

LITTLE EVIDENCE FOR LOCAL ADAPTATION TO SOIL TYPE BY *ACHILLEA MILLEFOLIUM* AND *HYPERICUM PERFORATUM* FROM DEER ISLES, MAINE, USA

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ABSTRACT

We investigated whether populations of two common forbs, *Achillea millefolium* subsp. *lanulosa* (Asteraceae) and *Hypericum perforatum* (Hypericaceae), found on serpentine and granite outcrops on Deer Isle, Maine are locally adapted to their ‘home’ soils. To test whether these two species show greater fitness in their ‘home’ soils compared to ‘away’ soils, and if substrate differences lead to divergent flowering times, we performed a reciprocal transplant experiment in a growth chamber using field-collected soils. We measured survival, growth and reproductive output for both species grown in both soil types. We found a significant substrate effect for belowground biomass of *H. perforatum*; serpentine populations had significantly more root biomass in ‘home’ soil compared to away ‘soil.’ Differences in survival, days to flower, root:shoot dry mass, total dry mass, number of flower heads, or total seed mass were not significant for either species grown in the two soil types. Our results suggest that the two species are not locally adapted to their home substrate and may represent general purpose genotypes broadly tolerant to edaphic conditions of both serpentine- and granite-based soils. Additional research, with larger sample sizes, is necessary to confirm the findings from the current study.

INTRODUCTION

Local adaptation is of fundamental importance in the process of ecological speciation. Divergent selection pressures can often lead to the evolution of ecotypes uniquely adapted to site-specific conditions. Local adaptation is often demonstrated when a population transplanted into their ‘home’ habitat outperforms a population from an ‘away’ habitat; in the strictest sense, a locally adapted population will exhibit higher mean reproductive fitness under ‘home’ compared to ‘away’ conditions (Cheplick 2015).

Local adaptation to soils is common although not ubiquitous (Beiger et al. 2014). Plants growing on serpentine soils have often been used as model systems for the study of local adaptation (Selby et al. 2014).

In this study, we test the hypotheses that populations of two common forbs, *A. millefolium* subsp. *lanulosum* (AM) and *H. perforatum* (HP), found on nearby serpentine and granite soils, are locally adapted to their ‘home’ substrate and have disparate flowering phenology. To do so, we conducted a reciprocal transplant study in a growth chamber using field collected soils. Specifically, we tested 1) whether AM and HP are locally adapted (i.e., show better growth or fitness) to serpentine and granite soils, showing evidence for ecotypic differentiation, and 2) whether the populations of these species demonstrate divergence in flowering phenology, either due to phenotypic plasticity or an inherent genetic difference, showing evidence for a mechanism of limiting gene flow between the edaphically distinct populations.

MATERIALS AND METHODS

Site description. Pine Hill Reserve (PH) on Little Deer Isle, Maine and Settlement Quarry (SQ) in Stonington, Deer Isle, Maine are two adjacent sites where the focal species are found. The two sites are approximately 17 km apart and are less than 1 km inland from Penobscot Bay. PH is a former serpentine quarry ~ 3 ha (44° 17'07.3" N, 68° 42'06.7" W, WGS 84). SQ is a former granite quarry (44° 10'37.6" N, 68° 38'20.7" W, WGS 84) and is ~ 30 ha. Previous work at these two sites suggest they have comparable climate, altitude, aspect, and land-use histories (Pope et al. 2010).

Reciprocal Transplant Experiment. In February (2015), we sowed 3 seeds each of 6-9 AM mothers and 5 seeds each of 6-9 HP mothers at 0.5 cm depth in 4"x4"x4" plastic pots with water saturated granite and serpentine soils. Approximately 3-7 days after the first seedlings appeared, we kept the largest seedling (height and width across the 2 leaves) and removed the rest so that each pot ended up with 1 individual plant. For AM from PH, we ended up with 9 replicates on serpentine and 11 on granite. For AM from SQ, we ended up with 10 replicates on serpentine and 9 on granite. For HP from PH, we ended up with 11 replicates each on serpentine and granite. Finally, for HP from SQ, we ended up with 9 replicates on serpentine and 8 on granite.

