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

Nicolas M. Alexandre[1,2,3,†], Parris T. Humphrey[2,3,4,†], Joseph Frazier[2], Andrew D. Gloss[2,3,5], Jimmy Lee[2], Henry A. Affeldt III[2], Noah K. Whiteman[1,2,3*]

[1] Department of Integrative Biology, University of California, Berkeley, CA 94720.

[2] Rocky Mountain Biological Laboratory, Crested Butte, CO 81224.

[3] Department of Ecology & Evolutionary Biology, University of Arizona, Tucson, AZ 85721.

[4] Department of Organismic & Evolutionary Biology, Harvard University, Cambridge, MA 02138.

[5] Department of Ecology & Evolution, University of Chicago, Chicago, IL 60637.

† *equal contribution.*

*Author for correspondence: NKW, whiteman@berkeley.edu, phone: +1 520-696-3950

Running title: Herbivore habitat choice shapes herbivory

Article type: Note

Author contributions: NMA, ADG, JL, HAA, and NKW designed the experiments; NMA, ADG, JL, HAA, and JF collected the data; PTH and NMA analyzed the data; NMA, PTH and NKW wrote the paper. All authors contributed to editing the submission.

Data accessibility: All data will be deposited in the Dryad data repository (doi pending).

Abstract

Habitat distributions of plants are often driven by abiotic factors, but growing evidence suggests an important role for consumers. A textbook example of consumers limiting the habitat distribution of a plant is in bittercress (*Cardamine cordifolia*). Bittercress is more abundant in shade than in sun habitats, and this is thought to arise because herbivore pressure is lower in the shade. Yet we still do not understand why herbivory is lower in the shade. Herbivores may avoid shaded bittercress because the plants are lower quality, or because herbivores simply prefer brighter, warmer habitats. We tested these alternative hypotheses through a series of herbivore choice experiments. *Scaptomyza nigrita*, a locally abundant specialist and dominant herbivore of bittercress, strongly preferred feeding and laying eggs on bittercress we collected from shade versus sun habitats. Thus, shaded bittercress are more, not less, palatable to these herbivores. Separately, *S. nigrita* strongly preferred feeding and laying eggs on leaves held in treatments that simulated sun rather than shade habitats—regardless of whether leaves came from sun or shade habitats originally. The underlying mechanism for an herbivore-driven plant distribution appears to be a simple behavioral preference of herbivores for brighter, warmer habitats.

Keywords: *Brassicaceae*, bittercress, *Drosophilidae,* leaf miner, *Scaptomyza*, herbivory.

Introduction

Habitat distributions of plants are thought to be shaped primarily by abiotic environmental gradients (Whittaker 1967, Sexton and Dickman 2016), but there is growing evidence that consumers can have a major impact as well (Maron and Crone 2006). A series of studies in the 1980–90s on bittercress (Brassicaceae: *Cardamine cordifolia*) in the Elk Mountains of Colorado

47 were among the first to explore the role of herbivory in shaping the fine-scale distribution of a
48 plant species (Collinge and Louda 1988, 1990; Louda 1984; Louda and Rodman 1983, 1996),
49 and this system serves as a textbook example of biotic drivers of plant distributions (Ricklefs and
50 Miller 2000). Adult females of *Scaptomyza nigrita* flies (Drosophilidae) are the primary
51 herbivores of bittercress and create feeding punctures in leaves with serrated ovipositors
52 (Collinge and Louda 1988). Their larvae, which form leaf-mines, can defoliate up to 75% of leaf
53 area from bittercress in sun habitats (Collinge and Louda 1988). Louda and Rodman (1996)
54 argued that fitness effects of high herbivory in sun habitats were strong enough to drive
55 bittercress into shade habitats, where herbivory was low. But over 20 years later, we still do not
56 understand the causes of differential herbivory across this sun–shade ecotone. Louda and
57 Rodman (1996) proposed two mechanisms to explain this pattern. Plants in sun habitats could be
58 less resistant to herbivores, likely due to water stress, than those in shade habitats. Herbivores
59 were also more abundant and active in sun habitats (Louda and Rodman 1996), leading them to
60 propose as an alternative that herbivores prefer warmer, brighter habitats (Louda and Rodman
61 1996). We addressed these alternative hypotheses in order to better understand the factors that
62 drive a textbook case of consumer-driven habitat limitation for a native plant.
63 Because previous herbivory data were collected nearly 30 years ago (Louda 1988), we
64 first verified that *S. nigrita* herbivory remained higher in sun relative to shade habitats. Second,
65 we tested the plant quality hypothesis by offering *S. nigrita* females a choice between sun- and
66 shade-derived bittercress under laboratory conditions. Finally, we conducted choice trials in
67 laboratory and field settings in which we manipulated the light and temperature environment of
68 plants within choice trials to measure how these abiotic variables impacted *S. nigrita* foraging
69 patterns, using plants collected from both sun and shade habitats. We found that *S. nigrita* prefer

70 bittercress from the shade, yet their strong attraction to plants held in bright and warm habitats is
71 sufficient to override this preference for shade-derived leaves. Our data lead us to reject the plant
72 quality hypothesis and instead conclude that the habitat preference of this herbivore for brighter,
73 warmer habitats underlies the higher rates of herbivory in sun habitats. Thus, the habitat
74 distribution of bittercress arises from the habitat preferences of its dominant herbivore.
75

76 **Materials & Methods**

77 *Herbivory surveys.* All experiments were conducted between 2010 and 2015 at the Rocky
78 Mountain Biological Laboratory (RMBL) in Gothic, CO, USA. In 2011, we conducted field
79 surveys of herbivore damage on bittercress in nine sun habitats (no tree canopy) and nine shade
80 habitats (dense evergreen tree canopy present) (Appendix S1: Fig. S1). Shade and sun sites were
81 interspersed geographically and in elevation and systematically differed in photosynthetically
82 active radiation, % shade cover, and nearby canopy tree size (Appendix S1: Table S1). We
83 recorded adult *S. nigrita* feeding punctures (stipples) and larval mines in two basal leaves from
84 each of ten ramets from the same bittercress patch (n=180 observations per habitat type).
85 We modeled stipple and mine counts using zero-inflated negative binomial (ZINB)
86 generalized linear models. We chose this model because a zero count can arise because local
87 herbivore abundance is too low ('false' zeros, Zuur et al. 2009). These are distinct from the
88 expected proportion of zeros arising from non-truncated count distributions such as Poisson or
89 NB ('true' zeros, Zuur at al. 2009). While all zeros in our dataset are meaningful, we modeled
90 whether a leaf belongs to the putatively un-sampled (i.e. 'false zero') class with probability π_0, as
91 a function of the fixed effects of source habitat (sun vs. shade) and leaf area (mm^2), using the
92 canonical logit link function in a binomial GLM. The count distribution containing the 'true'

93 zero class [with probability $(1-\pi_0)$] was simultaneously fit under a NB distribution with a log
94 link function, with habitat (sun vs. shade), leaf area, and an arbitrary leaf ID (two-levels)
95 modeled as fixed effects. Coefficients were estimated using maximum likelihood using **R** v3.3.3
96 (R Core Team 2017) package *pscl* v1.4.9. (Jackman 2015). We further describe our statistical
97 approach in detail in Appendix S3.

98 *Host choice experiment I: Sun versus shade-derived bittercress.* To test whether *S.*
99 *nigrita* adult females prefer feeding on individual bittercress derived from sun or shade habitats,
100 we transplanted bittercress plants from the field into soil within plastic pots and placed in the
101 laboratory under fluorescent lighting (16:8 light:dark) for < 24 h. In each of eight replicates, we
102 randomly assigned two shade-derived and two sun-derived bittercress plants to the four corners
103 of a mesh 35.5 x 35.5 x 61 cm cage (livemonarch.com). All ramets were un-mined, and we
104 subtracted pre-existing stipple damage from final counts. In each cage we placed two petri dishes
105 (100 mm diameter) containing 100% recycled paper towels: one moistened with a 5% sucrose
106 solution, and one with tap water. Four, field-collected adult female flies were introduced into
107 each cage and allowed to feed for 24 h, after which stipples and eggs were counted using a
108 dissecting microscope. See Appendix S2: Fig. S1A for a schematic.

109 To control for differences in plant architecture between sun- and shade-derived
110 bittercress, we conducted a detached leaf assay using cauline leaves clipped from the first or
111 second position from sun or shade habitats. For each of 15 replicate trials, two leaves each from
112 sun and shade plants were inserted by their petioles into a half liter-sized plastic container filled
113 with 1.5 cm of 2% Phytoblend (Caisson Laboratories, Logan, UT). Leaves were randomly
114 assigned to positions for each assay container, which was closed with a mesh lid. We introduced
115 one field-caught adult female fly into each container and allowed it to forage for 24 h, after

116 which we counted stipples and eggs as above. No flies were used for multiple trials. See
117 Appendix S2: Fig. S1B for a schematic.
118 For both assays, we modeled stipple and egg counts using NB GLMs with plant habitat
119 (sun vs. shade), number of cauline leaves (for whole-plants), and leaf width (for detached leaves;
120 mm) as fixed effects, and cage ID (i.e. replicate assay) as a random intercept. Coefficients were
121 estimated via maximum likelihood using **R** package *lme4* v1.1-13 (Bates et al. 2015). For
122 comparisons to Poisson and zero-inflated models see Appendix S3.
123 *Host choice experiment II: Effects of light and temperature.* In 2014 and 2015 we
124 conducted choice experiments to decouple the effects of light and temperature on *S. nigrita*
125 foraging behavior. In 2014, multiple sets of trials were conducted in a temperature-controlled
126 laboratory setting as well as in a field setting. In both settings, we manipulated light levels at one
127 end of large mesh cages (35.5 x 35.5 x 185 cm) and performed choice assays under warmer and
128 cooler air temperatures. For the field trials, we placed two mesh cages lengthwise on the ground
129 in adjacent sun and shade plots at a site at RMBL (Appendix S1: Fig. S1). Each cage was placed
130 under a large wooden picnic table wrapped in reflective Mylar to protect the surface of each
131 mesh cage from sun exposure. Shade cloth (70% opacity) was then used to wrap each cage until
132 ambient light was similar to that of shade habitats. We used a light meter to ensure ambient light
133 was equalized between the two cages. Within each cage, we established a light gradient by
134 affixing two LED lights (18–20 lumens, 7000K lights, LX-8058, Gemini, USA) via hooks at 14
135 and 21 cm from the bottom of one of two 1.25 cm thick plywood boards placed vertically against
136 both far sides of each cage. Both plywood boards had a 20 x 30 cm sheet of aluminum foil
137 affixed to them, which focuses the light beam on detached leaves on the light treatment side.
138 Adult female flies, when placed into the middle of the cage at the start of the experiments, were

139 not within line of sight of the source of the light source. Two data-loggers were mounted on each
140 board (Thermocron iButton DS1921G, Maxim, USA) to continuously measure temperature in
141 the cages. See Appendix S2: Fig. S3 for a schematic.

142 At two-day intervals, we conducted six trials using both sun-warmed and shade-cooled
143 cages at once. One side of each cage was randomized to receive the LED light treatment and the
144 other side without light. Ten un-damaged bittercress plants were collected near RMBL from
145 along the Copper Creek Drainage (Appendix S1: Fig. S1) and maintained in pots in the
146 laboratory for ≤ 4 d prior to each trial. Four leaves from each of the 10 plants were detached at
147 the petiole and randomized to each of four experimental conditions (two cage-level temperature
148 treatments X two light environments per cage). Each group of ten leaves was then placed into
149 petri dishes (100 mm) with petioles wrapped in a moistened 100% recycled paper towel. The two
150 sides of a cage contained ten petri dishes, each with a leaf (Appendix S3: Fig S2). Ten *S. nigrita*
151 adult females were collected near the RMBL along the Copper Creek drainage (Appendix S1:
152 Fig. S1) and released into the middle of each cage. Flies foraged for 24 hours starting at 1100 h.

