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Chinese mantids gut toxic monarch caterpillars: avoidance of prey defence?

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Abstract. 1. Monarch caterpillars, *Danaus plexippus* (Linnaeus), feed on milkweed plants in the genus *Asclepias* and sequester cardenolides as an anti-predator defence. However, some predators are able to consume this otherwise unpalatable prey.

2. Chinese mantids, *Tenodera sinensis* (Saussure), were observed consuming monarch caterpillars by 'gutting' them (i.e. removing the gut and associated internal organs). They then feed on the body of this herbivore without any apparent ill effects.

3. How adult *T. sinensis* handle and consume toxic (*D. plexippus*) and non-toxic [*Ostrinia nubilalis* (Hübner) and *Galleria mellonella* (Linnaeus)] caterpillars was explored. The differences in the carbon/nitrogen (C:N) ratio and cardenolide content of monarch tissue consumed or discarded by mantids were analysed.

4. Mantids gutted monarchs while wholly consuming non-toxic species. Monarch gut tissue had a higher C:N ratio than non-gut tissue, confirming the presence of plant material. Although there were more cardenolide peaks in the monarch body compared with gut tissue, the total cardenolide concentration and polarity index did not differ.

5. Although *T. sinensis* treated toxic prey differently than non-toxic prey, gutting did not decrease the mantid's total cardenolide intake. As other predators consume monarch caterpillars whole, this behaviour may be rooted in species-specific vulnerability to particular cardenolides or simply reflect a preference for high-N tissues.

Key words. Aposematism, cardenolides, chemical defence, *Danaus plexippus*, *Ostrinia nubilalis,* prey handling, *Tenodera sinensis.*

Introduction

Prey respond to predation risk with a variety of anti-predator defences including altered life history strategies, morphological defences, and behavioural changes in foraging behaviour and microhabitat use (Lima, 1998; Preisser & Bolnick, 2008). Prey without inducible strategies often compensate with constitutive defences, such as the production or sequestration of toxic substances, and frequently advertise their defence via aposematism (Duffey, 1980; Nishida, 2002; Ruxton *et al*., 2004). The stability of aposematic signals make it easier for predators to learn unpalatable prey (Gittleman & Harvey, 1980), thus allowing predators to consistently detect and avoid defended species.

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One well-known example of invertebrate aposematism involves the monarch butterfly, *Danaus plexippus* (Linnaeus). This species' black and yellow caterpillars feed on host plants in the genus *Asclepias* (Apocynaceae) that contain toxic cardenolides and sequester these toxins in their bodies (Agrawal *et al.*, 2012). These substances have an emetic effect in birds (Brower *et al.*, 1967). While aposematism provides an effective defence against some predators, other predators and parasitoids are able to prey upon *D. plexippus*. Birds such as Orioles (*Icterus* spp.) and Grosbeaks (*Pheucticus* spp.) learn to avoid the toxin-rich cuticle of adults and may develop a physiological insensitivity to the insect's sequestered toxins (Nishida, 2002). Predators such as ants (*Formica montana* Linnaeus) and ladybird beetles (*Harmonia axyridis* Pallas) also prey on eggs and early-instar larvae (Koch *et al.*, 2003; Prysby, 2004). In contrast, few predators attempt to consume late-instar caterpillars, although assassin bugs (Heteroptera: Reduviidae)

Fig. 1. Adult Chinese mantid (*Tenodera sinensis*) gutting a final-instar monarch (*Danaus plexippus*) caterpillar. For scale, mantid forelegs are ~3 cm in length. Photo credit: Alex Allaux.

can feed on them (Zalucki & Kitching, 1982) and predatory wasps, *Polistes dominulus* (Chirst), will attack and consume monarch caterpillars when more favourable prey types are unavailable (Rayor, 2004).

While carrying out an unrelated field experiment, we observed late-instar Chinese mantids [*Tenodera sinensis* (Saussure)], a generalist predator, consuming *D. plexippus* caterpillars by 'gutting' them and only eating their integument (Fig. 1). *Tenodera sinensis* reacts negatively to chemically defended insects such as *Diabrotica* beetles and milkweed bugs [*Oncopeltus fasciatus* (Dallas)], especially when these herbivores are raised on toxin-containing diets (Ferguson & Metcalf, 1985). They are also able to learn to avoid such encounters: naïve third-instar mantids presented with two toxic *O. fasciatus* in succession take less time to sample and reject the second prey item (Paradise & Stamp, 1991), and it takes fewer than six encounters for late-instar mantids to refuse to attack *O. fasciatus* (Berenbaum & Miliczky, 1984).

