Environmental and genetic variation in an asexual plant

Mark Davidson Jewell[1] and Graham Bell[1]

[1]Affiliation not available

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Mark Davidson Jewell[1] and Graham Bell[1,2]

[1]Department of Biology, McGill University; 1205 ave Docteur Penfield, Montreal, Quebec H3A 1B1, Canada.

[2]Redpath Museum, McGill University; 859 Sherbrooke St West, Montreal, Quebec H3A 0C4, Canada.

Abstract

Species may respond to variation in environmental conditions by modifying their phenotype to match local levels of resource availability. This phenotypic response can be driven by plastic physiological change, or by adaptive genetic change. Here we use*Lemna minor* (lesser duckweed), a small aquatic macrophyte that is increasingly used as a model in ecology and evolution, to investigate the source and maintenance of phenotypic variation in natural environments. We found substantial phenotypic variation in *L. minor* in the field, with its frond area and root length changing predictably over natural environmental gradients of resource availability. Separating environmental and genetic variation in these traits in a common garden, we attribute the majority of phenotypic variation we observed in the field to phenotypic plasticity. Despite this, there was substantial within-site genetic variation. We found evidence of strong purifying selection in the field, that is necessarily balanced by mutation and migration. Using measures of environmental and genetic variation in phenotype and fitness, we estimate rates of evolution of fitness, and dispersal necessary to sustain the observed levels of genetic variation.

Keywords: *Lemna minor, duckweed, phenotypic plasticity, local adaptation, purifying selection, migration-selection balance, multi-niche polymorphism, common garden*

Introduction

Separating environmental from genetic contributions to phenotypic variation is central to evolutionary ecology since it illuminates how species respond to their local environment and produce phenotypes capable of maintaining positive fitness and thus population persistence. When a population experiences new environmental conditions, either by environmental change or range expansion, existing genotypes may shift their phenotypic expression via physiological change (aka. adaptive plasticity), or evolution may shift genotype frequencies leading to local adaptation via genetic change (Sultan 2000, Kingsolver et al. 2002). Natural selection, acting on novel mutation and standing genetic variation, should reach beyond the limits of plasticity and maximize population mean fitness (Auld et al. 2010). The rate of evolutionary change is however dependent on the amount of genetic variation in a population, namely genetic variation in fitness (Burt 1995). Like phenotype, fitness itself is comprised of environmental and genetic components (Schoen et al. 1994). Genetic variation in fitness is the raw material natural selection acts on, and determines a population's rate of adaptive evolution in the face of environmental change and genetic degradation caused by deleterious mutation, maladaptive gene flow, genetic drift and inbreeding depression (Burt 1995, Hendry et al. 2018). Despite the critical role of intraspecific genetic variation to species adaptation and survival (Booy et al. 2000), the amount of genetic diversity in natural populations remains largely unknown. Whereas

much recent work has focused on the genetic structure of endangered populations, it is equally important to understand how genetic variation may contribute to the successes of populations that thrive.

In this study we quantified environmental and genetic variation in morphological traits and in fitness for the plant *Lemna minor* (lesser duckweed), a tiny floating aquatic plant in the family *Lemnaceae* found in eutrophic ponds and wetlands. Among the smallest of all angiosperms, *L. minor* consists of only a single leaf-like frond, a few mm across, to which a single unbranched root is attached. Its reproduction is almost exclusively asexual and vegetative with daughter fronds budding out the mother frond's lower surface (Landolt 1986, Lemon and Posluszny 2000). Daughter fronds remain attached to the mother for a certain period of time before splitting apart after abscission (Landolt 1986, Lemon et al. 2001). Their generation time may be as short as just a few days, and their small size results in populations of hundreds of thousands to millions of individuals in a single pond. Because they are widespread and abundant, are easily maintained and manipulated in the laboratory, and possess highly reduced morphology and simplified physiology, they are being increasingly used as a tractable model system in ecology and evolution (Laird and Barks 2018, Hart et al. 2019, Vu et al. 2019).

We quantified phenotypic variation in two morphological traits: frond area and root length. The extremely simplified morphology of *L. minor* means that these two traits essentially capture the totality of biomass allocation between shoot and root tissue, responsible for the capture of light and nutrients. The frond is essentially a photosynthetic sheet whose area may fluctuate to balance light capture and photosynthesis (growth) with the production of daughter fronds (reproduction) (Vasseur et al. 1995). Root length on the other hand has been shown to vary depending on nutrient levels, since uptake rates are proportional to root surface area (Cedergreen and Madsen 2002). Optimal phenotype in *L. minor* 's root-shoot ratio should then vary in the field as a function of local availability of light and nutrients, with the plant investing more biomass into the tissue responsible for the uptake of the limiting resource. Such phenotypic variation could arise via plasticity or local adaptation, or both, with consequences for within and among site genetic diversity. By measuring phenotypic expression in a common garden assay (Kawecki and Ebert 2004) we can quantify environmental and genetic components of variation in frond area and root length to determine if these traits have a genetic basis and result from local adaptation.

Our study had three main objectives. First, we ask if phenotypic variation in *L. minor* is correlated with natural gradients of resource levels. We hypothesize that biomass allocation and phenotype should match the environment to increase uptake rates of limiting resources, such that low light environments produce plants with larger fronds, and low nutrient environments produce plants with longer roots. Secondly, we ask if such phenotypic variation is due primarily to plasticity or local adaptations. We do this by quantifying the environmental and genetic contributions to phenotypic variation. Thirdly we aim estimate adaptive potential in natural populations of *L. minor* by quantifying the standing genetic variation in fitness.

