

CLIMATE VS. SOIL FACTORS IN LOCAL ADAPTATION OF TWO COMMON PLANT SPECIES

MIRKA MACEL,^{1,7} CLARE S. LAWSON,² SIMON R. MORTIMER,² MARIE ŠMILAUEROVA,³ ARMIN BISCHOFF,¹
 LISÈLE CRÉMIEUX,¹ JIŘÍ DOLEŽAL,³ ANDREW R. EDWARDS,² VOJTĚCH LANTA,³ T. MARTIJN BEZEMER,^{4,5}
 WIM H. VAN DER PUTTEN,⁴ JOSÉ M. IGUAL,⁶ CLAUDINO RODRIGUEZ-BARRUECO,⁶ HEINZ MÜLLER-SCHÄRER,¹
 AND THOMAS STEINGER¹

¹University of Fribourg, Department of Biology, Ecology and Evolution, Chemin du musée 10, CH-1700 Fribourg, Switzerland

²University of Reading, Centre for Agri-Environmental Research, Earley Gate, P.O. Box 237, Reading, RG6 6AR UK

³University of South Bohemia, Faculty of Biology, Branisovska 31, CZ-37005 České Budejovice, Czech Republic

⁴Netherlands Institute of Ecology, NIOO-KNAW, Multitrophic Interactions Department, P.O. Box 40,
 6666 ZG Heteren, The Netherlands

⁵Laboratories of Nematology and Entomology, Wageningen University and Research Centre, Wageningen, The Netherlands

⁶Instituto de Recursos Naturales y Agrobiología, Consejo Superior de Investigaciones Científicas, P.O. Box 257,
 37071 Salamanca, Spain

Abstract. Evolutionary theory suggests that divergent natural selection in heterogeneous environments can result in locally adapted plant genotypes. To understand local adaptation it is important to study the ecological factors responsible for divergent selection. At a continental scale, variation in climate can be important while at a local scale soil properties could also play a role. We designed an experiment aimed to disentangle the role of climate and (abiotic and biotic) soil properties in local adaptation of two common plant species. A grass (*Holcus lanatus*) and a legume (*Lotus corniculatus*), as well as their local soils, were reciprocally transplanted between three sites across an Atlantic–Continental gradient in Europe and grown in common gardens in either their home soil or foreign soils. Growth and reproductive traits were measured over two growing seasons. In both species, we found significant environmental and genetic effects on most of the growth and reproductive traits and a significant interaction between the two environmental effects of soil and climate. The grass species showed significant home site advantage in most of the fitness components, which indicated adaptation to climate. We found no indication that the grass was adapted to local soil conditions. The legume showed a significant home soil advantage for number of fruits only and thus a weak indication of adaptation to soil and no adaptation to climate. Our results show that the importance of climate and soil factors as drivers of local adaptation is species-dependent. This could be related to differences in interactions between plant species and soil biota.

Key words: climate; genetic differentiation; *Holcus lanatus*; local adaptation; *Lotus corniculatus*; nematodes; reciprocal transplant; soil; spatial heterogeneity.

INTRODUCTION

Local adaptation is the pattern of divergent selection of local environments that cause populations to evolve in response to specific ecological conditions (Williams 1966). Because plants are sessile and dispersal distances are often limited (Fenster 1991), selection by local environments may lead to genetically differentiated plant populations (Linhart and Grant 1996). Plants can also show phenotypic plasticity in response to their environment (Schlichting 1986) and both high levels of genetic variation and phenotypic plasticity have been found in many plant species with wide distributions (Bradshaw 1984). Common plant species may be best

characterized by many locally specialized plant genotypes rather than a few general-purpose genotypes (Van Tienderen 1990, Joshi et al. 2001). Local adaptation in plants has been detected in response to small-scale and large-scale environmental variation (e.g., Antonovics and Bradshaw 1970, Galen et al. 1991, Kindell et al. 1996, Nagy and Rice 1997, Joshi et al. 2001, Berglund et al. 2004). Nevertheless, local adaptation may not always be apparent (e.g., Levin and Schmidt 1985, Rice and Mack 1991, Leiss and Müller-Schärer 2001), particularly when strong gene flow prevents genetic differentiation among populations, when selection is constrained by lack of genetic variation, or when selection is fluctuating due to temporal environmental variability (Kawecki and Ebert 2004).

Divergent natural selection is the driving process of local adaptation, and, therefore, the study of the ecological factors responsible for divergent selection is important for understanding local adaptation (Kawecki and Ebert 2004). At large spatial scales, climate is one of

Manuscript received 17 April 2006; revised 2 August 2006; accepted 14 August 2006. Corresponding Editor: L. F. Galloway.

⁷ Present address: Evolution and Ecology, University of California, Davis, California 95616 USA.
 E-mail: mmacel@ucdavis.edu

the main sources of environmental variation (Santamaria et al. 2003). This is illustrated by the key role of climate in the global distribution of plant species (Woodward 1987) and the influence of climatic conditions such as precipitation and temperature on the functional composition of the vegetation (Box 1996). Evolutionary potential to adapt to climate will be important for persistence of plant populations with ongoing climate change (Etterson and Shaw 2001). Several studies have examined local adaptation in plants across strong climatic gradients and most, but not all, found latitudinal clines, which suggested that plant populations were adapted to climatic conditions (Joshi et al. 2001, Santamaria et al. 2003, Etterson 2004, Maron et al. 2004). However, in a European-scale study, climatic distance explained no more than 18% of the variation in selection indices related to geographic distance, suggesting that climate was not the only factor leading to local adaptation (Joshi et al. 2001).

While climate acts on a continental scale, soil may vary at a local scale (Van der Putten et al. 2004). Abiotic (chemistry, soil type, and soil structure) and biotic (root feeders, symbionts, decomposer organisms, and bioturbators) soil properties affect plant performance at small scales (Wardle 2002) and thus can act as selective agents. Indeed, local adaptation to highly toxic serpentine soils has been found in several plant species (Schat et al. 1996, Berglund et al. 2004, Wilcox Wright et al. 2006). Furthermore, leguminous species may be locally adapted to root-nodule-producing, nitrogen-fixing bacteria (Lie et al. 1987, Parker 1995). Although some plant traits, such as clonal growth, dispersal by seeds, or perennial growth forms, may be adaptations to soil pathogens or symbiotic mutualists (Van der Putten 2003), very few studies have explored local adaptation of plants to soil biota and the majority of, if not all, these studies have focused on specific groups of soil organisms, such as *Rhizobium* (Lie et al. 1987).

