

Female beetles facilitate leaf feeding for males on toxic plants

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Abstract. 1. Milkweed plants produce latex, which works as a defence against some herbivorous insects. The cerambycid beetles *Tetraopes femoratus* (LeConte) puncture the leaf midrib vein to extravasate the latex, and thus render the leaf edible. Nonetheless, it has not yet been investigated whether there is any further implication beyond food acquisition regarding this behaviour.

2. The present study examined the hypothesis that leaf latex drainage is done mainly by females of *T. femoratus*, and that the males opportunistically prefer to feed on those ‘disarmed’ leaves.

3. The experiments revealed that puncturing the leaf midrib vein to extravasate latex is done mainly by females of *T. femoratus* and not only serves their purpose of food acquisition, but also mediates an opportunistic feeding behaviour among males.

4. Males of *T. femoratus* preferred to feed on milkweed leaves that had previously been fed upon by females. In addition, field observations showed that mating occurred more frequently on recently ‘disarmed’ leaves, suggesting increased copulation opportunities for females during leaf feeding.

5. In sum, the results reveal that the feeding defence strategy of *T. femoratus* females to toxic milkweed foliage has created an opportunity for males to feed on milkweed leaves with a reduced risk of latex exposure and entanglement. Thus, this research provides empirical evidence of plant–insect interactions contributing to the appearance of an opportunistic adaptive feeding behaviour, which has broader implications for eco-evolutionary systems.

Key words. *Asclepias speciosa*, feeding behaviour, milkweed, opportunistic behaviour, *Tetraopes femoratus*.

Introduction

The production of latex by many plant species has been seen as a defence mechanism against herbivorous insects (Wittstock & Gershenson, 2002; Agrawal & Konno, 2009). Nonetheless, some insects have evolved a behaviour of vein cutting or puncturing to drain the leaf latex and thus circumvent the plant’s defence (Dussourd & Eisner, 1987). This behaviour has been documented for the cerambycid beetle *Tetraopes tetraophthalmus*, which feeds on milkweed plants *Asclepias syriaca* L. (Asclepiadaceae) in eastern U.S. (Dussourd & Eisner, 1987; Agrawal & Konno, 2009). By contrast, *Tetraopes*

femoratus is more common in the west, ranging east as far as Michigan (Lawrence, 1982), and is known to feed mainly on the milkweed *Asclepias speciosa* Torr. (Farrell, 1998). Although *Tetraopes* can feed on milkweed flowers, it is more common to encounter them feeding on the foliage (Matter *et al.*, 1999).

Like *Tetraopes tetraophthalmus*, *T. femoratus* also repeatedly punctures the midrib vein on the abaxial part of the leaf before feeding distal to the punctures (at the tip of the leaf). This feeding strategy blocks latex flow to the intended feeding sites and can be viewed as an insect counter-adaptation to the plant’s defensive exudate. The latex is stored under pressure within the laticifers (cellular tubes) arranged along the leaf venation (Williams, 1962). The laticifers easily exude latex upon puncturing by the insect’s mandibles. This defensive feeding behaviour may reduce latex

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ingestion by at least 92%, and thus protect the insect from taking in noxious chemicals such as alkaloids (Dussourd, 1999). However, after drainage, the latex drops congeal upon exposure to the air and may cause physical entanglement of *Tetraopes* (especially the mandibles) (Dussourd & Eisner, 1987), which could be more problematic for smaller beetles.

