



## Research

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# A nutrition–defence trade-off drives diet choice in a toxic plant generalist

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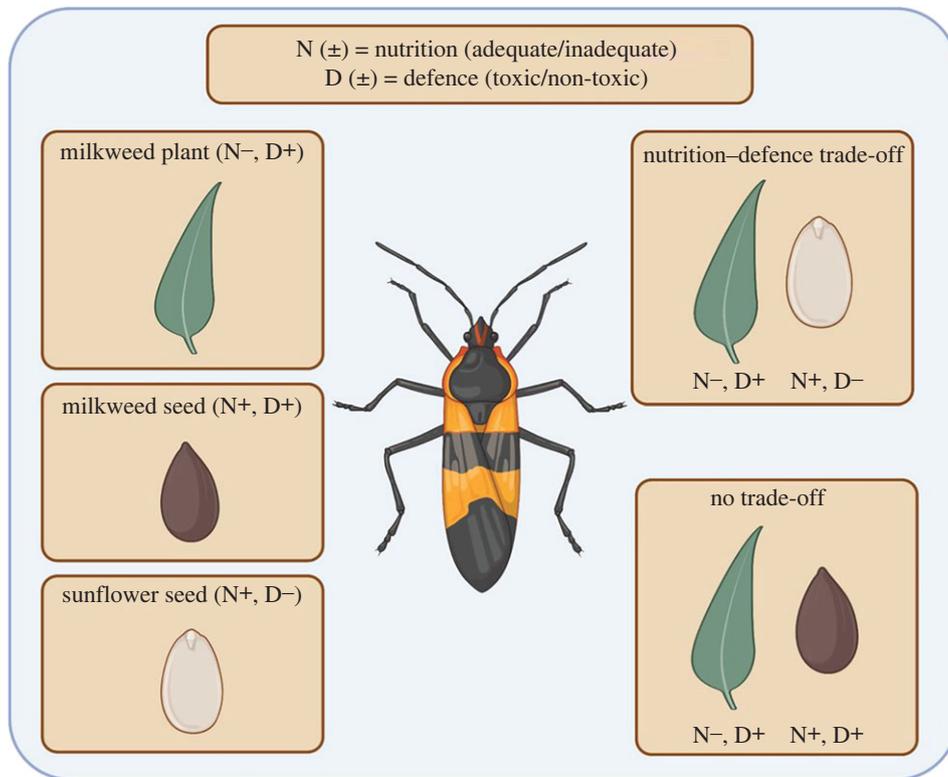
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Plant toxicity shapes the dietary choices of herbivores. Especially when herbivores sequester plant toxins, they may experience a trade-off between gaining protection from natural enemies and avoiding toxicity. The availability of toxins for sequestration may additionally trade off with the nutritional quality of a potential food source for sequestering herbivores. We hypothesized that diet mixing might allow a sequestering herbivore to balance nutrition and defence (via sequestration of plant toxins). Accordingly, here we address diet mixing and sequestration of large milkweed bugs (*Oncopeltus fasciatus*) when they have differential access to toxins (cardenolides) in their diet. In the absence of toxins from a preferred food (milkweed seeds), large milkweed bugs fed on nutritionally adequate non-toxic seeds, but supplemented their diet by feeding on nutritionally poor, but cardenolide-rich milkweed leaf and stem tissues. This dietary shift corresponded to reduced insect growth but facilitated sequestration of defensive toxins. Plant production of cardenolides was also substantially induced by bug feeding on leaf and stem tissues, perhaps benefitting this cardenolide-resistant herbivore. Thus, sequestration appears to drive diet mixing in this toxic plant generalist, even at the cost of feeding on nutritionally poor plant tissue.

## 1. Introduction

Herbivores balance ecological benefits (e.g. avoiding predation) with maximizing nutritional gains in their feeding strategies [1–5]. For herbivores that store plant toxins within their bodies as an anti-predator defence (sequestering herbivores), the quality of a particular food includes nutritional quality and secondary metabolites, some of which may be sequestered. The complex suite of physiological adaptations required for toxin resistance, and in turn sequestration, has typically led to the evolution of dietary specialization among sequestering herbivores [6]. Nonetheless, specialized sequestration can be a risky strategy, as these herbivores depend on a narrow range of host plants for both nutrition and defence, and thus are vulnerable to variations in host-plant abundance. Toxic plant generalists [5,7], which we define as herbivores that feed on plants containing toxic secondary metabolites, and typically sequester these toxins, but also feed substantially on non-toxic hosts, appear to reap the benefits of sequestration without the risks of dietary specialization.

For toxic plant generalists, there may often be a trade-off between the nutritional quality of a potential food and the availability of toxins for sequestration (i.e. highly nutritional foods often do not contain toxins for sequestration) [5,8–11]. It has been well demonstrated that, when faced with such a trade-off, toxic plant generalists engage in diet mixing, yielding protection from parasitism or predation [5,8]. Although there have been explicit tests of natural enemies as a behavioural driver of diet mixing in toxic plant generalists, we investigate the novel hypothesis that a nutrition–defence trade-off may drive diet mixing behaviour in such species [12]. Specifically, if acquiring toxins for sequestration is the driver of diet mixing in such species, we predict that alleviating a nutrition–defence trade-off by providing superior food that also contains toxins for sequestration should reduce diet mixing.



