

The Importance of Plant Provenance and Genotypic Diversity of Seed Material Used for Ecological Restoration

Armin Bischoff,^{1,2,3} Thomas Steinger,⁴ and Heinz Müller-Schärer¹

Abstract

The increased translocation of plant species for biodiversity restoration and habitat creation has provoked a debate on provenance and genotypic diversity of the used plant material. Nonlocal provenances are often not adapted to the local environmental conditions, and low population genotypic diversity may result in genetic bottlenecks hampering successful establishment. We tested provenance differentiation of four plant species used in agri-environment schemes to increase biodiversity of agricultural landscapes (wildflower strips). Provenances were collected close to the experimental field and at four further sites of different distances ranging from 120 to 900 km. In two of these provenances, different levels of genotypic diversity were simulated by sowing seed from a high and low number of mother plants. We found a large provenance differentiation in fitness-related traits, particularly in seedling emergence. There was no evidence for a general superiority of

the local population. The productivity was greater in populations of high genotypic diversity than in those of low diversity, but the effect was only significant in one species. Productivity was also more constant among populations of high diversity, reducing the risk of establishment failure. Our results indicate that the choice of an appropriate provenance and a sufficient genotypic diversity are important issues in ecological restoration. The use of local provenances does not always guarantee the best performance, but a spread of superior alien genotypes can be avoided. A sufficient genotypic diversity of the sown plants might be a biological insurance against fluctuations in ecosystem processes increasing the reliability of restoration measures.

Key words: competition, diversity–productivity relation, genetic diversity, population differentiation, seed origin, wildflower strips.

Introduction

Human impact has reduced species diversity in many ecosystems as a result of habitat damage or even destruction. During recent years, interest has increased to restore biodiversity and recreate habitats. Simply stopping negative impact and recreating appropriate environmental conditions are often not sufficient to reestablish species-rich communities of native plants because nearby populations that are required as propagule sources are not available any more (Bischoff 2002; Walker et al. 2004). Thus, sowing or planting of desired species has been increasingly used to improve restoration success. Fields of application range from reintroduction of rare species to sowing specifically designed seed mixtures for habitat creation (Montalvo et al. 1997; Bullock et al. 2001; Bischoff et al.

2006b). However, the required translocation of plant material has provoked a discussion on provenance and diversity of the introduced populations (Hamilton 2001; Wilkinson 2001; Hufford & Mazer 2003). Recent studies have shown that the approach has potential risks that may counteract the desired effects if nonlocal provenances are used and genotypic diversity of the source populations is too low (Keller et al. 2000; Williams 2001; McKay et al. 2005).

The risks of introducing nonlocal provenances include poor establishment of sown provenances owing to poor adaptation to the prevailing environmental conditions (Hamilton 2001; Hufford & Mazer 2003), superior alien genotypes invading and displacing local populations (Saltonstall 2002), and hybridization between introduced and local populations that may result in outbreeding depression, that is, a reduction of hybrid fitness relative to their parents (Fenster & Galloway 2000; Keller et al. 2000; Montalvo & Ellstrand 2001). Theory on local adaptation predicts that local genotypes should perform better than distant provenances when grown at their home site. Such an adaptive genetic differentiation is well documented in plant populations (Bradshaw 1984; Linhart & Grant 1996; Galloway & Fenster 2000; Etterson 2004). Generally, adaptation to a specific site is expected to decrease with increasing spatial distance from the source population due

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

² Address correspondence to A. Bischoff, email armin.bischoff@agrocampus-ouest.fr

³ Present address: Department of Biological Sciences, National Institute of Horticulture and Landscape Planning (INHP), Agrocampus West, Center of Angers, 2, rue André Le Nôtre, F-49000, Angers, France

⁴ Station de recherche Agroscope Changins-Wädenswil ACW, Case postal 1012, CH-1260 Nyon, Switzerland

to a concomitant increase in environmental distance and genetic isolation (Keller & Kollmann 1999; Galloway & Fenster 2000; Joshi et al. 2001). However, genetic differentiation has also been shown on very small scales or even within populations (Waser & Price 1985; Knight & Miller 2004; Lenssen et al. 2004; Bischoff et al. 2006a), reducing the correlation between fitness and spatial distance of a provenance.

In the present study, we analyzed effects of seed provenance in four plant species of an established wildflower seed mixture used to create wildflower strips in agricultural landscapes. Such wildflower strips have been implemented in Switzerland as ecological compensation areas to restore species richness and cover currently 2,400 ha on 2,600 different farms (Swiss Federal Office for Agriculture 2005). We tested five provenances of the four species in a field experiment, that is, one local provenance and four provenances collected at different geographical and environmental distances to the field site. Our aim was to analyze whether there is significant population differentiation between provenances and whether performance is negatively correlated with distance to the collection sites. Environmental maternal effects may contribute to population differentiation in plants (Roach & Wulff 1987; Donohue & Schmitt 1998). In a previous study on the same five populations of the test species, seed weight contributed little to among-provenance variation in germination and seedling performance, suggesting that maternal effects were relatively small (Bischoff et al. 2006b). To further check this assumption, we additionally grew F1 seeds of one test species produced under homogeneous conditions and compared the provenance ranking in the F1 generation with that of the parental generation.

Intraspecific diversity of the introduced plant material is a second issue that may be important for ecological restoration, but few empirical studies have addressed this issue. If seeds are collected from a limited number of sources, the level of genetic diversity is low and genetic bottlenecks may occur in the restored populations (Hufford & Mazer 2003). Procaccini and Piazzi (2001) observed that genotypic diversity of the founders may have stronger effects on the fitness of a population than the plant provenance. Recent studies have shown that intraspecific diversity, like species diversity, may be positively correlated with productivity and ecosystem functioning (Reusch et al. 2005; Crutsinger et al. 2006; Johnson et al. 2006). Similar to the species diversity–productivity relationship, a niche complementarity of different genotypes may be responsible for this correlation but also a “sampling effect” has been discussed as potential mechanism (Huston 1997; Hector et al. 1999; Crutsinger et al. 2006). The sampling effect predicts that a greater diversity increases the chance of containing a highly productive genotype. A “portfolio effect” (Tilman 1999) as a biological insurance has been proposed to explain the faster recovery of high-diversity stands of the sea grass *Zostera marina* after disturbance by herbivores (Hughes & Stachowicz 2004) and climatic

extremes (Reusch et al. 2005). Due to differences among genotypes in their resistance to herbivory and abiotic stress, the chance that populations comprise appropriate genotypes increases with genotypic diversity. Hence, variation in fitness among diverse populations is supposed to be lower than in nondiverse populations, and the risk of failure in establishment of single population may be smaller. In our study, we tested for effects of genotypic diversity on mean population productivity and among population variability in two plant species. We manipulated diversity levels by varying the number of seed families sown into experimental plots.