Given the long evolutionary history of *Tetraopes* beetles with milkweed plants (Farrell, 1998; Agrawal, 2004; Rasmann & Agrawal, 2011), it is expected that most individuals within a population would be able to perform the vein-puncturing behaviour in order to feed on the foliage. Nevertheless, Dussourd and Eisner's (1987) experiment showed that while 100% of artificially punctured leaves were fed upon by *T. tetraophthalmus*, about 40% of control milkweed leaves remained intact when exposed to individual beetles. The outcome of their experiment suggests that some individuals may prefer or require counterparts to drain the leaf latex prior to their feeding. Likewise, prior to this study, I sometimes also noticed in the field *T. femoratus* arriving to feed on a leaf that was already being fed upon by a counterpart, which may suggest a preference among some to feed on leaves that have already been 'disarmed' from latex. Differences in body size between females and males of *Tetraopes* may help to explain the discrepancy in the ability to feed on intact leaves. Females of both species *T. tetraophthalmus* and *T. femoratus* tend to be larger than males (Lawrence, 1986), and they could therefore be expected to grasp the leaf midrib more easily and exert stronger mandible pressure on the vein, thus facilitating latex drainage. Conversely, males are smaller (5–40%) and may face more difficulties (i.e. be less able to puncture the midrib and thus become entangled in the latex) when feeding on intact leaves, which might be circumvented by opportunistically feeding on leaves that are already punctured and/or that have recently been fed upon by females. In addition, the manifestation of such opportunistic male behaviour might also increase the chances of females mating during leaf feeding, as compared with females on intact leaves that are not feeding.

It is known that the leaf tissue distal to the midrib punctures is not completely consumed by specialists. This tissue may be exploited secondarily by generalist feeders that ordinarily would ignore intact milkweed leaves. For example, the generalist Japanese beetle (*Popillia japonica* Newman) has been observed feeding on leaf tissue distal to vein cuts made by the specialist feeder *Labidomera clivicollis* (Kirby) (Chrysomelidae) (Dussourd & Eisner, 1987). By contrast, the specialist feeders *Tetraopes* can feed both actively on intact leaves (by draining out the latex) and opportunistically on 'disarmed leaves' (Dussourd & Eisner, 1987); however, it is still unknown whether all individuals in a population adopt both feeding approaches, or whether some might prefer one instead of the other.

In this work, I explore the hypotheses that (i) leaf latex drainage (by puncturing of the leaf midrib) is done mainly by females of *T. femoratus*; and (ii) males of the same species prefer to feed on leaves already 'disarmed' (previously damaged) by females.

Materials and methods

Laboratory experiments

All the experiments were conducted in the Entomology Laboratory at the Tree Fruit Research and Extension Center (TFREC) in Wenatchee, WA, USA (July and August, 2011). The environmental conditions in the laboratory were as follows: temperature, $24 \pm 1^\circ\text{C}$; RH, $45 \pm 5\%$; fluorescent lights set at a photoperiod of LD 16:8 h. The adult beetles were collected from patches of milkweed *A. speciosa* surrounding the TFREC research station and/or from the Wenatchee city park. The beetles were collected and sexed on the evening before the beginning of each experiment (see later). The beetles were deprived of food overnight and kept in same-sex groups of two or three individuals in small plastic cups covered by organza. To make sure they were sexed correctly, these groups were watched intermittently (approximately every 5 min) for about 30 min to ensure no mating occurred (usually, mating occurs in less than 10 min when couples are paired in a small enclosure). Excised stems of milkweed *A. speciosa* with leaves completely expanded were used in five laboratory experiments (described later). All the stems (~20 cm long) were cut from the upper third of the plant main stem and placed immediately in a vase of water around 2 h before the beginning of the experiments. In a preliminary laboratory observation, leaves on excised milkweed stems were found to retain a considerable amount of latex for about 1 week (the midrib would still bleed a lot of latex after puncturing it with an insect pin, and, as such, remained a hurdle to the beetles trying to feed on it. During the experiments, each milkweed stem was kept individually in a glass bottle (8 × 4 cm) of water and sealed with parafilm to prevent evaporation of the water and to stop beetles falling into it. In each experiment, the excised stems were individually enclosed in a clear plastic cylindrical cage (25 × 20 cm) with an organza fabric glued to the top. All the statistical analyses were carried out using SAS v. 9.2 (SAS Institute, Inc, 2008).