We present research exploring predator–prey interactions between *T. sinensis*, *D. plexippus*, and two other species of non-toxic lepidopteran larvae. We observed the behaviour and consumption rates of field-collected adult *T. sinensis* when fed *D. plexippus*, non-toxic European corn borers [*Ostrinia nubilalis* (Hübner)], and wax moth larvae [*Galleria mellonella* (Linnaeus)]. We analysed differences in carbon/nitrogen (C:N) ratios and cardenolide content between caterpillar tissues consumed or discarded by mantids. We hypothesized that the gutting behaviour we observed for monarch caterpillars would not be employed for the two non-toxic prey and that cardenolide levels would be higher in the monarch gut than in the rest of the body.

Methods

Insect collection and maintenance

Adult praying mantids were collected in July 2011 from an abandoned agricultural field at East Farm (Kingston, Rhode Island), a research facility run by the University of Rhode Island (URI). They were returned to the lab and maintained at 25 °C in 50 × 25 × 30-cm plexiglass aquariums with plant material as perches. Each aquarium housed two mantids, separated from each other by a piece of cardboard. The mantids were fed house crickets, *Acheta domesticus*, and wax worms, *G. mellonella, ad libitum* until 3 days before the experiment (see below for details).

Monarch (*D. plexippus*) caterpillars and eggs were collected in August and September 2011 from milkweed plants (*Asclepias syriaca* L.) growing in a URI-managed farm. Caterpillars were removed from the leaf on which they were feeding; when eggs were found, the entire leaf was collected. Eggs and caterpillars were returned to the lab and kept in a 40 × 40 × 76-cm cage where they were fed cut milkweed. We reared a total of 21 caterpillars (all >0.5 g).

European corn borers (*O. nubilalis*) were collected in September 2011 from organically-grown flint corn (*Zea mays* L.) growing in a URI-managed farm. They were kept in the lab and fed ears of corn until the experiment. We reared a total of 15 caterpillars (all >0.3 g).

Wax worms (the larval phase of *G. mellonella*) were purchased from a local pet store and stored in the refrigerator at 10 °C prior to the experiment. At the start of the experiment, they had not consumed any food for 1–3 days.

D. plexippus*-only observation trials*

We followed standard experimental protocols (Reitze & Nentwig, 1991) and starved all praying mantids ($n = 11$) for 3 days prior to running the experiment. At the start of each trial, an individual mantid was placed in an 18 × 12.5 × 6-cm clear plastic container, and given 5 min to acclimate. We then placed a pre-weighed *D. plexippus* individual ($n = 10$) in the container. The interaction was video-recorded from the time the mantid attacked the prey until it was completely consumed or the mantid ceased feeding. We noted whether the mantid engaged in gutting behaviour, defined as the predator-induced expulsion of prey organs without any subsequent attempt at consumption.

Trials observing all three prey species

In order to determine if mantids exhibited gutting behaviour only when handling *D. plexippus* larvae, we conducted a series of no-choice trials in which we offered mantids non-toxic prey *O. nubilalis* ($n = 15$) and *G. mellonella* ($n = 8$) in addition

to *D. plexippus* ($n = 11$). These followed the procedures describe above but with the following modifications. First, the plastic container in which the trial was conducted was weighed prior to the start of each trial and after the trial was completed because we found that when the integument of larger caterpillars (i.e. *D. plexippus* and *O. nubilalis*) was punctured, haemolymph often dripped from the cadaver; we did not classify this incidental loss as gutting. We used this final mass to determine the amount of prey biomass discarded.

To determine why mantids gut *D. plexippus*, we disturbed mantids during these trials. As the mantid fed on *D. plexippus*, the gut content expelled from each larva was collected into a pre-weighed 1.5-ml eppendorf tube in order to determine the weight of the expelled material. After each *D. plexippus* larva was gutted, the remaining cadaver (i.e. the portion of the larva eaten by the mantid in the *D. plexippus*-only trials) was forcibly removed from the mantid and placed in a second pre-weighed Eppendorf tube. This tube was re-weighed to determine the weight of the remaining cadaver; both tubes were then frozen at -13 °C until their contents could be analysed. These data were used to determine the larval mass discarded by each mantid.