We tested both provenance and diversity effects in plants growing with and without surrounding vegetation in order to examine whether they depend on the competition regime. Most earlier studies have been conducted under noncompetitive conditions, assuming that the fitness ranking of the populations remains the same under natural competitive conditions (Bennington & McGraw 1995; Keller & Kollmann 1999; Joshi et al. 2001). However, such a correlation may be weak or absent, and other experiments have illustrated the importance of the surrounding vegetation as a potential driver of local adaptation (Prati & Schmid 2000; Ehlers & Thompson 2004; Bischoff et al. 2006a).

We addressed the following specific research questions:

- (1) How strong is population differentiation among five different European provenances in each of four species and do the local provenances perform the best?
- (2) What is the effect of genotypic diversity on mean population productivity and among population variability?

Methods

Study Species, Provenances, and Sampling

We selected four species of a standard seed mixture used to establish wildflower strips as ecological compensation areas in Swiss agricultural landscapes. They represent different stages of succession from early to late: the annual *Legousia speculum-veneris* (L.) Chaix (Primulaceae), the biennial *Echium vulgare* L. (Boraginaceae), the short-lived biennial or perennial *Cichorium intybus* L. (Asteraceae), and the late-successional perennial *Origanum vulgare* L. (Lamiaceae). No further taxonomic differentiation is known for central and western Europe except for the crop varieties *C. intybus* ssp. *sativus* and ssp. *foliosus*, which were excluded from the experiment (Tutin et al. 1976). All four species are predominantly outcrossing, although in *L. speculum-veneris*, *E. vulgare*, and *C. intybus*, selfing is also possible. *Legousia speculum-veneris* is endangered in many parts of Europe, and the other species are widespread.

We sampled one local and four nonlocal provenances across climatic gradient (Table 1). The local western Swiss (West CH) and the eastern Swiss (East CH) provenance are characterized by a Subatlantic climate with high

Table 1. Sampling scheme and characteristic of collection sites.

	Collection Site	Source	Distance (km)	Climate, Humidity	Diversity Experiment
West CH	Fribourg state	Wild	Local (<10)	Suboceanic, moist	Yes
East CH	Winterthur state	Stock	120–200	Suboceanic, moist	No
South D	South Hesse, North Baden	Stock	300–450	Suboceanic, medium	No
Central D	South Sachsen-Anhalt	Wild	600–650	Subcontinental, dry	Yes
West EUR	Sommerset ^a , Norfolk ^b , Bordeaux ^c	Stock	650–900	Oceanic, medium	No

Only the provenances collected from the wild (West CH and Central D) were used for the diversity experiment.

^a *Echium vulgare*.

^b *Origanum vulgare*.

^c *Cichorium intybus*.

rainfall (1,000–1,250 mm per annum). The southern German provenance (South D) originates from a Subatlantic climate with average rainfall (650–850 mm per annum) and the Central German one (Central D) from a subcontinental climate with low rainfall (450–500 mm per annum). A typical Atlantic climate with mild winters is represented by three subprovenances from western Europe (West EUR), one from western France (*C. intybus*), and two from the United Kingdom (*E. vulgare* and *O. vulgare*). *Legousia speculum-veneris* seeds were not available from central Germany and western Europe because the species has become extremely rare at the edge of the range.

Seeds of the local West CH and the Central D provenance were collected from the wild in populations of at least 150 individuals. The local provenance was sampled within a 10 km radius of the experimental site. To ensure a genotypic diversity representative of a population, seeds from 40 haphazardly chosen mother plants, separated by at least 5 m, were harvested. The seeds of each mother plant were kept separately and stored dry at room temperature until sowing in the following spring. Seeds of the remaining provenances were collected from populations in stock provided by botanical gardens or by companies specialized in wild seed production for conservation purposes. Only seed suppliers were chosen who could give information on the original collection site and who regularly replace stocks by wild collections. The suppliers guaranteed that seeds were not selected for size and that they were stored at room temperature for not longer than 2 years.

Testing for Provenance Effects

In April 2002, we sowed the seeds into $2 \times 2\text{-m}^2$ plots of a previously plowed and harrowed arable field 10 km north of Fribourg in western Swiss midlands. Each plot comprised seeds of one provenance \times species combination sown in a central grid of 24 cells ($0.2 \times 0.2\text{ m}^2$) with six seeds per grid cell. A higher number of 10 seeds per grid cell was chosen in *O. vulgare* because seeds are very small and are known to show a high mortality in the soil (Pons 1991). In the competition treatment, half of all plots were additionally sown with the usual wildflower strip

seed mixture that comprises 24 species in a total density of 600 seeds/m². In the other half of the plots (no competition), the space between the test plants was regularly weeded. For each species, the 10 different treatment combinations (5 provenances [*L. speculum-veneris*: 3] \times 2 competition) were arranged randomly within eight replicate blocks resulting in a total of 288 plots.

In order to test for maternal effects, seeds of a second generation of all *L. speculum-veneris* provenances were produced by plants grown under homogeneous conditions in the botanical garden of the University of Fribourg. We used nylon mesh cages to avoid cross-pollination between the provenances. Cages of the same provenance were opened for periods of 5 days to allow within-provenance pollination. The F1 seeds were sown 1 year later, in 2003, following the described procedure except that 10 instead of 6 seeds were placed into each grid cell.

Testing for Genotypic Diversity

A high- and a low-diversity treatment were established for provenances collected from the wild (West CH and Central D). The plots were divided into two subplots with 12 grid cells each. In the high-diversity subplot, each grid cell received seeds of a different mother plant, resulting in 12 seed families per subplot. We randomly selected seed families from the pool of 40 sampled mother plants per population, that is, each seed family occurred on average in 2.4 subplots. In the low-diversity subplot, two seed families were selected from the same pool, and seeds of each family were randomly assigned to six grid cells per subplot. Selection of the two seed families per subplot was random but contrary to the high-diversity treatment; multiple selection of the same family was prevented to achieve a maximum representation of the entire family pool, that is, altogether 16 families (8 subplots \times 2 families). The aim was to avoid a confounding of treatment and family (genotype identity) effects. Although not confirmed by genetic marker analysis, we assume that genetic diversity is a function of family number. Offspring from the same mother plants have a larger genetic similarity than those from different plants because they share at least one

parent (half-sib family). Because family sets are random subsamples of the same population and only the first generation of plants is tested, there are no inbreeding effects, and the average level of heterozygosity is expected to be the same in the high- and low-diversity treatment. A competition treatment was applied in the same way as in the test of seed provenance.

