

Detecting local adaptation in widespread grassland species – the importance of scale and local plant community

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Summary

1 Adaptation of plant populations to local environments has been shown in many species but local adaptation is not always apparent and spatial scales of differentiation are not well known. In a reciprocal transplant experiment we tested whether: (i) three widespread grassland species are locally adapted at a European scale; (ii) detection of local adaptation depends on competition with the local plant community; and (iii) local differentiation between neighbouring populations from contrasting habitats can be stronger than differentiation at a European scale.

2 Seeds of *Holcus lanatus*, *Lotus corniculatus* and *Plantago lanceolata* from a Swiss, Czech and UK population were sown in a reciprocal transplant experiment at fields that exhibit environmental conditions similar to the source sites. Seedling emergence, survival, growth and reproduction were recorded for two consecutive years.

3 The effect of competition was tested by comparing individuals in weeded monocultures with plants sown together with species from the local grassland community. To compare large-scale vs. small-scale differentiation, a neighbouring population from a contrasting habitat (wet-dry contrast) was compared with the ‘home’ and ‘foreign’ populations.

4 In *P. lanceolata* and *H. lanatus*, a significant home-site advantage was detected in fitness-related traits, thus indicating local adaptation. In *L. corniculatus*, an overall superiority of one provenance was found.

5 The detection of local adaptation depended on competition with the local plant community. In the absence of competition the home-site advantage was underestimated in *P. lanceolata* and overestimated in *H. lanatus*.

6 A significant population differentiation between contrasting local habitats was found. In some traits, this small-scale was greater than large-scale differentiation between countries.

7 Our results indicate that local adaptation in real plant communities cannot necessarily be predicted from plants grown in weeded monocultures and that tests on the relationship between fitness and geographical distance have to account for habitat-dependent small-scale differentiation. Considering the strong small-scale differentiation, a local provenance from a different habitat may not be the best choice in ecological restoration if distant populations from a more similar habitat are available.

Key-words: competition, geographical distance, *Holcus lanatus*, home-site advantage, *Lotus corniculatus*, *Plantago lanceolata*, population differentiation, provenance choice, reciprocal transplant, ecological restoration

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Introduction

The occurrence of adaptive genetic differentiation in populations of plant species that occupy different habitats and geographical regions is well documented (Bradshaw 1984; Linhart & Grant 1996; Galloway & Fenster 2000; Montalvo & Ellstrand 2000; Volis *et al.* 2002; Etterson 2004). In particular, widespread species are understood to be genetically diverse because they often cover large environmental gradients (Bradshaw 1984; Joshi *et al.* 2001; Santamaria *et al.* 2003). Local adaptation is expected if there is divergent selection acting on phenotypes in different habitats, if gene flow is low relative to the strength of selection, and if phenotypic plasticity is unable to produce a phenotype that is optimal in each environment (Van Tienderen 1997; Kawecki & Ebert 2004). As plants are sessile and gene flow through seeds and pollen is limited, local adaptation appears to be a common phenomenon in plant populations and has been found on small scales or even within populations (Waser & Price 1985; Hangelbroek *et al.* 2003; Knight & Miller 2004; Lenssen *et al.* 2004).

Experimental studies on local adaptation in plants have not always found a superiority of native genotypes in all fitness-related traits, at all transplant sites, or during the entire study period (Nagy & Rice 1997; Galloway & Fenster 2000; Joshi *et al.* 2001; Volis *et al.* 2002; Santamaria *et al.* 2003; Etterson 2004; Lenssen *et al.* 2004). In particular, for multisite reciprocal transplant experiments involving widespread species and large geographical or environmental scales, there were frequently sites where foreign genotypes perform the best (Galloway & Fenster 2000; Joshi *et al.* 2001; Santamaria *et al.* 2003). In *Phragmites australis*, a superior foreign genotype seems to be invading many natural sites in North America displacing local genotypes of this species (Saltonstall 2002).

A failure to detect local adaptation in experimental studies may also result from insufficient simulation of the local environment. Three major methodological constraints occur in classic reciprocal transplant experiments. First, the study period is too short to detect local adaptation. Experiments are often run for less than 1 year, rarely for more than 2 years. However, infrequent, but characteristic selective events, such as extreme frost or drought, may be the driving forces of adaptation (Millar & Libby 1989; Montalvo *et al.* 1997). To account for such events, study periods encompassing several decades may be required, a time scale that usually cannot be accomplished by experimental approaches. Second, seedlings germinated and grown under standardized greenhouse conditions rather than seeds are used as transplants in order to reduce within-population variation due to different germination dates. However, adaptive population differentiation has been found in seed survival, dormancy and germination (Nagy & Rice 1997; Keller & Kollmann 1999; Galloway & Fenster 2000; Bischoff *et al.* 2006). Third, experiments are often run under conditions of reduced competition (vegetation

removal and subsequent weeding), neglecting the local plant community as a potential driving force of local adaptation. The importance of the local plant community in detecting local adaptation has rarely been tested, but adaptation to grass competition (Prati & Schmid 2000) and to soil modifications mediated by different chemotypes of an associated plant species (Ehlers & Thompson 2004) illustrate the importance of competition.

In the present study, we tested local adaptation of three widespread grassland species in a reciprocal transplant experiment covering a large climatic gradient across three European countries. The importance of the local plant community in detecting local adaptation was examined by comparing the performance of the test species sown in weeded monocultures with the performance when sown together with species from the local grassland community. The experiment ran for 2 years to include two entire growing seasons. Sowing seed was selected in preference to planting seedlings in order to account for adaptation in germination traits.

Generally, adaptation to a specific site is expected to decrease with increasing geographical distance from the source population due to a concomitant increase in environmental distance (e.g. climatic differences) and genetic isolation (Keller & Kollmann 1999; Galloway & Fenster 2000; Joshi *et al.* 2001). This has led to the view that local genotypes should be used in habitat creation and restoration (Jones *et al.* 2001; Vergeer *et al.* 2003; Walker *et al.* 2004). However, strong adaptive genetic differentiation was also shown on very small geographical scales (see above) and fitness may be less strongly correlated with geographical distance than with environmental distance (Montalvo & Ellstrand 2000). So far, there is a lack of studies directly comparing small-scale variation, resulting from local habitat differentiation, and large-scale variation predominantly due to climatic effects. To fill this gap our experiment included a second local population from a contrasting habitat at each site. The following specific questions were addressed. (1) Is there local adaptation in three widespread grassland species on a European scale? (2) Does the detection of local adaptation depend on competition with the local plant community? And (3) Are local provenances also superior to neighbouring populations of contrasting habitats and how large is this small-scale differentiation compared with differentiation at a European scale?