153 For the six 2014 laboratory choice trials, the same two cages were placed without Mylar-
154 table overheads into temperature-controlled environmental chambers. One cage was placed into
155 each chamber, which was either cooled or held at ambient temperature (~16°C and ~21°C,
156 respectively; Appendix S4: Fig. S1). Plants, leaves, and flies were collected and utilized as
157 above, except that flies were allowed to feed for 8 hours (1100–1900 h) during each trial. LED
158 and data-logger placement in cages were the same for each cage as in the field trials.

159 We carried out similar trials in 2015 but in a single environmental chamber at two-day
160 intervals, alternating between two temperatures (approximately 20 °C and 24 °C; Appendix S4:
161 Fig. S1). Leaves were obtained from plants in sun and shade habitats along the Copper Creek

162 Drainage near the RMBL (Appendix S1: Fig. S1) and were randomized with equal representation
163 of sun and shade-derived leaves across treatments. Baseline temperatures in 2015 were elevated
164 by 4 °C relative to 2014 (Appendix S4: Fig. S2). In addition to stipples, we counted eggs
165 deposited by foraging *S. nigrita*, which were not counted in 2014 because our experiment began
166 later in the season when adult females were less likely to be gravid.

167 For all 2014 trials, we modeled stipple and egg counts using NB generalized linear mixed
168 models (GLMMs) with the following fixed effects: leaf width (mm^2), leaf position along stem
169 from which it was removed ('position'), light environment (light vs. dark), temperature (warm
170 vs. cool), as well a fixed interaction term between temperature and light environment, which
171 estimates how the effect of light differs depending on temperature. We modeled between-trial,
172 between-room, between-cage, and between-side-of-cage effects as a series of nested random
173 intercept terms. For both years, and for both stipple and egg intensity in 2015, we modeled
174 counts with NB GLMMs using **R** package *lme4*. For 2015 trials, we included plant source habitat
175 (sun vs. shade) as a fixed effect. For all of the statistical models, statistical significance of fixed
176 effects was assessed at the $p \leq 0.05$ level via asymptotic Wald tests (see Appendix S2 for details).
177

178 **Results**

179 *Herbivory surveys.* Bittercress plants in shade habitats had a lower prevalence of stippling (odds
180 ratio ['OR'] = 0.36 [0.16–0.82 c.i.]) and were less than one tenth as likely to have leaf mines
181 (OR = 0.08 [0.02–0.42 c.i.]) than bittercress in sun habitats (Table 1; Binomial model). Average
182 stippling intensity was over four times higher in sun (rate ratio ['RR'] = 4.45 [3.6–5.5 c.i.]), and
183 leaf miner damage was over 10 times in sun (RR = 11.5 [3.3–39.3 c.i.]; Table 1, 'NB model')
184 than in shade habitats (Fig. 1A, Table 1). Larger leaves were more likely to be damaged and had

185 a higher average stippling and mining intensity (Table 1). Leaf sizes substantially overlapped
186 between habitats but were slightly larger for shade habitats (Appendix S1: Table S2).
187 *Host choice experiment I: Sun versus shade-derived bittercress.* In laboratory choice
188 tests, *S. nigrita* female flies strongly preferred feeding and laying eggs on shade-derived
189 bittercress. Sun-derived plants received only a third as much stippling (RR = 0.33 [0.22–0.51
190 c.i.]), and a quarter as many eggs (RR = 0.25 [0.11–0.55 c.i.]) compared to shade-derived plants
191 (Fig. 1B, Table 1). Results were similar in the detached leaf assay (Appendix S3: Fig. S2, Table
192 1). Leaf area did not affect the results (Table 1).
193 *Host choice experiment II: Effects of light and temperature.* In the 2014 field trials,
194 stippling intensity was eight times higher on plants under lights compared to those not under
195 lights (RR = 7.9 [2.6–24.5 c.i.]; Fig. 2A, Table 2), and stippling intensity in warmer cages was
196 three times higher than in cooler cages (RR = 3.1 [1.00–9.54 c.i.]; Fig. 2A, Table 2). There was
197 no interaction of Light x Temperature on stippling intensity (Table 2).
198 *S. nigrita* exhibited a similarly strong light preference in both 2014 and 2015 laboratory
199 choice trials. Stippling intensity was six times higher in 2014 (RR = 6.0 [1.16–31.06 c.i.]) and
200 eight times higher in 2015 (RR = 8.7 [3.2–23.8 c.i.]) on leaves under lights compared to those
201 not under lights (Fig. 2A, Table 2). No effect of cage temperature was detected for stippling
202 (Table 2). In all models of stippling intensity, simpler models without an interaction term
203 between light and temperature were favored (Appendix S3: Table S5). When 2014 and 2015
204 were pooled, the results were unchanged (Appendix S3: Table S4).
205 Egg deposition intensity (measured in 2015 only) was over 30 times higher in leaves
206 under lights than those not under lights (RR = 36.5 [12.5–104.6 c.i.]) and over seven times
207 higher in warmer cages (RR = 7.1 [1.99–25.4 c.i.]) than in cooler cages (Fig. 2B, Table 2).

208 Overall, the model estimated similar egg laying intensities on leaves under lights at both
209 temperatures (Fig. 2B, Table 2), and the interaction term reflects that leaves not under lights
210 received more eggs in the warmed cages than in the cooler cages (Fig. 2B). Leaf size positively
211 impacted stippling intensity in 2014, and egg intensity in 2015 (Table 2). Plant source (sun vs.
212 shade) did not significantly impact stippling or egg intensity in the 2015 choice trials (Table 2).
213

214 **Discussion**

215 We found that herbivory was far higher in bittercress found in sun than in shade habitats (Fig.
216 1A, Table 1), the same pattern found nearly 30 years ago (Louda and Rodman 1996). To address
217 the two alternate hypotheses proposed by Louda and Rodman (1996) to explain this pattern—one
218 plant-centric (plant quality) and one herbivore-centric (herbivore habitat preference)—we
219 conducted a series of choice experiments allowing us to dissect the variables which may be
220 driving this pattern. We found that when given a choice between sun- and shade-derived
221 bittercress in the laboratory, female flies actually preferred bittercress from the shade (Fig. 1B,
222 Table 1). Thus, we did not find support for the hypothesis that herbivory is higher in sun habitats
223 because of higher plant quality. Given this result, we hypothesized that herbivores preferred the
224 brighter, warmer sun habitats. Choice tests in the field in which light levels and air temperature
225 were experimentally varied within cages allowed us to disentangle the effects of each variable on
226 herbivore preferences. We consistently found that female flies strongly preferred foraging on
227 leaves under lights compared to those not under lights when allowed to move across light
228 environments within cages (Fig. 2A, Table 2). In addition, stippling intensity higher in the
229 warmer habitat in the field and egg intensity higher at warmer temperatures in 2015 laboratory
230 trials (Fig. 2A, Table 2). The fewest eggs were laid on plants away from lights in cooler cages

231 (Fig. 2B, Table 2). Thus, warmer temperatures and high light levels combine to promote
232 herbivory in sun habitats, releasing bittercress in shade habitats from herbivory.

233 The preference of *S. nigrita* for sun habitats appears stronger than their preference for
234 shade-derived bittercress. We included leaves sampled from both sun and shade sites in our 2015
235 habitat choice trials. If host quality were a major driver of habitat specificity of *S. nigrita*
236 damage, we would expect to have observed higher damage levels on shade-derived than sun-
237 derived leaves placed under either light treatment.

238 The proximal mechanism behind this behavior could be a simple positive phototaxis or
239 thermotaxis. However, phototactic behavior is known to vary among individuals and strains of
240 *Drosophila melanogaster* and may be under active neuronal control (Gorostiza et al. 2016).
241 Given that attraction to light is a genetically labile trait yet persists in *S. nigrita,* we hypothesize
242 that there may be benefits to feeding in warm, sunlit habitats that outweigh any advantages to
243 feeding on the more palatable plants in the shade. However, it is completely unknown if the
244 observed preference of *S. nigrita* for sun habitats is adaptive. Habitat preference could be
245 adaptive due to phenological differences in host plant availability, lower parasitism in the shade,
246 or extended developmental times of larvae in the shade. Insects, as ectotherms, are highly
247 sensitive to the temperature of their environment (Sinclair et al. 2012). Cool temperatures restrict
248 the ability of insects to oviposit on available host plants, even when they are abundant, because
249 the temperature in such areas is too low for flight (Kingsolver 1989). This may explain why
250 insects are often restricted to sunny habitats (Huffaker and Kennett 1959; Kaufman 1968), areas
251 experiencing sunny weather (Whitman 1987), or areas within a plant exposed to the sun,
252 regardless of plant quality (Casey 1992). It could also be that *S. nigrita* uses visual cues to find
253 bittercress plants, its only known host, and as a result, has a reduced ability to find bittercress

growing under shade cover (Wallace 1958,Vernon and Gillepsie 1990). Bittercress plants in the shade are less clumped than those in the sun in distribution and often occur as single plants, potentially reducing encounters between *S. nigrita* and their hosts (Landa and Rabinowitz 1983, Finch and Collier 1994). Further studies are required to determine if light and temperature preferences of *S. nigrita* are adaptive or represent a constraint that limit the habitat distribution of this herbivore. The release of shade bittercress from herbivory could also promote adaptive divergence in plant defense strategies and responses to light between bittercress populations in sunlit versus shaded habitats, especially if the two habitats differ in flowering phenology; these questions await future study.

Our study adds to the evidence that herbivory has a major impact on fine-scale habitat distributions of plants. Even in the best-studied systems (e.g., Bruelheide and Schiedel 1999), the mechanisms driving differential herbivory have been difficult to ascertain. Here, we found a simple habitat preference of a specialist herbivore species for sunnier, warmer habitats in the sub-alpine environment of the Rocky Mountains. Regardless of the adaptive value for the flies, because of the high defoliation potential of larval leaf miners, this herbivore habitat preference may be sufficient to drive bittercress into the shade.

Acknowledgments

We thank Ian Billick (RMBL), Jennifer Reithel (RMBL), Kailen Mooney (UC-Irvine), Carol Boggs (University of South Carolina), Nick Waser (RMBL), Mary Price (RMBL), Brian Enquist (University of Arizona), and Tom Miller (Rice University) for feedback. Financial support was provided to N.K.W. by RMBL (Research Fellowships 2010–2013), the National Science Foundation Division of Environmental Biology (NSF DEB-1256758), the John Templeton

Foundation (41855) and the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R35GM119816; to P.T.H. by RMBL Graduate Fellowships (2011–2013), the University of Arizona Center for Insect Science, and the NSF (DEB-1309493); to A.D.G. by a NSF Graduate Research Fellowship and a RMBL Graduate Fellowship; and to H.A. and J.F. by RMBL undergraduate research awards.

Literature cited

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software, 67:1–48.

Bruelheide, H., and U. Scheidel. 1999. Slug herbivory as a limiting factor for the geographical range of *Arnica montana*. Journal of Ecology 87:839-848.

Casey, T.M. 1993. Effects of temperature on foraging of caterpillars. *Caterpillars: Ecological and Evolutionary Constraints on Foraging*. Chapman and Hall, New York.

