Journal of Applied Ecology

BRITISH ECOLOGICAL SOCIETY

Journal of Applied Ecology 2017, **54,** 127–136 doi: 10.1111/1365-2664.12645

Genetic differentiation and regional adaptation among seed origins used for grassland restoration: lessons from a multispecies transplant experiment

Anna Bucharova[1,*], Stefan Michalski[2], Julia-Maria Hermann[3], Karola Heveling[4], Walter Durka[2,5], Norbert Hölzel[4], Johannes Kollmann[3] and Oliver Bossdorf[1]

[1]Plant Evolutionary Ecology, Institute of Evolution & Ecology, University of Tübingen, Auf der Morgenstelle 5, 72076 Tübingen, Germany; [2]Department of Community Ecology (BZF), Helmholtz Centre for Environmental Research – UFZ, Theodor-Lieser-Str. 4, 06120 Halle, Germany; [3]Restoration Ecology, Department of Ecology & Ecosystem Management, Technical University Munich, Emil-Ramann-Str. 6, 85354 Freising, Germany; [4]Biodiversity and Ecosystem Research Group, Institute of Landscape Ecology, University of Münster, Heisenbergstr. 2, 48149 Münster, Germany; and [5]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

---

Summary

1. One of the key questions in ecosystem restoration is the choice of seed material for restoring plant communities. More and more scientists and practitioners are currently advocating the use of regional seed sources, based on the argument that plants are often adapted to local or regional environmental conditions, and thus, regional seed sources should provide the best restoration success. However, there is still substantial debate about this approach, partly because of a lack of solid empirical data.

2. We conducted a multispecies transplant experiment in which we compared the performance of eight seed origins of seven plant species frequently used in grassland restoration in four common gardens across Germany.

3. We found that, on average, plants of regional origins produced 10% more inflorescences and 7% more biomass than those of foreign origins. There were substantial differences among species in the strength of these effects, but in the majority of the study species fitness decreased with increasing geographical distance of seed origins or with increasing climatic differences between plant origins and experimental sites.

4. In addition to these effects on plant fitness, increasing geographical or climatic distances of origin were often also correlated with increasing differences in plant phenology. Since phenology is important for biotic interactions, especially with pollinators and seed predators, using foreign seed sources may have cascading effects on local ecosystems.

5. *Synthesis and applications*. Genetic differentiation is widespread in grassland species and often shows the patterns of regional adaptation. Our study thus supports the use of regional seed sources in restoration. Moreover, using non-regional seed sources in grassland restoration may not only decrease the performance of plants, but it will likely also affect their biotic interactions.

Key-words: fitness, genetic differentiation, grassland restoration, local adaptation, multispecies experiment, phenology, reciprocal transplant, regional adaptation, regional provenancing, seed sourcing strategy

Introduction

Ecologists and environmental managers often wish to restore the biodiversity and ecological integrity of degraded ecosystems (Clewell, Atonson & Winterhalder 2004). In many cases, the critical first step in such ecological restoration is to reestablish target plant communities. However, in modern fragmented landscapes, plant reestablishment can be seriously constrained by impoverished seed banks, particularly in species with a limited dispersal capability (Bakker & Berendse 1999). Because of

*Correspondence author. E-mail: anna.lampei-bucharova@uni-tuebingen.de

this, ecological restoration *in situ* frequently requires the introduction of seeds from other sources (Hölzel, Buisson & Dutoit 2012). In this context, the question of seed origin has become the subject of intense debate (McKay *et al.* 2005; Broadhurst *et al.* 2008; Sgrò, Lowe & Hoffmann 2011; Breed *et al.* 2012; Jones 2013a,b).

One main strategy for the choice of seed material for ecosystem restoration is so-called local provenancing (Hamilton 2001), that is the use of local or regional seed sources for plant community restoration. It is based on the observation that almost all plants show genetic and phenotypic differentiation among populations and regions of origin (Linhart & Grant 1996; Keller, Kollmann & Edwards 2000; Bossdorf *et al.* 2005; Jay *et al.* 2012), and this often appears to reflect adaptation to local or regional differences in environmental conditions such as soil, climate or biotic interactions (Keller, Kollmann & Edwards 1999). As a result, local or regional plants and their communities frequently perform better and have a higher fitness when compared to foreign origins (e.g. Joshi *et al.* 2001; Becker *et al.* 2008; Rice & Knapp 2008; Raabová, Münzbergová & Fischer 2011; Weißhuhn *et al.* 2012). Local or regional provenancing is supported by many scientists (reviewed e.g. in McKay *et al.* 2005; Kiehl *et al.* 2010; Vander Mijnsbrugge, Bischoff & Smith 2010), and it is increasingly adopted by practitioners.

The local provenancing approach has also been questioned (e.g. Broadhurst *et al.* 2008; Crowe & Parker 2008; Hoffmann & Sgrò 2011; Sgrò, Lowe & Hoffmann 2011; Breed *et al.* 2012; Jones 2013a,b). A meta-analysis of local adaptation studies showed that there was evidence of local adaptation in about half of them (Leimu & Fischer 2008). While some researchers consider this sufficient support for the use of local seeds, others use the same number to question the 'local is best' paradigm (Sgrò, Lowe & Hoffmann 2011). Another argument against local seed sources is that particularly in fragmented landscapes, local seeds may frequently be inbred and genetically impoverished and may thus not provide sufficient genetic diversity for populations to adapt to climate change (Broadhurst *et al.* 2008; Crowe & Parker 2008; Havens *et al.* 2015).

Throughout this debate about local provenancing, arguments are usually based on plant performance such as growth and fitness of different seed origins when planted in the same environments. However, plants of different origins may also differ in their phenology (Prendeville *et al.* 2013; Quilot-Turion *et al.* 2013). This will likely influence the interactions of plants with their pollinators and seed herbivores (Elzinga *et al.* 2007). The changes in herbivores and pollinators might in turn affect their predators (Benedek *et al.* 2015). Using different seed origins may thus have cascading effects on other ecosystem components (Keller, Kollmann & Edwards 1999).