Feeding and mating behaviour of T. femoratus. The mating and feeding behaviour of 22 pairs of *T. femoratus* was observed for 30 min over the course of 3 days (day 1, $n = 8$; day 2, $n = 8$; day 3, $n = 6$). Each pair was enclosed inside a cage containing an excised milkweed stem with two opposite intact leaves (as described earlier). All the observations were carried out between 08.00 and 15.00 hours. At the beginning of each observation, males and females were manually released onto the base of the milkweed stem at the same time. The couples that did not engage in any feeding or mating activity within the first 5 min were discarded. During each observation, I recorded which gender first punctured the midrib vein and fed on the leaf (male or female), the mating place, male feeding place (leaf recently punctured/fed or intact leaf), when mating started (before, during or after feeding), and when the male fed on the leaf (before or after mating).

No-choice and choice feeding tests for males of T. femoratus. In the no-choice test, 19 adult males were individually exposed

for 12 h (08.00–20.00 hours) on two dates (date 1, $n = 8$; date 2, $n = 11$) to an excised milkweed stem with two opposite intact leaves immersed in a water bottle and enclosed by a cage (as described earlier). In the choice test, 28 males were individually exposed for 12 h (08.00–20.00 hours) on three dates (date 1, $n = 10$; date 2, $n = 9$; date 3, $n = 9$) to an excised milkweed stem with one intact leaf and one leaf on which the vein-puncturing and feeding of a female were simulated. The leaf on which the female feeding was simulated had the upper third of the midrib vein on the abaxial part of the leaf punctured on both sides by an insect pin, and thereafter, the tip of leaf was cut out, making a V-shape as seen in the field. Therefore, the leaves mimicking female feeding had a residue of latex on its abaxial part. The differences in the results from the no-choice test and those from the choice test were analysed by pooling the data from the respective experiments and running a χ^2 test (PROC FREQ – SAS).

Effect of male proximity on initiation of female feeding. The effect of the proximity of male *T. femoratus* beetles on the initiation of feeding on intact leaves by females was investigated. Two treatments were tested: 1, female feeding in the proximity of a male; and 2, female feeding in the absence of male. There were 10 and eight replicates for the treatments 1 and 2, respectively. The replicates were run at two different dates (date 1, treatment 1, $n = 4$; date 1, treatment 2, $n = 4$; date 2, treatment 1, $n = 6$; date 2, treatment 2, $n = 4$). Each female was exposed to an excised milkweed stem with two intact leaves enclosed in a cage (described earlier). In treatment 1, all stems had a male *T. femoratus* tightly enclosed by a large-mesh organandy sleeve attached to one of the leaf petioles, and they were thus precluded from feeding, but maintained visual and olfactory cues for the female. The feeding status of the leaves (fed upon or not fed upon) was checked every 2 h during a 12-h period (08.00–20.00 hours). The difference between treatments regarding the time females initiated feeding was analysed by running a Kruskal–Wallis test (PROC FREQ – SAS).

Mandible size of T. femoratus. The maximum width between the open mandibles of *T. femoratus* was measured under a binocular scope using a micro ruler. This measurement was carried out for males and females (34 of each) used in some of the laboratory experiments. Each beetle was held between the thumb and index finger under the scope. The pronotum was gently squeezed a few times in order to induce the opening of the mandibles. The difference in maximum width between mandibles of males and females was analysed by running a *t*-test (PROC TTEST – SAS).

Female preference between 'old' and 'young' leaves (choice test). To test whether females prefer to feed on young leaves (top of the plant), the feeding choice between old and new leaves of 27 females was tested on three different dates (date 1, $n = 8$; date 2, $n = 10$; date 3, $n = 9$). Two milkweed stems with one intact leaf each were tied together with parafilm in a glass bottle of water such that the leaves were at the same height.

One of the stems was cut from the upper third of the plant (representing a young leaf), and the other was cut from the bottom of the plant canopy (representing old leaf). The feeding status of the leaves (fed upon or not fed upon) was checked every 2 h during a period of 12 h (08.00–20.00 hours). The difference in female choice regarding leaf age was analysed by pooling the data and running a χ^2 test (PROC FREQ – SAS).