Collinge, S.K., and S.M. Louda. 1988. Patterns of resource use by a drosophilid (Diptera) leaf miner on a native crucifer. Annals of the Entomological Society of America 81:733-741.

Collinge, S.K., and S.M. Louda 1989. *Scaptomyza nigrita* Wheeler (Diptera: Drosophilidae), a leaf miner of the native crucifer, *Cardamine cordifolia* A. Gray (Bittercress). Journal of the Kansas Entomological Society 62:1-10.

Finch, S., and R. Collier. 2000. Host-plant selection by insects–a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. Entomologia Experimentalis et Applicata 96:91-102.

Gorostiza, E.A., J. Colomb, and B. Brembs. 2016. A decision underlies phototaxis in an insect. Open Biology 6:160229.

Huffaker, C.B. and C.E. Kennett. 1959. A ten-year study of vegetational changes associated with biological control of Klamath weed. Journal of Range Management 12:69–82.

Humphrey, P.T., A.D. Gloss, N.M. Alexandre, M.M. Villalobos, M.R. Fremgen, S.C. Groen, L.N. Meihls, G. Jander, and N.K. Whiteman. 2016. Aversion and attraction to harmful plant secondary compounds jointly shape the foraging ecology of a specialist herbivore. Ecology & Evolution 6:3256-3268.

Jackman, S. 2015. *pscl*: Classes and methods for R developed in the political science computational laboratory, Stanford University. Department of Political Science, Stanford University, Stanford, CA. R package version 1.4.9.

Kingsolver, J.G. 1989. Weather and the population dynamics of insects: integrating physiological and population ecology. Physiological Zoology 2:314.

Landa, K., and D. Rabinowitz. 1983. Relative preference of *Arphia sulphurea* for sparse and common prairie grasses. Ecology 64:392-395.

Louda, S.M., and J. Rodman. 1983. Ecological patterns in the glucosinolate content of a native mustard, *Cardamine cordifolia*, in the Rocky Mountains insect-plant interactions, chemical defense, Colorado. Journal of Chemical Ecology 3:397-422.

Louda, S.M. 1984. Herbivore effect on stature, fruiting, and leaf dynamics of a native crucifer. Ecology 65:1379-1386.

Louda, S.M., and J.E. Rodman. 1996. Insect herbivory as a major factor in the shade distribution of a native crucifer (*Cardamine cordifolia* A. Gray, bittercress). Journal of Ecology 84:229-237.

Louda, S. M., P.M. Dixon, and N.J. Huntly.1987. Herbivory in sun versus shade at a natural meadow-woodland ecotone in the Rocky Mountains. Plant Ecology 72:141-149.

Louda, S.M. 1990. Predation in the dynamics of seed regeneration. In, *Ecology of Soil Seed Banks* 124: 105-113. Eds. Leck, M.A., T.V. Parker, and R.L. Simpson, Academic Press, Inc.

Maron, J.L., and E. Crone. 2006. Herbivory: effects on plant abundance, distribution and population growth. Proceedings of the Royal Society, London, Series B: Biological Sciences 273:2575-2584.

R Core Development Team 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Sexton, J.P., and E.E. Dickman. 2016. What can local and geographic population limits tell us about distributions? American Journal of Botany, 1031:129–39.

Sinclair, B. J., C. M. Williams, and J.S. Terblanche. 2012. Variation in thermal performance among insect populations. Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches 6:594-606.

Vernon, R.S., and D.R. Gillespie.1990. Spectral responsiveness of *Frankliniella occidentalis* (Thysanoptera: Thripidae) determined by trap catches in greenhouses. Environmental Entomology 19:1229-1241.

Wallace, G.K. 1958. Some experiments on form perception in the nymphs of the desert locust, *Schistocerca gregaria*. Journal of Experimental Biology 35:765-775.

Whittaker, R.H. 1967. Gradient analysis of vegetation. Biological Reviews 49:207-264.

Whitman, D.W. 1987. Thermoregulation and daily activity patterns in a black desert grasshopper, *Taeniopoda eques*. Animal Behavior 35:1814-1826.

Zuur, A., E.N. Ieno, N. Walker, A.A. Saveliev, and G.M. Smith. 2009. Mixed Effects Models and Extensions in Ecology with R. Statistics for Biology and Health. Springer Science+Business Media. ISBN 978-0-387-87458-6.

Table 1: Model coefficient estimates for herbivory field survey and lab choice assay of plants from sun and shade habitats.

			Herbivory type	
Dataset	Model type	Coefficient	Stipples	Mines
Herbivory survey	Binomial	Constant	−0.655 (0.486)	1.152 (0.884)
	(π_0)	Site type [sun]	-1.035^{**} (0.424)	-2.491^{***} (0.782)
		Leaf area (mm^2)	-0.027^{*} (0.015)	–
	NB	Constant	1.223^{***} (0.129)	-1.165^{*} (0.648)
	(count)	Site type [sun]	1.494^{***} (0.107)	2.438^{***} (0.629)
		Leaf area (mm^2)	0.004^{*} (0.002)	0.009^{***} (0.003)
		Leaf ID	0.051 (0.098)	-0.332^{**} (0.152)
			Stipples	Eggs
Choice assay	NB	Constant	1.777^{***} (0.393)	1.079^{*} (0.476)
(whole plants)	(count)	Leaf position	-0.179^{***} (0.035)	-0.432^{***} (0.075)
		Source type [sun]	-1.158^{**} (0.404)	-1.484^{**} (0.470)
Choice assay	NB	Constant	2.177^{***} (0.761)	0.856 (0.811)
(detached leaves)	(count)	Leaf area (mm^2)	0.034 (0.048)	0.019 (0.053)
		Source type [sun]	-1.743^{***} (0.286)	-0.567^{*} (0.304)

*p<0.1; **p<0.05; ***p<0.01

Table 2: Coefficient estimates for models of habitat choice assays.

	Stipples			Eggs
Year (setting)	2014 (field)	2014 (lab)	2015 (lab)	2015 (lab)
Constant	-2.038^{***}	-2.514^{***}	0.521	-4.870^{***}
	(0.603)	(0.870)	(0.885)	(0.824)
Leaf width (mm)	0.068^{***}	0.063^{**}	−0.003	0.054^{***}
	(0.012)	(0.026)	(0.022)	(0.019)
Plant source [sun]	–	–	−0.291	−0.002
	–	–	(0.702)	(0.19)
Light [light]	2.072^{***}	1.792^{**}	2.145^{***}	3.60^{***}
	(0.574)	(0.838)	(0.240)	(0.540)
Temp. [warm]	1.129^{**}	0.399	−0.125	1.960^{***}
	(0.575)	(0.885)	(0.501)	(0.650)
Light × Temp. [light:warm]	−1.263	1.123	0.325	-1.99^{***}
	(0.788)	(1.172)	(0.870)	(0.630)

$^{*}p<0.1$; $^{**}p<0.05$; $^{***}p<0.01$

352 **Figure captions**

353

354 **Fig. 1. Herbivory is higher on bittercress in sun versus shade habitats, but female *S. nigrita***
355 **prefer shade-grown bittercress when given a choice.** (**A**) Herbivory field survey results from
356 sun and shade habitats show higher stipples and mines on bittercress in sun habitats. Raw data
357 points for both stipples and leaf mines are shown in dark gray and are jittered for visual clarity;
358 medians are depicted as black bars, while light gray kernel-smoothed density underlay depicts
359 the distribution of the data. (**B**) Adult female *S. nigrita* stippled and laid more eggs in bittercress
360 derived from shade versus sun habitats in laboratory choice trials. Plotted are raw leaf-level
361 counts of stipples and eggs on leaves along bittercress stems. Statistical results are presented in
362 Table 1.

363

364 **Fig. 2. Female *S. nigrita* stippled (A) and laid more eggs (B) in bittercress leaves in**
365 **simulated sun compared to shade habitats in field and laboratory choice trials.** The field
366 and laboratory choice trials between light and dark sides of assay cages were conducted at two
367 temperatures (see Appendix S4: Fig. S1 for full temperature profiles), which are indicated below
368 each sub-plot. Eggs were counted only for laboratory trials conducted in 2015 (see Materials &
369 Methods). Plot features are depicted as in Fig. 1. Statistical results are presented in Table 2.

Figures

Fig. 1

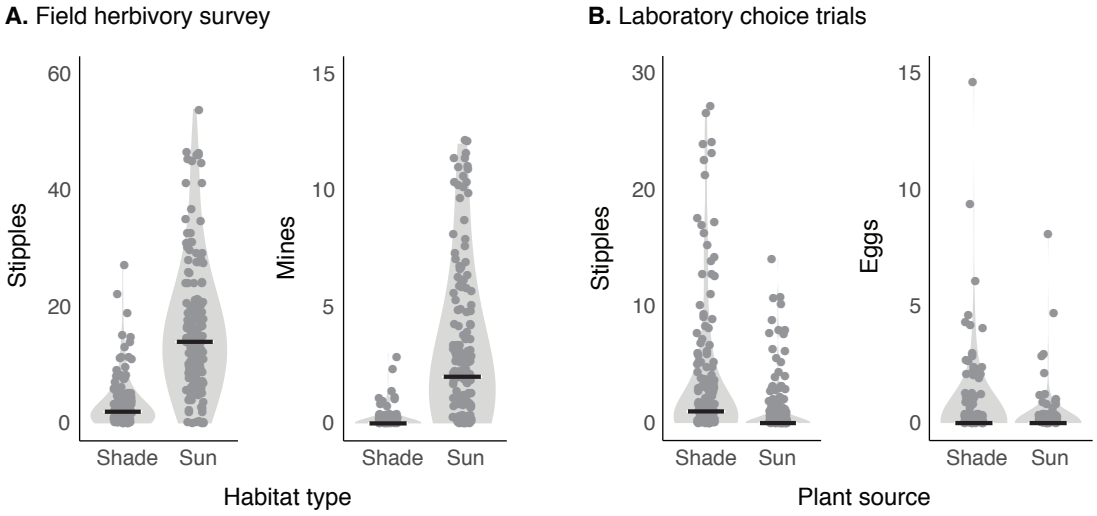
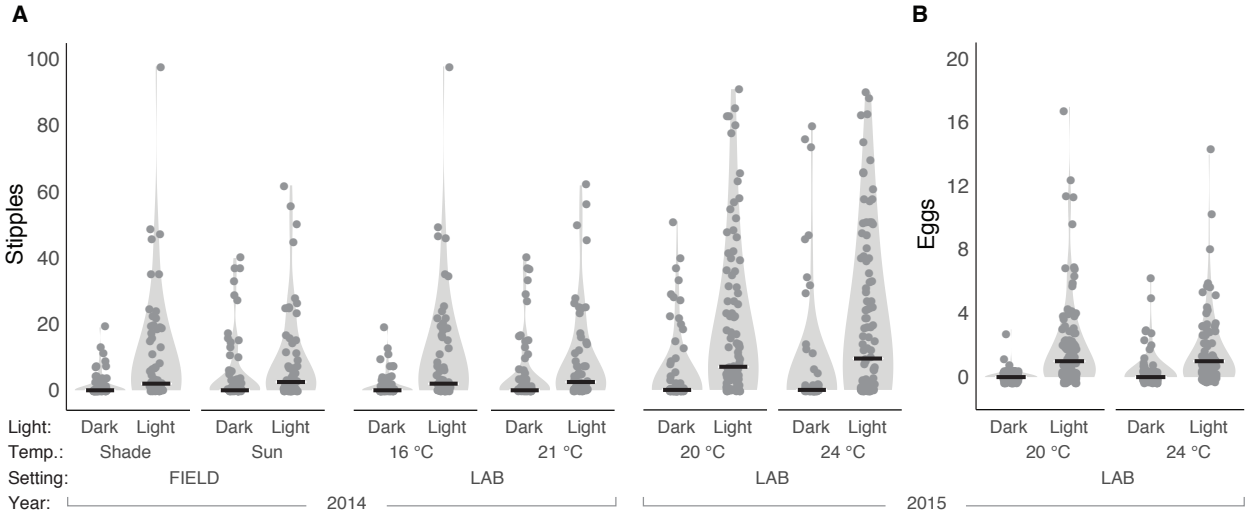


Fig. 2


A
B
Stipples
Eggs
100
80
60
40
20
0
20
16
12
8
4
0
Light:
Dark
Light
Temp.:
Shade
Sun
16 °C
21 °C
20 °C
24 °C
Setting:
FIELD
LAB
Year:
2014
2015

Appendix S1. Characteristics of source locations for bittercress herbivory survey.

