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Dynamics of host plant selection and host-switching by silver-spotted skipper caterpillars

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Abstract Investigations of host plant selection in herbivorous arthropods have emphasized the importance of oviposition site selection by adults; however, a more complete picture of this process requires additional consideration of the factors influencing host plant choice during the immature feeding stages. We conducted a series of larval choice experiments to examine both the innate and induced preferences of larvae of the Silver-spotted skipper (*Epargyreus clarus* L.) on three commonly used hosts (*Wisteria*, *Robinia*, and *Pueraria*). Late instar *E. clarus* larvae reared on each of the three host plants displayed an overall pattern of innate preferences that correlated well with larval performance measures and reflected differences in foliar nutrient concentrations. Larval preferences were also influenced by rearing host species, indicating a role for feeding-induced preferences. When larvae reared on low-quality *Wisteria* for the first four instars were switched to higher quality *Pueraria* for the final instar, they developed more quickly and attained significantly higher pupal mass than larvae maintained on *Wisteria* throughout development. Similarly, larvae switched from *Pueraria* to *Wisteria* for the final instar suffered increased development time and produced significantly smaller pupae than those maintained on *Pueraria* throughout. Thus host-switching, particularly during the more mobile final instars, appears to offer larvae an opportunity to recoup fitness losses associated with early development on a low-quality host. For an equal amount of consumption, larvae feeding on *Pueraria* gained 50% more mass than those feeding on *Wisteria*, reflecting measured differences in foliar nitrogen concentration; despite these overall differences in quality, larval growth efficiency was similar among hosts. Especially in the age of common exotic plant introductions, a full understanding of the behavioral component of host selection by herbivorous insects requires appreciation of the dynamic role that immatures can play in host selection and use.

Keywords Feeding efficiency · Host-switching · Host quality · Insect behavior · Larval choice

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Introduction

Understanding the process of host plant selection by herbivorous insects has been a major goal of insect evolutionary ecology (Bernays and Chapman 1994). In some herbivorous insect taxa, immature feeding stages (i.e., nymphs or larvae) select their own host plants. This process takes place in most Orthoptera and Phasmida, as well as in some Lepidoptera that hatch from eggs placed in or on non-plant substrates or on non-host plants, requiring hatchlings to locate appropriate hosts on their own (Bernays and Chapman 1994; Zalucki et al. 2002; Gómez-Jiménez et al. 2014). In addition to species exhibiting direct larval dispersal, early instar larvae from at least six families of Lepidoptera are reported to passively disperse away from

their oviposition sites by ballooning on a strand of silk, so that the larvae must engage in host selection once alighted (Zalucki et al. 2002). However, for most herbivorous insects, initial selection of larval or nymphal host plants is the province of the mother insect (Gripenberg et al. 2010; Garcia-Robledo and Horvitz 2012). She selects a plant to receive one or more eggs, and following eclosion, the hatchling(s) will generally feed on that host (Jaenike 1978; Renwick and Chew 1994; Gripenberg et al. 2010; König et al. 2016).

Implicit in many studies of host selection or diet breadth in insect herbivores is the presumption that once a choice is made (by either adults or offspring), the developing insect demonstrates fidelity to its selected plant, associating with that particular plant species and/or individual for the duration of its feeding stage. Nevertheless, in many situations larvae or nymphs may be required to switch host plants, either within or across host species. They may need to seek out a new plant if: (1) their original host is entirely consumed; (2) they fall off the plant or leave it in response to natural enemy threat; (3) the quality of the original host plant declines; (4) the larvae require complementary nutrients; or (5) the plant dies (Price et al. 1980; Bernays and Chapman 1994; Bernays and Bright 1993; Bernays 2001; Jonas and Joern 2008; Behmer 2009; Cunningham et al. 2011; McCormick et al. 2016). In addition, some generalists, such as *Grammia incorrupta* caterpillars (Arctiidae), wander and sample various hosts throughout their development, a behavioral strategy that may help the larvae to meet nutritional needs, avoid ingesting too much of any given defensive compound, and possibly obtain secondary compounds used in self-medication (i.e., pharmacophagy; Scriber and Slansky 1981; Singer and Stireman 2003; Singer 2008).

When selecting a new host plant, either for their first or a subsequent meal, herbivores often exhibit 'innate' preferences, selecting certain hosts with which they have had no prior experience, presumably in response to phagostimulants produced by the selected plant (Bernays and Chapman 1994, Jaenike 1990; Cunningham et al. 2011). Alternatively, an herbivore's preference may be affected by its perception of the nutritional quality of a host once sampled (Scriber and Slansky 1981; Schoonhoven 1987). Finally, host choice may be 'induced' by the herbivore's prior feeding experience such that it will prefer a familiar host over other acceptable (or even superior) host plants (Soler et al. 2012; Silva et al. 2014), sometimes to the point that only the familiar host will be tolerated (Bernays and Weiss 1996; Del Campo and Renwick 2000). Host plants often vary markedly in their effects on insect fitness measures due to intrinsic differences in nutritional quality, physical and chemical defenses, and/or risk of attack by natural enemies; as a consequence, host preferences are

expected to be the target of strong selection (Bernays and Chapman 1994; Mayhew 1997; McCormick et al. 2016). Because both primary host selection and host-switching can occur during the feeding stages of insect herbivores (Bernays and Chapman 1994; Soler et al. 2012), investigations of host selection choices and their consequences for insect performance during this critical stage are necessary for a complete view of the ecological and evolutionary determinants of these behaviors. In addition, it has long been hypothesized that in some systems, larval consumption of a host plant can directly influence adult preference for that species and thus play a role in diet breadth evolution via 'preimaginal conditioning' (Hopkins 1916); empirical tests of this hypothesis, however, have met with very limited support in Lepidoptera (reviewed in Barron 2001).