Measurements

We recorded seedling emergence 5 and 10 weeks after sowing in late May. Afterward, the number of seedlings was reduced to one per grid cell, that is, ideally 24 per plot. We kept plants of average size and removed later emerging seedlings. Moderate within-treatment transplanting from cells with spare seedlings was used to reduce the number of empty cells and to keep the number of plants constant among plots and treatments. Transplants were only considered for data analysis if they survived for at least 8 weeks until late summer.

We analyzed survival in all test plants and growth and reproduction in six randomly selected focal plants per plot. Plants were measured twice in 2002 and thrice in 2003 and 2004 (except for *L. speculum-veneris*). We regularly recorded the number of leaves and the length of the longest leaf, in *O. vulgare* additionally the number of basal shoots and the length of the longest shoot. In the other species, shoot length, shoot number (*E. vulgare* and *C. intybus*), and number of inflorescences (*L. speculum-veneris*) were measured after bolting. At the end of the flowering period, we recorded the number of capsules (*L. speculum-veneris*), cymes (*E. vulgare*), flower heads (*C. intybus*), and fertile shoots (*O. vulgare*). Seed production was estimated by counting the number of seeds in a subsample of these reproductive units. In the monocarpic species *L. speculum-veneris* and *E. vulgare*, aboveground parts of all focal plants were harvested after reproduction in 2002 and 2003, in *O. vulgare* at the end of the experiment in 2004. The plants were dried at 80°C for 48 hours and weighed. Due to potential differences in the life cycle of *C. intybus* populations (biennial vs. perennial), we decided to observe the plants until the end of the experiment instead of harvesting them after flowering in 2003.

Data Analysis

As an estimate of fitness combining several fitness-related traits, the dominant eigenvalues (λ , finite rate of population growth) of Leslie matrices were calculated based on seedling emergence, fecundity (seed production), and survival at plot level (Charlesworth 1994; Bischoff et al. 2006a). The calculation of λ was preferred over total reproduction because λ accounts for the higher contribution of first-year fecundity to population growth (McGraw & Caswell 1996). The calculated λ does not reflect the real growth rate because seeds were sown to bare ground and burial of seeds in the soil was much shorter than in nature,

reducing the risk of seed losses due to predation or harsh winter conditions. Thus, we will refer to the matrix eigenvalue as “fitness coefficient.” The matrix model could not be applied to the annual species *L. speculum-veneris*. Instead, the fitness coefficient was calculated as the product of seedling emergence, survival, and fecundity.

In the test of seed provenance, an analysis of variance (ANOVA) model was applied using provenance, competition, the provenance \times competition interaction, and block as fixed factors and block means ($n = 8$) as response variables. Tukey honestly significant difference (HSD) post hoc test was calculated for multiple comparisons of the means. All analyses were applied separately for each species because the full model revealed a significant species \times provenance interaction. Traits measured repeatedly on the same plants (vegetative growth) were analyzed using repeated measures ANOVA. Seedling emergence and survival were arcsine square root transformed, and growth traits, seed production, and fitness coefficients were log ($x + 1$) or square root transformed if necessary to meet the assumptions of ANOVA.

In the test of genotypic diversity, a hierarchical mixed-model ANOVA was applied to a subset of three species (*E. vulgare*, *C. intybus*, and *O. vulgare*) and the two provenances (local West CH and Central D) that were directly collected from the wild. The model included diversity, provenance, competition, and block as fixed factors and plot nested in provenance, competition, and block as random factors. For calculating F values, provenance, competition, the provenance \times competition interaction, and block were tested against plot, whereas diversity and all interactions with diversity were tested against the residuals. Coefficients of variation were calculated for plot productivity (vegetative growth and seed production) to analyze the effect of genotypic diversity on variability among populations of the different plots.

Results

Provenance Effects

In all four study species, significant differences in fitness coefficients were observed between provenances (Table 2). This was mainly due to differences in seedling emergence that were highly significant in all species. The provenance effect on survival was significant in *Echium vulgare*, *Cichorium intybus*, and *Origanum vulgare* but not in *Legousia speculum-veneris*. Seed production as the third variable included in calculation of fitness coefficients was only significant in *C. intybus*. Strong differentiation in vegetative traits was found for *O. vulgare* and *C. intybus*. In both species, leaf number and leaf and shoot length were significantly different but not the number of basal shoots. Significant differences in final biomass of *O. vulgare* did not result in significant differences in final seed number, indicating that provenance effects on reproduction are not necessarily

Table 2. *F* values and level of significance of the effects of provenance and competition on fitness-related traits; values resulting from repeated measures analysis are in italics if interaction with time is significant (*T*: number of measurements).

	Provenance (<i>P</i>), <i>df</i> 4(2)	Competition (<i>C</i>), <i>df</i> 1	<i>P</i> × <i>C</i> , <i>df</i> 4(2)	Block, <i>df</i> 7
<i>Legousia speculum-veneris</i>				
Fitness coefficient	16.11***	45.52***	2.87 ^(*)	4.09**
Seedling emergence	62.61***	0.19	1.44	3.17*
Final survival	1.27	0.25	2.71 ^(*)	3.64**
Capsule number	0.79	65.29***	1.37	4.14**
Leaf number	11.02***	0.96	1.85	3.40**
Inflorescence number	0.54	37.11***	1.67	4.12**
Shoot length	3.25 ^(*)	2.63	2.43	5.10***
Final biomass	0.14	43.68***	0.42	2.13 ^(*)
<i>Echium vulgare</i>				
Fitness coefficient	12.82***	26.27***	1.66	0.75
Seedling emergence	69.55***	23.22**	1.73	9.26***
Final survival	3.46*	24.02***	1.13	2.18*
Seed number	0.69	7.31**	0.75	2.11 ^(*)
Basal shoot number	1.40	15.41***	0.83	1.88 ^(*)
Shoot length	2.48 ^(*)	0.25	2.09 ^(*)	4.41***
Final biomass	0.92	11.40**	0.09	1.93 ^(*)
Leaf number (<i>T</i> = 3)	2.62*	19.02***	0.22	2.41*
Leaf length (<i>T</i> = 3)	2.91*	3.94 ^(*)	1.10	5.81***
<i>Cichorium intybus</i>				
Fitness coefficient	2.49 ^(*)	59.36***	0.49	4.90***
Seedling emergence	24.12***	6.06*	1.98	6.70***
Final survival	5.32***	4.53*	2.11 ^(*)	4.23***
Final seed number	3.89**	46.26***	0.87	3.10**
Basal shoot number	0.64	74.85***	1.72	0.84
Shoot length	29.16***	5.83*	1.41	3.04**
Leaf number (<i>T</i> = 3)	4.36**	131.15***	1.46	1.75 ^(*)
Leaf length (<i>T</i> = 3)	4.75**	17.92***	0.18	4.02**
<i>Origanum vulgare</i>				
Fitness coefficient	29.36***	136.97***	0.68	2.17*
Seedling emergence	19.16***	2.43	0.21	1.86 ^(*)
Final survival	2.67*	6.15*	0.16	0.35
Final seed number	1.93	127.79***	1.57	2.77*
Final biomass	3.13*	190.51***	0.62	4.56***
Leaf number (<i>T</i> = 7)	4.03**	30.16***	1.41	2.66***
Leaf length (<i>T</i> = 7)	8.83***	26.07***	2.78*	6.06***
Basal shoots (<i>T</i> = 8)	0.62	139.37***	0.65	0.71
Shoot length (<i>T</i> = 8)	7.58***	67.98***	1.87	8.91***

