

SHORT COMMUNICATION

The three criteria for resistance by plant carrion-provisioning: insect entrapment and predator enrichment on *Mimulus bolanderi*

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Abstract. 1. Many sticky plants provision mutualistic scavenging arthropod predators with carrion, which in turn protect the plant from insect herbivores. While insect entrapment is a common trait across plants, which plants attract these predators and may derive protection is still largely unknown.

2. Three conditions were proposed that must be satisfied for observational data to suggest this defensive strategy: (i) the consistent presence of scavenging predators, (ii) positive correlation between predator numbers and carrion, and (iii) suitability of these predators for controlling known herbivores.

3. As a case study, we examined the fire-following annual, *Mimulus* [*Diplacus*] *bolanderi* (Phrymaceae), which is part of a well-studied radiation of California monkeyflowers. Many monkeyflowers entrap insects, though attraction to predators has not been quantified in this genus.

4. A guild of scavenging arthropod predators on *M. bolanderi* (condition #1) was found, which correlated positively with carrion abundance (#2) and could consume the primary herbivore (#3), suggesting a carrion-mediated defensive strategy. Lastly, as *M. bolanderi* is variable in time and space, these interactions are facultative, and these predators are quick to adopt ephemeral carrion resources on novel host plants.

Key words. Indirect defense, insect–plant interactions, *Mimulus*, mutualisms, scavenging, true bugs.

Introduction

Many plants provision mutualistic insects for defensive functions. The most well studied of these interactions are those of ant plants, which provision sugars as extra-floral nectar and occasionally proteinaceous food bodies to ants. In turn, ants protect the plant from competitors and herbivores ranging in size from insects to large mammals (Kessler & Heil, 2011). Because of the number and largely consistent results of these studies, the presence of structures to provision mutualists – extra-floral nectaries, food bodies, and domatia – are generally regarded as adaptations for this mutualism. In the last decade, several research groups have found that entrapped insects on sticky plants provision scavenging predators which, like the aforementioned ants, reduce herbivory on the plant (Romero *et al.*, 2008; Krimmel & Pearse, 2013; LoPresti *et al.*, 2015). This effect has

been experimentally demonstrated in three plant species, though over 100 genera in dozens of families entrap insects (LoPresti *et al.*, 2015). Experimental removals or additions of carrion provide the most compelling causal evidence of this interaction. Unlike other plant structures necessary for plant–bodyguard mutualisms, glandular trichomes are extremely pleiotropic (Wagner *et al.*, 2004). Therefore, carrion entrapment may be incidental. Experimentally testing the effect across the hundreds of insect-entrapping plant species is unrealistic and many of these probably do not indirectly benefit from insect entrapment. Therefore, we propose observational natural history criteria to identify systems, for use in future field manipulations or comparative approaches, where this resistance mutualism may operate.

Three conditions must be satisfied for this observational proposal of a carrion provisioning system. The first condition is that scavenging predators are consistently present. While obvious, the predators must be present on the plant and must exploit the carrion resource (they may feed on still-alive but entrapped and struggling or slowed arthropods). The second

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condition is that the numbers of these predators correlate positively with the abundance of carrion. If no relationship between carrion and predators exists, there is no reason to believe that carrion mediates the interaction between predators and any predation on herbivores. The third condition is that the herbivory in the system is possibly controlled by the present predators. This last condition could be violated in several ways. Most obviously, the herbivore might be completely undeterred by the predators, such as if deer are the herbivore and small spiders the bodyguards. A second way would be a phenological mismatch if the herbivores and predators do not coexist in time on the plant. The best evidence of potential control would come from direct observations of predation or knowledge of the same relationships in other systems.