In this paper, we examine the dynamics of larval host choice and its consequences for an oligophagous herbivore. Specifically we ask: (1) to what extent do innate and/or induced preferences contribute to larval host selection, and how do these preferences interact? (2) will larvae reared on a poor quality host plant show fidelity to that host? (3) if larvae switch hosts for the last larval instar, is larval performance affected? and (4) what aspects of foliar quality correlate with larval preference and/or performance? We examine these questions using larvae of the silver-spotted skipper, *Epargyreus clarus* (Hesperiidae) and three of its leguminous host plants, all of which are used by adult females for oviposition and are fed on by larvae.

Materials and methods

Study organisms

Epargyreus clarus (Hesperiidae), commonly known as the silver-spotted skipper, is native to North America, and inhabits much of the United States (Scott 1986; Wagner 2005). *E. clarus* larvae feed on multiple host plant species within the Fabaceae, or pea family. Within Fabaceae, *E. clarus* has been reported to feed and oviposit on a number of different host plants, all of which belong to the subfamily Papilionoideae (Wojciechowski et al. 2004). The host plants vary in life form, and include vines, herbs, and trees (Scott 1986). We used three host plants in this experiment: black locust (*Robinia pseudoacacia* L.) wisteria (*Wisteria sinensis* (Sims) DC), and kudzu (*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen & S.M. Almeida ex Sanjappa & Predeep) (hereafter referred to solely by generic names). *Robinia* is native to upland areas of the eastern United States (Boring and Swank 1984), while the other two non-native hosts were introduced from Asia. *Pueraria* was introduced to the United States from

Japan in 1876. Between the 1930 and 1953, the US Soil Conservation Service advocated the planting of kudzu to control soil erosion, and the plant has since spread aggressively across the southern and central United States (Mitich 2000; USDA, NRCS 2016). *Wisteria* was introduced intentionally as a garden plant, but has since become naturalized throughout much of the eastern and southern U.S., where it frequently hybridizes with another naturalized exotic congener, *Wisteria floribunda* (Wildd.) DC (Trusty et al. 2008). Both *Pueraria* and *Wisteria* are considered to be invasive weeds, and are the targets of ongoing but mostly unsuccessful control efforts (Mitich 2000; Trusty et al. 2008). Throughout the study, leaves were collected from field locations on the Georgetown University Campus, in Silver Spring, MD, and in wooded areas of Washington DC.

Epargyreus clarus is known for its larval shelter-building behavior (Weiss et al. 2003). Once the larvae hatch from their eggs, they begin to construct shelters out of host plant leaves and silk; the larvae build 4 characteristic styles of shelters over their larval lifespans, and spend most of their time inside, exiting only to feed or to construct a new shelter (Lind et al. 2001; Weiss et al. 2003). *E. clarus* is well suited for studies of larval host preference because the location of the larvae can be easily determined on the host plant from one day to the next, and the presence of a shelter on a plant indicates a distinct larval "choice" of that host.

Our prior work has demonstrated that larvae reared on *Robinia* and *Pueraria* perform better than larvae reared on *Wisteria,* in terms of decreased development time and greater pupal mass (mean ± SE pupal mass: *Pueraria*, 724.39 ± 14.01 mg; *Robinia*, 649.94 ± 16.7 mg; *Wisteria*, 607.1 ± 23.2 mg; mean development time: *Pueraria*, 27.5 ± 0.40 days; *Robinia*, 24.9 ± 0.66 days; *Wisteria*, 30.8 ± 1.2 days) (MRW and JTL, *unpublished data*). Thus, these taxa provide an ideal system to investigate the dynamics of larval choice on poor and good-quality hosts.

Larval preference experiments

All larval preference experiments were conducted in June–August, 2014 and 2015. Caterpillars used in these experiments were derived from colonies established each year from a mix of field-collected larvae reared to adulthood and adults caught on or around the Georgetown campus in Washington, DC, USA. The butterflies used in creating and maintaining the colony were derived from larvae that had fed on all three hosts used in the experiments.

Eggs were collected daily from colonies consisting of multiple gravid females maintained in an outdoor flight cage and provided with fresh host plants. All eggs from a given day were batched, and upon hatching, larvae were haphazardly assigned to one of the three host plants (their initial rearing host). Subsequently, they were reared in small groups on cut foliage of their designated host plant until they reached the fourth instar, when they were selected for use in experimental trials.