Ultimately, the question of whether the use of local seed material has positive or negative effects can only be answered empirically, through molecular and quantitative genetic analyses and through experiments that directly compare the performance of different origins in the same test environment. However, so far our knowledge is very limited and much of it comes from experiments with single or few species, which usually differ in design and statistical power and thus cannot be directly compared. For many of the species commonly used in grassland restoration, we have no empirical data at all.

Here, we present the results of a multispecies and multi-site study in which we compared the performance of multiple seed origins of seven plant species frequently used in grassland restoration in four common gardens across Germany. Our study was based on the newly established German system of seed transfer zones, which allows comparison of plants of regional origin with those from foreign origins, and thus to test for regional adaptation. We asked the following questions: (i) How common and how strong is genetic differentiation in growth, fitness and phenology across the studied species? (ii) Do regional seed sources generally outperform foreign ones? (iii) If origins differ in their performance, does performance decrease with increasing geographical or climatic distance of seed origins?

Materials and methods

SEED MATERIAL

In Germany, commercial production of regional wild plant seed material is currently establishing, and 22 seed transfer zones ('Herkunftsregionen'), grouped into eight larger regions, have been delimited based on climate, geology and other criteria (Prasse, Kunzmann & der Schrö 2010). In future, seed transfer should take place only within the 22 smaller transfer zones (ErMiV 2011), but the current practice is that seeds are transferred within the eight larger regions (Fig. 1; see also Durka *et al.* (2016) for more details). This makes some sense, since the eight regions represent larger biogeographical units and may thus have some important adaptations in common. For instance, although region 1 (Fig. 1) is large and specific origins may be several hundred kilometres apart, they are all part of the northern German lowlands with a rather mild and wet oceanic climate. In our study, we mimicked the current restoration practice and worked with the eight regions.

We worked with seven common grassland species frequently used in restoration: *Arrhenatherum elatius* (L.) P.B. ex J. et C. Presl, *Centaurea jacea* L., *Daucus carota* L., *Galium album* Mill., *Hypochaeris radicata* L., *Knautia arvensis* (L.) Coult. and *Lychnis flos-cuculi* (L.) Greuter & Burdet, all species abbreviated by genus name hereafter. For each species, we obtained seeds from all (or most of) eight geographical regions (Fig. 1). All seeds were purchased from a certified regional seed producer (Rieger-Hoffmann GmbH; Blaufelden, Germany) in spring 2013. Seeds from certified producers were collected from multiple large (>1000 individuals) wild populations in a given region to ensure genetic variability. The seeds were then farm-propagated for up to five generations and stored under species-specific conditions to enhance germination. For further information, see http://www.natur-im-vww.de.

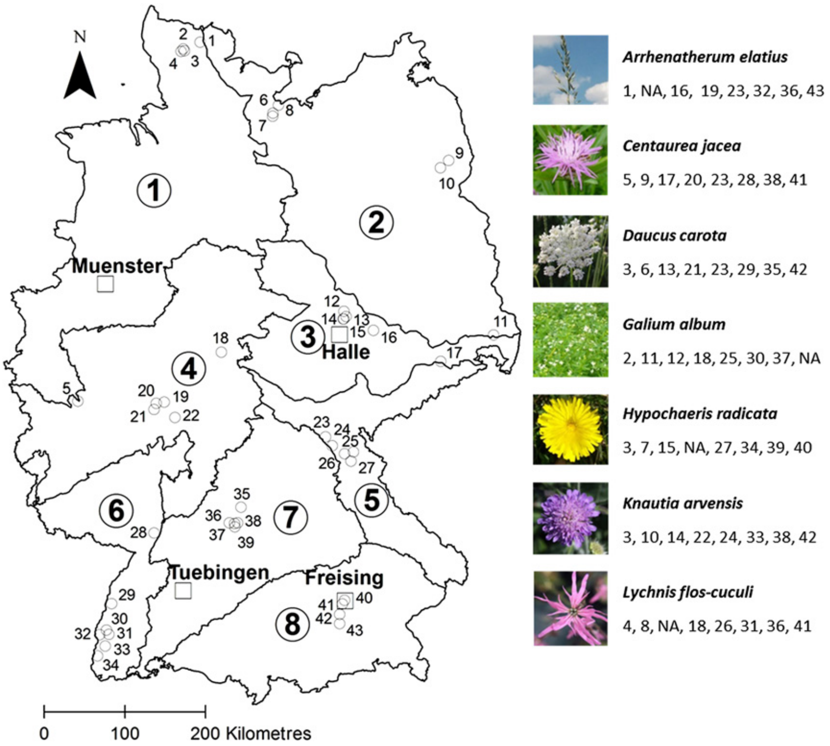

Fig. 1. Map of the main regions of seed production (1–8) for commercial wild plants in Germany, the locations of the four common gardens (☐) and all locations (○) where the seeds of the seven study species were originally collected. For each species, the eight locations of seed collection (= one or few neighbouring populations) are listed in the order of the regions (1–8). NA indicates that no seeds were available for a given species–region combination. [Colour figure can be viewed at wileyonlinelibrary.com]

COMMON GARDEN EXPERIMENT

In the summer of 2013, we carried out standardized and synchronized experiments in four common gardens in Germany, each located in a different region and close to one of the four participating research groups (Fig. 1). The common gardens were 200–550 km apart from each other and differed significantly in climate (Table S1 in Supporting Information). At the end of May, in each garden, we sowed 100 seeds of each of the 52 seed sources (seven species × eight seed origins, minus four missing species–origin combinations) into small trays filled with standard peat-based seeding substrate in an unheated glasshouse at each experimental site. When the majority of the seedlings had developed their first true leaves, we planted 12 randomly selected seedlings per species and origin into 2-L (14 × 14 cm upper surface) pots filled with a standard potting soil (Einheitserde Topferde, Patzer GmbH, Sinntal-Jossa, Germany). We kept the plants in the glasshouse for another week and then transferred them to the experimental gardens, where they were arranged in a fully randomized block design with four spatial blocks and three replicates per species and origin in each block. To make the experiment more realistic, we added 0·3 g grass seeds of a common grassland seed mixture (45% *Festuca rubra*, 35% *Festuca ovina*, 10% *Poa pratensis*, 5% *Agrostis capillaris*, 5% *Lolium perenne*) as a matrix of competitors to each pot after 2 weeks. The grasses never dominated the pots, though, and did not exceed 30% cover until the end of the experiment. To avoid drought-related mortality, the pots were watered when needed during the hottest summer period. In all gardens, we used the same pot size, the same substrate and the same seed material. The experimental set-up consisted of 624 plants in each common garden and a total of 2496 plants across all four gardens. Some plants died during the experiment, but the mortality rate was low at <5%. Throughout the experiment, we visited the plants three times per week and recorded their dates of first flowering. In early September 2013, we harvested all plants, counted the number of inflorescences produced by each plant (individual flowers for *Lychnis*), cut the biomass above-ground, dried it at 70 °C for 48 h and weighed it.