Materials and methods

STUDY SPECIES, SITES AND PROVENANCES

Three widespread grassland species were selected, each representing one of the major functional groups in grassland ecosystems: the grass *Holcus lanatus* L., the legume *Lotus corniculatus* L. and the non-leguminous herb *Plantago lanceolata* L. The species are abundant in temperate zones of Europe, occurring in a wide range of habitats and distributed over large parts of the northern

hemisphere (Cavers *et al.* 1980; Jones & Turkington 1986; Grime *et al.* 1988; Thompson & Turkington 1988). *Holcus lanatus* and *P. lanceolata* are wind-pollinated and incomplete self-incompatible, i.e. selfing is only occasional. *Lotus corniculatus* is insect-pollinated and predominantly outcrossing but selfing occurs more frequently than in the other species. All species are perennial and have a restricted capacity for vegetative regeneration but there are no specific features of vegetative spread (creeping runners, roots or rhizomes). Although sometimes sown to improve grasslands, their economic value is limited. Instead, they have become matrix species in seed mixtures for restoration and revegetation purposes (Jones & Turkington 1986; Bosshard 1999). Seeds were collected from grasslands where the species had not been sown in recent decades and where the population size was higher than 500 individuals. Randomly chosen plants, separated by at least 5 m, were sampled in late summer 2002 to produce a bulk sample of 3500 seeds per provenance. At least 40 *L. corniculatus*, 70 *P. lanceolata* and 80 *H. lanatus* mother plants were required to obtain this sample size. The seeds were stored dry at room temperature before sowing in November 2002.

In each of the three countries (Czech Republic, UK and Switzerland) two populations were sampled per species, one representing the environmental conditions of the field site ('home' population) and a second population from a contrasting habitat ('local different' population). At each collection and field site, aspect, slope, altitude and intensity of management were recorded to characterize the habitat type, and species composition was analysed to specify the plant community (Table 1). Long-term climatic data, as well as temperature and precipitation during the study period, were obtained from the closest meteorological station.

The home habitats of the three countries are characterized by pronounced macroclimatic differences with the UK climate being typically Atlantic and the Czech climate subcontinental (Table 1). Temperatures of the Swiss site are similar to those at the Czech site but precipitation is much higher than at both the Czech and

UK sites. Compared with the foreign provenances, the contrasting local habitats were in close proximity to the field site but the environmental conditions were different. The distances between habitats within the countries were 5–12 km in Switzerland, 44 (*H. lanatus*) to 100 km (*P. lanceolata*, *L. corniculatus*) in the Czech Republic and 28 km in the UK. The contrasting habitat was dryer than the home habitat in Switzerland and Czech Republic but wetter in the UK. For the Czech *H. lanatus* plants, the main within-country difference was altitude and its related climate. At all sites the first year of the experiment (2003, particularly the summer) was much drier and warmer than the average (CZ: 489 mm yr⁻¹, 21.5 °C in August; UK: 526 mm yr⁻¹, 20.0 °C; CH: 649 mm yr⁻¹, 21.9 °C) whereas 2004 was a typical year (slightly higher temperatures at all sites, precipitation average at UK, 12% higher than the average at CZ, 12% lower at CH site). The geographical distances between the field sites were 600 km (CH–CZ), 800 km (CH–UK) and 1100 km (CZ–UK).

DESIGN AND MEASUREMENTS

Seeds of the home populations were exchanged between the three countries and sown on previously ploughed arable fields in November 2002. Seeds from the contrasting habitats were only sown in the same country, resulting in four provenances tested at each site (e.g. at CH site: CH_{home}, CH_{different}, CZ_{home}, UK_{home}). At each site 2 × 2 m plots were established, each comprising seeds of one provenance × species combination sown in a central grid of 20 cells (0.2 × 0.2 m²) with 10 seeds per grid cell. To simulate the local plant community, an additional 10 characteristic grassland species of each site were sown in half of the plots (competition treatment, Table 2). The grasses were sown in a density of 400 seeds m⁻² per species, the forbs in a density of 200 seeds m⁻² resulting in a total of 3000 seeds m⁻². The eight different treatment combinations were arranged randomly within eight replicate blocks resulting in a total of 192 plots per site. All plots were mown once a year in July.

Table 1 Environmental conditions at the collection sites; the home provenance (X_{home}) reflects conditions of the experimental field, the second local provenance (X_{diff}) is from a contrasting habitat in each country; note that there are different CZ_{diff} habitats for *H. lanatus* (HI) and *P. lanceolata*/*L. corniculatus* (PI/Lc); temperature and rainfall are long-term averages

	Coordinates	Temperature Jan/Jul (°C)	Rainfall year ⁻¹ (mm)	Grassland habitat type	Plant community characterized by
CZ _{home}	49°0' N, 14°26' E†	-1.8/17.7	588	Mesotrophic, midlands	<i>Arrhenatherum elatius</i>
CZ _{diff} (PI, Lc)	49°56' N, 14°9' E	-1.5/18.0	573	Chalk grassland, midlands	<i>Bromus erectus</i>
CZ _{diff} (HI)	49°1' N, 14°0' E	-3.6/15.2	748	Montaneous, medium-moist	<i>Arrhenatherum elatius</i>
CH _{home}	46°52' N, 7°11' E‡	-1.0/17.6	1119	Mesotrophic, medium humidity	<i>Arrhenatherum elatius</i>
CH _{diff}	46°48' N, 7°10' E	-1.0/17.6	1119	Dry base-rich (23° WSW)	<i>Bromus erectus</i>
UK _{home}	51°40' N, 0°48' W	4.3/17.2	651	Chalk grassland (18° W)	<i>A. elatius</i> , <i>Centaurea nigra</i> subcommunity
UK _{diff}	51°26' N, 0°56' W	4.3/17.2	651	Mesotrophic, moist	<i>Lolium perenne</i> , <i>Cynosurus cristatus</i>

†CZ_{home} of *Holcus lanatus*: 48°57' N, 14°36' E.

‡CH_{home} of *Lotus corniculatus*: 46°52' N, 7°16'.

