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Long-term assessment of seed provenance effect on the establishment of the perennial grass *Bromus erectus*

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Abstract

Questions: Do short-term seed-addition experiments reliably tell us about seed limitation of perennial species? Does seed provenance affect recruitment success?

Locations: Three dry grassland sites (Negrentino, Poma, San Giorgio), southern Switzerland.

Methods: 18 000 seeds of *Bromus erectus* of three provenances were sown in a reciprocal design with temporal replication. Frequent checks were made of all emerged individuals of two seed cohorts over three years and continued annual checks of one cohort until year eight. Performance was determined by number and size of emerging and surviving individuals and a combined measure of population vigour.

Results: From a three-year perspective, all sites appeared to be seed limited, and differences due to seed provenance were small. Over five years, two sites showed an increasingly superior performance of the local over the foreign populations. At one of these two sites, average individuals remained small and a decrease in number and individual phytomass since the fifth year suggested complete microsite limitation. Reproductive individuals only occurred at the other sites and confirmed seed limitation after eight years. The best performing site had the first reproductive individuals in the fourth year.

Conclusions: *B. erectus* showed a long pre-reproductive phase during which the prediction of establishment success by individual counts can be misleading if plant size is not also measured. The effect of seed provenance was clearly indicated where populations established most successfully and local-over-foreign superiority increased with time.

Keywords: Grassland; Local adaptation; Microsite limitation; Reciprocal transplant experiment; Recruitment limitation; Seed addition; Seed limitation; Seed origin; Seedling establishment; Sowing experiment.

Introduction

Over the last decade, the number of studies concerned with the effects of seed, dispersal, microsite and recruitment limitation has strongly increased. The most reliable evidence for these forms of limitation comes from sowing experiments, i.e. the introduction of seeds into unoccupied habitats or the addition of seeds to existing populations (Münzbergova & Herben 2005; Zobel & Kalamees 2005). Seeds are introduced to find out whether the present local occurrence of a species is limited by seed availability (e.g. Stampfli & Zeiter 1999). Seed-addition studies have also been used to assess the role of seed availability on species population size (e.g. Eriksson & Ehrlén 1992) or on community properties, such as species richness (Vandvik & Goldberg 2005) or productivity (Zeiter et al. 2006).

The review of Turnbull et al. (2000) revealed that ca. 50% of the investigated plant species had seed-limited populations, i.e. they showed increased seedling recruitment in response to seed addition. Many ecological communities are unsaturated with species (Tilman 1997; Stampfli & Zeiter 1999; Zobel et al. 2000; Foster & Tilman 2003; Foster et al. 2004; Zeiter et al. 2006), but some studies found strong microsite limitation as species richness did not increase after seed addition (Kupferschmid et al. 2000; Wilsey & Polley 2003). The relative importance of seed vs. microsite limitation may depend on properties of the sites such as disturbance (Burke & Grime 1996; Austrheim & Eriksson 2003), water availability (Wilsey & Polley 2003), productivity (Burke & Grime 1996; Foster 2001; Stevens et al. 2004; Eskelinen & Virtanen 2005) and successional status (Turnbull et al. 2000), or on properties of the species such as seed size (Moles & Westoby 2002).

The role of seed provenance in sowing experiments has not been addressed so far. While several studies have used locally collected seeds, other experiments were conducted with commercially available seeds of unknown origin despite the fact that adaptive genetic variation may occur among plant populations from different sites (reviews by Bradshaw 1984; Venable 1984;

Linhart & Grant 1996). Effects of seed provenance on seed limitation cannot be deduced from classical reciprocal transplant experiments due to two methodological constraints. First, these experiments have often not been conducted with seeds, but with seedlings of different age or even with adult individuals after clonal propagation. Transplantation at the seedling stage eliminates adaptive population differences in seed survival, in seedling emergence and in early seedling survival, which can be considerable (Galloway & Fenster 2000; Bischoff et al. 2006a, b). Second, in reciprocal transplant studies conducted with seeds, the experimenter often exerted a more or less considerable impact on the target community by altering soil, water or light factors before or during the experiment, and/or reducing biotic interactions within the target communities. Therefore, the magnitude of genetic differences found may not be sustained in, or relevant to, natural communities (Antonovics & Primack 1982). Populations have indeed shown a different ranking of fitness in natural, competitive compared to non-competitive conditions (Hickey & McNeilly 1975; Turkington & Harper 1979; Bossdorf et al. 2004; Bischoff et al. 2006b). Until now, only few reciprocal seed-transplant experiments have used undisturbed target communities (Cheplick 1988; Jordan 1992, Raabová et al. 2007).

We reciprocally sowed seeds of *Bromus erectus* into dry, semi-natural meadows at three sites with the aim of assessing the effects of seed provenance on recruitment in natural target communities. *B. erectus* was chosen because it is a major component of this vegetation type. We repeated the field experiment in two successive years in an attempt to avoid the possible confounding of one-time seed additions with weather conditions in the year of sowing and measured establishment success of sown individuals of both temporal replicates over three years. We extended observations of the first-replicate individuals until year 8 for two reasons. First, unequivocal evidence of seed limitation, in the strict sense, requires showing that self-sustaining populations were successfully established, or at least showing that the sown populations can reach the reproductive stage (Turnbull et al. 2000). Sown individuals, however, have only rarely been monitored (e.g. Ehrlén et al. 2006) or measured in size over more than a few years, thus the role of seed limitation may often have been overestimated, particularly in the case of perennial species with a long pre-reproductive life span. Second, the diagnosis of local adaptation should ideally be based on a home-site advantage measured by life-time fitness (Kindell et al. 1996), which in reality is rarely feasible for long-lived perennials such as *B. erectus,* and thus studies are rarely run for more than two or three years (but see Waser & Price 1985). Studies may use short-term survival and juvenile plant size to predict relative cumulative fitness because size hierarchies establish early (Primack & Kang 1989; Montalvo 1994; Montalvo & Ellstrand 2000). However, fitness differences among populations may accumulate and become even stronger over time, as adaptation of populations to specific sites would integrate influential effects that occur only periodically (Montalvo & Ellstrand 2000).

