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Preference and performance of generalist and specialist herbivores on chemically defended host plants

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Abstract. 1. Both the physiological efficiency (PE) hypothesis and the preference–performance (PP) hypothesis address the complex interactions between herbivores and host plants, albeit from different perspectives. The PE hypothesis contends that specialists are better physiologically adapted to their host plants than generalists. The PP hypothesis predicts that larvae perform best on the host plant preferred by ovipositing females.

2. This study tests components of both hypotheses using the specialist checkerspot, *Euphydryas anicia*, the generalist salt marsh caterpillar, *Estigmene acrea*, and host plants in the genus *Penstemon*, which are defended by iridoid glycosides.

3. In laboratory experiments, the generalist preferred and performed significantly better on the less well defended host plant species. This is consistent with results from a common garden experiment where the less well defended *Penstemon* species received more damage from the local community of generalists. Larvae of the specialist checkerspot preferred the more chemically defended species in the laboratory, but performed equally well on both hosts. However, field experiments demonstrated that adult checkerspot females preferred to oviposit on the less well defended host plant.

4. Components of the physiological efficiency hypothesis were supported in this system, as the specialist outperformed the generalist on the more iridoid glycoside-rich host plant species. There was no support for the PP hypothesis, however, as there was no clear relationship between female preference in the field and offspring performance in the laboratory.

Key words. Herbivory, iridoid glycosides, Lepidoptera, physiological efficiency, plant–insect interactions, preference–performance.

Introduction

Individual plant species may be exposed to multiple herbivores that vary widely in their degree of dietary specialisation. The performance of specialist herbivores (monophagous or oligophagous species with adaptations to specific host plant defences) on particular plant species may be greater than, less than or the same as that of generalist herbivores (grazing species that feed on multiple plant species over the course of their lifetime). Several hypotheses have been proposed to explain these performance differences while providing insight into the evolution of host specificity (Krieger *et al.*, 1971; Whittaker & Feeny, 1971; reviewed in Cornell & Hawkins, 2003 and Ali & Agrawal, 2012). One of the most influential is the physiological efficiency (PE) hypothesis (also known as the feeding specialisation hypothesis or the trade-off hypothesis; Rausher, 1984, 1988; Noriyuki & Osawa, 2012; Friberg *et al.*, 2015), which posits that dietary specialists show superior performance (e.g. faster growth rates) compared with generalists on a shared host plant due to physiological adaptations for utilising the host plant as food (Dethier, 1954; Scriber, 1983, 2005; Singer, 2001). Additionally, the PE hypothesis predicts that variation in the chemical defences of host plant taxa used by generalists and specialists will result in larger differences in generalist herbivore performance than specialist performance (Cornell & Hawkins, 2003). However, this hypothesis has a history of mixed support from empirical studies, especially those studying ‘composite generalists’, which are relatively specialised at the population or individual level (Fox & Morrow, 1981), as

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opposed to a 'true' food-mixing, grazing generalist (Singer, 2001). A second hypothesis, the preference–performance (PP) hypothesis, also addresses host plant suitability, but in the context of maternal choice (see, e.g., review by Thompson, 1988; Gripenberg *et al.* 2010). Specifically, the PP hypothesis predicts that the oviposition preference of adult females corresponds to the performance of their offspring (Jaenike, 1978; Thompson, 1988). Thus, phytophagous females are expected to maximise their fitness by ovipositing on plants that will provide a better diet for their offspring (Dethier, 1959a,b; Singer, 1972; Jaenike, 1978). Yet empirical tests of the PP hypothesis have produced contradictory results, prompting the proposal of several theories addressing why female choice may not match offspring performance (e.g. Thompson, 1988; Courtney & Kibota, 1990; Thompson & Pellmyr, 1991; Mayhew, 1997; Craig & Itami, 2008; Gripenberg *et al.*, 2010). Together with physiological adaptations, female host plant choice is a necessary consideration when evaluating herbivore host plant suitability.

These two hypotheses are closely linked, as they both consider the importance of maternal choice on offspring performance for specialists and generalists. The physiological suitability of a plant species is probably a major component of a mother's host plant choice, particularly for specialists whose offspring may demonstrate a preference for the mother's preferred host species (e.g. Singer *et al.*, 1988). Although females are presented with a suite of external stimuli (e.g. multiple visual and olfactory cues) to assist with host plant choice, plant secondary metabolites, as detected by a female upon landing, often determine whether a female will oviposit (e.g. Bernays & Chapman, 1994; Macel & Vrieling, 2003; Nieminen *et al.*, 2003). Similarly, larvae also make host plant choices based on secondary metabolite content (Da Costa & Jones, 1971; Raybould & Moyes, 2001; Bowers, 2003), which may (e.g. Singer *et al.*, 1988; Gonzáles & Gianoli, 2003) or may not (e.g. Clark *et al.*, 2011) reflect their mother's preferences. Although the presence of particular secondary metabolites in host plants may harm or deter generalist herbivores, these same compounds could provide oviposition cues to specialist females with larvae capable of coping with these compounds (e.g. Bernays *et al.* 2003). Therefore, one might predict that the PP relationships are stronger for specialist herbivores, compared with generalists feeding on the same host, as specialists might be more 'finely tuned' to certain characteristics (e.g. secondary metabolites) that indicate host plant suitability (Gripenberg *et al.*, 2010).