Materials and Methods

Field Survey

In July 2019, we conducted a regional survey of natural populations of *Lemna minor* . We located 34 sites consisting of lakes, wetlands, ponds, and roadside ditches broadly distributed across southern Quebec, Canada (Fig. 1). Within each site, we sampled from three microsites, situated at least 10m apart and supporting a minimum of 10 *L. minor* colonies. From each microsite, we collected 10 free-floating colonies of *Lemna minor* . Colonies consisted of between 2-10 attached fronds. Since daughter fronds emerge from two meristematic pockets located on the frond's lower surface, frond genealogy is easily inferred. For each colony we measured both frond area and root length of the oldest frond by photographing the plant against a standard ruler and later analysing the photos using ImageJ. This resulted in 30 measurements of each trait per site.

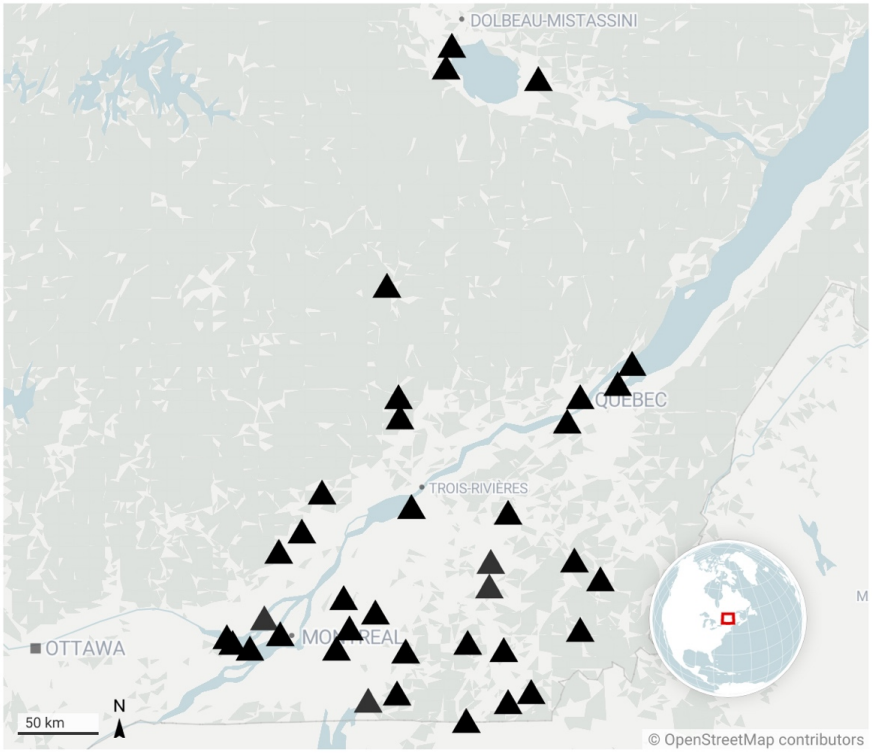

Fig. 1. Thirty-four sites broadly distributed across southern Quebec, supporting natural populations of *Lemna minor* . Map was produced with Datawrapper.

For each site we measured several environmental variables that we expected to be correlated with plant phenotype. Light availability, as percent transmittance of photosynthetically active radiation (PAR), was estimated *in situ* with the use of BF5 Sunshine Sensor (Delta-T, Burwell, Cambridge, UK) (Paquette et al. 2007). This instrument consists of an array of seven quantum sensors under a semi-shaded hemispherical dome to give estimates of diffused light under any meteorological condition. We took two simultaneous paired measurements, one at the sampling site in question, and a second reference point at a nearby open site (field or road) under full sun. Percent transmittance PAR was then estimated as the ratio of diffused light between the site and the reference measurement. This method has been demonstrated as a reliable and practical alternative to more standard measurement techniques including hemispherical image analysis (Rich et al. 1993, Paquette et al. 2007). Percent transmittance of PAR was estimated at each of the three microsites as well as the center of the site. All measurements were taken 1.5m above the water level, above any aquatic macrophytes or riparian herbaceous plants to obtain an estimate of shading from the canopy cover. These four measurements were then averaged to produce a single estimate of light availability for each site. To measure water nutrient content, we took eight water samples from the center of each site at a depth of 30cm. Total Nitrogen (TN), total Phosphorus (TP), dissolved Nitrogen (DN) and dissolved Phosphorus (DP) were estimated each from two replicate samples. Acid-washed tubes were first rinsed, and then filled with sample water, unfiltered for TN and TP samples, and sterile filtered at 0.45um for DN and DP samples. After sampling, all tubes were stored in a cooler on ice and brought back to the lab for analysis. Samples were then stored at 4°C and processed within 14 days. Water samples were analysed for TN and DN with a continuous flow analyser (OI Analytical Flow Solution 3100 ©) using an alkaline persulfate digestion method, coupled with a cadmium reactor (Patton and J.R. 2003) and for DP using a standard protocol (Wetzel and Likens 2000). TP was measured using colorimetric detection with a spectrophotometer at 890 nm, after digestion with potassium persulfate and the addition of an ammonium molybdate solution

(Wetzel and Likens 2000). All samples were analysed at the GRIL, Universite du Quebec a Montreal (UQAM) analytical laboratory. We used a YSI probe (YSI professional plus, Xylem Inc., Yellow Sprigs, OH, USA.) to measure water temperature and pH at the centre of each site at a depth of 30cm. Full list of environmental correlates can be found in the supplementary information (Table S1).

Common Garden Assay

To determine the sources of phenotypic variation in the field, we brought samples back to McGill University to use in a common garden growth assay. Whereas phenotypic variation in the field is due to a mixture of environmental and genetic sources, growth in a common garden removes environmental variation, isolating genetic variation. A single colony of *L. minor* was collected from each of 3 microsites for each site and preserved in tubes filled with natural sample water and stored in the dark during transport.