Table 2 Species sown to simulate competition with local plant community (competition treatment)

Czech Republic	United Kingdom	Switzerland
<i>Achillea millefolium</i>	<i>Achillea millefolium</i>	<i>Arrhenatherum elatius</i>
<i>Agrostis capillaris</i>	<i>Arrhenatherum elatius</i>	<i>Centaurea jacea</i>
<i>Alopecurus pratensis</i>	<i>Centaurea nigra</i>	<i>Crepis biennis</i>
<i>Anthoxanthum odoratum</i>	<i>Cynosurus cristatus</i>	<i>Dactylis glomerata</i>
<i>Betonica officinalis</i>	<i>Dactylis glomerata</i>	<i>Festuca rubra</i>
<i>Deschampsia cespitosa</i>	<i>Festuca ovina</i>	<i>Leucanthemum vulgare</i>
<i>Festuca rubra</i>	<i>Galium verum</i>	<i>Pimpinella major</i>
<i>Lychnis flos-cuculi</i>	<i>Leontodon hispidus</i>	<i>Poa pratensis</i>
<i>Prunella vulgaris</i>	<i>Sanguisorba minor</i>	<i>Prunella vulgaris</i>
<i>Rumex acetosa</i>	<i>Trisetum flavescens</i>	<i>Trisetum flavescens</i>

Seedling emergence was recorded for 8 weeks following occurrence of the first seedlings in late April 2003. Afterwards, the number of seedlings was reduced to one per grid cell, i.e. ideally 20 per plot. In some site \times species \times provenance combinations, a high proportion of cells remained without seedlings. Moderate transplanting from cells with spare seedlings was used to reduce the number of empty cells. Although watered at the start, there was a high mortality in the first 2 weeks after transplanting and therefore, transplants were only considered if they survived for at least 8 weeks until the second census in late August. Altogether three censuses were made in each year, one before mowing and two afterwards. The censuses included the measurement of survival, vegetative growth and reproduction. Survival was analysed in all test plants, growth and reproduction were recorded in five focal plants per plot that were randomly selected and labelled at the first census. In *P. lanceolata*, we measured number of leaves, length of the longest leaf, number of spikes and length of the longest spike; in *L. corniculatus* the number of shoots, length of the longest shoot, number of lateral shoots on the longest shoot, number of flowers and number of pods; and in *H. lanatus* the number of sterile tillers, number of fertile tillers (panicles) and, for both tiller types, the length of the longest. In the last census of each season, seeds of a subsample of spikes, pods and panicles were counted to obtain an estimate of seed production per plant. In September 2004, above-ground parts of all focal plants were harvested, dried at 80 °C for 48 h and weighed.

DATA ANALYSIS

To obtain a good estimate of fitness combining several fitness related traits, the dominant eigenvalues (λ) of Leslie matrices were calculated based on fecundity and survival at block level. The upper left value of the matrix is the net fecundity, i.e. the product of seedling emergence, survival to first reproduction and first-year fecundity (Charlesworth 1994). In addition, survival to the second year and second year fecundity are included. The calculation of λ was preferred against total reproduction because λ accounts for the higher contribution of first-year fecundity to population growth (McGraw & Caswell 1996). We used the number of spikes, pods

and panicles per plant as fecundity estimates because seed count data were less reliable. Early individuals had already shed seeds before the late individuals started to reproduce, and due to differences in phenology, the loss of seeds before counting was not completely random among provenances. As the fecundity estimates are lower than the actual fecundity and total life span of the test species is much longer than the study period of 2 years, the calculated λ does not reflect the real population growth rate, and we will refer to it as 'fitness coefficient' in the text. The 'fitness coefficient' F was used to calculate a selection coefficient (McGraw & Antonovics 1983) for each provenance P relative to the best performing provenance at each particular site as $S_p = 1 - (F_p/F_{max})$. S_p ranges from 0 for the most successful provenance to 1 indicating complete selection against the provenance.

Individual traits and fitness coefficients were analysed using hierarchical mixed-model ANOVAs with block as replicated units ($n = 8$). The analyses were calculated separately for each test species. To test for local adaptation, only the three exchanged 'home' populations were included. Site, provenance and competition were considered as fixed factors and block as a random factor, nested within site (Table 3). For calculating F -values,

Table 3 Basic statistical model to test for site, provenance, competition main effects, their interactions and the home vs. foreign contrast as the main criterion for local adaptation; ANOVA was calculated on block means ($n = 8$) of all measured traits and the fitness coefficient

Source of variation	d.f.	Mean Squares	F -value
Site	2	M_S	M_S/M_B
Provenance	2	M_P	M_P/M_{PB}
Competition	1	M_C	M_C/M_{CB}
Site \times Provenance	4	M_{SP}	M_{SP}/M_{PB}
Home vs. Foreign	1	M_{HA}	M_{HA}/M_{PB}
Site \times Competition	2	M_{SC}	M_{SC}/M_{CB}
Prov. \times Comp.	2	M_{PC}	M_{PC}/M_{PCB}
Site \times Prov. \times Comp.	4	M_{SPC}	M_{SPC}/M_{PCB}
Block	21	M_B	
Provenance \times Block	42	M_{PB}	
Competition \times Block	21	M_{CB}	
Residual (Prov. \times Comp. \times Block)	42	M_{PCB}	

site was tested against block, and provenance, competition and site-by-provenance interaction were tested against their interaction with block. Criteria for local adaptation were: (i) a significant site \times provenance interaction; (ii) a significant linear contrast of home (1 local \times 3 sites) vs. foreign (2 foreign \times 3 sites) that was specified within the site \times provenance interaction, and (iii) a superiority of the home provenance at least at two of the three sites. The third criterion was added to the common home vs. foreign criterion because the contrast may be significant even though one genotype is superior at all sites. In such cases the home vs. foreign contrast is not a sufficient diagnostic for local adaptation (Kawecki & Ebert 2004).

Significant three-way interactions (site \times provenance \times competition) were additionally considered because a differential response of provenances to different site conditions may depend on the competitive environment experienced. The 'home vs. foreign' contrasts are always presented separately for both competition treatments in order to show whether the expression of local adaptation depends on the surrounding vegetation. Traits that were measured more often than three times were tested using repeated measures analyses based on the same ANOVA model (primarily traits of vegetative growth). Seedling emergence and survival were generally arcsin square root transformed; growth traits, fecundity and fitness coefficients were $\log(x + 1)$ or square-root transformed if necessary to meet the assumptions of ANOVA. Due to low seedling emergence (*H. lanatus* at CZ and CH site) and high mortality (*P. lanceolata* at CZ site) there are empty cells (= block) in some analyses. They were replaced by treatment means, and degrees of freedom for block and interactions with block were reduced (Underwood 1997).

A slightly different ANOVA model was used to include the second local provenance from a contrasting habitat because this provenance was not exchanged between countries. We replaced 'provenance' by the factor 'distance' using the four levels 'home' (= local *sensu stricto*), 'local different' (contrasting habitat), 'foreign 1' and 'foreign 2'. As four instead of three provenances were included, 'distance' could be tested with higher statistical power than 'provenance' (d.f. 3, denominator (distance \times block interaction): d.f. 63). The distance main effect was decomposed into the linear contrasts 'home' vs. 'local different' and 'home' vs. 'foreign' in order to test the importance of the habitat where seeds were collected. All other factors and interactions in the model were the same as in the basic model (Table 3).