We carried out the experiment for 4 months. During the experiment, temperature, humidity, and light conditions were all gradually increased to mimic average day length, temperature, and humidity on Deer Isle from June to September. We watered the seedlings every 3 days for the first seven weeks. Three months into the experiment, we watered plants every 4-5 days. We recorded survival every 2 weeks. To avoid edge effects, pots were rotated within the growth chamber once a month.

Trait Measurements. We recorded the day of first flower for each replicate. For replicates with multiple inflorescences (i.e., flowering stalks), the day of first flower was recorded for each inflorescence. At the end of the season, we measured reproductive output by counting the flower heads for each replicate that produced flowers. For HP, we also counted the number of seeds produced and seed mass per replicate. We separated the aboveground (shoots and leaves) and belowground biomass (roots) and washed the roots to remove soil particles. We oven-dried the samples under 70°C for 24 hours. For aboveground biomass, we weighed the shoot with leaves (AM) or stem excluding the leaves (HP), since leaves may have dropped before the end of the experiment or when plants were harvested/processed. For belowground biomass, we weighed the dry root mass of each plant. We then calculated the root:shoot ratio for AM and HP.



Achillea millefolium subsp. *lanulosum* (L) and *Hypericum perforatum* (R).

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Statistical Analyses. All statistical analyses were conducted in R 3.1.2. using the R language and environment for statistical computing (R Core Team 2016). Results for all analyses were plotted as reaction norms, and interaction term significance for each species and trait was determined using a likelihood-ratio test comparing mixed models of the form:

Model 1. Trait ~ soil + source + soil*source + N(0, σ²mother);

Model 2. Trait ~ soil + source + N(0, σ²mother),

in which model 2 represents a null model that excludes interaction effects. Survival and flower phenology was only tested for HP due to low sample size of flowering and senescing AM during the experiment. Survival and flower phenology was analyzed with a Cox Mixed Effects model using the ‘coxme’ package (Therneau 2015), which compares the timing of events (survival analysis).

RESULTS

The only statistically significant interaction effect (i.e., differing slopes in reaction norm plots) was for belowground biomass in populations of HP (Fig. 1). Both HP populations had significantly more root mass in their ‘home’ soils compared to ‘away’ soils. For AM, there was no significant difference between populations grown in serpentine or granite soils (Fig. 2). No significant interactions were shown for the Cox mixed effects survival analyses ($P > 0.05$) for either species, suggesting no difference between survival or days to first flower on granite or serpentine soils. However, we treat our results with caution until we are able to repeat the experiment with a larger sample size, especially for AM.

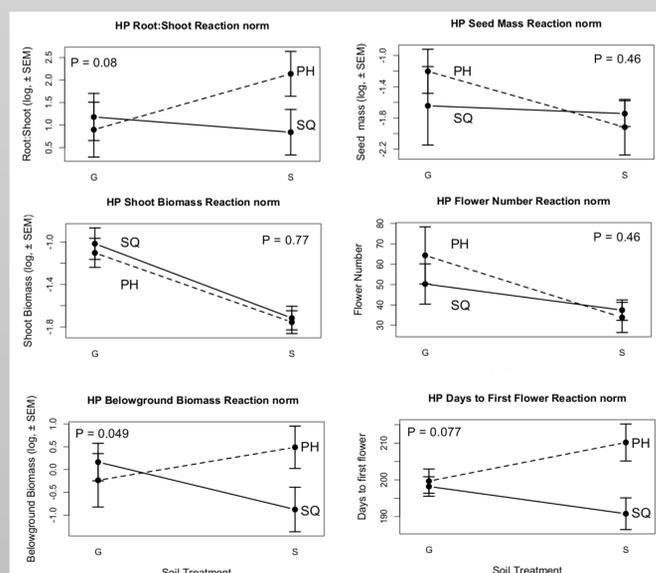


Fig. 1. Reaction norm plots for populations of *H. perforatum* (HP) grown in home and away soils (G=granite; S=serpentine). Solid line represents granite (SQ); dotted line represents serpentine (PH) source populations. Plotted values are means and standard errors after log-transforming the data to meet assumptions of normality, in most cases. Results show no evidence of local adaptation (no significant difference between reaction norm slopes), except in the case of belowground biomass, ($\chi^2(1) = 3.9, p < 0.05$).