DATA ANALYSIS

For each plant, we analysed four response variables: total above-ground biomass, the presence or absence of flowers in the first year, the number of inflorescences and the date of first flowering. We used biomass and the number of inflorescences as measures of overall plant performance and fitness, whereas the other two variables described different aspects of plant phenology. For the number of inflorescences, we included only flowering plants, because we were working with perennial plants and only over one growing season. Some plants might simply not flower yet in the first year to accumulate resources (represented by biomass) for flowering in the next year. Assessing performance as the number of inflorescences is thus meaningful only in plants that do already flower, whereas the lack of flowering does not mean low performance.

We asked a sequence of related questions: (i) Do plants from different regions differ in performance or phenology? If yes, do these differences depend on the experimental garden? (ii) For the fitness-related traits, we asked whether plants from the same region as the garden generally performed better than plants from other regions (Kawecki & Ebert 2004). However, since the eight regions are rather large (Fig. 1), the actual distances between seed origins and their 'home' gardens within the same region were 7–320 km, so that in some cases the origins of 'foreign' plants were geographically closer than those of plants from the same regions (Fig. 1). To account for this, (iii) we asked whether differences in plant performance were continuously related to the geographical or climatic distance between each individual seed collection locality and each experimental garden (Fig. 1). For the climatic distance, we focused on summer temperatures (June–August), because our experiments took place in summer and because this was the main climatic factor that was not experimentally controlled. To characterize the climates of seed origins, we used the average long-term (50-year) summer temperatures (June–August) for the respective locations provided by the WorldClim data base (Hijmans *et al.* 2005), and to characterize the experimental sites, we used the actual summer temperatures from the year 2013 (data from nearest meteorological station, www.dwd.de). Geographical distances (km) and temperature distances (°C) were only weakly correlated with each other (all correlation coefficients, Pearson's $r < 0{\cdot}2$, calculated separately for each species).

We analysed the data with generalized linear mixed-effects models, using the *lme4* package in R (R Development Core Team 2009). Corresponding to the three questions above, we fitted the following types of models for each species and response variable: (i) a model that contained region of origin, experimental garden and their interactions as fixed effects and block as a random effect; (ii) a model that tested the effect of regional origin (one ecotype per garden, from the same region where the garden was located) vs. non-regional origin (seven other ecotypes originating from other regions) while correcting for the fixed effect of planting date and the random effects of block, garden and region of origin. This model was performed for the fitness-related variables only; (iii) models testing for the influences of the continuous variables – geographical distance and absolute temperature distance – with the same additional fixed and random effects as in model 2. Total biomass and the time of first flowering were analysed with GLMMs with a normal error distribution, whereas for the number of inflorescences and the presence of flowers (binary variable), we used GLMMs with a Poisson (log-link function) or binomial error distribution (logit-link function), respectively. In the models with Poisson error, we corrected for overdispersion by fitting individual random effects (Bolker *et al.* 2009). For the fitness variables, we also did a cross-species version of model 2 where we combined the data from all species and included species as a random effect, to test whether regional plants generally perform better than foreign ones. We could not do the same cross-species tests for model 3, because the cross-species models did not converge.

To get an idea of the true magnitudes of the effects of regional origin, geographical or climatic distance, we used the parameter estimates from the GLMMs to calculate the absolute differences in biomass, reproduction, flowering probability or flowering time between plants of regional origin and the average of all other origins, or the absolute changes in the response variables per 100 km distance or per 1 °C temperature difference. In the linear variables – biomass and onset of flowering – the parameter estimates directly represented these differences, and thus, the estimated effects are absolute and in original units. In the other variables, for the number of inflorescences and probability of flowering that had been analysed with GLMMs with Poisson and binomial error distribution, respectively, the parameter estimates needed to be back-transformed in order to obtain values in the original units, and the effects could only be expressed as multiplicative change.

We calculated credible intervals, a Bayesian analogue of confidence intervals, for these effect sizes based on 10 000 simulations of the mean and variance of each estimate which are probable for given data, using the *sim* function in the R package *arm* (for details, see Gelman & Hill (2006)).

Finally, to describe the magnitude of heritable differentiation in phenotype among origins, we calculated broad-sense P_{ST} values (Leinonen *et al.* 2006; Kawakami *et al.* 2011) for all response variables as the ratio between the variance explained by origin and the total variance corrected for the effect of garden, that is among-origin variance plus residual variance. As variance estimates, we used the deviance values from the generalized linear models in R. We used P_{ST} as a measure of phenotypic differentiation because it allowed comparison across traits and species.

Results

GENETIC DIFFERENTIATION WITHIN SPECIES

All of the investigated species showed a significant genetic differentiation in growth and reproduction, as well as in phenology (Table 1 and Table S2). We found a significant region-of-origin effect in the number of inflorescences for all species, in biomass for all species except *Arrhenatherum* and in flowering time for all species except *Knautia* (here, the effect was only marginally significant). The probability of flowering in the first year was significantly differentiated in five out of the seven species.

There were significant interactions between region of origin and garden in all species for the number of inflorescences, and in five out of seven species for timing of flowering and total biomass (Table 1 and Table S2), indicating a variation in the plasticity of these traits. However, there were no such interactions for the probability of flowering (and hardly any garden main effects; Table S2), indicating that this trait is generally less plastic than the other traits.