Results

TEST OF LARGE-SCALE ADAPTATION

A strong effect of seed provenance was observed for many of the measured plant traits. The provenance effect was significant for final survival of *P. lanceolata* and *L. corniculatus*, for most growth and reproductive

traits, for seedling emergence and for the resulting fitness coefficient of all three species. There were also highly significant differences between the experimental sites and the site \times provenance interaction was significant for most traits. We found evidence for local adaptation in *P. lanceolata* and *H. lanatus* but not in *L. corniculatus* (Table 4, Fig. 1).

For *P. lanceolata*, a significant home vs. foreign contrast and a superiority of the local provenance were detected in seedling emergence, survival rate and reproduction (seed and spike number) resulting in a significantly higher fitness coefficient of the home plants (Table 4). At two out of three experimental sites, a fitness superiority of the home provenance was found for both competition treatments indicating local adaptation (Fig. 1). Parameters of vegetative growth such as leaf number, leaf length and final biomass did not differ significantly between home and foreign provenances.

For *H. lanatus*, statistical power to detect local adaptation was lower due to a low seedling emergence at the Czech and Swiss sites. Nevertheless, in both competition treatments, a significant home-site advantage was recorded in reproductive traits such as panicle and seed number (Table 4). Although survival and seedling emergence did not significantly differ between local and foreign plants, the fitness coefficient was higher in local plants as a result of large differences in reproductive traits. The significant home vs. foreign contrast for fitness coefficients and the greater fitness of the home provenance at two sites (Fig. 1) indicated local adaptation. In traits that were measured several times, the ranking of provenances was not the same in all censuses resulting in significant interactions with time in the repeated measures analyses (Table 4). For example, the tiller number of the home provenances was the highest in most censuses but not in the last census when plants were harvested, and hence home vs. foreign contrasts were not significant for biomass.

The fitness coefficient of *L. corniculatus* was not significantly different between home and foreign provenances (Table 4). The Swiss provenance was observed to have the highest fitness coefficient at the CZ and the UK site in both competition treatments (Fig. 1). The linear contrast of Swiss vs. the two other provenances was highly significant for most measured traits (fitness coefficient: $F_{\text{CHvsUK,CZ}} = 61.05$, $P < 0.001$) indicating an overall superiority of one provenance. Large differences occurred particularly in vegetative traits, whereas reproduction of the Swiss plants was not always higher than the other two provenances. This was probably the result between provenance differences in timing of reproduction (Fig. 2). The Swiss plants grew very large at all sites but started to reproduce later and hence not all plants reached the reproductive stage until the end of the season. The pod number was low early in the season but increased with time relative to the other provenances, resulting in highly significant interactions of provenance and time in the repeated measures analyses ($F_{\text{ProvxT}} = 22.76$, $P < 0.001$). In spite of the overall superiority of

Table 4 F-values of site, provenance and competition effects on seedling emergence, survival, growth and reproduction; block effect and all interactions were fitted (see Table 3) but only interactions including site and provenance are presented; linear contrasts of home vs. foreign combinations were specified separately for both competition treatments (Home (-C): without competition, Home (+C): competition, ↑: home > foreign) to test for local adaptation; F-values resulting from repeated measures analysis are in italics if interaction with time is significant (T: number of censuses)

	Site (S), d.f. = 2	Provenance (P), d.f. = 2	Competition (C), d.f. = 1	S × P, d.f. = 4	S × P × C, d.f. = 4	Home (-C), d.f. = 1	Home (+C), d.f. = 1
<i>Plantago lanceolata</i>							
Seedling emergence	6.56**	8.09**	0.44	9.97***	1.51	2.82(*)↑	12.42**↑
Final survival	154.25***	7.88**	0.88	7.20***	2.08	17.57***↑	3.47(*)↑
Seeds (03 + 04)	11.83***	26.48***	136.61***	3.81**	0.27	7.46**↑	2.72(*)↑
Biomass	42.74***	1.85	542.97***	1.58	0.21	–	–
Fitness coefficient	25.31***	3.57*	101.31***	7.95***	0.73	7.23**↑	14.39***↑
Leaf number (T = 5)	90.05***	7.44**	226.11***	2.44(*)↑	0.98	1.15	2.79(*)↑
Leaf length (T = 5)	72.62***	5.96**	0.67	0.59	1.91	–	–
Spike number (T = 5)	57.40***	3.82*	288.41***	2.64*	2.62	0.39	12.25***↑
<i>Holcus lanatus</i>							
Seedling emergence	148.45***	12.98***	1.95	4.90**	0.47	0.73	0.32
Final survival	63.81***	1.64	20.62***	0.51	2.21	–	–
Panicle number	153.63***	1.62	187.77***	8.34***	7.21***	6.77*↑	9.12**↑
Panicle length	118.02***	31.54***	21.30***	4.34**	6.82***	13.64***↑	1.53
Seeds	99.04***	6.25**	73.53***	8.16***	5.21**	6.35*↑	3.16(*)↑
Biomass	234.14***	4.70*	708.53***	2.43*	7.42***	1.34	0.53
Fitness coefficient	31.22***	12.58***	58.47***	18.71***	1.29	28.55***↑	4.45*↑
Tiller number (T = 5)	113.98***	17.94**	308.47***	6.02***	3.14*	18.44***↑	0.76
Tiller length (T = 5)	239.52***	1.56	7.07*	0.41	2.86*	4.06(*)↑	2.62
<i>Lotus corniculatus</i>							
Seedling emergence	54.82***	7.18**	0.85	9.25***	1.04	0.14	0.23
Final survival	13.28***	3.51***	0.08	0.87	1.83	–	–
Seeds (03 + 04)	0.05	37.46***	148.86***	4.83**	2.33(*)	0.88	0.81
Biomass	11.10***	104.27***	282.26***	3.35*	2.72*	0.00	3.72(*)↑
Fitness coefficient	2.00	62.66***	316.40***	7.81***	3.46*	2.63	0.51
Shoot number (T = 4)	52.81***	9.73***	518.69***	2.66*	3.77*	3.90(*)↑	3.55(*)↑
Shoot length (T = 5)	101.03***	122.71***	41.36***	2.87*	6.98***	6.79*↑	0.88
Lateral shoots (T = 5)	0.76	178.06***	231.63***	8.58***	7.35***	28.00***↑	1.30
Pod number (T = 5)	25.45***	9.12***	361.68***	6.46***	5.38**	0.04	0.00

(*) $P < 0.1$ * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

the Swiss provenance, a significant home vs. foreign contrast was found in several growth traits because the difference from the other provenances was larger at the 'home' (Swiss) site (Fig. 2).

THE EFFECT OF COMPETITION WITH THE LOCAL PLANT COMMUNITY

At all three sites, the sown competitor species established well in the competition treatment. Total vegetation cover was between 70% and 90% in 2003, and between 90% and 100% in the second year. At the start of the experiment, many arable weeds emerged from the soil seed bank, nevertheless, the sown grassland species became dominant after the first mowing in 2003. The presence of the local grassland community (competition treatment) had a significant negative effect on most growth and reproductive traits of the three test species but not on seedling emergence and survival (except for *P. lanceolata*, Table 4).