^(*)*p* < 0.1; **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

related to provenance effects on vegetative growth. For the two other species, *L. speculum-veneris* and *E. vulgare*, provenance differentiation was only significant for leaf traits but not for shoot length, shoot number, or biomass. The repeated measures analysis revealed significant provenance × time interactions for leaf length of *E. vulgare* and leaf number and shoot length of *O. vulgare*, demonstrating that magnitude of provenance differentiation and/or provenance ranking may depend on the time of measurement.

The most outstanding difference between provenances was found for *C. intybus*. The local plants (West CH) and plants from East CH and West EUR showed a biennial life cycle and died after flowering in the second year. In the South D and East D provenance, plants were perennial, and new shoots emerged in spring of the third year from the basis of the old flowering stems. Results presented in Table 2 and Figure 1 include only measurements

from the first 2 years because only second-generation seedlings remained in the biennial plants, and these seedlings most likely include interprovenance hybrids.

The ranking of provenances in fitness coefficients differed among species (Fig. 1), and there was no evidence for a general superiority of the local provenance from western Switzerland. The local plants of *E. vulgare* performed better than foreign provenances, but the difference was only significant in comparison with the West EUR provenance from the United Kingdom (*p* < 0.001, Tukey HSD). In *L. speculum-veneris* and *O. vulgare*, the local plants showed a lower performance than plants from East CH, South D, and Central D (*p* < 0.05, Tukey HSD). Plants from the most distant West EUR provenance had a significantly lower fitness than all other provenances in two out of three species (*E. vulgare*: *p* < 0.001; *O. vulgare*: *p* < 0.05, Tukey HSD).

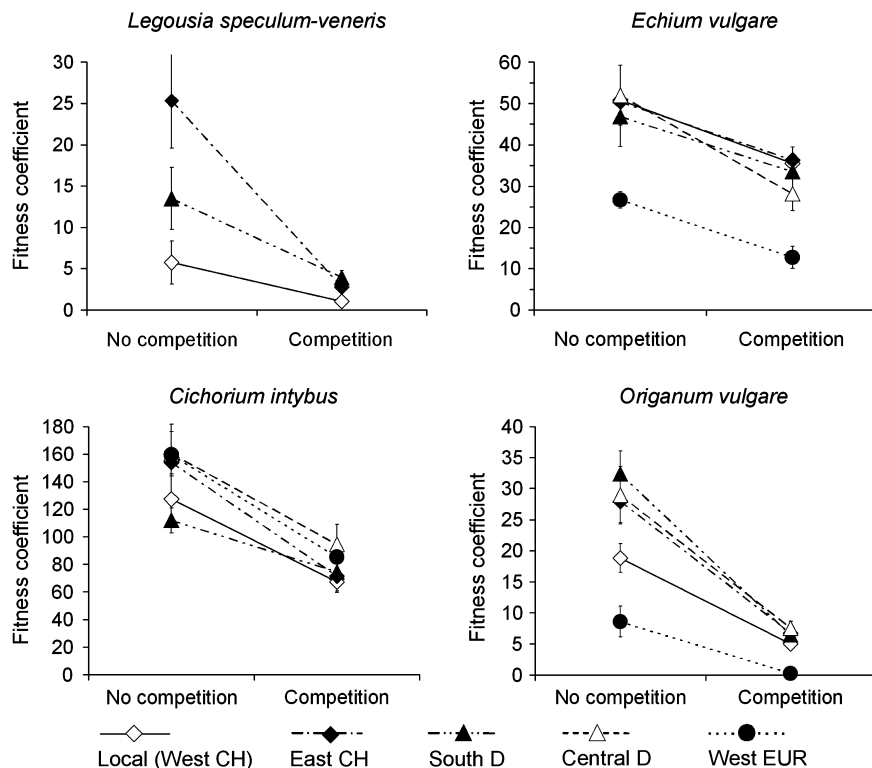


Figure 1. Performance of four provenances and species grown with addition of a seed mixture (competition) and in weeded monocultures (no competition); fitness coefficient = λ of Leslie matrices based on seedling emergence, survival, and seed production; \pm SE.

The competition treatment had a highly significant effect on survival (except for *L. speculum-veneris*) growth, reproduction, and resulting fitness coefficient, whereas seedling emergence was only affected in *E. vulgare* and *Ci. intybus* (Table 2). The interaction provenance \times competition was not significant for most measured traits indicating a high stability of provenance differentiation in the two competitive environments.

In a test for environmental maternal effects, second-generation plants of *L. speculum-veneris* from mothers grown under homogeneous conditions showed the same provenance ranking in fitness (pooled for competition) as first-generation plants from the original seed material, that is, West CH < South D < East CH. However, the provenance effect was only marginally significant in second-generation plants ($F = 2.70$, $p = 0.081$) and a significant generation \times provenance interaction occurred ($F = 9.63$, $p < 0.001$). The weakening of provenance differentiation in second-generation plants could be interpreted as a small contribution of maternal effects to provenance differentiation of first-generation plants.

