2017

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Citation/Publisher Attribution
Jamie L. Rafter, Justin F. Vendettuoli, Liahna Gonda-King, Daniel Niesen, Navindra P. Seeram, Chad M. Rigsby, Evan L. Preisser; Pretty Picky for a Generalist: Impacts of Toxicity and Nutritional Quality on Mantid Prey Processing, *Environmental Entomology*, Volume 46, Issue 3, 1 June 2017, Pages 626–632, https://doi.org/10.1093/ee/nvx038
Available at: https://doi.org/10.1093/ee/nvx038

Rafter et al: Mantids selectively process lepidopteran prey

Pretty picky for a generalist: impacts of toxicity and nutritional quality on mantid prey processing

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Abstract

Prey have evolved a number of defenses against predation, and predators have developed means of countering these protective measures. Although caterpillars of the monarch butterfly, *Danaus plexippus* L. are defended by cardenolides sequestered from their host plants, the Chinese mantid *Tenodera sinensis* Saussure guts the caterpillar before consuming the rest of the body. We hypothesized that this gutting behavior might be driven by the heterogeneous quality of prey tissue with respect to toxicity and/or nutrients. We conducted behavioral trials in which mantids were offered cardenolide-containing and cardenolide-free *D. plexippus* caterpillars and butterflies. In addition, we fed mantids starved and unstarved *D. plexippus* caterpillars from each cardenolide treatment and non-toxic *Ostrinia nubilalis* Hubner caterpillars. These trials were coupled with elemental analysis of the gut and body tissues of both *D. plexippus* caterpillars and corn borers. Cardenolides did not affect mantid behavior: mantids gutted both cardenolide-containing and cardenolide-free caterpillars. In contrast, mantids consumed both *O. nubilalis* and starved *D. plexippus* caterpillars entirely. *Danaus plexippus* body tissue has a lower C:N ratio than their gut contents, while *O. nubilalis* have similar ratios; gutting may reflect the mantid's ability to regulate nutrient uptake. Our results suggest that post-capture prey processing by mantids is likely driven by a sophisticated assessment of resource quality.

KEY WORDS *Danaus plexippus*, *Ostrinia nubilalis*, *Tenodera sinensis*, cardenolide, prey processing

Prey utilize an array of defenses against predation (reviewed in Lima and Dill 1990). Organisms can, for instance, avoid detection via crypsis or disruptive coloration that makes it difficult for predators to identify the boundaries of the prey's body. Prey can also employ behavioral measures to decrease their likelihood of attracting a predator: Veeries, *Catharus fuscescens* Stephens, respond to predation risk by decreasing the rate and length of their songs (Schmidt and Belinsky 2013). Once detected, prey can employ secondary defenses such as aggressive or escape behaviors as well as morphological and/or chemical defenses (Ruxton et al. 2004). The presence of trout, for example, can cause macroinvertebrates to alter their drift rates and foraging activity (Simon and Townsend 2003, Eby et al. 2006), as well as their microhabitat use (Lima 1998). Morphological changes are also possible: *Daphnia pulex* Leydig respond to predator cues by producing fewer, but larger, offspring with prominent neck spines (Luening 1994) that make the prey more difficult for predators to attack.

Organisms that lack behavioral and/or morphological defenses may instead deter predation via the production or sequestration of noxious chemical compounds. Prey that adopt this strategy typically possess aposematic coloration that advertises their toxicity (Duffey 1980, Nishida 2002, Ruxton et al. 2004). The nudibranch *Cratena peregrina* Gmelin, for example, uses bright coloration to display its unpalatability to fish predators (Aguado and Marin 2007). In insects, chemical defense and aposematism occurs in multiple orders, including the Hemiptera, Lepidoptera, Coleoptera, and Hymenoptera. Hemipteran milkweed bugs, *Oncopeltus fasciatus* Dallas, feed on cardenolide-rich host plants and sequester these toxins in their bodies; their contrasting orange-and-black coloration alerts predators to their toxicity (Scudder et al. 1986). Another insect that feeds on milkweed, the Oleander aphid *Aphis nerii* Boyer de Fonscolombe, also sequesters cardenolides and are brightly yellow-and-black colored (Malcolm 1990).