The monkeyflowers (*Mimulus* sensu lato) are one of the best-studied plant radiations, with the varied California climates hosting the vast majority of species (Beardsley *et al.*, 2004; Baldwin & Goldman, 2012). While certain interactions (e.g. pollination, Vickery, 1992; Schemske & Bradshaw, 1999; Gardner & Macnair, 2000; Streisfeld & Kohn, 2007; Grossenbacher & Stanton, 2014) have been well studied, little attention has been paid to their defensive strategies (but see Lincoln *et al.*, 1982; Ivey *et al.*, 2009; Holeski *et al.*, 2010, 2013). Many species host a variety of insect herbivores that feed primarily on the reproductive structures, often sterilising the plants (E. LoPresti and K. Toll, pers. obs.) and suggesting an important need for research into defensive strategies in these plants.

As a case study for the utility of this approach, and as an interesting and informative natural history investigation in its own right, we examined a natural population of the fire-following *Mimulus bolanderi* at the UC-Davis McLaughlin Reserve, and asked the three questions: Does *M. bolanderi* and its associated arthropod community satisfy the outlined criteria for a possible carrion-mediated defensive mutualism? How does this occur in an ephemeral plant? Could this be a common strategy among *Mimulus* species?

Materials and methods

On 25 June 2016, we censused every flowering *M. bolanderi* in a drying north-facing seep. Individual plants in this population are small and widely spaced. This spacing allowed us to count carrion and insects on plants on each plant without touching or otherwise disturbing the plant (which causes some predators to drop off or fly). As individual plants have multiple stems, we counted all stems of $> \sim 15$ cm as a proxy for plant size. We compared carrion and predator abundance with linear regression using R ver. 3.2.3. *Mimulus bolanderi* was keyed using Baldwin & Goldman (2012). The predator species were determined in previous investigations by B. A. Krimmel (UC-Davis, Entomology) and A. G. Wheeler (USDA), and *Heliothis phloxiphaga* was determined by J. A. De Benedictis (UC-Davis, Entomology) (Krimmel & Pearse, 2013; LoPresti *et al.*, 2015).

Results and Discussion

Mimulus bolanderi hosted a typical California sticky-plant scavenging predator community dominated by predatory true bugs

(Fig. 1). The most abundant predator found was *Hoplinus eschinatus* (Heteroptera: Berytidae), followed by *Dicyphus hesperus* (Heteroptera: Miridae), *Jalysus wickhami* (Heteroptera: Berytidae), *Pselliopus spinicollis* (Heteroptera: Reduviidae), *Mecaphesa schlingeri* (Araneae: Thomisidae), and *Geocoris* sp. (Heteroptera: Geocoridae), each of which are known protective sticky plant predators in other systems (e.g. Krimmel & Pearse, 2013; LoPresti *et al.*, 2015). These predators have a variety of adaptations allowing them to move easily over sticky glandular surfaces (Voigt & Gorb, 2008; Wheeler & Krimmel, 2015). The presence and abundance of these predators satisfies the first proposed condition of a protective mutualism.

The total abundance of predators was tightly correlated with carrion number (Fig. 1). The best fitting Poisson model included stem number interactively with carrion. This interaction results in a higher starting predator number and shallower slope for plants with more stems and *visa versa*; however, a positive correlation between carrion and predators was found for any size plant. The shape of the best-fitting curve is not biologically-realistic at the upper values of carrion, the number of predators will not continue to rise exponentially because of intra- or inter-specific negative interactions (e.g. competition or intraguild predation). Intraguild predation occurs commonly on sticky plants of several families (E.F. LoPresti and I.S. Pearse, unpublished) and during our censuses we observed *Geocoris* eating *Hoplinus* (Fig. 1e). Even with this caveat, the significant positive correlation between carrion and predators satisfies the second proposed condition of a protective mutualism.

