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Habitat preference of an herbivore shapes the habitat distribution of its host plant

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Abstract. Plant distributions can be limited by habitat-biased herbivory, but the proximate causes of such biases are rarely known. Distinguishing plant-centric from herbivore-centric mechanisms driving differential herbivory between habitats is difficult without experimental manipulation of both plants and herbivores. Here, we tested alternative hypotheses driving habitat-biased herbivory in bittercress (*Cardamine cordifolia*), which is more abundant under the shade of shrubs and trees (shade) than in nearby meadows (sun) where herbivory is intense from the specialist fly *Scaptomyza nigrita*. This system has served as a textbook example of habitat-biased herbivory driving a plant's distribution across an ecotone, but the proximate mechanisms underlying differential herbivory are still unclear. First, we found that higher *S. nigrita* herbivory in sun habitats contrasts sharply with their preference to attack plants from shade habitats in laboratory-choice experiments. Second, *S. nigrita* strongly preferred leaves in simulated sun over simulated shade habitats, regardless of plant source habitat. Thus, herbivore preference for brighter, warmer habitats overrides their preference for more palatable shade plants. This promotes the sun-biased herbivore pressure that drives the distribution of bittercress into shade habitats.

Key words: bittercress; *Brassicaceae; Drosophilidae;* herbivory; leaf miner; zero-inflated counts.

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INTRODUCTION

Abiotic gradients shape fine-scale patterns of plant distributions across the landscape (Whittaker 1967), but consumers, such as insect herbivores, can play a major role as well (Harley 2003, Fine et al. 2004, Maron and Crone 2006). Herbivores can drive plant distributions by reducing plant fitness in some habitats more than others, and this can occur both because of (A) differential impacts of a given level of herbivory or (B) differential rates of herbivory itself. Abiotic variation can impact susceptibility to herbivores (e.g., via growth–defense trade-offs; Fine et al. 2004, 2013), which leads herbivores to promote plant habitat specialization. But when the total intensity of herbivory itself also varies across habitats (Louda and Rodman 1996, Fine et al. 2006, 2013), it can be difficult to discern whether plant-centric or herbivore-centric mechanisms are responsible. Distinguishing among the mechanisms that shape herbivore distributions is vital for understanding their impacts on plant distributions as well as the likely responses of both herbivores and plants to changes to their abiotic environments.

What distinguishes plant- from herbivore-centric mechanisms is whether the plant or the herbivore response to abiotic gradients takes precedence in shaping realized patterns of herbivore pressure across habitats. Differential herbivore pressure between habitats can arise because herbivores seek out higher quality hosts, and in this case, plant-centric mechanisms (e.g., reduced plant defenses) ultimately shape herbivore distributions. Thus, the habitat effect on the herbivore is indirect, mediated instead by habitat-specific variation in plant traits. In contrast, herbivore-centric hypotheses posit that herbivores are more abundant in, or seek out, favorable abiotic habitat conditions (Huffaker and Kennett 1959), independent of how plant traits vary across habitats. In this case, herbivore habitat tolerance and/or preference is directly shaped by abiotic conditions, which creates enemy-free space exploitable by plants. Here, we addressed how plant- vs. herbivore-centric factors impact the habitat-specific herbivory pressure that is responsible for shaping the habitat distribution of a native subalpine plant.

Studies on bittercress (Brassicaceae: *Cardamine cordifolia*) in the Rocky Mountains of North America were among the first to explore how fine-scale variation in herbivory shapes plant fitness and abundance across habitats (Collinge and Louda 1988, Louda 1990, Louda 1984, Louda and Rodman 1983, 1996), and this system is a textbook example of an herbivore-limited plant distribution (Ricklefs and Miller 2000). *Scaptomyza nigrita* flies (Drosophilidae) are a major herbivore of bittercress: Female adults make feeding punctures (stipples) and oviposit in leaves, and the leaf-mining larvae can defoliate up to 70% of leaf area in sun habitats (Collinge and Louda 1988). Herbivory is higher in sun habitat, and the fitness effects are strong enough to drive bittercress into the shade (Louda and Rodman 1996). Surprisingly, the proximate drivers of differential herbivore pressure across this ecotone remain largely unknown. Collinge and Louda (1989*a,b)* found that plants growing in sun habitats suffer higher herbivory in part because of plant phenology: Immediately after snowmelt, flies only have access to plants that have emerged in sun habitats. However, flies are still abundant weeks after this period, and plants in the shade still suffer low herbivory (Collinge and Louda 1989*b*). We sought to address why this is the case.