Field observations

In the field, three patches of milkweed *A. speciosa* located on the grounds of the TFREC, with 78, 103 and 132 plants, respectively, were followed for 18–22 days (July–August, 2011). Two of the milkweed patches were growing in an area of grass, and the other was growing in the middle of a juniper shrub patch. The milkweed flowers started senescing in mid-July and by late August most of the flowers had fallen off. The milkweed plants had a height ranging from 20 to 162 cm, with an average of 90.3 cm. Each milkweed patch was checked every other day during the field observations. Leaves that showed characteristic signs of *T. femoratus* feeding (V-shape feeding at the tip of the leaf and vestiges of latex exudate) (Fig. 1a) were tagged on each patch visit, and followed every other day until the last day of observations. During each observation, I recorded the number of leaves per plant fed on by *T. femoratus*, the frequency at which a single leaf was fed upon by the beetles, the place of feeding (tip, lateral or bottom of the leaf), and the location within the plant canopy (top, middle or bottom) of the leaf that was fed upon. In order to inform leaf location, the plant canopy was visually divided into three equal thirds (top, middle and bottom). In addition, in each milkweed patch visit, I also searched, for approximately 20 min, for pairs of *T. femoratus* mating on the plants, and recorded the place of the leaf/flower on the plant where mating took place (top, middle or bottom) and the feeding status of that leaf (recently punctured/fed, old feeding or intact leaf). None of the other characteristic milkweed feeders, such as the chrysomelid beetle *Labidomera clivicollis* and larvae of the monarch butterfly *Danaus plexippus*, were observed feeding on the milkweed patches. Likewise, no generalist milkweed feeders were observed feeding on the plants.

Results

In the laboratory feeding and mating behaviour study, I found that females punctured the leaf midrib vein more frequently than males and always initiated the feeding of the leaf blade (Table 1). The mating always took place on the damaged leaf, and, in most of cases, it occurred while the female was feeding (Table 1, Fig. 1b). The males always fed on the leaf part previously fed on/damaged by the females and most of the time did so after mating (Table 1). Likewise, in the choice test, males of *T. femoratus* preferred to feed on the leaf that mimicked female feeding ($\chi^2 = 17.3$, $df = 26$, $P < 0.0001$). Twenty-five males (89.28%) fed on leaves mimicking female feeding, whereas only three males (10.72%) fed on intact leaves. Of those males that fed on intact leaves, only one

Table 1. Feeding and mating behaviour of *Tetraopes femoratus* on milkweed.

	Percentage (N)
Punctured leaf midrib vein	
Male	4.77% (1)
Female	95.23% (20)
First to feed on the leaf	
Male	0.00% (0)
Female	100.00% (22)
Mating place	
Intact leaf	0.00% (0)
Damaged leaf*	100.00% (20)
Male feeding place	
Intact leaf	0.00% (0)
Damaged leaf*	100.00% (16)
Mating started	
Before feeding [†]	15.00% (3)
After feeding [†]	5.00% (1)
During feeding [†]	80.00% (16)
Male fed on the leaf	
Before mating	37.50% (6)
After mating	62.50% (10)
During mating	0.00% (0)

*Leaf punctured and recently fed upon by females.

[†]First feeding of the leaf by females.

punctured the leaf midrib prior to feeding, and two fed on the lateral part of the leaf instead of the tip. In the no-choice test, most of the males did not feed on the intact leaves ($\chi^2 = 6.40$, $df = 17$, $P = 0.011$): 13 (68.42%) did not feed compared with six (31.58%) that did. Of those males that fed, only one punctured the leaf midrib prior to feeding, and three fed either on the lateral or bottom part of the leaf instead of the tip. In addition, those that fed without severing the veins appeared to consume less of the leaf blade than those feeding on leaves with severed veins. These results suggest that males prefer to feed on milkweed leaves that have had the latex previously drained out by the females. Although these tests were done with excised plant parts, the pattern in the preference of males for leaves with severed veins and avoidance of intact leaves indicates that the leaves still retained some amount of latex during the experiments. The presence (proximity) or absence of male beetles on the plant did not influence the time that females initiated leaf feeding ($\chi^2 = 3.07$, $df = 16$, $P = 0.080$). All females ($n = 18$), regardless of the treatment, had fed on a leaf by the eighth hour of the experiment, thereby indicating that the close proximity of the male is not the proximate cause of this female feeding behaviour. The females (2.82 ± 0.04 mm) had significantly ($t = 12.09$, $df = 66$, $P < 0.0001$) greater width between the open mandibles than the males (2.01 ± 0.04 mm), indicating larger mandibles, with possibly greater capacity to exert stronger pressure on the leaf midrib. There was no significant difference in the feeding choice of females regarding leaf age ($\chi^2 = 0.93$, $df = 25$, $P = 0.3360$). Sixteen females (59.25%) fed on the 'older' leaves, whereas 11 females (40.75%) fed on the 'young' leaves, suggesting that feeding on the leaves of the top of the canopy, as observed in the field (Table 2), may be due to preference of leaf location instead of age.

