You are what you eat: native versus exotic *Crotalaria* species (Fabaceae) as host plants of the Ornate Bella Moth, *Utetheisa ornatrix* (Lepidoptera: Erebidae: Arctiinae)

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*Crotalaria* plants and *Utetheisa ornatrix* are closely linked to each other: the larvae destroy the seeds, while the moth depends on hostplants for alkaloids. To better understand the ongoing co-evolution, the present study examines how native hostplants compare to exotic ones. Leaf-feeding on *Crotalaria pumila*, *C. rotundifolia*, and *C. incana*, native to the moth’s range, led to faster larval development than on the exotic *C. lanceolata*, *C. spectabilis*, and *C. pallida*. Seed-feeding on all species of *Crotalaria* led to accelerated larval development and a resultant larger adult moth, and correlates with a higher nitrogen content in the plant tissues. These results add a novel dimension to the previous studies of reproductive biology of this model organism. In controlled settings, mature larvae showed preference for leaves of *C. spectabilis* over those of other species, perhaps due to the higher alkaloid content. Differences in morphology and phenology of *Crotalaria* determine the ecology of *U. ornatrix* populations in Florida. The introduction of novel hostplants, on which *U. ornatrix* can have a significant negative effect and which are of concern to humans as invasive toxic weeds, has greatly expanded the niche occupied by this moth. Possible co-evolution of *Utetheisa sensu lato* and plant defences in the genus *Crotalaria* is discussed.

**Keywords:** chemical defence; pyrrolizidine alkaloids; trophic interactions; invasive exotic weeds; biological control; plant–herbivore co-evolution

Introduction

The genus *Crotalaria* (Fabaceae) includes over 700 mostly tropical and subtropical species, which have been frequently relocated by humans because of their useful properties, such as their ability to fixate nitrogen and their value as a green manure crop (e.g. Austin 2004; Uratani et al. 2004; Choi et al. 2008; le Roux et al. 2013). Many of these potentially invasive species contain different levels of pyrrolizidine alkaloids (PAs), which are toxic to mammals and birds (e.g. Williams and Molyneux 1987; Ribeiro et al. 2012). Insects rarely feed on *Crotalaria* plants due to their chemical defences, with the exception of the arctiine moth, *Utetheisa ornatrix* L., 1758, whose reproduction is intimately connected to *Crotalaria* plants (Figures 1, 2). For these moths, PAs sequestered by the larvae serve as both defence compounds for all stages of development and as precursors for pheromones. When present, the moths, too, have a substantial effect on the reproductive biology of their hosts as the caterpillars are very efficient in destroying the seeds. Hence, understanding this

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Figure 1. (A) In the wild population of *U. ornatrix*, adult moth landing on the flower of exotic *Crotalaria retusa*, Micanopy, Florida; (B) a typical size of a moth from a wild population at Cross Creek, Florida, resulting from larval feeding on *C. rotundifolia* leaves (top) and its offspring raised in the laboratory on beans of *C. spectabilis* (bottom) (fore wing length = 20 mm); (C) a single egg batch split in two (experimental and control groups) prior to hatching; (D) hostplant preference test using mature larvae of *U. ornatrix* inside a tray; (E) differences in pod size and seed volume in six *Crotalaria* species found in Florida; (F) difference in sprouting rate under similar conditions: native *Crotalaria pumila* shows much slower sprouting rate than introduced invasive *Crotalaria* species; (G) upland pine habitat on the University of Florida campus overtaken by thousands of exotic *Crotalaria lanceolata* plants with a sporadic native *C. pumila* in the midst (October 2014); (H) *U. ornatrix* eggs on *C. lanceolata*; (I) first instar larvae; (J) third instar larva.
Figure 2. (A) Understorey of the Florida hammock habitat occupied with invasive exotic Crotalaria spectabilis; (B) a clearing in a secondary Florida habitat, overgrown with exotic Crotalaria pallida; (C, D) mature larvae of U. ornatrix prefer pods of C. spectabilis over leaves; (E) carpenter ants are attracted to the extrafloral nectaries of C. lanceolata; (F, G) larva of U. ornatrix on C. pumila and a pod destroyed by it; (H) mature larva of U. ornatrix inside a pod of C. incana; (I, J) pods of C. pallida are numerous and large and provide ample food and shelter for U. ornatrix; (K) empty pods of C. spectabilis in December with all of their seeds consumed by U. ornatrix larvae; (L) in December, C. retusa becomes the preferred hostplant of U. ornatrix in the C. spectabilis-dominated habitat, when the latter declines; similarly, C. pumila becomes preferred for oviposition in C. lanceolata-dominated habitat; (M) the seeds of C. retusa are well protected by thick walls of the pod; here, a third instar larva is unable to penetrate it; (N) onset of the ultimate instar; (O–Q) prepupa-to-pupa development of U. ornatrix.
system of trophic interactions is important for those who want to propagate Crotalaria plants (e.g. DaCosta et al. 2012) and for those who want to control their spread (e.g. Fonseca et al. 2006), as well as, of course, for those interested in this moth’s fascinating biology, of which there is a growing number (e.g. Conner 2008 and references therein).