Although chemically-based antipredator defenses are often highly effective, predators have developed a variety of techniques for overcoming them. Floodplain death adders, *Acanthophis praelongus* Ramsay, prey on toxic frogs by biting the prey, injecting it with toxins, and then releasing it. The adder's toxins kill the frog, whose own defensive toxins degrade after it has died; the snake can then eat the formerly-toxic frog without any ill effects (Phillips and Shine 2007). Loggerhead Shrikes, *Lanius ludovicianus* Mearnsi, employ a similar strategy for feeding on chemically defended lubber grasshoppers, *Romalea guttata* Beauvois. Grasshoppers captured by the birds are impaled on thorns or barbed wire; the shrikes only return to feed on them once the grasshoppers' defensive toxins have been degraded and their aposematic coloration fades (Yosef 1992). Other predators process prey to feed selectively on the most palatable portion of the prey (Glendinning 2007) or regulate their toxicity burden (Skelhorn and Rowe 2007).

The monarch butterfly, *Danaus plexippus* L., is chemically defended and aposematically colored in both the black-and-yellow larval and black-and-orange adult stage. Their caterpillars sequester toxins when feeding on cardenolide-containing host plants in the genus *Asclepias* (Aponcynaceae) (Agrawal et al. 2012). Despite this generally effective chemical defense, *D. plexippus* is susceptible to predation across all life stages. Its invertebrate predators include ants, *Formica montana* Wheeler, ladybird beetles, *Harmonia axyridis* Pallas (Koch et al. 2003, Prysby 2004), and predatory *Polistes* (Rayor 2004), and *Vespula* wasps (Leong et al. 1990). Birds such as Orioles, *Icterus* spp., Grosbeaks, *Pheucticus* spp., (Nishida 2002) and other vertebrate predators such as *Peromyscus* mice also feed on *D. plexippus* (Glendinning 1990).

Danaus plexippus caterpillars are also preyed upon by an invasive generalist predator, the Chinese mantid, *Tenodera sinensis* Saussure (DJ Cox, personal observation). We have previously found (Rafter et al. 2013) that mantids consuming toxic *D. plexippus* caterpillars

actively reject the gut material, allowing it to fall from the body. However, they consume non-toxic lepidopterans such as European corn borers, *Ostrinia nubilalis* Hubner, and wax worms, *Galleria mellonella* L., in their entirety. These results suggest that the mantids' gutting behavior may be a behavioral mechanism for avoiding prey toxicity. A follow-up analysis of cardenolide levels, however, found that the mantid-discarded guts and mantid-consumed bodies of *D. plexippus* caterpillars contain similar cardenolide concentrations (although the two portions were composed of different individual cardenolides). We also found that gut material has a higher C:N ratio than body material, potentially making it less nutritious for this species (although nutrient requirements are unknown). As a result, the mantids' gutting behavior may reflect either their avoidance of individual cardenolides or their preference to feed selectively on the most nitrogen-rich portions of their prey (Rafter et al. 2013). Our aim was to test these specific hypotheses by conducting a series of behavioral trials in which we observed mantid prey handling behavior when presented with *D. plexippus* caterpillars reared on toxic cardenolide-containing and control no-cardenolide host plants. We paired the results of this experiment with other work in which we fed mantids starved and unstarved larval *D. plexippus* reared on the two host plants, adult *D. plexippus* reared on the two host plants, and non-toxic European corn borers. Unlike in our previous work, we reared all insects (except *O. nubilalis*) in the lab. Thus, the mantids were naive to each prey type. This allowed for further understanding of the innate behaviors exhibited by mantids when presented with a novel prey type. Our results suggest that post-capture prey processing by mantids is likely driven by an assessment of resource quality.

Methods

Mantid rearing and maintenance. We collected a single *Tenodera sinensis* egg mass in early April 2012 from an abandoned agricultural field at East Farm (Kingston, RI). It was

returned to the lab and maintained at 25ºC in a 50 x 25 x 30 cm Plexiglass aquarium until the eggs began to hatch. One day after hatching, nymphs were each placed in individual 1.9L mason jars; the top of each jar was replaced with mosquito netting for ventilation. Because they emerged from a single egg mass, all nymphs were either full- or half-sibs; using related individuals in controlled experiments is a commonly-used means for minimizing the magnitude of uncontrolled population-level variation (Beukeboom and Zwaan 2005). A single stick was provided for perching; when mantids reached the fourth instar, the stick was replaced with a mesh strip secured under the lid. Water was provided using a water wick made from capped soufflé cups and braided dental cotton inserted through a hole in the lid. The jars were held in a Percival growth chamber with a 16:8 L:D photoperiod and 60-80% humidity at 25˚C during lighted hours and 23˚C during dark hours. The remaining mantids from the egg mass were communally raised in two 50 x 25 x 30 cm aquaria. Each aquarium had several sticks arranged for perching sites. Mantids in both the jars and the aquaria were fed lab-reared apterous fruit flies, *Drosophila melanogaster* Meigen, for the first four instars; following this, they were fed appropriately-sized crickets (*Acheta domesticus* L.). Because crickets will prey on mantids during the molting process, we tested for satiation by using forceps to offer each mantid a cricket before adding crickets to its jar. If the mantid refused to attack the cricket we assumed it was preparing to molt and did not feed it that day. Mantids that accepted the cricket were fed two additional crickets; we deterred crickets from attacking the mantids by adding fruit flies to the jars for the crickets to eat. Because early-instar mantids have high mortality rates, we replaced any dead Percival-reared mantids with a communally-raised sibling of similar size and developmental stage; we stopped this replacement once a majority of Percival-reared mantids reached the sixth instar. Once mantids reached adulthood, they were fed three crickets daily and