Nearly all plants in the population lost some fruit to the polyphagous Heliiothiine noctuid caterpillar *Heliothis phloxiphaga* (Fig. 1c). This species is abundant on glandular plants and in later instars preferentially feeds on reproductive structures, resulting in a fitness cost to the plant. It feeds widely on glandular plants, and in manipulative experiments occurring nearby, the same predator species found on *M. bolanderi* (*H. eschinatus*, *P. spinicollis*, *J. wickhami*, and *M. schlingeri*) controlled damage from *H. phloxiphaga* on a sticky columbine, and damage from a closely related caterpillar (*Heliothodes diminutiva*) on a tarweed (Krimmel & Pearse, 2013; LoPresti *et al.*, 2015). A comparison of damage in *M. bolanderi* was not possible as the phenology of plants was extremely varied – a few were putting out their first flowers whereas the majority were nearly senescent, and thus the single snapshot of carrion and predators might not reflect the situation when the plant received herbivory or be consistent between plants. However, there were *H. phloxiphaga* caterpillars in instars 1–5 during the survey. The eggs and early instars of *H. phloxiphaga* are vulnerable to predation by at least *H. eschinatus*, *D. hesperus*, *P. spinicollis*, *M. schlingeri*, and *J. wickhami* (Krimmel & Pearse, 2013; LoPresti *et al.*, 2015; E. F. LoPresti, pers. obs.). *Pselliopus spinicollis* and *M. schlingeri* will consume them in mid- to late-instars as well (Krimmel & Pearse, 2013; E. F. LoPresti, pers. obs.), thus the final condition of a provisioning defense strategy was satisfied in *M. bolanderi*.

We found all three proposed conditions satisfied, thus we have observational evidence suggesting a carrion-mediated defensive system occurring in *Mimulus bolanderi*. This interaction is necessarily facultative. *Mimulus bolanderi* is a fire or