We analysed these videos for the following information. First we recorded whether or not the mantid engaged in the gutting behaviour. We also recorded the amount of time the mantid spent actively feeding in order to determine the predator consumption rate (g/m) of all prey. A total of eight mantids were tested in this experiment (one mantid refused to eat anything whereas another mantid escaped, consumed a smaller mantid, and refused to eat thereafter).

Carbon, hydrogen, and nitrogen and high-performance liquid chromatography analysis

Each gut and non-gut sample of *D. plexippus* was transferred into an individual 2-ml pre-weighed screw-cap tube and dried in a 45 °C drying oven for 5 days. After drying was complete, 1.0–2.2 mg of dried material was removed from each sample for carbon, hydrogen, and nitrogen (CHN) analysis. This material was sent to an analytic chemistry lab at URI (Narragansett, Rhode Island) for analysis. The remaining dry material from each sample was used for cardenolide analysis.

Cardenolide concentrations were assessed by high-performance liquid chromatography (HPLC), according to Bingham and Agrawal (2010). Briefly, oven-dried (40 °C) tissue from each sample was ground to a fine powder and extracted with 1.8 ml of methanol (MeOH). The sample mass ranged from 10 to 43 mg for gut tissue and 80–159 mg for body tissue. Each extract was spiked with 20 µg digitoxin as an internal standard and sonicated for 20 min at 55 °C in a water bath. After centrifugation, the supernatant was collected, dried, resuspended in 300 µl MeOH, and filtered through a 0.45-µm syringe-driven filter unit. About 15 µl of extract was then injected into an Agilent 1100 series HPLC (Agilent, Santa Clara, California) and compounds were separated on a Gemini C18 reversed phase column (3 µm, 150 × 4.6 mm; Phenomenex, Torrance, California). Cardenolides were eluted on a constant flow of 0.7 ml/min with an acetronile-0.25% phosphoric acid in water gradient as follows: 0–5 min 20% acetonitrile, 20 min 70% acetonitrile; 20–25 min 70% acetonitrile, 30 min 95% acetonitrile, and 30–35 min 95% acetonitrile. UV absorbance spectra were recorded from 200 to 400 nm using a diode array detector. Peaks with absorption maxima between 217 and 222 nm were recorded as cardenolides and quantified at 218 nm. Concentrations were calculated based on the dry mass and standardized by peak areas of the known digitoxin concentration. In addition to total cardenolides, we report cardenolide peak diversity (number of distinct cardenolide peaks per sample) and an index of cardenolide polarity (index $P = \text{sum}[P_i RT_i]$), where RT_i is the retention time of the ith peak, weighted by each peak's relative concentration P_i(Rasmann & Agrawal, 2011).

Statistical analysis

Data on mantid gutting behaviour (yes/no) for the three prey species were analysed using contingency analysis. Because some mantids consumed more than one individual of a given prey species, the effect of prey species on consumption rate (g/min) and per cent biomass discarded was tested using a mixed model for repeated measures analysis (Littell *et al*., 1996). This analytic method is suitable for use in cases where a portion of time series data is missing; in contrast, standard repeated measures ANOVAS exclude all subjects missing any time × treatment data (von Ende, 2001). A two-factor (treatment and time) repeated measures design was used, and 'mantid' was added as a random factor nested under 'treatment'. Because this design is unbalanced, i.e. not every mantid was fed two individuals from each prey species the test statistics did not follow an exact F distribution. Following a recommended procedure, we calculated P-values using the Satterthwaite method to generate an approximate F-value with fractional degrees of freedom (West *et al*., 2006). Although the data on prey handling time was normally distributed, the data on per cent biomass discarded was not even when transformed. Because using a non-parametric analysis would have prevented us from incorporating random effects (i.e. 'mantid' nested within 'treatment'), we chose to proceed with a parametric approach. We justify this decision by noting that ANOVAS are robust to departures from normality when per-treatment sample sizes are large (Underwood, 1997), a criterion that our 34-observation data set meets. We performed means separation tests, where appropriate, using Tukey's HSD at $\alpha = 0.05$. Data on the C:N ratio, total cardenolide content, number of cardenolide peaks, and the polarity index were analysed using a paired-sample t-test on gut and body tissue from each tested prey individual. We used the same approach to analyse data on individual cardenolides; because of the large number of comparisons, we present both the unadjusted P-value as well as the P-value corrected for multiple comparisons at $\alpha = 0.05$ using step-up FDR, a sequential Bonferroni-type procedure. Statistical analyses were performed using JMP 9.0.0 (SAS, 2010).

