

NOTES

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Costs and benefits of raising *Utetheisa ornatrix* on two different plant diets and the role of inbreeding depression in larval survival

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Abstract: The Bella Moth, *Utetheisa ornatrix*, has a strong affinity with *Crotalaria* plants, from which it derives alkaloids that are crucial for its defense and reproduction. While it has been suggested that there is no cost for sequestering alkaloids by caterpillars, there are, however, costs and benefits associated with using one diet versus another. While normally the larval mortality in the late instars in the lab is quite low, in the course of rearing inbred individuals on *Crotalaria lanceolata* beans and *Crotalaria spectabilis* leaves, a higher than normal mortality of larvae was observed. Depending on the diet, larvae showed a different survival pattern and the causes of mortality were different. This raises an interesting question: does testing plant defenses using heterozygous insects that is typical of most experiments mimic scenarios that realistically occur in nature, where the founder effect frequently generates inbreeding depression? Additionally, we report differences in developmental rate and final insect size resulting from feeding larvae on these two diets, simultaneously providing evidence for a linear positive correlation between pupal weight and the wingspan of adult moths.



Fig. 1. *Crotalaria spectabilis*, nr. Micanopy, Florida



Fig. 2. *Utetheisa ornatrix* larva consuming beans of *Crotalaria lanceolata*.

Table 1. Comparison of the two plants that provided diets for the experiment (after Sourakov 2014)

<i>Crotalaria</i> 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***
<i>C. lanceolata</i>	0.21%	4.9-5.2%	2.8-2.9%	3.5-3.9%	76%	87%	Sec. Hedriocarpaceae	Africa
<i>C. spectabilis</i>	3.81%	3.7-3.8%	3.3%	4.1%	61%	77%	Sec. Crotalaria	Asia

*** These plant species were introduced to Florida at the beginning of the 20th century as cover crops and became established. They are naturally used as hosts by *U. ornatrix*.

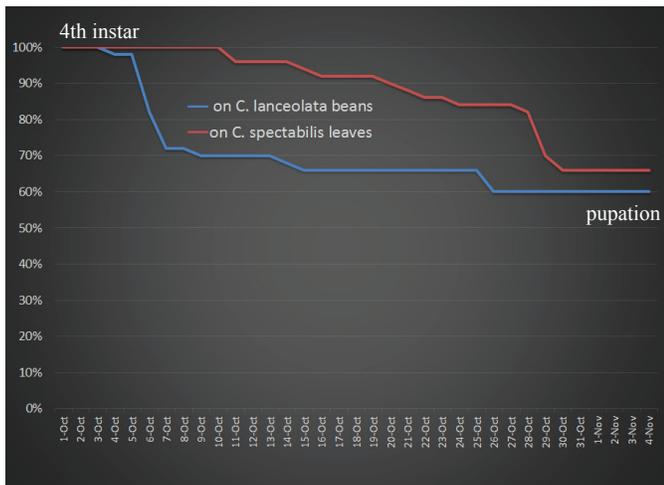


Fig. 3. *U. ornatrix* larval survival rate on two different diets (N=100).

Materials and Methods

Two lines of *Utetheisa ornatrix* (one from a batch of eggs laid by a female collected at North Brooksville, Hernando County, another from a female collected in Ordway, Putnam County, both localities being in Florida, about 100 miles apart, were crossed and the resulting siblings were crossed with each other. The F-1 sibXsib larvae were reared until the fourth instar on leaves of *Crotalaria lanceolata* (a low-alkaloid diet). Then, 100 of them were randomly divided into two groups: 50 were switched to beans of *C. lanceolata* (pods were split open for easy access) and 50 were switched to leaves of *C. spectabilis*. Cups (2 oz portion cups each containing a single larva) were arranged in a checkerboard manner on the lab table at 22°C and constant light. Experiments took place in September-October, when plants were available in the Natural Area, University of Florida campus. Food was replenished every two days with new cups replacing the old ones. While food was not sterilized, all other equipment was kept sterile.

Mortality

Larvae fed on beans experienced high mortality 3-4 days into the experiment (Fig. 3). The symptoms (Fig. 5) were consistent with an overdose of secondary plant compound (the second author frequently observed this type of mortality from alkaloid poisoning of *Automeris io* larvae fed on *Erythrina herbacea* or from cyanogenic compound-induced mortality on *Prunus serotina*). The symptoms were not consistent with viral, bacterial or fungal disease.