Combining the generalist/specialist performance component of the PE hypothesis and the importance of maternal host plant choice from the PP hypothesis, we can hypothesise that specialist herbivores will have a strong PP relationship and will also outperform generalists on host plant(s) preferred by the specialists. To test our hypothesis, we utilised a system involving a specialist herbivore, *Euphydryas anicia* Doubleday (Lepidoptera: Nymphalidae; the anicia checkerspot), two of its host plant species, and generalist herbivores, including *E. acrea* Drury (Lepidoptera: Erebidae). *Euphydryas anicia* specialises on plants containing iridoid glycosides (IGs) in several plant families (e.g. Plantaginaceae, Scrophulariaceae, Orobanchaceae, and Caprifoliaceae; Cullenward *et al.*, 1979; White, 1979) and can sequester these compounds (Stermitz *et al.*, 1986; Gardner & Stermitz, 1988; L'Empereur & Stermitz, 1990a). Here, we study a population of *E. anicia* that primarily uses two species, *Penstemon glaber* var. *alpinus* (Torr.) A. Gray (Plantaginaceae) and *Penstemon virgatus* A. Gray. *Penstemon* species contain IGs, a group of monoterpene-derived compounds found in over 50 plant families (El-Naggar & Beal, 1980; Boros & Stermitz, 1990; Bowers, 1991). These bitter compounds are important mediators of multitrophic interactions (e.g., Harvey *et al.* 2005; Lampert & Bowers, 2010). For example, IGs affect both the predators and parasitoids of sequestering caterpillar species (Smilanich *et al.*, 2009).

Here we investigate how differences in plant chemical defences mediate the host plant choice and performance of *E. anicia* and the generalist *E. acrea*. For this study, the palatability of host plants is considered from the perspective of a generalist insect herbivore (see Ali & Agrawal, 2012; Mooney *et al.*, 2012): 'better defended' plants contain higher concentrations and higher diversity of secondary metabolites, whereas 'less well defended' plants contain lower concentrations and less diversity of secondary metabolites. A more diverse suite of secondary metabolites is considered 'better defended' due to the potential for synergistic interactions, increasing the toxicity or unpalatability of the plant (McKey, 1979; Berenbaum, 1985; Nelson & Kursar, 1999; Richards *et al.*, 2012). First, we determined the relative levels of chemical defence, defined by IG content and composition, of *P. glaber* and *P. virgatus* when grown in a common environment. We then used a common garden to examine which of these two *Penstemon* species naïve generalists preferred to consume. We examined components of the PP hypothesis using the specialist checkerspot. We tested the prediction that female preference corresponds to larval performance by observing *E. anicia* oviposition choice in the field and measuring larval preference and performance when reared on either *P. glaber* or *P. virgatus*. To investigate components of the PE hypothesis, we compared these performance measures with those of the generalist *E. acrea*, testing the prediction that differences in host plant defences will result in greater variation in generalist preference and performance as compared with the specialist.

Materials and methods

Study system and field sites

Plants. Penstemon glaber var. *alpinus* and *P. virgatus* are herbaceous, long-lived perennials native to Colorado and the southwestern region of the U.S. (Shonle *et al.*, 2004). Both species grow in mountain meadows and road cuts and appear to prefer xeric, rocky, or sandy soils (Quintero & Bowers, 2013). *Penstemon virgatus* (upright blue beardtongue) features narrow leaves, tall stems and inflorescences with several small to medium purple flowers that bloom from June to August (Crosswhite, 1967; Quintero & Bowers, 2013). Previous research found two major IGs in *P. virgatus:* catalpol and scutellarioside-II (L'Empereur & Stermitz, 1990b; Fig. 1). *Penstemon glaber* var. *alpinus* (alpine sawsepal penstemon) is one of four known varieties of *P. glaber* and has broad and occasionally puberulent leaves, thick stems and inflorescences with