In this paper, we investigate the following questions: 1. Does seed provenance affect recruitment success, and in particular, do local individuals perform better than foreign individuals? 2. How reliable is a short-term assessment of seed limitation from an eight-year perspective on the establishment of a perennial species?

Material and Methods

Study sites

We used three experimental sites in the lower montane zone of Ticino (southern Switzerland), in dry, nutrient-poor meadows (*Mesobromion* type *sensu* Ellenberg 1996) which have declined in species richness during intermittent periods of abandonment over 1-3 decades. Since 1998/1999, all sites were mown and harvested twice a year, in the first half of July and in the second half of September. Poma (Monte Generoso, 45º53'56'' N, 9º00'54'' E, 990 m a.s.l.) and San Giorgio (Monte San Giorgio, 45º54'27'' N, 8º57'19'' E, 950 m a.s.l.) are close to one another (5 km) but probably isolated in terms of gene flow due to surrounding forests and location on slopes of disconnected mountains, whilst Negrentino (Valle di Blenio, 46°27'40'' N, 8°55'30'' E, 820 m a.s.l.) is separated from them both by 62 km. For a detailed description of the experimental sites see Zeiter et al. (2006). The climate is humid-temperate and shows relatively high annual precipitation, ca. 1300 mm at Negrentino, and ca. 1900 mm at the two other sites (Walter & Lieth 1964).

Relatively wet weather prevailed during the first four years, since the start of our seed-addition experiment in September 1998 (Zeiter et al. 2006). Later years had drier summers. At all sites, continuous measurements of soil water potential (Zeiter et al. 2006) revealed highest drought intensity in 2003; 2004 and 2006 still had intense droughts during several weeks. Ranked by drought intensity, the second summer was 2006 at Negrentino and 2004 at the other sites.

Target species

Bromus erectus is a densely tufted, perennial grass which occurs in many parts of Europe. It is wind-pollinated and self-incompatible (Frankel & Galun 1977).

We selected seeds of *B. erectus* of three provenances, viz. the neighbourhoods of the experimental plots at the three sites. Here, original populations extended over several hectares of grassland in which they represented one of the co-dominant species. In July of the two years 1998 and 1999, we collected seeds at random as mixed samples from 200-500 mature inflorescences of at least 30 individuals growing in an area of ca. 400 m^2. We cleaned the seeds and stored them at room temperature until time of sowing in September. Determination of seed mass by ten sets of 100 seeds revealed averages in the range of 5.0-6.3 mg for all provenances and years except for San Giorgio seeds collected in 1998 (4.2 mg). In August 1998 and September 1999, 100 seeds per provenance were placed on moist filter papers, pre-chilled for 7 days at 10°C, and transferred to a growth chamber providing a night-day temperature regime of 15°C / 25°C (8h / 16h). The number of germinated seeds was recorded after 14 days. This revealed high germination (89-96%) for seeds of all provenances in both years.

Experiment and sampling design

At each site we established ten blocks, each divided into two parts for the addition of seeds in 1998 or 1999. Both parts contained one plot of 60 cm × 90 cm that received the seeds, and a second plot of 60 cm × 30 cm that acted as a control. A buffer zone between plots of at least 15 cm was created. Before sowing in 1998, *B. erectus* occurred in nearly all plots at Negrentino and San Giorgio, but only outside plots at Poma. After mowing in mid-September, we dropped seeds individually to the ground from a height of ca. 5 cm by using forceps and a board with regularly spaced holes (Zeiter et al. 2006). We added 100 seeds of each of three provenances per plot. One set of ten individual seeds per provenance, placed along a line of 15 cm, was randomly allocated in each of ten subplots of 60 cm × 9 cm. The product of three provenances, three sites, ten replicates, 100 seeds and two years resulted in 18 000 added seeds.

Over 36 months after sowing, we recorded seedling emergence and subsequent survival at intervals of 1-3 months. We marked every seedling with a coloured ringlet, mapped its position on a grid and compiled the complete history of each individual from the maps. After recording length of the longest shoot and number of shoots twice a year before mowing in June/July and September/October, we clipped individuals at 5 cm above ground. Seedling phytomass was pooled per plot and dried at 80 °C for 24 h. Longest shoot length × number of shoots was taken as a measure of individual size, because phytomass values were not available for individuals < 5 cm tall. The summed individual size over all living individuals of each provenance in a plot was used as a measure of population vigour. In order to achieve an eight-year perspective on the population dynamics at annual intervals we extended censuses and clipping until autumn 2006 on the 30 plots sown in 1998. We counted spikelets per plot and used cumulative number of spikelets as a measure of reproductive output. We stopped shoot length measurements after five years.

Small differences in topography (anthills, tussocks, etc.) and/or dispersal of the seeds caused deviations from the regular positions aimed at in the sowing. Thus we had to define an area around the theoretical sowing line in which individuals could safely be allocated to the corresponding seed provenance. In a separate experiment with 400 coloured seeds sown in 40 lines of ten seeds as in the main experiment we determined the displacement from the theoretical sowing positions of the 364 rediscovered seeds and seedlings, three weeks after sowing. From the empirical distribution of these displacements, we defined an area around the theoretical sowing line (between 0.85 cm above and 4.35 cm below) in which an individual of another sowing line had a small probability of occurrence ($P < 0.02$). In analyses of seed provenance effects, we excluded 19% of all individuals that emerged outside this area because a wrong provenance allocation would have caused an increase in type-II error. An increase in type-I error, however, is very unlikely due to our sowing scheme.