Innate and induced preference

To determine whether *E. clarus* larvae showed innate or induced host plant preferences, we offered caterpillars that had been reared on each of the three hosts a bouquet of all three hosts, and assessed their plant choices. One freshly cut leaf or sprig each of *Robinia, Wisteria*, and *Pueraria* was placed in an individual aquapic to form a bouquet of the three host plants; each species presented a roughly equivalent leaf surface area. Nine larvae (three reared on each of the three host plants) were used in each of nine separate trials; individual larvae were used only once ($n = 81$ larvae). Each bouquet received a single fourth instar larva that was assigned to one of the three host plants in the bouquet such that all combinations of rearing host and placement host were represented in the 3×3 array. For example, in a given trial, of the three caterpillars initially reared on *Pueraria*, one would be placed on *Robinia*, one on *Wisteria*, and one on *Pueraria* in their respective bouquets. Following placement, each larva was free to either stay on its placement host or seek another among those provided in the bouquet. This factorial design allowed us to explicitly examine the contributions to host plant choice of both innate and induced preferences.

Once all the larvae were placed on their assigned plants, the location of each larva was recorded every 15 min over the course of an hour. Within that time period, if a larva left its bouquet entirely, it was returned to its originally assigned plant, in order to maintain an adequate sample size. Departures from the bouquet after than initial period were considered a choice. Larvae were allowed to explore the bouquets for 8–20 h (spanning a single day or overnight). Final locations of all larvae were recorded as the plant upon which they constructed their shelters, or, for the four larvae that did not build a shelter, the plant on which they were resting at the end of the trial period.

Larval fidelity to a poor quality host

As described above, *E. clarus* larvae exhibit the poorest performance (i.e., lowest survival, longest development time and smallest pupae) when reared on *Wisteria*, relative to the other two hosts. Given these results, we conducted a series of trials in the summer of 2015 to examine larval behavior when offered a choice between the poor quality host *Wisteria* and tulip poplar (*Liriodendron tulipifera* L.), a native non-host in the Magnoliaceae.

Three larvae, reared through the penultimate instar on each of the same three hosts (*Robinia*, *Pueraria*, and *Wisteria*), were used per trial; three trials were conducted, for a total of 27 larval choices; each larva was used only once. In this experiment, 2-leaf bouquets of freshly cut *Wisteria* and *Liriodendron* leaves were inserted in styrofoam blocks inside a 1 m³ mesh cage. For these trials, a single 4th instar larva was initially placed on either a *Wisteria* or *Liriodendron* leaf and its location noted every fifteen minutes for an hour, as in the host plant acceptance trials described above. Final locations of the larvae (*Wisteria*, *Liriodendron*, or 'no choice,' indicating that the larva had wandered off the bouquet and was resting on the mesh cage) were determined as above.

Host-switching experiment

To determine whether larval performance could be altered by switching host plant species for the final instar, we conducted an experiment using one low-quality host plant (*Wisteria*) and one high-quality host plant (*Pueraria*). Larvae were weighed just prior to molting to the fifth instar and then placed in rearing containers holding a single sprig of either their initial rearing host or a novel 'final' rearing host; this setup ensured that each larva's first bite as a fifth instar would be on the new sprig. Each experimental larva was housed in an inverted 32 oz. plastic deli container that held its assigned host plant in a water-filled aquapic. This no-choice feeding assay not only allowed monitoring of leaf consumption by individual larvae, but also maintained humidity in the chambers.

Host plant leaves were replaced every other day to prevent food limitation; at this time, larvae were removed from their shelters and placed on fresh leaves. Frass was collected daily in a separately marked container and left to air-dry. We noted the date of pupation for each individual, and sexed and weighed pupae (once hardened) within 5 days of pupation. Final accumulated frass for each individual was dried at 40 °C for a week and weighed to the nearest 0.1 mg. Total sample sizes for each initial × final host plant combination varied from 12 to 29 caterpillars due to reduced survivorship of larvae reared on *Wisteria;* larvae that died during the course of the experiment were not included in any analyses.

We assessed the growth efficiency of *E. clarus* on all four host-switching treatments by plotting the change in wet mass in the final instar (pupal mass—initial mass at 5th instar) against the mass of dried frass produced in the fifth instar (Singer 2001, Mason et al. 2011). We determined in a separate analysis that fifth instar larvae produce 12 times more frass in that stage alone than in all other instars combined (two-tailed Student's *t* test: $t_{22} = 15.56$, $p < 0.0001$).

Leaf nutrient analysis

To quantify differences in nutritional quality, we analyzed the elemental concentrations of carbon and nitrogen in leaves of all three of our host plant species. We collected fully expanded, undamaged leaves from five individual plants of each species, growing at our usual leaf collection sites, in the middle of the growing season. Fresh leaves were weighed, dried at 60 °C for >48 h, and reweighed to determine water content. Dried leaves were then ground to a fine powder using a Retsch 200 mixer mill. Two homogenized subsamples of leaf material from each of the five plant individuals per species were then analyzed for %C and %N (by mass) using a Costech EAS CN analyzer equipped with an autosampler (Costech Analytical Technologies Inc., Valencia, CA USA). The mean %C and %N of the two subsamples was used to characterize the elemental composition of the leaves from an individual plant. Using the same data, we also calculated the C/N ratio for each plant.

