

RESEARCH ARTICLES

ALKALOIDAL PROTECTION OF *UTETHEISA GALAPAGENSIS* (LEPIDOPTERA: ARCTIIDAE) AGAINST AN INVERTEBRATE AND A VERTEBRATE PREDATOR IN THE GALAPAGOS ISLANDS

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SUMMARY

The Galapagos endemic moth *Utetheisa galapagensis* has been shown to sequester pyrrolizidine alkaloids from its host plants in the genus *Tournefortia* (Boraginaceae). We here assess the palatability of *U. galapagensis* adults to sympatric invertebrate and vertebrate predators. Adult *U. galapagensis* and *Pilocrocis ramentalis* (Pyralidae) controls, were offered to orb-weaving spiders *Eustala vegeta* and female lava lizards, *Microlophus pacificus*. The spiders' reactions to the two types of prey were highly stereotyped; invariably the controls were eaten and the *U. galapagensis* were cut from the web and released. In comparison, when offered to female lava lizards both *U. galapagensis* and the pyralid controls were usually consumed. However, the lava lizards sometimes displayed rejection behavior with *U. galapagensis* and the time spent handling this species was significantly greater than for controls. Our results indicate that *U. galapagensis* relies on alkaloidal defense to protect it from nocturnal arachnid predators. Against diurnal lizards crypsis is likely their major defense since alkaloidal sequestration is only marginally effective in protecting them from these predators.

RESUMEN

Protección por alcaloides contra predadores vertebrados e invertebrados de *Utetheisa galapagensis* (Lepidoptera: Arctiidae) en las islas Galápagos. La mariposa endémica de Galápagos *Utetheisa galapagensis* secuestra alcaloides de su planta huésped del género *Tournefortia* (Boraginaceae). Hemos probado el sabor de adultos de *U. galapagensis* con predadores simpátricos de vertebrados e invertebrados. Adultos de *U. galapagensis*, y como control, de *Pilocrocis ramentalis* (Pyralidae), fueron ofrecidos a arañas tejedoras *Eustala vegeta* y a hembras de lagartijas de lava *Microlophus pacificus*. La reacción de las arañas a los dos tipos de presa fueron altamente estereotipadas; invariablemente el control fue comido y las *U. galapagensis* fueron liberadas luego de ser cortada la red. En comparación, cuando fueron ofrecidas a las hembras de lagartijas de lava, *U. galapagensis* y el control Pyralidae fueron usualmente consumidos. Sin embargo, la lagartija de lava algunas veces tuvo un comportamiento de regurgitación con *U. galapagensis* y el tiempo pasado manipulando esta especie fue significativamente mayor que para el control. Nuestros resultados indican que *U. galapagensis* tiene defensas por alcaloides para protegerse de predadores como arañas nocturnas. Contra las lagartijas diurnas la coloración críptica parece ser su mayor defensa desde que el secuestro de alcaloides es solo parcialmente efectivo para protegerlas contra esos predadores.

INTRODUCTION

Members of the cosmopolitan tiger moth genus *Utetheisa* are frequently brightly colored (Fig. 1) and are considered diurnal and aposematic (Holloway 1988). The latter contentions have been tested with larval and adult *U. ornatrix* L., a species known to sequester defensive pyrrolizidine alkaloids (PAs) from its larval host plants, legumes of the genus *Crotalaria* (Conner *et al.* 1981). By virtue of their bad taste, sequestered PAs protect larvae,

pupae, and adults from invertebrate and vertebrate predators (Eisner & Eisner 1991, Eisner *et al.* 2000, Eisner 2003, Eisner & Meinwald 2003, Rossini *et al.* 2004) and since the alkaloids can be passed transovarially, they protect the egg stage as well (Bezzerides *et al.* 2004, Hare & Eisner 1993). Diurnal predators are also apparently capable of learning to discriminate against the gold, black and white pattern of the larvae and the pink, black and white pattern of the adults as aposematic warnings of their underlying PA defense (Eisner 2003).