Reciprocal transplant experiments are often used to detect local adaptation. In such experiments contributions of environmental variation and genetic variation to plant performance can be assessed and indications for local adaptation are shown by significant home vs. away advantages (e.g., Joshi et al. 2001, Santamaria et al. 2003). The sources of environmental variation that contribute to the differences in selection regimes between sites are usually hard to detect in reciprocal transplants because plants are transplanted into the local field sites and thus effects of climatic environmental variation, soil, and biotic factors are often confounded.

In the present study, we aimed to disentangle the role of climate vs. soil in local adaptation of natural plant populations. We set up a reciprocal transplant experiment along an Atlantic–Continental central European transect in common gardens at three sites (United Kingdom, Switzerland, and Czech Republic). Plants of three populations in Europe were grown at the three sites in their home soil and in the soils of the other plant

populations, and thereby we were able to test for both home climate advantage and home soil advantage. We followed plant performance over two growing seasons. To our knowledge, this is the first full reciprocal transplant experiment involving both plants and soils. We used a grass and a legume for our experiment, because these species may have contrasting interactions with soil biota. Legumes associate with symbiotic, nitrogen-fixing rhizosphere bacteria (Lie et al. 1987). Grasses, on the other hand, can have typically negative feedback interactions with their rhizosphere community, driven by pathogen build-up, which also affects other grass species (Bezemer et al. 2006). Based on these differences in interactions with soil biota, we hypothesized that the legume would be locally adapted to soil but the grass would not. Assuming that parasites are ahead of their hosts in the evolutionary arms race (Hamilton et al. 1990), the potential negative interactions of the grass with its soil community could lead to a home soil disadvantage because pathogens could be adapted to the local host plant genotypes. In contrast, the positive interactions of the legume with nitrogen fixers could lead to local adaptation of the legume to local host strains (Lie et al. 1987) and vice versa and thus a home soil advantage. However, the strong spatial heterogeneity of soil at very small scales (Wardle 2002, Van der Putten et al. 2004) could hinder local adaptation to soil in plant species. Climate, on the other hand, is less variable over a regional scale, and, therefore, we expected both species to be adapted to climatic conditions. We addressed the following questions: (1) Are plants adapted to their local environments? (2) If so, are they adapted to climate or to soil or both? (3) Do the two plant species differ in their response to climate and soil?

MATERIAL AND METHODS

Reciprocal transplant

The grass *Holcus lanatus* L. and the N-fixing legume *Lotus corniculatus* L. (see Plate 1) were used for this experiment. Both are common perennial outcrossing grassland plant species that are native to most parts of Europe and occur on various soil types (Beddows 1961, Jones and Turkington 1986). Seeds of *L. corniculatus* and *H. lanatus* were collected from grasslands in the United Kingdom, Czech Republic, and Switzerland. In the United Kingdom, both species were collected from Bradenham, 51°40' N, 0°48' W. In the Czech Republic *L. corniculatus* was collected from České Budějovice, 49°0' N, 14°26' E and *H. lanatus* from České Budějovice–Ohrazení, 48°57' N, 14°36' E. In Switzerland *L. corniculatus* was collected from Wünnewil, 46°52' N, 7°10' E and *H. lanatus* from Dürdingen, 46°52' N, 7°11' E. Seed bulk samples were collected from 60 plants of each population and stored for 6 mo at room temperature in the dark. At the site of each plant population, six soil blocks of minimum 30 cm width, 60 cm long, and 20 cm deep were dug out. The six blocks at

each site were ~10 m apart. Large roots, rhizomes, and litter were removed from the soil. Per soil block, the soil was thoroughly homogenized and stored for 1 mo at 4°C in plastic bags.

Seed material and soils were exchanged among the three countries. The reciprocal transplant experiment was performed in common gardens in each country that were 1.3–22 km from the collection sites so that the local ambient climate matched the original site conditions. Climate data, both long-term and during the study period, were retrieved from the nearest meteorological station (0–8 km from experimental sites, maximum 22 km from the collection sites). Special care was taken to treat all soils and seeds the same way during transportation (e.g., all soils, including the “home” soils, were transported by car). The size of each experimental site was 51 m², 40 cm of the topsoil of which was removed. The bottom was covered with a water-permeable plastic root cloth (polypropylene), and the site was isolated by covering the sides with plastic barriers. The site was filled with sand to minimize migration of soil biota. The experimental design was a full factorial 3 × 3 × 3 design with the factors site (three countries), plant origin (three countries), and soil origin (three countries) with soil blocks nested within soil origin (six blocks). Positions of plant origin × soil origin combinations were randomized within each experimental site without further blocking (completely randomized design). Note that with this design, block effects only reflect spatial variation in soil traits at the grassland site where the soil was originally collected and not variation at the experimental site (common garden). For each species, there were a total of 12 replicates for each site × plant origin × soil origin combination (six soil blocks per soil origin × two replicates per plant origin, resulting in a total of 324 plants per species).

Seeds were surface-sterilized with KMnO₄ solution. *Lotus corniculatus* seeds were scarified with sand paper before sterilization to enhance germination. Seeds were germinated in autoclaved sand. Three weeks after germination, seedling height and number of leaves were measured and seedlings were transplanted into 3-L pots filled with 2-L of soil. Pots were sealed at the bottom with nylon mesh, with a mesh size of 60 µm, to prevent the roots from growing out of the pot. Each pot was put in another 3-L pot, and they were set 25 cm apart in the sand of the experimental site. Pots were hand-weeded but not protected from invertebrate herbivores or pathogens. The plots were fenced to prevent disturbance and grazing by mammals.

The experiment started in May 2003. During the two weeks after the start of the experiment dead seedlings were replaced. Plant survival, growth, and reproductive traits were measured over two growing seasons, at least three times a season. Roots and shoots were harvested at the end of the experiment, at the end of September 2004, to determine the dry mass of above- and belowground biomass. Infection of the main aboveground antagonist

on *H. lanatus*, the rust fungus (*Puccinia* sp.), was measured in the second growing season (2004). The number of infected tillers and the proportion of infection on five randomly chosen tillers were measured, and infection rates were calculated as (percentage of infected tillers) × (mean proportion of tiller infected).