Both plant- and herbivore-centric mechanisms can be proposed to explain sun habitat-biased herbivory (Louda and Rodman 1996). In addition to their earlier availability, plants in sun habitats may be less resistant, and thus more attractive or palatable to *S. nigrita*, than those in shade habitats. Under this plant-centric hypothesis, higher plant quality in the sun would cause sun-biased herbivory. Several lines of evidence are consistent with this hypothesis: Bittercress from sun habitats can have lower glucosinolate (GSL) content, the precursors of toxic mustard oils (isothiocyanates; Louda and Rodman 1996), and GSL-enriched bittercress can deter adult female *S. nigrita* and harm their larvae (Humphrey et al. 2016). Additionally, foraging adult females are more active and abundant in sun vs. shade habitats (Louda and Rodman 1996), which could arise if *S. nigrita* seek out higher quality plants as they forage across the ecotone.

Under an alternate hypothesis, sun habitat-biased herbivore abundance—and thus overall herbivore pressure—could arise because *S. nigrita* are attracted, or restricted, to sun habitats due to abiotic habitat features. Under this herbivore-centric hypothesis, higher herbivore pressure on sun plants arises because there are simply more flies in sun. Thus, a direct effect of the abiotic environment on herbivore behavior would release shade-associated bittercress from herbivore pressure, and this mechanism can operate with or without reinforcement from plant phenotypes. Whether the proximate driver of variation in herbivore pressure across this ecotone arises from plant- or herbivore-centric mechanisms has important implications for the types of natural selection faced in each habitat by both plants and herbivores (Fine et al. 2006).

Here, we provide a test of these alternate proximate causes of sun-biased herbivory in bittercress. We first revisited whether *S. nigrita* herbivore pressure is higher in the sun relative to shade habitats by conducting field herbivory surveys. Second, we tested whether *S. nigrita* preferentially forage on shade- or sun-source plants by offering *S. nigrita* females a choice of the two bittercress types under laboratory conditions. We then tested the hypothesis that abiotic features of sun and shade habitats drive feeding and oviposition behavior by manipulating light and temperature in a series of choice trials conducted under laboratory and field settings, using plants from both sun and shade habitats. Altogether, our experiments support an herbivore-centric behavioral explanation for the sun-biased herbivory pattern that shapes the habitat distribution of this textbook native interaction system.

Materials and Methods

Herbivory surveys

All experiments were conducted between 2010 and 2015 at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA. In 2011, we conducted field surveys of herbivore damage on bittercress in eight sun habitats (open meadows) and seven shade habitats (under dense evergreen tree canopies; Appendix S1: Fig. S1, Table S1). We recorded adult *S. nigrita* feeding punctures (stipples), larval mines, and leaf area of two basal leaves from each of 10 ramets from all 15 bittercress patches.

We modeled feeding punctures made by adult females (stipples) and larval mine counts using zero-inflated (ZI) negative binomial (ZINB) generalized linear mixed models. Zero-inflation (i.e., underdispersion) describes a notable excess of observed zero counts relative to the expected zero counts arising under non-truncated Poisson or NB processes (Zuur et al. 2009). In biological terms, zero-inflation can arise from patchily distributed herbivores and clustered feeding behavior, resulting in many undamaged leaves (zero counts) even while plants that are damaged may tend to have large amounts of damage (a typical negative binomially distributed pattern for parasites). Thus, herbivory intensity data can simultaneously exhibit both under- and overdispersion, and this can generate lack-of-fit and biased parameter estimates if ignored. Such was the case with our herbivore survey data (Appendix S3: Fig. S1).

We therefore constructed models that explicitly modeled ZI, as a single parameter and as a function of habitat type, using the canonical logit link function in a binomial GLM. The non-ZI count class was simultaneously estimated via NB GLM with a logit link function, with habitat (sun vs. shade) and leaf area (mm^2) included as population-level (fixed) terms, and site ID and plant ID as group-level (random) intercept terms. Coefficients were estimated via maximum likelihood using R v3.3.3 (R Core Development Team 2017) package glmmTMB v. 0.2.0 (Brooks et al. 2017, Magnusson et al. 2017).