In Florida, there are 14 species of Crotalaria, only four of which are native. Understanding how U. ornatrix adapted to feeding on these new species, and how the original populations of both moths and plants have been affected, can help in understanding co-evolutionary processes. All these species have different ecological requirements, chemistry, morphology and phenology. For example, Crotalaria pallida Aiton, (Figure 2B), relies on extrafloral nectaries to attract ants and hence is able to defend its seeds from U. ornatrix larvae (Guimarães et al. 2006). One can find such nectaries also in the widespread C. lanceolata E. Meyer, (Figures 1G, 2E), which is a close relative of C. pallida (le Roux et al. 2013).

Crotalaria species vary dramatically not only in their morphology, but also in their chemistry (e.g. Williams and Molyneux 1987), so it is not surprising that these plants can have very different effects on herbivores. Cogni (2010) examined C. incana L., 1753 and C. pallida both in the field and the laboratory, and found that the latter species, though introduced as an exotic species to his native Brazil, outperforms the native C. incana as a host. In that study, moths preferred to oviposit on C. pallida and the female larvae developed into heavier adults. Another recent study (Sourakov and Locascio 2013) compared the effectiveness of C. lanceolata and C. incana as hostplants of U. ornatrix in Florida and found a remarkable difference between the rates of larval development on the two hostplants. Cogni et al. (2011) point out that there is considerable intraspecific variation in plants on both a regional and continental scale and that the variable characteristics relate to herbivory. They showed that population differentiation in U. ornatrix may too occur, although this differentiation was found to be unstable over time, probably due to local extinction and migration of moths.

As habitats in Florida become more and more fragmented, it will be interesting to examine the differentiation in populations of U. ornatrix, especially when such populations are associated with different species of Crotalaria. The first step, however, is to establish the potential influence that these diverse hostplants have on U. ornatrix biology. For instance, the hostplant choice may be playing a role in the ongoing evolution of U. ornatrix, because both the rate of development and the size of offspring may be important factors in sexual selection in this species (e.g. Iyengar and Eisner 1999a, 1999b). Although exotic Crotalaria are much more prevalent in today’s Florida landscapes, from the conservation ecology point of view it seems important to understand how the native species fair against exotics as hostplants in attracting moths and in the capacity of a food source. Sourakov and Locascio (2013) showed that C. incana, which is supposedly native to Central and South America (e.g. Austin 2004; Cogni 2010), and hence has co-evolved with U. ornatrix there, is very well suited for the development of U. ornatrix larvae compared with the exotic African C. lanceolata, on which the development is much slower (Figure 3F). As a side note, Wunderlin and Hansen (2011) list C. incana as native to Africa. Perhaps it is a naturally pantropical species, since it is also widespread in the Neotropics, was described three centuries ago from Jamaica and is listed as a separate subspecies in Africa.
The studies presented below have tested two of the more common native Florida species, *C. pumila* Ortega and *C. rotundifolia* J. F. Gmelin as food source of *U. ornatrix*, as well as two additional exotic and widespread *Crotalaria*. One of them, *C. spectabilis* Roth, was introduced in 1921 from the Old World by David Fairchild as...
part of USDA efforts to improve soils (green manure) and to combat soil nematodes and garden pests (Austin 2004). Though the plant is toxic to cattle and horses (e.g. LSUAgCenter 2012) and is unlikely to be recommended to farmers today, it has persisted throughout Florida, especially in the former agricultural lands that have become urbanized or reforested. The plant is seasonal, but grows fast and can form dense stands of impressive 6–8 feet tall annuals (Figure 2A). *Crotalaria pallida*, a close relative of *C. lanceolata*, is another common exotic species found in Florida, and, like the latter, is native to Africa. Like *C. spectabilis*, *C. pallida* often forms dense stands along forest edges and clearings in Florida (Figure 2B).

In nature, all of the above-mentioned plants (as well as the exotic *C. retusa* L., 1753) support local populations of *U. ornatrix* around Gainesville, north central Florida (pers. obs.). In the present study, their effectiveness as hostplants was tested in the laboratory, including the effect of seed-feeding by late instar larvae on the growth rate and size of offspring. The latter is very important in *U. ornatrix* biology, as seeds have five times higher PA contents than leaves and were shown to have a significant effect on the rate of larval development, larval survival, resulting weight and chemical protection of adults, as well as the chemical protection of eggs of the next generation (Hare and Eisner 1993; del Campo et al. 2005; Ferro et al. 2006; DaCosta et al. 2012). Mature larvae were tested for food preference under controlled conditions.