Soil analysis

Nematodes represent an important part of the soil biota with plant-feeding nematodes being one of the most abundant group of root herbivores (Verschoor et al. 2002 and references therein). Nematode abundance was analyzed in 100 g soil of a soil core of 2.5 cm diameter and 20 cm deep collected from each soil block used in the experiment. This resulted in nematode data of six blocks of each plant population. Nematodes were extracted from 100 cm³ of soil by Oostenbrink elutriators (Oostenbrink 1960), counted in 10% of the total extracted soil volume, and determined up to genus or family level according to Bongers (1988) using an inverted-light microscope (10 × 20). Nematode analyses of all soils were performed in the same laboratory at the Netherlands Institute for Ecological Research in Heteren, The Netherlands.

Soil concentrations of P, K, NO₃[−], NH₄⁺, and pH and organic matter were determined in 600 g of dried soil collected from each soil block. The soil samples were air-dried (<30°C), sieved (<2 mm), and analyzed for pH, organic matter, inorganic N (NO₃[−] and NH₄⁺), P, and K contents, according to Tan (1996). Soil pH was measured in water at a soil:water ratio of 1:2.5. Total organic C was determined by the Walkley-Black Wet Combustion method, and soil organic matter content was then obtained by multiplying the C_{org} concentration by 1.724. Available P was determined by the Bray-Kurtz P1 method for acid and neutral soils and by the Olsen method for calcareous soils. Inorganic N was extracted with 2 mol/L KCl. P, NO₃[−], and NH₄⁺ concentrations in the extracts were determined in a Bran+Luebbe Autoanalyzer 3 with AA3 Digital Colorimeter (Bran+Luebbe, Norderstedt, Germany) following the manufacturer's instructions. Available K was determined by atomic absorption spectrophotometry after extraction with a solution of 1 mol/L ammonium acetate, pH 7.0. All chemical analyses were performed in a laboratory at the Consejo Superior de Investigaciones Científicas (CSIC) in Salamanca, Spain.

Data analysis

Whether nematode community composition differed between sites was analyzed with constrained multivariate analyses using CANOCO 4.5 (Ter Braak and Šmilauer 2002). Detrended Correspondence Analyses (DCA) by segments revealed a gradient of <2.0 SD units. Therefore, linear methods were considered most appropriate (redundancy analysis [RDA]; Legendre and Anderson 1999). Analyses were carried out for both plant species separately, with sites as explanatory

TABLE 1. Characteristics of the different soils used in the experiment.

Soil characteristics	<i>Holcus lanatus</i> soil origin			<i>Lotus corniculatus</i> soil origin		
	Swiss	Czech	UK	Swiss	Czech	UK
Nematodes (no./100 g dry mass soil)						
Plant feeding	2823 (408) ^a	2941 (445) ^a	3874 (728) ^a	2640 (345) ^{ab}	1500 (492) ^b	3874 (782) ^a
Bacterial feeding	2663 (537) ^a	594 (195) ^b	2291 (749) ^{ab}	1543 (277) ^a	3199 (1936) ^a	2291 (749) ^a
Fungivorous	186 (43) ^a	293 (80) ^a	337 (76) ^a	300 (36) ^a	152 (97) ^a	337 (76) ^a
Omnivorous	290 (101) ^a	717 (72) ^b	333 (53) ^{ab}	296 (41) ^a	724 (260) ^a	333 (53) ^a
Chemical properties						
pH	6.05 (0.06) ^b	5.02 (0.13) ^a	7.58 (0.02) ^c	7.25 (0.05) ^a	7.54 (0.03) ^{ab}	7.58 (0.02) ^b
NH ₄ ⁺ (mg/kg)	6.92 (0.88) ^a	21.93 (3.16) ^b	15.85 (1.15) ^b	8.60 (0.40) ^a	9.95 (2.75) ^a	15.85 (1.15) ^a
NO ₃ ⁻ (mg/kg)	13.60 (1.14) ^a	5.53 (0.83) ^a	17.68 (6.82) ^a	9.00 (0.35) ^b	1.40 (0.26) ^a	17.68 (6.82) ^a
P (ppm)	22.83 (3.66) ^b	2.48 (0.38) ^a	15.33 (0.84) ^{ab}	38.33 (11.37) ^a	27.63 (1.39) ^a	15.33 (0.84) ^a
K (ppm)	80.5 (10.6) ^a	164.7 (24.5) ^a	134.0 (9.6) ^a	69.3 (4.6) ^a	113.0 (11.6) ^b	134.0 (9.6) ^b
Organic matter (%)	4.98 (0.12) ^b	7.13 (0.64) ^a	8.28 (0.28) ^a	5.46 (0.36) ^b	2.43 (0.23) ^a	8.28 (0.28) ^c

Notes: At each site, six samples (one of each block; see *Material and methods*) were taken for soil analysis; values are means with SE in parentheses. The UK soil originated from one single field site, where both plant species co-occurred; for Switzerland and the Czech Republic, the soils of *Holcus lanatus* and *Lotus corniculatus* were collected from separate sites. Different superscript letters indicate significant differences in soil character between the soil origins (ANOVA, post hoc Tukey tests with sequential Bonferroni correction for multiple comparisons, $\alpha = 0.05$).

(dummy) variables. Significance was tested using a Monte Carlo permutation test (999 permutations). Soil chemistry and nematode abundance per feeding guild were analyzed with ANOVA and post hoc Tukey hsd tests for each plant species separately corrected for multiple comparisons with sequential Bonferroni tests.