Data analysis

We conducted statistical analyses using GENSTAT 6.0 (Payne 2000). The design was a three-factor split-plot arrangement with ten replicates. The factor site included three levels, year of sowing two levels and seed provenance three levels. The design had three strata: (a) site × block level, $n = 30$ with block nested within site; (b) site × block × plot level, $n = 60$ with plot (year of sowing) nested within block; (c) site × block × plot × subplot level, $n = 180$ with seed provenance nested within plot (year of sowing). We analysed individual size and population vigour using ANOVA. Individual size was log-transformed, population vigour $\log(x+1)$-transformed before analysis. We used GLM (binomial distribution and logit-link function) for the analysis of germination and survival data. The response variables were the number of germinated seeds (binomial totals = 100) or the number of surviving individuals (binomial totals = number of emerged seedlings). In the analysis of deviance, we produced an accumulated analysis of deviance table, built up the residuals from the interactions of block, year (plot) and seed provenance (subplot), and used quasi-F-tests based on the ratio of the deviances divided by their degrees of freedom (McCullagh & Nelder 1989; Egli & Schmid 2001; Zeiter et al. 2006). A linear contrast of 'local' (one local population × three sites) vs. 'foreign' (two foreign populations × three sites) provenances was specified within significant site × provenance interactions. We calculated the percentage variance accounted for by the factors (R^2 = (sum of square of factor / total sum of square) ×100) to compare their relative importance. For germination and survival data, deviance replaced sum of squares.

The effect of time on the development of local vs. foreign populations was tested with annual data of the 1998-cohort, measured over five years, 1999-2003. Repeated-measures ANOVA, with Greenhouse-Geisser corrected df, used annual difference in population vigour between local and foreign populations at each single site.

With data after eight years, the effect of seed provenance on reproductive output was tested in a single-site analysis using ANOVA, with seed provenance nested within plot. We applied Fisher's LSD tests to compare differences in population means. The effect of site on establishment success was also tested using ANOVA.

We calculated daily mortality rate as $m_d(t) = 100 \times [1-(1-(n_d/n_o))^{1/t}]$, where n_d individuals died in an original population of n_o over a census interval of t days (Alder 1995; Sheil & May 1996). We used seven one-year census intervals, starting in summer 1999 thus after emergence of the majority of individuals to calculate mortality rates for individuals with increasing age.

A few individuals (2.28%) escaped notice in single censuses and their exact date of germination or death was not clear. We excluded them from the analysis of temporal patterns in germination and mortality.

Results

Within three years after sowing, 8800 *Bromus erectus* seedlings emerged (sum; n = 60 plots). As only six seedlings emerged in all control plots we considered all seedlings in sown plots to have emerged from added seeds. Germination started soon after seed addition: 55.4% of all seedlings emerged within 66 days, 93.1% within one year, and 99.8% within two years.

Seed provenance effects and local adaptation

Three years after sowing, seed provenance affected seedling emergence, survival and individual size (Table 1). Seed provenance explained 5.3% of the total variance in emergence, 5.6% in survival and 2.2% in individual size. Seeds of San Giorgio provenance had a lower germination rate than those of Poma and Negrentino (App. 1a), and they also showed lowest survival and smallest size (App. 1b,c). Overall, the absolute differences between individuals of the three provenances, however, were still relatively small (emergence 6-7.5%, survival 6.3-9.9%, size 0.7-1.7 cm). Survival and individual size showed a site × provenance interaction, but seedling emergence did not (Table 1). The site × provenance interaction explained 2.6% of the variation in survival, and 2.7% of the variation in size. Survival and individual size showed a significant 'local-foreign' contrast (Table 1) with superior performance of local compared to foreign provenances in survival (30.8 ± 2.5% vs. 27.4 ± 1.8%) and size (13.4 ± 1.0 cm vs. 11.6 ± 0.7 cm). Local individuals showed largest relative size at Negrentino and San Giorgio (App. 1c), but individuals of Poma provenance showed highest survival at all sites (App. 1b). Population vigour, an integrative fitness measure, was influenced by seed provenance and site × provenance interaction (Table 1). Population vigour

Table 1. Effects of site, sowing year, seed provenance and their interactions on performance of *Bromus erectus* in reciprocal sowing experiment with two cohorts sown in 1998 and 1999. Emergence and survival over 3 years, individual size and population vigour in 3rd year after sowing. Population vigour is summed individuals sizes over all living individuals. A linear 'local-foreign' contrast was specified within significant provenance × site interactions. Asterisks indicate different significance levels, $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***). Reduced df's for size: [a]26, [b]98.

Source of variation	d.f.	Emergence *F*	Survival *F*	Size *F*	Vigour *F*
Site	2,27	13.8***	40.3***	61.8***	67.7***
Year	1,27[a]	3.0	0.3	3.7	10.9**
Site × year	2,27[a]	0.3	0.5	1.2	3.8*
Provenance	2,108[b]	26.5***	41.4***	16.7***	18.1***
Provenance × site	4,108[b]	0.4	9.8***	10.3***	5.3***
local vs. foreign	1,108[b]	-	18.3***	33.0***	18.7***
Provenance × year	2,108[b]	13.7***	1.7	1.1	3.7*
Provenance × site × year	4,108[b]	1.0	1.4	0.4	0.3

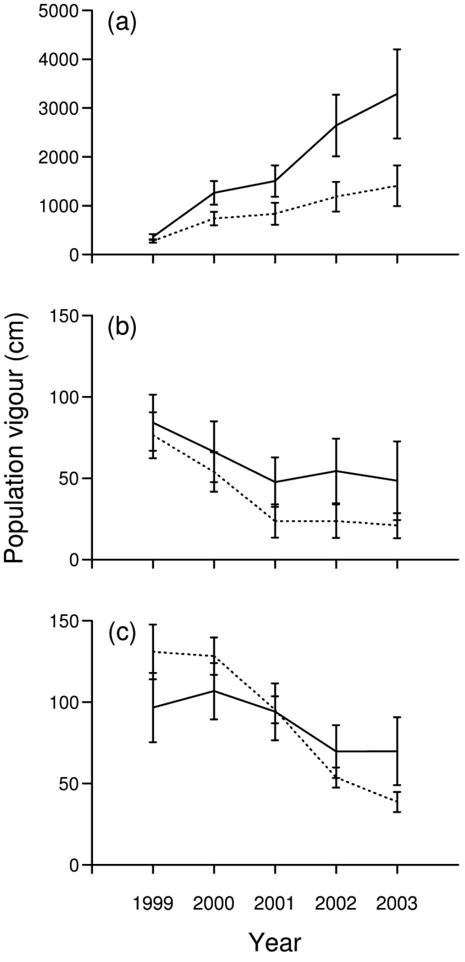

Fig. 1. Population vigour of local (line) vs. foreign (dotted line) seed provenance of *Bromus erectus* over 5 years after reciprocal sowing in 1998 (means ± SE, $n = 10$ plots) at (**a**) Negrentino, (**b**) Poma and (**c**) San Giorgio.