The detection of local adaptation in *P. lanceolata* and *H. lanatus* depended on the competitive environment. For *P. lanceolata* the home vs. foreign contrasts

of most traits were stronger with competition with the local plant community than without competition (Table 4). In the competition treatment the magnitude of home-site advantages in trait means was 16.8% for seedling emergence, 9.3% for leaf number (average of five censuses) and 22.3% for the spike number (average of five censuses) relative to foreign provenances, whereas without competition the corresponding values were 7.3%, 3.3% and 8.2%. An opposite effect was found for survival (competition: 9.8%, no competition: 18.9%) but the fitness coefficient combining survival, seedling emergence and reproduction showed again a higher home-site advantage in the competition treatment (Table 4, Fig. 1). In the presence of the local grassland community, the fitness coefficient of the home provenance was higher at all sites, whereas without competition, the home plants were only superior at the Czech and the Swiss site (Fig. 1).

For *H. lanatus*, the home vs. foreign contrasts were weaker (lower magnitude of difference, higher P -value) in the presence of competition with the local plant community than without competition (Table 4). In the competition treatment a higher fitness coefficient of the

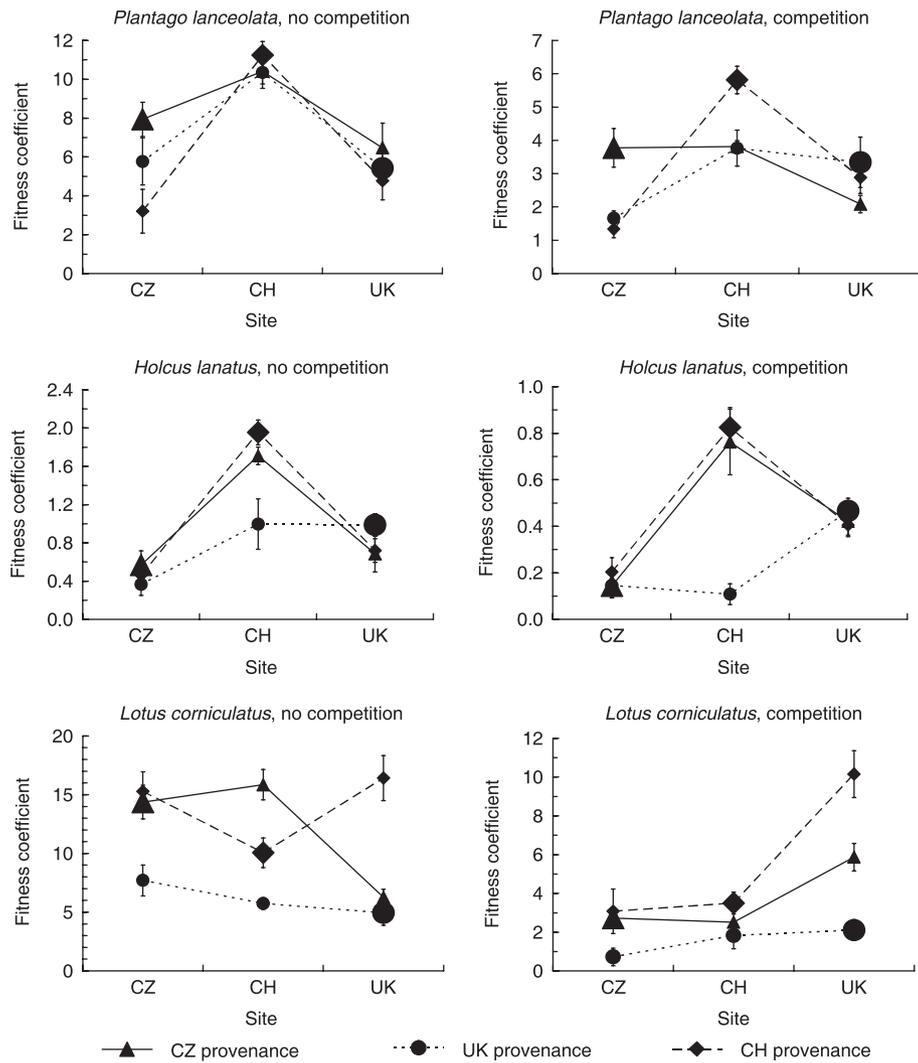


Fig. 1 Performance of three provenances in a reciprocal transplant experiment; larger symbols indicate populations growing at their home site; Fitness coefficients: λ of Leslie matrices based on seedling emergence, survival and number of spikes (*P. lanceolata*), pods (*L. corniculatus*) and panicles (*H. lanatus*) in 2003 and 2004, \pm SE.

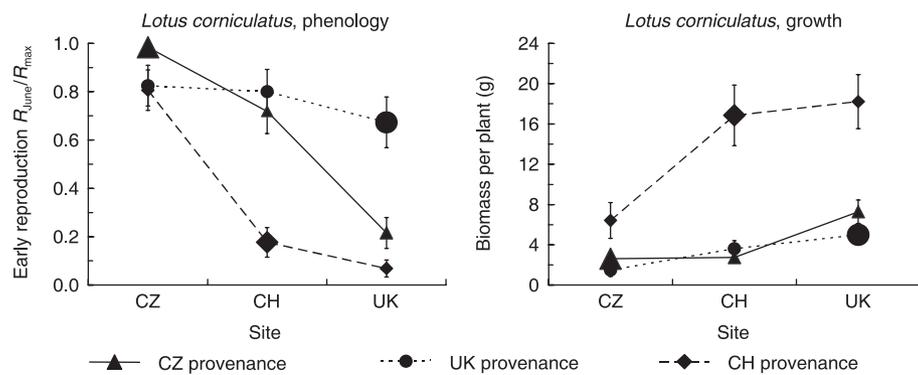


Fig. 2 Phenology and growth of *Lotus corniculatus*; phenology was measured as the number of reproductive plants in the first census (2004) divided by the maximum number of reproductive plants in the same year (usually third census); growth is represented by final above-ground biomass in the competition treatment; larger symbols indicate populations growing at their home site; \pm SE.

local plants was only found at the Swiss and the UK site, whereas without competition, the home provenance was superior at all sites (Fig. 1). Traits in which competition-dependent differences in the detection of local adaptation

occurred were tiller number and panicle length (competition: -8.1% and -1.4% , no competition: $+8.3\%$ and $+37.7\%$, *P*-values: Table 4). However, it has to be considered that survival was lower in the competition treatment,