**Materials and methods**

Plants for carbon/hydrogen/nitrogen (CHN) analyses were propagated from seeds (Figure 1E, F) and grown simultaneously in one-gallon pots, using similar potting soils and conditions. In the case of *C. rotundifolia*, these were also the plants used for the feeding experiments, while plant materials from other *Crotalaria* species in these experiments were obtained from naturally growing plants. A sample of host plant leaves (a mix of leaves from different plants and different parts of the plant) was dried, ground-up into powder using a manual grinder, and analysed by the staff of the Department of Chemistry, University of Florida, for nitrogen content using an Eager 200 CHN analyser (ThermoFisher Scientific, Waltham, MA, USA). Caterpillar food choice experiments were conducted inside 19 × 22 cm trays (Figure 1D) enclosed in zip-lock bags. Weighing of the larvae and hostplant materials was done using a Mettler Toledo AL104 (Mettler-Toledo AG, Laboratory & Weighing Technologies, Greifensee, Switzerland) analytical balance. Fore wing length (as illustrated in Figure 1B) was measured using electronic callipers. Hostplant materials for estimating water and nitrogen content in seeds was collected on the University of Florida campus on 25 October 2014, when females of *U. ornatrix* as well as mature larvae were present. Pods were collected at the ripening stage typically attacked by the larvae. Acquisition of eggs and their rearing, as well as chemical analysis, were performed according to methods described in Sourakov and Locascio (2013). For each of the experiments, neonate larvae from a single egg batch were randomly divided into feeding groups to minimize the influence of hereditary predisposition to slower/faster development or smaller/larger size. For the experiments that involved comparing development rate of ultimate instars, the larvae were separated into groups at the onset of the instar (as in Figure 2N). The parental females were netted at one of the four sites with the following corresponding dominant *Crotalaria* species:
experiment 1 – north Gainesville/C. pallida; experiment 2 – Micanopy/C. spectabilis; experiments 3, 4, 5, 6 and 7 – University of Florida/C. lanceolata; experiment 8 – Cross Creek/C. rotundifolia.

Results and discussion

Feeding on native versus non-native hostplants

(1) Crotalaria pumila versus C. lanceolata (Figure 3B, C)
When larvae were reared on C. pumila versus C. lanceolata foliage, it led to faster development on C. pumila. Two separate trials were conducted. In the first, the larvae resulting from the same egg batch were started immediately on different hosts and were weighed 18 days later when they reached third to fourth instar. The larvae developing on C. pumila were, by this time, twice as heavy (0.044 ± 0.016 g; n = 27) as those fed C. lanceolata (0.027 ± 0.012 g; n = 24); (p < 0.0002) (Figure 3B). This experiment was terminated at this point due to the lack of foliage of C. pumila. Nevertheless, the outcome of this trial unambiguously demonstrates faster development on the native C. pumila. During the second experiment, larvae that were raised throughout on C. pumila developed in 24.3 ± 1.5 days (n = 16), while larvae that were switched to C. lanceolata at the beginning of the ultimate instar developed significantly more slowly (26.8 ± 1.6 days; n = 13) (p = 0.0003) (Figure 3C), which supports the results of the first experiment. In contrast, larvae that developed exclusively on C. lanceolata in previous experiments (Sourakov and Locascio 2013) took c.44 days to pupate, while larvae on C. incana foliage finished their development in c.24 days (identically to larvae on C. pumila). These results correlate with the increased nitrogen content in C. pumila and C. incana leaves compared to C. lanceolata (Table 1).

(2) Crotalaria rotundifolia versus C. lanceolata (Figure 3A)
Similarly, feeding on C. rotundifolia versus C. lanceolata demonstrated that the native species (C. rotundifolia) led to faster development. The larvae, selected from the same egg batch, were started at the same time on C. rotundifolia and C. lanceolata and weighed 13 days later when they had reached the second to fourth instar. The larvae developing on C. rotundifolia were by this time three times heavier (0.028 ± 0.007 g; n = 16) than those fed C. lanceolata (0.009 ± 0.004 g; n = 10) (p < 0.0001). Again, development to pupation was not completed due to the lack of sufficient C. rotundifolia foliage. However, the results unambiguously suggested that larvae developed faster on the native C. rotundifolia.

(3) Crotalaria spectabilis versus C. lanceolata (Figure 3D)
On exotic Crotalaria spectabilis (occasionally supplemented with C. retusa leaves) the development from a neonate larva to pupa occurred more slowly than on C. lanceolata. For males, it took 48.3 ± 2.1 days (n = 9) (versus 42.3 ± 1.5 days on C. lanceolata; n = 12) (p < 0001); for females, it took 45.5 ± 2.6 days (n = 13) (versus 38.8 ± 3.5 days on C. lanceolata; n = 19) (p < 0001). The sizes of the resulting moths in this experiment showed no significant difference either between different hostplants or between sexes and in general varied little from the average: 20.0 ± 0.7 mm (n = 53).
Table 1. Pyrrolizidine alkaloid, N$_2$, and H$_2$O content in tissues of several native and introduced *Crotalaria* species in Florida.