Effects of Genotypic Diversity

In *O. vulgare*, the diversity effect on plot productivity was significant (Table 3). The cumulative height and the seed

number of all focal plants within one subplot were significantly higher in subplots of high genotypic diversity than in subplots of low genotypic diversity (Fig. 2). In *C. intybus*, a trend of higher plot productivity, in *E. vulgare* a trend of lower plot productivity, was found in high-diversity subplots compared with low-diversity subplots, but differences were not significant (Fig. 2). The interaction diversity \times competition was not significant (Table 3), indicating that diversity effects on plot productivity are relatively stable over the competition treatments. Therefore, only data pooled for competition are presented in Figure 2. The diversity \times provenance and the diversity \times competition \times provenance interaction were also not significant. However, data were not pooled for provenance in Figure 2 in order to compare the magnitude of diversity and provenance effects. In *E. vulgare*, the magnitude of the diversity effect was larger than that of provenance; in *C. intybus*, it was lower; and in *O. vulgare*, a similar magnitude was observed.

The coefficient of variation as a measure of among-plot variability was lower among high-diversity subplots than among low-diversity subplots (Fig. 3). The difference between high and low diversity was larger in the West CH than in the Central D provenance. When not corrected for mean values (variance and SE), no differences in among-subplot variation occurred between high and low diversity.

Table 3. Results of an ANOVA model testing the effect of genotypic diversity, provenance, and competition on plot productivity; *F* values and level of significance separately for cumulative height and seed production; block and plot were also fitted but not presented in the table (*df* = 1 for all factors and interactions).

	Diversity (<i>D</i>)	Provenance (<i>P</i>)	Competition (<i>C</i>)	<i>D</i> × <i>P</i>	<i>D</i> × <i>C</i>	<i>P</i> × <i>C</i>	<i>D</i> × <i>P</i> × <i>C</i>
<i>Echium vulgare</i>							
Cumulative height ^a (<i>F</i> _[1,28])	2.12	0.10	22.73***	0.53	1.68	2.96	1.23
Seeds (<i>F</i> _[1,28])	1.11	0.21	7.58*	0.06	2.43	0.69	0.29
<i>Cichorium intybus</i>							
Cumulative height ^a (<i>F</i> _[1,28])	0.01	36.64***	76.46***	0.99	0.25	0.67	0.30
Seeds (<i>F</i> _[1,28])	1.56	19.94***	56.18***	0.71	0.55	0.02	0.24
<i>Origanum vulgare</i>							
Cumulative height ^a (<i>F</i> _[1,28])	9.34**	22.80***	71.24***	3.77	1.03	1.99	0.53
Seeds (<i>F</i> _[1,28])	7.43**	10.47**	47.70***	1.24	0.58	0.46	0.08

^a Cumulative height = height of the longest shoot × number of basal shoots per plot.

* *p* < 0.05; ** *p* < 0.01; *** *p* < 0.001.

Discussion

Importance of Seed Provenance

We found a strong provenance differentiation in individual traits and fitness coefficients of all four test species. A similar provenance differentiation at a comparable geographic scale has been found in studies on perennial species (Joshi et al. 2001; Santamaria et al. 2003; Bischoff et al. 2006a). A smaller differentiation was observed in an early-successional species of disturbed habitats that experience frequent population turnover (Galloway & Fenster 2000). Our study species represent different stages of succession ranging from early (*Legousia speculum-veneris*) to late (*Origanum vulgare*) successional. However, even the annual *L. speculum-veneris* does not experience frequent turnover because the arable species survives regular disturbance in the soil seed bank (Schneider et al. 1994).

In all species, provenance differentiation was found to be much stronger in seedling emergence than in later stages of the plant development, indicating that particularly short- and medium-term restoration sites such as wildflower strips may be largely dependent on seed provenance. In *Cichorium intybus*, the life cycle was different being biennial in the two Swiss and the western France provenances and perennial in the two German provenances. Such an extreme within-taxon variation in life cycles has been found in several other species of the Asteraceae family (Huiskes et al. 2000 for *Aster tripolium*; Müller-Schärer et al. 2004 for *Centaurea maculosa*).

Population differentiation in plants is the result of various evolutionary processes such as differential selection, genetic drift, and different levels of inbreeding (Linhart & Grant 1996; Galloway & Fenster 2000). If differential selection is strong enough to overcome the homogenizing effect of gene flow, adaptation to local site conditions may occur (Linhart & Grant 1996). Such an adaptive genetic differentiation among plant populations was found in many studies (Bradshaw 1984; Linhart & Grant 1996; Joshi et al. 2001; Santamaria et al. 2003; Etterson 2004), and an overall superiority of local compared with alien provenances can be

expected. In our study, however, we did not find much evidence for a superiority of the local provenance. Only in *Echium vulgare*, the local provenance performed the best, and the difference was significant compared with the most distant provenance from the United Kingdom. In the three other species, the fitness coefficient of the local provenance was lower than that of most foreign provenances.

There are several reasons that may explain the lack of local adaptation. For example, methodological constraints can prevent the detection of a home site advantage. First, drivers of adaptation may be infrequent but characteristic selective events, such as extreme frost or drought (Montalvo et al. 1997). To account for such events, experiments would have to be run for several decades, which is usually not feasible. Second, in restoration approaches, the target sites are never identical with the collection sites. Small differences in local site conditions such as substrate type, soil humidity, and history of land use cannot be avoided even if seeds are collected in the neighborhood. Several studies have shown a small-scale adaptation to such local habitat differentiation (Waser & Price 1985; Knight & Miller 2004; Lenssen et al. 2004; Bischoff et al. 2006a), which may reduce the correlation between population differentiation and geographical distance. Third, environmental maternal effects might have contributed to the observed provenance differentiation. In particular, differences in germination can be inflated by heterogeneous environmental conditions among sites at which seeds were collected (Roach & Wulff 1987; Donohue & Schmitt 1998). In one species, *L. speculum-veneris*, we further compared the performance of second-generation plants from mothers grown under homogeneous conditions with that of plants grown from the original seed material. There was some weakening of provenance differentiation, but the ranking was the same and differences remained significant, confirming that the contribution of environmental maternal effects was probably small. In an earlier study on the same populations, controlling for maternal effects by using seed mass as covariate in the analysis did not decrease among-provenance differences in germination,