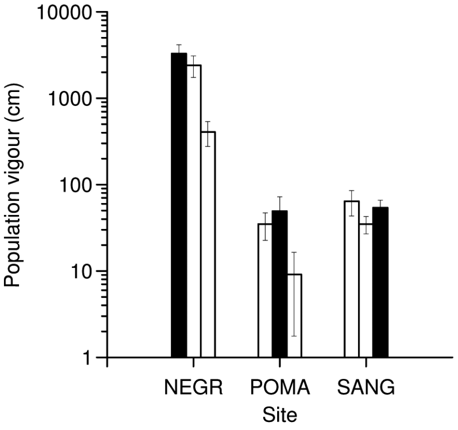

Fig. 2. Population vigour of *Bromus erectus* from three provenances (Negrentino, Poma, San Giorgio) at three sites, Negrentino (NEGR), Poma (POMA), San Giorgio (SANG), 5 years after reciprocal sowing in 1998 (means ± SE, $n = 10$ plots). Bars denote provenance in same sequence as site. Black bars indicate populations growing at their home site.

also showed a significant 'local-foreign' contrast (Table 1) with superior performance of local than foreign provenances (593 ± 141 cm vs. 384 ± 70 cm). Nevertheless, the Negrentino population was clearly superior at all three sites (App. 1d).

Over time, population vigour only increased at Negrentino, nevertheless, superiority of local over foreign populations developed at two sites, Negrentino ($F_{4,36} = 9.9$, $P\varepsilon < 0.01$, Greenhouse-Geisser adjusted probability) and San Giorgio ($F_{4,36} = 5.6$, $P\varepsilon < 0.01$), as differences between both populations increased with time (Fig. 1).

Five years after sowing in 1998, population vigour showed an effect of seed provenance ($F_{2,54} = 13.1$, $P < 0.001$) and a site × provenance interaction ($F_{4,54} = 3.8$, $P < 0.01$). Population vigour showed a 'local-foreign' contrast ($F_{1,54} = 9.9$, $P < 0.01$) with a superior performance of local than foreign provenances (1101 ± 385 cm vs. 498 ± 160 cm). Local populations were superior at Negrentino and Poma (Fig. 2).

Eight years after sowing in 1998, individuals of San Giorgio provenance were fewer in number than individuals of the two other sites (Table 2), but this could not be tested because the number of individuals was zero in many single plots at two sites. Individuals of all provenances reached the reproductive stage (Table 2), but due to insufficient flowering individuals at two sites (San Giorgio and Poma), provenance effects cannot be compared in an

Table 2. Performance of *Bromus erectus* from provenances N, P, S at three sites, Negrentino, Poma, San Giorgio, 8 years after reciprocal sowing in 1998 (sums, $n = 10$ plots). Number of living individuals in summer 2006 and cumulative number of individuals reaching reproductive stage in parentheses.

	Seed provenance			
	N	P	S	Sum
Negrentino	113 (30)	113 (10)	18 (3)	264 (43)
Poma	9 (1)	15 (2)	1	25 (3)
San Giorgio	14	10	13	37
Sum	136 (31)	138 (12)	32 (3)	

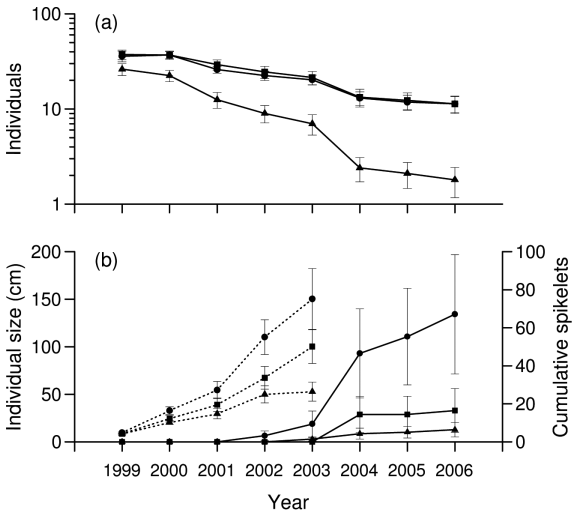

Fig. 3. Long-term establishment success of populations of *Bromus erectus* originating from Negrentino (circles), Poma (quadrats) and San Giorgio (triangles) over 8 years after sowing in 1998 at Negrentino, the site with highest establishment success (means ± SE, n = 10 plots). (**a**) Number of living individuals. (**b**) Individual size in June (dotted lines) and cumulative number of spikelets (lines).

overall analysis. At Negrentino, provenance affected the cumulative number of spikelets per plot ($F_{2,18} = 35.3$, $P < 0.0001$) and the local population performed best (Fig. 3b). The weaker performance of the foreign populations was composed of a lower number of living individuals (Fig. 3a), mainly due to low survival of individuals of San Giorgio provenance, and/or a smaller individual size (Fig. 3b), and was also reflected by the number of individuals in reproductive stage (Table 2).

Site effects

After three years, site affected seedling emergence, survival and individual size (Table 1). The factor site explained 28.8% of the total variance in seedling emergence, 53.0% in survival and 68.6% in individuals' size.

Seedling emergence was lower at Poma than at the two other sites (App. 1a).