| *Crotalaria* species | PAs in seeds* | N$_2$ in foliage, potted | N$_2$ in foliage, wild | N$_2$ in seeds, wild | H$_2$O in foliage | H$_2$O in unripe seeds | Taxonomic position** | Origin*** |
|----------------------|---------------|--------------------------|-----------------------|---------------------|------------------|------------------------|-------------------|----------|
| *C. incana*          | 0.07%         | 4.7–5.0%                 | 3.4–3.6%              |                     |                  |                        | Sec. Crysocalycinae | New World, native in U. ornatrix range |
| *C. lanceolata*      | 0.21%         | 4.9–5.2%                 | 2.8–2.9%              | 3.5–3.9%            | 76%              | 87%                    | Sec. Hedriocarpace | Africa |
| *C. pallida*         | 0.26%         | 5.9–6.3%                 | 3.8–4.2%              | 5.4–5.5%            | 57%              | 79%                    | Sec. Hedriocarpace | Africa |
| *C. pumila*          | 0.04%         | 4.2–4.3%                 | 4%                    | 3.6–3.9%            | 95%              | 89%                    | Sec. Geniculatae  | Native to Florida |
| *C. retusa*          | 2.69%         | 1.6–2.3%                 |                       |                     |                  |                        | Sec. Crotalaria   | Africa |
| *C. rotundifolia*    | n/a           | 2.3–2.5%                 |                       |                     |                  |                        | Sec. Calycinae   | Native to Florida |
| *C. spectabilis*     | 3.81%         | 3.7–3.8%                 | 3.3%                  | 4.1%                | 61%              | 77%                    | Sec. Crotalaria   | Asia    |

Notes: *After Williams and Molyneux (1987).
**After le Roux et al. (2013).
***After Polhill (1982), Cogni (2010), Wunderlin and Hansen (2011).
The nutritional properties of *Crotalaria* change seasonally and locally (e.g. Uratani et al. 2004; Cogni et al. 2011). This factor can therefore affect the rate of development. When a batch of larvae was reared on young tender leaves of pre-blooming *C. spectabilis* and on *C. lanceolata* in September 2013, the rate of development was shown to be faster on the former than on the latter (they developed in 34.5 ± 3.2 days (n = 20) – about 4 days faster than on *C. lanceolata* in the previous trial), but still significantly slower than on the native species described above.

(4) *Crotalaria pallida* versus *C. lanceolata* (*Figure 4A*)

Despite the superficial differences between the shrubby *C. pallida* and the more delicate *C. lanceolata*, these two species are very closely related, belonging to the ‘lanceolata alliance’ of Hedriocarpae section (subsection Macrostachyae) (le Roux et al. 2013). It is not surprising, therefore, that their beans have similar levels of alkaloids. While the nitrogen content of *C. pallida* leaves seems to consistently slightly exceed that of *C. lanceolata*, the nitrogen level in these plants can be quite variable depending on the growing conditions (Table 1). Moths that developed on these two plants showed no significant differences in the rate of larval development and the resulting adult size (males raised on *C. lanceolata* were 19.8 ± 0.9 mm (n = 8) versus 20.2 ± 0.8 mm (n = 18) on *C. pallida, p = 0.3; females on *C. lanceolata* were 19.5 ± 0.9 mm (n = 15) versus 20.2 ± 0.8 mm (n = 7) on *C. pallida, p = 0.1).

The interactions of *C. pallida* with *U. ornatrix* has been a focus of several investigations (e.g. Ferro et al. 2006; Cogni 2010; Cogni et al. 2011), which showed that in Brazil it can be a more effective food source than the native *C. incana*, leading to faster development and higher pupal weight of females.

*Leaves versus beans as food source of mature larvae*

(5) *Crotalaria spectabilis/C. retusa* beans versus *C. lanceolata* (*Figures 3E, 4C*)

Larvae fed *C. lanceolata* foliage still developed faster than those fed *C. spectabilis/C. retusa* leaves, even when the latter diet was replaced with beans in the last instar. Males developed on *C. lanceolata* in 42 ± 2.7 days (n = 19) versus 45.5 ± 2.4 days on *C. spectabilis/C. retusa* (n = 10), p < 002; females developed on *C. lanceolata* leaves in 38.8 ± 2.5 days (n = 19) versus 44.4 ± 1.8 days on *C. spectabilis/C. retusa* (n = 8), p < 0001 (*Figure 3E*). However, there is a marked difference between the results of this trial and those described above (experiment 3); moths whose larvae fed on beans were larger: males were 21.0 ± 0.7 mm (n = 11) versus 20.0 ± 0.9 mm (n = 19) raised on *C. lanceolata* leaves, p < 0.005; females were 21.2 ± 0.7 mm (n = 8) versus 19.9 ± 0.6 mm (n = 19), p = 0.0001 (*Figure 4C*).

From other studies (e.g. Ferro et al. 2006), we know that beans have higher concentrations of PAs than leaves. However, it is doubtful that these compounds are responsible for the larger size of the resulting progeny in this and other experiments that involve using beans as a diet and that are presented below. Cogni et al. (2012) demonstrated that significantly increasing levels of PAs in an artificial diet slightly slowed down larval development, but was not causing any noticeable changes in adults. It is more likely that the observed larger wing size was caused by higher
Figure 4. Fore wing size of *Utetheisa ornatrix* raised on different species of native and exotic *Crotalaria* and effect of leaves versus beans in the diet (see text for details): (A) Fore wing size of...
nitrogen content of *C. spectabilis* seeds (c.4%) versus that of *C. lanceolata* leaves (c.3%) (Table 1).