no fruit flies. Jars containing adult mantids were removed from the Percival and kept in the lab at ambient room temperature with a 16:8 L:D photoperiod.

Experiment 1: Do mantids handle toxic (cardenolide-containing host plant) and non-toxic (no-cardenolide host plant)* D. plexippus *caterpillars differently? This experiment tested whether mantids varied in their behavior towards *D. plexippus* caterpillars raised on toxic (i.e., cardenolide-containing) and non-toxic (no-cardenolide) host plants. It tests the hypothesis that the mantids' gutting behavior is a response to the presence of cardenolides in *D. plexippus* gut tissue. Two hundred *D. plexippus* eggs were purchased from Flutterby Gardens (Bradenton, FL, USA) and reared in 50 x 25 x 30 cm aquaria. Half of the emerging larvae were reared on a cardenolide-containing host plant, the common milkweed *Asclepias syriaca* L.; the other half of the emerging larvae were reared on a zero-cardenolide host plant, the swamp milkweed *A. incarnata* L. *Asclepias syriaca* plants were grown from seed while *A. incarnata* plugs were purchased from Northcreek Nursery (Landenberg, PA, USA).

Twenty lepidopteran-naïve adult mantids were randomly assigned to consume late-instar *D. plexippus* larvae raised on either *A. syriaca* (ten mantids) or *A. incarnata* (ten mantids) host plants. All mantids were starved for three days prior to the trial. At the start of each trial, each mantid was weighed, placed into a pre-weighed 23.3 x 15.5 x 16.5 cm plastic container, and allowed to acclimate for five minutes. After the five-minute acclimation period, a pre-weighed caterpillar was placed into the enclosure. We video-recorded each trial from the moment the prey item was placed in the enclosure until the end of the trial. The mantid was given ten minutes to orient on the prey. If the mantid did not orient within this period, the trial ended. Mantids that oriented were given an additional ten minutes to attack the prey. If the mantid did not attack during this period, the trial ended. If the mantid attacked, we recorded five minutes of video

following the attack. At the same time, we recorded whether or not the mantid gutted the prey. Every mantid was tested every day for six days during the experiment. Once an individual mantid had attacked prey in two separate trials, we disturbed the remaining trials in which the mantid attacked so that we could collect mantid-dissected gut and body material for CNH analysis. Gut material was collected in a 2 ml pre-weighed screw-cap tube as it fell from the caterpillar. We then pried the remaining cadaver from the mantid and placed it into a second tube. This material was frozen at -13˚C until analyzed.

Experiment 2: Does the presence of plant material in the caterpillar gut affect how mantids handle 'toxic' (cardenolide-containing host plant) and 'non-toxic' (no-cardenolide host plant)* D. plexippus *caterpillars? This experiment tested whether mantid behavior varied as a function of the presence or absence of plant material in the gut of *D. plexippus* caterpillars reared on cardenolide-containing and no-cardenolide host plants. It tests the hypothesis that mantid gutting behavior is driven by the presence of plant material *per se* rather than by cardenolide concentrations. This experiment was conducted identically to experiment one (and used the same mantids), but added an additional experimental factor: the presence (‘unstarved’) or absence (‘starved’) of plant material in the caterpillar gut. The ten mantids that had previously been fed cardenolide-containing *D. plexippus* caterpillars were split into two groups of five mantids. Mantids in one of the five-mantid groups were fed starved *D. plexippus* caterpillars whose guts were free of plant material ('starved' treatment); mantids in the other five-mantid group were fed *D. plexippus* caterpillars whose guts were filled with plant material ('unstarved' treatment). This design was replicated for the ten mantids that had previously been fed no-cardenolide *D. plexippus* caterpillars, for a total of four five-mantid treatments: starved toxic caterpillars, unstarved toxic caterpillars, starved non-toxic caterpillars, and unstarved non-toxic