Survival over three years was lowest at Poma and highest at Negrentino (App. 1b). The number of living individuals declined most during the first two summers at Poma, and during the exceptionally hot and dry summer 2003 at Negrentino (Fig. 4a). However, unlike at San Giorgio where populations showed constant mortality over eight years, mortality declined with age of the sown individuals (Fig. 5). Eight years after sowing in 1998, the number of living individuals still reflected a site effect ($F_{2,22} = 83.7$, $P < 0.001$); the number of individuals was higher at Negrentino than at the two other sites (Fig. 4a), and most plots within these sites did not show living individuals of all provenances. Between-site differences

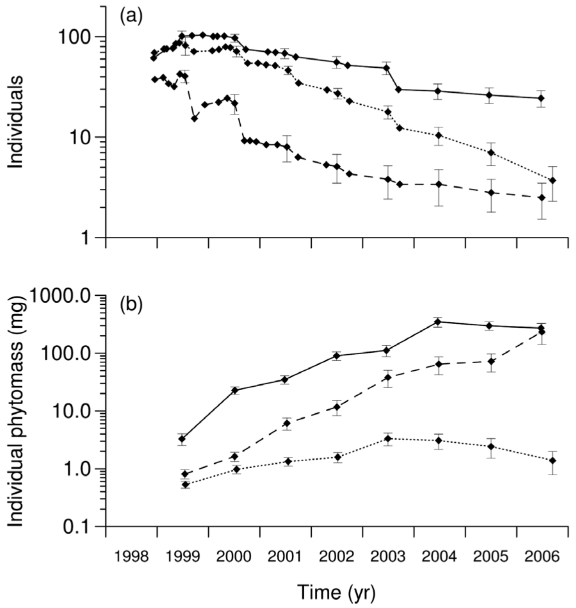

Fig. 4. Performance of *Bromus erectus* individuals at Negrentino (line), Poma (broken line) and San Giorgio (dotted line) across 8 years after sowing in autumn 1998. (**a**) Number of living individuals per plot (means ± SE, n = 10 plots). For simplicity, error bars are shown once a year. (**b**) Mean phytomass of individuals clipped at a height of 5 cm (means ± SE, n = 10, except n = 9 plots in 2001-2005 at Poma and in 2006 at San Giorgio, and n = 8 plots in 2006 at Poma). Mean individual phytomass is based on low number of individuals in most plots (three plots had > 5 individuals) from 2003 onwards at Poma, and in 2006 at San Giorgio.

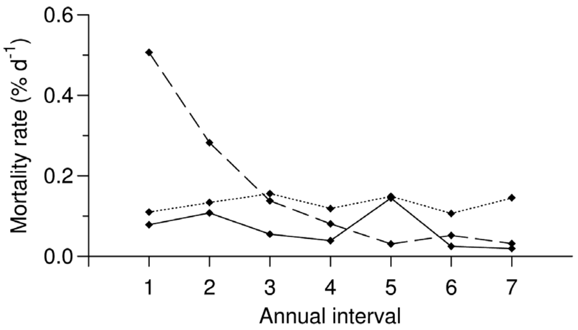

Fig. 5. Mortality rate of *Bromus erectus* individuals at Negrentino (line), Poma (broken line) and San Giorgio (dotted line) having emerged from seeds sown in 1998, based on number of living individuals recorded in annual intervals, with first interval starting in summer 1999.

in number of established individuals were mainly due to differences in survival.

Individual size was largest at Negrentino and smallest at San Giorgio three years after sowing (App. 1c). Differences in individual size between sites were even more pronounced in the phytomass data (Fig. 4b) as horizontal growth was similarly affected as vertical growth (proportion of individuals with more than one shoot: Negrentino 41.1%, Poma 15.0%, San Giorgio 1.4%). Individual phytomass increased in the first five years after sowing at all sites (Fig. 4b), reached its maximum in 2003 at San Giorgio, in 2004 at Negrentino, and continuously increased until 2006 at Poma. Thus, the discrepancy between San Giorgio and the two other sites increased over eight years (Fig. 4b), and in 2006 the effect of site ($F_{2,27} = 30.2$, $P < 0.001$) was reflected by 169- or 196-fold differences in mean individual phytomass. At San Giorgio 51% of all individuals were still < 5 cm in size and only 5.6% of them had more than one shoot.

A few individuals started flowering in the fourth year after sowing, but eight years after sowing, only a minority of individuals had reached the reproductive stage (Table 2) at all sites, Negrentino (17.6%), Poma (12.0%) and San Giorgio (0%).

Discussion

Seed provenance effect and local adaptation

The significant seed provenance effect found for all measures of establishment success indicates population differentiation. Increasing differences in population vigour between local and foreign provenances show native superiority at two sites (Negrentino, San Giorgio). Following the criteria of Kawecki & Ebert (2004) and Bischoff et al. (2006b), population vigour in our experiment would have indicated overall local adaptation five years after sowing, because the significant 'local-foreign' contrast was combined with absolute local superiority at least at two out of three sites (Negrentino, Poma). At San Giorgio, the local population did not show highest establishment success. Jakobsson & Dinnetz (2005) argue that, due to initial founder effects and genetic drift, local adaptations do not require native superiority, as long as locals perform equally well as foreigners and the negative difference in fitness between the populations considered is smaller at the local population's home site. Accordingly, despite its lower overall establishment success, the San Giorgio population performed equally well at San Giorgio as the two other populations. Therefore, adaptation would be indicated by 'relative performance' (*sensu* Jakobsson & Dinnetz 2005). The reduced performance of the San Giorgio populations, which is strongly affected by low survival, is likely related to their reduced root-shoot ratio (Zeiter 2005). Eight years after sowing, approval of local adaptation in an overall analysis was no longer possible because insufficient individuals had survived at two sites to test effects of site and seed provenance on size. At Negrentino, the only site where enough individuals had survived for a comparison, native superiority was found in reproductive output.

