

INSECT BEHAVIOR DETERMINES PLANT DISTRIBUTION IN ALPINE HABITAT

By

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Abstract:

Specialist leaf-mining fly *Scaptomyza nigrita* is a major herbivore of its host plant *Cardamine cordifolia*. Host plants are found along a gradient from sun-exposed bog to deep spruce shade habitat where herbivory decreases as shade increases. Prior studies have failed to support the hypothesis that plant quality determines plant distribution. A series of outdoor cage experiments utilizing a factorial design tested whether herbivore choice may instead drive plant distribution. It was found that adult female *S. nigrita* exhibited a significant preference for feeding and ovipositing on *C. cordifolia* leaves exposed to light, regardless of habitat origin of plant, leaf width, or presence of prior herbivore damage. This study does not support the phytocentric explanation that host plant distribution is determined by herbivore preference for plants based on their quality. My findings instead support the hypothesis that herbivores drive plant distributions into the shade by creating enemy-free space for host plants through their preference for light.

Introduction.

Spatial heterogeneity of herbivore damage is common in plants (Collinge and Louda 1989). If such variation persists among populations or patches of a plant species over time, fitness differentials in both host plants and herbivores will affect the distribution and population dynamics of the system. *Scaptomyza nigrita* is an alpine drosophilid leaf-mining fly that specializes on host plant *Cardamine cordifolia* (Collinge and Louda 1988). This herbivore subjects its host plant to damage during its larval stage as a leaf miner where larvae have the ability to travel through the petioles to other leaves within the same plant. Adult female *S. nigrita* use a dentate ovipositor to cut into leaves before then feeding on the juice that seeps into the wound. They oviposit in some of these wounds, therefore, adult female flies largely determine the environment a larvae will experience for the majority of its life. Previous studies in Gothic, Colorado have shown that damage from *S. nigrita* and other herbivores on *C. cordifolia* is found in a predictable fashion among particular patches of plants. Plants exposed to full sunlight experience the most herbivory whereas those in the shade experience little to none.

C. cordifolia is more abundant in intermediate willow shade than in adjacent full sun habitat. Furthermore, herbivory is more pronounced in full sun than shaded habitats. Prior studies have posited that variation in herbivory pressure is the primary factor determining the distribution of *C. cordifolia* (Louda and Rodman 1996). Evidence for this came from data showing that plant fitness was reduced in sun-exposed plants subjected to herbivory, but not when sun-exposed plants were treated with insecticide. Furthermore, it was suggested in this study that the discordance in distribution of herbivory between the sun and shade habitat was potentially due to a preference for higher quality plants in the

sun as a result of lower defenses or that plants were of higher nutritive quality relative to those from the shade habitat. This is an example of Plant Vigor Hypothesis, the idea that herbivores choose plants based on their quality, thus driving them into lower quality habitat. However, the fact that that two plant ecotypes are differentially attacked does not actually illuminate why there is such a discrepancy.

In contrast to the Plant Vigor Hypothesis, we hypothesize that plant distribution is driven by herbivore preference for environment. At high altitude, insects should have diminished flight ability due to a decrease in mean air temperature and its alteration of flight metabolism and muscle physiology (Dillon and Dudley 2006). Thus, insects in cold environments may have evolved behaviors that allow them to seek out optimal conditions for movement. Furthermore, the ability to seek out warmer conditions may be accompanied by the ability to increase body temperature in said conditions.

Previous work on *C. cordifolia* has not found support for the Plant Vigor Hypothesis (Louda and Rodman 1996). This summer we conducted research at the Rocky Mountain Biological Laboratory in Gothic, Colorado. We tested the alternative hypothesis that herbivore preference for environment is driving plant distribution rather than plant quality, effectively providing *C. cordifolia* with an anti-herbivore defense in intermediate and deep shade habitat. Given that *C. cordifolia* patches experience different environments along a gradient from full sun in bogs to deep shade under spruce and fir canopy, and these habitats differ and overlap in environmental factors such as air temperature and sun exposure, one must examine abiotic/biotic factors independently to discover which are most important for herbivore choice. Additionally, in order to examine an insect's ability to heat itself in various conditions, one must examine

immediate internal body temperature change during exposure to said conditions.

Methods.

