

Rocky Mountain Biological Laboratory

Local Adaptations and Fitness of Transplants in the Lewis Flax – Flax Rust Coevolutionary System

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Abstract

Disease progresses differently in varying environments. In coevolutionary interactions, species that interact frequently may adapt to their environment and to each other in a process called local adaptation. However, there may be trade-offs between adapting to the environment and to a coevolving partner. We used the host–pathogen system of Lewis flax and its coevolved fungal pathogen, flax rust, to study how the environment mediates adaptation to locally common pathogens. To separate these environmental and genetic effects, we placed sibling seedlings in their home environments, in different environments of similar elevation, and in environments of different elevations, and compared disease resistance to the local flax rust spores at the test site. We predicted that: 1) regardless of genetic resistance, lower elevation populations would present with less disease on average than higher elevation populations because of inhibitory effects of temperature; and 2) that seedling transplants would be more adapted to the locally common pathogens in their sites of origin than foreign transplants would be. This research will help us to disentangle the effects of the environment from geography on the Lewis Flax- Flax Rust system in the high mountain elevations at the Rocky Mountain Biological Laboratory in Colorado. It will also contribute to the broader study of how hosts balance adapting to both pathogens and the abiotic environment. Building on previous studies of local adaptations across large scales, our experiment attempted to study local adaptation on a smaller scale in an effort to isolate the effects of genetic lineage from environmental changes to phenotypes.

Introduction

Local Adaptation, Coevolution and the Geographic Mosaic of Coevolution Theory

Local adaptation is an evolutionary process in which a population evolves to become more suited to its local environment through natural selection. When it occurs, local phenotypes will have a home site advantage (a higher fitness in their home environment) compared to individuals that originate from other locations. However, local adaptations to some factors, such as abiotic factors, can restrict local adaptations to other factors like pollinators and herbivores. This results in a fitness trade-off between adaptation to the abiotic environment and adaptations to natural enemies.

Local adaptations can also occur in the context of coevolutionary interactions. Coevolution occurs when two or more species that have regular interactions reciprocally affect each other's evolution by applying selection pressure on each other. For example, a study on the erosional effects of the fungal pathogen *Pseudocercospora fijiensis* on bananas in Cuba and the Dominican Republic found that there was a local adaptation pattern for resistant cultivars in both countries due to the existence of specific host–pathogen interactions, and the data also suggested the existence of host selection on those resistant cultivars (Dumartinet et al. 2019).

Additionally, the geographic mosaic of coevolution theory suggests that local adaptation and geography can shape coevolutionary dynamics. Coevolution proceeds by natural selection acting on three sources of variation that affect interactions among species: organism 1's genotype, organism 2's genotype, and the environment. The structure of natural selection on interactions differs among environments, such as high vs. low temperatures, high vs. low nutrient conditions, and a surrounding species web that is species-rich vs. species-poor. This variation

occurs because genes are expressed in different ways in different environments (genetic x environment interactions) and how one species affects the evolutionary fitness of another species depends on the environment the interaction takes place in (Percy 2007). Reciprocal selection between interacting organisms can vary spatially, resulting in local adaptation and speciation.

Illustrating this, a study testing the patterns of the plant-pathogen coevolution between prairie tallgrass *Integrifolium Michx* and its foliar pathogens found that on average, eastern populations had greater resistance to disease than western populations, consistent with the evolutionary legacy of more rapid coevolution due to high precipitation. Additionally, the rust pathogen was better able to infect plant populations from the same region, which is consistent with local adaptation of specialist pathogens to their local hosts (Cassetta et al. 2023).

Another study used a measured fitness trait of the hatching of cysts to test for local adaptation between the potato cyst nematode *Globodera pallida* and wild potatoes in Peru (Gautier et al. 2020). Although it did not find a strong pattern of local adaptation of the parasite, it did find that the sympatric combinations induced better hatching of cysts than allopatric combinations and that hatching percentage decreased with the host's distance from the nematode origin. Furthermore, the environment had a strong effect: the south of Peru induced better hatching than the north.