We cannot exclude that the observed population differences between provenances are influenced by environmental maternal effects (Roach & Wulff 1987; Schmid & Dolt 1994; Weiner et al. 1997) mediated by seed size, as we used seeds that were directly collected in the field for this study. Nevertheless, we suppose that the population differences mainly reflect genetic variation because they continuously increased over eight years, while environmental maternal effects usually become less pronounced with age (Roach & Wulff 1987; Miao et al. 1991; Weiner et al. 1997). This is also supported by a parallel greenhouse experiment with populations of the same provenances (Zeiter 2005), in which we found a transitory positive effect of seed size on seedling weight that disappeared in 4-month-old individuals.

Local adaptation is predicted to result from a habitat-specific natural selection strong enough to override the homogenizing effect of gene flow (Linhart & Grant 1996). As we conducted this experiment with a long-lived, wind-pollinated and out-breeding species at geographically close sites and in similar habitats, we expect even higher impacts of seed provenance in seed-addition experiments conducted with short-lived and/or inbreeding species and/or when seeds were collected in ecologically more distinct habitats and/or at geographically more distant sites.

Site effects

The germination pattern following seed addition confirms that most *Bromus erectus* seeds are non-dormant and only transient in the soil (Thompson et al. 1997). Increased numbers of emerging seedlings in response to sowing preliminarily indicated seed limitation at all three sites. Based on the number of surviving recruits, this seemed to be a valid judgement over a period of six years. Along with a further declining number of recruits, differences in size between individuals became more apparent and a sizeable minority of recruits reached reproductive stage. From an eight-year perspective, the shorter-term judgement of seed limitation has to be revised. This shows seed limitation in the strict sense at Negrentino and Poma, but not at San Giorgio where only a low number of small-sized individuals had survived. A constant mortality rate and a decreasing trend in size since the fifth year suggest that these individuals will die in the near future without ever reaching reproductive stage. Therefore, although seedlings may survive for a decade, complete microsite limitation is most likely for *B. erectus* at the San Giorgio locality. The concurrent presence of old *B. erectus* populations at this site rises the question whether suitable conditions for establishment of this long-lived species occur intermittently, e.g. depending on unknown soil factors and/or ant activity, or whether the habitat conditions have shifted since the past, possibly connected with stopped haymaking since the 1950s causing a time-delayed extinction of *B. erectus* (extinction debt *sensu* Tilman et al. 1994). At the two other localities, the number of living individuals can now anticipate a definite judgement by cumulative number of reproductive individuals in the future because individuals have reached relatively large size and show declining mortality rates with age. Microsite limitation is stronger at Poma than at Negrentino, where sparse recruitment is rather due to seed limitation caused by early haymaking (Stampfli & Zeiter 2008). The ranking of two sites (Poma, San Giorgio) by strength of microsite limitation was ambiguous in the first five years because number and size of living individuals implied different establishment success.

Our results exemplify that short-term experimental assessment of recruitment limitation by number of surviving individuals can indeed be misleading (Zobel & Kalamees 2005). Clearly, long-term monitoring of sown individuals is required when perennial species with a long pre-reproductive life span are involved (Ehrlén et al. 2006). However, as mortality declined with age at sites where plant size strongly increased with time, our study suggests that preliminary estimates of recruitment limitation can be improved by measurement of plant size and/or growth rate.

Conclusion

From an eight-year perspective, seed provenance effects on recruitment were confirmed at Negrentino, but became obsolete at San Giorgio due to unsuccessful establishment. An increasing difference in population vigour between local and foreign provenances suggested local superiority over five years at both sites, but local superiority was only accompanied by an increase in population vigour at Negrentino. This illustrates that short-term assessments of seed provenance effects should be interpreted with care. A correct assessment of provenance effects requires considering the chance that populations reach the reproductive stage by checking if population vigour increases over time.

In our study, the strength of seed limitation was affected by seed provenance at the site of best recruitment. Under harsh conditions for establishment, the importance of seed provenance was not clear, or seed provenance was not relevant. Hence, with foreign seeds showing lower establishment success, the strength of seed limitation is underestimated when environmental conditions for recruitment are favourable. But, non-local seeds may theoretically also lead to an overestimation of the strength of seed limitation in sowing experiments, e.g. if local seeds are from small isolated populations showing reduced fitness (Ellstrand & Elam 1993; Leimu et al. 2006).

We conclude that studies of recruitment limitation conducted with seeds of unknown origin may confound environmental effects with seed provenance. We therefore recommend adding a consideration of seed provenance to Turnbull's (1999) protocol for seed-addition experiments. Plant ecologists should be aware of the possibility that seed provenance and duration of monitoring may affect the recognition of the strength of seed limitation. The use of local seeds becomes the more important the more reliably evidence is sought in experiments of long duration.

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References

Alder, D. 1995. Growth modelling for mixed tropical forests. In: Anonymus (eds.) *Forestry Papers, 30,* p. 231. Oxford Forestry Institute, Oxford, UK.

Antonovics, J. & Primack, R.B. 1982. Experimental ecological genetics in *Plantago*. 6. The demography of seedling transplants of *P. lanceolata*. *Journal of Ecology* 70: 55-75.

Austrheim, G. & Eriksson, O. 2003. Recruitment and life-history traits of sparse plant species in subalpine grasslands. *Canadian Journal of Botany* 81: 171-182.

Bischoff, A., Vonlanthen, B., Steinger, T. & Müller-Schärer, H. 2006a. Seed provenance matters – effects on germination of four plant species used for ecological restoration. *Basic and Applied Ecology* 7: 347-359.

Bischoff, A., Crémieux, L., Šmilauerová, M., Lawson, C.S., Mortimer, S.R., Doležal, J., Lanta, V., Edwards, A.R., Brook, A.J., Macel, M., Lepš, J., Steinger, T. & Müller-Schärer, H. 2006b. Detecting local adaptation in widespread grassland species – the importance of scale and local plant community. *Journal of Ecology* 94: 1130-1142.

Bossdorf, O., Prati, D., Auge, H. & Schmid, B. 2004. Reduced competitive ability in an invasive plant. *Ecology Letters* 7: 346-353.