The group fed on *Crotalaria spectabilis* exhibited mortality towards the end of larval development and during the pupal stage. The symptoms (Fig. 6) were consistent with fungal disease very likely occurring as a result of ingestion of fungal spores located on *C. spectabilis* leaves. While the total mortality on two diets was similar, with the overall survival rate of about 70% (Fig. 3), it had different



Fig. 5. Mortality of *U. ornatrix* larvae feeding on *C. lanceolata* beans occurred between 4th and 7th days of feeding and most likely was plant diet-related.

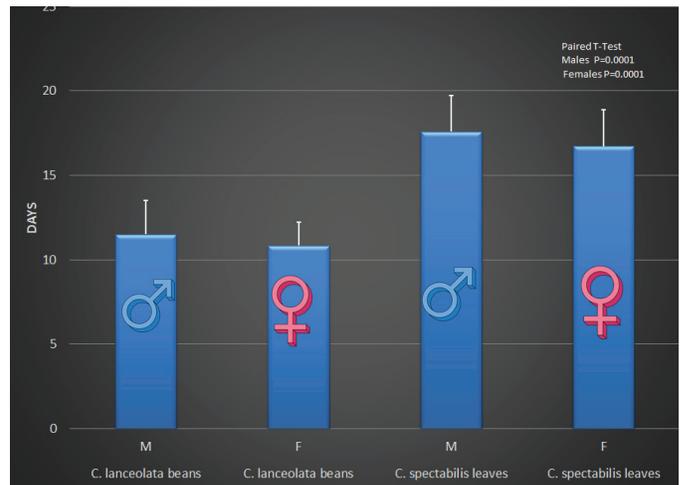


Fig. 4. Average length of development of *U. ornatrix* larvae from 4th instar to pupation on two different diets.

patterns and causes.

We strongly suspect that inbreeding contributed to the mortality observed on the bean diet, which is supported by additional observations from two other broods that were raised in a separate lab in parallel with the experiment described above. One brood represented similarly inbred individuals with the same ancestry (a cousin line), but raised on diets of *Crotalaria pallida* beans and leaves. While the leaf diet caused no mortality in late stages of larval development, eight among 25 larvae switched to beans died with the same symptoms as caused by *C. lanceolata* beans in the experiment above. The other brood of 50 individuals that represented a first generation cross between two separate genetic lineages (and hence likely much more heterozygous) was raised identically to the experiment described above, with only two larvae dying from feeding on *C. lanceolata* beans while 10 died on the *C. spectabilis* leaf diet.

Development rate and size of adults

Development occurred faster on beans than on leaves and the resulting adults were larger (Figs. 6-7). While normally bean diet results in faster development and larger adults than leaves of the same plant (e.g. Sourakov 2014), it was largely attributed to the difference in nitrogen content. Here, however, the nitrogen content was likely comparable in both diets (Table 1), and the greatest easily measurable difference is in the amount of water: *C. lanceolata* beans have 1.5 times more water than *C. spectabilis* leaves. The amount and the chemical composition of alkaloids was certainly different between the two diets, but this analysis is beyond the scope of the present note.

We also tested whether the pupal weight, as one measurement of the adult size, positively correlated with wing span. Figure 7 convincingly demonstrates that it does and that either measurement can be adequately used to assess the influence of environmental factors



Fig. 6. Mortality of larvae feeding on *C. spectabilis* leaves occurred throughout feeding, but mostly towards pupation, and was likely pathogen-related.