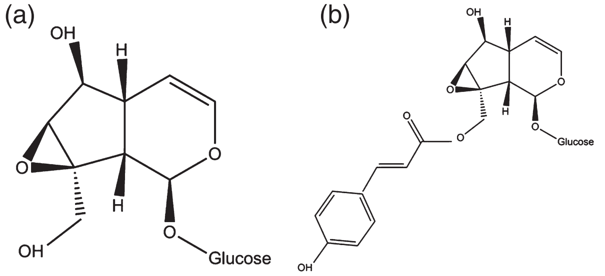

Fig. 1. Chemical structures of: (a) catalpol and (b) scutellarioside.

blue or blue-purple flowers. Although the defensive chemistry of *P. virgatus* has been previously examined (L'Empereur & Stermitz, 1990b; Quintero & Bowers, 2013), the chemical defences of *P. glaber* are less well known (Stermitz *et al.*, 1994). There is only one other published study on the chemistry of *P. glaber* (Stermitz *et al.*, 1994), which found the IGs catalpol, lamiide, globularin, and durantoside-I. However, those individuals were collected from the northernmost part of the range in central Wyoming and the variety of *P. glaber* was not specified. Preliminary analyses with Colorado populations in recent years suggest that *P. glaber* var. *alpinus* from our field site contains large amounts of catalpol but not other IGs (M.D. Bowers, unpublished). Both species naturally co-occur at the field site used in this study. All plants used in experiments were obtained from a local nursery and were maintained in 1 gallon pots outside of a greenhouse until used in experiments.

Insects. Euphydryas anicia occurs throughout the western half of the United States, including the mountains of Colorado (Cullenward *et al.*, 1979; White, 1979; Ferris & Brown, 1981). Females lay large egg masses (50–200 eggs) on the underside of leaves. Larvae are gregarious in early instars and form a web on their host plant. They enter diapause in the fourth instar, and then overwinter in this stage, emerging in spring to complete development. At our field site, Crescent Meadows (Eldorado Canyon State Park, Boulder County, Colorado; 39°55′51.60″N, 105°20′16.80″W, elevation 2258 m), adults are typically found in late June to early or mid-July. *Euphydryas anicia* is known to sequester the IGs catalpol, macfadienoside, and aucubin (Gardner & Stermitz, 1988; L'Empereur, 1989; L'Empereur & Stermitz, 1990a), but there is currently no evidence that they sequester scutellarioside. Analyses to be presented elsewhere showed that larvae only sequester catalpol when reared on either *P. glaber* or *P. virgatus* (C.A. Kelly, unpublished). Depending on the location and host plant, *E. anicia* can sequester 7.1–18.0% dry weight IGs as larvae and 0.5–10.4% dry weight IGs as adults (Gardner & Stermitz, 1988; L'Empereur, 1989; L'Empereur & Stermitz, 1990a). Sequestered IGs are retained through metamorphosis and both larvae and adults are aposematically coloured (Stermitz *et al.*, 1986; Gardner & Stermitz, 1988). Larvae used in these experiments were reared from eggs collected at Crescent Meadows in Eldorado Canyon State Park and were maintained as separate family groups in a growth chamber at the University of Colorado.

The salt marsh moth, *E. acrea*, is a conspicuous grazing generalist found throughout the United States, including Colorado (Bernays *et al.*, 2004). Populations have been recorded feeding on at least 88 plant species in 33 families, and are pests on many crops (Singer *et al.*, 2004). They can tolerate feeding on IG-rich plants (Bowers, 2009; Lampert & Bowers, 2010). Females lay 400–1000 eggs in one or more clusters. Larvae complete five to seven instars and typically overwinter as mature larvae. They can complete between one and three generations year^{-1}, depending on location. Larvae are poor sequesterers of IGs (Lampert & Bowers, 2010). Larvae used in these experiments were from a laboratory colony maintained at the University of Colorado. The colony was started with eggs and larvae collected in Austin, Texas. The colony was maintained on dandelion (*Taraxacum* spp.) leaves collected in Boulder, Colorado.

Field site. The field experiment was conducted at the Crescent Meadows site. This location features a large population of the IG specialist, *Euphydryas anicia*, and several of its potential host plants. Multiple *Penstemon* species, including *P. virgatus* and *P. glaber*, co-occur at this site and *E. anicia* uses both species as host plants at this site (C.A. Kelly, pers. obs.).

Common garden site. The common garden experiment took place in a 14 × 12 m plot with sandy soil located in Longmont, Colorado (40°9′24.29″N 105°12′11.87″W, elevation 1566 m). This site was chosen because it is outside of the range of herbivores naturally found on *Penstemon*, which allowed us to examine the interaction of naïve herbivores with the two *Penstemon* species grown under identical conditions.

