tr>
<td>d</td>
<td>Longevity of males (categories i and iii–vi)</td>
<td>171</td>
<td>— —</td>
<td>—</td>
<td>0.065</td>
</tr>
<tr>
<td>e</td>
<td>Fecundity of mated females (categories iii–vi)</td>
<td>110</td>
<td>Female diet &gt;0.05, Male mass &lt;0.001,</td>
<td>Mating status &gt;0.05</td>
<td>0.538</td>
</tr>
<tr>
<td>f</td>
<td>Fecundity of females (categories ii–vi)</td>
<td>170</td>
<td>— —</td>
<td>—</td>
<td>0.574</td>
</tr>
<tr>
<td>g</td>
<td>Egg viability (categories iii–vi)</td>
<td>110</td>
<td>Male diet &lt;0.005, Male mass &gt;0.05,</td>
<td>—</td>
<td>0.029</td>
</tr>
</tbody>
</table>
Thus, the two bars given for mated females in Fig. 2 include the lumping the results from some of our experimental categories. For purposes of presentation, data pertinent to experimental categories iii–vi are lumped as shown (see text). Numbers above columns are sample sizes.

Longevity, but the positive correlation was only marginally significant.

It is clear also from Table 1 (regressions a–d) that there were no interactions between the various parameters assessed. This finding justified our lumping the data from some of our experimental categories. Thus, the two bars shown for mated females in Fig. 1A contain the females from experimental categories iii and iv (light bar) and v and vi (dark bar). Likewise, the two bars for mated males in Fig. 1B contain the males of categories iii and v (light bar) and iv and vi (dark bar).

**Fecundity.** As is evident from Fig. 2 and Table 1 (regressions e and f), female fecundity was affected by female pupal mass and mating status, indicating, as one might expect, that larger mated females tend to lay more eggs than small virgins. However, fecundity was unaffected by either female or male larval diet, meaning that PA acquisition on the part of the female, whether from the diet, her mate, or from both sources, did not result in a change in the lifetime egg output. Again, given the lack of interactions (Table 1, regressions e and f), we felt justified in lumping the results from some of our experimental categories. Thus, the two bars given for mated females in Fig. 2 include the females from experimental categories iii and iv (light bar) and v and vi (dark bar).

**Oviposition Rate.** As is clear from Fig. 3 and Table 2 (repeated measure analyses a and b), Utetheisa responded to mating with an outburst of egg deposition, which peaked on the next day and subsided gradually in the days following. The peak was highest for the females of category vi (Fig. 3), in other words, for females of the highest PA content in possession of both diet-derived and male-derived PA. No such peak occurred in females that remained unmated (Fig. 4). Utetheisa is not unusual in responding in this fashion. Mating is known to trigger a sharp transient rise in oviposition in some other insects (16).

Given that PA possession shortened the lifespan of the Utetheisa female without curtailing her fecundity, it was clear that PA possession had to have an elevating effect on the ovipositional rate of the moth. Therefore, the prominence of the ovipositional output by category vi females on the day after mating came as no surprise. However, category vi females continued laying eggs at a significantly higher rate for several days after that of peak output, when there was a gradual decline of the ovipositional rate for all females. During that period, there was no significant difference in the ovipositional rate of females of categories iii–v (Fig. 3 and Table 2, repeated measure analysis c), but the egg-laying rate of the category vi females consistently exceeded that of the females of the other categories. Maximization of ovipositional output during what amounts to, essentially, the first half of the females’ life, was therefore contingent upon possession of PA of both dietary and seminal origin. Possession of PA from either source alone did not, during that period, have a promotional effect on egg laying (total number of eggs laid on average by the females of categories iii to vi over the span of 4 to 13 days after emergence were: iii = 333 ± 19; iv = 343 ± 19; v = 317 ± 18; vi = 418 ± 16).

A positive correlation between female mass and oviposition rate was also established (Table 2, repeated measures analyses a–c): quite aside from PA content, larger females tended to have higher oviposition rates.