At each site, we recorded leaf area of all sampled leaves, photosynthetically active radiation (PAR) using a light meter (Spectrum Technologies, Inc.), percent canopy cover using a densiometer, diameter at breast height (*dbh*) of the four largest trees within four meters, and latitude, longitude, and elevation using a GPS unit (Garmin) (Table S1). Environmental variables at each site were compared using one-way ANOVAs. Sun habitats had higher average PAR and % open canopy than shade habitats (both $p < 0.001$) and did not systematically differ in elevation ($p > 0.8$, Table S2).

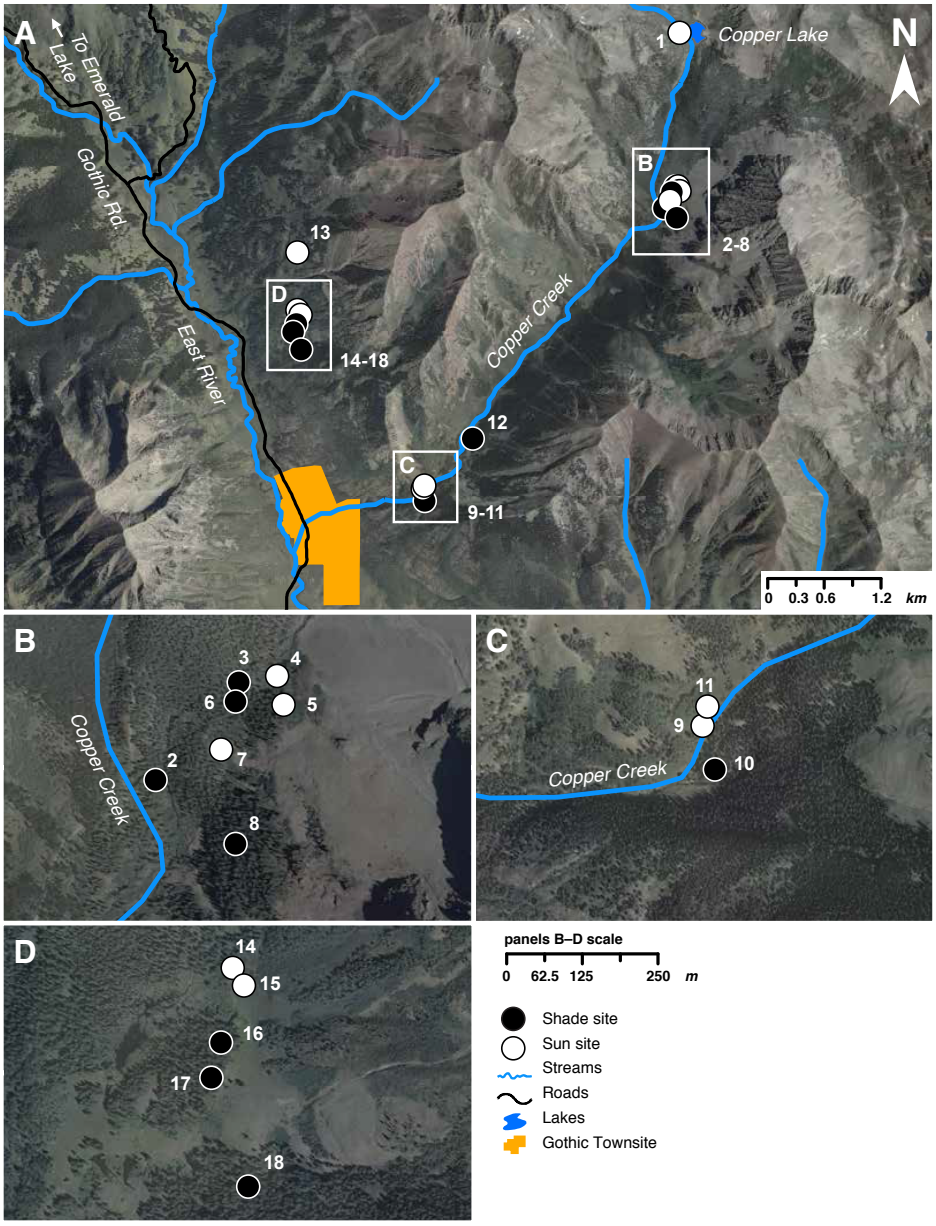


Fig. S1. Map of source sites used in the herbivory surveys in the East River Valley and Copper Creek drainages, near the RMBL in Gothic, CO. A. Base map showing all sites within region (1:48,000). **B–D**. Maps showing detail of site locations (all same scale, 1:7500).

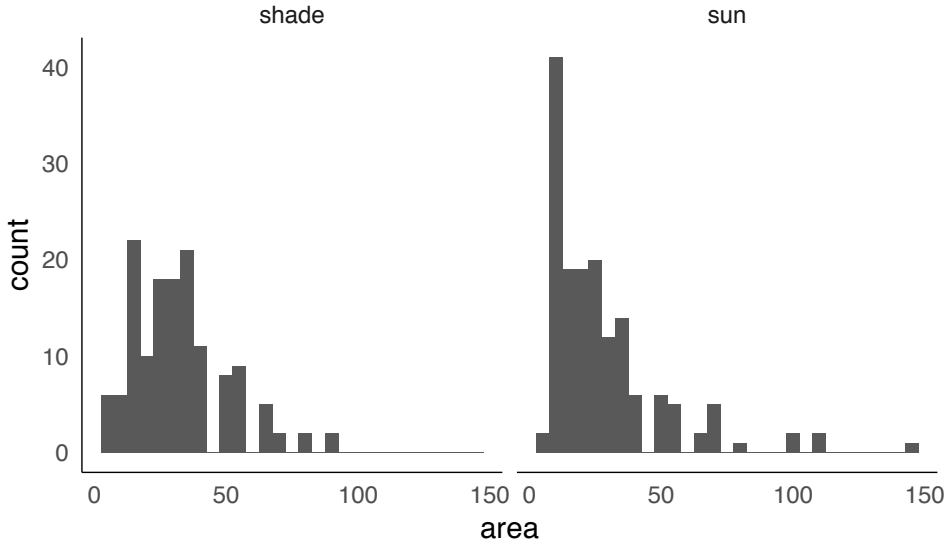


Fig. S2. Distribution of bittercress leaf area observed across all shade and sun habitats.

Table S1. Attributes of sites used for herbivory survey.

Site #	Date sampled	Lat.	Long.	Elevation (m)	Soil Moisture	Light Environment	PAR (μmol•s•m-1)[1]	% Canopy Open	Average DBH (cm)
1	10-Jul-11	39.0050602776535	-106.943317166893	3461	Very Wet	Sun	1980	99.84	0
2	10-Jul-11	38.9885304491745	-106.945774321867	3218	Moist Loamy	Shade	300	6.76	10
3	11-Jul-11	38.9900162385746	-106.944210201638	3240	Moist Loamy	Shade	130	8.06	121.75
4	11-Jul-11	38.9900829890862	-106.943450029132	3253	Very Wet	Sun	2030	82.94	0
5	11-Jul-11	38.9896982273227	-106.9432894133	3261	Very Wet	Sun	2034	95.42	0
6	11-Jul-11	38.9897805369006	-106.94429611421	3244	Moist Loamy	Shade	130	4.16	83.25
7	11-Jul-11	38.9890385630266	-106.944483622637	3220	Very Wet	Sun	2134	99.84	0
8	11-Jul-11	38.9876108211294	-106.944190554921	3250	Moist Loamy	Shade	28	3.38	81.75
9	12-Jul-11	38.9607056556777	-106.973679741745	3023	Very Wet	Sun	2026	88.14	0
10	12-Jul-11	38.9600423351315	-106.973476684125	3013	Wet Loamy	Shade	35	5.72	72.25
11	12-Jul-11	38.9609507737634	-106.973571138078	3994	Very Wet	Sun	1933	86.84	0
12	12-Jul-11	38.9655594715881	-106.968516958823	3994	Very Wet	Shade	32	3.9	101
13	13-Jul-11	38.9828665210329	-106.989822099201	4058	Very Wet	Sun	1780	96.2	0
14	13-Jul-11	38.9774145794955	-106.98986568487	4082	Very Wet	Sun	1870	88.4	0
15	13-Jul-11	38.9771831850899	-106.989697600334	4057	Very Wet	Sun	1850	99.84	0
16	13-Jul-11	38.976356744414	-106.990078474138	4052	Very Wet	Shade	54	4.68	98
17	13-Jul-11	38.975757817399	-106.990327194402	4046	Moist Loamy	Shade	60	4.16	140.5
18	13-Jul-11	38.974195183581	-106.989498527449	4051	Very Wet	Shade	40	7.28	78.75

[1] Light meter was positioned above the center of each source collection plot, at times without cloud cover between 1:00 pm and 3:00 pm during the week of July 17, 2011.

Table S2. Environmental attributes of sun and shade sites where herbivory survey was conducted

	Sun Sites (N = 9)		Shade sites (N = 9)		ANOVA	
Site attributes	*μ*	se	*μ*	se	*F*	*P*
PAR ($\mu mol \bullet s^{-1} \bullet m^{-2}$)	1959.7	37.0	89.0	29.4	1564	$<10^{-10}$
% Canopy Open	93.1	2.2	5.3	0.6	1527	$<10^{-10}$
DBH (*cm*)	0.00	0.00	87.5	12.2	51.77	$<10^{-10}$
Elevation (*m*)	3601	146	3568	150	0.025	0.875
	Sun leaves (N = 157)		Shade leaves (N = 140)		ANOVA	
Sample attributes	*μ*	se	*μ*	se	*F*	*P*
Leaf size (*mm*)	4.98	0.15	5.47	0.13	5.95	0.015

Appendix S2: Design schematics for choice experiments.