Statistical analyses

All statistical analyses were conducted using R (R Development Core 2015). For both the host plant acceptance and larval fidelity experiments, larval preferences were evaluated with *Chi*-square statistics against a null model of no preference generated using a Monte Carlo simulation. Because host plant acceptance data are categorical, we used multinomial log-linear models (nnet package in R, Venables and Ripley 2002) to test for the effects of rearing host, placement host, and their interaction on the likelihood of choosing a particular host. During the host plant acceptance and larval fidelity trials, in the few instances in which larvae made shelters using leaves from two host plants, one of the two plants was randomly assigned as the larva's final choice. For the host-switching experiment, we compared growth efficiency of the larvae in each combination of treatments using an analysis of covariance (ANCOVA) model, with initial and final host plant (and their interaction) as fixed main effects and dry mass of frass produced as a covariate. We used general linear models to examine the effects of initial and final host plant (and their interaction) on final instar development time, assuming a Poisson error distribution and using the likelihood ratio *Chi*-square (LR χ^2) as the test statistic. Pupal mass was log-transformed and analyzed with standard two-way ANOVA. A Student's t-test was used to determine the differences in mass of frass produced by larvae feeding on *Pueraria* during the early instars (1–4) with that produced in the ultimate instar (5).

Results

Host plant acceptance

Overall, larvae showed a clear preference hierarchy for the three host plants, with *Robinia* the most preferred, *Pueraria* intermediate, and *Wisteria* the least preferred by the combined set of larvae reared from all three hosts ($\chi^2_2 = 19.81$, $p < 0.0001$; compare the total heights of the three stacked bars in Fig. 1). This preference hierarchy incorporates both innate and induced preferences.

Larvae demonstrated an innate preference for *Robinia* over *Pueraria* and *Wisteria*. Individuals reared on *Robinia* (red bars) were more likely to choose *Robinia* over the other two hosts, while those reared on *Pueraria* (blue bars) were equally likely to choose *Robinia* or *Pueraria* over *Wisteria*. In contrast, individuals reared on *Wisteria* were most likely to choose *Robinia*, followed by *Pueraria*, over their own rearing host.

In addition to innate preference, larval rearing host plant also influenced a larva's choice of final location (likelihood ratio *Chi*-squared: $\chi^2_4 = 9.80$, $p = 0.044$); the largest number of larvae choosing any given host were themselves reared on that host. (Compare rearing host plant contributions within a given bar, Fig. 1).

Final larval location was not a function of initial placement in the bouquet (LR *Chi*-squared: $\chi^2_4 = 6.27$, $p = 0.18$) and the rearing host × placement host interaction was not significant (LR *Chi*-squared: $\chi^2_8 = 13.00$, $p = 0.11$). Many larvae moved amongst the three host plant sprigs in the bouquet within the first hour; 44% of larvae placed on *Wisteria*, 37% of those placed on *Robinia*, and 19% of those placed on *Pueraria* contacted or visited at least one other plant during this observation period.

Larval fidelity to a poor quality host

When offered a choice between the low-quality *Wisteria* and *Liriodendron*, a non-host plant, the majority of caterpillars (56%) declined to choose either *Wisteria* or *Liriodendron*, and instead left the plant bouquet entirely. Final larval location was not affected by either rearing plant ($\chi^2_4 = 8.267$, $p = 0.083$; Fig. 2) or the plant on which the larva was initially placed ($\chi^2_2 = 4.747$, $p = 0.093$). However, of the larvae that did choose *Wisteria*, more than half (56%) had been reared on *Wisteria*.

Host-switching experiment

The duration of the last instar was a function of both the initial (LR $\chi^2_1 = 3.991$, $p = 0.046$) and final (LR $\chi^2_1 = 22.594$, $p < 0.0001$) rearing host (Fig. 3a). The duration of the final instar for individuals switched from *Pueraria* to *Wisteria* lasted ~30% longer than it did for
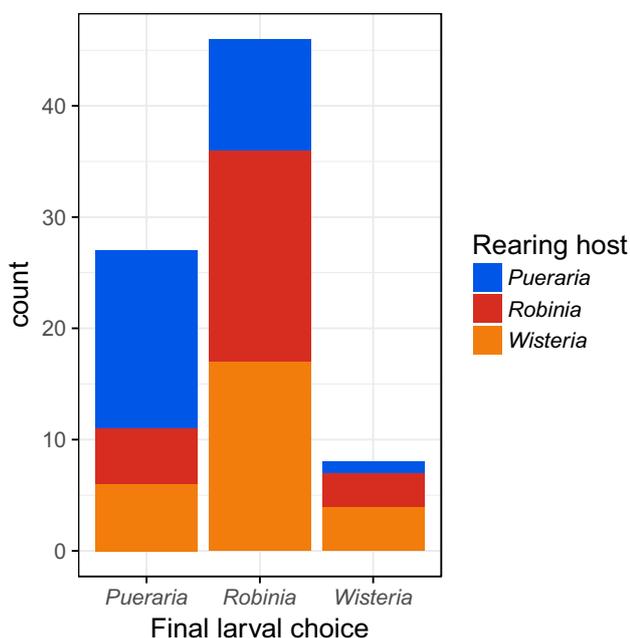