Choice Experiments in Field and Laboratory. During the 2015 summer season from June 1-July 1 at the Rocky Mountain Biological Laboratory in Gothic, Colorado, two large cloth mesh cages (35.5 x 35.5 x 185 cm) with a clear plastic top were placed lengthwise on the soil in adjacent canopy-free and deep evergreen shade plots. Canopy-free habitat was area in which there was no tree cover and full exposure to sunlight. Evergreen shade was habitat in which tree cover was between 90 and 100 percent. All measurements were confirmed via use of a densiometer. Each cage was placed under a large table wrapped in reflective Mylar, and 70% shade cloth was then used to wrap the cages. Shade cloth was used to remove effects of ambient light within each cage whereas tables wrapped in mylar were placed over each cage to remove effects of surface temperature altering the atmosphere within each cage. At this point, the temperature within each cage was the same as air temperature in full sun and deep shade environments surrounding either cage. We used a light meter to ensure ambient light within cages was equalized. Within each cage, we established a steep light gradient by affixing two LED lights (375 lumens, 7000k) via hooks at 14 and 21 cm from the bottom of 1.25 cm thick plywood boards placed vertically against both far sides of each cage. On each board two data-loggers were also mounted (ds1921g Thermocron Ibutton) in order to continuously measure temperature within the cages.

For each of six choice trials conducted at two-day intervals, one side of each cage was randomized to receive the LED light treatment while the other side remained dark.

Prior to beginning a trial, ten plants were selected from Copper Creek: five from deep shade, five from full sun. Four leaves from each plant were snipped at the petiole; these 40 leaves were randomized into four groups of ten, which were then placed inserted into 100 m petri dishes filled with Phytoblend (1.5%). Each petri dish containing ten leaves was randomized to the sides of each cage and placed 8 cm from the base of each board. For each trial, ten *S. nigrita* adult females were collected from along the Copper Creek drainage and released into the middle of each cage. During each trial, LED lights were angled down so as to illuminate the petri dish on the lighted side of the cage. Flies were allowed to forage for twelve hours during daylight and were then recollected via aspirator while all leaves were collected for analysis. Data-loggers were employed during all trials to record temperature.

These trials were then replicated in temperature-controlled rooms in the RMBL Laboratory in order to replicate field data in a controlled setting. For the six laboratory choice trials, the same two cages were placed without Mylar or shade cloth into temperature-controlled environmental chambers. Leaves were collected and measured before the experiment for width, number of stipples, number of eggs laid, and psyllid or other insect damage prior to being randomized to a side of each cage. Trials were done at two-day intervals sequentially, alternating between 15°C or 21.5°C as these were temperatures found to be the high averages that field cages in the sun and shade experienced. Plants, leaves, and flies were collected and utilized as above, and the LED and data-logger placement was the same.

Fly Internal Temperature by Environment. Flies were collected via aspirator from vegetation living along the East River, which flows out of Emerald Lake. Flies were either used the day of collection or were housed overnight in a 14x14x24 butterfly castle with leaves and water provided in damp paper towels. Flies were killed via cold shock in the -80°F freezer for three minutes an hour before use. Pins were prepared with small points to accommodate the small body size of the insects used in order to avoid piercing the body cavity. The tips of the points were bent slightly and coated with Elmer's glue in order to adhere the insect's thorax with minimal surface area exposed to the glue; 10 minutes were allowed for glue to harden. At the RMBL laboratory, raised pins were placed on a small corkboard under the full-shaded canopy of the laboratory building to mimic deep shade conditions or out in full sun on grass adjacent to the laboratory building to mimic full sun conditions. All measurements were taken behind a wooden barrier to airflow to control for small fluctuations in temperature from wind. Because temperatures did not change over a range of 5-20 minutes when placed in shade or sun based on a test run with one *S. nigrita* female and one unknown species of dipteran as a control, each individual was measured for temperature in the thorax and abdomen after 5 minutes. A Bailey Instruments Thermocouple Model BAT-12 Needle Adapter was used to pierce the thorax and abdomen of female flies for a total of two measurements per individual. Only females were collected because all experiments performed have utilized female flies. Two potted *Cardamine* plants were placed in each respective environment at the same location near the RMBL Laboratory and leaf surface temperatures were recorded using the adaxial surface after 5 minutes of acclimation time.

Results.

Light and temperature herbivore choice experiments. Choice trials with adult *S. nigrita* in the field between plants in the light and dark side of each outdoor cage revealed a significant preference of the flies for leaves in the light across both cage types (Light treatment $P < 0.001$, Fig. 1A, Table 1). The preference appeared to be stronger when the choice arena was placed under evergreen canopy (Cage X Light treatment, $P < 0.001$, Fig. 1A, Table 1), and overall feeding activity was higher in the warmed (i.e. sun-exposed) cage (Cage type, $P < 0.001$, Table 1). The cage under evergreen canopy had a lower maximum and higher minimum daily temperature across all trials. In the light/dark choice tests conducted in the lab at constant temperatures, *S. nigrita* adult females fed significantly more on plants in the light than the dark at both temperatures (Light treatment, $P = 0.024$, Fig. 1B, Table 1); no significant interaction between light and temperature was detected for the lab choice trials (Table 1).