Back in the lab, all tubes were placed under artificial grow lights (100 μmols/m^2/s) until the plants had doubled in number, consisting of at least two detached colonies which would be used to found two clonal replicates for each microsite. The common garden assay was done using 500mL Erlenmeyer flasks. There was a total of 204 flasks (34 sites x 3 microsites x 2 replicate flasks). Flasks were filled with 350mL of growth media, diluted Hoagland's E-media ([N]=5000 μgL^{-1}, [P]= 780 μgL^{-1}, pH=7.0 +/-0.05) (recipe in Supplementary information, Table S2), plugged with a foam stopper, and then autoclaved. A single colony (3-4 attached fronds) was used to inoculate each flask. These initial fronds were first marked on their ventral surface with a small dot with a permanent marker to later track generations. This was to ensure that phenotypes were only measured on fronds at least two generations younger than those sampled from the field.

All flasks were placed in one of two identical controlled growth chambers of the McGill phytotron (200 μmols/m^2/s light, 20^0C, 70% relative humidity, with a 14/10 light-dark cycle). The two replicates were blocked, with one replicate of each microsite in each chamber. The 102 flasks in each growth chamber were randomly positioned, leaving a 15cm boundary from the chamber wall on all sides. The common garden assay was broken into three 10-day phases, separated by two transfers. Transferring the plants to fresh media every 10 days prevented nutrient depletion, all-the-while limiting the growth of phytoplankton whose differential abundance among flasks could influence nutrient availability and plant growth. To remove any maternal or carry over effects, we tracked generations to ensure that we only measured the phenotypes of plants at least two generations younger than the initial plants brought back from the field. The first, 10-day preliminary acclimation phase served to ensure an equal physiological starting point of all plants before we began to track population growth rates, and to ensure the removal of all fronds initially present in the assay. After these 10 days of growth, all fronds marked with a black dot were discarded, and a single younger colony was randomly selected and used to inoculate identical flasks with fresh growth media, after the oldest frond in this colony was again marked. After a second 10 days of growth, flasks were again removed, and all plants were transferred to fresh media before being returned to the chambers for a final 10 days of growth. After each of the two transfer dates, all flasks were returned to the same growth chambers, but their positions within each were independently randomized. At the end of the experiment, the total number of fronds was counted in each flask and used to calculate rates of exponential population growth (over the final 20 days). From each flask, we randomly sampled 10 individuals (on average ˜15% of the population) for whom we measured frond area and root length by imaging (plants were pressed onto a sheet including a reference ruler and photographed at a standard 20cm distance) and subsequent image analysis using Image J (Abràmoff et al. 2004). Second generation fronds (marked with a black dot) were excluded, as were immature fronds (that didn't yet have two daughter fronds budding from them).

Statistical analysis

To test whether there were differences in phenotype among sites and microsites in the field, we used a 2-way nested analysis of variance (ANOVA) with microsite nested within site. Both site and microsite were analysed

as type II random factors. This was done for both response variables (frond area and root length). Since the environmental correlates were measured at the site level, all 30 measures of phenotype (10 individuals x 3 microsites) were averaged to produce a single value per site for frond area and root length. We then regressed site mean phenotype (both frond area and root length) against the environmental correlates (light availability, TN, TP, DN, DP, and pH) using linear regression and simplified the models by removing non-significant terms. To test whether there were differences in phenotype among sites and microsites after the common garden assay we used a similar nested ANOVA as that used for the field data, but with a 3rd level (replicate flask), nested within microsite, using the 10 individuals per flask as the error variance. Growth rate (fitness) was calculated for each flask over the final 20 days of the common garden assay using the standard formula for exponential growth$r = \frac{\ln\left(\frac{N_t}{N_0}\right)}{t}$ where N_0 is initial population size, t is time in days, and N_t is population size at time t. To test for differences in fitness among sites and microsites we used a similar nested ANOVA with microsites nested within sites. However, since there is only a single measure of fitness per flask, replicate flask was used as the error variance.

Results

We observed substantial phenotypic variation in the field for both frond area and root length. Frond area varied significantly among sites ($F_{33,68}$=5.39, p<0.0001) and among microsites within sites ($F_{68,918}$=15.26, p<0.0001) with roughly equal contributions to variation from each level (Table 1). Similarly, root length varied significantly among sites ($F_{33,68}$=14.12, p<0.0001) and among microsites within sites ($F_{68,918}$=3.46, p<0.0001), although the majority of this variation was at the among site level (Table 1).

Table 1. Variation in frond area (mm^2) and root length (mm) measured in the field. Ten individual plants were sampled in each of three microsites, for 34 sites situated broadly across southern Quebec, Canada.

Trait	Source	df	SumSq	MeanSq	*F*	*p*	Variance Component
Frond Area	Site	33	13844	419.52	5.39	<0.0001	11.389
	Microsite	68	5294	77.853	15.26	<0.0001	7.2753
	Error	918	4682	5.1002			5.1002
	Total	1019	23820				
Root Length	Site	33	335062	10153.39	14.12	<0.0001	314.47
	Microsite	68	48861	718.54	3.46	<0.0001	51.10
	Error	918	190561	207.58			207.58
	Total	1019	239422				

Variation in frond area was correlated with natural levels of light availability ($F_{1,32}$ = 16.99, p=0.0002, β= -9.663, R^2=0.35), with plants growing in more heavily shaded sites with thicker canopy cover expressing larger fronds (Fig. 2A). Variation in root length was correlated with water nutrient levels (both dissolved and total Nitrogen and Phosphorus), with plants growing longer roots in lower nutrient conditions (Fig. 2B). The variable with the most explanatory power was total Phosphorus content ($F_{1,32}$ = 27.59, p<0.0001, β= -0.10, R^2=0.46). PH failed to explain additional variation in these traits.