In addition, the flexibility of package glmmTMB allowed us to estimate the NB dispersion parameter (ϕ) as a function of source type, with and without simultaneous accounting for ZI. Both parameters help account for unequal variance across residuals and improve goodness of fit (higher values of ϕ correspond to lower variance in the present NB variance formulation; Appendix S3). We evaluated fixed effects by stepwise model reduction (Zuur et al. 2009) and model comparisons via Akaike's information criterion (AICc; Hurvich and Tsai 1989). We compared best-fitting models to those with additional parameters that estimated ZI and NB dispersion as a function of site type using AIC_c and with predictive model checks to inspect goodness of fit. Model details, diagnostics, and comparisons can be found in Appendix S3.

Host choice experiment I: Sun- vs. shade-derived bittercress

In 2010, we tested whether *S. nigrita* adult females prefer feeding on individual bittercress derived from sun or shade habitats. We transplanted bolting bittercress ramets from the field into soil within plastic pots in the laboratory under fluorescent lighting (16-h:8-h light:dark) for up to 24 h. All field-collected plants retained intact belowground tissue and were discarded if any signs of wilting were visible after 12 hr after transplanting. In each of eight replicates, we randomly assigned two shade-derived and two sun-derived bittercress plants to the four corners of a mesh 35.5 × 35.5 × 61 cm cage (livemonarch.com; see Appendix S2: Fig. S1A). All leaves were unmined, and we subtracted preexisting stipple damage from final counts. Four field-collected adult female flies were introduced into each cage and allowed to feed for 24 h, after which stipples and eggs were counted with a dissecting microscope.

To control for differences in plant architecture between sun- and shade-derived bittercress, we conducted a detached leaf assay using cauline leaves clipped from the flowering stalk of first or second lowest position of plants from sun or shade habitats. For each of 15 replicate trials, two leaves each from sun and shade plants were inserted by their petioles into a half liter-sized transparent plastic container filled to a depth of 1.5 cm with 2% Phytoblend (Caisson Laboratories, Logan, Utah, USA). Leaves were randomly assigned to positions for each assay container, which was closed with a plastic and mesh lid (see Appendix S2: Fig. S1B for a schematic). We introduced one field-caught adult female fly into each container and allowed it to forage for 24 h, after which we counted stipples and eggs. No flies were used for multiple trials.

For both assays, we modeled stipple and egg counts with NB mixed models using *glmmTMB*, with plant habitat (sun vs. shade), number of cauline leaves (for whole plants), and leaf width (for detached leaves; mm) as fixed effects, and cage ID (i.e., replicate assay) as a group-level random intercept term. We evaluated support for habitat-specific estimates of θ as above. We assumed that adult female *S. nigrita* flies had sufficient time to potentially visit all available plant tissue; thus, a priori we favored models without ZI terms.

Host choice experiment II: Effects of light and temperature

In 2014 and 2015, we conducted choice experiments in the laboratory and field to decouple the effects of light and temperature on *S. nigrita* foraging behavior. At two-day intervals, we conducted six trials using both sun-warmed and shade-cooled large mesh cages (35.5 × 35.5 × 185 cm) where one side of each cage was randomized to receive a lighting treatment. Ten undamaged bolting bittercress ramets were collected near RMBL along the Copper Creek Drainage (CCD; Appendix S1: Fig. S1) and were potted and maintained in the laboratory as above for up to 4 d prior to each trial. Four leaves from each of the 10 plants were detached at the petiole and randomized to each of four experimental conditions (2 cage-level temperature treatments × 2 light environments per cage). Leaf petioles were fixed with a moist paper towel in 100-mm Petri dishes placed at either end of the cages (Appendix S2: Fig S2). Ten *S. nigrita* adult females were collected along the CCD (Appendix S1: Fig. S1), released into the middle of each cage, and allowed to forage for 24 h starting at 1100 hours. Additional details on the methods and design of these cage experiments can be found in Appendix S2.