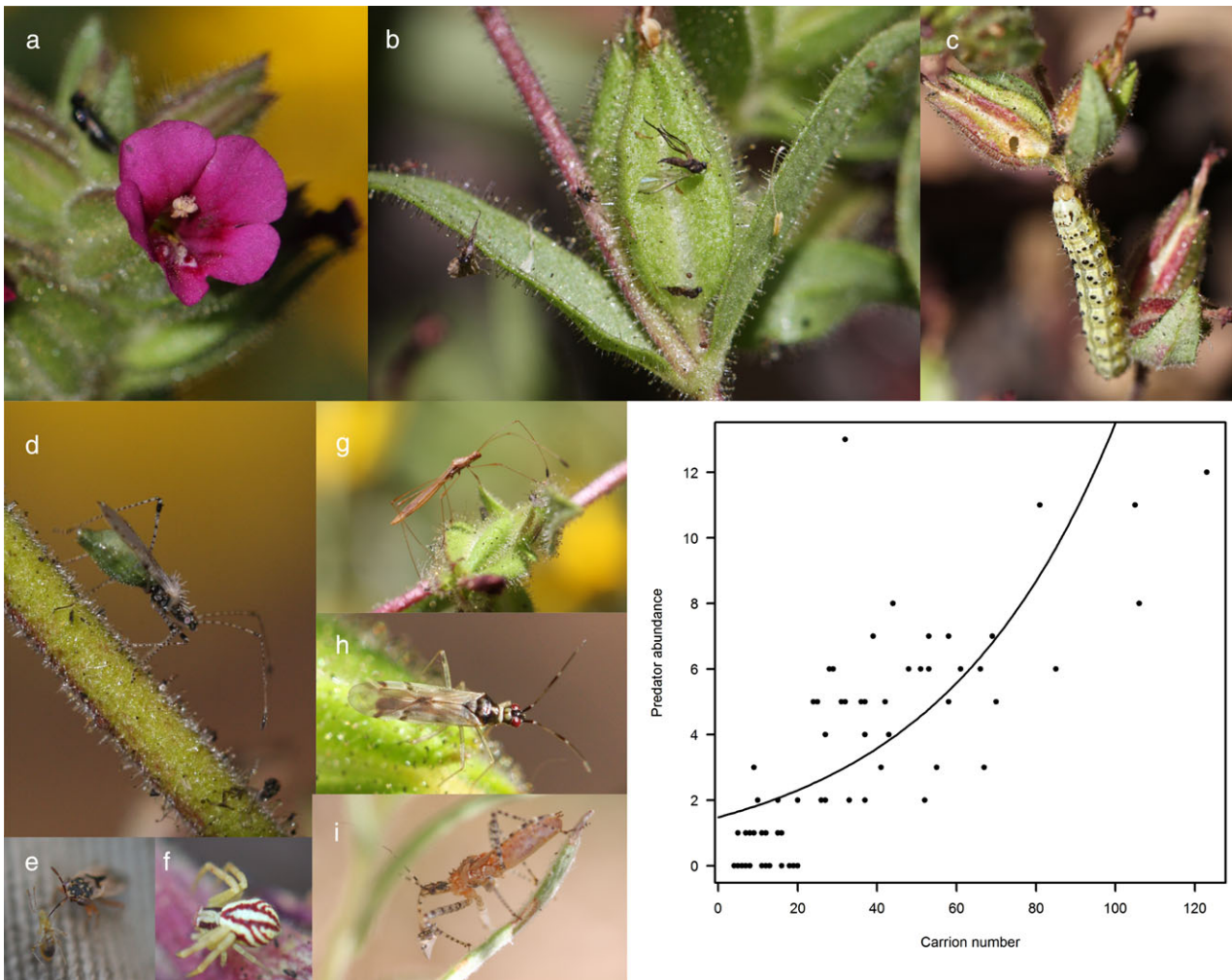


Fig. 1. Photos, from top left. (a) *Mimulus bolanderi* flower, note entrapped wasp in background; (b) bud and bracts, with entrapped flies; (c) *Heliothis phloxiphaga* caterpillar, with characteristic damaged fruit; (d) *Hoplinus eschinatus*, the most common scavenging predator ($n = 144$ individuals observed); (e) the noted example of a *Geocoris* sp. ($n = 2$) feeding on an *H. eschinatus* nymph (pictured on the author's pants after they dropped off the plant); (f) female *Mecaphesa schlingeri* ($n = 3$); (g) adult *Jalysus wickhami* ($n = 35$); (h) adult *Dicyphus hesperus* ($n = 48$); (i) *Pselliopus spinicollis*, pictured on *Hemizonia congesta*, another sticky plant in the area. At the bottom right, the relationship between carrion number and total predator abundance (all of the pictured species summed). Line of best fit is the predictions of a generalised linear model with Poisson error distribution for a plant with a mean number of stems (4.01). This model explained $\sim 52\%$ of variance (pseudo- r^2) and had significant predictors of amount of carrion (coefficient $0.029 + 0.004$; $z = 7.142$; $P < 0.001$), the number of stems ($0.133 + 0.048$; $z = 2.785$; $P = 0.005$) and the interaction between them ($-0.002 + 0.001$; $z = -3.634$; $P < 0.0001$). This interaction results in a shallower slope and higher intercept for larger plants and a steeper slope and lower intercept for smaller plants. [Colour figure can be viewed at wileyonlinelibrary.com].

disturbance following annual (Baldwin & Goldman, 2012) and came up in areas which previously had not had it after the Rocky and Jerusalem fires, which burned the study site in late summer 2015. In a normal year, these predators associate with many other glandular-sticky plants in the area, including two other *Mimulus* species (*M. layneae* and *M. nudatus*). The abundance of carrion on the phylloplane of this species presents a more concentrated carrion resource than most other plants in the area (with the notable exception of *Aquilegia eximia*).

Mimulus species have a wide variety of exudates. Some, like *M. bolanderi* are classically glandular-sticky, others are slimy (e.g. *mephiticus*), still others are resinous (e.g. *aurantiacus*),

and others are non-glandular hairy or glabrous (e.g. *ringens*). The former two categories entrap insects to varying degrees (Plate S1), whereas the latter do not. However, insect entrapment occurs in the majority of subgroups (Table 1). This trait has been noticed and put to use by *M.* researchers: *floribundus* has been used in greenhouses as a flytrap (J.H. Willis, Duke University, pers. comm.). The insect-entrapping species in *Mimulus* sections *Erythranthe* and *Simiolus* are model systems for ecological and evolutionary genomics (Wu *et al.*, 2008), yet relatively little is known about the ecological importance of insect entrapment or relationships with predatory insects in these groups. The sticky *cardinalis*

Table 1. List of *Mimulus* species that entrap insects, organised by section per Barker *et al.* (2012).