Fig. 1 Host plant selection by *E. clarus* larvae reared on *Wisteria*, *Pueraria*, or *Robinia* and offered a choice of all three plants. *Colors* denote the rearing plant. Note that for each host plant, the largest number of larvae that chose each plant were reared on that plant
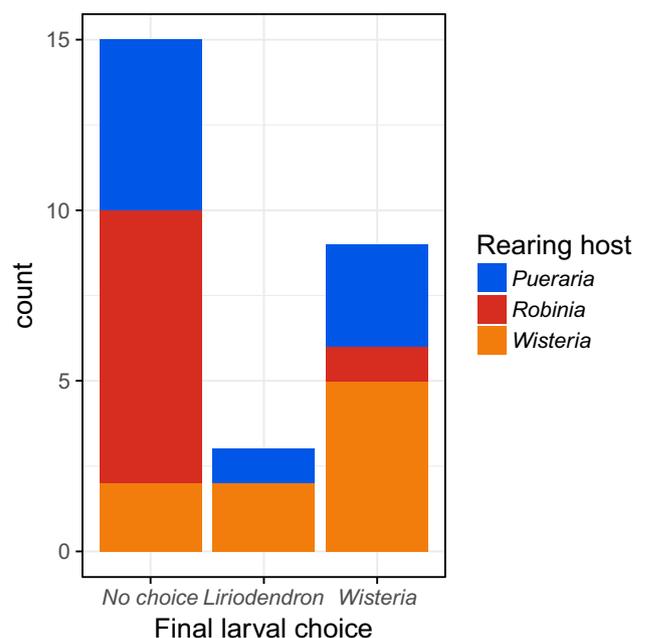

Fig. 2 Host plant selection by *E. clarus* larvae reared on *Wisteria*, *Pueraria*, or *Robinia* and offered a choice of *Wisteria* or *Liriodendron* (a non-host). 'No choice' indicates larvae that wandered off the bouquet and rested on the wall or floor of the mesh cage. *Colors* indicate initial rearing host plant prior to choice experiment
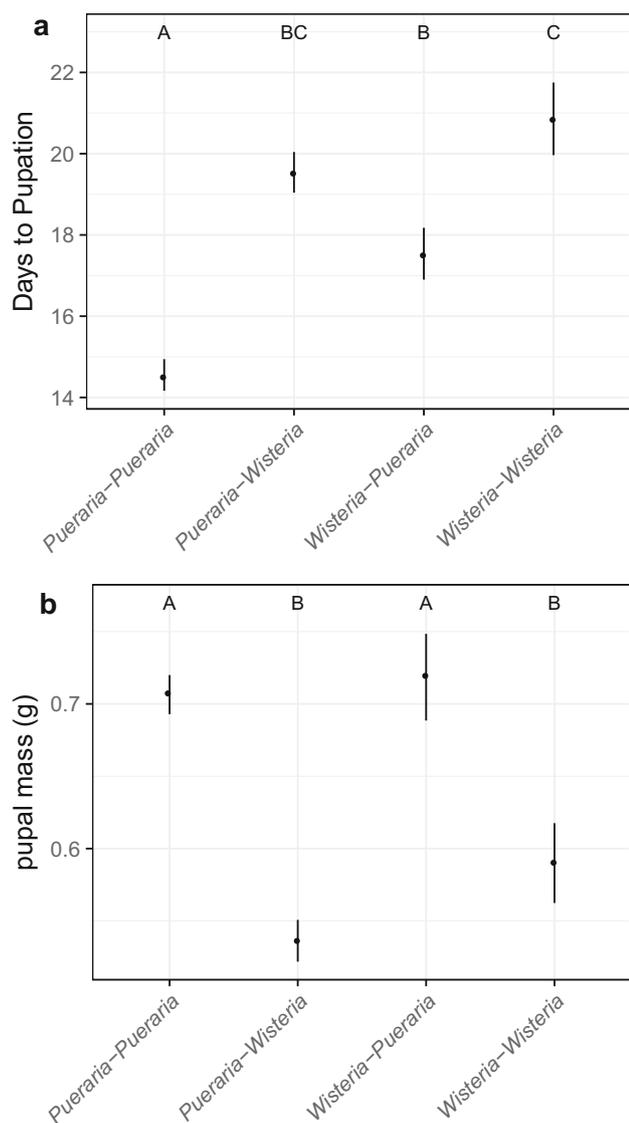
Fig. 3 Performance of *E. clarus* larvae in no-choice host-switching experiment conducted during the last larval instar. Each treatment (mean ± SE) indicates the initial and final rearing host combinations for larvae reared initially on either *Wisteria* or *Pueraria*. For each treatment combination, we plotted the duration of the final instar (**a**) and the final pupal mass (**b**). Treatment means with the *same letter* were not significantly different based on post hoc Tukey HSD tests

Although initial rearing host (during instars 1–4) did not influence final pupal mass ($F_{1,74} = 0.613$, $p = 0.436$), larvae reared on *Pueraria* during the last larval instar weighed 30% more (as pupae) than larvae reared on *Wisteria* ($F_{1,74} = 65.359$, $p < 0.0001$; Fig. 3b), regardless of initial rearing host. There was no significant interaction between initial and final rearing host ($F_{1,74} = 1.855$, $p = 0.177$).