**Figure 1.** To test the prediction that *Oncopeltus fasciatus* engages in increased diet mixing to gain toxins for sequestration under a nutrition–defence trade-off, we rear *O. fasciatus* under a such a trade-off (top right box), where it has access to one food with toxins for sequestration (a milkweed plant) and another that is nutritionally adequate but non-toxic (sunflower seeds). We also alleviate this trade-off, providing a food that is nutritionally adequate and contains toxins for sequestration (milkweed seeds) in addition to a milkweed plant (lower right box). We hypothesize that plant feeding would be reduced under this alleviated trade-off. We also rear *O. fasciatus* on all three foods alone (left boxes) as a basis for comparison with mixed treatments. Created with biorender.com.

The large milkweed bug, *Oncopeltus fasciatus* (Hemiptera: Lygaeidae: Lygaeinae), is a toxic plant generalist that sequesters cardenolide toxins (inhibitors of animal Na<sup>+</sup>/K<sup>+</sup>-ATPases, the dominant defensive secondary metabolite produced by milkweeds (*Asclepias* spp.) as an anti-predator defence [13–17]. *Oncopeltus fasciatus* is a piercing–sucking herbivore that feeds on a diversity of plants and plant parts. Although milkweed seeds appear to be the most important food of *Oncopeltus* and related lygaeines, these bugs also feed on milkweed herbaceous tissue (leaves and stems of the plant), and seeds of species lacking cardenolides; in fact, laboratory colonies are commonly reared on common sunflower (*Helianthus annuus*) seeds [18–20]. *Oncopeltus fasciatus* is adapted to milkweed cardenolides, expressing four highly tolerant copies of Na<sup>+</sup>/K<sup>+</sup>-ATPase and storing sequestered cardenolides in a specialized dorso-lateral compartment [17,21–24]. While a variety of seeds are nutritionally adequate (which we define as being able to support development to adulthood), milkweed plant tissue alone is nutritionally insufficient for *O. fasciatus* development and reproduction (but contains cardenolides for sequestration) [25,26]. As *O. fasciatus*' natural dietary regime encompasses foods that are nutritionally variable and chemically diverse, it is a well-suited system for studying diet mixing in toxic plant generalists.

This study tests the hypothesis that shifts to feeding on toxic foods that are nutritionally inadequate may be driven by a lack of toxins when feeding on nutritionally superior diets. We incorporate toxic (milkweed seeds) and non-toxic (sunflower seeds) nutritionally adequate food sources in addition to a toxic, nutritionally inadequate food

(milkweed plants) in an ecologically relevant experimental design. Bugs were reared to adulthood separately with access to either type of seed with or without access to a milkweed plant (figure 1). We calculate indices representing the diversity and polarity of cardenolides sequestered by bugs, which reflect uptake, modification and potential consequences for defence. For example, cardenolide polarity impacts uptake and potentially defence (non-polar cardenolides are more bitter tasting), and differences in diversity of cardenolides between a bug and its food may reflect selective excretion or modification [27–30]. Metrics of the composition of sequestered toxins (aside from total concentration) have not been employed in studies of toxic diet mixing to date and allow us to ask whether diet mixing drives functional differences in sequestered toxins in such species.

Diet mixing induced by the availability of food with particular quality (nutrition or defence), may carry consequences for future species interactions. Milkweed cardenolides are highly inducible by feeding on leaves and stems, and induction of cardenolides appears to be specific to the extent, duration and type of herbivory [31,32]; nonetheless, induction by the piercing–sucking herbivory of *O. fasciatus* has not been previously investigated. Thus, we assessed plant induction in our main experiment where we measured insect diet shifts and conducted an additional experiment where milkweed plants were subject to more controlled damage. We hypothesized that if cardenolides are induced by *O. fasciatus* herbivory, there may be reciprocal impacts of diet mixing on the defences of both the bug and the plant: *O. fasciatus* may benefit from a higher concentration of cardenolides to sequester as an anti-predator defence, and

the plant may benefit from increased toxicity against later arriving, sensitive herbivores.

## 2. Methods and materials

### (a) Performance and sequestration of *Oncopeltus*

#### *fasciatus* on non-preferred food sources

We examined performance and sequestration of seed bugs on the tropical milkweed (*Asclepias curassavica*), an important host plant in the southern end of the bug's range. All bugs used in this experiment were from a colony ( $\approx 30$  adult bugs) of wild-collected *O. fasciatus* from Tompkins County, NY, USA. The colony had been reared for several generations on common milkweed (*Asclepias syriaca*) seeds. First instar bugs (from a pool of clutches laid by several females) were divided into five experimental treatment groups within 24 h after hatching: bugs with access to milkweed seeds (nutritionally adequate, toxic), bugs with access to milkweed seeds and milkweed plant tissue (nutritionally adequate, toxic + nutritionally inadequate, toxic), bugs with access to sunflower seeds (nutritionally adequate, non-toxic), bugs with access to sunflower seeds and milkweed plant tissue (nutritionally adequate, non-toxic + nutritionally inadequate, toxic), and bugs with access to milkweed plant tissue with no seeds (nutritionally inadequate, toxic). These treatments were chosen to test all factorial combinations of nutritionally adequate toxic and non-toxic foods (both seed types), and nutritionally inadequate toxic foods (plant tissue) (figure 1). Plants were reared following the method outlined in electronic supplementary material, appendix SI.