**Egg Viability.** Viability of eggs (Fig. 5) from mated females was consistently high, and was unaffected by the PA content of either father or mother (Table 1, regression g).

The eggs produced by virgin females were consistently nonviable.

**Discussion**

Our initial thought, when we noted that PA-possession in female Utetheisa cuts back on longevity, was that PAs are toxic to the moth and that a shortening of the lifespan was the price the female paid for making defensive use of the compounds. However, we grew skeptical when it became apparent that PA-possession had no corresponding effect on the male. If toxicity was at issue, why should the male be spared the negative consequences of PA possession? It was not until we learned that the female incurs no loss of fecundity when PA-laden, that it occurred to us that the female might actually profit from PA possession.

We now view PAs as serving in two beneficial capacities in Utetheisa, as defensive agents in both sexes and as systemic ovipositional stimulants in the female. The early death induced by PA-possession in the female, we now think, is not evidence of toxic action on the part of the compounds, but merely of the fact that death comes earlier to the female if she is induced more quickly to lay her eggs and exhaust her reserves. Indeed, in our current study, it was the females with the highest PA content (category vi) that showed the highest ovipositional rates in the...
days immediately following the initial burst of egg laying that is the typical sequel to copulation.

Does it make sense for the female to pace her ovipositional rate in accord to her PA possession? We are inclined to suggest that it does. As regards the provisioning of her offspring, a female cannot “do better” than to have come into possession, preparatory to egg laying, of substantial quantities of PA. Females such as those of category \( vi \), which bore both diet-derived and male-derived PA, were clearly the “best off” among those we tested, and therefore driven to lay their eggs the quickest. We know from previous studies that being richly provisioned with PA on the part of females is a mark of fitness in \( U. \). A female rich in diet-acquired PA is likely not only to be larger, on average, than one not so endowed, but likely also, given that body size is heritable in \( U. \) (15) and associated with increased fecundity, to be the carrier of good genes. Should she, while thus advantaged, also be richly PA-endowed by the male, she could potentially be in possession of good genes from him as well, given that the magnitude of seminal PA-bestowal by the male correlates with male body size, which in turn correlates with male success in courtship (3, 14, 15–20). Thus, there was “no point” for \( U. \) females of category \( vi \) to hold back on egg deposition. There was no way for them to “do better,” or to improve upon the quality of their mate. Females of the other categories not so generously PA-endowed laid eggs at lesser rates, as if programmed to keep opportunities open for subsequent receipt of improved seminal gifts. Being promiscuous, female \( U. \) do indeed face the reality of encountering males of differing “value.” Experimental evidence indicates that they are capable of discriminating between these on the basis of precopulatory and postcopulatory choice (3, 18, 21, 22).

Therefore, is one to conclude that PAs are nontoxic to \( U. \)? We suggest, tentatively, that this might indeed be the case. With >370 described examples, PAs make up one of the largest categories of plant-produced secondary metabolites.

Table 2. Repeated measures analyses performed on females’ daily egg output for the period of 13 days after adult emergence

<table>
<thead>
<tr>
<th>Repeated measure analysis</th>
<th>Dependent variable</th>
<th>( n )</th>
<th>Independent variable</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Oviposition pattern of mated females (categories iii–vi)</td>
<td>102</td>
<td>Effects over time (within)*</td>
<td>Female diet</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Averaged effect independent from time (between)*</td>
<td>(&gt;0.05)</td>
</tr>
<tr>
<td>b</td>
<td>Oviposition pattern of females (categories ii–vi)</td>
<td>161</td>
<td>Effects over time (within)*</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Averaged effect independent from time (between)*</td>
<td>(&lt;0.01)</td>
</tr>
<tr>
<td>c</td>
<td>Oviposition pattern of females (categories iii–v)</td>
<td>77</td>
<td>Effects over time (within)*</td>
<td>(&lt;0.05)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Averaged effect independent from time (between)*</td>
<td>(&lt;0.05)</td>
</tr>
</tbody>
</table>

*The within effects refer to effects of the independent variable on the repeated measures (daily egg output).
†The between effects refer to the effects of the independent variables on the specified levels within them.