Effective fecundities (F) per plant were calculated as the total number of pods or panicles over both seasons including plants not surviving to reproduction and vegetative plants ($F = 0$ for those plants). This fecundity is a limited estimate because it is based on reproduction over a two-year period and not on lifetime fitness. Survival data were analyzed using a three-way log-linear model with G^2 statistics performed in R freeware version 2.3.1 (*available online*).⁸ Total biomass (root + shoot), number of pods (*L. corniculatus*), effective fecundity, and rust fungus infection rates (*H. lanatus*) were analyzed using a mixed-model ANOVA based on restricted maximum likelihood (REML) performed in JMP 5.0.1 (SAS Institute, Cary, North Carolina, USA). Climate, seed origin, and soil origin were included in the models as fixed factors; blocks were nested in soil as a random factor (not included for survival data). Initial size (height \times number of leaves) was considered as a covariate to correct for possible maternal effects. Local adaptation to climate and/or soil conditions was detected in linear contrasts of “home” (1 home \times 3 climates/soils) vs. “away” (2 away \times 3 climates/soils) within (near) significant interactions of climate \times seed origin and soil \times seed origin, i.e., contrast between the mean of populations growing in their home soil or climate vs. the mean of populations growing in foreign soils or climates. Criteria for local adaptation were (1)

significant home vs. away contrast and (2) the home population had the highest mean value within a climate or soil in at least two of the three climates or soils. In addition, post hoc pairwise t tests were performed on significant interactions to determine which seed origins differed from one another at a particular climate or soil. Variables were transformed (square-root transformation) prior to the analyses when necessary to meet the assumptions of normality and homoscedasticity.

RESULTS

Climate and soil characteristics

The soils of the three sites of each plant species differed in nematode abundance and chemical properties (Table 1, Appendix A). Nematode communities differed significantly between the sites, amongst others due to differences in the dominant plant-feeding nematodes (Appendix A). Soil pH ranged from 5.02 in the Czech *H. lanatus* soil to 7.58 in the UK soil. Available K, available P, NO₃⁻, and NH₄⁺ also differed between the soils. P was very low in the Czech *H. lanatus* soil, whereas total available nitrogen was relatively high in the UK soil.

During the study period, mean temperature was highest in the UK site (12.4°C) compared to the Swiss site (11.4°C) and the Czech site (11.5°C; Appendix B). The Swiss site was the wettest site with a total precipitation of 1275 mm compared to the UK site (808 mm) and the Czech site (954 mm; Appendix B). Long-term climate data of the sites showed a similar pattern. Winter temperatures were higher in the UK site (4.3°/17.2°C January/July) compared to the Swiss site (−1.0°/17.6°C) and the Czech site (−1.8°/17.7°C). Mean annual precipitation was higher at the Swiss site (1119 mm) compared to the UK site (652 mm) and the Czech site (588 mm).

⁸ <http://www.R-project.org>

TABLE 2. Effects of soil, climate, and seed origin on plant growth and fitness traits.

Trait	Initial size (1, 236–280)	Climate (2, 236–280)	Soil (2, 15)	Seed origin (seed) (2, 236–280)	Climate \times soil (4, 236–280)	Climate \times seed (4, 236–280)
<i>Holcus lanatus</i>						
Total biomass	1.32	45.73***	47.21***	11.10***	6.19***	3.09*
Fecundity	1.14	31.16***	4.77*	4.83**	8.30***	2.92*
Infection rate	0.01	74.88***	2.16	7.59***	2.39†	3.87**
Survival	0.56	25.10***	1.09	6.74*	12.58*	0.77
<i>Lotus corniculatus</i>						
Total biomass	1.72	84.28***	18.86***	81.12***	6.93***	2.32†
Pod number 2003	4.63*	0.43	54.63***	21.26***	8.08***	0.61
Pod number 2004	1.01	1.29	5.79*	24.07***	2.33†	3.89**
Fecundity	0.34	0.12	9.53**	4.90**	3.16*	0.39
Survival	0.02	7.76*	0.23	2.87	8.43†	5.78

Notes: Table entries are F values, with asterisks indicating significance levels of restricted maximum-likelihood estimation. Survival data were analyzed with a log-linear model (G^2 deviance and significance levels are shown). Degrees of freedom are reported in parentheses beneath column headings (factor df, error df). Degrees of freedom in the error term vary due to missing values in some dependent variables. Initial size was added to the models as a covariate. Linear contrasts of “home” vs. “away” were tested in significant climate \times seed and soil \times seed interactions. Arrows show the direction of significant “home” vs. “away” contrasts (an upright arrow indicates home > away). Models also included a random factor “block” nested within soil, but this result is not presented here.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; † $P < 0.10$.

Performance of *Holcus lanatus*

At all sites, *H. lanatus* plants flowered only in the second year of the experiment. We found significant environmental (climate, soil) and genetic (seed origin) effects for most of the performance traits measured, as well as a significant interaction between climate and soil (Table 2). There was no effect of soil block nested within soil type on plant performance (all tests, $P > 0.10$). Initial size did not affect survival, growth, or reproduction of the species (Table 2). The interaction between seed origin and climate was significant for most traits, but there was no significant three-way interaction between seed origin, climate, and soil for any trait. This allowed us to examine local adaptation to climate without having to refer to specific soil types. We found that the significant seed origin \times climate interactions could be explained by significant home vs. away climate contrasts (Table 2), indicating that seed origins generally performed best in their respective home climates. Swiss and UK plants had highest biomass in their home climates compared to the other origins while Czech and UK plants had highest fecundity in their respective home climates (Fig. 1A, C). Although overall home vs. away contrasts were significant, pairwise comparisons per site showed that in some cases the home origins did significantly better than one but not the other away origin (Appendix C). On the contrary, all soil environment \times seed origin effects were not significant (Table 2, all data), and there were no indications for local adaptation to soil (Fig. 1B, D shows data for fecundity and biomass). During the experiment, plants showed few signs of herbivore attack at any study site but some infection by a rust fungus did occur. The infection rates of the fungus differed between the climates (environmental effect) and between the seed origins (genetic effect), and the interaction climate \times seed origin was

highly significant (Table 2, Fig. 2). The home vs. away contrast was also significant, which could be explained by the low infection rate of the UK seed origin in the Swiss and Czech climates compared to the Swiss and Czech seed origins (Fig. 2). There is, however, no indication of local adaptation of the plants to the rust fungus.