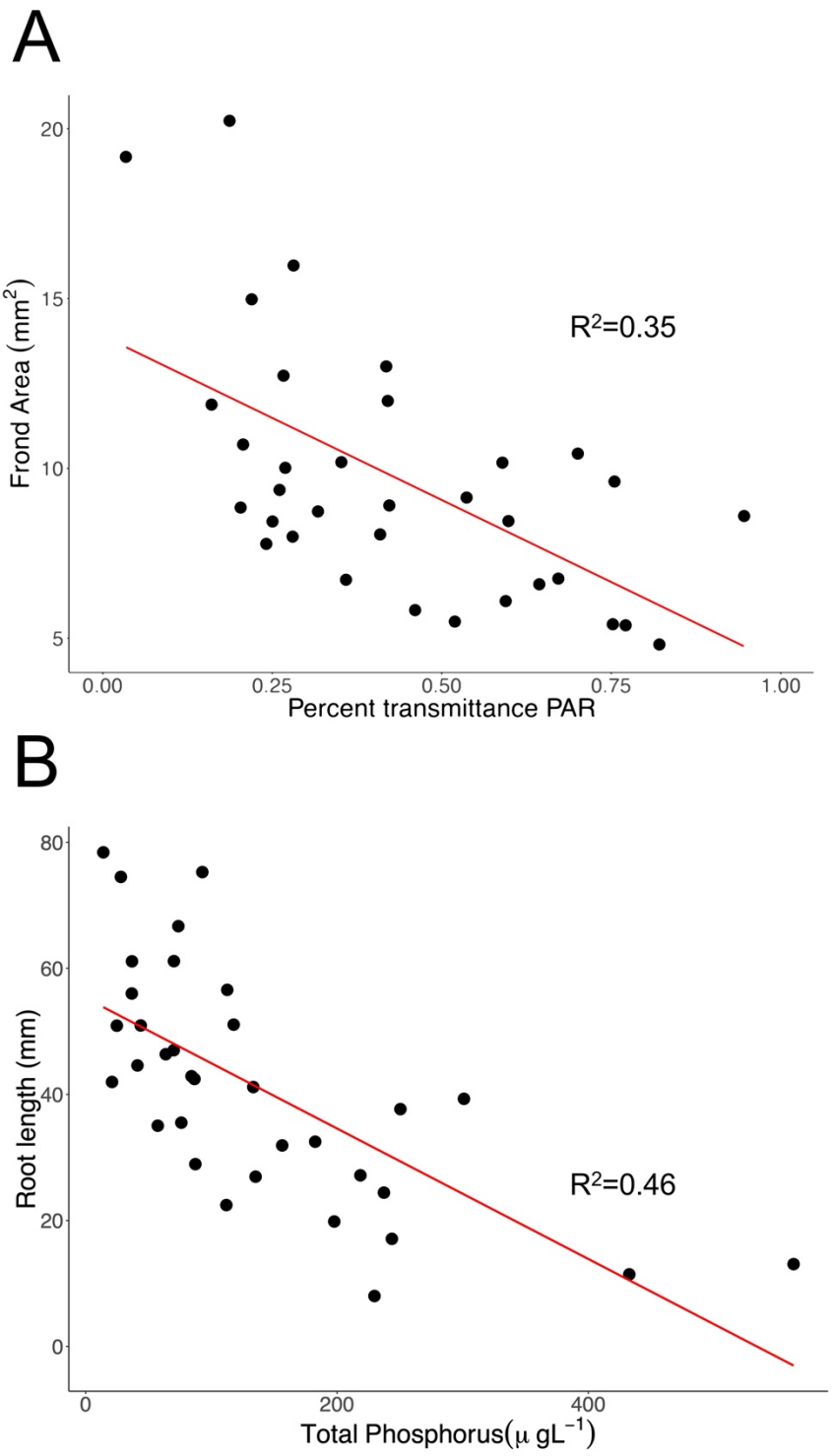

Fig. 2. Correlations between phenotypes measured in the field and natural levels of resource availability. A. Average frond area (mm^2) as a function of local light availability. Each point is the average of 10 individuals in each of 3 microsites to give a single value of average frond area per site. Light availability is measured as

percent transmittance of photosynthetically active radiation. B. Average root length (mm) as a function of water total Phosphorus (μgL^{-1}). Each point is the average of 10 individuals in each of 3 microsites to give a single value of average root length per site.

Plants from the field were taken back to the lab and grown in a common garden assay. Mean generation time in the common garden was 4.1 days which resulted in a total of ˜7 generations for the full 30-day common garden assay. Although diverse protists and cyanobacteria were observed in the flasks with microscopy, their densities remained low as the growth media never became green to the naked eye.

Whereas phenotypic variation in the field is due a mixture of environmental and genetic sources, any persistent variation in the common garden can be attributed to genetic differences. There was a major reduction in phenotypic variation among sites, comparing measurements from the field to those in the common garden, for both frond area (Fig. 3 A&B), and root length (Fig. 3 C&D). Frond area generally increased in the common garden compared to field measurements, likely due to the vastly lower irradiance provided by artificial light in the growth chambers (200 $\mu mols/s/m^2$) compared to natural irradiance, even in shaded sites.