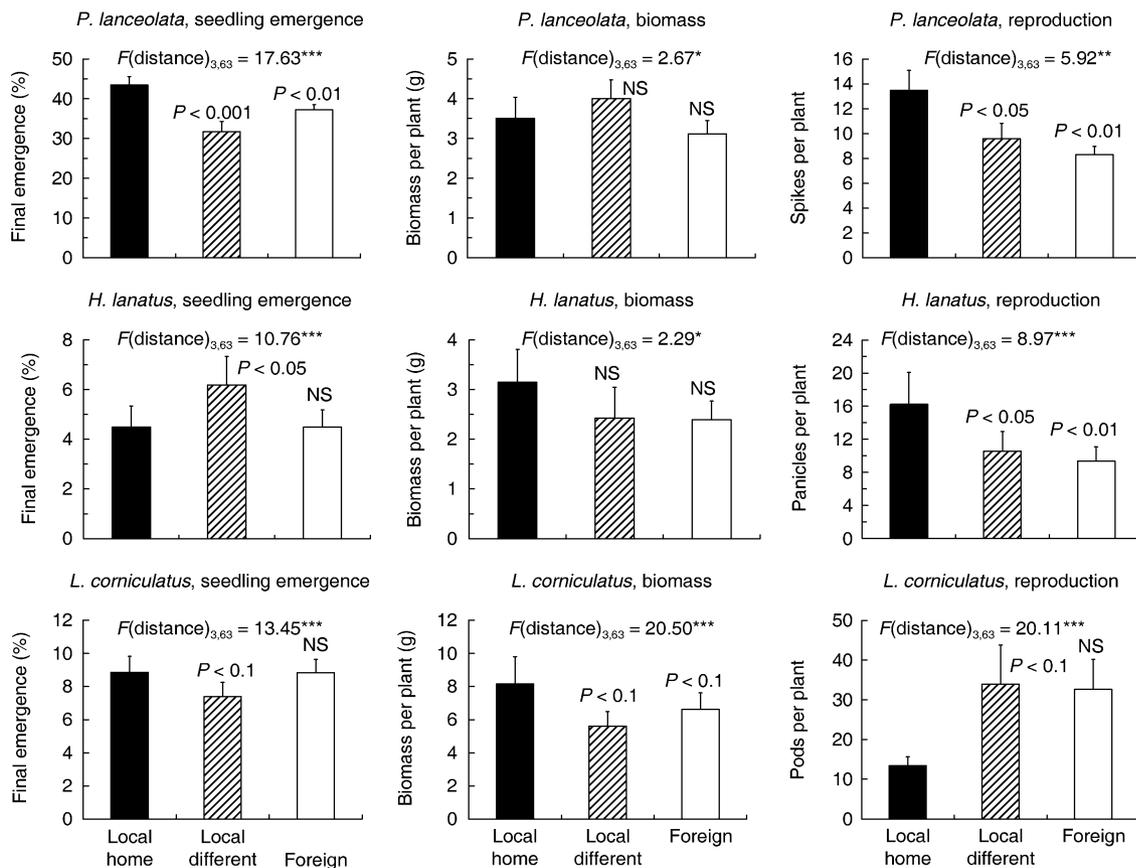


Fig. 3 Seedling emergence, final biomass and reproduction (sum of 2003 and 2004) of the two local provenances (home and different habitat) and the foreign provenances (data of the two foreign provenances pooled) in the competition treatment; the ANOVA model of Table 3 was fitted, except that 'provenance' was replaced by 'distance' (home, different habitat, foreign 1 and foreign 2, F-values above the graphs); the distance effect was decomposed into the linear contrasts home vs. 'local different' habitat and home vs. foreign (P -values of the contrasts presented), \pm SE.

thereby increasing the within-treatment variation due to a lower number of measured plants and decreasing the probability of finding significant differences.

In *L. corniculatus*, we found no evidence for a fitness superiority of the home provenances in either competition treatment.

ADAPTATION TO SMALL-SCALE VS. LARGE-SCALE ENVIRONMENTAL DIFFERENCES

The 'local different' provenance was included to analyse the spatial scale of differentiation and adaptation. Here, we only present the data of the competition treatment but similar results were obtained for plants grown without competition.

The main effect of the factor 'distance' comprising the home, 'local different' and two foreign provenances at each site was significant for seedling emergence and reproduction of all species, and for the biomass of *L. corniculatus* (F_{distance} in Fig. 3). Differences between home and 'local different' provenances were significant in nearly all traits that showed significant differences between home and foreign populations (Fig. 3). In some traits, this small-scale differentiation was even larger than between home and foreign. For example, the home

vs. 'local different' advantage in seedling emergence was 37.0% ($F_{1,63} = 41.46$, $P < 0.001$) for *P. lanceolata* and 20.1% ($F_{1,63} = 2.81$, $P = 0.098$) for *L. corniculatus* whereas the corresponding home vs. foreign contrasts were only +16.8% ($F_{1,63} = 12.42$, $P = 0.001$) and +0.1% ($F = 0.23$, $P = 0.635$).

There was no superiority of the *P. lanceolata* home populations in biomass but seedling emergence and reproduction were much larger than in the 'local different' population (Fig. 3) resulting in a significantly higher fitness coefficient ($F_{1,63} = 13.34$, $P < 0.001$). A comparison of selection coefficients demonstrated that at the Czech and UK sites, selection against populations from the contrasting habitat 'local different' was stronger than selection against the distant foreign populations (Table 5). At the Swiss site, the selection coefficient of the 'local different' population was intermediate between that of home and foreign provenances.

Biomass and reproduction of local populations of *H. lanatus* and *L. corniculatus* from the contrasting habitats were closer to the foreign than to the 'local home' populations (Fig. 3). Seedling emergence differed between the two local populations ('local different' was higher than 'local home' in *H. lanatus* but lower in *L. corniculatus*) although no significant differences

Table 5 Selection coefficients derived from fitness coefficients (λ of Leslie matrices based on seedling emergence, survival and fecundity, only competition treatment) relative to the best performing provenance at each site ($S=0$); the 'home' provenance (bold) is compared with two foreign provenances and to a second contrasting local provenance (Local (diff.))