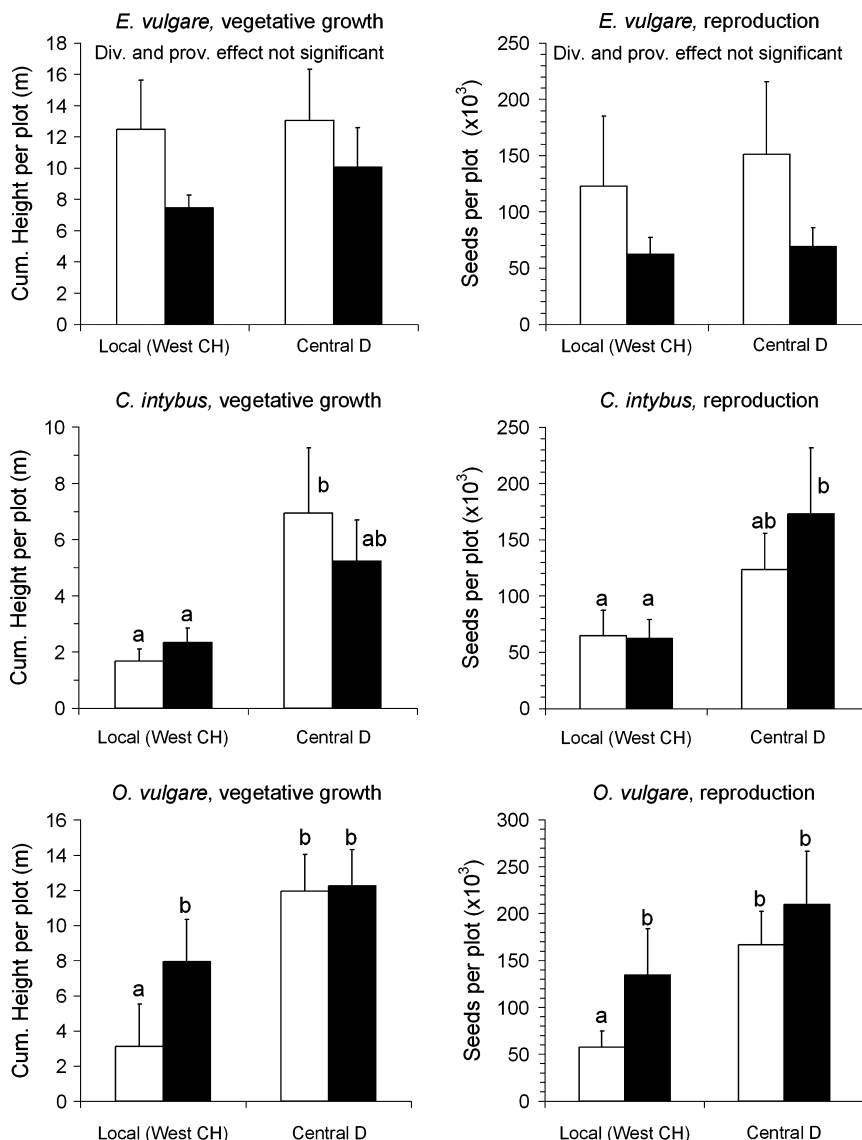


Figure 2. The effect of genotypic diversity on vegetative growth and reproduction in a subset of two provenances; open bars: low diversity (seeds from 2 mother plants per plot) and filled bars: high diversity (seeds from 12 mother plants per plot); data pooled for competition treatment; different letters indicate significant differences of Tukey HSD post hoc test applied separately for competition treatments; \pm SE.

indicating that population differentiation is largely genetically determined (Bischoff et al. 2006b).

Besides methodological constraints preventing a detection of the superiority of local provenances, maladaptation may also be real (Crespi 2000). A superiority of nonlocal genotypes at particular sites has repeatedly been observed in reciprocal transplant experiments (Galloway & Fenster 2000; Leiss & Müller-Schärer 2001; Santamaria et al. 2003). Environmental changes like global warming or conversion of management might have resulted in a disruption of local adaptation. Our test species predominantly occur in anthropogenic or seminatural habitats where management has largely changed in recent decades (e.g., Joyce & Wade 1998 for grasslands), and it is possible that they are no lon-

ger adapted to the current environmental conditions. Evidence for a real superiority of nonlocal provenances came from a study on North American *Phragmites australis* populations. A foreign genotype has invaded many natural sites and completely displaced the local genotypes of this species (Saltonstall 2002).

The provenance differentiation was not much affected by the competition treatment, and provenance ranking was largely stable in both environments. A relatively better performance of the local compared with alien provenances could be expected in the competition treatment if the competitive environment mimics more closely the local conditions. For example, resource availability and microclimate depend on the competitive environment. In

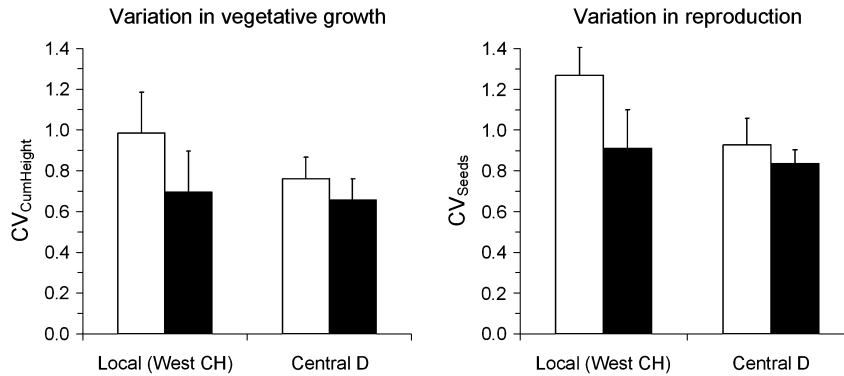


Figure 3. The effect of genotypic diversity on among-plot variation (coefficient of variation) in vegetative growth and reproduction; open bars: low diversity (2 mother plants per plot) and filled bars: high diversity (12 mother plants per plot); data pooled for species and competition; \pm SE.

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). However, the effects are not always strong, and a home site advantage may also be less pronounced in competition to a plant community than in weeded monocultures (Kindell et al. 1996; Bischoff et al. 2006a).

Importance of Genotypic Diversity

We found large differences in productivity between stands of high and low genotypic diversity. The magnitude of difference was as high as between the two tested provenances, but due to the high scatter of data, the diversity effect was only significant in one species, *O. vulgare*. In this species, seed production and biomass per plot were significantly higher in the high-diversity treatment. A negative effect of low genotypic diversity on average performance of a population has been observed in many studies, but in most cases, it has been explained by genotypic drift or inbreeding, resulting in a low degree of heterozygosity (Procaccini & Piazzini 2001; Williams 2001; Vergeer et al. 2003). In our study, we assumed that the level of heterozygosity was the same because we sampled the same populations to establish a high- and low-diversity treatment, and our experiment was restricted to first-generation offspring of parental seed. Thus, inbreeding depression and drift effects were excluded, and the treatments only differed in the number of seed families. Such an approach to test the effect of genotypic diversity on plant performance has rarely been used so far. In two recent studies testing three and four diversity levels, a greater genotypic diversity led to higher productivity (Crutsinger et al. 2006; Johnson et al. 2006). The authors found that qualitative and quantitative effects of genotypic diversity can be similar to those of plant species diversity.