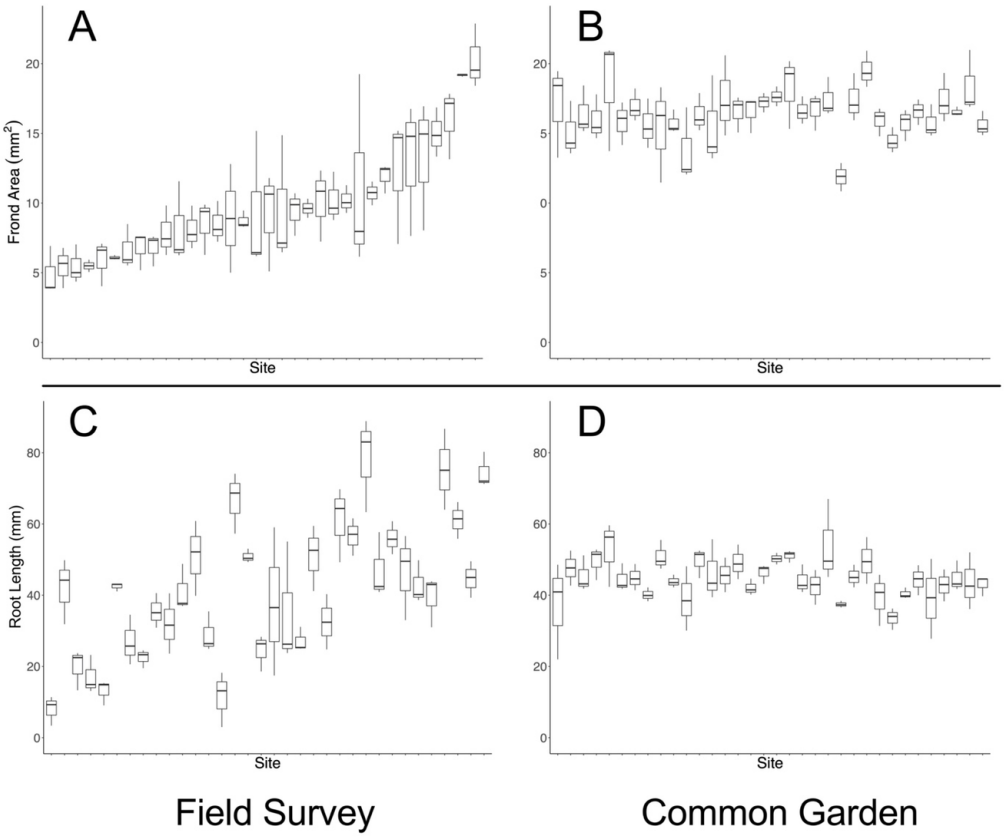

Fig. 3. Phenotypic variation for plants in the field and grown in a common garden assay. Boxes and whiskers show among microsite variation for each site. Sites are ordered by mean frond area and are consistent for the four panels. A&C. Phenotypic variation in the field. Phenotype was measured on 10 plants per microsite and averaged to produce a single estimate per microsite. B&D. Phenotypic variation in the common garden. Phenotype was measured on 10 plants in each of two replicate flasks for each microsite and averaged to produce a single estimate per microsite.

Despite the overall marked decrease in phenotypic variation, variation among sites and microsites persisted in the common garden assay. Frond area varied significantly among sites ($F_{33,68}$=1.71, p=0.03) and among microsites within sites ($F_{68,102}$=2.15, p=0.0002) with roughly twice as much variation within sites among microsites, than among sites (Table 2). Similarly, root length varied significantly among sites ($F_{33,68}$=1.98, p=0.009) and among microsites within sites ($F_{68,102}$=1.44, p=0.05), with roughly equal amounts of variation among sites and among microsites (Table 2).

Table 2. Variation in frond area (mm^2) and root length (mm) among plants grown in a common garden assay. Ten individual plants were measured in each of two replicate flasks for each of three microsite, for 34 sites situated broadly across southern Quebec.

	Source	df	SumSq	MeanSq	*F*	*p*	Variance Component
Frond Area	Site	33	4028	122.05	1.711	0.0313	0.845
	Microsite	68	4851	71.34	2.154	0.0002	1.911
	Replicate	102	3379	33.12	5.862	<0.0001	2.747
	Residuals	1836	10373	5.65			5.65
Root Length	Site	33	45204	1369.8	1.979	0.008923	11.295
	Microsite	68	47063	692.1	1.440	0.0472	10.58
	Replicate	102	49010	480.5	3.076	<0.0001	32.43
	Residuals	1836	286724	156.2			156.2

Comparing variance components calculated in the field and then in the common garden, we estimate that 93% of the among site variation in frond area in the field was environmental, with only 7% genetic, which persisted in the common garden (Fig. 3A&B, Table 3). Likewise, 96% of the among site variation in root length in the field was environmental, with only 4% due to genetic variation, which persisted in the common garden (Fig. 3C&D). Whereas the vast majority of among site phenotypic variation was environmental in origin, within site phenotypic variation had a more substantial genetic component (Frond area: 26%, Root length: 21%), (Table 3).

Table 3. Environmental and genetic components of phenotypic variation. Variation in the field survey (FS) consists of the combined contributions of environmental and genetic variance, whereas variation in the common garden (CG) isolates the genetic component.

Trait	Source	Variance Component	Variance Component	% Environmental *(FS-CG)/FS*	% Genetic *CG/*
		(FS)	*(CG)*		
Frond Area	Site	11.389	0.845	0.926	0.074
	Microsite	7.275	1.911	0.737	0.263
Root Length	Site	314.47	11.295	0.964	0.036
	Microsite	51.10	10.58	0.793	0.207

Fitness was estimated for each replicate flask in the common garden assay as population exponential rate of increase. There was added variance in fitness among sites ($F_{33,68}$ = 2.03, p=0.007) and among fronds within sites ($F_{68,102}$ = 3.26, p<0.0001) (Table 4). The variance among fronds within sites was roughly twice as large as the variance among sites.

Table 4. Variation in fitness measured in the common garden assay

Source	df	SumSq	MeanSq	F	p	Variance Component
Site	33	0.0383	0.001160	2.0314	0.0069	0.000098
Microsite	68	0.0388	0.000571	3.2561	<0.0001	0.000198
Error	102	0.0179	0.000175			0.0001753
Total	203	0.0950				