It has been shown previously that sexual selection favours larger *U. ornatrix* males (Iyengar and Eisner 1999a), and that the size of adult moths is inherited paternally (Iyengar and Eisner 1999b). These studies, conducted in controlled conditions using artificial diets, provided a framework for our understanding of the biology of *U. ornatrix*. The fact that the expression of the size trait also depends on the larval diet adds new insights into how this system functions in nature.

(6) *Crotalaria spectabilis/C. retusa* beans versus leaves (Figure 4C)

We can adequately compare the moths raised solely on the foliage of *C. spectabilis/retusa* (experiment 3) with those that were fed beans in their ultimate instar (experiment 5), because both groups were offspring of the same female and were raised simultaneously in similar conditions. Bean-feeding led to the increase in size of adults (*p = 0.01* (males), *p = 0.03* (females)). This increase, modest, but statistically significant, correlates with the increase in nitrogen content, which in *C. spectabilis* is 3.3% in leaves and 4.1% in seeds (Table 1). The results are supported by similar

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experiments with *Crotalaria pallida* and *U. ornatrix* in Brazil by Ferro et al. (2006), results of which are illustrated in Figure 5C, and additional similar experiments presented below. This increase in size, resulting from feeding on the reproductive organs of the plants versus other tissues, is not unique to *Utetheisa*. For instance, the larvae of *Terastia meticulosalis* Guenee (Crambidae), which attack both the stems and the beans of the coral bean (*Erythrina herbacea* L.), were significantly larger in the early summer generation, which was raised on beans (their natural diet at that time), than in the late summer/autumn generations, which normally burrow the stems as beans are no longer available (Sourakov 2011). For *U. ornatrix*, this difference is important because it confirms that the size of moths, which has been shown to be a genetically controlled characteristic (Iyengar and Eisner 1999b), can also depend not only on the choice of hostplant species (as described above) but even on the individual plant chosen for oviposition. For instance, ovipositing on a hostplant with formed or forming seed pods rather than a hostplant with no or less developed reproductive organs can lead not only to faster development of more chemically protected individuals, but also to larger, more reproductively successful ones. It has been noted (pers. obs.) that mature larvae of *U. ornatrix* can travel between plants, and hence such choice of hostplant may rest not only with the ovipositing female, but also with the individual larvae.

(7) *Crotalaria lanceolata* beans versus leaves (*Figures 4B, 5A*)

In a separate trial, roughly half of the larvae feeding on *C. lanceolata* foliage were switched to feeding on the beans of this hostplant at the onset of the last instar. The resulting adult moths again were larger: the fore wing length of males raised on beans was 20.9 ± 0.7 mm (n = 18) versus 19.9 ± 0.4 mm (n = 11) raised on leaves throughout their development, \( p = 0.0006 \); females raised on beans were 20.3 ± 0.9 mm (n = 11) versus 19.5 ± 0.6 mm (n = 9) raised on leaves, \( p = 0.03 \). The development of the last instar was also c.7% faster on beans: 12.7 (n = 26) days versus 13.9 (n = 22) (\( p = 0.0077 \)). Hence, *C. lanceolata* shows similar properties, as far as the nutritional value of its reproductive organs for *U. ornatrix* larvae is concerned, to *C. spectabilis* and *C. pallida*, and this correlates with the c.25% increase in nitrogen from leaves to seeds (*Table 1*).

(8) *Crotalaria rotundifola* leaves (wild parental population) versus beans of *C. spectabilis* (F1 laboratory generation) (*Figures 1B, 4D*)

In April 2014, a sample of nine *U. ornatrix* specimens (four females and five males) were collected from a Cross Creek site outside of Gainesville, FL. This population corresponds to the native hostplant, *C. rotundifolia*. Offspring of three of the females were raised in captivity, where they were first fed on *C. lanceolata* leaves, but starting with the third instar were raised through to pupation on previously frozen *C. spectabilis* beans. The laboratory-raised offspring were significantly larger than the parental wild population (e.g. *Figure 1B*): laboratory-raised males had fore wing size of 20.7 ± 1.1 mm (n = 13) versus 18.7 ± 0.4 mm (n = 5) of the wild males, \( p = 0.001 \); females: 20.0 ± 1.1 mm (n = 10) versus 18.6 ± 0.9 mm (n = 4), \( p = 0.05 \).
Crotalaria pallida beans versus leaves (Figure 5B, C – after data from Ferro et al. 2006)

Ferro et al. (2006) experimented with this species and found that feeding on beans led to faster development and resulted in female pupae of higher weight than feeding on leaves. My observations concerning the rate of development of *U. ornatrix* on leaves versus frozen beans of *C. pallida* support their conclusion that larval feeding on beans of this species, as in the case of other species described above, leads to faster development time.