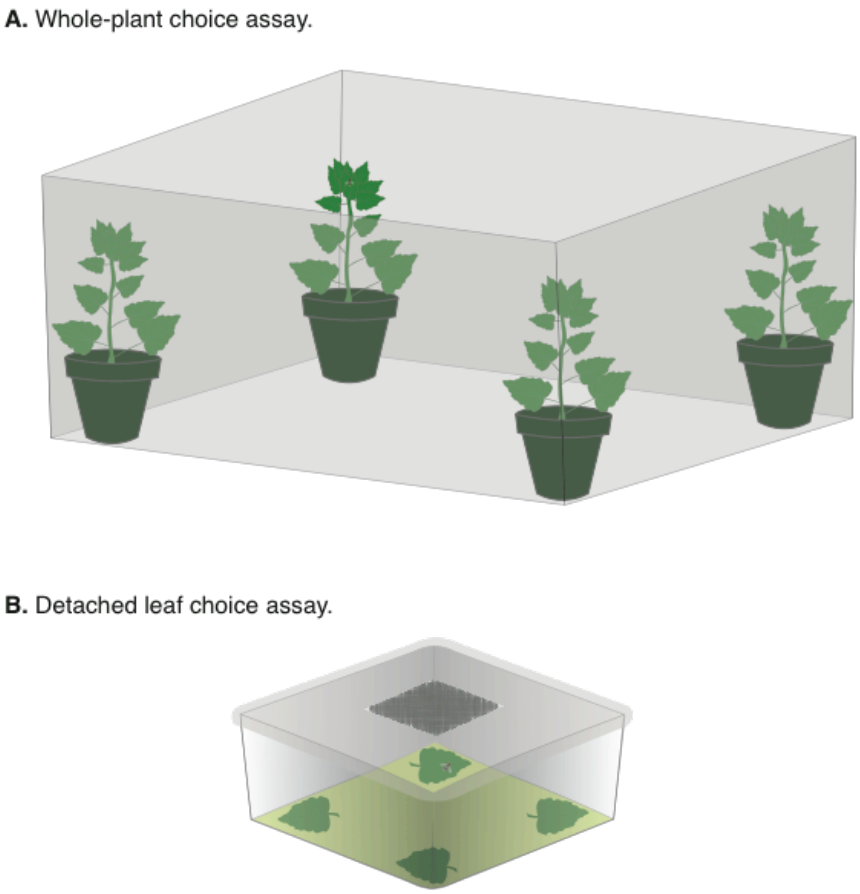


Fig. S1. Schematic of experimental design for Host choice experiment I: Sun versus shade-derived bittercress. (**A**) Whole-plant assay depicted (eight replicate trials were conducted; see Materials & Methods, main text). (**B**) Detached leaf assay (fifteen replicate trials were conducted; see Materials & Methods, main text).

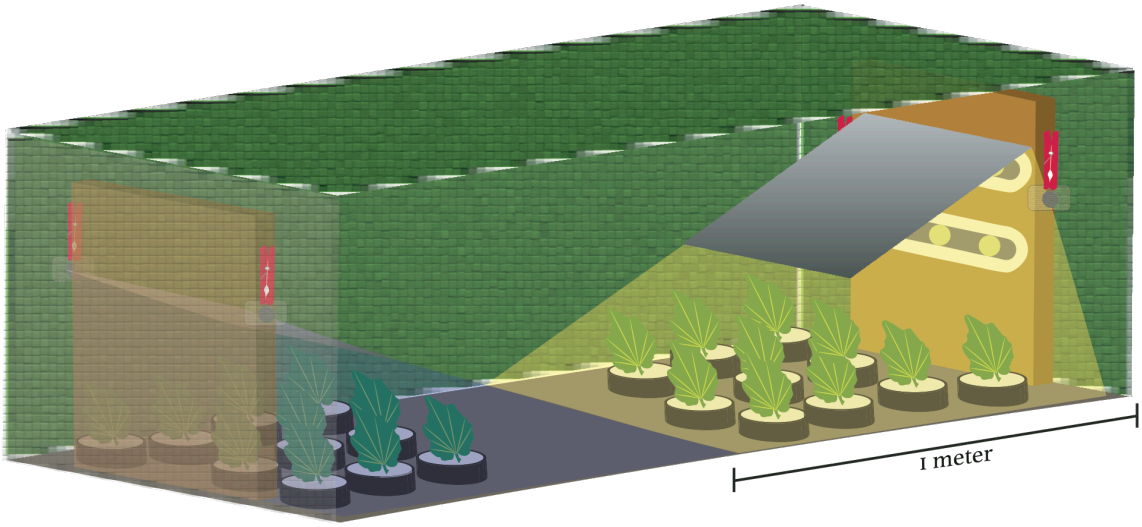


Fig. S2. Schematic of experimental design for Host choice experiment II: Effects of light and temperature.

Appendix 3: Statistical Supplement

Alexandre et al.

Contents

1 Herbivory survey **1**

1.1 Modeling the host selection process 2

1.2 Modeling the damage intensity process 4

1.3 Model comparisons 6

2 Herbivore choice tests I: Sun versus shade derived bittercress **7**

2.1 Models for choice experiments 7

3 Herbivore choice tests II: Effects of light and temperature. **8**

3.1 2014 Trials 8

3.2 2015 Trials 9

3.3 Combined 2014-2015 Analysis 10

3.4 Model Selection 11

4 References **13**

1 Herbivory survey

Herbivore damage from *Scaptomyza nigrita* on its host plant, *Cardamine cordifolia* arises from a sequence of contingent events: (i) the arrival by an herbivore, usually a female, at a host plant (or leaf), and (ii) the damage that results from acceptance of such a host by an adult female or a larva. The former process can be modeled as the probability that an herbivore arrives at a plant (prevalence),

while the latter captures the probability that the resulting damage is of a particular extent (intensity). We model each of these steps explicitly by considering herbivore damage count data as a mixture of these two processes in a way that is directly compatible with standard generalized linear regression approaches. Specifically, herbivore damage (in this case, counts of feeding punctures made by adult females ['stipples'], leaf mines made by larvae, and eggs laid by adult females) is always recorded as positive integer counts, and such count data typically exhibit under-dispersion (i.e. 'zero-inflation'), over-dispersion ('excess variance'), or both, with respect to expectations of Poisson or Poisson–Gamma mixture (i.e. negative binomial, 'NB', models.

Our understanding of the foraging ecology of *S. nigrita* leads us to expect that dispersion of both of these types is plausible. Female flies are choosy, often visiting several leaves before making feeding punctures; females may also avoid leaves entirely, either actively or due to stochasticity in the host sampling process, or to differences in local abundances of foraging *S. nigrita*. Once a host has been preliminarily accepted, we expect that variation in the intensity of feeding damage arises from factors perceived subsequent to the initiation of damage. We thus assume that separate (but potentially related) biological processes govern the host acceptance vs. the host damage stages of herbivory as measured in our study.

Below we plot the distribution of stipple and mine counts from our herbivory survey, broken down by habitat type.

1.1 Modeling the host selection process

Practically, our herbivore survey data contain both an excess of zeros as well as an over-dispersed count distribution relative to expectations of a Poisson error model This makes zero-inflated count models a natural choice [10], which are mixture models composed of a binomial component that models the zero-inflation, and a count component (typically Poisson or negative binomial). In our case, a simplest example of the binomial component of such a mixture model assumes that the number of damaged leaves y_i in group i of size n_i, each with probability p_i, is a realization of a binomially distributed random variable Y_i:

$$Y_i \sim B\Big(n_i, p_i\Big)$$

In the context of a zero-inflated GLM, it is not p_i that is estimated but rather $1 - p_i$, or the probability of zero (i.e. failure), which we define as π_0. Thus, our expression for the binomial probability distribution of y zeros, dropping the i subscript, is

$$Pr\Big\{Y(0) = y\Big\} = \binom{n}{y}\pi_0^y(1-\pi)^{n-y} \tag{1}$$

To describe the influence of habitat type and leaf area on $\pi_{0,i}$ we construct a binomial GLM which estimates the linear effects of habitat and leaf area on the logit of $\pi_{0,i}$ (i.e. the log odds), which we call η_i:

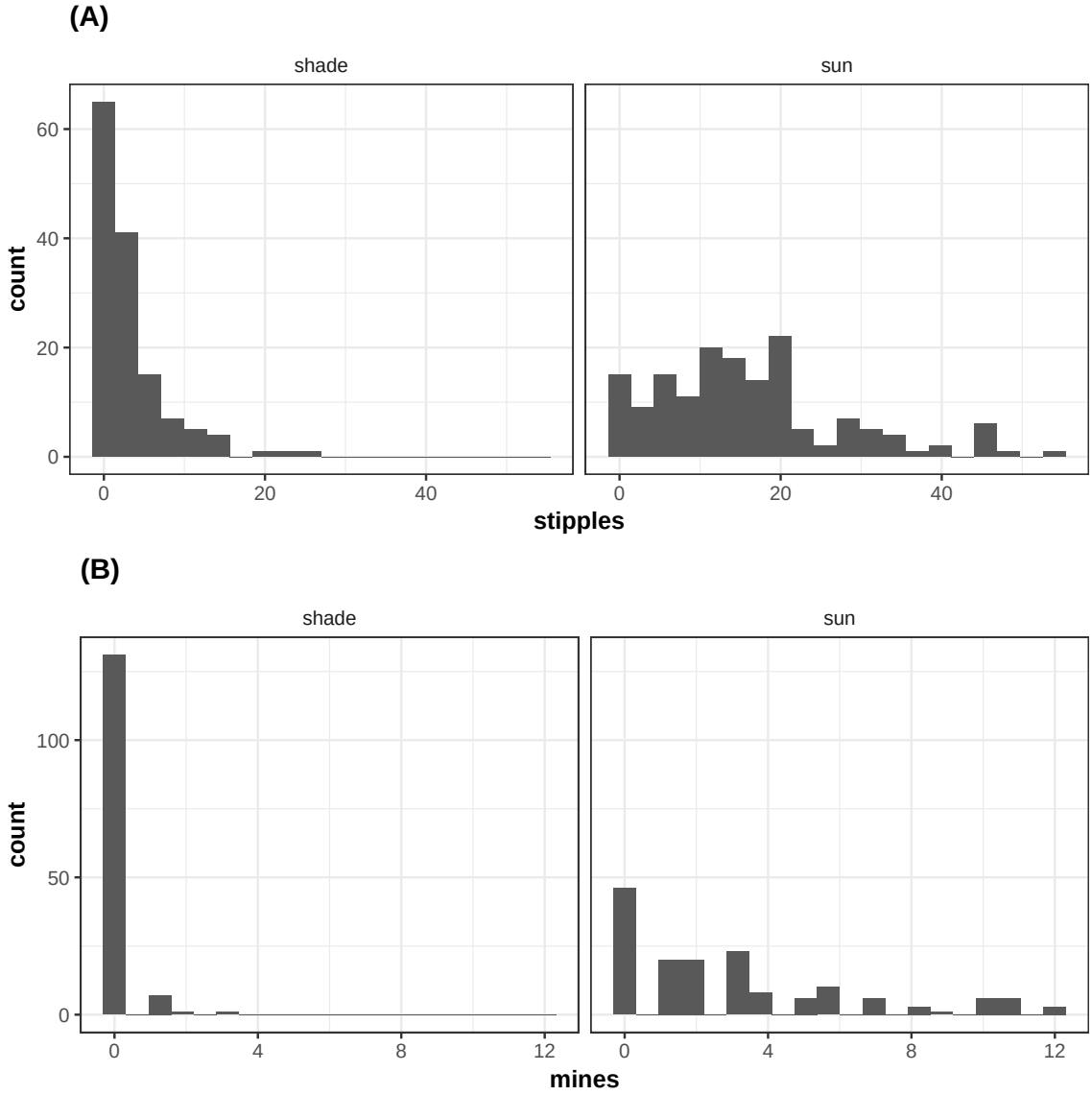


Figure 1: Distribution of (**A**) stippling and (**B**) leaf miner damage on sun and shade-grown bittercress.

$$\text{logit}(\pi_{0,i}) = \log\left(\frac{\pi_{0,i}}{1-\pi_{0,i}}\right) = \eta_i$$

Our binomial GLM for stipple abundance takes on the following linear expression for η_i:

$$\eta_i = \begin{cases} \alpha_0 + \beta x_i, & \text{if habitat = shade.} \\ \alpha_0 + \alpha_1 + \beta x_i, & \text{if habitat = sun.} \end{cases} \tag{2}$$

where α_0 is the coefficient for the shade habitat, taken as the reference level (i.e. the 'Constant' or intercept), to which the effect of the sun habitat (α_1) is added; β captures the effect of the area of each leaf (x_i).