Common garden experiment – chemistry of P. glaber *and* P. virgatus *and preference of generalists*

To examine the IG content of both plant species and the host preference of naïve, generalist herbivores, we planted 30 *P. glaber* and 30 *P. virgatus* plants in a common garden in Longmont, Colorado, in May 2010. The plot was rototilled prior to planting. All 60 plants were equally spaced, 1.5 m apart, in a randomly assigned order and then mulched. The garden was weeded and watered regularly. Plants were allowed to grow uninhibited and free of pesticide until late August 2010, approximately 15 weeks. Herbivores observed on the plants were noted throughout the season and collected when possible. There are no known IG specialists that occur at the garden site and none were observed in the garden. Therefore, generalist herbivores were presumed to be the source of all damage. At the end of the growing period, the proportion of leaf material missing due to herbivore consumption was calculated according to methods in Stamp and Bowers (1996). Leaves were then harvested and dried for chemical analysis.

Field experiment – oviposition choice by the specialist, E. anicia

Twenty pairs of potted *P. glaber* and *P. virgatus* plants were placed in sites at Crescent Meadows where *E. anicia* had been observed flying. Each *P. glaber* was paired with a similarly sized *P. virgatus*. Plants were checked for egg masses every other day. If a plant contained one or more egg masses, the leaves containing eggs were removed and the same number of leaves were also removed from its partner plant, so as not to bias female choice. Each egg mass was considered a single choice by a female. Plants remained at the field site for about 3.5 weeks, until adult females could no longer be found, after which the total number of choices was tallied for each *Penstemon* species. Plants were watered by hand every other day. Leaves containing eggs were kept in a growth chamber (Percival model LLVL, 25: 20 °C day:night, 14 h day length) until the eggs hatched. The oviposition preference of *E. acrea* was not tested since females are known to lay eggs on non-plant surfaces (Castrejon *et al.*, 2012).

Laboratory experiments – host plant choice and suitability for a generalist and a specialist

Food plant preference was determined by presenting groups of 10 newly hatched *E. acrea* ($n = 16$) and 10 newly hatched *E. anicia* ($n = 26$) with two leaf discs 5 mm in diameter, one *P. virgatus* and one *P. glaber*, for 24 h. Groups of larvae were used because these species are gregarious in early instars (White, 1979; Singer *et al.*, 2004). At the beginning of the experiment, caterpillars had not previously been exposed to any food. Each trial was conducted inside a rectangular plastic container (5 × 3 cm). For half of the trials, *P. virgatus* discs were placed on the left and *P. glaber* discs were placed on the right, switching the locations for the other half of the trials. Groups of caterpillars were placed on one end of the container approximately 2 cm away from the leaf discs placed at the opposite end. After the 24 h feeding period, the leaf discs were photocopied, magnified 400×, and the amount of each leaf sample missing was quantified by hand using a sampling grid.

To compare the growth of the generalist and specialist caterpillars on the two *Penstemon* species, groups of 10 newly hatched first-instar larvae were fed either *P. glaber* or *P. virgatus ad libitum.* There were 11 groups of *E. anicia* larvae and 20 groups of *E. acrea* larvae reared on each of the two *Penstemon* species. After 6 days, groups of larvae were weighed to determine the relative growth rate ($\text{mg mg}^{-1}\ \text{day}^{-1}$) and the number of living caterpillars in each group was counted to determine survivorship. As *E. anicia* larvae enter diapause upon reaching the fourth instar, the experiment could not continue beyond the initial 6 days, as some larvae began to diapause on day 7. The intrinsic growth rates of *E. acrea* and *E. anicia* are quite different, and thus we did not compare them statistically.

Chemical analyses

Plant IG content was analysed by gas chromatography (Gardner & Stermitz, 1988; Bowers & Collinge, 1992; Bowers & Stamp, 1992; Fajer *et al.*, 1992). Leaf samples were oven-dried at 50 °C and ground to a fine powder in a mortar. To prepare material for analysis, each sample was extracted overnight in methanol. The solid material was filtered out, and the extract evaporated to dryness. An internal standard, phenyl β-D-glucopyranoside (0.500 mg), was added to each sample. The sample was partitioned between water and ether. The ether fraction, which contains lipophilic substances, was discarded, and the water fraction, containing primarily the IGs and sugars, was evaporated to dryness. An aliquot of this was derivatised using Tri-Sil Z (Thermo-Fisher Chemical Company, Waltham, Massachusetts), prior to injection onto an HP 7890A gas chromatograph (Agilent Technologies, Santa Clara, California) using an Agilent DB-1 column (30 m, 0.320 mm, 0.25 mm particle size; Gardner & Stermitz, 1988; Bowers & Collinge, 1992; Fajer *et al.*, 1992). The gas chromatograph was calibrated using standards of purified catalpol and scutellarioside II (hereafter scutellarioside). Amounts of catalpol and scutellarioside were quantified using ChemStation B-03-01 software and data were analysed as percentage of dry mass (concentration).