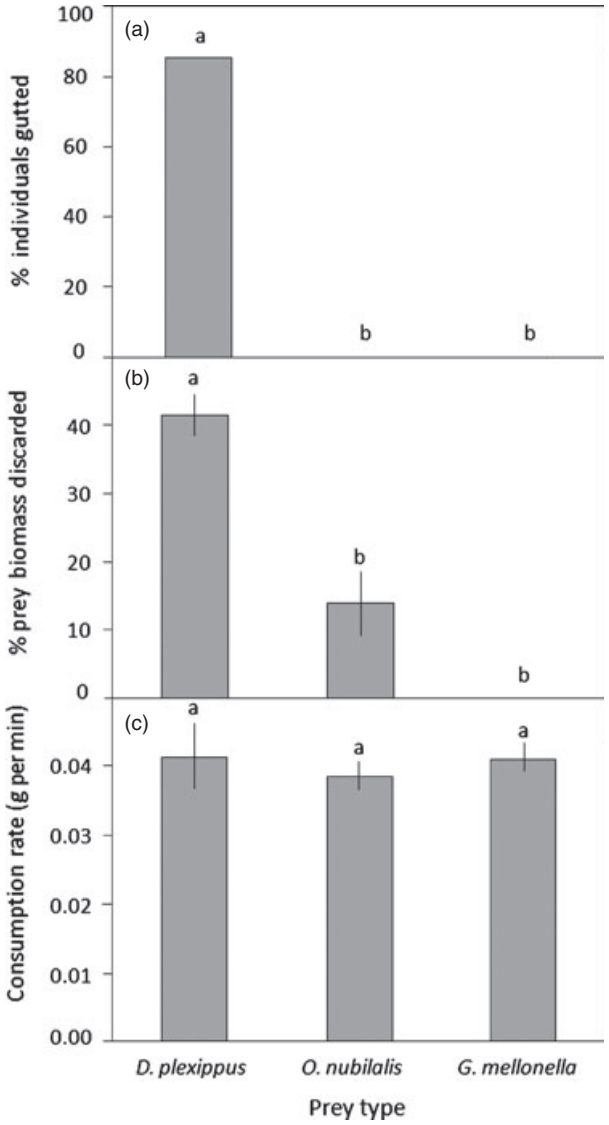

Fig. 2. (a) Per cent individuals of each prey type gutted by *Tenodera sinensis*. (b) Per cent mass of each prey type discarded ± SE. (c) Consumption rates for each prey type ± SE.

Results

We observed 44 predator–prey encounters between *T. sinensis* and the 3 prey species. Upon detecting their prey, the mantids would orient on it, grasp the prey with their forelegs, and begin consuming it. Mantids encountering *G. mellonella* or *O. nubilalis* caterpillars ($n = 8$ and 15, respectively) ate these prey in their entirety (excluding any haemolymph that fell from *O. nubilalis* prey). In contrast, mantids encountering *D. plexippus* would allow the gut content to fall from the cadaver while feeding, and would not attempt to consume it even after finishing the rest of the cadaver (Fig. 1). Mantids encountering *D. plexippus* larvae gutted 18 of 21 (86%) caterpillars (Fig. 2a; $X^2 = 42.3$, $P < 0.001$). Two of the three individuals that were not gutted were each parasitised by a single late-instar larvae of a tachinid fly; the remaining larvae was extensively infected with a fungal pathogen (probably *Beauveria bassiana* Balsamo Vuillemin).