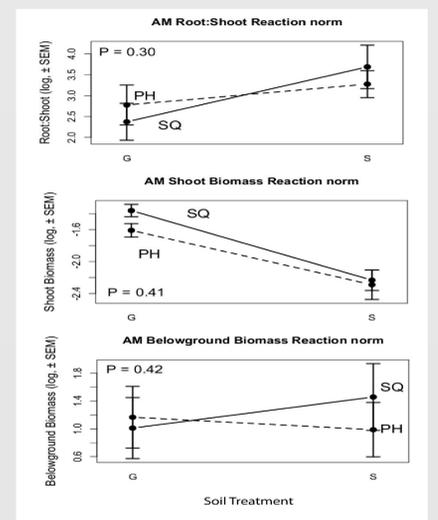


Fig. 2. Reaction norm plots for populations of *A. millefolium* (AM) grown in home and away soils (G=granite; S=serpentine). Solid line represents granite (SQ); dotted line represents serpentine (PH) source populations. Plotted values are means and standard errors after log-transforming the data to meet assumptions of normality.

DISCUSSION

Our study revealed little to no evidence for local adaptation to serpentine or granite soils by either AM or HP. Previous studies on AM, however, have documented ecotypes on serpentine soils (Kruckeberg 1967; Higgins and Mack 1987; O’Dell and Claassen 2006), with greater tolerance to ion imbalances and enhanced growth on serpentine, compared to populations collected from non-serpentine soils. Similarly, ecotypic differentiation has been documented for calcareous and acidic populations of HP (Ramakrishnan 1969). In our experiment, AM plants did not flower in either serpentine or granite soils, therefore we were unable to assess whether there would be any evidence for local adaptation to either soil via increased reproductive fitness. Additional work is necessary to further explore if and how soil type affects reproductive effort in AM.

The only evidence for adaptive differentiation was found in belowground biomass of HP. Selection for an increased belowground allocation can be a result of local adaptation to nutrient poor and water stressed soil conditions such as those found on serpentine soil (Rajakaruna et al. 2003). Greater carbon allocation to root has been observed in several serpentine-associated plants, including herbs (Rajakaruna et al. 2003) and seedlings of woody plants (Burgess et al. 2014).

Our data also suggest several trends pointing to possible differences in the extent of phenotypic plasticity (e.g., Root:Shoot, Seed Mass for HP from PH and Shoot Mass for HP, AM from both sites) when grown on ‘home’ vs ‘away’ soils (Fig. 1, 2). We also detect a noticeable difference in the days to flower in HP from PH, flowering 10-20 days later than HP from SQ when on S soil (Fig. 1). We will delay our discussion of the possible adaptive significance of these observations until we repeat the study with larger sample sizes, particularly for AM, and include leaves in Shoot Mass for HP, to get a better measure of how soil type affects aboveground mass.

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LITERATURE CITED

Beiger, A. et al. (2014) *Plant Ecology and Diversity* 7: 411-420; Burgess, J. L. et al. (2015) *Australian Journal of Botany* 63: 308-323; Cheplick, G.P. (2015) *Approaches to Plant Evolutionary Ecology*, Oxford University Press, New York.; Higgins, S. S. & R. N. Mack (1987) *Oecologia* 73:591-597; Kruckeberg, A. R. (1967) *Brittonia* 19: 133-151; O’Dell, R. E. & V. P. Claassen (2006) *Plant Soil* 279: 253-269; Pope, N., et al. (2010) *Rhodora* 112:105-141; R Development Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0; Rajakaruna, N. et al. (2003) *New Phytologist* 157: 93-103; Ramakrishnan, P. S. (1969) *Can. J. Bot.* 47: 175-181; Selby J. P. et al. (2014) In: *Plant Ecology and Evolution in Harsh Environments*. Pp. 243-265. NOVA Science Publishers, Inc. Hauppauge, NY, USA; Therneau, T. M. (2015) *Mixed Effects Cox Models – R package ver. 2.2-5*.