Fly Temperature Experiments. Thorax and abdomen temperature measurements with adult *S. nigrita* females in the field between full sun and shade at the RMBL laboratory showed a pronounced difference in average internal body temperature with internal temperatures in the sun reaching almost twice that of internal temperatures in the shade (Full Sun Abdomen 33.9°C, Full Shade Abdomen 15.54°C, Full Sun Thorax 35.99°C, Full Shade Thorax 15.4°C, Fig. 2).

Statistics.

Host choice experiments. For sun vs. shade host choice cage experiments in the field and laboratory, we utilized GLMMs with negative binomial distributed errors using R

function *glmer.nb* in package *lme4* (Bates et al. 2012). When analyzing stipple and egg count data from sun/shade plant and leaf choice experiments, plant origin (full sun/deep shade) was modeled as a fixed factor, cage number as a random factor, and leaf area or number of cauline leaves as covariates. When analyzing light/dark habitat choice experiments, we modeled Trial as having a random slope (with respect to light treatment) while evaluating the main fixed effects of the two categorical variables Light (yes/no) and Temperature (sun- or shade-exposed in the field; approx. 16°C or 21°C in the laboratory growth chambers), as well as their interaction.

Discussion.

Shade and sun habitat vary in abiotic factors such as temperature and light throughout the day as well as biotic factors such as leaf width and presence of prior herbivore damage, making abiotic and biotic stressors nonexclusive to one habitat. This experiment is unique in that it focuses on herbivore behavior rather than plant quality and decouples environmental factors underlying these distributions by examining each factor independently. Our results support the hypothesis that herbivores are driving plant distributions into the shade through behavioral preference for light. Support for insect preference driving host plant distribution comes from cage choice experiments where adult female *S. nigrita* exhibited a significant preference for feeding and ovipositing on *C. cordifolia* leaves exposed to light regardless of habitat origin of plant, leaf width, or presence of prior herbivore damage. Flies showed an even more pronounced preference for leaves exposed to light as temperature decreased. By utilizing a factorial design and using cages that isolated air temperature in the field from ambient light and surface

temperature, this study has effectively shown that herbivore preference is primarily light-driven. Furthermore, supporting evidence for ability to increase internal body temperature comes from ancillary data collected showing that after only minutes in the sun, adult females can reach internal body temperatures of up to 38°C which can decrease by almost 50% in the shade.

C. cordifolia may persist in full-sun habitat exposed to herbivores as a result of trade-offs in fitness experienced by various patches of plants. Plants in the sun may exhibit faster growth from increased levels of sunlight, allowing them to offset some fitness disadvantages of herbivory in increased levels of sunlight. However, plant abundance still may be lower than that of willow shade plants due to the heavy pressure of herbivory in these areas. Conversely, plants restricted to intermediate willow shade may experience the densest populations because they experience scattered light conditions. In this case, having less sunlight might also mean less herbivory because of lower temperatures. Furthermore, plants found in the deep-shade may experience little to no herbivory, but will get the least sunlight and will probably grow more slowly than the two former ecotypes.

Louda and Rodman's 1996 study proposed that the discordant distribution in herbivore pressure may be due to shade plant quality being lower than that of sun plants. Rather than a phytocentric explanation, my results suggest that plant distribution is likely primarily herbivore-driven as a result of simple phototactic behavior. This interaction is an example of how herbivores drive plant distributions into the shade by creating enemy-free space for host plants.

References.

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Data and Tables.

Figure 1. *S. nigrita* adult female foraging patterns in light/dark choice trials conducted at different temperatures.