caterpillars. As in experiment one, toxic *D. plexippus* caterpillars were raised on *A. syriaca* and non-toxic *D. plexippus* caterpillars were raised on *A. incarnata*. Starved caterpillars were kept without food for 24 hours in order to clear their guts of any plant material; any mantid-attacked 'starved' caterpillars whose guts still contained trace amounts of plant material (apparent as undigested green material within the gut) were excluded from our analysis. Mantid-*D. plexippus* interaction trials were conducted for six days following the same procedure as in the first experiment. We collected caterpillar biomass for chemical analysis once individual mantids attacked twice.

Experiment 3: Do mantids handle toxic (cardenolide-containing host plant) and non-toxic (no-cardenolide host plant) adult* D. plexippus *differently? This experiment tested whether mantids differed in their handling behavior of adult *D. plexippus* butterflies reared on cardenolide-containing versus no-cardenolide host plants. Adult *D. plexippus* are nectar feeders that no longer consume cardenolides; the experiment tested the hypothesis that this ontogenic shift affected how mantids responded to *D. plexippus* reared on different hosts. Twelve *D. plexippus* caterpillars were reared to adulthood, six on *A. syriaca* and six on *A. incarnata*. Twelve mantids used in experiments one and two (six that were fed *A. syriaca* caterpillars, and six that were fed *A. incarnata* caterpillars) were each fed a single *A. syriaca*-reared adult butterfly or a single *A. incarnata*-reared adult butterfly, respectively. For each trial, we noted if the butterfly was gutted and which body parts were discarded by the mantid; all twelve trials took place on the same day.

Experiment 4: Do mantids handle larval* O. nubilalis *differently than* D. plexippus*? This experiment repeated previously-published work (Rafter et al. 2013) finding that non-toxic *O. nubilalis* larvae were consumed in their entirety by mantids that would gut *A. syriaca*-reared

202 *D. plexippus* caterpillars. The current experiment was designed to confirm the results of the 2011
203 experiment and provide more precise information on how mantids handle prey that do not
204 sequester toxic compounds from their host plant and that may be of higher nutritional value (i.e.,
205 lower C:N ratio). Because of the difficulty in finding sufficient late-instar caterpillars, the
206 experiment was conducted in two stages (=trials). In trial one of this experiment, we presented
207 each of 16 lepidopteran-naïve mantids with one late-instar *O. nubilalis* caterpillar collected from
208 organically-grown flint corn, *Zea mays* L., growing in an experimental farm. The second trial
209 was essentially identical to the first, but took place two weeks later: in it, we presented each of
210 12 naïve mantids with one late-instar *O. nubilalis*. Caterpillars were always collected on the day
211 of the trial; both trials lasted one day. Data collection procedures were as above. If mantids did
212 not gut the caterpillars, we froze whole caterpillars and later dissected the caterpillars to isolate
213 the gut and body portions for chemical analysis.

214 ***Chemical analysis:*** All of the preserved caterpillar biomass was stored in plastic tubes
215 and dried in a 45^{o}C drying oven for five days. After drying was complete and samples were
216 ground and homogenized 1.0-2.0 mg of dried material was removed from each sample and sent
217 for CHN analysis to the Analytic Chemistry lab at the University of Rhode Island's Graduate
218 School of Oceanography (Narragansett RI).

219 In order to ensure that cardenolide content differed between *A. syriaca* and *A. incarnata*,
220 and between caterpillars reared on these two host plants, we analyzed the cardenolide content of
221 plant tissue from both *Asclepias* species and body tissue from monarch caterpillars fed
222 exclusively on either *A. syriaca* or *A. incarnata*. Fresh leaf and caterpillar tissue was stored,
223 dried, ground, and homogenized as above. Powdered tissue was extracted at 2°C in 95% ethanol
224 at a ratio of 1 mL to 100 mg tissue for 48 hours with occasional vortexing, and the 9,000 x g

supernatant was used directly as the source of cardenolides. The commercially available 3,5-dinitrobenzoic acid (Sigma 121258; Rowson 1952, Dobler and Rowell-Rahier 1994) was used in place of 2,2',4,4'-tetranitrodiphenyl (e.g., Brower et al. 1984). In triplicate wells of a Griner UV-Star® 96 well microplate (Monroe, NC), 50 µL sample was mixed with 50 µL 2% (w:v) 3,5-dintrobenzoic acid in 100% ethanol, allowed to incubate at room temperature for 1 min, and then 100 µL 3% NaOH in 100% ethanol was added to each well. The plate was incubated at room temperature for 10 min and then the absorbance quantified at 535 nm with a Spectramax M2 Multi-Mode spectrophotometer (Molecular Devices, Sunnydale, CA). Triplicate control wells with 100% ethanol replacing 2% 3,5-dinitrobenzoic acid in 100% ethanol were used to correct for background absorbance, and cardenolide content was expressed as µg digitoxin equivalents per mg dry weight (µg mg^{-1} DW).