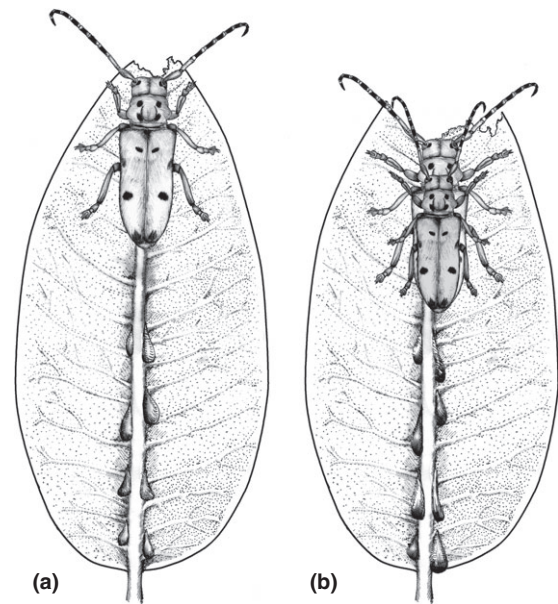


Fig. 1. (a) Female of *Tetraopes femoratus* punctures the abaxial leaf midrib and thereafter feeds distal to the punctures. (b) Male of *T. femoratus* copulates prior to feeding on a leaf already partially consumed ('disarmed') by the female. Drawing courtesy of Douglas Santos.

Fifty-four couples of *T. femoratus* were observed mating on the milkweed plants during the field observations. Most of the couples were seen to mate on milkweed leaves that were recently punctured/fed on or being fed (recent feeding) (Figs 1b and 2). In addition, most of those couples (92.6%) were observed to mate on leaves at the top of the plants compared with leaves at the middle (5.55%) and bottom (1.85%). Most of the plants in the field had only one or two leaves fed upon by the beetles (Table 2). Most of the leaves were fed upon only on one date, and the feeding mostly took place at the tip and at the top of the plant canopy (Table 2).

Discussion

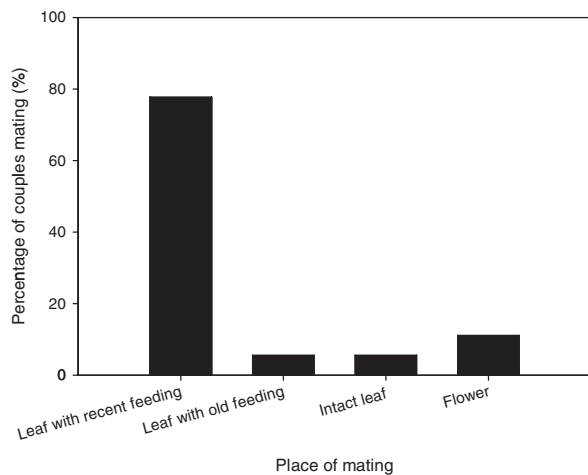
The data reveal that latex drainage and leaf first feeding, hereafter called 'feeding facilitation', are done chiefly by females of *T. femoratus*, and that these have allowed the manifestation of an opportunistic behaviour where males prefer to feed upon those leaves previously damaged by females. Most of the few males that fed on intact leaves did not sever the leaf midrib and, unusually, most of them fed on the lateral or bottom of the leaf, suggesting that males may be less able to drain out the leaf latex – this is perhaps why they prefer to feed on leaves already 'disarmed' by the females. This opportunistic feeding behaviour can be advantageous for the males. Feeding facilitation can reduce the risk of males ingesting noxious chemicals (i.e. allelochemicals) and being exposed to latex exudate. For instance, Dussourd and Eisner (1987) observed that latex congeals upon contact with air and can gum up the beetle's mandibles. Since the males have smaller mandibles,

Table 2. Feeding behaviour of *Tetraopes femoratus* on milkweed observed in the field.