Statistical analyses

All statistical analyses and figures were done using R software version 3.1.2 (R Development Core Team, 2011). Welch's two-sample *t*-tests were used to compare common garden herbivory and chemistry data on the two plant species (the main effect was plant species and the dependent variable was either leaf damage or IG content). Model I regressions were used to determine the relationship between the IG content of both *Penstemon* species and the amount of plant damage. Oviposition data from the field experiment were analysed with a χ^2 test comparing the number of egg masses on each host plant species. Laboratory food choice data were analysed with paired *t*-tests comparing the area of the leaf disc eaten for each plant species. The growth rate and survivorship data were analysed with Welch's two-sample *t*-tests comparing either the relative growth rate or proportion of survivors (out of the 10 larvae initially put on each plant species) on the two host plant species.

Results

Common garden experiment – chemistry of P. glaber *and* P. virgatus *and preference of generalists*

Chemical analyses of common garden plants showed that *P. glaber* did indeed contain catalpol (verified by retention times and co-injection with a standard of catalpol) and no other IGs were detected. *Penstemon glaber* leaves contained significantly more catalpol than *P. virgatus* leaves ($t = 18.64$, d.f. = 20.23, $P < 0.001$; Fig. 2), with an average of 18.12% dry weight. This was within the range seen previously in plants from Crescent Meadows (17–23% dry weight catalpol in 2007; M.D. Bowers, unpublished), but was more concentrated than previous studies have found in other populations of *P. glaber* (Stermitz *et al.*, 1994). *Penstemon virgatus* leaves from these common garden plants contained two different IGs, catalpol and scutellarioside, as well as a significantly higher overall IG concentration (average of 30.56% dry weight; $t = -9.55$, d.f. = 38.86, $P < 0.001$; Fig. 2), largely due to the high scutellarioside content (average

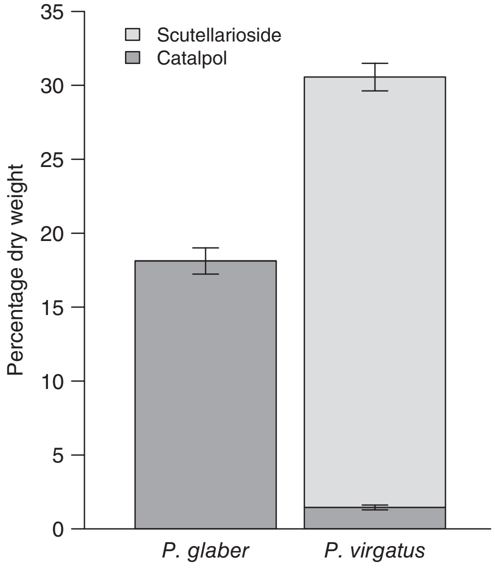

Fig. 2. The iridoid glycoside (IG) composition of *Penstemon glaber* and *Penstemon virgatus* leaves from plants reared in the common garden. Data are means ± SE.

of 29.10% dry weight), which is not found in *P. glaber*. The IG content of *P. virgatus* fell within the range of variation recently seen in natural populations, including Crescent Meadows (14–32% dry weight scutellarioside, 1–5% dry weight catalpol; C.A. Kelly, unpublished). However, the *P. virgatus* in this study contained noticeably higher amounts of IGs than previous work in other populations (L'Empereur & Stermitz, 1990b) or with plants in earlier developmental stages (Quintero & Bowers, 2013). Thus, from the perspective of a generalist, *P. virgatus* is considered the more defended (less palatable) plant because it contains both larger amounts of IGs and a higher diversity of IGs.

In the common garden experiment, *P. glaber* leaves received significantly more damage by generalist herbivores than *P. virgatus* ($t = 10.88$, d.f. $= 29.09$, $P < 0.001$; Fig. 3). Unidentified species of *Phyllotreta* (Coleoptera: Chrysomelidae) were the most commonly observed herbivore in the garden. This result provided ecologically relevant support for our designation of the relative palatability of these two host plants, as the less chemically defended plant species was the preferred choice of generalists. The catalpol concentration of *P. glaber* showed a marginally significant negative linear association with the amount of damage ($F_{1,18} = 4.082$, $r^2 = 0.1396$, $P = 0.0585$). However, *P. virgatus* showed no significant linear associations between IG content and amount of damage (total IGs, $F_{1,18} = 0.010$, $r^2 = 0.055$, $P = 0.922$; catalpol only, $F_{1,18} = 1.808$, $r^2 = 0.041$, $P = 0.196$; scutellarioside only, $F_{1,18} = 0.103$, $r^2 = 0.050$, $P = 0.753$).