We conducted 10 replicates of each treatment, and each replicate received five bugs. All trials were conducted in 10 cm diameter pots filled with potting soil. After bugs were added, all pots were covered with semi-transparent mesh bags to prevent bugs from escaping. We also conducted a 'no herbivory' treatment, which consisted of a milkweed plant that was bagged, but received no bugs or additional seeds ( $n = 9$ ; one plant was removed due to infestation with thrips). For seed treatments, 0.5 g of seed was presented on the soil surface and replenished with another 0.5 g of seeds once per week. This amount of seed was determined to be well in excess of the amount required for the growth and development of five *O. fasciatus* nymphs. Seeds that germinated over the course of the experiment were removed and replaced with fresh, ungerminated seeds. *Asclepias curassavica* seeds were used for milkweed seed treatments and organic shelled sunflower seeds from a local supermarket were used as a cardenolide-free food source. All pots were watered approximately once every 2 days, regardless of the presence of a milkweed plant.

The experiment consisted of five blocks, each consisting of two replicates of each experimental treatment (10 pots per block). Bugs were allowed to feed and develop to adulthood. Bugs were collected from pots within 24 h of reaching adulthood, starved alone in a Petri dish for 24 h (with access to water), frozen at  $-80^{\circ}\text{C}$ , and freeze-dried. Thirty-one bugs (15 males and 16 females) survived to adulthood on milkweed seeds alone,  $n = 18$  bugs (seven males and 11 females) survived to adulthood on sunflower seeds alone,  $n = 35$  bugs (19 males and 16 females) survived to adulthood that had access to both milkweed seeds and plants, and  $n = 36$  bugs (23 males and 13 females) survived to adulthood that had access to sunflower seeds and milkweed plants. These

samples were used in analyses of development time to adulthood and adult dry mass, while a smaller subset was used for chemical analyses. The sex of adult bugs was noted, as *O. fasciatus* bugs are known to be sexually dimorphic in terms of adult dry mass and cardenolide sequestration (with females growing to larger sizes and sequestering higher concentrations of cardenolides) [25].

For treatments in which bugs had access to a milkweed plant, all aboveground biomass was collected once all bugs had reached adulthood and the crown of the plant (the top six fully expanded leaves and all intervening stem) was freeze-dried for chemical analysis. All bugs with access to milkweed plants but without seeds died before reaching adulthood. For plants with no adult bugs (i.e. the 'no herbivory' treatment, which received no bugs and the treatment where bugs had access to milkweed plants only), collection of the plants of a given treatment was timed with the collection of the first and last plants in the same block on which all bugs reached adulthood.

### (b) Impacts of *Oncopeltus fasciatus* feeding on milkweed performance and defensive chemistry

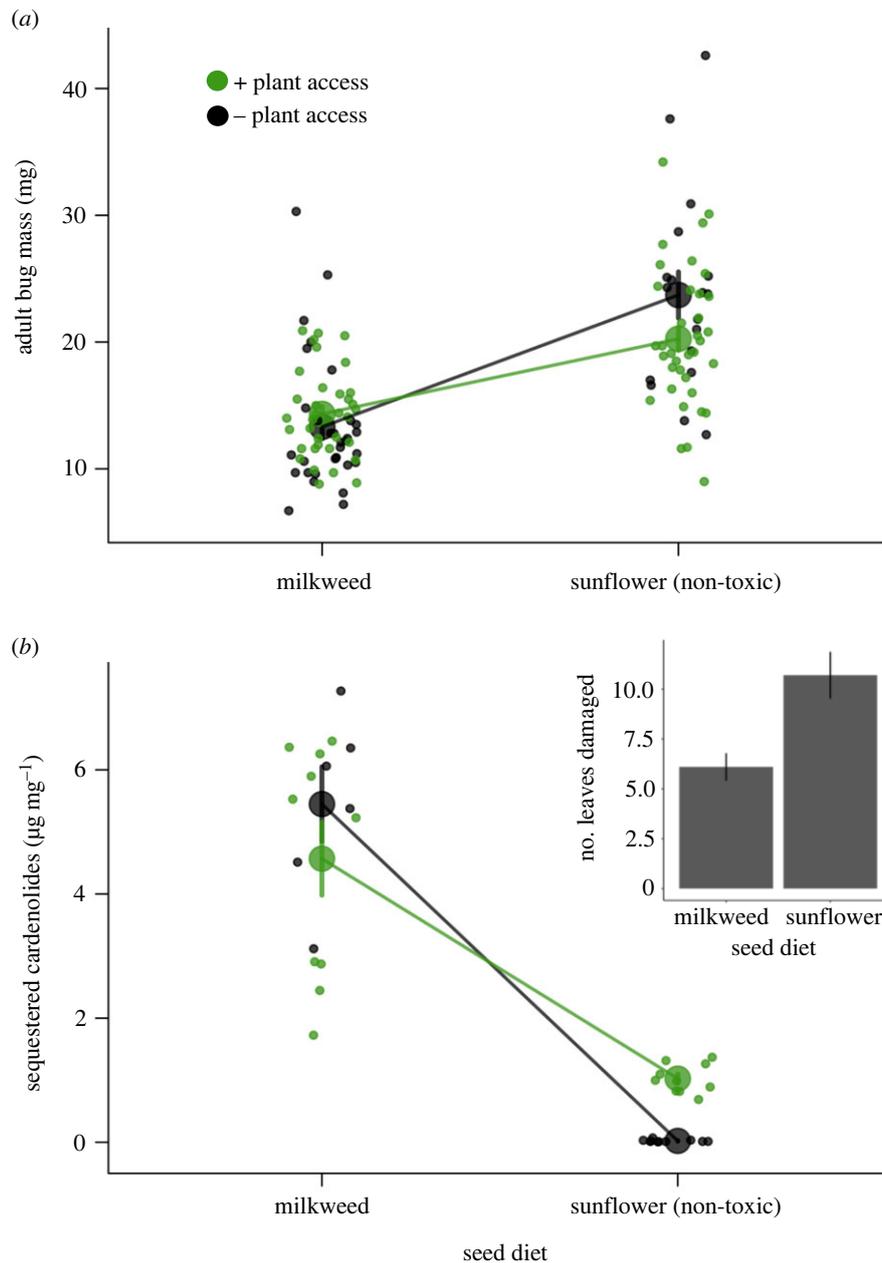
Due to low bug survivorship when feeding on milkweed plant tissue alone, a separate experiment was conducted to assess the impacts of bug feeding on milkweed growth and defensive chemistry. Eighteen milkweed plants were placed in bags at the 10–12 leaf stage as above and separated into three blocks of six plants each. Half the plants within each block received five first-instar milkweed bugs, and control plants received no bugs. One control and one herbivore-damaged plant were collected from each block at one, two, and three weeks after the start of treatments. The crown of each plant was collected for chemical analysis following the same method used in the previous experiment. The number of leaves damaged by bugs, and total number of leaves were recorded for all plants.