Number of leaves fed upon per plant	
1	64.55% (122 plants)
2	25.90% (49 plants)
≥ 3	9.55% (18 plants)
Frequency a single leaf was fed upon*	
1	95.30% (264 leaves)
2	4.35% (12 leaves)
≥ 3	0.35% (1 leaf)
Place where feeding happened on the leaf	
Tip	97.50% (270 leaves)
Lateral	2.50% (7 leaves)
Bottom	0.00% (0 leaf)
Leaf location where feeding happened within plant canopy†	
Top	96.00% (266 leaves)
Middle	2.50% (7 leaves)
Bottom	1.50% (4 leaves)

*Feeding done on different dates.

†Plant canopy divided into three sections.

**Fig. 2.** Part of milkweed plant where mating of *Tetraopes femoratus* took place during field observations. $N = 54$ pairs of *T. femoratus* were observed mating. ‘Recent feeding’ refers to leaves that still had fresh latex running along the leaf midrib vein or were still being fed upon. ‘Old feeding’ refers to the place of previous feeding – latex vestiges on the leaf blade were dried out and presented a brown coloration.

they could be more prone to physical entanglement if exposed to latex. In addition, this feeding preference of males appears to increase the chances of copulation among females during feeding time, as I noticed most of the couples mating on leaves recently fed on or being fed on by the beetles at the time of the field search.

The female feeding facilitation behaviour may also elicit milkweed to release volatiles that help males to locate food, and possibly also a mate. For example, males of the beetle

cockchafer and Japanese pine sawyer are aided by herbivory-induced plant volatiles to encounter mates (Ruther *et al.*, 2000; Fan *et al.*, 2007). Females of *Tetraopes* are also known to copulate multiple times with different males over the season (McCauley, 1983), and as pheromones do not play a major role in mate attraction (Reagel *et al.*, 2002), females that ‘disarm’ milkweed leaves more often and/or do so in a more conspicuous place on the plant (i.e. the top of the plant) may have a greater chance of copulating with males searching for food. The fact that *Tetraopes femoratus* feeds only on one date on the same leaf (Table 2) suggests that males may prefer to feed on a leaf currently being fed upon or recently fed upon, which could consequently increase the likelihood of encountering a female at the time of leaf feeding. In addition, the fact that *T. femoratus* feeds more frequently on the leaves at the top of plants (Table 2) may also provide visual cues that facilitate food and mate encounter for the males flying above dense milkweed patches. Nevertheless, more studies are warranted to better understand whether the males were in fact attracted to feed on disarmed leaves and opportunistically mated, or whether the reverse was the case: they were attracted to mate and opportunistically fed.

The proximity of males did not affect the feeding initiation by females, suggesting that female leaf feeding is not induced by mating, as suggested for other insect species (Perry, 2011). It is known that some generalist feeders can opportunistically exploit milkweed leaf tissue that has been partially consumed by specialists such as *Tetraopes*; however, this study is the first to report on one sex taking advantage of the latex removal made by the other sex within the same species. Despite the evolutionary similarity among *Tetraopes* species considering their association with milkweed plants, the results observed here should not be extended to other species beyond *T. femoratus* without prior experimentation.

In summary, the results reveal a novel opportunistic adaptive feeding behaviour of males of *T. femoratus* that is mediated by another behaviour, the female defence to milkweed leaf latex. Thus, the study provides empirical evidence for plant–insect interactions contributing to the shaping of behaviour evolution in insects. Moreover, these findings suggest that midrib vein puncturing done by females might serve as both a counter-defence strategy to leaf latex and a means of facilitating mate access. Further investigation is necessary to confirm this.