Study System

Lewis flax (*Linum lewisii*) is a perennial plant native to western North America, and is abundant in the sunny subalpine meadows in the Rocky Mountains of Colorado. Lewis flax can be infected by flax rust (*Melampsora lini*), a coevolved fungal pathogen that completes its life cycle on living flax tissue. Flax rust affects Lewis flax by limiting photosynthesis and weakening it by taking its nutrients. High levels of infection are correlated with low levels of fruit production (Jiranek, unpublished data).

Flax rust has been developed for over 80 years as a model rust to understand the mechanisms underlying the process by which disease develops, progresses, and persists or is resolved. In his study with *Melampsora lini*, Flor determined that genes in the host that expressed resistance and genes in the rust that expressed avirulence were highly specific in their interaction and corresponded to each other in a one-to-one relationship (Flor 1971).



Figure 1: Lewis flax



Figure 2: Lewis flax infected with Flax Rust

Both Lewis flax and flax rust are found across an elevational gradient in the area surrounding the Rocky Mountain Biological Laboratory. We were interested in the local adaptations of Lewis Flax populations in various elevations, and wanted to test the fitness of transplants of different populations by placing sibling transplants in their local populations and in foreign populations of varying elevations, and documenting their health and/or disease progressions. We predicted that the transplants would have a higher fitness in their local environment (Kalske et al. 2012) and would show differences in their flax rust infection rates, as past work showed that disease increased across an elevational gradient (see figure 3).

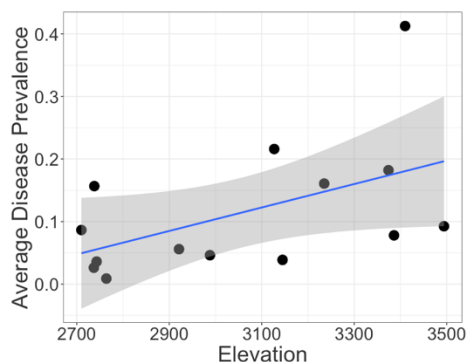


Figure 3: Disease Prevalence and Elevation

Hypotheses

We were interested in studying two questions within this system: 1) what are the effects of elevation on *Linum lewisii*'s disease resistance patterns to *Melampsora lini*; and 2) does Lewis Flax adapt to locally common pathogens? Our hypotheses were as follows:

Hypothesis 1: hosts would be more adapted to locally common pathogens in their sites of origin.

Hypothesis 2: high elevation sites are more conducive to pathogen growth, so we predicted greater infection at higher elevations regardless of the genetic backgrounds of the hosts.

Methods

Experimental Design

Seeds were collected from eleven of Juliana Jiranek's long-term study sites and were germinated in the greenhouse at the University of Virginia in sterilized potting mix. The seedlings were transported to RMBL by vehicle and were placed in a weather port at RMBL to prevent pathogen exposure. Plant families were selected for inclusion in the experiment based on their production of enough sibling plants to test at multiple sites. Families were classified as either "low" (< 3200 m) or "high" (> 3200 m) elevation.

We wanted to observe how the seedlings' resistance to disease varies with 1) adaptation to abiotic conditions (e.g., elevation, weather), and 2) adaptation to local pathogen populations. To do this, we performed the following at each of our sites: we transported each set of seedlings to their sites in individual crates that kept them in their cone-tainers. We placed the crates on the outside lower-left section of the transects. Then we collected five rust-infected leaves of similar rust coverage from multiple Lewis flax plants from near or within our transects. These flax leaves were chosen to be a representative sample of the local rust population. We put those leaves in a glass spray bottle with 10 mL of distilled water, shook the bottles to get a rust-water solution, and evenly and equally sprayed all of the transplants with that solution. This spraying supplemented the natural exposure of our plants to the local pathogen populations at each test site. The plants were watered on site and before being inoculated, to prevent the rust-solution from being washed off during watering.