Final instar larvae feeding on the low-quality *Wisteria* produced significantly more frass during the final instar of development than larvae feeding on *Pueraria* (Fig. 4a). However, within both final host treatments, the total amount of frass produced by individual caterpillars (an
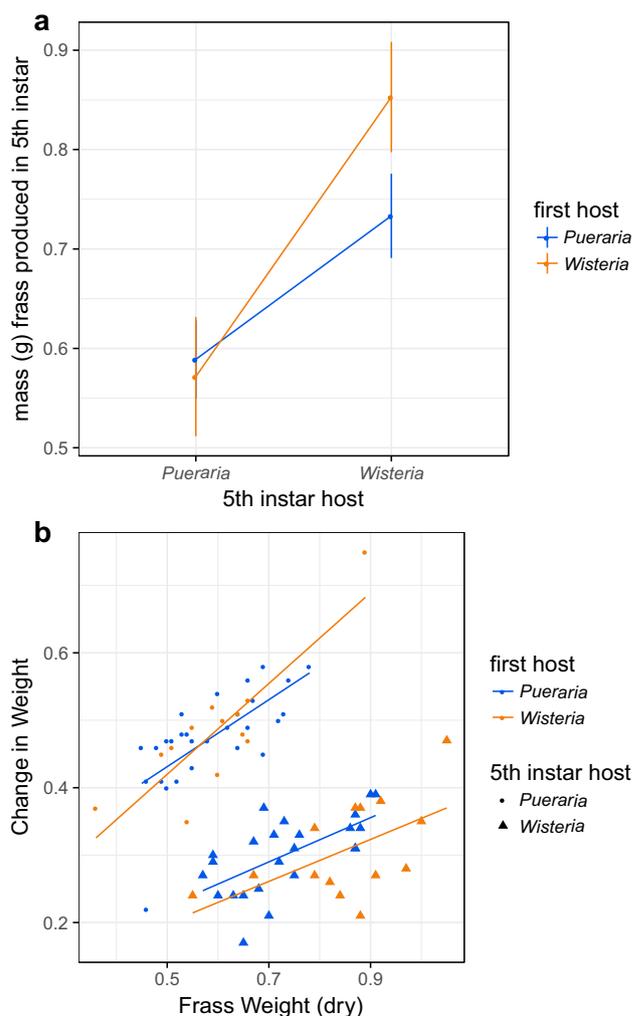

Fig. 4 Frass production (**a**) and growth efficiency (**b**) of *E. clarus* larvae in no-choice host-switching experiment conducted during the last larval instar. In (**b**) the mass gain of larvae in the final instar is plotted against the total dried mass of frass produced during this period. While larval feeding efficiencies on the two host plants are similar (i.e., the slopes do not differ among treatments), the overall mass gain for a fixed amount of feeding (frass production) is significantly higher for larvae reared on *Pueraria*, regardless of the initial rearing host (see Table 1 for statistics)

individuals that remained on *Pueraria*, whereas for larvae switched in the other direction, from *Wisteria* to *Pueraria*, the fifth instar was ~20% shorter than it was for individuals that remained on *Wisteria*. Larvae initially reared on *Wisteria* had longer development times than those initially reared on *Pueraria*, regardless of final feeding host. The effects of initial rearing host on the duration of the 5th instar did not depend on the identity of the final rearing host (i.e., there was no rearing host × assigned host interaction; LR $\chi^2_1 = 0.944$, $p = 0.331$).

index of feeding effort) varied almost twofold. Final host plant was also a significant predictor of weight gain, with larvae feeding on *Pueraria* gaining ∼50% more weight that those feeding on *Wisteria* for any given amount of feeding (Fig. 4b; Table 1, significant effect of final host). However, growth efficiency, measured as the change in larval wet mass in the final instar per gram of dried frass produced over that period, was similar among host plant treatment combinations (i.e., compare the slopes of best-fit lines among treatments in Fig. 4b; all interaction terms $p > 0.05$, Table 1). Thus, the baseline expectation of mass gain differed by final host plant, and caterpillar feeding rate predicted further increases similarly across host plants.

Leaf nutrient analysis

All three measures of leaf quality differed significantly among host plants (Fig. 5). Average foliar nitrogen content (%N) of *Wisteria* was significantly lower than for *Robinia* and *Pueraria* (Fig. 5a; $F_{2,12} = 17.936$, $p < 0.001$). Similarly, the C:N ratio was significantly higher for *Wisteria* than for the other two host plants, which were not different from one another (Fig. 5b; $F_{2,12} = 21.994$, $p < 0.001$). For the same samples, leaf water content also varied significantly across hosts ($F_{2,12} = 17.774$, $p < 0.001$). Again, water content was also higher for *Robinia* and *Pueraria* relative to *Wisteria* (Fig. 5c; Tukey HSD post hoc comparison $p < 0.05$).