The mantid's gutting behaviour led to large differences in the mean per cent of prey mass discarded (i.e. unconsumed at the end of the feeding bout) by the mantids (Fig. 2b; $F_{2,26.8} = 16.3$, $P < 0.001$). While mantids discarded 41 ± 3.1% [mean (SE); $n = 11$] of *D. plexippus* larval mass, they only discarded 14 ± 4.6% of *O. nubilalis* larval mass and 0% of *G. mellonella* larval mass (Fig. 2b; $P < 0.05$). Mantids that consumed multiple caterpillars of a given prey species did not differ over time in the proportion discarded ($F_{1,11.3} = 0.44$, $P = 0.52$), and there was no time × prey species interaction ($F_{2,11.2} = 0.13$, $P = 0.88$). While the mass discarded from *D. plexippus* caterpillars consisted primarily of its gut, the discarded mass from *O. nubilalis* consisted entirely of haemolymph; mantids never discarded any tissue from either *O. nubilalis* or *G. mellonella* caterpillars. In spite of the species-specific differences in gutting behaviour, mantids consumed the 'edible' portion of all three prey species at an equal rate (Fig. 2c; $F_{2,21.8} = 0.36$, $P = 0.70$). Again, mantids that consumed multiple prey items of a given species did not differ in their prey consumption rate over time ($F_{1,8.94} = 0.09$, $P = 0.77$), and there was no time × prey species interaction ($F_{2,8.90} = 0.04$, $P = 0.96$).

Gut tissue from *D. plexippus* had a marginally lower concentration of C [38.1 ± 1.30 (SE) μmol mg^{-1}] than did non-gut tissue (46.2 ± 3.94 μmol mg^{-1}; $t_8 = 2.18$, $P = 0.061$). However, there was 58% less N in the gut (3.1 ± 0.34 μmol mg^{-1}) versus non-gut tissue (7.5 ± 0.33 μmol mg^{-1}; $t_8 = 14.97$, $P < 0.001$). As a result, gut tissue had a higher C:N ratio (13.2 ± 1.22) than non-gut tissue (6.15 ± 0.44; $t_8 = 4.77$, $P = 0.001$). This suggests that the mantid-discarded *D. plexippus* material consisted mainly of macerated plant tissue, which was low in nutritive value.

In spite of the large amount of plant material in the *D. plexippus* gut, there were no differences in total cardenolide content [body: 1.90 ± 0.77 (SE) μg cardenolides mg^{-1}; gut: 1.74 ± 0.88] or polarity index (body: 19.2 ± 2.66; gut: 18.9 ± 3.46) between mantid-consumed and mantid-discarded herbivore tissue (both $P > 0.10$). There were nearly three times as many cardenolide peaks in *D. plexippus* body versus gut tissue ($t_8 = 11.8$, $P < 0.001$), probably reflecting the breakdown of plant-derived cardenolides into differentially sequestered compounds. For example, the large cardenolide peak in the gut tissue at 10.8 min is twice as concentrated as in the body tissue; the three subsequent peaks, however, are absent from the gut and only in the body tissue, potentially suggesting transformation during sequestration (Fig. 3).

Discussion

We found that adult *T. sinensis* can capture and consume late-instar *D. plexippus* caterpillars with no apparent ill effects. The fact that *T. sinensis* handled *D. plexippus* caterpillars differently than *O. nubilalis* and *G. mellonella* larvae appears to