In nature, it is unusual to find young larvae feeding on beans. Eggs are laid in batches of a few to many (Figures 1C, H, 2L). At first, larvae feed gregariously on leaves (Figures 1I, 2L), and then they disperse by the second–third instar, but are often too small to penetrate the bean pods (Figure 2M). Experiments show that pod penetration at younger stages holds considerable risk of mortality (Ferro et al. 2006). My observations suggest that pods of different *Crotalaria* shield their seeds from *U. ornatrix* larvae to different degrees, and that sometimes even larger larvae have a difficult time breaking through their tough (and perhaps otherwise mechanically/chemically protected) outer surface (e.g. Figure 2M). However, sometimes larvae as young as the 3rd instar can be found inside pods. This can potentially offer the larvae a great advantage: in the laboratory, larvae that were given fresh-frozen beans of *C. spectabilis* as neonates developed relatively faster (c.22 days) compared to the fastest development time of c.34 days on leaves of this species. This, the protection offered by pods from natural enemies and harsh environmental conditions, and the fact that larvae feeding on seeds can sequester pyrrolizidine alkaloids faster, explain the remarkable ability of *U. ornatrix* larvae to penetrate not only seed pods, but also man-made surfaces that are normally impenetrable to other larvae. For instance, in the laboratory, hungry *U. ornatrix* larvae were observed to chew through the walls of plastic containers, and even to search out and ‘break into’ other containers with food and larvae by chewing holes through them. The mature *U. ornatrix* larvae can travel long distances in search of plants. For instance, in a screened enclosure, larvae were observed to travel easily between potted plants located several metres apart. They can survive a long time without food, and switch easily between species of *Crotalaria*. All of these characteristics make *U. ornatrix* a very interesting object for studying herbivore–host interactions.

**Nitrogen and pyrrolizidine alkaloid contents of Crotalaria**

Plant secondary chemicals vary significantly at the intraspecific level, as for instance does caffeine in coffee beans, where a worldwide survey found the maximum difference of 50% (Fox et al. 2013). This variation can be a driving force of plants’ co-evolution with herbivores (Moore et al. 2014). *Crotalaria* is no exception, as was shown in the case of *C. pallida* (Cogni et al. 2011) when it comes to intraspecific variation. What is especially interesting for the present study is that interspecific variability in the PA content of *Crotalaria* seeds is much higher than the intraspecific variability. For instance, the PA content in *C. spectabilis* seeds is 100 times higher than in *C. pumila* and 50 times higher than in *C. incana* (Williams and Molyneux 1987). At the same time, it does not appear from the present study that PA presence slows down or speeds up the development of larvae, supporting Cogni et al. (2012), who tested under controlled conditions the effect of PA concentration on *U. ornatrix* fitness, and found
that though the expression of genes responsible for PA detoxification can increase as much as 40 times, no significant cost is incurred by the larvae or the resulting adults. In my experiments, it is the nitrogen content in leaves and seeds, evaluated using a CHN analyser, that seems to correlate with the rate of development. For instance, *C. lanceolata*, on which the development is usually slower, tends to have 20–30% less nitrogen in its leaves (Table 1). *Crotalaria retusa* leaves, on which development is also quite slow (pers. obs.), also shows low nitrogen content. The nitrogen in plants is known to fluctuate not only between species, but also within a species and seasonally, and *Crotalaria* is no exception. For instance, it was shown (Uratani et al. 2004) that *C. pallida* consistently exceeded *C. incana* in nitrogen content, although in both species the levels fluctuated significantly throughout the growing season. It appears that the nitrogen content can be increased for all *Crotalaria* species by growing the plants in rich potting soil (Table 1, columns 3 and 4), but this increase is not the same in all species, and some species, such as *C. retusa* and *C. rotundifolia*, are relatively low in nitrogen even when grown in rich potting soil.

**Larval feeding preference**

Ultimate instar larvae, previously raised on *C. lanceolata* leaves (a relatively low PA diet), were released into trays with randomly positioned fresh-cut foliage of different *Crotalaria* species (*C. lanceolata, C. incana, C. pallida, C. spectabilis, C. pumila, C. retusa* and *C. rotundifolia*) (Figure 1D). After crawling around, five out of 10 times the larvae ended up feeding on *C. spectabilis*, two times on *C. lanceolata*, two times on *C. incana*, and once on *C. pallida*. These trials, although limited in number and constrained by artificial set-up, indicate that there is no single hostplant that is unambiguously more attractive to the larvae, while the preference for *C. spectabilis* may be due to the higher PA content in this species (Table 1). This explanation is supported by experiments with artificial diets, which showed that *U. ornatrix* larvae raised on low PA diet prefer diets with higher PA contents (Hoina et al. 2013).

While growing plants in a large walk-in screened enclosure, several larvae that were placed as neonates on one plant, after consuming it, moved freely 3–4 m away, locating additional potted plants for their development. It is quite clear from this, as well as from field observations, that *U. ornatrix* larvae can migrate long distances in search of food and have an excellent ability to cue in on their hostplants in general and on high-PA plants and plant organs, in particular. More experiments in different settings are needed to fully understand how larvae locate plants in the field and whether they prefer one species of *Crotalaria* over another.