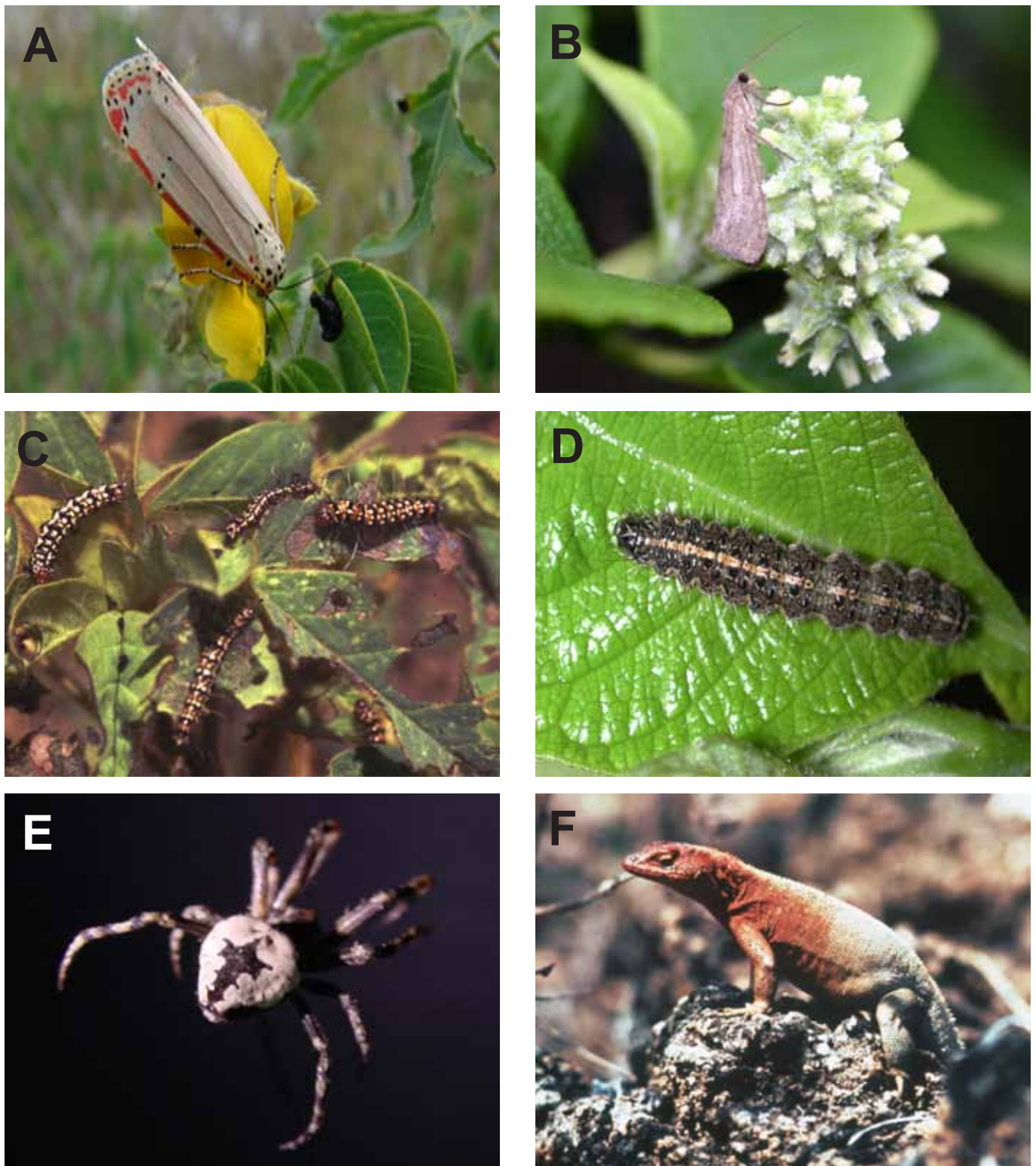


Figure 1. Comparison of the aposematic adult and larval stages of *Utetheisa ornatrix* (A and C) with the cryptic adult and larval stages of *Utetheisa galapagensis* (B and D). Predators used to assess moth palatability: (E) the nocturnal arachnid *Eustala vegeta* (alcohol preserved specimen) and (F) the diurnal lava lizard *Microlophus pacificus*.

In the Galapagos Archipelago there are three endemic species of *Utetheisa* that do not fit the general *ornatrix* pattern. *U. galapagensis* (Wallengren), *U. perryi* Hayes and *U. devriesi*

Hayes can all be considered cryptic (Fig. 1). Larvae of all three species are drab in color and secretive in habit. Adults are brownish grey and blend in with their environs.