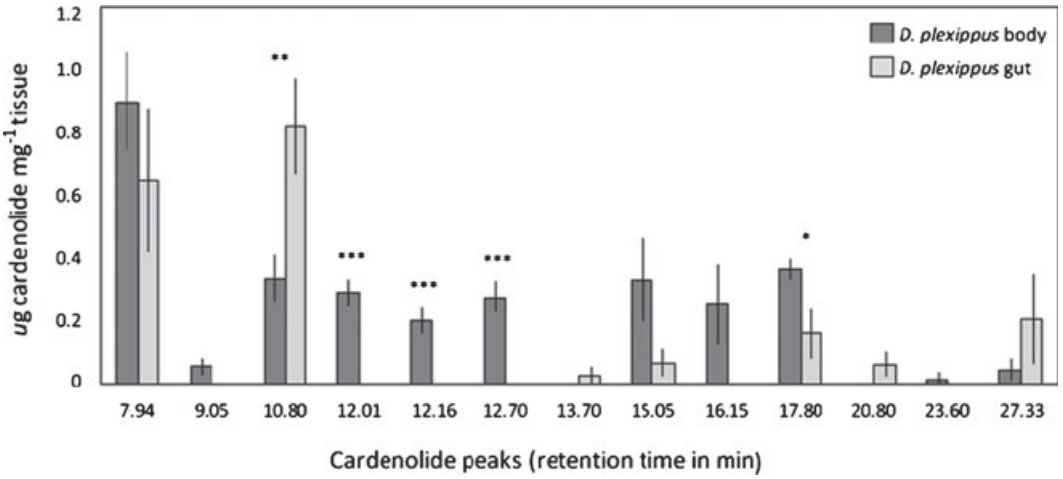

Fig. 3. Concentration of individual cardenolides in *Danaus plexippus* gut and body (i.e. non-gut) tissue ± SE. For initial values, $*P < 0.05$, $**P < 0.01$, and $***P < 0.005$. For adjusted values, § = significant at $\alpha = 0.05$ after step-up FDR Bonferroni-type correction.

be a behavioural mechanism to reduce exposure to prey toxicity. This interpretation is supported by the fact that all of the tested mantids treated *D. plexippus* caterpillars very similarly (Fig. 2a): chewing open the integument and letting the gut fall out while consuming the remains (Fig. 1). Given that gutted caterpillars were consumed in their entirety, the discarding of ∼40% of prey biomass (Fig. 2b) cannot be attributed to mantid satiation. In contrast, *T. sinensis* never gutted either *O. nubilalis* or *G. mellonella* larvae and consumed all non-haemolymph biomass (Figs 2a, b). While it is possible that larger prey are easier to gut, mantids consumed a substantial amount of *O. nubilalis* gut material that could easily have been avoided by the mantid. In addition, large *O.nubilalis* larvae (e.g., one weighing 0.73 g) were not gutted, whereas smaller *D. plexippus* larvae (e.g., one weighing 0.63 g) were gutted. Once the mantids had gutted the *D. plexippus* caterpillars, they consumed all three prey types at a similar rate (Fig. 2c). This suggests that the mantid considered all three prey types equally palatable.

The gutting behaviour we documented in *T. sinensis* is similar to that observed in other predators, many of which are capable of identifying and selectively consuming the least noxious body parts of chemically-defended prey (reviewed in Glendinning, 2007). The European paper wasp *P. dominulus*, for instance, will gut *Pieris napi* (Linnaeus) caterpillars reared on toxic host plants, but not those that were reared on non-toxic plants (Rayor *et al*., 2007). Conversely, Tanagers (*Pipraeida melanonota* Vieillot) avoid the toxic integument of ithomiine moths by chewing them until the abdominal content is expelled; they then eat the abdominal contents and discard the rest (Brown & Neto, 1976). Predators that cannot separate the toxic and non-toxic fractions of unpalatable prey often learn to avoid them entirely. In experiments with cardenolide-containing milkweed bugs, naïve third-instar *T. sinensis* fed a single *O. fasciatus* nymph took much less time to reject a second one (Paradise & Stamp, 1991); similarly, sixth-instar mantids quickly (three to four exposures) learned to ignore mature *O. fasciatus* (Berenbaum & Miliczky, 1984).

In spite of the behavioural data, mantid-consumed and -discarded *D. plexippus* tissue had equal cardenolide concentrations and a similar polarity index. There were more distinct cardenolide peaks in the consumed tissue, probably owing to the breakdown of plant-derived cardenolides (e.g. at 10.80 nm in Fig. 3) into other forms (e.g. at 12.01, 12.16, and 12.70 nm in Fig. 3). Overall, these results are consistent with previous work showing that cardenolide sequestration occurs in the haemolymph and epidermis of *D. plexippus* (Duffey, 1980) in concentrations equal to or exceeding those of host plant foliage (Malcolm *et al*., 1989; Agrawal *et al*., 2012). Our findings appear, however, to reject the hypothesis that mantid gutting of *D. plexippus* caterpillars allows them to avoid cardenolide-rich gut material while consuming the less-defended integument. Below, we discuss potential resolutions to the apparent mismatch between the behavioural data (Fig. 2) and cardenolide analyses.