When we quantified phenotypic differentiation through P_{ST} values (Table 2), we found that the phenotypic differentiation among regions was generally higher in the phenology-related traits (on average, 0·20 and 0·17 for probability of flowering and timing of flowering, respectively) than in the fitness-related traits (0·08 and 0·11 for biomass and the number of inflorescences, respectively).

The species differed substantially in their degree of phenotypic differentiation. With regard to the fitness traits, the most differentiated species were (in that order) *Knautia*, *Galium*, *Lychnis* and *Centaurea*, often with more than twofold differences in biomass and the number of inflorescences between different seed origins (Tables S2 and S3), whereas the other three species were much less differentiated. Phenology traits were strongly differentiated in many of the studied species, particularly in *Centaurea*, *Galium* and *Lychnis* (Tables S2 and S4). For example, the probability of flowering varied from 0·25 to 0·93 among different *Lychnis* origins and from 0·02 to 0·6 among *Knautia* origins. *Galium* and *Centaurea* plants from different origins differed by up to 23 and 17 days, respectively, in their time of flowering.

Table 1. Genetic differentiation and regional adaptation in (A) fitness- and (B) phenology-related traits of seven common grassland species (ARR, *Arrhenatherum elatius*; CEN, *Centaurea jacea*; DAU, *Daucus carota*; GAL, *Galium album*; HYP, *Hypochaeris radicata*; KNA, *Knautia arvensis*; and LYC, *Lychnis flos-cuculi*). Cell colours and values indicate the direction and magnitude of effects and a statistical significance of different hypotheses. In fitness-related traits, the last three hypotheses are directional (regionals do better: if true, effect size is positive. Performance decreases with increasing geographical or climatic distance: if true, effect size is negative), and colour indicates whether the results are in the predicted direction (= green) or not (= red), with significant effects in dark and non-significant ones in light colours. In all other tests with non-directional hypotheses, blue indicates a statistical significance. The values in the cells indicate the predicted effect sizes, obtained from back-transformed model coefficients. For biomass and onset of flowering, the numbers represent the absolute differences between regional and non-regional plants, per 100 km distance or per 1 ℃ (negative values mean decrease, positive increase). For the other two traits – the number of inflorescences and probability of flowering – the values indicate the multiplicative change for the same comparisons/tests as above (values < 1 represent decrease, and values > 1 represent increase)

(A) FITNESS-RELATED TRAITS	ARR	CEN	DAU	GAL	HYP	KNA	LYC
Biomass							
Origins differ		***	***	**	***	***	***
Origin × Garden	(*)	*	**	***		***	***
Regional plants do better (+)	-0.4%	+14.2%	-1.3%	+38.9%***	+12.3%(*)	-3.3%	+15.9%**
Biomass decreases with geographical distance (-)	+1.6%	-4.8% (*)	-4.1%	-8.8%**	-3.4%*	-0.6%	+1.9%
Biomass decreases with climatic distance (-)	-0.05%	-16.6% *	+8.7%	-8.0%**	+2.8%	+34.8%	-2.5%
#Inflorescences							
Origins differ	**	***	***	***	***	***	***
Origin × Garden	***	***	***	***	***	***	***
Regional plants do better	1.05	1.34*	1.01	0.81	1.08	0.97	1.37 *
#Inflorescences decreases with geographical distance	1.00	0.90**	0.92*	1.09	0.98	1.06	1.04
#Inflorescences decreases with climatic distance	0.92(*)	0.87*	1.08	1.26	0.99	1.74	0.80 **

(B) PHENOLOGY	ARR	CEN	DAU	GAL	HYP	KNA	LYC
Probability of flowering							
Origins differ		***	**	***		***	***
Origin × Garden							
Phenology changes with geographical distance	1.03	0.97*	0.97*	0.97	1.03(*)	0.84**	1.02
Phenology changes with climatic distance	0.99*	1.03	1.03	1.04	1.00	1.09	0.96
Timing of flowering							
Origins differ	***	***	***	***	***	(*)	***
Origin × Garden	*		*	*	(*)	*	**
Phenology changes with geographical distance (days)	+0.1	+0.3	+0.8	+0.5	-0.1	-0.4	+0.0
Phenology changes with climatic distance (days)	+1.1(*)	-2.4	+1.3	-5.5**	+1.9*	+0.5	+1.7*

Levels of significance are indicated by (*) for $P < 0{\cdot}1$; $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; and $***P < 0{\cdot}001$.

Table 2. Phenotypic differentiation (P_{ST}) among plants from different regions of seed origin in seven common grassland species

	Biomass	Inflorescences	Probability of flowering	Timing of flowering
Arrhenatherum elatius	0·01	**0·01**	0·06	**0·12**
Centaurea jacea	**0·05**	**0·14**	**0·26**	**0·20**
Daucus carota	**0·03**	**0·06**	**0·10**	**0·08**
Galium album	**0·08**	**0·18**	**0·23**	**0·45**
Hypochaeris radicata	**0·03**	**0·04**	0·30	**0·10**
Knautia arvensis	**0·28**	**0·14**	**0·21**	0·02
Lychnis flos-cuculi	**0·07**	**0·17**	**0·23**	**0·19**

Values in boldface indicate cases where the log-likelihood ratio test for the effect of seed origin was significant ($P < 0{\cdot}05$, for P-values, see Table S2).