For the 2014 laboratory-choice trials, two large mesh cages were placed into temperature-controlled environmental chambers that were either cooled or held at ambient temperature (~16°C and ~21°C, respectively; Appendix S4: Fig. S1). Plants, leaves, and flies were collected and utilized as above, and flies were allowed to feed for 8 hours (1100–1900 hours) during each trial. We carried out similar trials in 2015 but in a single environmental chamber at 2-day intervals, alternating between approximately 20° and 24°C (Appendix S4: Fig. S1). Leaves were obtained along the CCD (Appendix S1: Fig. S1) and were randomized across treatments as before. Baseline temperatures in 2015 were elevated by 4°C relative to 2014 (Appendix S4: Fig. S2). In addition to stipples, we counted eggs deposited by *S. nigrita*, which were not counted in 2014 because our experiment began later in the season when adult females were not often gravid.

We modeled stipple and egg counts using NB mixed models using *glmmTMB* with the following fixed effects: leaf width (mm^2), leaf position along stem from which it was removed (“position”), light environment (light vs. dark,), temperature (warm vs. cool,), and an interaction term between temperature and light environment. We modeled between-trial, between-room, between-cage, and between-side-of-cage effects as a series of nested random intercept terms. For 2015 trials, we also included plant source habitat (sun vs. shade) as a fixed effect. Finally, we evaluated support for inclusion of condition-specific NB dispersion parameters via *AICc* comparisons to the best-fit model without such terms, as above.

For all analyses, statistical significance of fixed effects was assessed at the $P < 0.05$ level via asymptotic Wald’s z-tests. Average differences in herbivore damage counts reported in the results are predicted mean (with predicted 95% confidence interval) of the response variable generated via 1000 simulations from the best-fitting model, using the maximum-likelihood point estimate of model coefficients.

RESULTS

Herbivory surveys

Across 15 field sites, naturally occurring stippling and leaf miner damage from *S. nigrita* was strongly biased toward bittercress in sun habitats compared to shade habitats (Fig. 1A). Compared to plants in shade habitats, leaves of plants in the sun showed an overall fivefold higher stipple abundance (16.1 [12.5–20.4, 95% CI] vs. 3.2 [2.3–4.3] mean stipples per leaf, $P < 0.001$; Table 1). We estimated that 29% (20–37%, 95% CI) of leaves in the shade avoided stippling altogether, compared to only 9% (5–14%) of leaves in the sun. Sun plants also had a > 40-fold higher overall leaf mine abundance (3.7 [2.0–6.7] vs. 0.09 [0.03–0.21] mean mines per leaf; $P < 0.001$; Table 1), and 93% (88–98%) of leaves in shade habitats had no leaf mines at all, compared to only 29% (19–40%) in the sun.