Performance of *Lotus corniculatus*

There were significant environmental (soil) and genetic (seed origin) effects for most plant performance traits, as well as a significant interaction between the two environmental effects, climate and soil (Table 2). Soil block nested within soil type did not affect plant performance (all tests, $P > 0.05$). There was no effect of climate on pod number and fecundity, but biomass and survival did differ significantly between the climates (Table 2). Initial size affected pod number in the first year but had no effect on the other traits (Table 2). In contrast to the results for *H. lanatus*, the interaction between climate and seed origin was only significant for pod number in the second year and marginally significant for biomass, and this could not be explained by significant home vs. away climate contrasts (Table 2, all data; Fig. 1E, G shows data for fecundity and biomass). For pod number and fecundity, the soil environment \times seed origin interactions were significant (Table 2, Fig. 1F), and significant home vs. away soil contrasts were found for the number of pods produced in the first year (Table 2; see Appendix C for pairwise comparisons). Even though overall home vs. away soil contrasts were not significant, Czech and UK seed origins had the highest fecundity and pod number in the second year on their respective home soils (Fig. 1F, Appendix C). Swiss plants showed a superior vegetative growth, i.e., shoot and root biomass, number of shoots,

TABLE 2. Extended.

Home vs. away climate (1, 236–280)	Soil \times seed (4, 236–280)	Home vs. away soil (1, 236–280)	Climate \times soil \times seed (8, 236–280)
9.08*** \uparrow	0.23	...	0.65
8.41*** \uparrow	1.11	...	0.42
4.16* \uparrow	0.81	...	1.55
...	2.12	...	4.12
2.25	1.47	...	0.66
...	2.46*	6.46* \uparrow	0.87
0.14	3.71**	0.03	0.56
...	2.65*	2.22	0.78
...	2.85	...	5.72

and shoot length, compared to the other seed origins, which was independent of site or soil environment (Fig. 1G, H shows data for total biomass).

DISCUSSION

Our approach to reciprocally transplant both seed material and soils among the three study sites allows disentangling the importance of climate vs. soil conditions in local adaptation. We found evidence for adaptation to local climatic conditions in *H. lanatus*, one of the two widespread grassland species examined here. In the comparison across climates, home populations of *H. lanatus* generally had a higher performance than away populations. In contrast, we found no evidence for adaptation to local soils of this grass species. The legume *L. corniculatus* showed strong genetic differentiation and differential responses of populations to climate and soil conditions, but there was no consistent pattern of superiority of home populations over away populations. Similarly, Smith et al. (2005) also found no indications of local adaptation in different British populations of *L. corniculatus*. We cannot rule out that maternal environmental effects may have contributed to the observed patterns of genetic differentiation, but this seems unlikely, given that in both species the covariate initial seedling size had little influence on the performance traits we measured. Environmental maternal effects are typically most apparent in the early stages of plant growth (Roach and Wulff 1987, Schmid and Dolt 1994). Genetic differentiation can also be caused by genetic drift and founder effects (Linhart and Grant 1996). However, genetic drift and founder effects are not likely to result in a recurring pattern of superior performance of the home populations as is the case for *H. lanatus* (Linhart and Grant 1996).

Climate and soil had interactive effects on most performance traits of both species, indicating that the influence of climate depended on soil type and vice versa. Populations of *H. lanatus* differed in their response to climate but, importantly, there were no



PLATE 1. *Lotus corniculatus*. Photo credit: C. S. Lawson.

significant three-way interactions between plant population, climate, and soil. Thus, we could examine adaptation to one environmental factor (climate) in the variable background of the second environmental factor (soil). Factors causing differential selection across study sites may not only include climate but also communities of herbivores and pathogens (Sork et al. 1993, Fornoni et al. 2004). However, in our experiment we found little damage by herbivores on *H. lanatus*, but symptoms of infection by an unidentified rust fungus were frequent. Severity of infection varied between sites and among genotypes with UK genotypes being more resistant than the other genotypes at the Swiss and Czech site but not at the home site. Therefore, climate rather than biotic factors is the most likely selective force driving local adaptation in *H. lanatus*. Nevertheless, replication of this experiment with more plant populations per climate region could increase confidence in our results.

In contrast to the results for *H. lanatus*, we found no evidence of adaptation to climate of *L. corniculatus* at the scale of our experiment. The geographic scale of our experiment was large enough to detect local adaptation to climate in *H. lanatus*, but might have been too small to