Conclusions

This study reports on males of *T. femoratus* opportunistically feeding beyond vein cuts in milkweed leaves produced by females of the same species. Previous work has shown that generalist feeders opportunistically feed beyond vein cuts or trenches produced by other species, but this work is the first to document one sex taking advantage of the vein cuts of the other. The study also provides important natural history information on sexual relations between insects integrated with plant defence strategies, which have broader implications for eco-evolutionary systems. Furthermore, this study can

provide a functional model for those interested in understating how ecological interactions can influence the evolution of behaviour.

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References

- Agrawal, A.A. (2004) Resistance and susceptibility of milkweed: competition, root herbivory, and plant genetic variation. *Ecology*, **85**, 2118–2133.
- Agrawal, A.A. & Konno, K. (2009) Latex: a model for understanding mechanisms, ecology, and evolution of plant defense against herbivory. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 311–331.
- Dussourd, D.E. (1999) Behavioral sabotage of plant defense: do vein cuts and trenches reduce insect exposure to exudate? *Journal of Insect Behavior*, **12**, 501–515.
- Dussourd, D.E. & Eisner, T. (1987) Vein cutting behavior: insect counterploy to the latex defense of plants. *Science*, **237**, 898–901.
- Fan, J., Kang, L. & Sun, J. (2007) Role of host volatiles in mate location by the Japanese pine sawyer, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae). *Environmental Entomology*, **36**, 58–63.
- Farrell, B.D. (1998) The timing of insect/plant diversification: might *Tetraopes* (Coleoptera: Cerambycidae) and *Asclepias* (Asclepiadaceae) have co-evolved? *Biological Journal of the Linnean Society*, **63**, 553–577.
- Lawrence, W.S. (1982) Sexual dimorphism in between and within patch movements of a monophagous insect: *Tetraopes* (Coleoptera: Cerambycidae). *Oecologia*, **53**, 245–250.
- Lawrence, W.S. (1986) Male choice and competition in *Tetraopes tetraophthalmus*: effects of local sex ratio variation. *Behavioral Ecology and Sociobiology*, **18**, 289–296.
- Matter, S.F., Landry, J.B., Greco, A.M. & Lacourse, C.D. (1999) Importance of floral phenology and florivory for *Tetraopes tetraophthalmus* (Coleoptera: Cerambycidae): tests at the population and individual level. *Environmental Entomology*, **28**, 1044–1051.
- McCauley, D.E. (1983) Gene flow distances in natural populations of *Tetraopes tetraophthalmus*. *Evolution*, **37**, 1239–1246.
- Perry, J.C. (2011) Mating stimulates female feeding: testing the implications for the evolution of nuptial gifts. *Journal of Evolutionary Biology*, **24**, 1727–1736.
- Rasmann, S. & Agrawal, A.A. (2011) Evolution of specialization: a phylogenetic study of host range in the red milkweed beetle (*Tetraopes tetraophthalmus*). *American Naturalist*, **177**, 728–737.
- Reagel, P.F., Ginzel, M.D. & Hanks, L.M. (2002) Aggregation and mate location in the red milkweed beetle (Coleoptera: Cerambycidae). *Journal of Insect Behavior*, **15**, 811–830.
- Ruther, J., Reinecke, A., Thiemann, K., Tolasch, T., Francke, W. & Hilker, M. (2000) Mate finding in the forest cockchafer, *Melolontha hippocastani*, mediated by volatiles from plants and females. *Physiological Entomology*, **25**, 172–179.
- SAS Institute, Inc (2008) *SAS Version 9.2*. SAS Institute, Inc, Cary, North Carolina.
- Williams, L. (1962) Laticiferous plants of economic importance: I sources of balata, chicle, gutta-percha and allied guttas. *Economic Botany*, **16**, 17–24.
- Wittstock, U. & Gershenzon, J. (2002) Constitutive plant toxins and their role in defense against herbivores and pathogens. *Current Opinion in Plant Biology*, **5**, 300–307.

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