REGIONAL ADAPTATION

In two of the studied species, plants of regional origins generally produced a significantly greater biomass than those of non-regional origin: *Galium* (average gain of +38·9% biomass, and *Lychnis* +15·9% biomass). In *Hypochaeris*, the region vs. foreign effect was marginally significant (+12·3% biomass) (Table 1, Fig. S1a). Moreover, in two species, plants of regional origins had generally a greater reproductive output: in *Centaurea* and *Lychnis*,

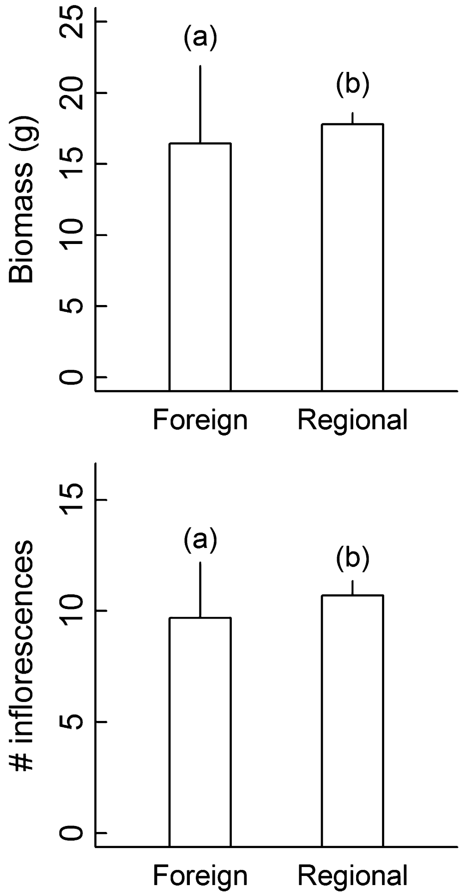

Fig. 2. The performance of regional plants vs. foreign ones across all species and gardens. Plotted values of both biomass and the number of inflorescences are obtained from fitted values of the respective models (model 2). Vertical bars represent standard errors. Local plants perform marginally better than foreign ones in terms of biomass ($P = 0{\cdot}07$) and the number of inflorescences ($P = 0{\cdot}09$).

the number of inflorescences of regional plants was on average 1·3 times and 1·4 times higher, respectively, than that of non-regional plants. Taken together, we found evidence for regional adaptation in the fitness-related traits in three out of the seven studied species. There was no evidence in the opposite direction, that is that non-foreign plants would generally outperform regional ones, in any of the species (Table 1A). Across all species, regional plants produced 7·5% more biomass ($P = 0{\cdot}07$, credible interval −1·4 to 16·1%) and produced 10% more inflorescences ($P = 0{\cdot}09$, 0·99–1·24; Fig. 2).

GEOGRAPHICAL AND CLIMATIC DISTANCE

In four out of the seven studied species – *Centaurea, Daucus*, *Galium* and *Hypochaeris* – we found a significant decrease in fitness-related traits (biomass or the number of inflorescences) with increasing geographical distance of origin (Table 1A, Fig. S1b). The strongest effect was in *Galium* where biomass decreased by 8·8% per 100 km, resulting in a 61·6% decrease over the 700-km range of our study. In *Centaurea*, the number of inflorescences decreased strongly with increasing geographical distance: 0·9 times per 100 km, corresponding to a decrease to 0·48 of the maximum reproduction over the entire range of our study. In addition to these relationships with geographical distance, we also found significant correlations between fitness-related traits and climatic distances of plant origins in three out of the seven studied species. In *Centaura*, *Galium* and *Lychnis,* either biomass or the number of inflorescences significantly decreased with increasing climatic distance (Table 1A, Fig. S1c).

When we examined the effects of geographical and climatic distance on phenology traits, we found that in three species geographical distance was significantly related to the probability of flowering in the first year, but there was no relationship with the timing of flowering (Table 1B, Fig. S1b). In contrast to geographical distance, climatic distance was related to the timing of flowering, in three out of the seven species: *Galium*, *Hypochaeris* and *Lychnis* (Table 1B, Fig. S1c). The strongest effect was in *Galium* with a 5·5-day shift of flowering date per 1 °C difference between origin and common garden and with a predicted shift of flowering time by 19·2 days over the entire range of our study.

The degree to which plant fitness or phenology was related to geographical vs. climatic distance was highly species and trait specific (Table 1). In some species, climatic distance of origin was the stronger predictor of fitness (*Centaurea*: 58% decrease in biomass over the whole temperature range of 3·5 °C, but only 34% decrease across the geographical range of 700 km). In other species, geographical distance was a better fitness predictor (*Galium*: 56% biomass decrease over geographical range, but only 31% over temperature range).

Discussion

The local provenancing approach in plant restoration is based on the assumption that plants are generally geographically differentiated and adapted to regional environmental conditions. In a multi-site common garden experiment, we indeed found a substantial genetic differentiation, both in fitness-related traits and in phenology, among different seed origins of most of the studied species. In several of the species, there was evidence of regional adaptation, as plants of regional origins or from geographically closer or climatically more similar origins often performed better than plants from non-regional origins or those from more geographically or climatically distant origins.

GENETIC DIFFERENTIATION WITHIN SPECIES

In all of the seven studied species, we found a significant genetic differentiation among plant origins in at least two out of the four measured traits. However, the degree of differentiation was species and trait specific. Fitness-related traits appear to be generally less differentiated than phenological traits. This is not surprising since the variation in fitness-related traits is under strong directional selection limiting differentiation among populations (Geber & Griffen 2003; Kingsolver & Diamond 2011). The only species with a high P_{ST} in fitness-related traits was *Knautia*, a species that turned out to occur as both diploid and tetraploids in our study (Durka *et al.* 2016). Plants with different ploidy levels are known to show different vigour and fitness (Otto & Whitton 2000).

The stronger differentiation of phenology-related traits likely reflects plant adaptation to different latitudes, climate and seasonality, or different plant strategies in different environments. It is well known that flowering time is often strongly differentiated along geographical gradients, even within perennial plants (e.g. Montague, Barrett & Eckert 2008; Kawakami *et al.* 2011). Moreover, traits related to life span, such as the probability of flowering in the first year, are often also strongly genetically differentiated (VanDijk *et al.* 1997). Accumulating further resources through extended growth before flowering may only be adaptive in some environments (Hesse, Rees & Müller-Schärer 2008; Chamorro & Sans 2010).

REGIONAL ADAPTATION

Across all species and experimental sites, plants of regional origin produced on average 7% more biomass and 10% more inflorescences than plants of non-regional origins. All of the study species are common and frequently used in grassland restoration, and to our knowledge, and although only marginally significant, this is some of the strongest and most general evidence for regional plant adaptation to date.