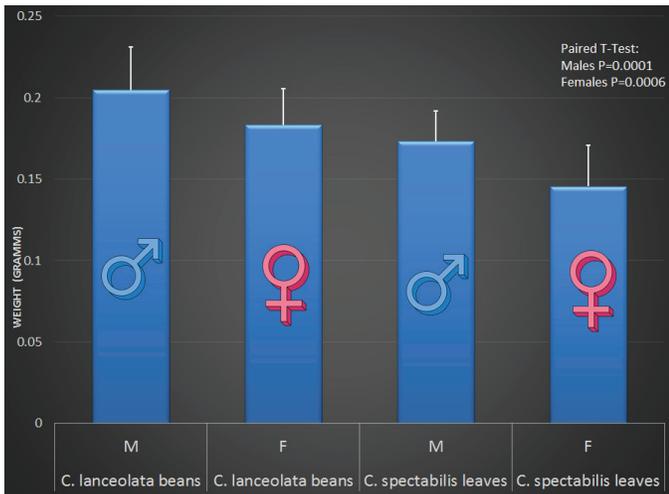


Fig. 6. Pupae of *U. ornatrix* resulting from larval feeding on *C. lanceolata* beans were significantly heavier than those fed on *C. spectabilis* leaves.

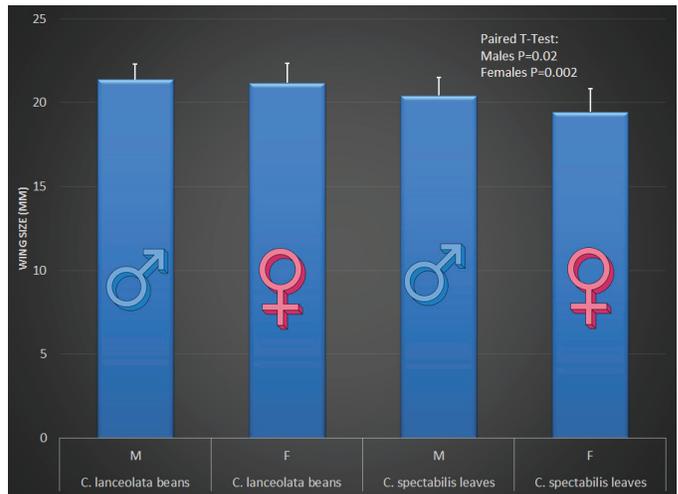


Fig. 6. Wingspan of *U. ornatrix* resulting from larval feeding on *C. lanceolata* beans was significantly larger than those fed on *C. spectabilis* leaves.