Roque-Albelo *et al.* (2002) posed four possible explanations for this deviation from the aposematic norm: (1) the endemic species may be chemically protected (unpalatable) yet primarily nocturnal, rendering warning coloration unnecessary; (2) they may be chemically protected and diurnal and use crypsis as a second line of defense; (3) they may be palatable (not chemically protected), diurnal and employ crypsis; (4) they may be palatable, nocturnal and non-aposematic. Roque-Albelo *et al.* (2002) found that one of the endemics, *U. galapagensis*, sequesters PAs not from *Crotalaria* but instead from *Tournefortia* species (Boraginaceae), including *T. rufo-sericea* Hook. f. Both plant and insect contain the PA indicine and related alkaloids that are potentially protective. However, *Tournefortia* alkaloid-laden *U. galapagensis* have never been tested to determine their degree of protection against natural predators. We here describe simple spider and lizard bioassays with predators and prey in their natural habitat.

METHODS

Adult *U. galapagensis* were offered as prey items to an endemic orb-weaving spider, *Eustala vegeta* (L. Koch) Simon (Araneidae), on Isabela island, as well as to an endemic lava lizard, *Microlophus pacificus* Steindachner (Kizirian *et al.* 2004), on Pinta island (Fig. 1). Lava lizards were chosen because they are a diurnal predator known to include adult moths in their diet (Stebbins *et al.* 1966). All bioassays were conducted in the species' natural habitats. Spiders were identified by Dr. Léon Baert, Departement Entomologie, Koninklijk Belgisch Instituut voor Natuurwetenschappen.

Spider Bioassays

For the first set of experiments, adult *U. galapagensis* and controls (*Pilocrosis ramentalis*, Lederer: Pyralidae) were collected on 12 and 13 March 2005, at an ultraviolet light at 850 m elevation on the eastern slope of Alcedo volcano, Isabela island. The habitat was the humid zone of the volcano dominated by *Psidium galapageium* Hook. f. (Myrtaceae), *Tournefortia pubescens* Hook. f. and *Scalesia microcephala* B.L. Rob. (Asteraceae). Moths were collected directly before their use in spider bioassays. *Eustala* were located in the immediate surroundings of the moth collecting site. Only spiders with fresh webs were used in the bioassays.

Bioassays were carried out after dusk. Each began by placing a randomly chosen *U. galapagensis* or pyralid within the lower half of a spider's web. Once the spider had either cut the moth from its web or consumed the moth, in part or in whole, and had returned to the hub of its web, the alternate prey species was placed in the web. The reaction of each spider to both prey items was recorded in infrared digital video for later analysis (Sony Digital Handycam DCR-TRV36). No spider was used for more than one presentation of *U. galapagensis* and one presentation of a control pyralid.

Lizard Bioassays

Adult *U. galapagensis* and pyralid controls were collected on 17 and 18 March 2006, at an ultraviolet light at 421 m elevation on the southern slope of Pinta island. The habitat was transition forest dominated by *Pisonia floribunda* Hook. f. (Nyctaginaceae), *Zanthoxylum fagara* (L.) Sarg. (Rutaceae), and *Tournefortia rufo-sericea* Hook. f. (Boraginaceae). Moths were collected and housed in plastic vials until bioassays could be performed the following day.

Female lava lizards *Microlophus pacificus* were located in our camp and along adjacent trails. Although *U. galapagensis* are primarily nocturnal they frequently take flight when disturbed during the day. This renders them susceptible to attack by diurnal predators like lava lizards.

Bioassays were carried out at midday. Each began by offering a lizard one control pyralid moth and one *U. galapagensis* in random order. One wing was clipped from each moth to prevent escape. The alternate moth was then presented to the lizard approximately 1 min. after the first interaction had terminated. The reaction of each lizard to both prey items was videotaped using the video camera described above, but with natural illumination. Handling time was determined by field-by-field analysis of the videotape and was defined as the time between a lizard's first contact with the prey and when the prey was completely consumed by the lizard (defined as the disappearance of any body parts protruding from the lizard's moth).

Statistics

A Fisher Exact Test (Sokal & Rohlf 1981) was used to compare the reaction of both lizards and spiders to *U. galapagensis* and pyralid controls. To compare mean handling times we used a paired *t*-test assuming unequal variance (SPSS® 14.0).

RESULTS

Spiders

The spiders' reactions to the two types of prey item were stereotyped. When a pyralid was placed in the web, the spider tensed the web and located the prey item. It then rushed to the moth, palpated it, attacked and immediately began to consume the moth. After a feeding bout, lasting a variable amount of time, the spider wrapped the moth in silk and then either continued to feed or carried the prey to the hub of the web where it hung the moth for later feeding. The reaction to *U. galapagensis* was strikingly different. The spider again rushed to the moth, contacted the moth with its mouthparts, apparently tasting it, and instantly withdrew a variable distance. After a moment the spider began to manipulate the moth with its legs and mouthparts to detach the moth from the web. *U. galapagensis* specimens were invariably released (Table 1). The probability of this distribution of results is highly significant (Fisher Exact Test $P < 0.01$).