Most of our previous knowledge on local and regional adaptation comes from studies of individual or few species. A meta-analysis of these studies found evidence for local adaptation in 45% of them (Leimu & Fischer 2008). This ratio is rather similar to our study where regional origins outperformed foreign ones in four out of seven species. Most importantly, we found no evidence *against* regional adaptation. Foreign plants never performed significantly better than regional plants, and non-significant trends mostly pointed towards regional adaptation. The magnitude of the advantage of regional origins we found in our study was strongly species specific, with effect sizes from 16% to almost 40% increased performance (Table 1). These effect sizes are within the range of the values reported in previous studies on local adaptation (e.g. Joshi *et al.* 2001; Becker *et al.* 2006, 2008; Leimu & Fischer 2008; Raabová, Münzbergová & Fischer 2011). Together with the evidence from previous studies, our results suggest that regional adaptation at a scale relevant for restoration practice is indeed fairly common among grassland plants.

Regional adaptation of plants affects the performance of the plants as well as influences the performance of interacting organisms. Plant populations have often co-evolved with their co-occurring herbivores (Low, Ellner & Holden 2013), pollinators (Elzinga *et al.* 2007) or pathogens (Thrall *et al.* 2012). One of the key traits in this context is plant phenology because it determines the synchrony of these interactions and thus their intensity. We found that in most of the study species, plants of different origins substantially differed in their probability of flowering in the first year, as well as in their timing of flowering: up to 17 days in *Centaurea* and up to 23 days in *Galium*. This phenology difference is large enough to affect pollinator densities (Westphal, Steffan-Dewenter & Tscharntke 2003; Steffan-Dewenter & Schiele 2008) and to increase risk of extinction for specific pollinators (Memmott *et al.* 2007). Additionally, if flowering time of restored plant populations differs from that of adjacent local populations, gene flow from and into local populations may be hampered. This might have two consequences. First, the lack of gene flow may prevent outbreeding depression both in local and in novel population (Montalvo & Ellstrand 2001; Caruso *et al.* 2015). Secondly, isolated novel populations, especially if they are small, will face negative effects of genetic drift and inbreeding, ultimately leading to a decrease in plant performance (Walisch *et al.* 2012).

A common argument against the use of regional provenances in ecological restoration is that these plants may not be able to cope with warmer climates in future (Parmesan 2006; Kreyling *et al.* 2011; Sgrò, Lowe & Hoffmann 2011), and therefore, ecotypes adapted to the predicted future climates might be more suitable. However, the year 2013, in which we conducted our study, was one of the warmest years on record (http://www.ncdc.noaa.gov/sotc/global/2013/13), with temperatures in our experimental gardens 1·5–2·0 °C higher than the long-term means. In spite of this, regional plants still tended to perform better on average than non-regional ones, and plants from warmer climates did not appear to have any particular advantage (A. Bucharova, unpublished data), indicating that regional adaptation must involve other climatic or non-climatic factors, such as range and variability of temperature, photoperiod or regional microbial communities, which together override a possible advantage of adaptation to mean temperature. Given that the introduction of novel ecotypes from warmer climates is likely also going to disrupt plant biotic interactions, we thus cannot advocate the strategy of deliberately introducing plant ecotypes from warmer origins.

GEOGRAPHICAL AND CLIMATIC DISTANCE

Although fitness comparisons of local vs. foreign origins, as discussed above, represent classic tests for local adaptation, a more precise approach is to relate a continuous measure of the geographical, environmental or ecological distances of plant origins to their relative fitness or other important traits. Therefore, we also tested for relationships between geographical and climatic distance of plant origin and fitness-related traits and phenology in the common gardens. In most of the seven studied species, we found that either biomass or reproduction decreased with increasing geographical or climatic differences between plant origin and testing site. In many species, we found that phenological differences increased with increasing climatic distance. The further away or the more climatically different the seed origins were, the higher the risk of maladaptation or of mismatch in biotic interactions.

Although both geographical and climatic distances were significantly correlated with fitness-related traits or phenology in several of the studied species, there was no general pattern as to whether climatic or geographical distance generally was a better predictor of plant performance. In previous studies, the geographical distance between plant origin and planting site proved to be a good predictor of plant performance (e.g. Galloway & Fenster 2000; Joshi *et al.* 2001; Becker *et al.* 2006, 2008), whereas in others it was the climatic difference between origin and planting site (Weißhuhn *et al.* 2012), the similarity of habitats (Bischoff *et al.* 2006) or the general ecological similarity of the two sites (Smith *et al.* 2009). The effects of geographical and climatic distance on plant performance and phenology are thus highly species specific.

Our experiment did not cover the full spectrum of potential climatic effects. First, we watered the plants, so that the main climatic factor that differed and likely determined differential plant success within the growth season was temperature. Secondly, while restoration of grasslands is commonly done via seed sowing, we started our experiments with seedlings. We thus missed seedling establishment as one of the most environmentally sensitive life stages of plants (Gimenez-Benavides, Escudero & Iriondo 2007). Thirdly, our experiment ran only over one summer, and we thus did not capture winter hardiness and cold resistance of seedlings as one of main adaptations to climatic variability (Lu, Joyce & Sinclair 2003). All these effects of climate considerably contribute to plant adaptation, and we may thus have even underestimated the true magnitude of regional climate effects and estimates of regional adaptation. With natural differences in rainfall, sowing seeds earlier in the spring directly outside and including overwintering of plants, the differences between regional and non-regional may have been even larger.

Conclusions

The goal of our study was to test the frequency and strength of genetic differentiation and regional adaptation in common grassland species. Populations of the seven studied species showed a strong genetic differentiation across a range of several hundred kilometres. In several of the species, plants of regional origins outperformed non-regional plants, and often, fitness decreased with increasing geographical or climatic distance of origin. This indicates that many plant species are indeed adapted to regional climates and other environmental factors and that the risk of introducing maladapted genotypes will increase with increasing distance of the seed source. Moreover, non-regional plants often differed substantially in their phenology, which suggests that using non-regional seed sources may also have cascading effects on pollinators and other biotic interactions and thus far-reaching ecosystem consequences.