In analogy to the effects of species diversity on ecosystem functioning, there might be two mechanisms through which genotypic diversity within species can affect productivity or resistance to disturbance. First, positive interactions

between different genotypes may increase productivity. Niche complementarity of different species was found to improve the utilization of resources, resulting in overyielding (Hector et al. 1999; Tilman 1999). The productivity of multigenotype mixture would then be higher than the average productivity of monocultures of those genotypes. There may also be positive mutualistic interactions between species, and the probability of such interaction increases with diversity (Hector et al. 1999). However, in our study, interactions between individuals of different genotypes can only be assumed in the weeded monocultures where intraspecific competition became apparent in the second growing season. In competition to the usual wildflower strip mixture (competition treatment), interspecific competition was dominant, and interactions between genotypes of our test species can be neglected. Because high genotypic diversity increased productivity also in the competition treatment, the diversity effect was unlikely the result of positive interactions between different genotypes.

Second, diversity effects can also be explained without assuming competitive or mutualistic interactions between different genotypes. Noninteractive or “additive” effects of species diversity on productivity have sometimes been subdivided into two similar mechanisms, the sampling effect and the portfolio effect. The sampling effect predicts that a greater genotypic or species diversity increases the chance of containing a highly productive species or genotype (Huston 1997; Hector et al. 1999). The portfolio effect takes into account that plant populations and communities face fluctuations in environmental conditions (Tilman 1999). A higher diversity increases the chance that under different conditions including disturbances appropriate species or genotypes are available. So, species diversity can have a buffering and stabilizing effect on the productivity of plant communities (Loreau 2000). A similar effect of genotypic diversity within a population was observed in a study on the sea grass *Zostera marina* (Hughes & Stachowicz 2004). A higher productivity as a result of greater diversity was only found after disturbance by grazing geese, indicating that this was not only a sampling effect. A positive effect of

genotypic diversity on population recovery of *Z. marina* was also observed after climatic extremes (Reusch et al. 2005). In our study, the greater productivity in high-diversity subplots compared with low-diversity ones was probably the result of such a sampling or portfolio effect. Considerable fluctuations in environmental conditions occurred during the study period including a wet summer in 2002 and a dry and hot one in 2003.

Some evidence for a stabilizing effect of genotypic diversity derives from the comparison of among-plot variability in productivity. This variability is smaller under high than under low diversity if corrected for mean values (coefficient of variation). In restoration practice, the risk of a complete failure in establishment at a particular site increases with decreasing genotypic diversity of the founders. However, it has to be considered that a lower variability among high-diversity subplots may be partly due to its larger genotype identity compared with the low-diversity subplots. In the high-diversity treatment, an average of 20% of the founders were identical among subplots, whereas there was no overlap in the low-diversity treatment.

The approach of using the number of mother plants or seed families as an indicator of genetic diversity could be easily applied in restoration practice but it has some limitations in small and inbred populations. Inbreeding and genetic drift can reduce within-population genetic diversity (Procaccini & Piazzi 2001; Vergeer et al. 2003) and may result in small diversity differences among sets of high and low numbers of seed families. In our study, a low genetic diversity of the source populations may be an explanation for the lack of significant differences in *E. vulgare* and *C. intybus*. If within-population diversity is low, a sampling from different populations is required to increase genetic diversity.

Implications for Practice

- Seed provenance and genotypic diversity may have strong effects on the performance of populations introduced for biodiversity restoration or habitat creation.
- The use of local provenances is recommended. It does not guarantee best performance and establishment of the restored populations, but the risk of spreading undesired alien genotypes can be avoided.
- The number of sampled mother plants should be maximized to obtain a high genotypic diversity of the restored populations. If source populations are small and/or inbred, a sampling from several different populations has to be considered. A sufficient genotypic diversity of the sown plants might be a biological insurance against fluctuations in ecosystem processes and thus increases the success of restoration measures.
- Further studies are required to improve knowledge on the relationship between fitness and genetic architecture. Simplified assemblages of plants grown without competition allow a good prediction of provenance and diversity effects in restoration practice.

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LITERATURE CITED

- Bennington, C. C., and J. B. McGraw. 1995. Natural selection and ecotypic differentiation in *Impatiens pallida*. *Ecological Monographs* **65**:303–323.
- Bischoff, A. 2002. Dispersal and establishment of floodplain grassland species as limiting factors of restoration. *Biological Conservation* **104**: 25–33.
- Bischoff, A., L. Crémieux, M. Smilauerova, C. Lawson, S. Mortimer, J. Dolezal, et al. 2006a. Detecting local adaptation in widespread grassland species—the importance of scale and local plant community. *Journal of Ecology* **94**:1130–1142.
- Bischoff, A., B. Vonlanthen, T. Steinger, and H. Müller-Schärer. 2006b. Seed provenance matters—effects on germination of four plant species used for ecological restoration. *Basic and Applied Ecology* **7**: 347–359.
- Bradshaw, A. D. 1984. Ecological significance of genetic variation between populations. Pages 213–228 in R. Dirzo and J. Sarukhan, editors. *Perspectives on plant population biology*. Sinauer Associates, Sunderland, Massachusetts.
- Bullock, J. M., R. F. Pywell, M. J. W. Burke, and K. J. Walker. 2001. Restoration of biodiversity enhances agricultural production. *Ecology Letters* **4**:185–189.
- Charlesworth, B. 1994. *Evolution in age-structured populations*. University Press, Cambridge, New York.
- Crespi, B. J. 2000. The evolution of maladaptation. *Heredity* **84**:623–629.
- Crutsinger, G. M., M. D. Collins, J. A. Fordyce, Z. Gompert, C. C. Nice, and N. J. Sanders. 2006. Plant genotypic diversity predicts community structure and governs ecosystem process. *Science* **313**:966–968.
- Donohue, K., and J. Schmitt. 1998. Maternal environment effects in plants: adaptive plasticity? Pages 137–158 in T. A. Mousseau and C. W. Fox, editors. *Maternal effects as adaptations*. University Press, Oxford, United Kingdom.
- Ehlers, B. K., and J. Thompson. 2004. Do co-occurring plant species adapt to one another? The response of *Bromus erectus* to the presence of different *Thymus vulgaris* chemotypes. *Oecologia* **141**:511–518.
- 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.
- Fenster, C. B., and L. F. Galloway. 2000. Population differentiation in an annual legume: genetic architecture. *Evolution* **54**:1157–1172.
- Galloway, L. F., and C. B. Fenster. 2000. Population differentiation in an annual legume: local adaptation. *Evolution* **54**:1173–1181.
- Hamilton, N. R. S. 2001. Is local provenance important in habitat creation? A reply. *Journal of Applied Ecology* **38**:1374–1376.
- Hector, A., B. Schmid, C. Beierkuhnlein, M. C. Caldeira, M. Diemer, P. G. Dimitrakopoulos, et al. 1999. Plant diversity and productivity experiments in European grasslands. *Science* **294**:843–845.