Statistical analysis: Since post attack prey handling behavior by mantids (all of which fed multiple times in respective trials) did not vary (see results) statistical analysis was unnecessary for these data. Results from the CHN analysis were used to determine the percent carbon and nitrogen in both gut and body tissues and calculate their carbon/nitrogen (C:N) ratios. We analyzed the *D. plexippus* data using a two-way ANOVA that crossed the main factors toxicity (cardenolide-containing or cardenolide-free caterpillars) and body tissue (gut versus body). We analyzed the *O. nubilalis* data using a one-way ANOVA with the main factor body tissue (gut versus body). Where appropriate, we determined among-treatment differences using Tukey-Kramer HSD. All analyses were performed using JMP 9 (SAS Institute, Inc).

Results

Cardenolide concentrations in A. syriaca, A. incarnata, and the body tissues of monarch larvae fed exclusively on either plant species. Cardenolide content is expressed as µg

digitoxin equivalents per mg of dry weight (μg mg^{-1} DW). *Asclepias syriaca* tissue contained 5.32 ± 0.60 [SE] μg mg^{-1} DW; the body tissue of larvae fed on *A. syriaca* also contained cardenolides (3.19 ± 0.35 μg mg^{-1} DW). Neither *A. incarnata* nor the body tissue of larvae fed on *A. incarnata* contained cardenolides at levels detectable with our assay (both 0.0 μg mg^{-1} DW).

Experiment 1: Do mantids handle toxic (cardenolide-containing host plant) and non-toxic (no-cardenolide host plant)* D. plexippus *caterpillars differently? We observed 117 predator-prey interactions; predators attacked the prey in 64/114 cases (three caterpillars infected with a fungal pathogen were excluded from the analysis). Regardless of treatment, mantids gutted all the *D. plexippus* caterpillars they attacked (31/31 non-toxic and 33/33 toxic caterpillars, respectively).

Experiment 2: Does the presence of plant material in the caterpillar gut affect how mantids handle toxic (cardenolide-containing host plant) and non-toxic (no-cardenolide host plant)* D. plexippus *caterpillars? We observed 113 predator-prey interactions; mantids attacked the prey in 20 of the 113 interactions. As in Experiment 1, mantid behavior was unaffected by toxicity and they gutted all (12/12) of the unstarved prey but none (0/8) of the starved prey.

Experiment 3: Do mantids handle toxic (cardenolide-containing host plant) and non-toxic (no-cardenolide host plant) adult* D. plexippus *butterflies differently? We observed 12 predator-prey interactions (six for each toxicity treatment). Mantids did not gut any of the adult butterflies regardless of the larval host plant. In each case, mantids consumed the body while discarding the wings, antennae, and legs. Some mantids appeared to 'taste' the wings, but stopped and returned to feeding on the body.

Experiment 4: Do mantids handle* O. nubilalis *differently than* D. plexippus*? We observed a total of 28 predator-prey interactions; mantids attacked the prey in 13 of the 28

interactions. In the first trial, six of seven caterpillars were not gutted, and in the remaining case the mantid stopped feeding entirely. In the second trial, 6/6 caterpillars were not gutted.