### (c) Analysis of plant and insect cardenolides

To analyse cardenolides, we used high-performance liquid chromatography (HPLC) on freeze-dried leaves, adult bugs ( $n = 10$  per treatment, five male and five female; except for the treatment that only received milkweed seeds, for which three male and three female bugs were run), and seeds ( $n = 5$  sunflower and *A. curassavica*). Cardenolides were extracted from samples and analysed following the method established by Züst *et al.* [33] and outlined in electronic supplementary material, appendix SI. The commercially available cardenolide digitoxin, which is not found in milkweed, was used as an internal standard. HPLC chromatogram peaks with a single absorption maximum between 214 and 222 nm were considered to be cardenolides [34], and cardenolide concentrations were calculated from peak areas at 218 nm and were standardized by the concentration of the internal standard digitoxin in each sample and sample dry mass.

### (d) Statistical analyses

For each sample, the Shannon–Wiener diversity index ( $H'$ ) was calculated by taking the natural logarithm of the proportion each cardenolide comprised the total cardenolides in a sample and summing this across all cardenolides in the sample. Additionally, a polarity index was calculated by



**Figure 2.** *Oncopeltus fasciatus* performance is highest on non-toxic sunflower seeds alone, but when a plant is available, bugs supplement their diet with milkweed leaves at a cost to growth performance. (a) Dry mass of bugs with (green) and without (black) access to *A. curassavica* plants reared on *A. curassavica* seeds (left) or reared on sunflower seeds (right). Bugs have lower growth performance when they have access to a milkweed plant in addition to sunflower seeds, achieving lower adult masses. (b) Total cardenolide concentration of bugs in treatments as determined by HPLC. Note that bugs sequester greater than three times more cardenolides when they have access to milkweed seeds. Inset: number of milkweed leaves damaged by bug feeding when the bugs had access to cardenolide-rich milkweed seeds versus cardenolide-free sunflower seeds. Means  $\pm$  SE are shown. Datapoints representing individuals were jittered for clarity.

expressing each cardenolide as a proportion of the total cardenolides in a sample, weighting this by the cardenolide's retention time, and summing this across all cardenolides in the sample [35]. Higher polarity index corresponds to a more non-polar assemblage of cardenolides. Statistics were performed in R version 4.1.2. Total cardenolide concentration, polarity, diversity, and metrics of bug performance were compared among bugs using fixed effects ANOVAs (response variable~seed type\*plant presence + bug sex). Bugs that only fed on sunflower seeds were excluded from polarity and diversity analyses, as these bugs contained only trace amounts of cardenolides. Plant growth and cardenolide diversity in the second experiment (examining plant induction over time) were also analysed using ANOVAs (response variable~week\*bug presence). Pairwise comparisons were performed

using Student's *t*-tests. The relationships between bug feeding and plant cardenolide concentration, polarity, and diversity were evaluated using Pearson correlations.

### 3. Results

#### (a) Impacts of diet mixing on performance and sequestration

When bugs lacked cardenolides from a nutritionally adequate food source (milkweed seeds), they engaged in substantial feeding on milkweed plants, causing over 40% more damage to milkweed leaves than bugs with access to milkweed seeds ( $p < 0.001$ , figure 2b). This plant feeding corresponded to

sequestration of cardenolides, although they had only 20% of the cardenolides found in bugs reared on milkweed seeds ( $p < 0.001$ , figure 2b). This gap is consistent with the 63% lower cardenolide concentration in milkweed leaves ( $2.7 \pm 0.36 \mu\text{g mg}^{-1}$ ) compared to seeds ( $7.3 \pm 0.14 \mu\text{g mg}^{-1}$ ) ( $p < 0.001$ ). In line with prior research, we found differences in total sequestered cardenolide concentration across sex, with females sequestering 30% more than males across all diets containing cardenolides (females  $4.03 \pm 0.7 \mu\text{g mg}^{-1}$  and males  $2.79 \pm 0.56 \mu\text{g mg}^{-1}$ ,  $F_{1,31} = 6.26$ ,  $p = 0.0178$ ; electronic supplementary material, table S1) [25]. However, this effect was not uniform across treatments, with females sequestering only 5% more than males on milkweed seeds alone ( $F_{3,28} = 5.29$ ,  $p < 0.01$ ; electronic supplementary material, table S1).