![Fig. 4. Daily egg output of virgin females, plotted as a function of time (days since emergence from pupa) and female diet. Sample sizes for the (+) and (−) females were 30 and 29, respectively, for the 13-day period analyzed.](image)

![Fig. 5. Egg viability, plotted as a function of the parental categories shown. Numbers above columns are sample sizes.](image)
The biosynthesis of these compounds has been worked out in great detail, and much has been learned about PA sequestration and storage in insects, including lepidopterans and coleopterans, that derive protection from acquisition of the compounds (23). PAs are generally thought to be defensive in plants, partly as a consequence of their toxicity (24). Interestingly, vis à vis vertebrates at least, the compounds are relatively innocuous in the form in which they are stored in plants. They become toxic only after ingestion, when they are converted into pyrolic alkylating agents by the P450 enzymes of the liver (25). Oddly, P450 enzymes are viewed as detoxifying agents, as part of the defensive arsenal of animals. Vis à vis most phytotoxins, they are in fact defensive, for they render the compounds innocuous by transforming them in various ways (26). Their accomplishing the exact opposite in their action upon PAs is of coevolutionary interest, in that it suggests that plants may have fashioned PAs specifically to subvert the herbivore’s P450 enzyme system, in other words, to use the animal’s counterdefense to bolster their own defense (3).

Tolerating PAs, on the part of a herbivore, may involve no more than preventing the ingested PA from exposure to P450 enzymes. One could imagine this being achieved either by shielding the acquired PAs in some way, or by modifying or entirely foregoing possession of the P450 system. Whichever the explanation, it is clear that Utetheisa stores acquired PAs in unaltered form, without converting them into alkylating agents. In a sense, the moth can be thought of as having countered the plant’s counterplay, by having evolved the means for preventing the plant from coopting its detoxification mechanism. Utetheisa is thus enabled to retain PAs in unaltered form when it acquires the compounds from plants, and to put the chemicals to defensive use just as they are put to such use in plants. The PAs remain untransformed into toxic alkylating agents in the course of their uptake, and their storage, as a result, may entail no cost.

Does it make sense to consider the life-shortening effect induced by PA-possession in female Utetheisa to be adaptive? It could be argued, of course, that Utetheisa females are not really benefiting, if by ridding themselves of their eggs more quickly they hasten their own demise. One could imagine situations where a curtailing of the lifespan, if incurred with no loss of fecundity, could pay off. Just shortening the life cycle could bring the advantage of accelerated offspring production, or a decrease in the probability that the female will fall victim to predation, disease, or other lethal causes before she had the opportunity to oviposit. In Utetheisa, there may actually be special factors at play. As we have learned from observation, Utetheisa are effective colonizers. Their Crotalaria food plants in central Florida occur in widely scattered patches, in which they may lie dormant for years, after a natural decline, until revived by fire. Fire, in fact, may play a major role in triggering seed germination in Crotalaria microronata and Crotalaria spectabilis, the principal food plants of Utetheisa in the region. We have noted emergent patches of Crotalaria plants to be quickly colonized by Utetheisa, indicating that the exploitation of newly available resources may be a matter of some competition in this moth. Females of optimal fitness, driven by high PA content to oviposit at accelerated rates, may well be the ones to gain an early numerical edge in such competition.

We also know now, as we shall report elsewhere, that PA-possession shortens the duration of the larval period in Utetheisa. The larvae are quicker to reach the pupal stage when given a PA-laden diet, and they grow to a larger size when thus fed. The effect has a behavioral cause. Larvae raised on PA-laden diet are driven to invest more time in eating, and to eat faster, than those kept on PA-free food. What triggers this increased commitment to feeding are the PAs themselves. PAs are potent phagostimulants in Utetheisa.

It is tempting to predict that Utetheisa will turn out not to be unique. Reduced female longevity, such as is known to be the sequel to mating in a number of arctiids (27), could well be reflective, at least in cases where the phenomenon is a correlate of accelerated oviposition, of adaptive reproductive decision-making on the part of the female, rather than cost incurred as a consequence of mating.

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