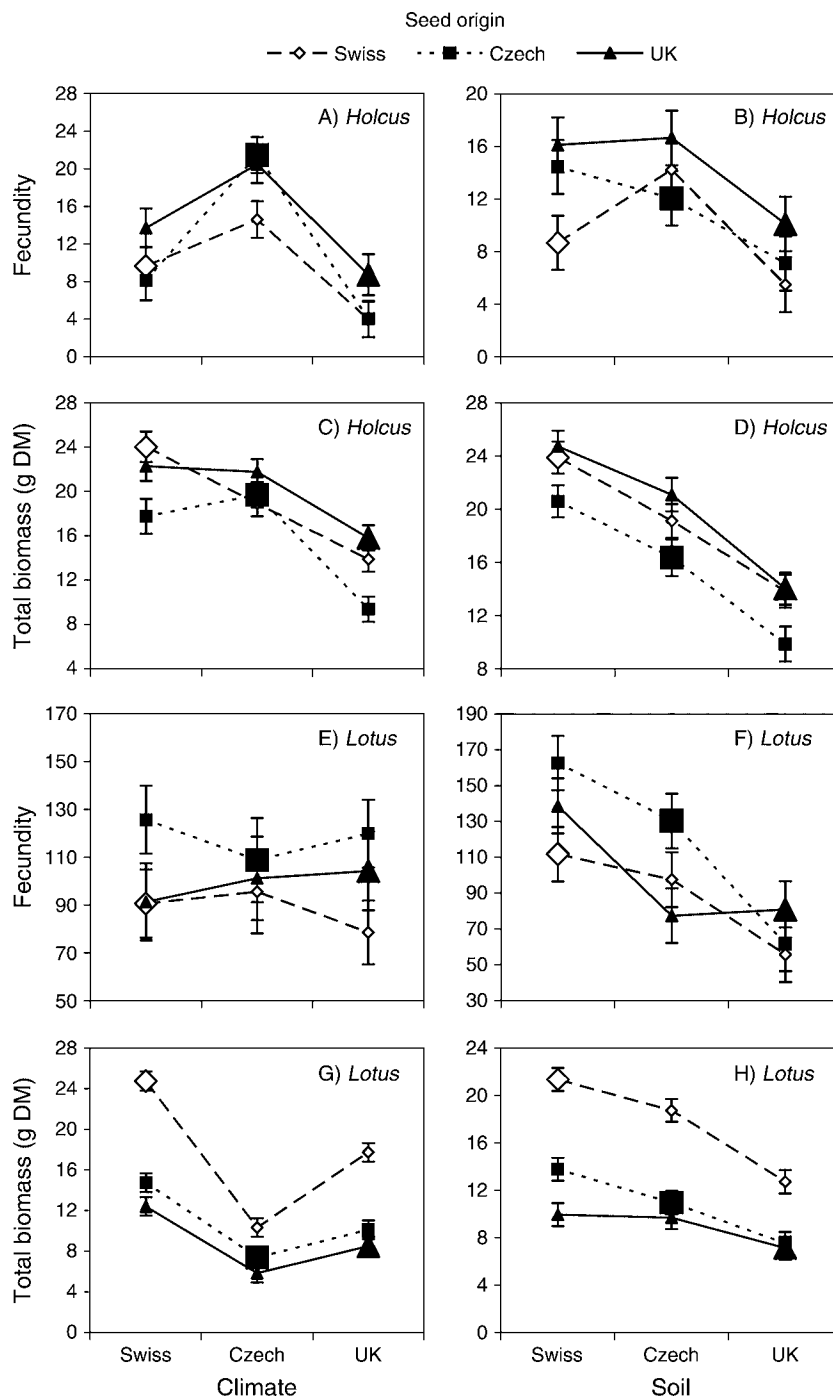


FIG. 1. Examples of performance traits of seed origins of *Holcus lanatus* and *Lotus corniculatus* on the Swiss, Czech, and UK climates and soils. (A, C) Least-square means of fecundity (survival \times no. panicles) and shoot biomass (DM, dry mass) of *H. lanatus* in the different climates; (B, D) fecundity and shoot biomass of *H. lanatus* on the different soils; (E, G) fecundity (survival \times pods year 1 + year 2) and shoot biomass of *L. corniculatus* on the different climates; (F, H) fecundity and shoot biomass of *L. corniculatus* on the different soils. Larger symbols indicate plants in their home climate or home soil. Error bars indicate standard errors of least-square means (untransformed data).

detect a similar pattern in *L. corniculatus*. The native distribution range of *L. corniculatus* by far exceeds that of *H. lanatus* (Beddows 1961, Jones and Turkington 1986). For the aquatic plant *Potamogeton pectinatus*,

there was also little evidence for local adaptation to climate but populations at the edges of the species range showed changes in life-history traits that suggested adaptation to more extreme climates (Santamaria et al. 2003).

A surprising result of our study is that there is no clear evidence for adaptation to soil in both plant species, even though there was a strong effect of soil type on plant performance. One potential explanation for this could be that the soils used in our experiment changed compared to the original soils. Mixing, transporting, and storing the soils may have influenced the soil structure and biotic conditions to some extent. However, all soils were handled early in the season when most soil organisms were in a state of dormancy and the soils were stored at temperatures similar to the field conditions. The gentle mixing of soils will have had a short-term effect on soil fauna (nematodes and arthropods) but not on soil microorganisms. In other studies in which soils have been intensively homogenized, root-feeding nematodes re-colonized the soil from eggs within weeks (De Rooij-Van der Goes et al. 1997). Soil chemistry (e.g., nutrient availability) and soil biota in our pot experiment could also have changed over time and thus could have altered the plant–soil interactions. It is possible that natural soil feedbacks in which plants can accumulate pathogens and/or mutualists over time (Bever 1994) could obscure local adaptation to specific soil organisms. For example, plants could be adapted to their local microbial symbionts but when pathogens accumulate over time, the net effect of the soil community may change from positive to neutral or even negative. Plant–soil feedbacks have been studied almost exclusively at the interspecific level (e.g., Klironomos 2002), and relatively little is known on how such feedbacks affect the performance of different genotypes of the same species. Higher pod production of *L. corniculatus* populations growing in their home soil was only found in the first season and not in the second season, which may have been the result of changes in the soils in the second year. In the case of *H. lanatus*, when the first- and second-year data were analyzed separately, there was no indication of a home soil advantage in both years, suggesting that the lack of local adaptation to soil of *H. lanatus* is not likely to be the result of changes in soils during the experiment. However, future studies could use more preserving techniques, for example transplanting entire soil blocks, to minimize artificial changes of the soils in preparation of and during the experiment that could conceal local adaptation to soil.

Based on the assumed differences in interactions with soil biota, we predicted adaptation to soil of *L. corniculatus* but not in *H. lanatus*. Interestingly, the result of the first-season pod data suggested local adaptation of *L. corniculatus* to soil. It is unclear to which soil components plants may be adapted. Microbial symbionts possibly play a role (Lie et al. 1987, Parker 1995). *Lotus corniculatus* forms mutualistic interactions with Rhizobia and mycorrhizal fungi, and in the absence of a soil biota community, *L. corniculatus* grows poorly (M. Macel, unpublished data). Indeed, there seems to be some specificity in the interaction between *L. corniculatus* and *Rhizobium loti* bacterial

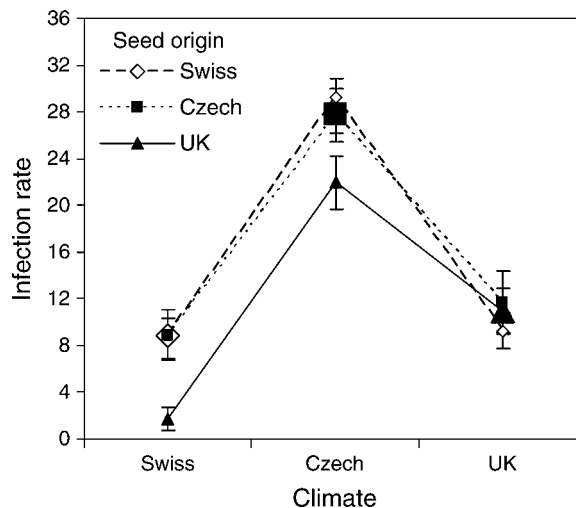


FIG. 2. Infection rates (percentage of infected tillers \times proportion of leaf infected; mean \pm SE) of rust fungus, *Puccinia* sp., on seed origins of *Holcus lanatus* on the Swiss, Czech, and UK experimental sites (climates). Larger symbols indicate plants in their home climate.

strains (Lieven-Antoniou and Whittam 1997). In contrast, the effects of soil biota community on the performance of *H. lanatus* are null to weakly negative (M. Macel, unpublished data), suggesting that selection of soil biota differs in strength and direction between the two plant species. We predicted that potential negative interactions with soil biota could lead to a home soil disadvantage if the pathogens were adapted to their local host plant genotypes. However, we found no evidence for home soil disadvantage of *H. lanatus*. Studies on the specificity of plant–soil biota interactions in the two plant species we investigated are currently ongoing.