Acknowledgements

We are grateful to Ernst Rieger and Johann Krimmer for their expert advice and to the numerous helpers in Freising, Halle, Münster and Tübingen who participated in the set-up, maintenance and harvest of the experiments. We thank Dierk Kunzmann for providing GIS data for the map. Our study was supported by a DFG grant (KO 1741/3-1) and TUM Internal grant to JK and JMH.

Data accessibility

Data are archived in Dryad Digital Repository doi: 10.5061/dryad.1fj40 (Bucharova *et al.* 2016).

References

Bakker, J. & Berendse, F. (1999) Constraints in the restoration of ecological diversity in grassland and heathland communities. *Trends in Ecology & Evolution*, **14**, 63–68.

Becker, U., Colling, G., Dostal, P., Jakobsson, A. & Matthies, D. (2006) Local adaptation in the monocarpic perennial *Carlina vulgaris* at different spatial scales across Europe. *Oecologia*, **150**, 506–518.

Becker, U., Dostal, P., Jorritsma-Wienk, L.D. & Matthies, D. (2008) The spatial scale of adaptive population differentiation in a wide-spread, well-dispersed plant species. *Oikos*, **117**, 1865–1873.

Benedek, K., Bálint, J., Salamon, R.V., Kovács, E., Ábrahám, B., Fazakas, C., Loxdale, H.D. & Balog, A. (2015) Chemotype of tansy (*Tanacetum vulgare* L.) determines aphid genotype and its associated predator system. *Biological Journal of the Linnean Society*, **114**, 709–719.

Bischoff, A., Crémieux, L., Smilauerova, M., Lawson, C.S., Mortimer, S.R., Dolezal, J. *et al.* (2006) Detecting local adaptation in widespread grassland species – The importance of scale and local plant community. *Journal of Ecology*, **94**, 1130–1142.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.-S.S. (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution*, **24**, 127–135.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W.E., Siemann, E. & Prati, D. (2005) Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, **144**, 1–11.

Breed, M.F., Stead, M.G., Ottewell, K.M., Gardner, M.G. & Lowe, A.J. (2012) Which provenance and where? Seed sourcing strategies for revegetation in a changing environment. *Conservation Genetics*, **14**, 1–10.

Broadhurst, L.M., Lowe, A., Coates, D.J., Cunningham, S.A., McDonald, M., Vesk, P.A. & Yates, C. (2008) Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Applications*, **1**, 587–597.

Bucharova, A., Michalski, S., Hermann, J.-M., Heveling, K., Durka, W., Hölzel, N., Kollmann, J. & Bossdorf, O. (2016) Data from: Genetic differentiation and regional adaptation among seed origins used for grassland restoration: lessons from a multi-species transplant experiment. Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.1fj40.

Caruso, C.M., Benscoter, A.M., Gale, N.V., Seifert, E.K., Mills, E.R. & Case, A.L. (2015) Effects of crossing distance on performance of the native wildflower *Lobelia siphilitica*: implications for ecological restoration. *Journal of the Royal Statistical Society*, **142**, 140–151.

Clewell, A., Aronson, J. & Winterhalder, K. (2004) The SER International primer on ecological restoration. *Ecological Restoration*, **2**, 206–207.

Chamorro, L. & Sans, F.X. (2010) Life-history variation in agricultural and wild populations of *Erucastrum nasturtiifolium* (Brassicaceae). *Flora - Morphology, Distribution, Functional Ecology of Plants*, **205**, 26–36.

Crowe, K.A. & Parker, W.H. (2008) Using portfolio theory to guide reforestation and restoration under climate change scenarios. *Climatic Change*, **89**, 355–370.

Durka, W., Michalski, S., Berendzen, K., Bucharova, A., Hermann, J., Hölzel, N. & Kollmann, J. (2016) Genetic differentiation within multiple common grassland plants supports seed transfer zones for ecological restoration. *Journal of Applied Ecology*. doi: 10.1111/1365-2664.12636

Elzinga, J.A., Atlan, A., Biere, A., Gigord, L., Weis, A.E. & Bernasconi, G. (2007) Time after time: flowering phenology and biotic interactions. *Trends in Ecology & Evolution*, **22**, 432–439.

ErMiV (2011) Regulation about the placing on the market of seed of conservation mixtures. Bundesgesetzblatt I S. 2641.

Galloway, L.F. & Fenster, C.B. (2000) Population differentiation in an annual legume: local adaptation. *Evolution*, **54**, 1173–1181.

Geber, M.A. & Griffen, L.R. (2003) Inheritance and natural selection on functional traits. *International Journal of Plant Sciences*, **164**, S21–S42.

Gelman, A. & Hill, J. (2006) *Data Analysis Using Regression and Multilevel/hierarchical Models*. Cambridge University Press, New York.

Gimenez-Benavides, L., Escudero, A. & Iriondo, J.M. (2007) Local adaptation enhances seedling recruitment along an altitudinal gradient in a high mountain Mediterranean plant. *Annals of Botany*, **99**, 723–734.

Hamilton, N.R.S. (2001) Is local provenance important in habitat creation? A reply. *Journal of Applied Ecology*, **38**, 1374–1376.

Havens, K., Vitt, P., Still, S., Kramer, A.T., Fant, J.B. & Schatz, K. (2015) Seed sourcing for restoration in an era of climate change. *Natural Areas Journal*, **35**, 122–133.

Hesse, E., Rees, M. & Müller-Schärer, H. (2008) Life-history variation in contrasting habitats: flowering decisions in a clonal perennial herb (*Veratrum album*). *The American Naturalist*, **172**, 196–213.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hoffmann, A.A. & Sgrò, C.M. (2011) Climate change and evolutionary adaptation. *Nature*, **470**, 479–485.

Hölzel, N., Buisson, E. & Dutoit, T. (2012) Species introduction – a major topic in vegetation restoration. *Applied Vegetation Science*, **15**, 161–165.