After the seedlings were inoculated, we left them in the field for two weeks. Pilot data suggests that disease pustules manifest within 10-12 days, so this timeframe would have been long enough for symptoms to develop, but not long enough for the transplants to spread disease. Since the seedlings wouldn't be of flowering age during this period, they also could not introduce any new genetic material to the nearby populations. Plants were watered once a week to prevent drying. After two weeks, we put all of our transplants and their soils into trash bags, carried them back down the sites, and autoclaved (treated with high heat and high pressure) the material in the bags to destroy the spores.

If for all populations the local transplant seedlings outperformed the foreign transplants, the local seedlings would have been understood to be locally adapted (Hereford 2009).



Figure 4: Experimental Set-up



Figure 5: Pilot Seedling Infected with Rust (Orange pustules circled in purple)

Sites

We had 12 sites in total: three off of Cement Creek Trail (south of Crested Butte South), three off of Avery Trail, three off of Belleview, and three off of the 403 Gothic Trail. The three sites in each trail started at low, then medium, then high elevation, and were at approximately the same elevation across transects. Seeds were collected from 10 of these 12 sites. These sites were chosen because they were across an elevation gradient, provided variation in landscapes, and captured temperature and weather condition gradients.



Figure 6: Cement Creek Sites



Figure 7: Belleview Sites

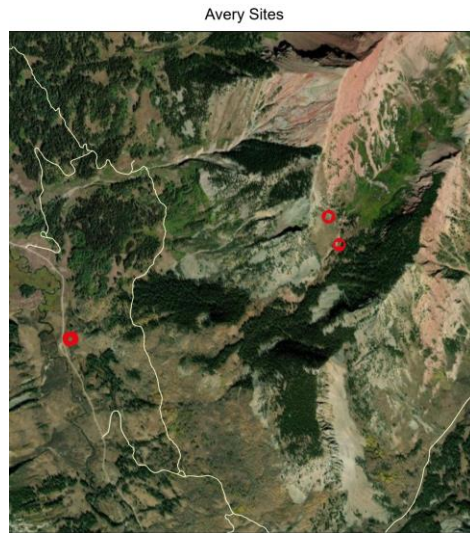


Figure 8: Avery Sites

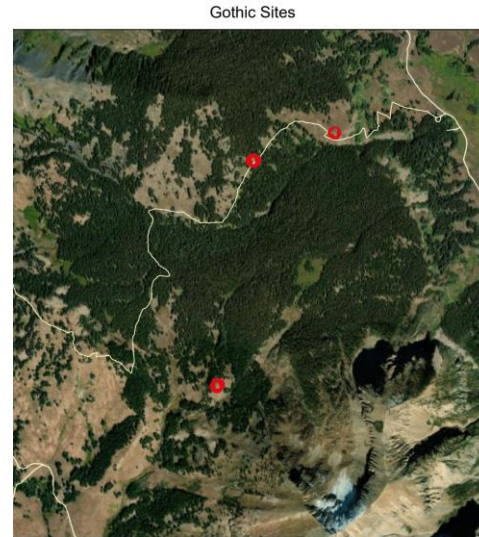


Figure 9: Gothic Sites

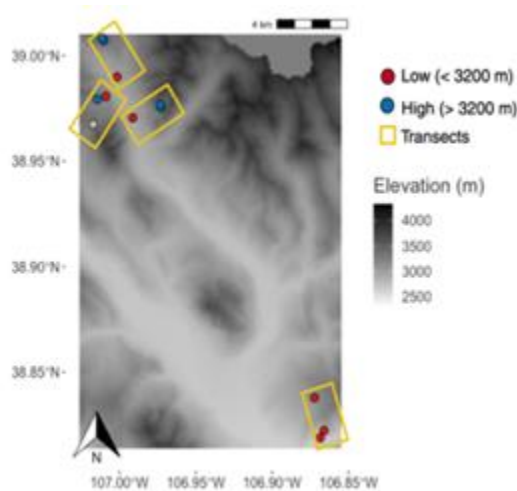


Figure 10: All Sites and Elevation Categories

Site	Latitude	Longitude	Elevation
CC3	38.84057	-106.873	3127
CC2	38.82501	-106.867	2921
CC	38.82156	-106.869	2440
AV2	38.97788	-106.978	3478
AV1	38.97641	-106.977	3416
BT	38.9713	-106.996	2940
BV2	39.00869	-107.017	3494
BV1	39.00759	-107.016	3410
RG1	38.99054	-107.007	2988
GM	38.97969	-107.019	3220