Another explanation for the overall lack of adaptation to soil could be that the strong spatial variability of soil hampers the process of local adaptation. Soil is spatially heterogeneous at small scales (Ettema and Wardle 2002, Wardle 2002), specifically with regard to soil biota, and thus selection of soil might fluctuate even with limited dispersal. At the scale of our experiment, variation within soil type was indeed considerable, especially for nematode abundance. However, the lack of significant soil block effects (nested within soil type) on performance traits of both species suggests that small-scale variation in selection regimes was considerably lower than large-scale variation among the different soil types. Examples of adaptation to soil are mostly known of plants growing in extreme soil types. Local adaptation to serpentine soils has been found in several plant species (e.g., Schat et al. 1996, Berglund et al. 2004, Wilcox Wright et al. 2006). Differences between non-serpentine and serpentine soils are likely to be much more pronounced than differences among the non-

serpentine grassland soils in our experiment, thus providing much stronger selection.

To summarize, we found little evidence of local adaptation of the legume *L. corniculatus*. It is possible that phenotypic plasticity and strong gene flow hinder local adaptation of this extremely common species. We cannot exclude the possibility that plants are locally adapted to other factors that were not tested in this experiment, such as local competitors (Kindell et al. 1996; Bischoff et al. 2006). In addition, effects of climate and soil on seed survival, germination, and early seedling performance were not included in this study. In contrast to *L. corniculatus*, we found adaptation to climate, but not to soil, of the grass *H. lanatus*. Adaptation to climate will be important for population growth under global climate change. Even in moderate scenarios of climate change, the risk of extinction for European plants could be large (Thuiller et al. 2005). Today, both plant species we examined are common in most parts of Europe (Beddows 1961, Jones and Turkington 1986). However, selection by climate is changing, and different plant traits may be favored. Whether populations will be able to adapt to and persist under changing climatic conditions will depend on genetic variation and metapopulation processes such as gene flow.

ACKNOWLEDGMENTS

We thank all other members of the TLinks team for their contribution to the project and especially Franziska Leuenberger, Blanka Divišová, Alex Brook, and Victoria Chapman for technical assistance and Henk Duyts for identifying nematodes. We thank two anonymous reviewers for helpful comments on the manuscript. This project was supported by the Swiss Federal Office for Education and Science (Project 01.0086 to H. Müller-Schärer) to join the TLinks project, EVK2-CT-2001-00123, funded by the Framework V Program of the European Community.

LITERATURE CITED

- Antonovics, J., and A. D. Bradshaw. 1970. Evolution in closely adjacent plant populations. VIII. Clinal patterns at a mine boundary. *Heredity* 25:349–362.
- Beddows, A. R. 1961. Biological flora of the British Isles: *Holcus lanatus* L. *Journal of Ecology* 49:421–430.
- Berglund, A. N., S. Dahlgren, and A. Westerbergh. 2004. Evidence for parallel evolution and site-specific selection of serpentine tolerance in *Cerastium alpinum* during the colonization of Scandinavia. *New Phytologist* 161:199–209.
- Bever, J. D. 1994. Feedback between plants and their soil communities in an old field community. *Ecology* 75:1965–1977.
- Bezemer, T. M., C. S. Lawson, K. Hedlund, A. R. Edwards, A. J. Brook, J. M. Igual, S. R. Mortimer, and W. H. van der Putten. 2006. Plant species and functional group effects on abiotic and microbial soil properties and plant–soil feedback responses in two grassland systems. *Journal of Ecology* 94: 893–904.
- Bischoff, A., et al. 2006. Detecting local adaptation in widespread grassland species—the importance of scale and local plant community. *Journal of Ecology* 94:1130–1142.
- Bongers, T. 1988. *De Nematoden van Nederland*. Stichting Uitgeverij van de Koninklijke Nederlandse Natuurhistorische Vereniging, Utrecht, The Netherlands.
- Box, E. O. 1996. Plant functional types and climate at a global scale. *Journal of Vegetation Science* 7:309–320.
- Bradshaw, A. D. 1984. Ecological significance of genetic variation between populations. Pages 213–228 in R. Dirzo and J. Sarukhán, editors. *Perspectives in plant population ecology*. Sinauer, Sunderland, Massachusetts, USA.
- De Rooij-Van der Goes, P. C. E. M., C. Van Dijk, W. H. Van der Putten, and P. D. Jungerius. 1997. The effects of sand movement by wind in coastal foredunes on nematodes and soil-borne fungi. *Journal of Coastal Conservation* 3: 133–142.
- Ettema, C. H., and D. A. Wardle. 2002. Spatial soil ecology. *Trends in Ecology and Evolution* 17:177–183.
- Etterson, J. R. 2004. Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. I. Clinal patterns of selection along an environmental gradient in the Great Plains. *Evolution* 58:1446–1458.
- Etterson, J. R., and R. G. Shaw. 2001. Constraint to adaptive evolution in response to global warming. *Science* 294: 151–154.
- Fenster, C. B. 1991. Gene flow in *Chamaecrista fasciculata* (Leguminosae) II. Gene establishment. *Evolution* 45:410–422.
- Fornoni, J., P. L. Valverde, and J. Nunez-Farfan. 2004. Population variation in the cost and benefit of tolerance and resistance against herbivory in *Datura stramonium*. *Evolution* 58:1696–1704.
- Galen, C., J. S. Shore, and H. Deyoe. 1991. Ecotypic divergence in alpine *Polemonium viscosum*: genetic structure, quantitative variation, and local adaptation. *Evolution* 45:1218–1228.
- Hamilton, W. D., R. Axelrod, and R. Tanese. 1990. Sexual reproduction as an adaptation to resist parasites (a review). *Proceedings of the National Academy of Sciences (USA)* 87: 3566–3573.
- Jones, D. A., and R. Turkington. 1986. Biological flora of the British Isles: *Lotus corniculatus* L. *Journal of Ecology* 74: 1185–1212.
- Joshi, J., et al. 2001. Local adaptation enhances performance of common plant species. *Ecology Letters* 4:536–544.
- Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. *Ecology Letters* 7:1225–1241.
- Kindell, C. E., A. A. Winn, and T. E. Miller. 1996. The effects of surrounding vegetation and transplant age on the detection of local adaptation in the perennial grass *Aristida stricta*. *Journal of Ecology* 84:745–754.
- Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417: 67–70.
- Legendre, P., and M. J. Anderson. 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs* 69:1–24.
- Leiss, K. A., and H. Müller-Schärer. 2001. Performance of reciprocally sown populations of *Senecio vulgaris* from ruderal and agricultural habitats. *Oecologia* 128:210–216.
- Levin, D. A., and K. P. Schmidt. 1985. Dynamics of a hybrid zone in Phlox: an experimental demographic investigation. *American Journal of Botany* 72:1404–1409.
- Lie, T. A., D. Görkan, M. Engin, J. Pijnenborg, and E. Anlarsal. 1987. Co-evolution of the legume–Rhizobium association. *Plant and Soil* 100:171–181.
- Lieven-Antoniou, C. A., and T. S. Whittam. 1997. Specificity in the symbiotic association of *Lotus corniculatus* and *Rhizobium loti* from natural populations. *Molecular Ecology* 6: 629–639.
- Linhart, Y. B., and M. C. Grant. 1996. Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* 27:237–277.
- Maron, J. L., M. Vilà, R. Bommarco, S. Elmendorf, and P. Beardsley. 2004. Rapid evolution of an invasive plant. *Ecological Monographs* 74:261–280.