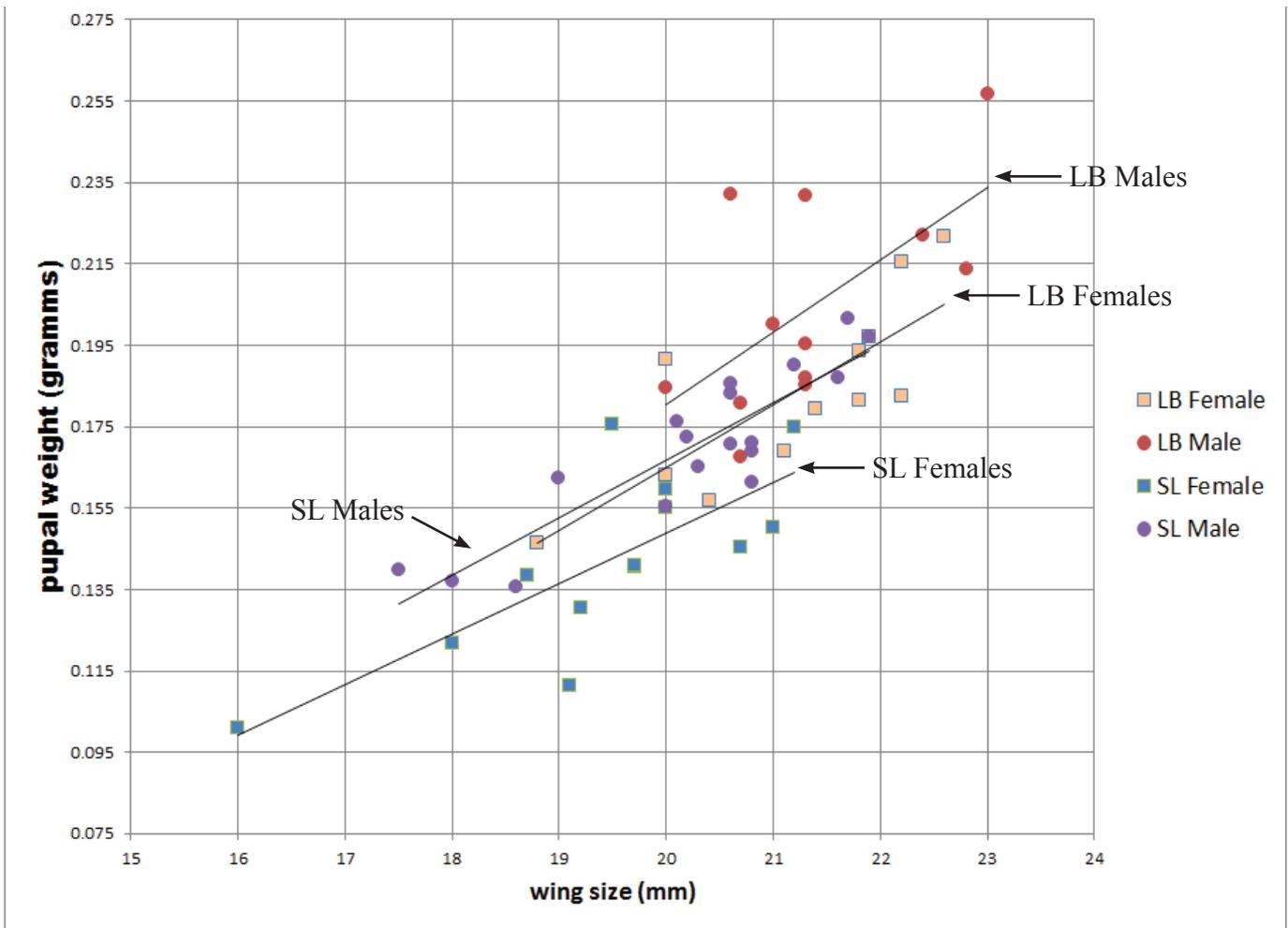


Fig. 7. There is a clear linear relationship between pupal weight and wingspan, although wingspan increases slightly slower with pupal weight on LB diet. (LB - larvae raised on diet of *C. lanceolata* beans; SL - larvae raised on diet of *C. spectabilis* leaves)

on moth size.

The present note provides another window into the interactions between exotic *Crotalaria* hostplants and the native moth, *Utetheisa ornatrix*. The system, while interesting from the biocontrol point of view, is also full of potential when it comes to basic ecological research.

References

Sourakov, A. 2015. You are what you eat: Native versus exotic *Crotalaria* species (Fabaceae) as host plants of the Ornate Bella Moth, *Utetheisa ornatrix* (Lepidoptera: Erebiidae: Arctiinae). *Journal of Natural History* 49:2397-2415. and references therein.

ELEN V and ELEN VI

I was fortunate to attend the Fifth Meeting on the Neotropical Lepidoptera in November 2015 that took place in Tucumán, Argentina. Just like the previous meeting in Montevideo four years ago, this was the most delightful experience.

To begin, I had a layover in Córdoba on the flight from Miami, and enjoyed several hours wondering around this old city, the home of the oldest University in Argentina. Upon arrival in Tucumán, one of the organizers, Germán San Blas, met me in his car and took me to a biological reserve belonging to the University of Tucumán where the meeting was taking place.

The setting for the conference was the best a naturalist could wish for, as the building where participants stayed and attended talks was right next to the forest, and everyone was able to enjoy the unique nature of these mountains between sessions. The dry river bank nearby and the forest trails provided for a chance to take photos of the local Lepidoptera fauna.

Talks and posters were diverse and so were the participants, who came from as far as USA and Spain. Talks were grouped into symposia on *Ecology, Biogeography, Conservation and Evolution; Lepidoptera of Economic Importance; and Systematics, Morphology, and Biodiversity*. There were also numerous poster presentations and opportunities for informal interactions, such as joint meals, banquet and trips. Several of us visited the Institute Miguel Lillo in Tucumán - an important collection containing many type and historical specimens.

One can find the program, photo gallery and other information about ELEN V at <http://www.elen5.com.ar/>. The next ELEN meeting is planned for 2018 in Concepción, Chile.

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A roost of *Heliconius erato phyllis*, nr. Tucumán, Argentina.



nr. *Semniomima tristrigalis* (Crambidae: Pyraustinae), nr. Tucumán, Argentina.



Heterusia cruciata (Geometridae: Larentiinae), nr. Tucumán, Argentina.