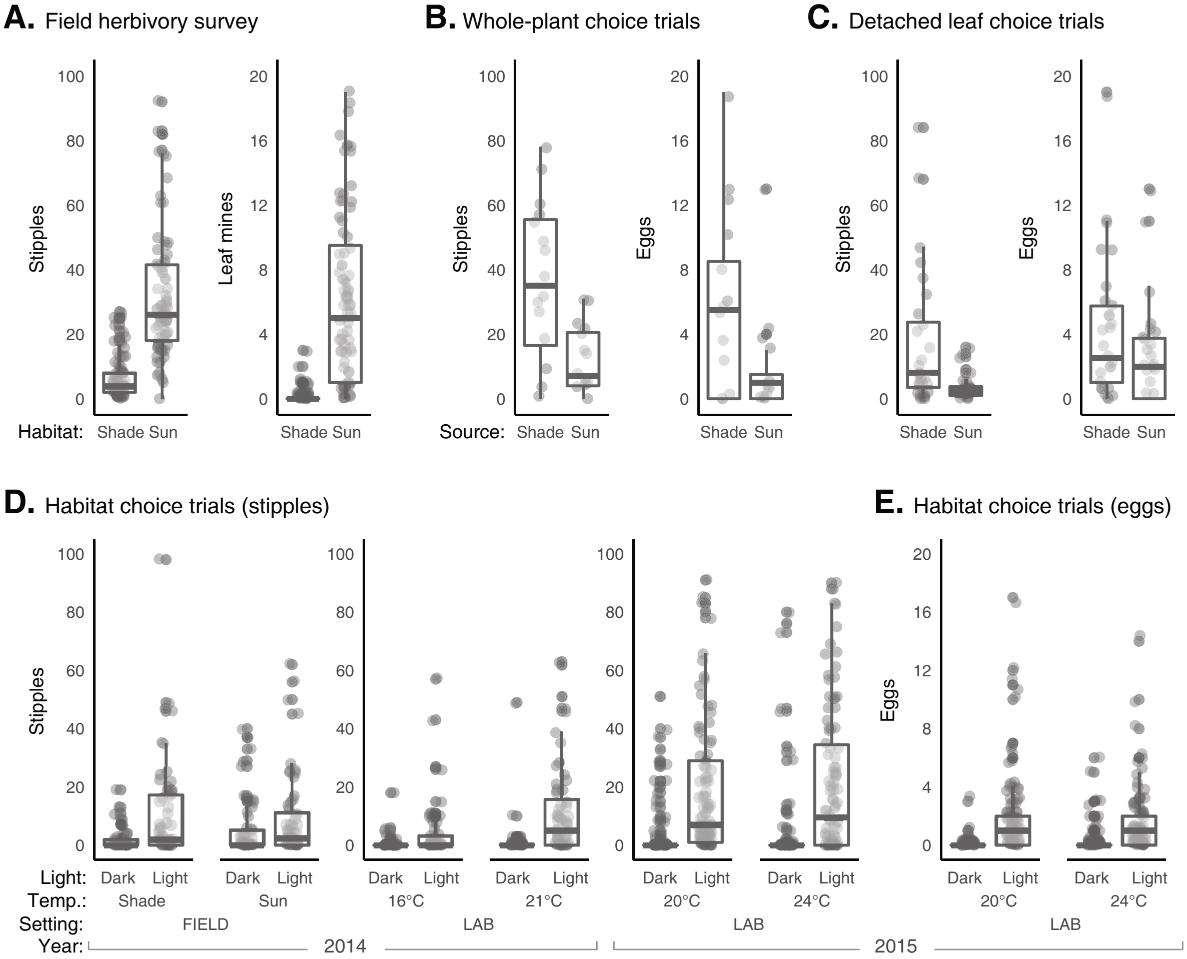

Fig. 1. Choice experiments reveal that habitat selection, and not host selection, by foraging *Scaptomyza nigrita* underlies the pattern of sun-skewed herbivory in nature, despite higher palatability of bittercress from the shade. (A–C) Herbivory is higher on bittercress in sun vs. shade habitats, but female *S. nigrita* prefer shade-grown over sun-grown bittercress when given a choice under uniform lighting. (A) Herbivory field surveys show higher stipples and mines on bittercress in sun vs. shade habitats. (B–C) Adult female *S. nigrita* stippled and laid more eggs in bittercress derived from shade vs. sun habitats in laboratory-choice trials. (D–E) Female *S. nigrita* stippled more (D) and laid more eggs (E) in bittercress leaves in simulated sun compared to shade habitats in field and laboratory-choice trials. Choice trials between light and dark sides of assay cages were conducted at two temperatures (see Appendix S4: Fig. S1 for full temperature profiles) indicated below subplots. Eggs were measured only for trials in 2015 (see *Materials and methods*). Statistical results are presented in Table 1.

Host choice experiment I: Sun- vs. shade-derived bittercress

In contrast to patterns revealed in the herbivory survey, *S. nigrita* female flies strongly preferred feeding and laying eggs on bittercress from shade habitats when given a choice of whole plants from shade or sun habitats under uniform light conditions (Fig. 1B). Compared to shade-source plants, overall stipple abundance on sun-source plants was 60% lower (1.4 [0.6–3.1] vs. 4.5 [1.9–9.9] mean stipples per leaf, $P < 0.01$). Female flies left sun-source leaves free of stipples 71% (61–80%) of the time, compared to only 43% (30–55%) of the time for shade-source plants. Overall egg abundance was 75% lower on sun-source plants (0.13 [0.05–0.25] vs. 0.64 [0.32–1.10] mean eggs per leaf, $P < 0.01$; Table 1), and 93% (88–96%) of sun-source leaves remained free of eggs compared to 80% (74–87%) of shade-source leaves.