- Nagy, E. S., and K. J. Rice. 1997. Local adaptation in two subspecies of an annual plant: implications for migration and gene flow. *Evolution* 51:1079–1089.
- Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. Pages 85–102 in J. N. Sasser and W. R. Jenkins, editors. *Nematology*. University of North Carolina Press, Chapel Hill, North Carolina, USA.
- Parker, M. A. 1995. Plant fitness variation caused by different mutualist genotypes. *Ecology* 76:1525–1535.
- Rice, K. J., and R. N. Mack. 1991. Ecological genetic of *Bromus tectorum*. III. The demography of reciprocally sown populations. *Oecologia* 88:91–101.
- Roach, D. A., and R. D. Wulff. 1987. Maternal effects in plants. *Annual Review of Ecology and Systematics* 18:209–235.
- Santamaria, L., et al. 2003. Plant performance across latitude: the role of plasticity and local adaptation in an aquatic plant. *Ecology* 84:2454–2461.
- Schat, H., R. Voijis, and E. Kuiper. 1996. Identical major gene loci for heavy metal tolerances that have independently evolved in different local populations and subspecies of *Silene vulgaris*. *Evolution* 50:1888–1895.
- Schlichting, C. D. 1986. The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics* 17: 667–693.
- Schmid, B., and C. Dolt. 1994. Effects of maternal and paternal environment and genotype on offspring phenotype in *Solidago altissima* L. *Evolution* 48:1525–1549.
- Smith, B. M., A. Diaz, L. Winder, and R. Daniels. 2005. The effect of provenance on the establishment and performance of *Lotus corniculatus* L. in a re-creation environment. *Biological Conservation* 125:37–46.
- Sork, V. L., K. A. Stowe, and C. Hochwender. 1993. Evidence for local adaptation in closely adjacent subpopulations of Northern red oak (*Quercus rubra* L.) expressed as resistance to leaf herbivores. *American Naturalist* 142:928–936.
- Tan, K. H. 1996. Soil sampling, preparation and analysis. Marcel Dekker, New York, New York, USA.
- Ter Braak, C. J. F., and P. Šmilauer. 2002. CANOCO reference manual and CanoDraw for Windows. User's guide: software for canonical community ordination. Version 4.5. Microcomputer Power, Ithaca, New York, USA.
- Thuiller, W., S. Lavorel, M. B. Araújo, M. T. Sykes, and I. C. Prentice. 2005. Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences (USA)* 102:8245–8250.
- Van der Putten, W. H. 2003. Plant defense belowground and spatiotemporal processes in natural vegetation. *Ecology* 84: 2269–2280.
- Van der Putten, W. H., et al. 2004. Ecosystem processes in natural and managed terrestrial soils and the role of soil organisms in the sustainable delivery of ecosystem goods and services. Pages 15–43 in D. H. Wall, editor. *Sustaining biodiversity and ecosystem services in soils and sediments*. Island Press, Washington, D.C., USA.
- Van Tienderen, P. H. 1990. Morphological variation in *Plantago lanceolata*: limits of plasticity. *Evolutionary Trends in Plants* 4:35–43.
- Verschoor, B. C., T. E. Pronk, R. G. M. de Goede, and L. Brussaard. 2002. Could plant-feeding nematodes affect the competition between grass species during succession in grasslands under restoration management? *Journal of Ecology* 90:753–761.
- Wardle, D. A. 2002. *Communities and ecosystems: linking the aboveground and belowground components*. Princeton University Press, Princeton, New Jersey, USA.
- Wilcox Wright, J., M. L. Stanton, and R. Scherson. 2006. Local adaptation to serpentine and non-serpentine soils in *Collinsia sparsiflora*. *Evolutionary Ecology Research* 8:1–21.
- Williams, G. C. 1966. *Adaptation and natural selection*. Princeton University Press, Princeton, New Jersey, USA.
- Woodward, I. 1987. *Climate and plant distribution*. Cambridge University Press, Cambridge, UK.

APPENDIX A

Constrained redundancy analysis (RDA) biplot of nematode taxa and sites for *Holcus lanatus* and *Lotus corniculatus* soils (*Ecological Archives* E088-027-A1).

APPENDIX B

Climate (precipitation and temperature) data during the experiment (*Ecological Archives* E088-027-A2).

APPENDIX C

Table of least-square means of plant performance traits of *Holcus lanatus* and *Lotus corniculatus* in the different climates and soils (*Ecological Archives* E088-027-A3).