Discussion

Environmental and genetic variation in phenotype

In this study we set out to explain the nature, origin, and maintenance of phenotypic variation in *L. minor* in the field. Phenotype varied widely among sites, with mean frond area varying by a factor of two (Fig. 2A), and mean root length by a factor of more than eight (Fig. 2B). This variation was overwhelmingly the result of phenotypic plasticity. Although there were persistent differences in phenotype among sites in the common garden assay, the reduction of variation in frond area by 93% and in root length by 96% (Fig. 3, Table 2) reveals that among site phenotypic variation is almost exclusively environmental. This is consistent with previous work that has shown a large degree of plasticity in these traits, (Vasseur and Aarssen 1992, Cedergreen and Madsen 2002) and the absence of local adaptation (Vámos and van Moorsel 2022). Both among and within sites, the environmental contribution to phenotypic variation was larger for root length than frond area, which is also consistent with previous work reporting root length as *L. minor* 's most plastic trait (Vasseur and Aarssen 1992). Phenotypic variation in *L. minor* in the field is largely explained as a plastic response to the abiotic environment, shifting its phenotype to levels of resource availability. 35% of among site variation in frond area is explained by light availability, with plants producing larger fronds in more heavily shaded environments. The production of larger leaves in low light environments is a standard ecophysiological response in plants (Meziane and Shipley 1999, 2001), that influences fitness through photosynthesis, transpiration and thermoregulation (Anten et al. 1995, Hirose et al. 1997). Similarly, 46% of among site variation in root length is explained by nutrient availability with a dramatic increase for plants growing in sites with low levels of dissolved N and P. This is consistent with previous experimental work that has documented a plastic increase in root length in *L. minor* in response to nutrient limitation (Cedergreen and Madsen 2002). Although *L. minor* can uptake inorganic nutrients through both the root and the frond (Landolt 1986, Cedergreen and Madsen 2002), this balance shifts depending on both nutrient availability (Cedergreen and Madsen 2002), and irradiance (Cedergreen and Madsen 2004) with the production of longer roots resulting in an increase in root N uptake and NO_3 reduction. Variation in frond area and root length in *L. minor* can be conceptualised as a simplified root-shoot ratio (Cedergreen and Madsen 2002). A well-studied trait in land plants (Brouwer 1962, Poorter and Nagel 2000), *L. minor*seems to respond to resource limitation by investing more biomass into increasing the surface area of the tissue responsible for the uptake of the limiting resource.

In addition to among-site phenotypic variation, we observed significant phenotypic variation within sites. Whereas frond area varied substantially both among sites and among microsites within sites, the majority of variation in root length was at the among site level. Given the largely environmental origin of this variation, it is perhaps uprising that frond area would vary within sites due to the patch-like variation in light availability caused by fine-scale shading from macrophytes and riparian plants (Bell et al. 1991). In contrast, water nutrient availability is likely much more homogenous within sites due to mixing and diffusion resulting in most variation in root length manifesting among and not within sites. For both frond area and root length, the proportion of phenotypic variation with a genetic origin was much higher within sites (26% and 21%) than among sites (7% and 4%). The larger contribution of environmental variation to phenotype among sites can be explained by the greater environmental variation at the higher geographical resolution.

However, we observed a surprisingly large amount of within site genetic variation. Environmental variation aside, the absolute amount of genetic variation in frond area was twice as large within sites than among sites, and equal within and among sites for root length. Whereas among site genetic variation is easily explained by adaptation to local conditions or genetic drift given limited gene flow, the large amount of within site genetic diversity is surprising, especially in the absence of sexual reproduction.

In the common garden assay, the contribution of replicate flask to overall phenotypic variation was significant and second only to residual variation. This is perhaps surprising since replicate flasks consisted of clones, descending from the same ancestor sampled from the field. However, replicate flasks confounded several sources of variation including flasks effect, chamber effect (from the blocked design), and birth order effects from the original parental frond which have been shown to persist over several generations (Barks and Laird 2015, 2016, Mejbel and Simons 2018). Removing this variation from the residuals enabled us to detect the higher-level effects of microsite and site.

Genetic variation in fitness and evolutionary potential

In asexual, clonal populations, fitness can be directly measured as the population's exponential rate of increase (Bell 2008). Like phenotype, fitness also consists of environmental and genetic components that can be separated in a common garden assay. There is strong evidence for a large amount of genetic variation in fitness among different genotypes of *L. minor* , and in some cases, even greater variation among genotypes of the same species of *Lemnaceae* than among closely related species (Ziegler et al. 2015). However, how this variation maps onto the landscape remains unclear (Xu et al. 2015). Although many studies have reported considerable among-site genotype diversity (Vasseur et al. 1993, Cole and Voskuil 1996, Xue et al. 2012, Xu et al. 2015, Ho 2018), it is sometimes thought that *L. minor* possess poor levels of within site genetic diversity (William C . Jordan 1996, Xu et al. 2015). To our surprise, we found that there was twice as much genetic variation in fitness within sites (among microsites) than among sites (Table 4). This is consistent with studies quantifying intraspecific genetic variation in *L. minor* using allozymes (Vasseur et al. 1993, Cole and Voskuil 1996, El-Kholy et al. 2015) and amplified fragment length polymorphisms (Bog et al. 2022) that have reported between 4-20 genotypes per site. The source of this genetic variation remains unclear given the low estimates of gene flow (Cole and Voskuil 1996), mutation rates (Sandler et al. 2020), and frequency of sexual reproduction (Hillman 1961, Landolt 1986, Vasseur et al. 1993, Ho 2018) in *L. minor* .

Genetic variation in fitness is arguably the most important parameter in evolutionary biology since it is what natural selection acts upon, and is therefore directly related to the adaptive potential of a population (Burt 1995). Fisher formalised this relationship in his 1930 fundamental theorem of natural selection (Fisher 1930, Crow 2002) by equating the standardized additive genetic variance in fitness ($\mathrm{SV_A}$) with the per generation change in ln mean fitness,w (Equation 1).

$$\mathrm{SV}_A = \frac{var(w)}{w^2} = \ln\left(w\right) \tag{1}$$

In a constant environment, all populations experience genetic degradation due to deleterious mutations (Lynch and Gabriel 1990), maladaptive gene flow (Lenormand 2002), and genetic drift (Barton and Partridge 2000). The amount of genetic variation in fitness then represents the population's ability to counteract these processes and predicts the per-generation increase in mean fitness expected to result from natural selection (Fisher 1930) Likewise, this rate of evolution of fitness, represents the evolutionary potential of a population to respond to maladaptation caused by environmental change. Despite nearly 100 years since Fisher first recognized the crucial importance of this relationship, how much genetic variation in fitness exists in natural populations is a question that still sees considerable debate (Burt 1995, Shaw and Shaw 2014, Hendry et al. 2018).