Figure 11: All Sites' Coordinates and Elevations

Data Collection and Tests

On the second (final) week of the experiment, we scored the seedlings as dead or alive, and as either 1, infected, or 0, uninfected. We rated the resistance responses on a binary scale, with zero indicating complete susceptibility and one indicating resistance. Resistance responses were scored using the methodology described in Thrall et. al's 2002 study. We planned to analyze infection results in R with a generalized linear mixed model with a binomial family, using the lme4 package. This model would have tested whether resistance responses were affected by the plant being at its home site (sympatry) and being an environmental match (low/medium/high elevation). We would also have included random effects for plant family and exposure site.

This would have tested for local adaptation by determining if seeds isolated from their home sites fared better in their local population and against local pathogens than seeds isolated

from other sites. This will also have tested for genetic conditions and/or environmental conditions contributing to the patterns of disease distribution. We hypothesized that conditions for rust growth would have been better at high elevations: since plants are stressed at higher elevations, they may have shown higher fitness (including resistance) when moved to lower elevations. Also, lower elevation plants, being larger and presumably more genetically diverse, could be more resistant to disease on average. Furthermore, previous data from Juliana Jiranek's lab indicate that flax-rust infection levels are greater at higher elevations (see Figure 3).

Results

We transplanted 263 seedlings across the 10 test sites, evaluating between 22 and 34 seedlings per site. The following number of seedlings were transported to the highest, middle and lowest elevation sites, respectively: 27, 22, and 24 at Cement Creek; 34 at Gothic Mountain; 26, 23, and 27 at Avery; 24, 28, and 28 at Bellview. None of our seedlings were infected by any amount of visible rust, and many of the seedlings dried out and died. The following number of seedlings died at each site in the order of highest, middle and lowest elevation: 2, 9, and 15 at Cement Creek; 22 at Gothic Mountain; 6, 0, and 5 at Avery; and 7, 2, and 15 at Bellview. This corresponds to the following percent mortality rates, in the same order: 7.41, 40.91, and 62.50 at Cement Creek; 64.71 at Gothic Mountain; 0, 23.08, and 18.52 at Avery; and 25, 8.33, and 53.57 at Bellview, as seen in Figures 10 and 11. Percent mortality was greater in lower elevation transects than in higher elevation transects.