Bradshaw, A.D. 1984. Ecological significance of genetic variation between populations. In: Dirzo, R. & Sarukhan, J. (eds.) *Perspectives on plant population ecology,* pp. 213-228. Sinauer Associates, Sunderland, MA, US.

Burke, M.J.W. & Grime, J.P. 1996. An experimental study of plant community invasibility. *Ecology* 77: 776-790.

Cheplick, G.P. 1988. Influence of environment and population origin on survivorship and reproduction in reciprocal transplants of amphicarpic peanutgrass (*Amphicarpum purshii*). *American Journal of Botany* 75: 1048-1056.

Egli, P. & Schmid, B. 2001. The analysis of complex leaf survival data. *Basic and Applied Ecology* 2: 223-231.

Ehrlén, J., Münzbergová, Z., Diekmann, M. & Eriksson, O. 2006. Long-term assessment of seed limitation in plants: results from a 11-year experiment. *Journal of Ecology* 94: 1224-1232.

Ellenberg, H. 1996. *Vegetation Mitteleuropas mit den Alpen*. Ulmer Verlag, Stuttgart, DE.

Ellstrand, N.C. & Elam, D.R. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217-243.

Eriksson, O. & Ehrlén, J. 1992. Seed and microsite limitation of recruitment in plant populations. *Oecologia* 91: 360-364.

Eskelinen, A. & Virtanen, R. 2005. Local and regional processes in low-productive mountain plant communities: The roles of seed and microsite limitation in relation to grazing. *Oikos* 110: 360-368.

Foster, B.L. 2001. Constraints on colonization and species richness along a grassland productivity gradient: The role of propagule availability. *Ecology Letters* 4: 530-535.

Foster, B.L. & Tilman, D. 2003. Seed limitation and the regulation of community structure in oak savanna grassland. *Journal of Ecology* 91: 999-1007.

Foster, B.L., Dickson, T.L., Murphy, C.A., Karel, I.S. & Smith V.H. 2004. Propagule pools mediate community assembly and diversity-ecosystem regulation along a grassland productivity gradient. *Journal of Ecology* 92: 435-449.

Frankel, R. & Galun, E. 1977. *Pollination mechanisms, reproduction and plant breeding*. Springer, Berlin, DE.

Galloway, L.F. & Fenster, C.B. 2000. Population differentiation in an annual legume: Local adaptation. *Evolution* 54: 1173-1181.

Hickey, D.A. & McNeilly, T. 1975. Competition between metal tolerant and normal plant populations – field experiment on normal soil. *Evolution* 29: 458-464.

Jakobsson, A. & Dinnetz, P. 2005. Local adaptation and the effects of isolation and population size – the semelparous perennial *Carlina vulgaris* as a study case. *Evolutionary Ecology* 19: 449-466.

Jordan, N. 1992. Path-analysis of local adaptation in 2 ecotypes of the annual plant *Diodia teres* Walt (Rubiaceae). *American Naturalist* 140: 149-165.

Kawecki, T.J. & Ebert, D. 2004. Conceptual issues in local adaptation. *Ecology Letters* 7: 1225-1241.

Kindell, C.E., Winn, A.A. & Miller, T.E. 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.

Kupferschmid, A.D., Stampfli, A. & Newbery, D.M. 2000. Dispersal and microsite limitation in an abandoned calcareous grassland of the southern Prealps. *Folia Geobotanica* 35: 125-141.

Leimu, R., Mutikainen, P., Koricheva, J. & Fischer, M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology* 94: 942-952.

Linhart, Y.B. & Grant, M.C. 1996. Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* 27: 237-277.

McCullagh, P. & Nelder, J.A. 1989. *Generalized linear models*. 2nd ed. Chapman and Hall, London.

Miao, S.L., Bazzaz, F.A. & Primack, R.B. 1991. Persistence of maternal nutrient effects in *Plantago major* – the 3rd generation. *Ecology* 72: 1634-1642.

Moles, A.T. & Westoby, M. 2002. Seed addition experiments are more likely to increase recruitment in larger-seeded species. *Oikos* 99: 241-248.

Montalvo, A.M. 1994. Inbreeding depression and maternal effects in *Aquilegia caerulea*, a partially selfing plant. *Ecology* 75: 2395-2409.

Montalvo, A.M. & Ellstrand, N.C. 2000. Transplantation of the subshrub *Lotus scoparius*: Testing the home-site advantage hypothesis. *Conservation Biology* 14: 1034-1045.

Münzbergová, Z. & Herben, T. 2005. Seed, dispersal, microsite, habitat and recruitment limitation: Identification of terms and concepts in studies of limitations. *Oecologia* 145: 1-8.

Payne, R.W. (ed.) 2000. *The guide to Genstat*. Lawes Agricultural Trust, Rothamsted, UK.

Primack, R. B. & Kang, H. 1989. *Measuring fitness and natural-selection in wild plant-populations*. Lawes Agricultural Trust, Rothamsted. 20: 367-396.

Raabová, J., Münzbergová, Z. & Fischer, M. 2007. Ecological rather than geographic or genetic distance affects local adaptation of the rare perennial herb, *Aster amellus*. *Biological Conservation* 139: 348-357.

Rice, K.J. & Mack, R.N. 1991. Ecological genetics of *Bromus tectorum*. 3. The demography of reciprocally sown populations. *Oecologia* 88: 91-101.

Roach, D.A. & Wulff, R.D. 1987. Maternal effects in plants. *Annual Review of Ecology and Systematics* 18: 209-235.

Schmid, B. & Dolt, C. 1994. Effect of maternal and parental environment and genotype on offspring phenotype in *Solidago altissima* L. *Evolution* 48: 1525-1549.

Schmidt, K.P. & Levin, D.A. 1985. The comparative demography of reciprocally sown populations of *Phlox drummondii* Hook. 1. Survivorships, fecundities, and finite rates of increase. *Evolution* 39: 396-404.