- Hufford, K. M., and S. J. Mazer. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology and Evolution* **18**:147–155.
- Hughes, A. R., and J. J. Stachowicz. 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences of the United States of America* **101**:8998–9002.
- Huiskes, A. H. L., B. P. Koutstaal, A. Wielemaker-Van dem Dool, and M. M. Markusse. 2000. A study on polymorphism in *Aster tripolium*. *Plant Biology* **2**:547–557.
- Huston, M. A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* **110**:449–460.
- Johnson, M. T. J., M. J. Lajeunesse, and A. A. Agrawal. 2006. Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecology Letters* **9**:24–34.
- Joshi, J., B. Schmid, M. C. Caldeira, P. G. Dimitrakopoulos, J. Good, R. Harris, et al. 2001. Local adaptation enhances performance of common plant species. *Ecology Letters* **4**:536–544.
- Joyce, C. B., and M. W. Wade. 1998. European wet grasslands: biodiversity, management and restoration. John Wiley, Chichester.
- Keller, M., and J. Kollmann. 1999. Effects of seed provenance on germination of herbs for agricultural compensation sites. *Agriculture, Ecosystems and Environment* **72**:87–99.
- Keller, M., J. Kollmann, and P. J. Edwards. 2000. Genetic introgression from distant provenances reduces fitness in local weed populations. *Journal of Applied Ecology* **37**:647–659.
- 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.
- Knight, T. M., and T. E. Miller. 2004. Local adaptation within a population of *Hydrocotyle bonariensis*. *Evolutionary Ecology Research* **6**:103–113.
- 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.
- Lenssen, J. P. M., M. van Kleunen, M. Fischer, and H. de Kroon. 2004. Local adaptation of the clonal plant *Ranunculus reptans* to flooding along a small-scale gradient. *Journal of Ecology* **92**:696–706.
- 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.
- Loreau, M. 2000. Biodiversity and ecosystem functioning: recent theoretical advances. *Oikos* **91**:3–17.
- McGraw, J. B., and H. Caswell. 1996. Estimation of individual fitness from life-history data. *American Naturalist* **147**:47–64.
- McKay, J. K., C. E. Christian, S. Harrison, and K. J. Rice. 2005. How local is local?—a review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology* **13**:432–440.
- Montalvo, A. M., and N. C. Ellstrand. 2001. Nonlocal transplantation and outbreeding depression in the shrub *Lotus scoparius* (Fabaceae). *American Journal of Botany* **88**:258–269.
- Montalvo, A. M., S. L. Williams, K. J. Rice, S. L. Buchmann, C. Cory, S. N. Handel, G. P. Nabhan, R. Primack, and R. H. Robichaux. 1997. Restoration biology: a population biology perspective. *Restoration Ecology* **5**:227–290.
- Müller-Schärer, H., U. Schaffner, and T. Steinger. 2004. Evolution in invasive plants: implications for biological control. *Trends in Ecology and Evolution* **19**:417–422.
- Pons, T. L. 1991. Dormancy, germination and mortality of seeds in a chalk-grassland flora. *Journal of Ecology* **79**:765–780.
- Prati, D., and B. Schmid. 2000. Genetic differentiation of life-history traits within populations of the clonal plant *Ranunculus reptans*. *Oikos* **90**:442–456.
- Procaccini, G., and L. Piazzini. 2001. Genetic polymorphism and transplantation success in the Mediterranean seagrass *Posidonia oceanica*. *Restoration Ecology* **9**:332–338.
- Reusch, T. B. H., A. Ehlers, A. Hämmerli, and B. Worm. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences of the United States of America* **101**:2826–2831.
- Roach, D. A., and R. D. Wulff. 1987. Maternal effects in plants. *Annual Review of Ecology and Systematics* **18**:209–235.
- Saltonstall, K. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences of the United States of America* **99**:2445–2449.
- Santamaria, L., J. Figuerola, J. J. Pilon, M. Mjelde, A. J. Green, T. de Boer, R. A. King, and R. J. Gornall. 2003. Plant performance across latitude: the role of plasticity and local adaptation in an aquatic plant. *Ecology* **84**:2454–2461.
- Schneider, C., U. Sukopp, and H. Sukopp. 1994. Biologisch-ökologische Grundlagen des Schutzes gefährdeter Segetalpflanzen. *Schriftenreihe für Vegetationskunde* **26**:1–356.
- Swiss Federal Office for Agriculture. 2005. Agrarbericht 2005. RDV, Berneck.
- Tilman, D. 1999. The ecological consequences of changes in biodiversity: a search for general principles. *Ecology* **80**:1455–1474.
- Tutin, T. G., V. H. Heywood, N. A. Burges, D. H. Valentine, S. M. Walters, and D. A. Webb. 1976. *Flora Europaea*, vol. 4. University Press, Cambridge, United Kingdom.
- Vergeer, P. R., C. Rengelink, C. Copal, and N. J. Ouborg. 2003. The interacting effects of genetic variation, habitat quality and population size on individual performance of *Succisa pratensis*. *Journal of Ecology* **91**:18–26.
- Walker, K. J., P. A. Stevens, J. O. Mountford, S. Manchester, and R. F. Pywell. 2004. The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biological Conservation* **119**:1–18.
- Waser, N. M., and M. P. Price. 1985. Reciprocal transplant experiments with *Delphinium nelsonii* (Ranunculaceae): evidence for local adaptation. *American Journal of Botany* **72**:1726–1732.
- Wilkinson, D. M. 2001. Is local provenance important in habitat creation? *Journal of Applied Ecology* **38**:1371–1373.
- Williams, S. L. 2001. Reduced genetic diversity in eelgrass transplantations affects both population growth and individual fitness. *Ecological Applications* **11**:1472–1488.