Field experiment – oviposition choice by the specialist, E. anicia

Females of the specialist, *E. anicia*, laid significantly more egg masses on leaves of *P. glaber* (41 masses) than on *P. virgatus* (two masses) ($\chi^2 = 35.37$, d.f. $= 1$, $P < 0.001$). Although, in July 2010, the existing population of *P. virgatus* at Crescent Meadows appeared to be larger than that of *P. glaber*, there were very few naturally occurring *P. virgatus* plants found with egg masses (C.A. Kelly, pers. obs.). Conversely, nearly every *P. glaber* observed at Crescent Meadows had at least one egg mass.

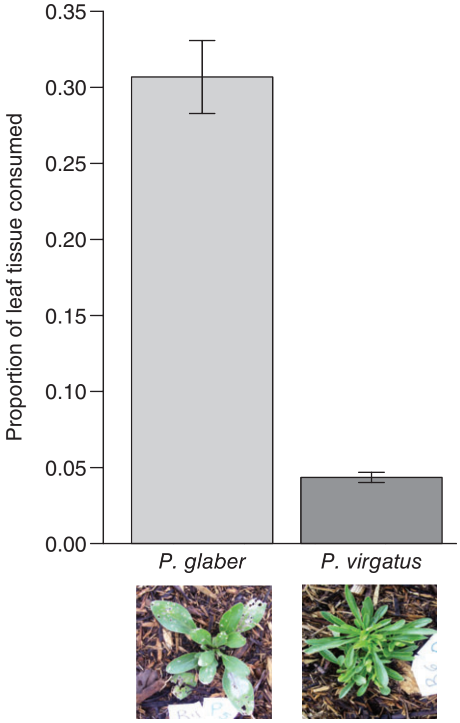

Fig. 3. Leaf consumption by herbivores on *Penstemon glaber* and *Penstemon virgatus* plants in the common garden. A representative picture of a typical plant after the experiment is below the matching bar. Data are means ± SE.

Laboratory experiments – host plant choice and suitability for a generalist and a specialist

Larvae of the specialist and generalist behaved differently in the food choice experiments. Larvae of the generalist, *E. acrea*, preferred to eat *P. glaber*, consuming a significantly larger proportion of the leaf disc (mean ± SE: *P. glaber* $= 0.117 \pm 0.015$, *P. virgatus* $= 0.0 \pm 0.0$; paired $t = -7.45$, d.f. $= 15$, $P < 0.01$). In contrast, larvae of the specialist *E. anicia* preferred to consume *P. virgatus* (*P. glaber* $= 0.043 \pm 0.011$, *P. virgatus* $= 0.101 \pm 0.015$; paired $t = -2.35$, d.f. $= 25$, $P = 0.027$).

As expected given their food preference, *E. acrea* larvae had significantly higher growth rates when fed *P. glaber* compared with *P. virgatus* in the no-choice feeding experiment ($t = -8.03$, d.f. $= 23.68$, $P < 0.001$; Fig. 4a). In contrast, although *E. anicia* preferred *P. virgatus*, there was no difference in growth rate when reared on either plant species ($t = -0.68$, d.f. $= 10.47$, $P = 0.51$; Fig. 4b). Consistent with the growth rate data, survivorship by day 6 for *E. acrea* was significantly higher on *P. glaber* than on *P. virgatus* ($t = -2.64$, d.f. $= 30.29$, $P = 0.013$;