Discussion

Larvae of *E. clarus* clearly discriminate amongst host plants, and their choices appear to be shaped by a combination of innate and induced preferences. Across all rearing hosts, fourth instar *E. clarus* larvae exhibit a clear preference for the native *Robinia*, with the exotic host *Pueraria* intermediate and the exotic host *Wisteria* the least

preferred. Given that *Robinia* is locally abundant and geographically widespread (Scott 1986; Boring and Swank 1984), coupled with the fact that *E. clarus* has a much longer evolutionary history with *Robinia* than the other two introduced hosts, a strong larval preference for *Robinia* is perhaps unsurprising. Despite the strong innate preference for *Robinia*, at least some *E. clarus* larvae built shelters (our proxy for host plant choice) on each of the three host plants, suggesting the larvae recognize all three plant species as acceptable hosts.

In addition to innate preferences, larval choices also reflected prior feeding experience, with clear evidence of feeding induction in both the host acceptance and larval fidelity experiments. Initial rearing host was a significant predictor of host choice; the largest fraction of larvae choosing a particular host had been reared on that host. This was true even for the least preferred host, *Wisteria*. In the host plant acceptance experiment, although the majority of larvae reared on *Wisteria* (from hatching to the fourth instar) ultimately chose one of the other host plants, reflecting a strong innate preference, more than half of those that did select *Wisteria* had been reared on it. Additionally, in the larval fidelity experiment, although the majority of the larvae declined to choose either plant offered, a significantly higher proportion of larvae that did choose *Wisteria* had been reared on *Wisteria*, rather than on the other hosts. We note that unlike some caterpillars that regularly 'sample' foliage, potentially gaining important information about foliar quality, host selection decisions by later instar *E. clarus* are typically made as they build their shelters, prior to feeding, suggesting that chemical and/or physical properties of foliage play a primary role in host acceptance. The complete absence of feeding on the non-host *Liriodendron tulipifera* by *E. clarus* larvae in the larval fidelity experiment further supports this supposition.

Induced host plant preferences are fairly common in Lepidoptera (Jermy et al. 1968), and often the mechanisms underlying this particular sort of learning (Papaj and

Table 1 ANCOVA table for growth efficiency of *E. clarus* larvae reared either on their original host or switched to a different final host during the last larval instar

Factor	Sum Sq	Df	*F* value	Pr(>*F*)
Original host	0.003	1	1.138	0.290
Final host	0.620	1	246.307	<0.001
Initial larval weight (g)	0.009	1	3.759	0.057
Frass weight (dry g)	0.124	1	49.340	<0.001
Initial host × final host	0.002	1	0.936	0.337
Initial host × initial larval weight (g)	0.002	1	0.633	0.429
Final host × initial larval weight (g)	0.003	1	1.382	0.244
Initial host × final host × initial larval weight (g)	0.000	1	0.158	0.693
Residuals	0.174	69		

The mass gained during the last instar was only a function of the final rearing host and the mass of frass produced
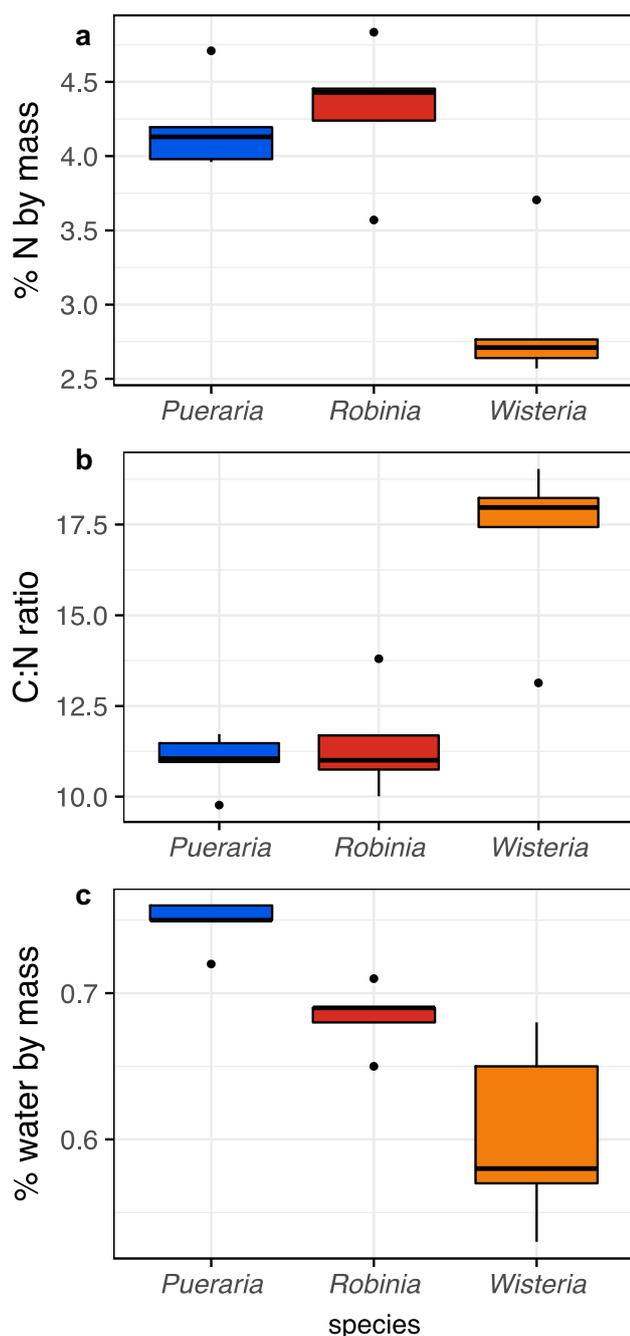
Fig. 5 Boxplots depicting the nutrient content of foliar samples from each of the three host plants used in the larval acceptance and no-choice feeding experiments. Total leaf nitrogen (**a**), carbon:nitrogen ratio (**b**), and water content (**c**) are plotted for five representative leaf samples collected from field sites in the Washington, DC metropolitan area. *Boxes* indicate first and third quartiles and contain medians while whiskers indicate 95% CI and points are outliers