One explanation for our findings is that the gutting behaviour of *T. sinensis* avoids plant-produced cardenolides present in the gut that *D. plexippus* larvae metabolises into different compounds before sequestering them in their haemolymph and integument. This explanation is consistent with the fact that the three monarch caterpillars that mantids ate whole (individuals containing either tachinid larvae or a fungal pathogen) consumed virtually no *A. syriaca* in captivity. When mantids punctured the integument and began feeding on these three larvae, no green plant material was present; in contrast, the other 18 monarch guts were green with plant material. Evidence suggests that monarch predators differ in their preference for (or avoidance of) particular body parts. The mouse *Peromyscus melanotis* Allen & Chapman avidly consumes even high-cardenolide monarch adults, for instance, by opening the abdomen and eating the internal contents while avoiding the integument (Glendinning, 1990); yellowjacket wasps (*Vespula vulgaris* Linnaeus) use similar techniques to prey on adult monarchs (Leong *et al.*, 1990). Although *P. dominulus* wasps prefer more palatable prey over *D. plexippus* larvae (Rayor, 2004), there are reports that

they do not gut or otherwise 'selectively process' late-instar monarch caterpillars before eating or feeding them to their offspring (L. Rayor, unpublished, cited in Rayor *et al*., 2007). Our results may thus be explained by mantids' greater tolerance for monarch-metabolised cardenolides in the integument than for plant-derived chemicals in the gut.

The gutting behaviour of *T. sinensis* might also be explained by this obligate carnivore's distaste for partially-digested plant tissue. The digestive system and enzymatic pathways of carnivores are optimised for a heterotrophic diet, and the autotrophic biomass differs substantially in a wide range of parameters (reviewed in Price *et al*., 2011). This explanation is inconsistent with the fact that the mantids consumed similarly-sized *O. nubilalis* larvae in their entirety. Even so, the low nutrient content and likely equal (or greater) toxicity of the gut tissue may help explain the mantid's behaviour if the predators can tolerate consumption of the integument but not the material in the gut. Avian insectivores are able to regulate their exposure to toxins by consuming fewer individuals as prey toxicity increases (Skelhorn & Rowe, 2007); mantids may be similarly able to regulate their toxin loads. In spite of being a fairly recent arrival to the east coast of the U.S., *T. sinensis* has rapidly become the dominant invertebrate predator in many old-field ecosystems (reviewed in Snyder & Evans, 2006). The gutting behaviour we describe may enable this mantid to utilise otherwise inaccessible prey and thrive in their invaded range. The similar polarity index and total cardenolide content of mantid-consumed versus -discarded tissue also adds an intriguing twist to the monarch–cardenolide–predator interaction first elucidated nearly 50 years ago (Brower *et al*., 1967). Although total cardenolide content alone may not entirely explain the mantids' behaviour, we speculate that the context of the gut's character, largely low nitrogen-containing milkweed tissue, may be critical to the gutting of monarchs.

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References

Agrawal, A., Petschenka, G., Bingham, R., Weber, M. & Rasmann, S. (2012) Toxic cardenolides: chemical ecology and coevolution of specialized plant-herbivore interactions. *New Phytologist*, **194**, 28–45.

Berenbaum, M. & Miliczky, E. (1984) Mantids and milkweed bugs: efficacy of aposematic coloration against invertebrate predators. *American Midland Naturalist*, **111**, 64–68.

Bingham, R. & Agrawal, A. (2010) Specificity and trade-offs in the induced plant defence of common milkweed *Asclepias syriaca* to two lepidopteran herbivores. *Journal of Ecology*, **98**, 1014–1022.

Brower, L., van Brower, J. & Corvino, J. (1967) Plant poisons in a terrestrial food chain. *Proceedings of the National Academy of Sciences of the United States of America*, **57**, 893–898.

Brown, K. & Neto, J. (1976) Predation on aposematic ithomiine butterflies by tanagers (*Pipraeidea melanonota*). *Biotropica*, **8**, 136–141.

Duffey, S. (1980) Sequestration of plant natural products by insects. *Annual Review of Entomology*, **25**, 447–477.

von Ende, C. (2001) Repeated-measures analysis: growth and other time-dependent measures. *Design and Analysis of Ecological Experiments* (ed. by S. Scheiner and J. Gurevitch), pp. 134–157. Oxford University Press, New York, New York.

Ferguson, J. & Metcalf, R. (1985) Cucurbitacins: plant-derived defense compounds for *Diabroticites* (Coleoptera: Chrysomelidae). *Journal of Chemical Ecology*, **11**, 311–318.

Gittleman, J. & Harvey, P. (1980) Why are distasteful prey not cryptic? *Nature*, **286**, 149–150.