Carbon and nitrogen concentrations: Percent carbon (Fig. 1A) was significantly higher in the mantid-consumed body tissue than in the mantid-discarded gut tissue of *D. plexippus* caterpillars ($F_{3,53} = 31.3$, $p < 0.001$). This did not differ between toxic and non-toxic *D. plexippus* ($F_{3,53}=1.03$, $p=0.31$), and there was no interaction between these factors ($F_{3,53}=0.10$, $p=0.75$). Percent nitrogen (Fig. 1B) was also higher in body versus gut tissue, and in non-toxic *D. plexippus* ($F_{3,53}=94.0$, $p<0.001$ and $F_{3,53}=7.47$, $p<0.001$, respectively); however, the interaction was not significant ($F_{3,53}=1.64$, $p=0.21$). The resulting C:N ratio (Fig. 1C) for *D. plexippus* was higher in the gut versus body tissue, higher in toxic versus non-toxic caterpillars ($F_{3,53}=57.3$, $p<0.001$ and $F_{3,53}=10.6$, $p=0.002$, respectively), and there was a significant interaction ($F_{3,53}=9.27$, $p=0.004$). In contrast, there was no difference in the percent carbon, nitrogen, and C:N ratio in *O. nubilalis* guts and bodies ($F_{1,9}=4.52$, $p=0.066$; $F_{1,9}=0.83$, $p=0.39$; and $F_{1,9}=0.24$, $p=0.64$, respectively). For *D. plexippus*, the C:N ratio of mantid-consumed body tissue was lower than the C:N ratio of mantid-discarded gut tissue; however, mantids eagerly consumed *O. nubilalis* tissue with C:N ratios equal to or greater than those of the *D. plexippus* gut. In other words, mantids consumed tissues with both a higher and lower C:N ratio than the *D. plexippus* guts they rejected.

Discussion

We found no evidence that *D. plexippus*-sequestered cardenolides affected mantid prey handling behavior. Specifically, *T. sinensis* behaved similarly towards *D. plexippus* larvae (experiments 1-2) and adults (experiment 3) reared on cardenolide-containing *A. syriaca* versus no-cardenolide *A. incarnata*. Since these mantids were lab-reared, their inability/unwillingness to

294 discriminate between cardenolide-containing versus no-cardenolide *D. plexippus* gut tissue must
295 be innate. The lack of a behavioral response to *D. plexippus* adults seems appropriate given that
296 mantids experienced no apparent ill-effects from consuming the cardenolide-laden bodies (Rafter
297 et al. 2013) of *D. plexippus* caterpillars fed *A. syriaca*.

298 The addition of a starved/unstarved caterpillar treatment to experiment 2 revealed that the
299 mantids' gutting behavior reflects the active rejection of partially-digested plant material found
300 within the gut. This suggests that rather than avoiding cardenolides, mantids may instead be
301 avoiding the lower-quality (higher C:N ratio) plant material found in the gut tissue (Fig. 1C).
302 This interpretation is further supported by the third experiment that found mantids did not gut
303 adult *D. plexippus*, nectar feeders whose guts are free of plant material. While our three *D.*
304 *plexippus* experiments support the 'food quality' hypothesis for the mantids' gutting behavior,
305 the results of our fourth experiment (*O. nubilalis* trials) do not. In this experiment, which was
306 intended to confirm results first reported in Rafter et al (2013), we again found that mantids
307 readily consume *O. nubilalis* gut and body tissue. The results of our first three experiments led us
308 to hypothesize that the gut material of *O. nubilalis* caterpillars would be of higher nutritional
309 quality (as indicated by the C:N ratio) than the mantid-discarded portions of *D. plexippus*
310 caterpillars. While we found that both *O. nubilalis* gut and body tissue was relatively high in C
311 and N (Figs. 1A and 1B, respectively), the C:N ratio of mantid-accepted *O. nubilalis* gut tissue
312 equaled or exceeded those of mantid-rejected *D. plexippus* gut tissue (Fig. 1C). Researchers
313 commonly use C:N ratios as a proxy for nutritional quality of food types and have been able
314 relate nutrient quality to prey selectivity (Zandonà et al. 2011). However, given the inconsistency
315 in mantid preference for tissues in relation to their respective C:N ratios, this metric does not
316 appear to explain the gutting behavior. It may be that mantids are not responding to a specific

C:N ratio *per se*, but rather are processing prey based on detectable differences in the nutritional quality of prey gut content versus body tissues. The gut content of *D. plexippus* is largely undigested leaf material low in nutrients and high in indigestible cellulose, while that of *O. nubilalis* is largely undigested corn that is higher in nutrients and lower in cellulose. The gut and body tissues of *D. plexippus* differ markedly in their chemical signatures with respect to carbon, nitrogen and the resulting C:N ratio while those of *O. nubilalis* do not (Fig. 1). Mantids may gut *D. plexippus* larva to maximize intake of high quality body tissues, but consume *O. nubilalis* entirely because the nutritional quality of their guts and body tissue are similar.