In Table 1 of the main text, we report the values of the coefficients α_0 as log odds (the Constant), and α_1, and β as log odds ratios for the factor levels listed in the table. In the Results section of the main text, for ease of interpretation, we report the exponentiated coefficients (e.g. $\exp[\alpha_0]$) to communicate risk of herbivory in terms of odds ($\frac{\pi_i}{1-\pi_i}$) and odds ratios (ORs); we also discuss differences between habitats in terms of the probability of zero counts, π, calculated as:

$$\pi_i = \text{logit}^{-1}(\eta_i) = \frac{e^{\eta_i}}{1+e^{\eta_i}}$$

Linear estimators ($\hat{\eta}_i$) were generated by maximum likelihood fits of coefficients, as well as the standard error of each coefficient estimate, using **R** [7] package *pscl* [3, 9, 5] using function `zeroinfl`, which jointly estimates these parameters along with those for the count process (see below). Model code in **R** syntax is presented in section 1.3 below.

1.2 Modeling the damage intensity process

We model counts of herbivore damage (stipples and leaf mines) as a Poisson random variable (Y_i) where the Poisson mean (Θ_i) is itself a random variable, which gives rise to the following expression for the conditional probability of Y_i given Θ_i:

$$Pr\left\{Y_i = y_i | \Theta_i = \theta_i\right\} = \begin{cases} \pi_{0,i} + e^{-\theta}, & \text{if } y_i = 0. \\ (1-\pi_{0,i}) \cdot \dfrac{\theta_i^{y_i}}{y_i!} e^{-\theta_i}, & \text{if } y_i > 0. \end{cases} \tag{3}$$

Conceptually, this means that the variance of our random variable Y includes the Poisson variance associated with each Poisson mean Θ as well as additional variance in the distribution of θ itself. We adopt the conventional probability density function for θ as a Gamma distribution with scale parameter α and rate parameter β [10]:

$$g(\theta) = \frac{\alpha^\beta}{\Gamma(\beta)} \theta^{\beta-1} \cdot e^{-\alpha\theta}$$

We recover the the unconditional probability of Y_i by integrating out the θ (i.e. the variable Poisson mean) to recover the parameterization of the negative binomial (NB) that we will use [10]:

$$\begin{aligned} Pr\Big\{Y_i = y_i\Big\} &= \int_0^\infty Pr\Big\{Y_i = y_i | \Theta_i = \theta_i\Big\} \cdot g(\theta) \cdot d\theta \\ &= \frac{\Gamma(\alpha + y_i)}{y_i!\Gamma(\alpha)} \cdot \left(\frac{\mu_i}{\mu_i + \beta}\right)^{y_i} \left(\frac{\beta}{\beta + \mu_i}\right)^\beta \end{aligned} \tag{4}$$

Note that the mean of $g(\theta)$ is $\frac{\alpha}{\beta}$ and its variance is $\frac{\alpha}{\beta^2}$. Setting $\alpha = \beta = r$ allows us to re-write the mean as equal to 1 and the variance σ^2 as $\frac{1}{r}$. This gives the expectation of Y_i equal to μ_i and the variance equal to $\mu + \sigma^2\mu^2$. Thus, the 'dispersion parameter' of the NB, σ^2 (or alternatively, $\frac{1}{r}$), can be interpreted as the variance of the Gamma distribution from which the Poisson means (θ_i) are drawn. When $\sigma^2 \to 0$ the negative binomial collapses to the Poisson, where $\mathrm{E}[Y_i] = \mathrm{Var}[Y_i] = \mu_i$. Note that the μ_i of the NB is not equivalent to the $\frac{\alpha}{\beta}$ from the Gamma distribution.

When combined with the binomial probability of zero (π_i), the full expression for the unconditional probability of Y_i becomes (using the $\sigma^2 = \frac{1}{r}$ parameterization to be consistent with **R**):

$$Pr\Big\{Y_i = y_i\Big\} = \begin{cases} \pi_{0,i} + (1 - \pi_{0,i}) \cdot \left(\frac{r}{r + \mu_i}\right)^r, & \text{if } y_i = 0. \\ (1 - \pi_{0,i}) \cdot \frac{\Gamma(r + y_i)}{y_i!\Gamma(r)} \cdot \left(\frac{\mu_i}{\mu_i + r}\right)^{y_i} \left(\frac{r}{r + \mu_i}\right)^r, & \text{if } y_i > 0. \end{cases} \tag{5}$$

Thus, $Pr\{Y_i\}$ depends on the three parameters, $\pi_0, \mu,$ and r. In our zero-inflated negative binomial (ZINB) GLM framework, the mean of the NB μ_i for each group i is modeled, via the log link function, as a linear function of our predictor variables. Thus, we model $\log(\mu_i)$ as a function of bittercress habitat, leaf area, as well as an additional 'structural' fixed effect, the arbitrary leaf ID (levels A or B):

$$\log(\mu_i) = \begin{cases} \alpha_0 + \beta x_i + \gamma_i, & \text{if habitat = shade.} \\ \alpha_0 + \alpha_1 + \beta x_i + \gamma_i, & \text{if habitat = sun.} \end{cases} \tag{6}$$

Here, α_1 indicates the fixed effect of the sun habitat type; γ_i represents leaf ID, which has two levels (arbitrarily 'A' and 'B'). We set γ_1 to 0 because the reference level of this factor is embedded in the Constant (α_0); β captures the effect of leaf position along the stem (x_i). In the main text, we report model coefficient estimates corresponding to each level of α_i and the single β term for the stipple model. The residual error term is implied.

Table S1: GLM comparisons for **stipple** counts from herbivory survey.

		Model			
Model type	Coefficient	Poisson	NB	ZIP	ZINB
Count model (μ_i)	Constant (α_0)	1.00^{***}	0.92^{***}	1.36^{***}	1.22^{***}
		(0.06)	(0.14)	(0.06)	(0.13)
	Leaf area (β)	0.005^{***}	0.007^{*}	0.003^{***}	0.004
		(0.001)	(0.003)	(0.001)	(0.002)
	Habitat [sun] (α_1)	1.60^{***}	1.63^{***}	1.39^{***}	1.49^{***}
		(0.05)	(0.12)	(0.05)	(0.11)
Binomial model (π_i)	Constant (α_0)			-0.37	-0.66
				(0.36)	(0.49)
	Leaf area (mm^2) (β)			-0.02^{*}	-0.03
				(0.01)	(0.02)
	Habitat [sun] (α_1)			-1.35^{***}	-1.04^{*}
				(0.35)	(0.42)
	AIC	2999.90	1847.35	2490.04	1820.66
	Log Likelihood	-1495.95	-918.68	-1238.02	-902.33

$^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$

1.3 Model comparisons

To justify our use of a ZINB model for the herbivory survey data, we constructed ZI-Poisson models and the non-ZI versions of both NB and Poisson models and compared model fits. For stipple data, all GLMs contained the same terms (Eq. 2, both with logit link functions; Eq. 6 for count models, both with log link function). The same was true for the leaf mine models, but we removed the leaf area term (β) from the binomial component because its inclusion increased overall model *AIC* by 1.95. In contrast, for the ZINB stipple models, adding leaf area to the binomial component reduced AIC by > 2.5 points and was thus retained. In table S2 below and in Table 1 in the main text, we report the leaf mine model results without the leaf area term.

We compared non-nested models (Poisson, NB-only, ZIP, ZINB) using an analog of the likelihood ratio test (Vuong test) as implemented in **R** package `pscl` [5]. This test calculates $p_i = \hat{Pr}(y_i|M_1)$, the predicted probabilities of each data point from model 1, evaluated under the maximum likelihood estimates of the coefficients, as well as q_i, the corresponding probabilities from model 2. The Vuong (Z) statistic is $Z = \sqrt{N}\bar{m}/\sigma_m$ where $m_i = log(p_i) - log(q_i)$ and σ_m is the sample standard deviation of m_i. P-value of the Vuong test is taken as the $Z < \alpha$, $Z > (1 - \alpha)$ quantile of the standard Normal distribution; here, and throughout this study, we use $\alpha = 0.05$. We report a version of Z which scales $\bar{m}$ according to an AICc finite sample size correction [5]. Tables 1 and 2 show coefficient estimates for stipples and leaf mines (respectively) for each of the four candidate models, as well as their log likelihoods and AIC scores.

Notice that the coefficient estimates are largely similar for both ZINB and NB-only models, while both Poisson family models are far worse fits to the data, indicated by the very large ΔAIC between these and the NB models. Overall, the ZINB model has the lowest AIC (ΔAIC > −20 between ZINB

Table S2: GLM comparisons for **larval leaf mine** counts from herbivory survey.

		Model			
Model type	Coefficient	Poisson	NB	ZIP	ZINB
Count model (μ_i)	Constant (α_0)	-2.60^{***}	-2.65^{***}	-0.82	-1.19
		(0.30)	(0.34)	(0.56)	(0.67)
	Leaf area (mm^2) (β)	0.006^{***}	0.009^{*}	0.008^{***}	0.009^{**}
		(0.002)	(0.004)	(0.002)	(0.0003)
	Habitat [sun] (α_1)	3.61^{***}	3.64^{***}	2.19^{***}	2.46^{***}
		(0.29)	(0.32)	(0.56)	(0.65)
Binomial model (π_i)	Constant (α_0)			1.65^{*}	1.24
				(0.71)	(0.75)
	Habitat [sun] (α_1)			-2.57^{***}	-2.44^{**}
				(0.67)	(0.63)
	AIC	1003.99	802.94	839.12	792.92
	Log Likelihood	-497.99	-396.47	-412.56	-389.46

$^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$

and NB-only models). Adding the binomial coefficient estimates of $\pi_{0,i}$ to a NB count model is thus justified on both biological and statistical grounds.

Comparing the ZINB model to the NB-only model gave an AIC-corrected Vuong statistic of $Z = 2.45$ ($p < 0.01$) for stipples and $Z = 1.41$ ($p = 0.07$) for leaf mines; Z values were well above 5 when ZINB models were compared to ZIP or Poisson models (all $p < 0.001$).

Full **R** code for all figures, models, and calculations can be found in the Dryad data repository (doi pending).

2 Herbivore choice tests I: Sun versus shade derived bittercress

2.1 Models for choice experiments

Using NB generalized linear mixed models (GLMMs), we model variation in stipple and egg counts on plants arising from source habitat (sun versus shade) and leaf attributes (as fixed effects), as well as 'structural' random effects of plant ID nested within cage ID (i.e. replicate) to capture experimental design constraints that determine the level of independence among datapoints. Our mixed model for stipple and eggs counts, for both the whole-plant and detached leaf assays, takes the following form:

$$\log(\mu_{ijk}) = \begin{cases} \alpha_0 + \beta x_i + \gamma_{(jk)} + \gamma_k, & \text{if habitat = shade.} \\ \alpha_0 + \alpha_1 + \beta x_i + \gamma_{(jk)} + \gamma_k, & \text{if habitat = sun.} \end{cases} \tag{7}$$

In this model, μ_{ijk} is the estimate for each leaf i, α_0 and α_1 are as in Eqn. 6, and β is leaf position along stem (low to high) for the whole-plant model or leaf area (mm^2) for the detached leaf assay; finally, γ_k represents a random effect of cage ID (k) and $\gamma_{(jk)}$ represents a random effect for plant ID j nested within each level of cage ID k; $\gamma_j \sim N(0, \sigma_1^2)$ and $\gamma_{(jk)} \sim N(0, \sigma_2^2)$.