Glendinning, J. (1990) Responses of three mouse species to deterrent chemicals in the monarch butterfly. II. Taste tests using intact monarchs. *Chemoecology*, **1**, 124–130.

Glendinning, J. (2007) How do predators cope with chemically defended foods? *The Biological Bulletin*, **213**, 252–266.

Koch, R., Hutchison, W., Venette, R. & Heimpel, G. (2003) Susceptibility of immature monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by *Harmonia axyridis* (Coleoptera: Coccinellidae). *Biological Control*, **28**, 265–270.

Leong, K., Frey, D. & Nagano, C. (1990) Wasp predation on overwintering monarch butterflies (Lepidoptera: Danaidae) in central California. *Pan-Pacific Entomologist*, **66**, 326–328.

Lima, S. (1998) Nonlethal effects in the ecology of predator-prey interactions. *Bioscience*, **48**, 25–34.

Littell, R., Miliken, G., Stroup, W. & Wolfinger, R. (1996) *SAS System for Mixed Models.* SAS Institute Inc., Cary, North Carolina.

Malcolm, S., Cockrell, B. & Brower, L. (1989) Cardenolide fingerprint of monarch butterflies reared on common milkweed, *Asclepias syriaca* L. *Journal of Chemical Ecology*, **15**, 819–853.

Nishida, R. (2002) Sequestration of defensive substances from plants by Lepidoptera. *Annual Review of Entomology*, **47**, 57–92.

Paradise, C. & Stamp, N. (1991) Prey recognition time of praying mantids (Dictyoptera, Mantidae) and consequent survivorship of unpalatable prey (Hemiptera, Lygaeidae). *Journal of Insect Behavior*, **4**, 265–273.

Preisser, E. & Bolnick, D. (2008) The many faces of fear: comparing the pathways and impacts of nonconsumptive predator effects on prey populations. *Public Library of Science ONE*, **3**, e2465.

Price, P., Denno, R., Eubanks, M., Finke, D. & Kaplan, I. (2011) *Insect Ecology: Behavior, Populations and Communities.* Cambridge University Press, New York.

Prysby, M. (2004) Natural enemies and survival of monarch eggs and larvae. *The Monarch Butterfly: Ecology and Conservation* (ed. by K. Oberhauser and M. Solensky), pp. 27–37. Cornell University Press, Ithaca, New York.

Rasmann, S. & Agrawal, A. (2011) Latitudinal patterns in plant defense: evolution of cardenolides, their toxicity and induction following herbivory. *Ecology Letters*, **14**, 476–483.

Rayor, L. (2004) Effects of monarch larval host plant chemistry and body size on *Polistes* wasp predation. *The Monarch Butterfly: Ecology and Conservation* (ed. by K. Oberhauser and M. Solensky), pp. 39–46. Cornell University Press, Ithaca, New York.

Rayor, L., Mooney, L. & Renwick, J. (2007) Predatory behavior of *Polistes dominulus* wasps in response to cardenolides and

glucosinolates in *Pieris napi* caterpillars. *Journal of Chemical Ecology*, **33**, 1177–1185.

Reitze, M. & Nentwig, W. (1991) Comparative investigations into the feeding ecology of six Mantodea species. *Oecologia*, **86**, 568–574.

Ruxton, G., Sherratt, T. & Speed, M. (2004) *Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals and Mimicry.* Oxford University Press, Oxford, U.K.

SAS (2010) *JMP User's Guide, Version 9.0.* SAS Institute Inc., Cary, North Carolina.

Skelhorn, J. & Rowe, C. (2007) Predators' toxin burdens influence their strategic decisions to eat toxic prey. *Current Biology*, **17**, 1479–1483.

Snyder, W. & Evans, E. (2006) Ecological effects of invasive arthropod generalist predators. *Annual Review of Ecology and Systematics*, **37**, 95–122.

Underwood, A. (1997) *Experiments in Ecology.* Cambridge University Press, New York, New York.

West, B., Welch, K. & Galecki, A. (2006) *Linear Mixed Models: A Practical Guide Using Statistical Software.* CRC Press, New York, New York.

Zalucki, M. & Kitching, R. (1982) Temporal and spatial variation of mortality in field populations of *Danaus plexippus* L. and *D. chrysippus* L. larvae (Lepidoptera: Nymphalidae). *Oecologia*, **53**, 201–207.

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