Although *T. sinensis* appears to be insensitive to the presence of cardenolide in *D. plexippus* caterpillars, it does exhibit an adverse reaction when consuming cardenolide-sequestering milkweed bugs, *Oncopeltus fasciatus*. They quickly learn to reject and will eventually completely avoid this prey after few encounters (Berenbaum and Miliczky 1984, Paradise and Stamp 1991). This suggests that the Chinese mantid is tolerant of, rather than unaffected by, cardenolide consumption. Milkweed bugs uptake cardenolides more efficiently and at substantially higher concentrations than do *D. plexippus* (Scudder et al. 1986, Agrawal et al. 2012); mantids may be intolerant to the higher cardenolide concentrations found in milkweed bugs.

An alternate hypothesis for the mantid's gutting behavior is that they may be responding to the presence of other secondary plant compounds found in prey biomass. Adult *D. plexippus* have been shown to feed on plants containing pyrrolizidine alkaloids and sequester these compounds; these compounds may play a role in defending adult *D. plexippus* against both vertebrate and invertebrate predators (Kelley et al. 1987, Stelljes and Seiber 1990). These compounds are sequestered during the adult stage, however, and *D. plexippus* butterflies were

fed sugar water in this experiment. To our knowledge, there are no reports of *D. plexippus* caterpillars sequestering toxins other than cardenolides. However, plants often employ a suite of defenses against herbivory and maintain multiple defense strategies with little cost (Koricheva et al. 2004). Thus, there are a number of potential toxins that mantids could be responding to in the plant material found in the caterpillar's gut. Many cardenolide-containing plants in the Apocynaceae, including genus *Asclepias*, also contain alkaloids (Agrawal et al. 2012). In addition, although *A. incarnata* is cardenolide-free, it is not undefended. Both the roots and aboveground biomass contain pregnane glycosides (Warashina and Noro 2000a, b) that are inducible defenses against herbivory (A. Agrawal, personal communication). If mantids are unable to tolerate compounds found in undigested plant material, they might respond by gutting the caterpillar.

Our results may also be influenced by the fact that *D. plexippus* caterpillars and European corn borers feed on different parts of their respective host plants; *D. plexippus* feed on leaves, while corn borers feed on seeds. Corn has been selectively bred for human consumption and is thus relatively undefended compared to milkweed leaves. This further supports the idea that mantids may be gutting *D. plexippus* because of their intolerance to plant compounds found in the leaves of *Asclepias* plants. A number of other species are able to process food items in response to toxicity. Tanagers, *Pipraeida melanonota* Vieillot, reduce the toxicity of ithomiine moths by chewing on them until the abdominal content is expelled; they then eat the abdominal contents while leaving the rest behind (Brown and Neto 1976). The European paper wasp *Polistes dominula* Christ will gut *Pieris napi* L. caterpillars that were reared on toxic host plants, but not those that were reared on non-toxic plants (Rayor et al. 2007). Herbivores such as the meadow vole will cut branches from conifers and leave them uneaten for several days until

363 tannins and phenolic concentrations are reduced sufficiently for the vegetation to be palatable
364 (Roy and Bergeron 1990). Mantids may be similarly reducing their toxin burden by processing
365 prey.

366 The results of our work illustrate the unexpectedly complex mechanisms determining
367 how Chinese mantids process lepidopteran prey. This predator is responding to a number of
368 chemical cues as it consumes prey items that are heterogeneous in nutritional value and degree of
369 toxicity. Because mantids did not respond to cardenolides in *D. plexippus*, it seems most likely
370 that their gutting behavior is driven instead by other plant secondary compounds and/or the
371 nutritional quality of prey tissue. Irrespective of mechanism, this mantid's ability to efficiently
372 process toxic and non-toxic prey is likely important in allowing this non-native generalist
373 predator to utilize a wide array of prey taxa.

374 **Acknowledgements**

375 We gratefully acknowledge L. Hurd, whose willingness to discuss these ideas and offer
376 suggestions led to this manuscript. R. Casagrande and L. Tewksbury assisted with insect
377 husbandry; S. Alm, N. Castrataro, and E. Roberts assisted with plant propagation and
378 maintenance; L. Elder, J. Hayward, C. Klein, N. Le Claire-Conway, L. Rafter, and L. Westbrook
379 assisted with mantid rearing; A. Agrawal, R. Casagrande, M. Hickin, and C. Thornber provided
380 comments to improve the manuscript. Funding was provided by a Sigma Xi Grant-in-Aid of
381 Research, a University of Rhode Island Enhancement of Graduate Research Award, and NIFA
382 2011-67013-30142 to EP.