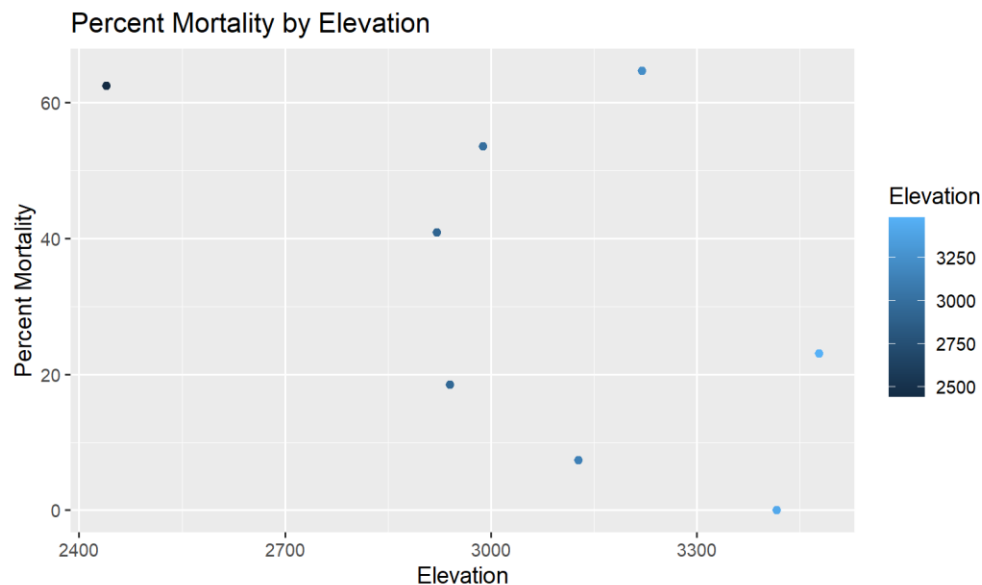


Figure 12: Percent Mortality by Elevation

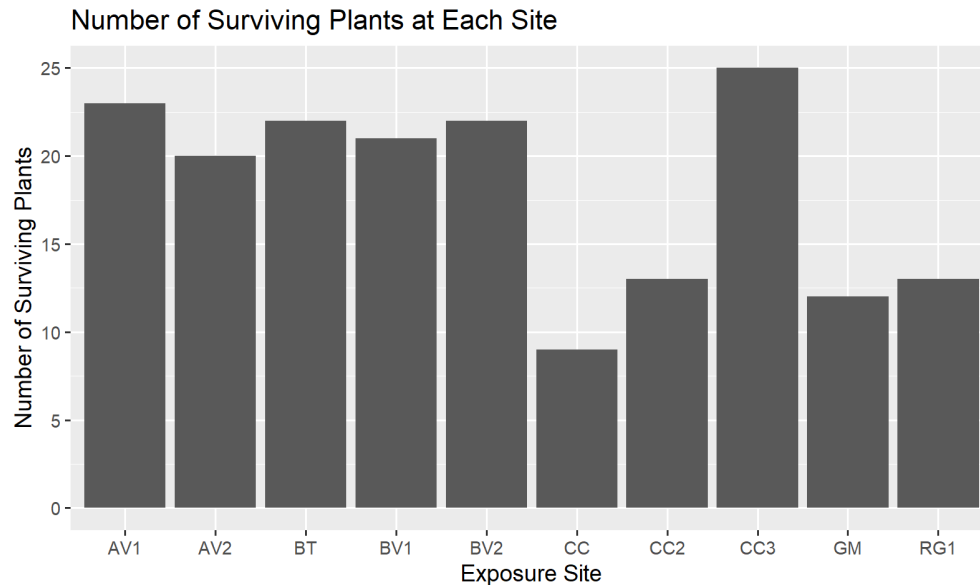


Figure 13: Plant Survival by Exposure Site (Experiment Site)

Discussion

We are not certain as to why rust did not grow on our experimental seedlings, but we believe it is likely because the plants dried out in their small pots between waterings. Our pilot inoculations succeeded with the same method, so it is unlikely that the spray method was inefficient or that the inoculum was too low. We watered them once a week as planned, but given the hot and dry conditions during our 2-week testing period and the relatively small amount of moisture the cone-tainers could hold, this was likely too infrequent. The seedlings either exhibited the purple pigmentation characteristic of stressed plants, or dried out completely and died.

This total lack of disease symptoms suggests that *M. lini* may require a minimum level of host health for infection. *M. lini* is an obligate biotroph, and thus requires living plant tissue in order to reproduce. It may also suggest a host temperature range that is suitable for rust. Since our seedlings were very dehydrated, they likely could not cool themselves off through evapotranspiration. This could have made them too hot and dry for rust grow on, as high humidity or free water conditions are necessary for germination, and temperatures above 23-27 Celsius will inhibit germination (Lawrence et al. 2007).

Our experiment may differ from other local adaptation experiments in that our plants weren't planted in the soil and so did not have access to the water table, and this is something we will consider changing in future experiments. If the experiment were repeated using plant hosts in conetainers, the plants would have to be watered more frequently. However, these changes present their own trade-offs between realism and invasiveness, since watering as often as necessary means that our seedlings' watering conditions may not match that of the rest of the transect, and planting the seedlings requires further disrupting the ecosystem.

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