Although genetic variation in fitness is the result of dominance and epistatic variance in addition to additive variance (Burt 1995, Matsui et al. 2022), this course measure can be used to approximate the upper limit of

SV_A and therefore rates of evolutionary change. Taking the microsite variance component from the common garden analysis of variance (Table 4), and standardizing it by dividing it by the square of mean fitness, we estimate SV_A as 0.0094, or about 1%. This means that fitness is degraded by up to 1% each generation by mutation and immigration, and then restored via purifying selection. Empirical estimates of SV_A in wild populations are exceeding scarce. From the 30 estimates in the literature, including just five on plants, SV_A seems to range from 1-10% (Burt 1995, Hendry et al. 2018), which is consistent with our findings.

Migration-selection balance

Whereas genetic variation is constantly removed each generation via purifying selection, it is continually renewed by mutation and migration. A multi-niche polymorphism describes how genetic variation can be maintained in a population though spatially-variable selection, where low-fitness alleles persist in a population given gene flow between niches that favour different optimal phenotypes (Maynard Smith 1970, Bulmer 1972). Having obtained estimates of environmental variance (V_E) and additive genetic variance (V_A) for traits in addition the genetic variance in fitness (γ), we can estimate the rate of migration (m) necessary to sustain these observed levels of variation given a range in the selection difference among niches (Bulmer 1985). If ϑ_1 is the optimal phenotype in niche 1, and ϑ_2 is the optimal phenotype in niche 2, then we can solve for m, the proportion of the population that must migrate between niches each generation to maintain the polymorphism (Equation 2, from Bulmer 1985, Eq 10.65, pg. 181):

$$\left[V_A + 2m\left(V_E + \gamma\right)^2\right] = m\left(1 - m\right)\left(\theta_1 - \theta_2\right)^2\left(V_A + V_E + \gamma\right) \tag{2}$$

We calculated the rate of dispersal necessary to maintain the observed variation in frond area (Fig. 4A) and root length (Fig. 4B), over a range of selection differences ($\theta_1 - \theta_2$), in the absence of mutation. The hyperbolic function indicates that, in the absence of mutation, there rate of dispersal of about 1% that is sufficient to sustain the observed diversity given a selection gradient of 7mm^2 for frond area, and 15mm for root length). In a study on the genetic structure of *L. minor* populations in central Minnesota, Cole and Voskuil (1996) estimated much lower rates of gene flow, Nm =0.3, which suggests that mutation must play a critical role in maintaining the genetic variation we observed.

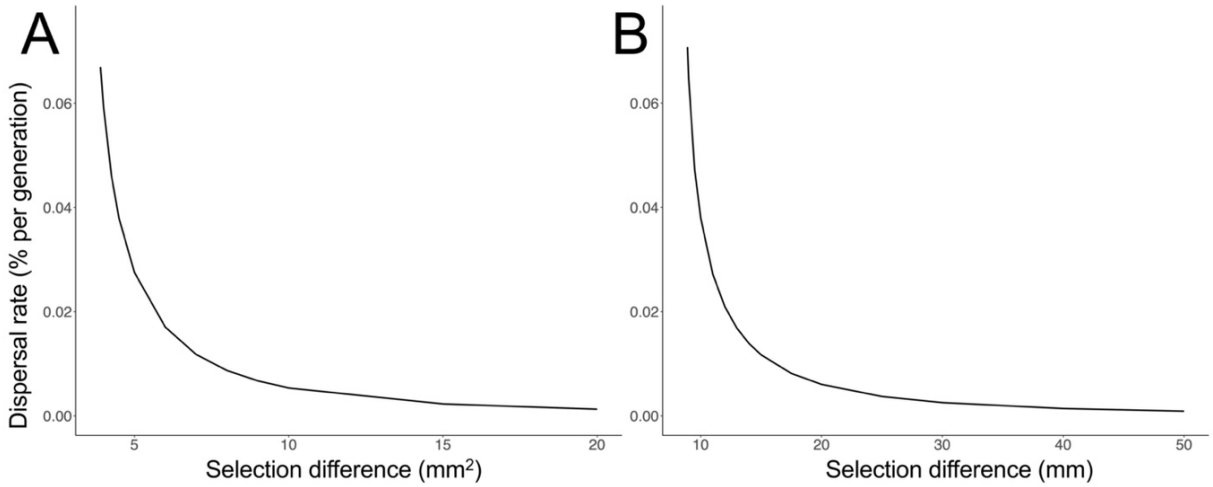

Fig. 4. Migration-Selection balance. Dispersal rate (% per generation) between two environments required to maintain the observed average within site phenotypic variation in the absence of mutation, as a function

of selection difference (the difference in optimal phenotype between the two environments). A. Migration-selection balance for to maintain variation in frond area (mm^2). B. Migration-selection balance for to maintain variation in root length (mm).

Conclusions

Frond area and root length varied widely in the field and were correlated with natural levels of resource availability, with plants investing more biomass into the tissue responsible for the uptake of the resource that is in short supply. This was most striking for root length, for which variation among sites was more than sixfold, and strongly correlated with levels of dissolved phosphorus. This large phenotypic variation in the field was overwhelmingly a result of phenotype plasticity, and not local adaptation. Despite the predominance of environmental variation in both traits, there was also a genetic basis to these traits that persisted when environmental variation was removed. We recorded surprisingly high levels of genetic variation in phenotype and fitness within sites, which itself indicates the presence of strong purifying selection of about 1% per generation and the potential to counter environmental change. Future work should focus on uncovering mechanisms responsible for maintaining such high levels of genetic variation in *L. minor*. The continued development of*Lemnaceae* as a model system in experimental population genetics (Acosta et al. 2021), community ecology (Laird and Barks 2018) and eco-evolutionary dynamics (Hart et al. 2019) promises illumination in understanding the larger mechanisms responsible for maintaining diversity more generally.