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476

477

478 **Figure Legend**

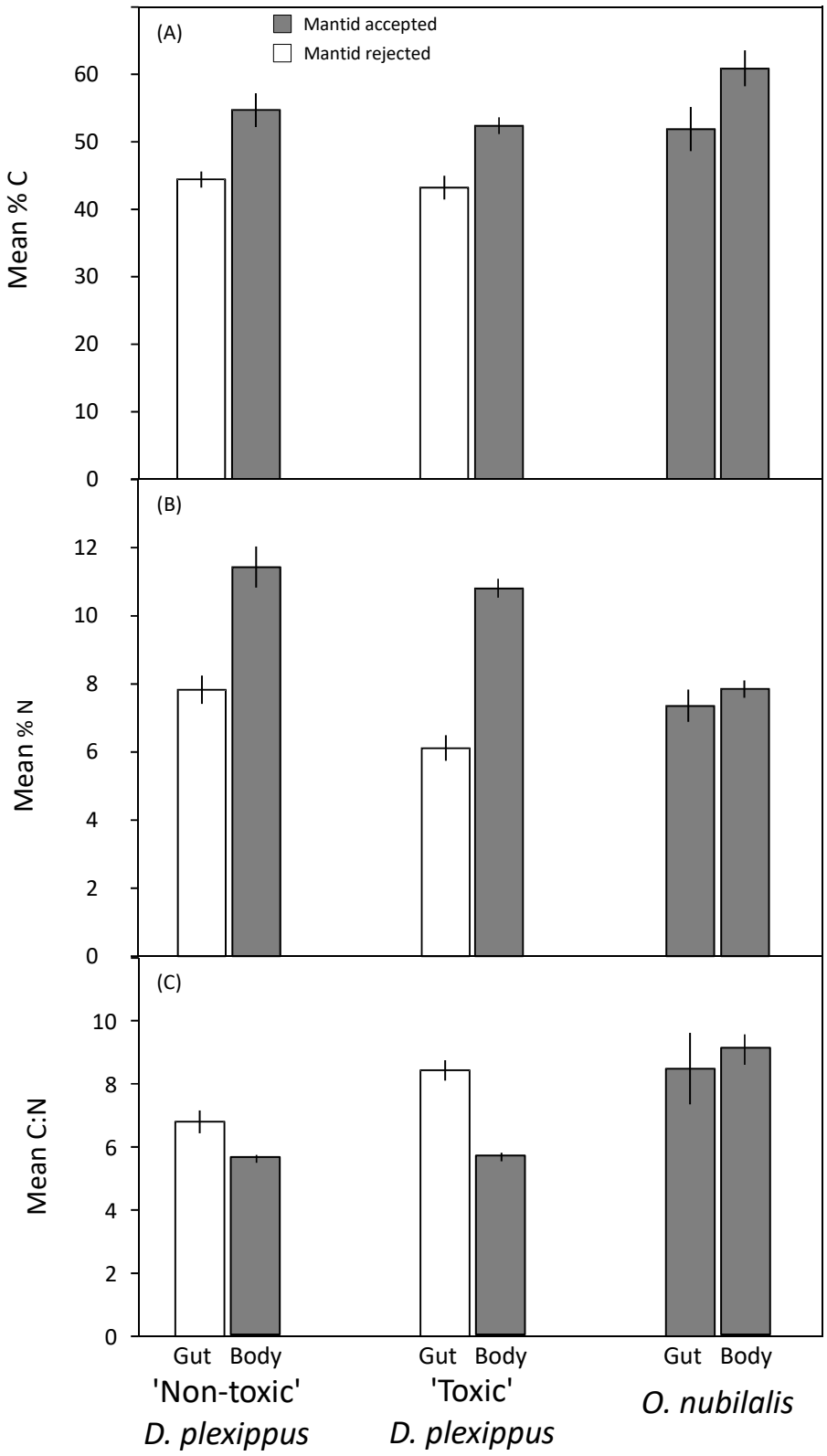
479 Figure 1: (a) Mean percent of carbon (C) present in each prey and tissue type ± 1 SE. (b)

480 Mean percent of nitrogen (N) present in each prey and tissue type ± 1 SE. (c) Mean C:N ratio of

481 each prey and tissue type ± 1 SE.

482 Fig 1

483


(A)
Mantid accepted
Mantid rejected
Mean % C
0
10
20
30
40
50
60
(B)
Mean % N
0
2
4
6
8
10
12
(C)
Mean C:N
0
2
4
6
8
10
Gut Body
Gut Body
Gut Body
'Non-toxic'
D. plexippus
'Toxic'
D. plexippus
O. nubilalis