In our assay cages, we suspect that adult females *S. nigrita* flies had sufficient time to potentially visit all available plant tissue; thus, *a priori* we favor using NB-only compared to ZINB models; we do not consider Poisson models further in this analysis on the basis of far worse model fits observed for stipple and leaf mine data, above. We directly compared NB-only models to a ZINB version to justify this approach. We found that adding a ZI term did not improved model fit for stipples (ΔAIC = 1.24) nor for eggs (ΔAIC = 1.94). For the detached leaf assay, the ZINB model was also a marginally worse fit for both stipples (ΔAIC = 2) and eggs (ΔAIC = 1.77) as well (all ΔAIC are ZINB − NB; Table 3).

ZINB and NB mixed models were fit using **R** package *glmmADMB* with the NB parameterization described in section 1 [2, 8]. *glmmADMB* handles ZI mixed models by fitting a single constant π_0 term across all groups, rather than a $\pi_{0,i}$ for each designated group, making it less flexible than the ZINB implementation in package *pscl*; however, *glmmADMB* handles NB and ZINB mixed models, making it more appropriate for fitting models where the random effects define the appropriate level of experimental replication required to avoid pseudoreplication. Coefficient estimates for NB and ZINB models for stipple and egg counts were nearly indistinguishable for both assay types (Table 3); in the main text, we report the NB-only model results.

3 Herbivore choice tests II: Effects of light and temperature.

The NB model structures for this set of choice experiments were similar to those described in above except for an expanded random effects structure. All models described below are NB-only GLMMs; ZINB models will not be evaluated further in this section. The choice trials in 2014 and 2015 were conducted slightly differently, which means that each year's data calls for slightly different random effects. We present the analyses of each dataset separately, then jointly.

3.1 2014 Trials

The 2014 field and lab trials were conducted in two temperature environments simultaneously, with one cage held in each. This gives a structure of temperature environment ($\gamma_{(lk)}$, n = 2) nested within trial (γ_l, n = 6 each for the 2014 field and lab assays). Nested within temperature environment is cage, but since we have only a single level of cage for each temperature environment per trial, this level is irrelevant for the 2014 dataset. However, we include side-of-cage ($\gamma_{(kj)}$, n = 2; left or right, arbitrarily) to control for pseudo-replication at the level of the main treatment effect (i.e. Light environment, light versus dark), which was applied with randomization to the sides of each cage. Each random effect is $\sim N(0, \sigma_i^2)$. The full model is given below using the above notation for the random effects. The number of independent data points in the 2014 field and lab trials is 24 each (2 sides per cage, 1 cage per trial, 2 temperature settings per trial, and 6 trials).

Table S3: Model estimates for plant source choice experiments.

	stipples		eggs	
Whole-plant assay	NB	ZINB	NB	ZINB
Constant (α_0)	1.777 (0.393)***	1.927 (0.412)***	1.079 (0.476)*	1.285 (0.802)
Leaf position (β)	−0.179 (0.035)***	−0.184 (0.036)***	−0.432 (0.075)***	−0.430 (0.075)***
Plant source (α_1)	−1.158 (0.404)**	−1.127 (0.406)**	−1.484 (0.470)**	−1.469 (0.469)**
Cage (σ_1^2)	0.323	0.331	0.0004	0
Plant:Cage (σ_2^2)	0.919	0.938	0.473	0.466
AIC	1143.024	1144.268	386.976	388.920
Log Likelihood	−565.512	−565.134	−187.488	−187.460
Num. obs.	356	356	356	356
Zero inflation: parameter		0.138		0.200
Zero inflation: SD		0.121		0.589
Detached leaf assay				
Constant (α_0)	2.177 (0.761)**	2.273 (0.770)**	0.856 (0.811)	1.433 (0.718)*
Leaf position (β)	0.034 (0.048)	0.034 (0.049)	0.019 (0.053)	0.009 (0.047)
Plant source (α_1)	−1.743 (0.286)***	−1.770 (0.291)***	−0.567 (0.304)	−0.368 (0.305)
Cage (σ_1^2)	1.35	1.135	0.561	0
AIC	395.818	397.554	280.105	281.934
Log Likelihood	−192.909	−192.777	−135.052	−134.967
Num. obs.	60	60	60	60
Num. groups: cage	15	15	15	15
Zero inflation: parameter		0.000		0.217
Zero inflation: SD		0.000		0.103

$^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$

$$\log(\mu_{ijkl}) = \begin{cases} \alpha_0 + \alpha_{1,i} + \alpha_{2,i} + \alpha_{12} + \beta x_i + \gamma_l + \gamma_{(lk)} + \gamma_{(kj)}, & \text{if Light = light \& Temp = warm} \\ \alpha_0 + \alpha_{1,i} + \alpha_{2,i} + \beta x_i + +\gamma_l + \gamma_{(lk)} + \gamma_{(kj)}, & \text{otherwise.} \end{cases} \tag{8}$$

We set the first levels of coefficients for the fixed effects of Light ($\alpha_{1,1}$ = dark) and Temperature ($\alpha_{2,1}$ = cool) equal to zero since these two states are incorporated into the Constant (α_0, i.e. the reference level of the model). Our experiment was designed to allow us to fit an interaction term α_{12} which estimates how much the effect of Light is impacted by the Temperature environment (thus α_{12} has a single level). NB models were estimated using **R** package *lme4* [1]. We fit models with the same structure for field and laboratory trials.

3.2 2015 Trials

In the 2015 trials, we used bittercress leaves collected from both sun and shade habitats, and our randomization scheme ensured equal representation of sun- and shade-derived leaves across all

treatments. We did this to test whether *S. nigrita* would exhibit preference for shade-derived bittercress in the context of our temperature and light manipulations. In our analysis, we included leaf source (sun v. shade) as an additional fixed factor. Our model structure was thus

$$\log(\mu_{ijkl}) = \begin{cases} \alpha_0 + \alpha_{1,i} + \alpha_{2,i} + \alpha_{12} + \alpha_{3,i} + \beta x_i + \gamma_l + \gamma_{(lk)} + \gamma_{(kj)}, & \text{if Light = light \& Temp = warm} \\ \alpha_0 + \alpha_{1,i} + \alpha_{2,i} + \alpha_{3,i} + \beta x_i + \gamma_l + \gamma_{(lk)} + \gamma_{(kj)}, & \text{otherwise.} \end{cases} \tag{9}$$

All coefficients are as in the 2014 trials above, except that the term $\alpha_{3,i}$ is for source habitat, and the first level ($\alpha_{3,1}$ = shade) was set to 0 and was thus incorporated into the intercept term α_0. Thus, this model estimates only a single coefficient for the effect of habitat.

The experimental procedures also differed slightly in 2015. We placed two cages in one environmental chamber which was set to a single temperature per trial, and individual trials were conducted at different temperatures sequentially. This implies a structure of cage (γ_{kl}, $n = 2$) nested within trial (γ_l, $n = 5$), along with the side-of-cage nested within cage ($\gamma_{(kj)}$, $n = 2$). The number of independent data points in the 2015 trials is thus 40 (2 sides per cage, 2 cages per room, 10 trials [5 in each temperature regime]). While the model structure is the same as in Eqn. 8, the meaning of the random effects (and their coding in the design matrix) are slightly different. Below we discuss how we reconciled the random effects to analyze both years' data together.

3.3 Combined 2014-2015 Analysis

We reconcile the slight distinctions between 2014 and 2015 datasets by including trial-year as a composite random effect, now re-coded to reflect each experiment conducted in a given room (compared to a trial containing two separate temperature settings, as in the 2014-only analysis). Nested within the new trial factor is cage, which has n = 1 for 2014 and n = 2 for 2015; side-of-cage is modeled in the same way as above. This model structure is now identical to Eqn. 8.

For the combined analysis, we drop the term for plant source habitat (α_3) since the 2014 trials were not designed to examine plant source habtat. Additionally, we model both a continuous and discrete versions of the Temperature factor: the discrete model is the same as Eqn. 8, while in the continuous form $\alpha_{2,j}$ is replaced with $\beta_2 y_j$, where y_j is the temperature measured at the level of cage for each trial separately (Appendix B, Fig. 2C). Additionally, the fixed interaction term α_{12} is now replaced with an interaction modeled by the expression $\beta_3 y_j(\alpha_{1,j})$, which captures how the effect of light ($\alpha_{1,j}$) changes as a function of cage temp (y_i); since $\alpha_{1,i}$ has one level (the first level is set to 0), estimating this interaction term β_3 adds only a single parameter to the model. The full model is thus:

$$\log(\mu_{ijkl}) = \alpha_0 + \alpha_{1,i} + \alpha_{2,i} + \beta_1 x_i + (\beta_2 + \beta_3 \alpha_{1,i}) y_j + \gamma_l + \gamma_{(lk)} + \gamma_{(kj)} \tag{10}$$

3.4 Model Selection

In the main text, we report the results from the NB GLMMs fit to the 2014 and 2015 datasets (separately) using **R** package `lme4` using function `glmer.nb`. The full results for the combined analysis is displayed below (Table S4). For each dataset, We also analyzed a series of nested models to evaluate the relative performance of simpler models lacking our source habitat (α_4) and the Light $\times$ Temperature interaction term (Table S5). As indicated in Table S5, the model reported in the main text is not typically the best model as judged by AIC or other metrics. Nonetheless, we designed our trials to provide a test of the null hypothesis that $\alpha_{12} = 0$ (or $\beta_3 = 0$, in the case of the model specified in Eqn. 9) and justify the inclusion of this term in the main text model results because of this intention, so that the coefficient estimates are apparent.

To perform model selection, we began by fitting a null model with random effects only to capture the structure of the experiment. We explicitly model batch effects, room effects, cage effects, and side-of-cage effects as random effect terms in our model. We then add the fixed factors we hypothesize will explain the variance otherwise observed at each of these random effects levels: first, the relevant leaf traits (width, in this case) common to all models; the source habitat (sun or shade) from which leaves were derived (for 2015 trials only); Light treatment,(i.e. side-of-cage level factor); Temperature treatment (cage-level treatment); and then their interaction. We evaluated model fits by calculating ΔAIC values of models with and without source habitat term and the interaction term between light and temperature. We also conducted likelihood ratio tests, which generally agreed with ΔAIC results. Additionally, we evaluated overall model fit for the fixed effects-only portion with $R^{2(M)}_{GLMM}$ and for all model terms combined with $R^{2(C)}_{GLMM}$ using which were calculated as derived in [6] using **R** package `piecewiseSEM` [4] using the function `sem.model.fits`.

Table S4: Coefficient estimates for all light–temp choice experiments.