Table 1. Palatability of *Utetheisa galapagensis* and pyralid controls to *Eustala vegeta* spiders. Left column gives the number of the individual spider tested. + indicates the spider fed on and wrapped the moth; – indicates it rejected the moth.

Spider	Pyralid control	<i>Utetheisa galapagensis</i>
1	+	no data
2	no data	-
3	+	-
4	+	-
5	no data	-
6	+	-
7	+	-
8	+	-
9	+	-
10	+	-
11	+	-

The behavior of each moth species may also have contributed to the interaction. The pyralids clearly struggled with the spider, and the spider responded by wrapping the moths in silk. The *U. galapagensis* froze, rarely struggled and were consequently not wrapped in silk.

Lava Lizards

When a moth specimen was offered the lizard either responded immediately or at the first sign of fluttering of the moth. The attack was swift and visually directed. The lizard proceeded to mouth the specimen while reorienting it for swallowing. All control pyralids were eaten and all but two of the *U. galapagensis* were also consumed (Fisher Exact Test $P > 0.05$). However, the lizards showed greater reluctance to swallow the *U. galapagensis*, as manifest in a significantly longer handling time (4.97 ± 1.64 s for pyralid controls and 12.79 ± 5.51 s for *U. galapagensis*; t -test, $P < 0.01$; Table 2). The two lizards that did not eat the *U. galapagensis* showed classic rejection behavior. They attempted to scrape the specimen from their mouths with their forelegs

Table 2. Palatability of *Utetheisa galapagensis* and pyralid controls to lava lizards *Microlophus pacificus*. Left column gives the number of the individual lizard tested. The other columns indicate the time (seconds) between the lizard's first contact with the moth and the disappearance of the prey item.

Lava lizard	Pyralid control	<i>Utetheisa galapagensis</i>
1	4.58	7.94
2	4.80	13.17
3	2.67	12.40
4	5.60	not eaten
5	4.93	24.57
6	2.07	12.13
7	5.49	11.00
8	6.14	14.80
9	7.77	not eaten
10	5.64	6.27

and frequently wiped their mouths on the substrate after rejecting the specimen. However, if the released moth fluttered again, the lizard reinitiated the attack sequence. One *U. galapagensis* was attacked and released three times in rapid succession. No learning was evident.

DISCUSSION

It is clear from even this small number of presentations that *U. galapagensis* is highly unpalatable to *Eustala vegeta*. It seems probable that the unpalatability is due to sequestered PAs, which have previously been shown to be present in both the food plant and bodies of male and female *U. galapagensis* (Roque-Albelo *et al.* 2002). PAs are extremely effective repellents for arachnids (Brown 1984, Eisner 2003) and the behavioral responses of *Eustala vegeta* are identical to those seen with *U. ornatatrix* and the orbweaving spider *Nephila clavipes* L. (Eisner 2003). The PAs of *U. galapagensis* were apparently less effective against the lava lizards. It is unlikely that the moth would survive the intense attack of a lava lizard even if rejected. The rapid, motion-triggered attack of the lava lizard places a premium for the moth on resting motionless during the day. Handling of *U. galapagensis* also frequently elicits "freezing" behavior, which may be adaptive in this context.

Thus, *U. galapagensis* appears to incorporate components of hypotheses 1 and 2 (in our Introduction) proposed to explain loss of aposematism in Galapagos *Utetheisa* (Roque-Albelo *et al.* 2002). The moths are primarily nocturnal and chemically defended: strongly against a nocturnal orb weaving spider and mildly against a diurnal lizard. They appear to employ crypsis against diurnal vertebrate predators since the alkaloidal defense is less effective against them. Future experiments with insectivorous birds, such as Galapagos mockingbirds, may clarify the degree of unpalatability of this moth to diurnal predators.

It remains to be seen whether the aposematically colored *U. ornatatrix* is the closest relative of the three endemic, cryptic species. If this is the case, the switch to PA-containing *Tournefortia* would be a simple transition from one PA hostplant species (*Crotalaria*) to another (*Tournefortia*), with the loss of aposematism and the species becoming nocturnal on the islands. Alternatively, *U. galapagensis* may have arisen from a cryptically colored nocturnal ancestor with characteristics similar to those of all three endemic species. Phylogenetic and biogeographic analyses may ultimately answer these questions.

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