Prokopy 1989; Silva et al. 2014) remain elusive (reviewed in Bernays and Weiss 1996). In some cases, however, such preferences have been shown to reflect induced detoxification of plant secondary metabolites characteristic of specific host plant species (e.g., Snyder and Glendinning

1996). The responses recorded here for *E. clarus* are in keeping with those reported recently for *Heliconius* butterfly larvae that similarly were shown to exhibit a mixture of innate and mildly inducible preferences for various species of *Passiflora* (Silva et al. 2014). Some authors have suggested that the 'inducibility' of alternative hosts may be tied to host nutritional quality, wherein an herbivore's induction response is positively correlated with host quality, thereby reinforcing the use of higher quality hosts in an area (Silva et al. 2014). In this study, *E. clarus* exhibited a modest degree of induction on all three host plants, despite rather large differences in host quality between *Wisteria* and the other two hosts. While the association between larval experience and adult preference was not examined explicitly as part of this study, we note that individual *E. clarus* females provided access to larval host plants typically lay eggs on multiple host plant species and thus do not appear to be strongly 'conditioned' by larval feeding experience (i.e., *E. clarus* does not appear to follow Hopkins host selection principle; M. Weiss, unpublished data).

The host plant hierarchy recorded in our larval preference trials closely matches patterns of performance for *E. clarus* reared under laboratory conditions on cut foliage of these three hosts, with larvae reared on *Robinia* and *Pueraria* performing better than larvae reared on *Wisteria*, in terms of decreased development time and greater pupal mass. Basic measures of leaf nutrients reported in this study correspond well with both larval preference and performance, as *Robinia* and *Pueraria* foliage contained significantly higher water content, leaf nitrogen, and lower C:N ratios than *Wisteria* foliage.

Results of our host-switching experiments demonstrate a potentially important fitness consequence of shifting hosts later in larval life. Final instar host strongly predicts pupal mass, regardless of initial rearing host. Larvae switched from a poor quality host to a better host at the start of the final instar can attain a pupal mass equal to that of larvae reared throughout on a good quality host, and larvae switched to a poor quality host can lose any earlier advantage. Development time is also affected by final host plant; larvae switched from *Wisteria* to *Pueraria* for the last larval instar pupate more rapidly than those maintained on *Wisteria*, but still lag a couple of days behind those reared throughout on *Pueraria*, suggesting a modest physiological cost to switching, as has been shown previously in both *Spodoptera eridania* (Karowe and Martin 1989) and *Colias philodice* (Karowe 1989). Final host significantly affected growth increment and frass production in the last instar, with larvae reared on the high-quality *Pueraria* gaining significantly more mass for the same amount of feeding effort compared with larvae reared on *Wisteria*. While *Wisteria* is clearly a lower quality host for *E. clarus*, increased feeding on this plant allows larvae to

gain mass at the same rate as those feeding on *Pueraria* (note parallel slopes in Fig. 4b).

Because *E. clarus* larvae consume twelve times more food in the final (fifth) instar than they do in all other instars combined, this developmental stage is a major determinant of adult fitness via the well-documented positive correlation between size at metamorphosis and potential or realized female fecundity (Slansky and Scriber 1985; Awmack and Leather 2002; Murphy et al. 2011). Additionally, Barton Brown and Raubenheimer (2003) showed that the increased feeding rate during the fifth instar had a large impact on size and fecundity. Late instar *E. clarus* larvae tend to be much more mobile than earlier instars, traveling significantly greater distances both to feed and to construct new shelters (Lind et al. 2001). In our field sites, we frequently see the three host plants used in this study intertwined and on several occasions we have observed late instar larvae that had been feeding on *Wisteria* shift to feeding on *Pueraria* or *Robinia*, in accordance with the larval preference hierarchy documented in our laboratory trials. This switch could be considered an adaptive response enabling larvae to compensate for suboptimal oviposition choices made by their mothers (Shikano et al. 2010; Soler et al. 2012; Gómez-Jiménez et al. 2014).

Finally, given that *Wisteria* appears to be nutritionally inferior to other commonly used hosts, and larvae generally avoid it when given a choice of other acceptable hosts, why has *E. clarus* incorporated and maintained this novel plant in its diet? Beyond its nutritional value, a host plant can provide other fitness benefits, such as decreased mortality from predators and parasitoids (Denno et al. 1990; Murphy 2004; Singer 2008). Field trials comparing tritrophic fitness measures for *E. clarus* on a variety of hosts including *Wisteria* are currently underway and may help elucidate the selective forces acting to retain *Wisteria* in the diet of *E. clarus*.

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