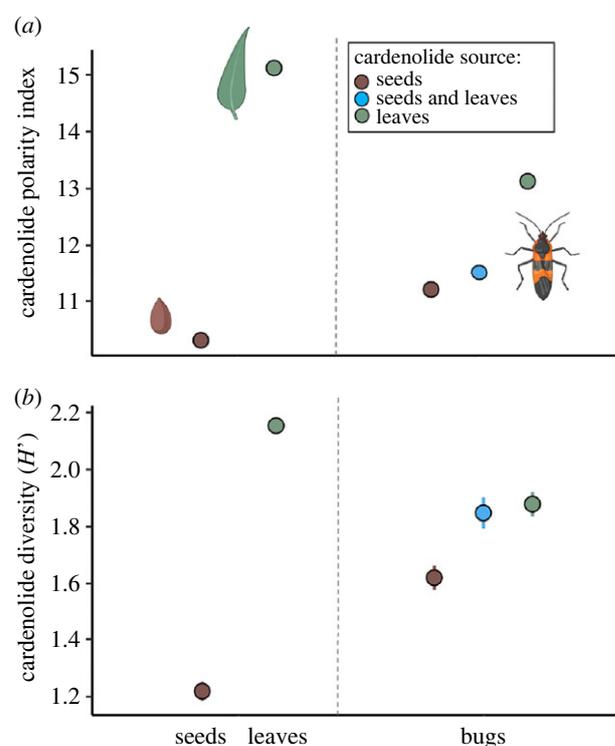
Plant feeding came at a cost to insect growth. Increased feeding on milkweed plant tissue when reared with sunflower seeds corresponded to a 16% reduction in adult dry mass relative to bugs raised on sunflower seeds alone ( $p = 0.028$ , figure 2a). However, bugs raised on milkweed seeds achieved comparable adult dry masses in the presence and absence of milkweed plants (seed type by plant access interaction:  $F_{1,115} = 3.94$ ,  $p = 0.049$ , plant versus no plant on milkweed seed:  $p > 0.1$  figure 2a).

As expected, we found that milkweed plant tissue was nutritionally inadequate for *O. fasciatus* development, as all bugs reared on milkweed plants alone died before reaching adulthood (typically in the second instar). Across all other treatments, the presence of a plant substantially increased bug survivorship to adulthood, with 73% ( $3.65 \pm 0.27$  bugs out of five per-replicate) surviving when they had access to a plant compared to 52% ( $2.60 \pm 0.38$  bugs) surviving when no plant was present ( $F_{1,36} = 5.24$ ,  $p = 0.028$ ). Across most of our experimental treatments, females grew larger than males, a finding in line with prior research [25]. However, when reared with access to both milkweed seeds and plants, females grew 11.26% smaller than males ( $F_{3,112} = 6.59$ ,  $p < 0.001$ , electronic supplementary material, table S1). There was an effect of both seed-type and plant presence on bug development time, but no interaction effect (seed type:  $F_{1,115} = 207.88$ ,  $p < 0.001$ , plant presence:  $F_{1,115} = 35.25$ ,  $p < 0.001$ ), with bugs reared with sunflower seeds taking 35% longer to reach adulthood than bugs with milkweed seeds and bugs with access to plants taking 18% longer to reach adulthood than bugs on seeds alone.

Sunflower seeds contained no cardenolides, and bugs that had access to sunflower seeds were only able to substantially sequester cardenolides in the presence of a milkweed plant (seed type by plant access interaction:  $F_{1,31} = 6.692$ ,  $p = 0.015$ , figure 2b). As bugs reared on sunflower seeds alone contained only trace amounts of cardenolides (less than  $0.03 \mu\text{g mg}^{-1}$ , electronic supplementary material, table S1), maternally transmitted cardenolides do not appear to be important in our experiment.

### (b) Cardenolide polarity and diversity

In addition to effects on toxin concentration, the polarity and diversity of cardenolides sequestered by bugs depended on their diet. Cardenolides in milkweed seeds had a 32% lower polarity index ( $p < 0.001$ , figure 3a) and a 43% lower diversity index ( $p < 0.001$ , figure 3b) compared to milkweed leaves. The availability of seed and plant cardenolides impacted the polarity of cardenolides sequestered by bugs (seed type:

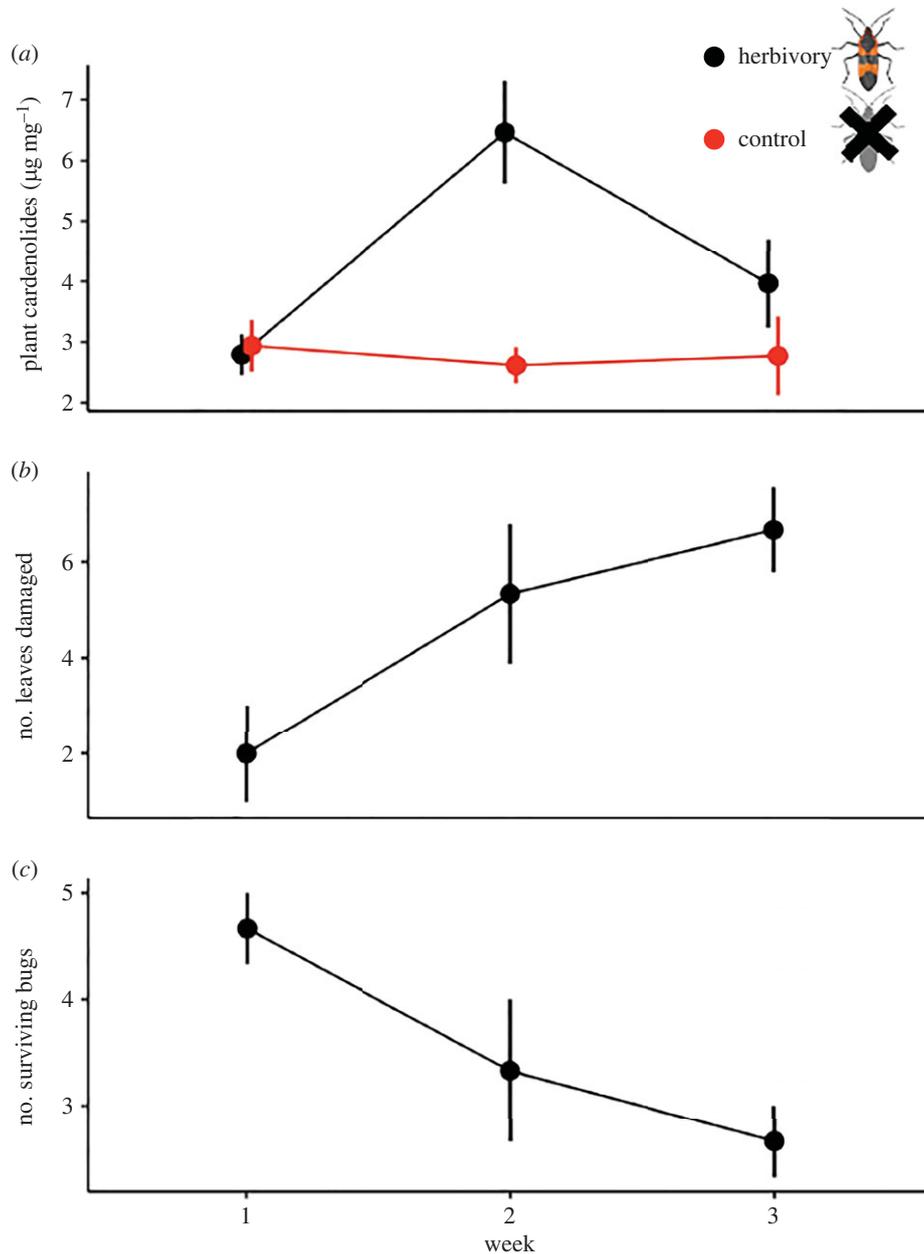


**Figure 3.** Chemical profile of cardenolides from *A. curassavica* seeds and leaves (left) and sequestered cardenolides in *O. fasciatus* bugs (right). In the figure key, ‘seeds’ refers to milkweed seeds. Bugs whose cardenolide source was ‘leaves’ alone were reared with supplemental sunflower seeds. (a) Polarity index of cardenolide samples. Values were determined as concentration-weighted HPLC retention times. A lower polarity index indicates a higher proportion of polar compounds, and higher index indicates a higher proportion of non-polar compounds (thus, seeds have much more polar cardenolides than leaves). Note that bugs converge to intermediate polarities of sequestered cardenolides, but the polarity sequestered is still dependent both on plant and seed diet. (b) Shannon–Wiener diversity index of cardenolide samples (higher scores indicate greater diversity). Bugs sequester a comparable, high diversity of cardenolides whenever they have access to a plant, regardless of access to seed cardenolides. Means  $\pm$  SE are shown. Icons from biorender.com.

$F_{1,22} = 151.332$ ,  $p < 0.001$ , figure 3a; plant access:  $F_{1,22} = 4.92$ ,  $p = 0.037$ , figure 3a). Despite selectively sequestering more polar cardenolides from plants (electronic supplementary material, figure S2), bugs that only had access to plant cardenolides still sequestered a more non-polar assemblage ( $p = 0.001$ , figure 3a) of cardenolides than bugs with access to cardenolides from seeds alone. However, differences in the diversity of sequestered cardenolides were driven only by the availability of plant cardenolides ( $F_{1,22} = 8.0$ ,  $p < 0.01$ , figure 3b). Accordingly, it appears that plant access alone drives the sequestration of a higher diversity of cardenolides, while the polarity of sequestered cardenolides (which may impact palatability to predators) is sensitive to the relative amount of seed and plant tissue in the diet.