**Field observations concerning phenologies of Crotalaria and U. ornatrix**

In Brazil, Cogni (2010) showed that exotic *C. pallida* can be more attractive for oviposition than the native *C. incana*: females laid c.2.5 times more eggs on cuttings from pot-grown plants of *C. pallida* than of *C. incana*. It is possible that this was due to the higher N\(_2\) content: when grown under controlled conditions in rich soil, N\(_2\) in *C. pallida* can be much greater than in *C. incana* (Table 1). One must note, however, that in nature, these two species may prefer to grow in different soils, and hence the natural N\(_2\) contents may prove to be different. They also may have different phenologies which will depend not only on their growing conditions, but also on the latitude.
It was proposed (Cogni et al. 2011; Franco and Cogni 2013; Sourakov and Locascio 2013) that the differences in phenology of *Crotalaria* will affect the resulting moth populations. My observations made on various *U. ornatrix* populations around Gainesville, Florida, during 2011–2014 support this supposition. For instance, at a site dominated by *Crotalaria lanceolata* (the upland pine habitat behind the Florida Museum of Natural History on University of Florida campus; Figure 1G), the first adult moths appeared on 1 October two years in a row, even though the plants were present in great abundance throughout the summer. By late November, *C. lanceolata* had almost disappeared, and with them, the moths. The small patch of *C. pumila* within this habitat, which had no infestations by *U. ornatrix* in the fall, became the site of larval development (Figure 2F, G), with plants and larvae thriving through December, until this plant, too, declined.

At another site in north Gainesville, where only *C. pallida* grows in a large and dense stand in the woods (Figure 2B), the first larvae were found in June, and adult moths were flying throughout the fall, until the first freeze in January sent this population into a diapause by obliterating the plant foliage.

At a site occupied by *C. spectabilis* and *C. retusa* in Micanopy Hammock (about 25 km south of Gainesville), the extensive stands of *C. spectabilis* (Figure 2A) were utilized by the moths as hostplants in July–October. However, by late November, *C. spectabilis* was dying out, following the maturation of its beans, which were favoured by the larvae (Figure 2C, D, K). Sympatric *C. retusa*, on the other hand, which had only an occasional larva on it in the summer/fall, became the primary hostplant for this population in December (Figure 2L, M), when some of its plants were still in full bloom (Figure 1A). At this *U. ornatrix* population, while *C. spectabilis* has gone to seed and died off in winter, a number of *C. retusa* plants survived the freeze, as they are able to grow in denser woods and perhaps are also more freeze-tolerant. In late March 2014, a search of the few tiny *C. spectabilis* sprouts at this site yielded viable *U. ornatrix* eggs, while a much larger number of *C. retusa* plants (including flowering and fruiting individuals), which survived the winter freezes under denser tree cover, yielded no *U. ornatrix*, again supporting the existence of preference of one hostplant species over another by the female moths for oviposition.

The moths began to fly in early March 2014 at a Cross Creek site, where only the native *C. rotundifolia* was re-sprouting from roots out in the open field, and became abundant by the end of the month. On the other hand, the populations sustained by other *Crotalaria* species, such as *C. lanceolata, C. pallida*, and *C. pumila*, showed no sign of new hostplant growth or the moths throughout the spring.

In addition to differences in the phenology of the hostplants, which are due to different sprouting regimes and requirements, in growing rate, and in freeze tolerance, there are differences in their physical properties which may affect the biology of *U. ornatrix*. Indeed, the seeds of different *Crotalaria* species sprout differently under similar conditions. The seeds of the native *C. pumila*, for example, require a much longer exposure to moisture before they sprout than those of several exotic species (Figure 1F). At the same time, the native species, *C. pumila* and *C. rotundifolia*, proved to be more drought-tolerant: they survived better than *C. lanceolata* and all three performed much better than *C. spectabilis* and *C. pallida* during short-term desiccation of pots in the heat of Florida summer. The latter two species proved to be not only less heat tolerant, but also much more subject to mortality from nematodes, leaf-mining wasps and fungi.
Differences in rates of development affect the speed with which the moths can cycle through generations. These differences can be due not only to the higher nutrition value of different hostplant tissues as demonstrated above, but also due to their morphology. For instance, the amount of foliage on a single plant of *C. rotundifolia* or *C. lanceolata* is frequently insufficient to support even a single larva, which forces larvae to travel between plants before they complete their development. On the other hand, a single plant of *C. spectabilis* or *C. pallida* offers a substantial amount of foliage sufficient to sustain numerous larvae throughout their development. The large pods of *C. pallida*, *C. retusa*, *C. incana* and *C. spectabilis* are able to conceal larvae, providing shelter during the cold conditions and from predators and parasitoids, while pods of *C. pumila*, *C. lanceolata*, and *C. rotundifolia* are of a more modest size and offer no such protection (Figures 1E, 2C–K). For instance, on 15 March 2013, when no hostplant foliage had been available for at least two months due to prior freezing, I found a hibernating larva inside a dry pod of *C. spectabilis* which, once brought inside, was promptly raised on frozen *Crotalaria* seeds to adult stage, emerging in April. Such hibernation of larvae during the winter has been recorded for other species of Lepidoptera in north-central Florida (e.g. *Agraulis vanillae* L.; see Sourakov 2009), and perhaps is much more common than we think in subtropical regions.