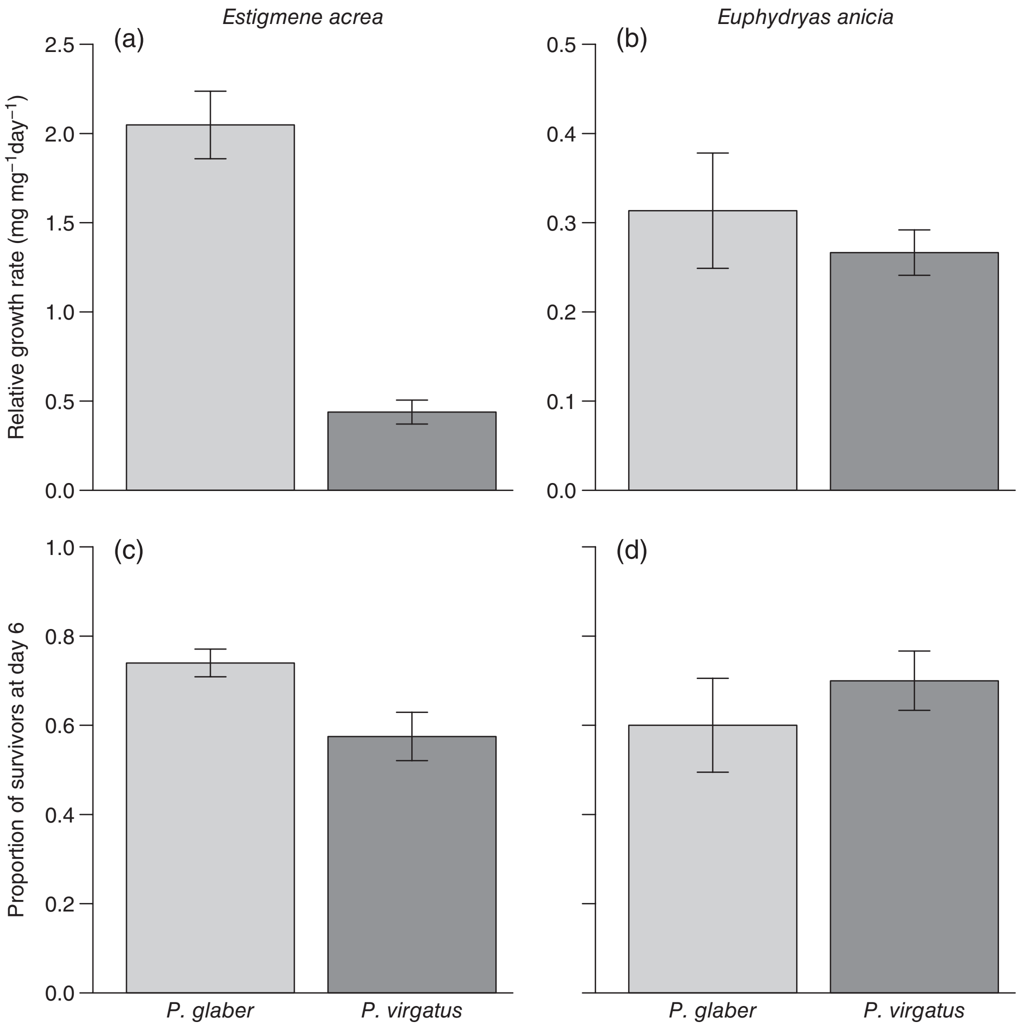

Fig. 4. Larval performance: the relative growth rates of *Estigmene acrea* (a) and *Euphydryas anicia* (b) when reared exclusively on either *Penstemon glaber* or *Penstemon virgatus* and survivorship by day 6 for *E. acrea* (c) and *E. anicia* (d) reared on each plant diet. Data are means ± SE.

Fig. 4c), whereas survivorship for *E. anicia* was similar for both host plant species ($t = -0.80$, d.f. = 16.65, $P = 0.434$; Fig. 4d).

Discussion

This study provides mixed support for our initial hypothesis that specialist herbivores will have a strong preference-performance relationship and will also outperform generalists on host plants species preferred by the specialists. *Penstemon glaber* had a lower concentration of total IGs and fewer IG types than *P. virgatus*. Our data suggest that these chemical differences may play an important role in driving host plant preferences in the herbivores *E. anicia* and *E. acrea*. Both the common garden and laboratory experiments indicate that generalist herbivores prefer *P. glaber* to *P. virgatus.* The physiological efficiency hypothesis predicts that generalist herbivores will be more sensitive to variation in plant defences than specialist herbivores. Indeed, the performance of the generalist, *E. acrea* was notably decreased when reared on *P. virgatus* (the better defended host), whereas specialist performance did not vary with the host plant species. In contrast, support for components of the PP hypothesis was lacking. Ovipositing females of the specialist *E. anicia* demonstrated a preference for *P. glaber*; however, larvae performed equally well on the two host plants. Furthermore, specialist offspring in the laboratory preferred to feed on a different host species than the mothers preferred for oviposition in the field. Thus, we found no clear relationship between specialist female preference and offspring performance.

That host plant preference differed between parent and offspring of the IG specialist, *E. anicia*, was unexpected, given that female herbivores, particularly those with more specialised diets, are predicted to preferentially oviposit on host plants that enable their offspring to attain the highest performance (reviewed in Gripenberg *et al.*, 2010). Although the PP relationship of *E. anicia* was not negative, neither do the results suggest a strong positive relationship for this specialist. These results contrast with Jaenike's (1978) prediction that if females prefer to oviposit on one host species despite the presence of other suitable hosts, then the preferred host species will be most suitable for larval development. As there was no difference in *E. anicia* performance on the two plants in the laboratory, it is possible that maternal host plant preferences may be influenced by other factors, such as host plant nutritional quality. Non-chemical differences between these *Penstemon* species, including leaf structure, plant size, and visual detectability (Singer *et al.*, 2004; Reudler Talsma *et al.*, 2008), may also drive host plant choice in the field. Furthermore, the PP hypothesis assumes that larvae are unable to disperse to other plants (Clark *et al.*, 2011), but many caterpillar taxa, including checkerspots, are able to relocate to other host plants (e.g. Cain *et al.*, 1985). Host plant availability and enemy-free space are also important components of host plant choice (Fraenkel, 1953; Jeffries & Lawton, 1984; Thompson, 1988; Denno *et al.*, 1990; Osier & Lindroth, 2001; Holton *et al.*, 2003; Murphy, 2004; Wiklund & Friberg, 2008) and could be factors in our system as well. Larvae preferred *P. virgatus* in the laboratory, an environment devoid of natural enemies. Adult females showed a strong preference to oviposit on *P. glaber* in their natural habitat, which probably includes predators and parasitoids. Thus, larval fitness may be higher on *P. glaber* when the threat of natural enemies is present.