	Stipples					Eggs
	Field (2014)	Lab (2014)	Lab (2015)	Both years (1)	Both years (2)	Lab (2015)
Fixed effects						
α_0 (Constant)	-2.038^{***}	-2.514^{**}	0.364	−1.147	−2.195	-4.870^{***}
	(0.603)	(0.870)	(0.885)	(0.661)	(1.599)	(0.812)
β_1 leaf width (*mm*)	0.068^{***}	0.063^{*}	−0.003	0.033	0.033	0.054^{**}
	(0.012)	(0.026)	(0.022)	(0.018)	(0.018)	(0.019)
$\alpha_{1,i}$ [light]		1.792^{*}	2.160^{***}	2.019^{***}	−0.048	3.598^{***}
		(0.838)	(0.515)	(0.470)	(1.965)	(0.537)
$\alpha_{2,j}$ [warm]		0.399	−0.100	0.109		1.960^{**}
		(0.885)	(0.880)	(0.667)		(0.650)
α_{12} [light:warm]		1.123	0.293	0.636		-1.994^{**}
		(1.172)	(0.718)	(0.654)		(0.628)
α_3 [sun]			−0.291			−0.002
			(0.701)			(0.19)
β_2 [temp]					0.102	
					(0.081)	
β_3 [light:temp]					0.091	
					(0.096)	
Random effects						
σ^2_{kj} [trial/cage/side]	0.559	1.397	0.720	1.102	0.126	0.152
σ^2_{lk} [trial/cage]	0.000	0.099	2.451	1.687	0.321	0.170
σ^2_{l} [trial]	0.204	0.000	0.000	0.000	0.000	0.163
σ^2_{m} [year]				0.036		
AIC	1152.412	874.431	2125.715	2995.843	3044.220	861.166
Log Likelihood	−567.206	−428.216	−1053.858	−1487.921	−1513.110	−421.583
Num. obs.	240	240	398	638	638	398

$^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$

Table S5: Model comparisons for different fixed effect combinations.

Dataset	Fixed effects	$R^{2(M)}_{GLMM}$	$R^{2(C)}_{GLMM}$	AIC	ΔAIC	log Lik
2014 Field	β	0.31	0.91	1158.50	6.43	-573.25
(Stipples)	β, α_{1i}	0.52	0.91	1152.07	**0.00**	-569.03
	$\beta, \alpha_{1i}, \alpha_{2i}$	0.53	0.91	1152.81	0.75	-568.41
$\longrightarrow$	$\beta, \alpha_{1i}, \alpha_{2i}, \alpha_{12}$	0.58	0.90	1152.41	0.34	-567.21
2014 Lab	β	0.06	0.87	883.03	9.69	-435.52
(Stipples)	β, α_{1i}	0.40	0.87	873.34	**0.00**	-428.67
	$\beta, \alpha_{1i}, \alpha_{2i}$	0.47	0.85	873.34	**0.00**	-428.67
$\longrightarrow$	$\beta, \alpha_{1i}, \alpha_{2i}, \alpha_{12}$	0.48	0.85	874.43	1.09	-428.22
	$\beta, \alpha_{1i}, \beta_2$	0.44	0.86	874.61	1.28	-429.31
	$\beta, \alpha_{1i}, \beta_2, \beta_3$	0.49	0.85	873.85	0.51	-427.93
2015 Lab	β	0.00	0.95	2142.79	20.90	-1065.40
(Stipples)	β, α_{1i}	0.28	0.95	2121.89	**0.00**	-1053.94
	$\beta, \alpha_{1i}, \alpha_{2i}$	0.28	0.95	2123.88	1.99	-1053.94
$\longrightarrow$	$\beta, \alpha_{1i}, \alpha_{2i}, \alpha_{12}$	0.28	0.95	2125.72	3.83	-1053.86
	$\beta, \alpha_{1i}, \beta_2$	0.28	0.95	2123.89	2.00	-1053.94
	$\beta, \alpha_{1i}, \beta_2, \beta_3$	0.28	0.95	2125.83	3.94	-1053.92
2015 Lab	β	0.02	0.64	902.03	40.86	-445.01
(Eggs)	β, α_{1i}	0.43	0.64	867.95	6.78	-426.97
	$\beta, \alpha_{1i}, \alpha_{2i}$	0.46	0.65	867.88	6.71	-425.94
$\longrightarrow$	$\beta, \alpha_{1i}, \alpha_{2i}, \alpha_{12}$	0.56	0.68	861.17	**0.00**	-421.58
	$\beta, \alpha_{1i}, \beta_2$	0.46	0.64	867.56	6.39	-425.78
	$\beta, \alpha_{1i}, \beta_2, \beta_3$	0.51	0.66	866.07	4.91	-424.04
Combined Lab	β	0.02	0.93	3022.43	29.04	-1504.21
(Stipples)	β, α_{1i}	0.31	0.92	2993.43	0.04	-1488.71
	$\beta, \alpha_{1i}, \alpha_{2i}$	0.32	0.92	2994.78	1.40	-1488.39
	$\beta, \alpha_{1i}, \alpha_{2i}, \alpha_{12}$	0.32	0.92	2995.84	2.46	-1487.92
	$\beta, \alpha_{1i}, \beta_2$	0.35	0.92	2993.38	**0.00**	-1487.69
	$\beta, \alpha_{1i}, \beta_2, \beta_3$	0.35	0.92	2994.27	0.89	-1487.14

Notes: ΔAIC is each model minus model with lowest AIC.
$\longrightarrow$ indicates model reported in main text, Table 3.

4 References

References

[1] Douglas Bates, Martin Mächler, Ben Bolker, and Steve Walker. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1):1–48, 2015.

[2] D. A. Fournier, H. J. Skaug, J. Ancheta, J. Ianelli, A. Magnusson, M.N. Maunder, A. Nielsen, and J. Sibert. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optim. Methods Softw.*, 27:233–249, 2012.

[3] Simon Jackman. *pscl: Classes and Methods for R Developed in the Political Science Computational Laboratory, Stanford University*. Department of Political Science, Stanford University, Stanford, California, 2015.

[4] Jonathan S. Lefcheck. piecewisesem: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, 7(5):573–579, 2016.

[5] Tom Loeys, Beatrijs Moerkerke, Olivia De Smet, and Ann Buysse. The analysis of zero-inflated count data: beyond zero-inflated poisson regression. *Br J Math Stat Psychol*, 65(1):163–80, Feb 2012.

[6] Shinichi Nakagawa and Holger Schielzeth. A general and simple method for obtaining r2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4(2):133–142, 2013.

[7] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2017.

[8] Hans Skaug, Dave Fournier, Ben Bolker, Arni Magnusson, and Anders Nielsen. *Generalized Linear Mixed Models using 'AD Model Builder'*, 2016-01-19.

[9] Achim Zeileis, Christian Kleiber, and Simon Jackman. Regression models for count data in r. *Journal of Statistical Software, Articles*, 27(8):1–25, 2008.

[10] A. Zuur, E.N. Ieno, N. Walker, A.A. Saveliev, and G.M. Smith. *Mixed Effects Models and Extensions in Ecology with R*. Statistics for Biology and Health. Springer New York, 2009.

Appendix S4. Temperature profiles for field and laboratory habitat preference choice tests.

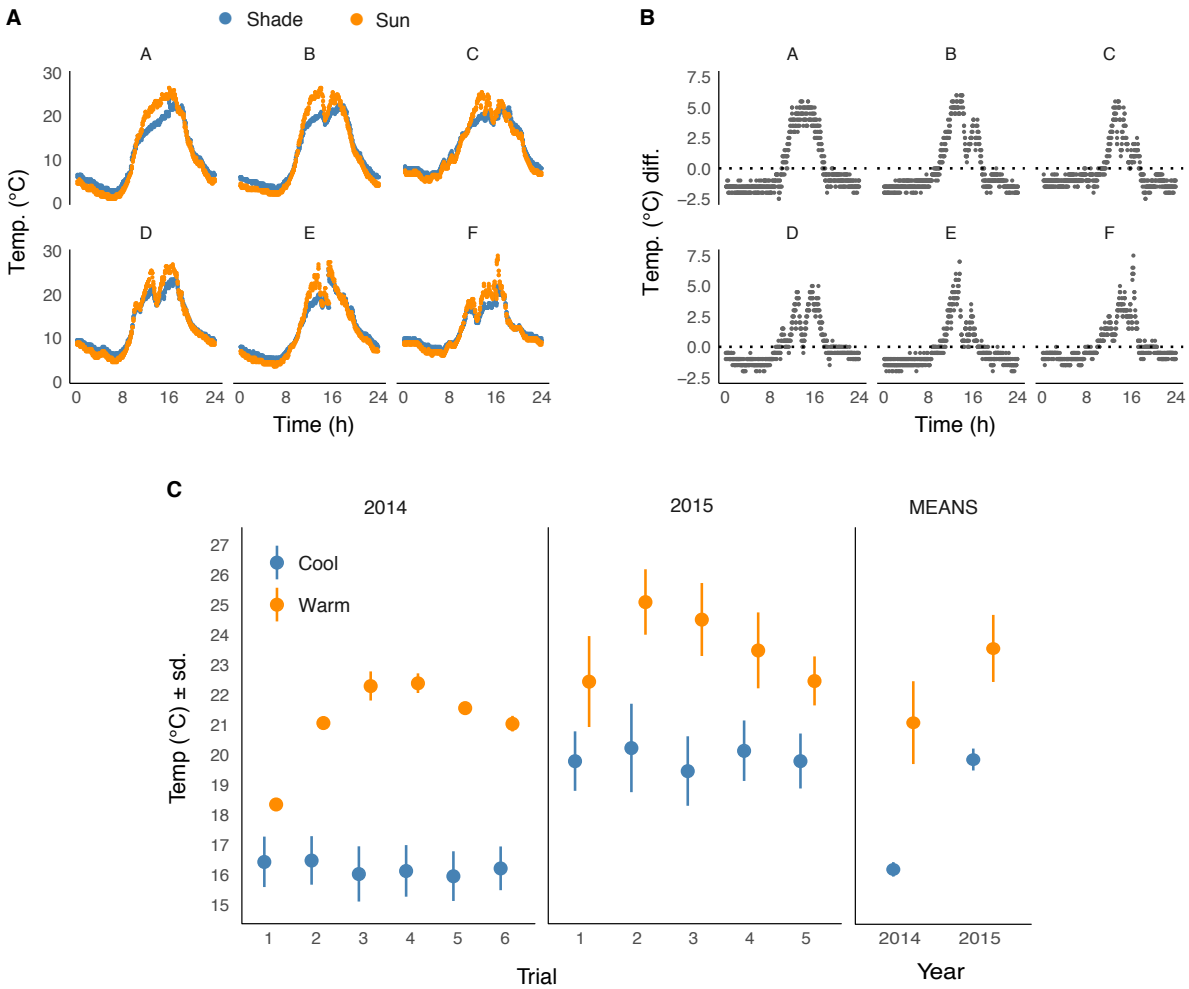


Fig. S1. Temperature profiles for field and laboratory habitat preference choice tests. (**A**) Temperature profiles for field cages (from 2014). Both 'Sun' and 'Shade' cages were equally masked from natural sunlight but were either sun-exposed or canopy shaded in order to confer different temperature profiles. We collected a full 24 h of temperature data during each trial (ordered in time and labeled A–F), which took place for 24 h beginning at 1100 h. 0 h represents midnight. (**B**) Differences in temperature between sun-exposed and canopy-shaded assay cages (sun – shade), showing a maximal difference of 5 °C in mid-afternoon during each trial. (**C**) Average temperature for each laboratory trial for 2014 and 2015 (left; ordered in time and labeled 1–6), and the mean (right; ± 1 standard deviation) over all trials for each year. 2014 trials used two environmental chambers, while 2015 trials alternated between warm and cool trials at two-day intervals (see Methods in the main text for details).