**The impact of larvae on hostplants**

Although the present study mostly focuses on the ways hostplants influence the biology of *U. ornatrix*, the moths’ effect on its hosts should also be noted. From a practical point of view, *U. ornatrix* can be very important as both a pest and a biocontrol agent (depending on whether one is growing rattlebox plants as cover crops, or is suffering from their effects as toxic and highly invasive weeds). Cogni et al. (2011) already noted the dramatic impact that *U. ornatrix* can have on *Crotalaria* plants. To illustrate the influence of *U. ornatrix* on a *Crotalaria* population in Florida, I can cite my recent observation from the *C. pallida* site in Gainesville (Figure 2B), where, in the summer of 2013, there were two groups of *C. pallida* located within 100 m from each other in a small nature preserve within the city. The first group, located in a clearing inside the forest, consisted of hundreds of plants, with a thriving population of *U. ornatrix* present, while the second group, located on the forest edge, consisted of a dozen plants and was devoid of *U. ornatrix*. It should be noted that *C. pallida* is an extremely prolific seed producer (Figures 1E, 2B, I), where 2000 seeds can easily be produced by a single plant, and these seeds sprout readily in moist soil (Figure 1F). Despite that, and the fact that 2014 had conditions extremely favourable for *Crotalaria*, with most populations of these plants around the city thriving, the first group saw a significant reduction of the number of plants by the following fall, probably because most of the seeds were destroyed by moth larvae. Conversely, the second group, which had just a few *C. pallida* plants in 2013, by fall of 2014 had hundreds of plants and a thriving population of *U. ornatrix*. From this example, one can see a great potential for the herbivore-driven evolution of traits related to plant defence within the genus *Crotalaria* (see below).
Co-evolution of Utetheisa and Crotalaria

Old World in its origin (DaCosta 2010), the genus Utetheisa has accompanied Crotalaria plants in their evolution and distribution throughout their pantropical range for a long time; the larval specialization on Crotalaria PAs in this genus evolved early during the history of larval pharmacophagy in the tiger moth subfamily (Zaspel et al. 2014). Crotalaria as a genus is partially defined by the accumulation of PAs (Wink and Mohamed 2003; le Roux et al. 2013), and that is what the moths are seeking. Hence, the genus has likely had to adapt to the pressures placed by the moth larvae onto the reproductive organs of its hosts. Judging by my observations in Florida, where Crotalaria belonging to distant clades are all attacked by a single species of Utetheisa, it is likely that none of 702 species of Crotalaria is immune from being attacked by this moth or its relatives. Worldwide, these include not only members of the genus Utetheisa sensu lato, which encompasses c. 60 species (e.g. DaCosta 2010), but its close relatives, such as the genus Amphicallia Aurivillius, which in Africa seriously attack Crotalaria cover crops (e.g. Girma et al. 2006), and where the origin of Crotalaria likely lies (Subramaniam et al. 2013).

Moths may be the main cause for variability found in the amounts of PAs among different Crotalaria species. Other strategies to escape the pressure from moths may include changes in plant morphology and ecology; the mode of growth found in different Crotalaria species can vary from densely growing tall plants with extremely prolific seed production, perhaps aimed at overwhelming the demand from the moths (e.g. C. pallida, C. lanceolata and C. spectabilis; Figures 1G, 2A, B), to sporadic plants with smaller seed pods, where a single larva would have to penetrate and consume numerous pods to develop into a pupa, and hence a viable population of moths would be harder to sustain (e.g. C. pumila; Figure 1G). It is notable that the latter species, which is native to Florida and hence likely to have coexisted with U. ornatrix for a long time, also exhibits extremely low PA content (0.04%) and extremely high water content (95%) in its seeds, which are enclosed into a small and hard pod, perhaps to make itself (or at least its reproductive organs) even less attractive to the larvae. Perhaps because of their relative attractiveness to Utetheisa and its relatives, some Crotalaria species, such as C. pallida and C. lanceolata, developed extrafloral nectaries that attract and feed ants (Figure 2E) and that in return provide protection from the larvae (Guimarães et al. 2006).

Conclusions

To conclude, the introduction into Florida of numerous exotic Crotalaria species with different morphology, ecology and chemistry manifests itself in the substantial expansion of habitat for Utetheisa ornatrix as well as in effect on its phenology, likely expanding this species’ activity periods as adults and larvae throughout the year. The hostplant choice on the intra and interspecific levels may influence the size of the resulting moths, creating new dimensions for our understanding of the biology and evolution of U. ornatrix. These novel manmade herbivore–hostplant associations provide a remarkable opportunity to study micro-evolutionary processes in this beautiful, economically and ecologically important, and highly researched species of moth.
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