Provenance	Experimental site		
	CZ	CH	UK
<i>Plantago lanceolata</i>			
CZ	0.00	0.57	0.57
CH	0.49	0.00	0.16
UK	0.61	0.47	0.00
Local (diff.)	0.75	0.20	0.57
<i>Holcus lanatus</i>			
CZ	0.29	0.17	0.15
CH	0.00	0.09	0.18
UK	0.28	0.88	0.06
Local (diff.)	0.33	0.00	0.00
<i>Lotus corniculatus</i>			
CZ	0.42	0.59	0.42
CH	0.34	0.43	0.00
UK	0.84	0.70	0.79
Local (diff.)	0.00	0.00	0.88

occurred between home and foreign. The 'local home' vs. 'local different' contrasts were not significant for fitness coefficients (*H. lanatus*: $F_{1,63} = 0.16$, $P = 0.695$; *L. corniculatus*: $F_{1,63} = 0.38$, $P = 0.385$). 'Local different' populations of *H. lanatus* populations had almost the same selection coefficient as home populations (Table 5). They were well adapted to the Swiss and UK site and maladapted to the Czech site. *Lotus corniculatus* populations from the contrasting habitat 'local different' performed best at the Czech and Swiss site but had the highest selection coefficient at the UK site. Only at the Swiss site, selection coefficients of the two local provenances were more similar than selection coefficients of the 'local different' and foreign provenances. However, it has to be considered that fitness coefficients do not account for differences in vegetative growth. Selection coefficients based on growth instead of reproduction would reveal a strong superiority of the Swiss home population of *L. corniculatus* at all sites.

Discussion

POPULATION DIFFERENTIATION AND LARGE-SCALE LOCAL ADAPTATION

At all sites and in all test species, large differences between plant provenances were found, thus indicating strong population differentiation. We cannot entirely exclude that maternal environmental effects may have contributed to the observed differentiation, as seeds used in this study were collected directly in the field. However, we found a similar degree of differentiation and provenance ranking in a parallel pot experiment in which we analysed the same populations of two test

species (*H. lanatus* and *L. corniculatus*) at the same sites and in which we corrected for initial size (Macel *et al.* in press). Such a correction for initial size can be used to minimize maternal effects (Hangelbroek *et al.* 2003) and therefore, we are confident that the observed differentiation has a genetic basis.

Other large-scale studies on widespread perennial species have also found strong heritable population differentiation (Joshi *et al.* 2001; Santamaria *et al.* 2003). A smaller differentiation may occur in early successional or colonizing species of disturbed habitats that experience frequent population turnover (Galloway & Fenster 2000). Such habitats favour the selection for phenotypic plasticity as an appropriate response to a range of different environmental conditions rather than ecological specialization.

Two of our three study species, *P. lanceolata* and *H. lanatus*, showed clear evidence of adaptation to the local site conditions (Table 4, Fig. 1). In accordance to the local adaptation hypothesis, the local 'home' populations performed better than foreign ones. At large scales, climate is a major source of environmental variation and an important driving force of local adaptation (Joshi *et al.* 2001; Santamaria *et al.* 2003; Etterson 2004). Soil (Snaydon & Davies 1982) and biotic conditions (Turkington & Harper 1979; Sork *et al.* 1993) are further factors to which the populations may have adapted. We found a stronger superiority of the home provenance than other studies that used comparable geographical scales. They failed to detect a home-site advantage (Santamaria *et al.* 2003) or differences between home and away were smaller and not always significant (Galloway & Fenster 2000). A similar home-site advantage was observed in reciprocal transplant experiments involving particularly large environmental gradients (Nagy & Rice 1997; Joshi *et al.* 2001). As in the latter studies, we found evidence for local adaptation in reproductive traits rather than in vegetative growth. In *P. lanceolata*, significant differences between home and foreign provenances were observed in seedling emergence which demonstrates the importance of considering early plant stages in studies on local adaptation.

The third species in our study, *L. corniculatus*, showed no evidence of local adaptation. Instead, the Swiss population was found to be superior at all sites, particularly when considering vegetative growth (Fig. 2). Even though a significant overall home-site advantage was found in most studies, the superiority of non-local genotypes at particular sites has repeatedly been observed in transplant experiments (Rice & Mack 1991; Galloway & Fenster 2000; Joshi *et al.* 2001; Leiss & Müller-Schärer 2001; Santamaria *et al.* 2003). However, studies that reveal a general superiority of one provenance at several sites are rare. In a reciprocal transplant experiment on a European scale, the German provenance of *P. lanceolata* performed better than the local provenance at all six foreign sites and was the best performing population at three sites (Joshi *et al.* 2001). Agricultural populations of *Senecio vulgaris* were found to have a higher fitness at

ruderal and agricultural sites than ruderal populations (Leiss & Müller-Schärer 2001).

The superiority of non-local genotypes may be the result of complete genetic isolation from local genotypes. Such isolation allows the independent adaptation to similar habitats and the evolution of different adaptive strategies and traits. Genotypes may have evolved that are also superior in other regions but colonization has been prevented by limited gene flow. When introduced, such alien genotypes have the potential to replace the local ones, as demonstrated by the cryptic invasion of a foreign *Phragmites australis* genotype in North America (Saltonstall 2002). However, it is also possible that populations of grassland species are no longer adapted to the current environmental conditions because the management of grasslands has changed in recent decades (Joyce & Wade 1998), and further environmental changes such as global warming or eutrophication might have contributed to the disruption of adaptation. This may also be the reason for not finding any evidence for local adaptation in a study on 30 British populations of *L. corniculatus* (Smith *et al.* 2005). Unlike the other two species, the growth of *L. corniculatus* strongly depends on *N*-fixing soil bacteria and local adaptation was found between plant provenances and *Rhizobium* strains (Lie *et al.* 1987). Hence, soil conditions might be more important for population differentiation in this species than climatic differences that distinguish the test sites of the three countries.

The duration of the experiment may have been insufficient in finding local adaptation of *L. corniculatus* although two full growing seasons are included. Driving forces of adaptation may be occasional events that are difficult to study experimentally. For example, in a reforestation programme non-local *Pseudotsuga menziesii* populations initially did well but were killed later on by a harsh frost which, although infrequent, is typical at the site (Millar & Libby 1989). Nevertheless, our study demonstrated that a superiority of non-local provenances is possible, at least on intermediate time scales.

Last but not least, superior performance of a particular species is not the only way to ascertain adaptive processes at the level of plant communities. Adaptation of conspecific neighbours to allelopathic compounds may counteract the selection for superiority of local genotypes of a target species if such compounds are different between local and foreign populations. Disrupting this coevolutionary relationship by introducing foreign genotypes can lead to competitive exclusion and a superiority of foreign provenances, an effect that has been shown for invasive species (Callaway *et al.* 2005). In the following paragraph, interactions of adaptive processes at population and community level will be discussed in more detail.

THE IMPORTANCE OF COMPETITION WITH THE LOCAL PLANT COMMUNITY

We found evidence in one species, *P. lanceolata*, that the expression of local adaptation was higher in the com-

petitive environment of local plant communities than in the absence of interspecific competitors (Table 4, Fig. 1). Few field studies have examined the importance of interspecific competition in local adaptation of plants. In a reciprocal transplant experiment with the perennial grass *Aristida stricta*, the expression of local adaptation was hardly affected by vegetation removal and if anything, the evidence pointed to greater local adaptation in the absence of competition (Kindell *et al.* 1996). In a field study on *Hydrocotyle bonariensis* plants from high and low elevations of sand dunes, the home-site advantage in total biomass was larger in presence of the 'natural' vegetation than in a vegetation removal treatment (Knight & Miller 2004).