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Authors' contributions: MDJ lead the field survey and performed the common garden experiment. Analysis was done jointly by MDJ and GB. The manuscript was written by MDJ. GB contributed substantially to revisions. The study was conceived by GB with input from MDJ.

Competing interests: The authors declare no competing interests.

Data availability: Raw data from which all figures were generated will be stored in the Dryad repository before publication of the article.

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Supplementary Information

Table S1. Environmental variables measured at 37 sites in a regional field survey across southern Quebec. We measured pH, total and dissolved phosphorus (TP, TN, μgL^{-1}), total and dissolved Nitrogen (TN, DN, mgL^{-1}) and light availability (% PAR).

Site	Date	Long	Lat	pH	DP	TP	DN	TN	Light
1	3.07	45°35'32.1"N	73°50'10.9"W	7.23	76.87	133.34	0.59	0.72	0.28
2	4.07	45°31'41.0"N	74°12'36.7"W	8.75	88.19	117.74	0.88	1.06	0.76
3	5.07	45°58'94.6"N	73°54'74.9"W	7.45	13.75	57.32	1.67	2.54	0.54
4	6.07	46°07'68.1"N	73°30'51.5"W	8.35	7.89	14.01	1.6	1.66	0.7
5	6.07	46°19'25.9"N	73°21'54.7"W	7	32.64	135.18	0.69	0.78	0.64
6	7.07	46°19'25.8"N	73°21'54.5"W	7.95	9.41	27.97	0.25	0.54	0.19
7	7.07	46°51'43.0"N	72°46'69.9"W	7.46	40.8	73.71	0.77	0.87	0.95
8	7.07	47°26'60.8"N	72°46'95.8"W	8.58	44.95	76.08	0.18	0.32	0.67
9	8.07	47°26'60.8"N	72°46'96.0"W	6.9	42.51	92.79	1.19	1.25	0.22
10	8.07	48°43'4.0"N	72°24'11.7"W	7.22	26.06	43.76	0.36	0.51	0.41
11	9.07	48°38'14.7"N	71°43'30.0"W	7.51	125.85	243.63	0.31	0.36	0.52
12	9.07	48°38'14.6"N	71°43'30.3"W	8.79	7.23	20.92	0.33	0.37	0.77
13	10.07	46°57'51.7"N	70°58'5.4"W	9.71	68.64	250.42	0.69	0.9	0.42
14	10.07	46°39'40.9"N	71°34'49.8"W	8.62	24.15	197.77	1.55	2.59	0.75
15	12.07	45°26'17.5"N	73°19'55.5"W	8.04	477.6	563.31	0.93	1.27	0.46
16	12.07	45°32'39.6"N	73°08'50.1"W	9.63	17.68	112.62	0.25	0.43	0.35
17	12.07	45°26'17.6"N	73°19'55.6"W	7.91	27.2	182.61	1.82	2.1	0.27
18	13.07	45°06'23.3"N	72°58'46.9"W	7.43	57.42	229.79	0.9	1.53	0.82
19	13.07	45°11'31.8"N	72°46'15.5"W	7.78	134.0	300.99	0.86	1.29	0.42
20	13.07	45°08'80.3"N	72°38'69.6"W	7.39	113.97	218.59	1.58	2.26	0.26
21	14.07	45°28'21.7"N	72°10'38.5"W	7.05	18.54	70.05	0.54	0.65	0.21
22	14.07	45°46'53.7"N	72°00'20.5"W	9.01	63.38	111.92	0.57	0.68	0.36
23	14.07	45°50'36.9"N	72°01'51.9"W	7.8	21.12	86.52	0.59	0.88	0.59
24	14.07	45°50'54.6"N	72°02'38.1"W	7.12	362.53	432.56	1.7	2.33	0.6
25	15.07	45°24'93.2"N	71°53'65.7"W	7.36	13.51	84.21	2.16	2.13	0.27
26	15.07	45°24'79.9"N	71°53'52.3"W	6.64	281.41	237.3	2.42	2.97	0.2
27	16.07	45°07'22.7"N	71°58'45.1"W	7.44	19	36.66	0.3	0.34	0.16
28	16.07	45°07'50.7"N	71°59'31.3"W	7.4	24.89	70.19	2.17	2.19	0.28
29	16.07	45°08'34.1"N	71°53'48.2"W	7.39	19.99	63.71	0.54	0.8	0.42
30	16.07	45°48'73.0"N	71°10'1.1"W	7.05	14.77	41.15	0.52	0.67	0.03
31	17.07	45°48'74.4"N	71°10'0.4"W	8.56	14.7	24.75	0.59	0.67	0.32
32	17.07	46°14'83.2"N	72°34'43.5"W	7.45	41.55	156.36	0.66	0.76	0.24
33	18.07	45°28'92.4"N	73°44'79.2"W	7.05	23.43	87.25	0.81	1.18	0.25
34	18.07	45°28'93.1"N	73°44'77.1"W	8.55	19.53	36.79	0.41	0.51	0.59

Table 5: This is a caption

Table S2. Recipe for Hoagland's E Medium used in the common garden assay. The pH was set to 7.0 before autoclaving the media.

	Concentration
$MgSO_4$	12.300 mg/L
$Ca(NO_3)$ x 4 H_2O	27.140 mg/L
KH_2PO_4	4.3530 mg/L
KNO_4	12.625 mg/L
H_3BO_3	71.50 µg/L
$MnCl_2$ x $4H_2O$	45.50 µg/L
$ZnSO_4$ x 7 H_2O	5.500 µg/L
$NaMoO_4$ x 2 H_2O	2.250 µg/L
$CuSO_4$ x 5 H_2O	3.500 µg/L

	Concentration
$FeCl_3$ x 6 H_2O	0.484 mg/L
EDTA	1.500 mg/L