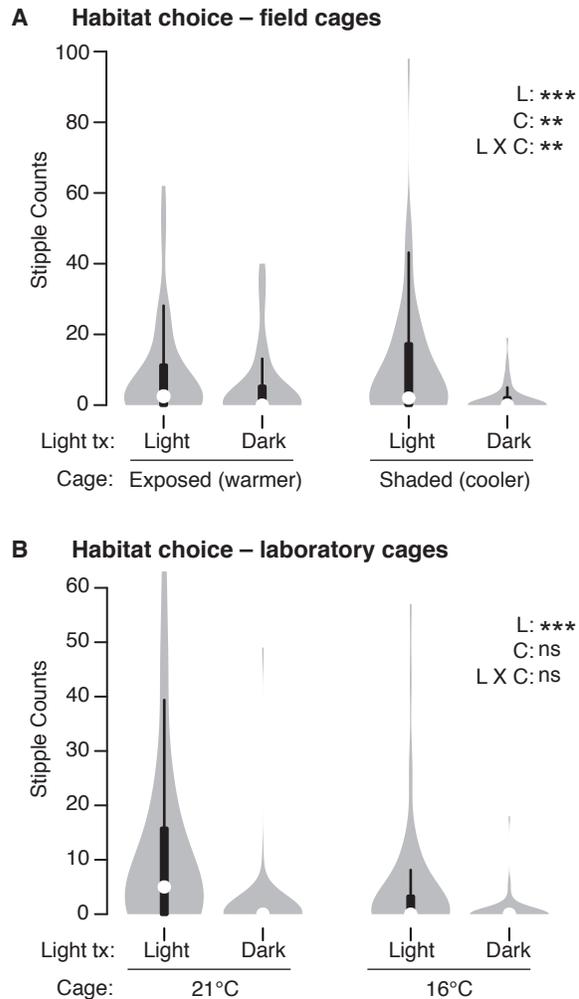


Figure 1. *S. nigrita* adult female foraging patterns in light/dark choice trials conducted at different temperatures. A. Light/dark choice trials conducted in sun-exposed (warmed) vs. shaded field cages. **B.** Light/dark choice trials conducted under controlled temperatures in the laboratory. L = light level (lighted vs. dark), C = cage type (warmer vs. cooler), L X C = interaction between light level and cage type.

Figure 2. Results of internal thorax and abdominal temperature assays.

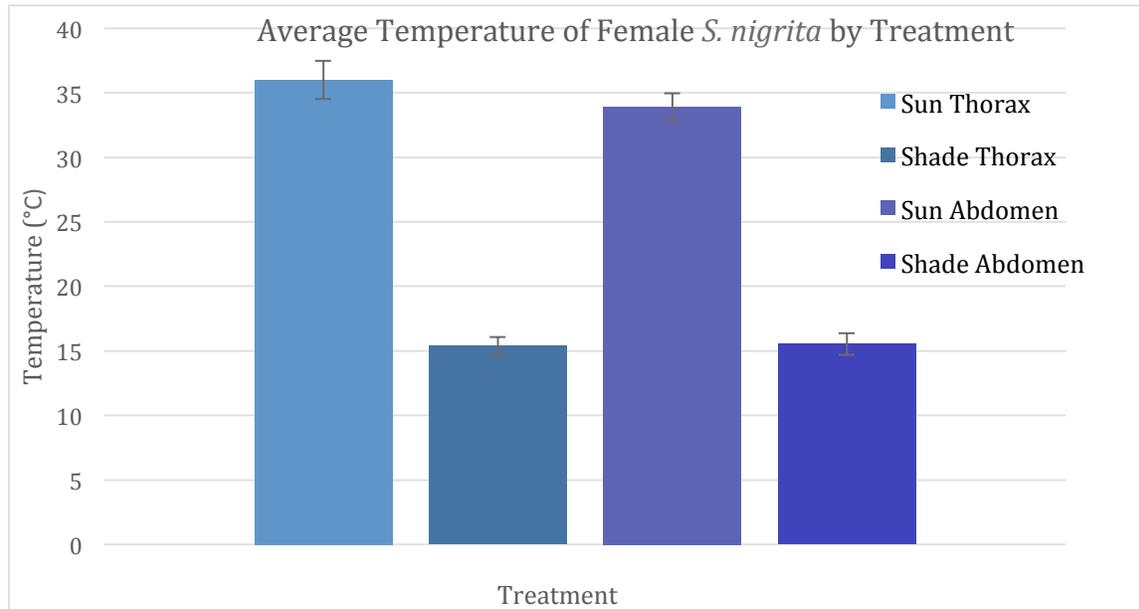


Figure 2. *S. nigrita* adult female thorax and abdomen temperature assays conducted in full sun and shade near the Rocky Mountain Biological Laboratory.

Table 1. Results of light vs. dark leaf choice experiments at different temperatures near Copper Creek (field) and in temperature-controlled rooms in the RMBL Laboratory.

Experiment	Predictor variable	Estimate	Std. Error	t	P-value
Field	Intercept	-1.797	0.496	-3.626	< 0.001
	Leaf width	0.063	0.010	6.547	< 0.001
	Cage – Sun	1.070	0.326	3.279	0.001
	Light tx – Light	2.230	0.464	4.811	< 0.001
	Cage X Light tx – Sun, Light	-1.333	0.441	-3.022	0.003
Lab	Intercept	-1.411	0.766	-1.842	0.065
	Leaf width	0.050	0.028	1.809	0.070
	Cage – Sun	0.304	0.584	0.520	0.603
	Light tx – Light	1.618	0.717	2.256	0.024
	Cage X Light tx – Sun, Light	0.652	0.791	0.825	0.410