### (c) Impacts of herbivory on milkweed performance and defensive chemistry

Feeding by *O. fasciatus* induced cardenolide defences in damaged milkweed plants. Across the four treatments with milkweed plants, plants with more bug damage had higher total concentrations of foliar cardenolides ( $r = 0.22$ ,  $n = 37$ ,  $p < 0.01$ , electronic supplementary material, figure S1). In a



**Figure 4.** Bug feeding induces plant cardenolides over time. (a) Total cardenolide concentration in leaves after one, two or three weeks with (black) or without (red) insect feeding (points jittered between treatments to avoid overlap). (b) Number of leaves visibly damaged by bug feeding over three weeks. Note that the amount of bug feeding peaks between weeks 1 and 2, which corresponds to induction of cardenolides in week 2. (c) Number of surviving bugs out of five first-instar *O. fasciatus* nymphs originally introduced to each plant. There was high mortality of bugs over the course of this experiment, as milkweed plant tissue is nutritionally inadequate, corresponding to a reduction in feeding over time. Means  $\pm$  SE are shown. Icons from biorender.com.

separate experiment to test for induced cardenolide responses by bug feeding, the number of leaves damaged by bugs increased over the course of the experiment ( $r = 0.56$ ,  $n = 9$ ,  $p = 0.02$ , figure 4b), with the greatest amount of damage occurring between weeks 1 and 2. We found a decline in the amount of herbivory between weeks 2 and 3, likely due to bug mortality (figure 4c). Bug feeding in week 2 caused twofold induction of plant cardenolides compared to controls ( $p < 0.001$ , figure 4a). Nonetheless, there was no detectable difference in total cardenolide concentration between control and bug-damaged plants after three weeks ( $p > 0.1$ , figure 4a).

#### 4. Discussion

If sequestration is the driver of diet mixing in toxic plant generalists, we predicted that a toxic plant generalist should engage

in more diet mixing under a nutrition–defence trade-off than when this trade-off is alleviated. Accordingly, we found that *O. fasciatus* engaged in more feeding on a toxic, nutritionally inadequate food when reared under such a trade-off than when this trade-off was alleviated by adding a nutritionally adequate, toxic food (milkweed seeds) (figure 2b inset). Furthermore, this compensatory diet mixing corresponded to sequestration of plant toxins: although bugs that sequestered toxins from milkweed plant tissue were unable to sequester the high levels of cardenolides found in bugs that had access to milkweed seeds, they were able to sequester, on average,  $1.12 \mu\text{g}$  cardenolides per milligram of dry mass (figure 2b)—an amount comparable to adult monarch butterflies reared on the same milkweed species (approximately  $2 \mu\text{g mg}^{-1}$ ) [35]. However, it appears that this increased plant feeding comes at the expense of bug growth. Thus, we find our major prediction supported: sequestration appears to be associated

with increased feeding on a toxic, nutritionally inadequate food in this toxic plant generalist.

The total concentration (but not composition as measured by polarity and diversity) of plant cardenolides was induced proportionally to the extent of plant feeding by *O. fasciatus*, which may have impacts on *O. fasciatus* sequestration from milkweed plant tissue. It has been shown that the performance of *O. fasciatus* is largely insensitive to the concentration of cardenolides in its diet [21,25,36]. Accordingly, we speculate that such induction may be adaptive for the bug, as induced plants will have higher concentrations of defensive cardenolides for sequestration than uninduced plants. Further work is needed to test this hypothesis, as well as downstream consequences of plant induction for more sensitive milkweed feeders.

It appears that achieving sequestered toxicity in the absence of milkweed seeds is not the sole driver of plant feeding in *O. fasciatus*. Survivorship was substantially higher when bugs were reared with access to plants than when they had seeds alone across both seed types, indicating that there may be benefits to plant feeding aside from sequestration. Furthermore, bugs that had access to both plant and seed cardenolides still engaged in feeding on milkweed plants despite having access to cardenolides from a nutritionally adequate source. All experimental replicates received a vial of water on the soil surface, suggesting that benefits of plant feeding were not derived from hydration alone. More research is needed to identify these additional benefits of plant feeding.

Some prior work has shown that toxic plant generalists preferentially engage in feeding on nutritionally poor, toxin-containing foods over nutritionally adequate, non-toxic foods. For the saltmarsh caterpillar *Estigmene acrea*, growth was lower on a mixed toxic/non-toxic diet than on a completely non-toxic diet, but caterpillars raised on the mixed diet were more resistant to parasitoids [5]; additionally, a toxic plant generalist leaf beetle (*Oreina elongata*) showed oviposition preference for a more toxic, less nutritive host plant species, indicating that sequestration is associated with dietary preference among such species [8]. Collectively, these results indicate that, among toxic plant generalists, sequestration is facilitated not only by physiological adaptations, but also by a complex suite of behaviours by which herbivores balance nutritional needs with the ecological benefits of sequestration. Nonetheless, while toxic plant generalists face a nutrition–defence trade-off at the species scale of diet choice, the opposite may be true within plants: the most nutritive plant tissues (seeds, young leaves, etc.) are often the best defended [37–39]. Still, diet mixing as a means of navigating a nutrition–defence trade-off appears to be quite general, across three taxonomic orders of herbivorous insects. There has long been interest in the inducibility of diet-mixing behaviour in toxic plant generalists by predator pressure. Our study provides the first evidence that diet mixing in a toxic plant generalist is additionally inducible by the nutritional quality and toxicity of available foods, and we show that this diet mixing corresponds to chemical sequestration in a system where it is known to be an effective predator deterrent [14].