When individual leaves were offered instead of whole plants (Fig. 1C), overall stipple abundance was >80% lower for sun-source leaves (4.8 [1.8–10.9] vs. 27.9 [12.1–60.8] mean stipples per leaf, $P < 0.001$). While a similar proportion of leaves from each habitat received zero eggs (shade, 24% [10–40%]; sun, 35% [20–53%]), overall egg abundance for sun-source plants was 45% lower than for shade-source plants (2.5 [1.2–4.3] vs. 4.4 [2.3–8.0] mean eggs per leaf). This difference was marginally significant ($P = 0.065$; Table 1), and an intercept-only model was favored via model comparisons ($\Delta AIC_c = -1.2$).

Host choice experiment II: Effects of light and temperature

In the 2014 field trials, *S. nigrita* strongly preferred feeing in the lighted sides of the cages over the unlighted sides of the cages (Fig. 1E). Overall stipple abundance was fourfold higher on plants under lights compared to those under shade (18.1 [6.3–52.8] vs. 4.0 [1.5–9.9] mean stipples per leaf, $P < 0.001$; Table 1). We detected no effect of cage warming, and removal of temperature terms was statistically favored via model comparisons ($\Delta AIC_c = -1.2$). *S. nigrita* preference for feeding in the light was strong in both 2014 and 2015 laboratory-choice trials (Fig. 1E): Average stipple abundance was eightfold higher in 2014, and fivefold higher in 2015, on leaves in the light compared to the unlighted sides of the cages (2014, 9.7 [4.5–19.3] vs. 1.1 [0.3–2.9] mean stipples per leaf; 2015, 38.5 [13.8–93.9] vs. 6.9 [1.5–20.7]; both $P < 0.001$, Table 1). For 2014, warmed cages exhibited a marginally significant 2.5-fold increase in stipple abundance ($P = 0.094$, Table 1), while for 2015 trials we detected no temperature effect and removal of this term was statistically favored ($\Delta AIC_c = -2.0$). When both years' data were pooled, temperature remained non-significant, and all other results were qualitatively unchanged (Appendix S3: Table S2).

In warmed cages, egg abundance was 4.5-fold higher on leaves under lights than those in the unlighted sides of cages (Fig. 1F; 1.6 [0.8–2.7] vs. 0.36 [0.1–0.8] mean eggs per leaf, respectively; $P < 0.001$, Table 1). Cooler temperatures reduced egg laying sixfold in the dark (down to 0.05 [0–0.12] mean eggs per leaf) but had no effect in the light (light-by-temperature interaction term $P < 0.01$; Table 1). Notably, plant source habitat (sun vs. shade) did not impact stippling ($P > 0.2$) or egg ($P > 0.7$) abundances (Appendix S5: Fig. S1), and inclusion of this term was never supported via model comparisons using AICc.

Discussion

Here, we report evidence of a proximate explanation for a textbook case of an herbivore-driven habitat distribution: Insect behavioral taxis strongly biased herbivore foraging toward sun habitats, causing the increased herbivore pressure on sun plants that drives bittercress into the shade. Consistent with the pattern found by Louda and Rodman (1996), we found that overall herbivore pressure was higher on bittercress naturally growing in sun than in shade habitats (Fig. 1A). Contrary to this sun-biased herbivory pattern in the field, *S. nigrita* strongly preferred bittercress from shade when given a choice (Fig. 1B–C). But when we manipulated the abiotic conditions under which herbivores foraged, a strong preference for bright habitats emerged (Fig. 1E) that overrode their preference for plants from shade habitats. In fact, warmer temperatures and high light levels combined to drive herbivory into simulated sun habitats: The fewest eggs were laid on leaves away from lights and in cooler cages (Fig. 1F). Thus, the distribution of herbivore pressure across the sun/shade ecotone that drives bittercress into the shade (Louda and

Table 1. Coefficient estimates for herbivory models across habitats and choice trials.

