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Pretty Picky for a Generalist: Impacts of Toxicity and Nutritional Quality on Mantid Prey Processing

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Pretty Picky for a Generalist: Impacts of Toxicity and Nutritional Quality on Mantid Prey Processing

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1 Rafter et al: Mantids selectively process lepidopteran prey

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

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40**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

41 Prey utilize an array of defenses against predation (reviewed in Lima and Dill 1990).
42 Organisms can, for instance, avoid detection via crypsis or disruptive coloration that makes it
43 difficult for predators to identify the boundaries of the prey's body. Prey can also employ
44 behavioral measures to decrease their likelihood of attracting a predator: Veeries, *Catharus*
45 *fuscescens* Stephens, respond to predation risk by decreasing the rate and length of their songs
46 (Schmidt and Belinsky 2013). Once detected, prey can employ secondary defenses such as
47 aggressive or escape behaviors as well as morphological and/or chemical defenses (Ruxton et al.
48 2004). The presence of trout, for example, can cause macroinvertebrates to alter their drift rates
49 and foraging activity (Simon and Townsend 2003, Eby et al. 2006), as well as their microhabitat
50 use (Lima 1998). Morphological changes are also possible: *Daphnia pulex* Leydig respond to
51 predator cues by producing fewer, but larger, offspring with prominent neck spines (Luening
52 1994) that make the prey more difficult for predators to attack.

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

64 Although chemically-based antipredator defenses are often highly effective, predators
65 have developed a variety of techniques for overcoming them. Floodplain death adders,
66 *Acanthophis praelongus* Ramsay, prey on toxic frogs by biting the prey, injecting it with toxins,
67 and then releasing it. The adder's toxins kill the frog, whose own defensive toxins degrade after it
68 has died; the snake can then eat the formerly-toxic frog without any ill effects (Phillips and Shine
69 2007). Loggerhead Shrikes, *Lanius ludovicianus* Mearnsi, employ a similar strategy for feeding
70 on chemically defended lubber grasshoppers, *Romalea guttata* Beauvois. Grasshoppers captured
71 by the birds are impaled on thorns or barbed wire; the shrikes only return to feed on them once
72 the grasshoppers' defensive toxins have been degraded and their aposematic coloration fades
73 (Yosef 1992). Other predators process prey to feed selectively on the most palatable portion of
74 the prey (Glendinning 2007) or regulate their toxicity burden (Skelhorn and Rowe 2007).

75 The monarch butterfly, *Danaus plexippus* L., is chemically defended and aposematically
76 colored in both the black-and-yellow larval and black-and-orange adult stage. Their caterpillars
77 sequester toxins when feeding on cardenolide-containing host plants in the genus *Asclepias*
78 (Apocynaceae) (Agrawal et al. 2012). Despite this generally effective chemical defense, *D.*
79 *plexippus* is susceptible to predation across all life stages. Its invertebrate predators include ants,
80 *Formica montana* Wheeler, ladybird beetles, *Harmonia axyridis* Pallas (Koch et al. 2003, Prysby
81 2004), and predatory *Polistes* (Rayor 2004), and *Vespula* wasps (Leong et al. 1990). Birds such
82 as Orioles, *Icterus* spp., Grosbeaks, *Pheucticus* spp., (Nishida 2002) and other vertebrate
83 predators such as *Peromyscus* mice also feed on *D. plexippus* (Glendinning 1990).

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

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

107 **Methods**

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

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

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

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

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

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

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

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

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

199 ***Experiment 4: Do mantids handle larval O. nubilalis differently than D. plexippus?***
200 This experiment repeated previously-published work (Rafter et al. 2013) finding that non-toxic
201 *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°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

225 supernatant was used directly as the source of cardenolides. The commercially available 3,5-
226 dinitrobenzoic acid (Sigma 121258; Rowson 1952, Dobler and Rowell-Rahier 1994) was used in
227 place of 2,2',4,4'-tetranitrodiphenyl (e.g., Brower et al. 1984). In triplicate wells of a Griner UV-
228 Star[®] 96 well microplate (Monroe, NC), 50 μ L sample was mixed with 50 μ L 2% (w:v) 3,5-
229 dinitrobenzoic acid in 100% ethanol, allowed to incubate at room temperature for 1 min, and then
230 100 μ L 3% NaOH in 100% ethanol was added to each well. The plate was incubated at room
231 temperature for 10 min and then the absorbance quantified at 535 nm with a Spectramax M2
232 Multi-Mode spectrophotometer (Molecular Devices, Sunnydale, CA). Triplicate control wells
233 with 100% ethanol replacing 2% 3,5-dinitrobenzoic acid in 100% ethanol were used to correct
234 for background absorbance, and cardenolide content was expressed as μ g digitoxin equivalents
235 per mg dry weight (μ g mg⁻¹ DW).

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

245 **Results**

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

248 digitoxin equivalents per mg of dry weight ($\mu\text{g mg}^{-1}$ DW). *Asclepias syriaca* tissue contained
249 5.32 ± 0.60 [SE] $\mu\text{g mg}^{-1}$ DW; the body tissue of larvae fed on *A. syriaca* also contained
250 cardenolides (3.19 ± 0.35 $\mu\text{g mg}^{-1}$ DW). Neither *A. incarnata* nor the body tissue of larvae fed on
251 *A. incarnata* contained cardenolides at levels detectable with our assay (both 0.0 $\mu\text{g mg}^{-1}$ DW).

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

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

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

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

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

273 **Carbon and nitrogen concentrations:** Percent carbon (Fig. 1A) was significantly higher
274 in the mantid-consumed body tissue than in the mantid-discarded gut tissue of *D. plexippus*
275 caterpillars ($F_{3,53} = 31.3$, $p < 0.001$). This did not differ between toxic and non-toxic *D. plexippus*
276 ($F_{3,53}=1.03$, $p=0.31$), and there was no interaction between these factors ($F_{3,53}=0.10$, $p=0.75$).
277 Percent nitrogen (Fig. 1B) was also higher in body versus gut tissue, and in non-toxic *D.*
278 *plexippus* ($F_{3,53}=94.0$, $p<0.001$ and $F_{3,53}=7.47$, $p<0.001$, respectively); however, the interaction
279 was not significant ($F_{3,53}=1.64$, $p=0.21$). The resulting C:N ratio (Fig. 1C) for *D. plexippus* was
280 higher in the gut versus body tissue, higher in toxic versus non-toxic caterpillars ($F_{3,53}=57.3$,
281 $p<0.001$ and $F_{3,53}=10.6$, $p=0.002$, respectively), and there was a significant interaction
282 ($F_{3,53}=9.27$, $p=0.004$). In contrast, there was no difference in the percent carbon, nitrogen, and
283 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 $F_{1,9}=0.24$,
284 $p=0.64$, respectively). For *D. plexippus*, the C:N ratio of mantid-consumed body tissue was
285 lower than the C:N ratio of mantid-discarded gut tissue; however, mantids eagerly consumed *O.*
286 *nubilalis* tissue with C:N ratios equal to or greater than those of the *D. plexippus* gut. In other
287 words, mantids consumed tissues with both a higher and lower C:N ratio than the *D. plexippus*
288 guts they rejected.

289 Discussion

290 We found no evidence that *D. plexippus*-sequestered cardenolides affected mantid prey
291 handling behavior. Specifically, *T. sinensis* behaved similarly towards *D. plexippus* larvae
292 (experiments 1-2) and adults (experiment 3) reared on cardenolide-containing *A. syriaca* versus
293 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

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

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

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

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

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

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Figure Legend

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

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

481

each prey and tissue type \pm 1 SE.

482

Fig 1

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