Section	Species
Cleisanthus	<i>torreyi</i> *
Erythranthe	<i>cardinalis</i> *
	<i>lewisii</i> †
	<i>parishii</i> ‡
Erimimimulus	<i>parryi</i> *
Eunanus	<i>bigloveii</i> *
	<i>bolanderi</i> *
	<i>brevipes</i> *
	<i>constrictus</i> §
	<i>johnstonii</i> ‡
	<i>layneae</i> *
	<i>mephiticus</i> *
	<i>rattanii</i> ‡
	<i>viscidus</i> *.‡
	<i>whitneyi</i> *
Mimulosma	<i>floribundus</i> ¶
	<i>moschatus</i> *
	<i>norrisii</i> §
	<i>evanescens</i> *
Monimanthus	<i>breweri</i> *
Oenoe	<i>tricolor</i> *
Simiolus	<i>guttatus</i> *
	<i>nudatus</i> *

*pers. obs.

†Spomer (1999).

‡S. Schoenig (pers. comm.).

§D. Grossenbacher (pers. comm.).

¶J. Willis (pers. comm.).

and *lewisii* (sect. Erythranthe) trap insects, and the leaves and calyces of *lewisii* exhibit proteinase activity, which may function in the digestion of entrapped arthropods (Spomer, 1999). It is quite likely, given the extremely facultative nature of the interactions we observed on *bolanderi* that these interactions occur in many of these other species, as all of the observed predator species (e.g. *Mecaphesa*, *Jalysus*, *Hoplinus*) occur widely in California on glandular plants, including other *Mimulus* (E. F. LoPresti, pers. obs.).

As sticky plant provisioning mutualisms are a new topic of research, it is important to identify systems where this interaction may be occurring, both for comparative evolutionary studies and ecological ones. Unlike other plant traits mediating indirect resistance (e.g. EFNs), glandular trichomes have a great diversity of functions, and insect-entrapment should not be an automatic assumption of an indirect defensive strategy. In the introduction, we proposed three conditions for observational natural history data to suggest this specific interaction. While experimental evidence is always necessary to prove a defensive function, it is not always realistic or possible (e.g. *M. bolanderi* will likely not return to our study site next year) and these criteria can be used to identify systems for manipulations. In this fire-following monkeyflower, we found support for all three conditions, suggesting an ephemeral, facultative defensive strategy though experimental work should be directed to this end. Finally, our observations of other *Mimulus* species suggest that these interactions may be common across this well-studied

genus. The data on *M. bolanderi* should be taken as a case study for the way to gather these data on and apply these conditions in other sticky-plant insect systems.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12377

Plate S1. Insect entrapment in other *Mimulus* species. (a) *moschatus*, El Dorado County, CA, entraps small numbers of insects in slimy exudates on leaves, stems, and calyxes. (b) *viscidus*, El Dorado County, CA, remains viscid and insect-entrapping even after senescence! (c) *torreyi*, El Dorado County, CA, entraps much carrion even when rather small (d) *layneae*, Lake County, CA. (e) *Jalysus wickhami*, a common sticky plant predator, on *torreyi*, El Dorado County, CA. (f) viscid *guttatus*, Nevada County, CA. Some populations examined in the Sierra Nevada mountains and coast range are viscid, whereas coastal perennials we have examined are largely glabrous. (g) *breweri*, El Dorado County, CA. (h) *nudatus* in lab acting as a flytrap for large numbers of fungus gnats – in field the plants entrap fewer insects, but host the sticky plant predators *Jalysus* and *Hoplinus*.

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