An increase in the pattern of local adaptation under competition with the local plant community suggests that the competitive environment mimics more closely the conditions to which populations are adapted. Three factors may have contributed to such a site-specific selection mediated by plant neighbours. First, the contribution of competition to local adaptation could be the result of community-specific abiotic conditions to which species are adapted (resource availability and microclimate). In a glasshouse experiment standardizing all other environmental factors, *Ranunculus reptans* populations from an open 'lake shore' and a dense 'land' habitat performed best in a competitive environment of the home habitat simulated by growth with and without competition by one grass species (Prati & Schmid 2000).

Second, coevolution of plant species or genotypes of local plant communities may have led to mutual adaptation (specific plant–plant interactions, Callaway *et al.* 2005). Evidence for such plant–plant interactions as drivers of local adaptation was found in a study of *Trifolium repens* (Turkington & Harper 1979). In a reciprocal transplant experiment, subpopulations from four patch types each dominated by a different grass species performed best when transplanted back to the patch of their origin. One possible mechanism involved in such adaptation might be an effect of the grass species on type and number of the *T. repens* associated *Rhizobium* strains (Thompson *et al.* 1990). Adaptation to certain plant species or genotypes may also result from allelopathic interactions. Populations of *Bromus erectus* originating from sites differing in the dominance of either a phenolic or a non-phenolic chemotype of *Thymus vulgaris* performed best when grown in the soil of the native *T. vulgaris* chemotype (Ehlers & Thompson 2004). Evolution of tolerance to the allelopathic effects of the secondary compounds produced by *T. vulgaris* could well be the ultimate cause of the observed adaptive pattern.

Third, differential attack by herbivores and pathogens may have contributed to differences between competition treatments in the expression of local adaptation. A study on northern red oaks has shown that herbivores can be an important driving forces of local adaptation (Sork *et al.* 1993). Differences in plant species composition may cause differences in assemblages of associated

herbivores and pathogens, and local plant provenances may have evolved higher resistance to these antagonists.

Our experimental approach does not allow to distinguish between the above mentioned factors that may have contributed to a higher expression of local adaptation in the competition treatment. There is some evidence for the third mechanism from our study on leaf beetle damage in *P. lanceolata* (L. Crémieux *et al.*, unpublished data). The level of damage was larger in the competition treatment than without competition, and the levels of resistance were higher in the home provenances compared with foreign ones.

In our second test species, *H. lanatus*, the evidence for local adaptation was weaker in competition with the local plant communities than without competition. There is some evidence that causality of the three mechanisms described above may also act in the opposite direction. Studies on invasive plant species have demonstrated that in the home range plant communities are often less susceptible to allelopathic compounds than in the invasive range where adaptation and tolerance have not evolved (Callaway *et al.* 2005). In the case of considerable population differentiation in such compounds, similar effects may be found at genotype level. Plant communities in the home range of a genotype may be less susceptible to genotype-specific allelopathic compounds than outside the range and competition by the surrounding vegetation would then be higher for the local genotype. In our study on pathogen attack in *H. lanatus*, the degree of leaf infection by a specialist rust fungus was lower in the competition treatment and the home populations of the plant species were less resistant than the foreign ones (L. Crémieux *et al.*, unpublished data). Hence, the pathogen may have reduced home-site advantages but this effect was smaller without competition.

THE IMPORTANCE OF SMALL-SCALE VS. LARGE-SCALE ADAPTATION

A negative relationship between distance from the source population and the level of adaptation to a given site has typically been found in studies testing local adaptation at different geographical scales (Galloway & Fenster 2000; Joshi *et al.* 2001). However, populations from similar habitats were used in these studies, which raises the question whether this negative relationship can also be observed if local populations from contrasting habitats are included. Such a small-scale adaptation to different local habitat types has been reported for several species, although population differentiation was not always very strong (Waser & Price 1985; Van Tienderen 1992; Kindell *et al.* 1996; Hangelbroek *et al.* 2003; Knight & Miller 2004; Lenssen *et al.* 2004). The tested habitats differed in slope, elevation, humidity, flooding regime or substrate type.

To our knowledge, our study is the first in which small-scale differentiation between local populations from contrasting habitats is compared with large-scale

differentiation between different countries (600–1100 km). In all three test species, small-scale differentiation in growth and reproduction was found to be high (Fig. 3). In general, differences between the two local populations (home and 'local different') were nearly as large as between home and foreign populations. In some trait \times species combinations small-scale differentiation was even larger. Although power of the home vs. 'local different' comparisons was lower (one provenance at each site) than that of home vs. foreign (two provenances at each site), the proportion of traits showing significant linear contrasts was similar. For *P. lanceolata*, the small-scale differentiation was also found in fitness and selection coefficients, thus indicating strong selection against local populations from a contrasting habitat (Table 5). Selection coefficients of *H. lanatus* were similar at the small scale because lower reproduction of the 'local different' population was compensated for by higher seedling emergence and survival. For *L. corniculatus* considerable small-scale differentiation was found but the direction of contrasts was inconsistent. At two sites the selection coefficient of the 'local different' population was the lowest, much lower than in the home and foreign populations whereas at the third site it was the highest.

Our finding that population differentiation and local adaptation on small geographical scales can be as strong as on large scales supports that of Montalvo & Ellstrand (2000) who only found a weak correlation of fitness to geographical distance but a much stronger one to environmental distance. Studies comparing populations within and among regions often reveal larger within-region differences confirming the importance of small-scale population differentiation (Santamaria *et al.* 2003; Berg *et al.* 2005).

Conclusions

Our study demonstrates the importance of competition with the local plant community in shaping local adaptation. The home vs. foreign contrasts were affected by the competition treatment, indicating that local adaptation cannot necessarily be predicted from plants grown without competition. Local adaptation may be underestimated in such situations because surrounding vegetation might be an important agent of selection (Turkington & Harper 1979; Prati & Schmid 2000; Ehlers & Thompson 2004; Lortie *et al.* 2004) but it may also be overestimated due to factors reducing home site advantages in a competitive environment (e.g. smaller allelopathic effects of the home population on conspecific neighbours, Callaway *et al.* 2005). We found the small-scale differentiation between different habitats to be great and in some traits even greater than large-scale differentiation between different countries. Thus, the view that local genotypes should be preferred in habitat creation or restoration has to be modified. Populations that are further away but from similar habitats may be better adapted than neighbouring populations from a

contrasting habitat. Environmental distances (Montalvo & Ellstrand 2000) are therefore more appropriate for selecting suitable provenances than only geographical distances.

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