Consistent with the predictions of the PE hypothesis (Cornell & Hawkins, 2003; Mooney *et al.*, 2012), *E. acrea* demonstrated inferior performance on the better defended host (defined by IG amount and diversity), whereas *E. anicia* performed similarly on the two *Penstemon* species. The PE hypothesis suggests that the benefits of dietary specialisation, such as faster larval development and increased survival rates, should be more pronounced on more highly defended host plants. Differences in host plant defences were not reflected in specialist performance on the two plant species, yet, as predicted, noticeably affected the generalist's survivorship and performance. What we did not predict, however, was that the specialist would prefer *P. virgatus*, yet grow equally well on both plant species. Although performance differences may only be detectable in post-diapause larvae or later life stages, the focus of these experiments was female oviposition and early-instar larval performance. Additionally, caterpillars in these experiments were coping with extremely high levels of IGs in the plants on which they were feeding. Data from a different experiment showed that *E. anicia* larvae contained only catalpol when reared on *P. virgatus*, and in relatively large amounts (mean $= 8.83 \pm 0.64\%$ dry weight; C.A. Kelly, unpublished), while *P. virgatus* contains only a mean of 1.45% catalpol. This suggests that the larvae are metabolically converting scutellarioside into catalpol by hydrolysing the side chain. A previous study proposed that *E. anicia* is capable of metabolically converting the catalpol ester, 6-isovanillylcatalpol, into catalpol via hydrolysis and then sequestering the catalpol (Gardner & Stermitz, 1988). A similar conversion of scutellarioside may be occurring here. Such a conversion could incur a metabolic cost; thus it was unexpected that larvae performed similarly on the two *Penstemon* species. Perhaps metabolising or eliminating scutellarioside incurs a fitness cost to *E. anicia* that is not apparent with our chosen metrics, which could partially explain the female preference for *P. glaber*. Likewise, compensatory feeding could have allowed *E. anicia* to overcome any potential host plant deficiencies, resulting in no measurable difference in performance on the two hosts.

In agreement with our results, previous studies have also produced mixed support for the PE and PP hypotheses (e.g. Friberg *et al.*, 2015). Although several studies have failed to find a strong relationship between female preference and larval survival and performance (reviewed in Futuyma, 2008; Friberg & Wiklund, 2009), field experiments (in the presence of a third trophic level, natural enemies) often show tighter PP relationships (Damman & Feeny, 1988; Doak *et al.*, 2006; Wiklund & Friberg, 2008). Yet a meta-analysis found support for the PP hypothesis in a strictly bitrophic setting (Gripenberg *et al.*, 2010). Our study features a combination of field and laboratory studies, with female preference tested in the presence of natural enemies, and larval preference and performance tested in the absence of natural enemies. Our results, together with those of past studies, seem to emphasise the need for different sets of predictions within the PP hypothesis: one for tritrophic environments and another for bitrophic. It is possible the traditional specialist–generalist paradigm confounds the predictions of the PP and PE hypotheses, thus highlighting the need to reconsider the classifications of herbivore diet breadth.

The goal of understanding the ecology and evolution of diet breadth in herbivorous insects has resulted in the development of several hypotheses that attempt to explain the observed patterns and processes. Here we combined components of both the PE and PP hypotheses to test the hypothesis that specialist herbivores will show a strong PP relationship and will also outperform generalists on host plant(s) preferred by the specialists. The results of our experiments only partially agree with these predictions. Given the increasing amount of mixed support for these seminal hypotheses, considered both separately and in combination, we suggest that consideration of the third trophic level may be important in refining them (e.g. Mooney *et al.*, 2012). Preliminary tests of such a unified hypothesis, the tritrophic interactions (TTI) hypothesis, showed that the interaction between herbivore diet breadth and plant quality may be altered by the presence of natural enemies (Mooney *et al.*, 2012). Thus, the future development of theories that better address the complexities of trophic interactions may be in the synthesis of these foundational hypotheses.

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