Given the high mobility of both immature and adult *O. fasciatus*, it has been difficult to assess the extent of diet-mixing by *O. fasciatus* in the field. Because of this, the relative importance of plant tissue, milkweed seeds, and non-milkweed seeds to wild populations of *O. fasciatus* remains unknown. However, the bug's ability to develop to adulthood

on a taxonomically diverse array of seeds in the laboratory makes it relatively unique among insects that sequester specific plant toxins. Additionally, milkweed phenology and the semi-migratory life history of the large milkweed bug likely necessitate some diet mixing: *O. fasciatus* typically migrates from southern to northern habitats in the late spring and early summer, when milkweed plants are not yet producing seeds [40]. As such, these bugs likely depend on a combination of relatively scarce older milkweed seeds and non-milkweed seeds on the soil surface for nutrition when milkweed plants are not yet producing mature fruit [26]. This inference is supported by the fact that cardenolide concentrations in field-collected *O. fasciatus* adults range from 0 to 300 µg per bug (with most bugs containing some cardenolides), suggesting either extreme physiological variation in sequestration (which we find no evidence for in our study) or variation in dietary cardenolide intake [41,42]. Indeed, scarcity of cardenolide-containing seeds may drive the use of plant tissue as a source of defensive cardenolides.

### (a) Chemical ecology

Despite large differences in the cardenolide profiles of milkweed plant tissue and seeds, cardenolides sequestered by bugs converged on an intermediate polarity and diversity relative to their two food sources, supporting previous findings that sequestration of cardenolides by *O. fasciatus* is selective and involves chemical modification [36]. This result is consistent with work on other milkweed herbivores; when fed on the leaves of multiple species of milkweeds, monarch butterflies (*Danaus plexippus*) converged in terms of the polarities of sequestered cardenolides [35,43]. Differences in the composition of sequestered toxins between an insect and its food source likely occur for three reasons: (1) insects avoid or break down compounds that are more toxic to them [44], (2) physiological constraints (as opposed to toxicity) prevent storage of certain toxins [43], and (3) sequestration of certain toxins may be adaptive (e.g. specific toxins may be more effective deterrents of natural enemies, or may be modified as pheromones) [45,46]. This convergence may buffer the impacts of diet mixing in *O. fasciatus* on subsequent predation: the diversity and polarity of cardenolides experienced by a predator of *O. fasciatus* may not be strongly dependent on a bug's dietary regime. In particular, polarity of sequestered cardenolides appears to determine the palatability of a toxic plant generalist to its predators [28].

However, this convergence was incomplete, and there were substantial differences in the profile of cardenolides sequestered by bugs based on diet. Specifically, plant presence always increases the diversity (but not polarity) of sequestered cardenolides. We hypothesize that this is due to the conversion of dominant, highly toxic, non-polar cardenolides from milkweed plant tissue to more polar forms in bugs. For example, the non-polar cardenolide voruscharin (which is demonstrably toxic to the resistant Na<sup>+</sup>/K<sup>+</sup>-ATPase; retention time 18.6 min in this study) was not sequestered by *O. fasciatus* bugs that had access to milkweed plant tissue, despite its high concentration in *A. curassavica* leaves (electronic supplementary material, figure S2). Voruscharin is dominant in *A. curassavica* leaves but is not found in its seeds. In monarch butterflies, this compound is converted into more polar cardenolides [44], while other non-polar compounds from milkweed leaves are excreted in monarch frass [35].

## 5. Conclusion

We speculate that the evolutionary lability in diet and sequestration seen within the lygaeine seed bugs [19,21] may be linked to their status as toxic plant generalists. For toxic plant generalists (as opposed to more host-specific sequesterers), nutritional requirements are uncoupled from the acquisition of toxins for sequestration. This might buffer the impacts of shifting availability of preferred sources of toxins for sequestration (see also [47]). Indeed, reliance on toxic plants for anti-predator defence, without the reduced dietary niche seen in other sequesterers, may allow toxic plant generalists to engage with a wider range of potential host plants, escaping an evolutionary dead end.

Sequestration appears to be a physiologically costly strategy in this system: it requires the maintenance of a less efficient cardenolide-resistant Na<sup>+</sup>/K<sup>+</sup>-ATPase and a specialized compartment for storage and delivery of a defensive secretion [21,48]. Such an investment is only worthwhile if a sequesterer is sufficiently toxic to avoid predation. In the face of environmental variability, the ability to feed on multiple toxic and non-toxic foods may allow toxic plant generalists to achieve toxicity to avoid predation—offsetting the costs of sequestration while also optimizing growth and reproduction. In this way, toxic plant generalism might expand the total niche space in which sequestration is an adaptive strategy.

In the large milkweed bug, this strategy has likely facilitated the insect's complex life cycle, which involves long migrations and seasonal fluctuations in the availability of its preferred food source. Strong tolerance of toxins, finely tuned adaptations to specific cardenolides, and the ability to feed on many toxic and non-toxic hosts allow the large milkweed bug to achieve high levels of sequestration in a

wide range of environmental conditions. We provide evidence that *O. fasciatus* favors sequestration of cardenolides from inadequate food sources even at some cost to growth. As such, this bug might be considered a dietary generalist but a chemical specialist: although it interacts with a diversity of toxic and non-toxic foods, it still prioritizes defensive sequestration in its feeding choices.

**Ethics.** This work did not require ethical approval from a human subject or animal welfare committee.

**Data accessibility.** All original data and code used to generate figures and statistical analyses are available from the Dryad Digital Repository at <https://doi.org/10.5061/dryad.pzgmbscs5> [49].

Supplementary figures, appendix methods, and a table of sex-level ANOVA results are available in the electronic supplementary material [50].

**Declaration of AI use.** We have not used AI-assisted technologies in creating this article.

**Authors' contributions.** N.J.C.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, visualization, writing—original draft, writing—review and editing; A.A.A.: conceptualization, funding acquisition, methodology, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

**Conflict of interest declaration.** We declare we have no competing interests.

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