Sheil, D. & May, R.M. 1996. Mortality and recruitment rate evaluations in heterogeneous tropical forests. *Journal of Ecology* 84: 91-100.

Stampfli, A. & Zeiter, M. 1999. Plant species decline due to abandonment of meadows cannot easily be reversed by mowing. A case study from the southern Alps. *Journal of Vegetation Science* 10: 151-164.

Stampfli, A. & Zeiter, M. 2008. Mechanisms of structural change derived from patterns of seedling emergence and mortality in a semi-natural meadow. *Journal of Vegetation Science* 19: 563-574.

Stevens, M.H.H., Bunker, D.E., Schnitzer, S.A. & Carson, W.P. 2004. Establishment limitation reduces species recruitment and species richness as soil resources rise. *Journal of Ecology* 92: 339-347.

Thompson, K., Bakker, J. & Bekker, R. 1997. *The soil seed banks of North West Europe: methodology, density and longevity*. Cambridge University Press, Cambridge, UK.

Tilman, D. 1997. Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology* 78: 81-92.

Tilman, D., May, R.M., Lehman, C.L. & Nowak, M.A. 1994. Habitat destruction and extinction debt. *Nature* 371: 65-66.

Turkington, R. & Harper, J.L. 1979. Growth, distribution and neighbor relationships of *Trifolium repens* in a permanent pasture. 4. Fine-scale biotic differentiation. *Journal of Ecology* 67: 245-254.

Turnbull, L.A., Crawley, M.J. & Rees, M. 2000. Are plant populations seed-limited? A review of seed sowing experiments. *Oikos* 88: 225-238.

Vandvik, V. & Goldberg, D.E. 2005. Distinguishing the roles of dispersal in diversity maintenance and in diversity limitation. *Folia Geobotanica* 40: 45-52.

Venable, D.L. 1984. Using intraspecific variation to study the ecological significance and evolution of plant life-histories. In: Dirzo, R. & Sarukhan, J. (eds.) *Perspectives on plant population ecology,* pp. 166-187. Sinauer Associates, Sunderland, MA, US.

Walter, H. & Lieth, H. 1964. *Klimadiagramm Weltatlas. Alpenraum*. Fischer, Jena, DE.

Waser, N.M. & Price, M.V. 1985. Reciprocal transplant experiments with *Delphinium nelsonii* (Ranunculaceae) – evidence for local adaptation. *American Journal of Botany* 72: 1726-1732.

Weiner, J., Martinez, S., Müller-Schärer, H., Stoll, P. & Schmid, B. 1997. How important are environmental maternal effects in plants? A study with *Centaurea maculosa*. *Journal of Ecology* 85: 133-142.

Wilsey, B.J. & Polley, H.W. 2003. Effects of seed additions and grazing history on diversity and productivity of subhumid grasslands. *Ecology* 84: 920-931.

Zeiter, M. 2005. *Regeneration by seeds in semi-natural grasslands*. Inauguraldissertation Universität Bern, CH.

Zeiter, M., Stampfli, A. & Newbery, D.M. 2006. Recruitment limitation constrains local species richness and productivity in dry grassland. *Ecology* 87: 942-951.

Zobel, M. & Kalamees, R. 2005. Diversity and dispersal – can the link be approached experimentally? *Folia Geobotanica* 40: 3-11.

Zobel, M., Otsus, M., Liira, J., Moora, M. & Mols, T. 2000. Is small-scale species richness limited by seed availability or microsite availability? *Ecology* 81: 3274-3282.

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App. 1. Performance of *Bromus erectus* from three provenances (Negrentino, Poma, San Giorgio, Switzerland) at three sites, Negrentino, Poma, San Giorgio, over three years after reciprocal sowing in 1998 and 1999 (means ± SE, $n = 20$ plots). (**a**) Percentage of emerged individuals; (**b**) Percentage of surviving individuals; (**c**) Individual size in third year and (**d**) Population vigour in third year.

		Seed provenance			
		Negrentino	Poma	San Giorgio	Mean
(a) Emergence (%)					
	Negrentino	49.0 ± 2.4	47.7 ± 2.2	41.6 ± 2.9	46.1 ± 1.5
	Poma	31.2 ± 2.2	30.8 ± 2.2	23.8 ± 2.0	28.6 ± 1.3
	San Giorgio	47.6 ± 3.5	44.9 ± 3.0	39.9 ± 3.4	44.1 ± 1.9
	Mean	42.6 ± 1.9	41.1 ± 1.7	35.1 ± 1.9	
(b) Survival (%)					
	Negrentino	49.7 ± 3.3	54.1 ± 3.3	32.2 ± 2.8	45.5 ± 2.2
	Poma	9.1 ± 1.8	13.3 ± 2.0	5.9 ± 1.6	9.4 ± 1.1
	San Giorgio	30.0 ± 2.4	32.7 ± 2.9	29.4 ± 2.5	30.7 ± 1.5
	Mean	29.6 ± 2.6	33.4 ± 2.7	22.7 ± 2.1	
(c) Size (cm)					
	Negrentino	55.7 ± 7.5	44.1 ± 7.5	31.8 ± 4.5	43.9 ± 4.0
	Poma	14.9 ± 2.1	13.9 ± 1.7	9.5 ± 1.6	12.9 ± 1.2
	San Giorgio	6.3 ± 0.4	5.6 ± 0.3	6.9 ± 0.4	6.3 ± 0.2
	Mean	26.4 ± 4.0	21.4 ± 3.4	16.7 ± 2.4	
(d) Population vigour (cm)					
	Negrentino	1619.1 ± 451.5	1395.0 ± 418.4	602.7 ± 196.4	1205.7 ± 277.3
	Poma	72.4 ± 38.4	60.8 ± 16.7	28.9 ± 16.5	54.0 ± 18.5
	San Giorgio	109.3 ± 19.3	94.6 ± 10.9	99.6 ± 15.8	101.2 ± 11.0
	Mean	600.4 ± 244.0	516.8 ± 218.8	243.7 ± 98.1	

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