Dataset	Term type	Coefficient	Response A†	Response B
Field survey				
NB (count)	Fixed	Intercept [shade]	0.79 [0.41, 1.18]***	−3.26 [−4.27, −2.251]***
		Site type [sun]	1.63 [1.23, 2.04]***	3.75 [2.73, 4.77]***
		Leaf area (mm^2)	0.01 [0.004, 0.02]***	0.02 [0.01, 0.03]***
	Random	Stem ID	0.44 [0.34, 0.559]	0.13 [0.001, 14.72]
		Site ID	0.31 [0.18, 0.536]	0.65 [0.39, 1.08]
	Dispersion	ϕ_{shade}	1.45 [0.84, 2.483]	0.24 [0.04, 1.38]
	Dispersion	ϕ_{sun}	16.36 [7.86, 34.075]***	4.02 [1.62, 9.98]***
	Zero-inflation	π_0	0.09 [0.06, 0.14]***	0.14 [0.08, 0.26]***
Host choice				
Whole plants	Fixed	Intercept [shade]	1.82 [1.06, 2.60]***	1.08 [0.15, 2.01]*
NB (count)		Source type [sun]	−1.10 [−1.90, −0.30]**	−1.48 [−2.4, −0.56]**
		Leaf position	−0.19 [−0.26, −0.12]***	−0.43 [−0.58, −0.29]***
	Random	Stem ID	0.94 [0.57, 1.56]	0.69 [0.21, 2.28]
		Cage ID	0.57 [0.21, 1.60]	0 [0, ∞]
	Dispersion	ϕ_{shade}	0.59 [0.42, 0.86]	0.27 [0.14, 0.54]
		ϕ_{sun}	0.30 [0.19, 0.47]	$\phi_{sun} = \phi_{shade}$
Leaves	Fixed	Intercept [shade]	2.27 [0.76, 3.78]**	0.93 [−0.69, 2.54]
NB (count)		Source type [sun]	−1.77 [−2.34, −1.2]***	−0.57 [−1.18, 0.04]‡
		Leaf area (mm^2)	0.03 [−0.06, 0.13]	0.02 [−0.09, 0.13]
	Random	Cage ID	1.07 [0.6, 1.89]	0.75 [0.31, 1.79]
	Dispersion	ϕ	1.15 [0.71, 1.86]	1.15 [0.56, 2.4]
Habitat choice	Fixed	Intercept [shade]	−1.29 [−2.36, −0.22]*	−2.39 [−3.97, −−0.80]**
2014		Light environment [sun]	1.31 [0.46, 2.17]**	2.12 [1.02, 3.22]***
NB (count)		Temp [warm]	–	0.90 [−0.15, 1.96]•
		Light × temp [light:warm]	–	–
		Leaf width (mm)	0.07 [0.04, 0.09]***	0.07 [0.02, 0.12]**
	Random	Side of cage	0.81 [0.43, 1.52]	0.86 [0.39, 1.93]
		Cage ID	0 [0, ∞]	0 [0, ∞]
		Batch ID	0.40 [0.08, 2.04]	0 [0, ∞]
	Dispersion	ϕ_{shade}	0.24 [0.15, 0.38]***	0.06 [0.03, 0.12]***
		ϕ_{sun}	0.45 [0.32, 0.62]***	0.19 [0.11, 0.34]***
		δ_{warm}§	–	0.94 [0.28, 1.59]**
2015	Fixed	Intercept [shade]	0.47 [−0.86, 1.80]	−4.86 [−6.59, −3.13]***
NB (count)		Light environment [sun]	1.67 [1.04, 2.30]***	3.62 [2.40, 4.84]***
		Temp [warm]	–	2.09 [0.54, 3.64]**
		Light × temp [light:warm]	–	−2.13 [−3.62, −0.65]**
		Leaf width (mm)	0.02 [−0.02, 0.06]	0.06 [0.02, 0.09]**
		Source habitat [sun]	−0.27 [−0.67, 0.14]	−0.06 [−0.44, 0.32]
	Random	Side of cage	0 [0, ∞]	0.33 [0.03, 3.57]
		Cage ID	1.41 [0.94, 2.12]	0.36 [0.04, 3.07]
		Batch ID	0 [0, ∞]	0.44 [0.15, 1.32]
	Dispersion	ϕ_{shade}	0.07 [0.05, 0.10]***	0.11 [0.05, 0.27]
		ϕ_{sun}	0.52 [0.41, 0.65]***	0.89 [0.59, 1.34]***

Notes: NB refers to negative binomial.Term types are as follows: Fixed coefficients are log rates (intercept) or log rate ratio estimators; random effect estimators are given on standard deviation scale; dispersion parameter estimators are given on data scale; (P values for ϕ are under the null hypothesis that $\ln(\phi) = 0$; zero-inflation estimators are given on probability scale [0–1]. Responses are as follows: For field surveys, response A is stipples and response B is leaf mines; for host choice for both whole plants and leaves, response A is stipples, and response B is eggs; for habitat choice in 2014, response A and response B are stipples; for habitat choice in 2015, response A is stipples and response B is eggs.

† Response *A* for 2014 habitat choice trials corresponds to data collected under simulated field conditions; all other choice trials were done in laboratory settings (see *Methods*).

‡ $0.05 \leq P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; 95% Wald-type CIs appear in square brackets.

§ Coefficient (log rate ratio) for effect of temperature on dispersion parameter.

• $0.05 \leq P < 0.10$.

Rodman 1996) results from a direct effect of the abiotic environment on herbivore behavior.

A variety of adaptive and non-adaptive explanations can be posited to explain a strong habitat preference for feeding and oviposition by *S. nigrita*. In particular, oviposition preference should reflect the habitat distribution where larvae have the greatest probability of survival (Craig et al. 2000). While adult herbivore feeding and oviposition preference do not necessarily predict larval performance (Craig et al. 1999, 2000), evidence from a recent study supports the notion that shade-source bittercress are, in fact, higher quality for *S. nigrita* than sun-source plants: Short-term larval performance was higher in shade-source plants compared to sun-source plants when both were regrown in shade habitats (Humphrey et al. 2018). This is consistent with preferences of adult *S. nigrita* for shade-derived plants reported in this study and adds to the evidence that *S. nigrita* foraging preferences for certain plant tissues over others generally reflect differences in plant quality for larvae (Humphrey et al. 2016, 2017). Measuring fitness through the entire life cycle will be essential for testing the hypothesis that choosing shade-source plants is (or would be) adaptive for *S. nigrita*.

Alternatively, a non-adaptive (or maladaptive) explanation is that herbivore attraction to light is too strong to permit foraging on the higher quality plants in nearby shade habitats. We regard the constraint hypothesis as intriguing but implausible, because phototactic behavior can vary plastically and genetically both between and within species of drosophilids (Gorostiza et al. 2016). The fact that it persists in *S. nigrita* suggests that there may be benefits to feeding in warm, sunny habitats at >3000 m in elevation that outweigh any advantages to feeding on the more palatable host plants in the shade. Cool temperatures restrict the ability of insects to oviposit on available host plants, even when abundant, because temperatures are too low for flight (Kingsolver 1989). This may explain why insects are often restricted to sunny habitats (Huffaker and Kennett 1959), areas experiencing sunny weather (Whitman 1987), or areas within a plant exposed to the sun, regardless of plant quality (Casey 1993). Separate from thermal tolerance, perception may simply be more efficient in the sun, where flies may rely on visual cues or the clumped distribution of host plants (Wallace 1958, Vernon and Gillespie 1990). Exploring how thermal tolerance, insect perception, and variation in host-plant quality interact to reinforce herbivore habitat preferences is a promising future research direction in this system.

Establishing whether herbivore- or plant-centric mechanisms shape the distribution and/or abundance of herbivores is crucial for understanding the nature of the selective forces that promote habitat specialization. Even in well-studied systems (Bruelheide and Scheidel 1999, Fine et al. 2004, 2006, 2013), the mechanisms (plant-centric or herbivore-centric) responsible for the differential herbivore pressure that shapes plant habitat distributions have been difficult to ascertain. In the Amazon, for example, insect herbivores promote habitat specialization by polarizing the allocation strategies best suited toward resource-rich clay vs. resource-poor white-sand habitats (Fine et al. 2004). Clay habitats which favor increased plant growth over anti-herbivore defenses also tend to have higher herbivore pressure (Fine et al. 2006) and herbivore abundances (Fine et al. 2013), but whether this is a consequence of reduced plant defenses or herbivore-centric mechanisms has not been addressed. Our study on a textbook system is novel because it provides a direct test of whether habitat-biased herbivory arises from herbivores tracking plant quality or from herbivore behaviors that arise independent of (or even in spite of) differences in plant quality. Our work suggests that the abiotic environment has a direct effect on maintaining enemy-free space in shade habitats. This, in combination with early-season plant phenological escape from herbivory (Collinge and Louda 1989*a,b*), drives the distribution of bittercress toward shade habitats. As a consequence, strong herbivore habitat preference—regardless of its adaptive value for the insect—likely alters the nature and strength of selection on plant defense strategies across this sun/shade ecotone (Humphrey et al. 2018).

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