Jay, F., Manel, S., Alvarez, N., Durand, E.Y., Thuiller, W., Holderegger, R., Taberlet, P. & François, O. (2012) Forecasting changes in population genetic structure of alpine plants in response to global warming. *Molecular Ecology*, **21**, 2354–2368.

Jones, T.A. (2013a) Ecologically appropriate plant materials for restoration applications. *BioScience*, **63**, 211–219.

Jones, T.A. (2013b) When local isn't best. *Evolutionary Applications*, **6**, 1109–1118.

Joshi, J., Schmid, B., Caldeira, M.C., Dimitrakopoulos, P.G., Good, J., Harris, R. *et al.* (2001) Local adaptation enhances performance of common plant species. *Ecology Letters*, **4**, 536–544.

Kawakami, T., Morgan, T.J., Nippert, J.B., Ocheltree, T.W., Keith, R., Dhakal, P. & Ungerer, M.C. (2011) Natural selection drives clinal life history patterns in the perennial sunflower species, *Helianthus maximiliani*. *Molecular Ecology*, **20**, 2318–2328.

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

Keller, M., Kollmann, J. & Edwards, P.J. (1999) Palatability of weeds from different European origins to the slugs *Deroceras reticulatum* Müller and *Arion lusitanicus* Mabille. *Acta Oecologica*, **20**, 109–118.

Keller, M., Kollmann, J. & Edwards, P.J. (2000) Genetic introgression from distant provenances reduces fitness in local weed populations. *Journal of Applied Ecology*, **37**, 647–659.

Kiehl, K., Kirmer, A., Donath, T.W., Rasran, L. & Hölzel, N. (2010) Species introduction in restoration projects – evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. *Basic and Applied Ecology*, **11**, 285–299.

Kingsolver, J.G. & Diamond, S.E. (2011) Phenotypic selection in natural populations: what limits directional selection? *The American Naturalist*, **177**, 346–357.

Kreyling, J., Bittner, T., Jaeschke, A., Jentsch, A., Jonas Steinbauer, M., Thiel, D. & Beierkuhnlein, C. (2011) Assisted colonization: a question of focal units and recipient localities. *Restoration Ecology*, **19**, 433–440.

Leimu, R. & Fischer, M. (2008) A meta-analysis of local adaptation in plants. *PLoS ONE*, **3**, e4010.

Leinonen, T., Cano, J.M., Mäkinen, H. & Merilä, J. (2006) Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *Journal of Evolutionary Biology*, **19**, 1803–1812.

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

Low, C., Ellner, S.P. & Holden, M.H. (2013) Optimal control and cold war dynamics between plant and herbivore. *The American Naturalist*, **182**, E25–E39.

Lu, P., Joyce, D.G. & Sinclair, R.W. (2003) Geographic variation in cold hardiness among eastern white pine (*Pinus strobus* L.) provenances in Ontario. *Forest Ecology and Management*, **178**, 329–340.

McKay, J.K., Christian, C.E., Harrison, S. & Rice, K.J. (2005) 'How local is local?' - A review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology*, **13**, 432–440.

Memmott, J., Craze, P.G., Waser, N.M. & Price, M.V. (2007) Global warming and the disruption of plant-pollinator interactions. *Ecology Letters*, **10**, 710–717.

Montague, J.L., Barrett, S.C.H. & Eckert, C.G. (2008) Re-establishment of clinal variation in flowering time among introduced populations of purple loosestrife (*Lythrum salicaria*, Lythraceae). *Journal of Evolutionary Biology*, **21**, 234–245.

Montalvo, A.M. & Ellstrand, N.C. (2001) Nonlocal transplantation and outbreeding depression in the subshrub *Lotus scoparius* (Fabaceae). *American Journal of Botany*, **88**, 258.

Otto, S.P. & Whitton, J. (2000) Polyploid incidence and evolution. *Annual Review of Genetics*, **34**, 401–437.

Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 637–669.

Prasse, R., Kunzmann, D. & der Schrö, R. (2010) *Development and Practical Implementation of Minimal Requirements for the Verification of Origin of Native Seeds of Herbaceous Plants*.

Prendeville, H.R., Barnard-Kubow, K., Dai, C., Barringer, B.C. & Galloway, L.F. (2013) Clinal variation for only some phenological traits across a species range. *Oecologia*, **173**, 421–430.

Quilot-Turion, B., Leppälä, J., Leinonen, P.H., Waldmann, P., Savolainen, O. & Kuittinen, H. (2013) Genetic changes in flowering and morphology in response to adaptation to a high-latitude environment in *Arabidopsis lyrata*. *Annals of Botany*, **111**, 957–968.

R Development Core Team (2009) R: A language and environment for statistical computing.

Raabová, J., Münzbergová, Z. & Fischer, M. (2011) The role of spatial scale and soil for local adaptation in *Inula hirta*. *Basic and Applied Ecology*, **12**, 152–160.

Rice, K.J. & Knapp, E.E. (2008) Effects of competition and life history stage on the expression of local adaptation in two native bunchgrasses. *Restoration Ecology*, **16**, 12–23.

Sgrò, C.M., Lowe, A.J. & Hoffmann, A.A. (2011) Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications*, **4**, 326–337.

Smith, B.M., Diaz, A., Daniels, R., Winder, L. & Holland, J.M. (2009) Regional and ecotype traits in *Lotus corniculatus* L., with reference to restoration ecology. *Restoration Ecology*, **17**, 12–23.

Steffan-Dewenter, I. & Schiele, S. (2008) Do resources or natural enemies drive bee population dynamics in fragmented habitats? *Ecology*, **89**, 1375–1387.

Thrall, P.H., Laine, A.-L., Ravensdale, M., Nemri, A., Dodds, P.N., Barrett, L.G. & Burdon, J.J. (2012) Rapid genetic change underpins antagonistic coevolution in a natural host-pathogen metapopulation. *Ecology Letters*, **15**, 425–435.

Vander Mijnsbrugge, K., Bischoff, A. & Smith, B. (2010) A question of origin: where and how to collect seed for ecological restoration. *Basic and Applied Ecology*, **11**, 300–311.

VanDijk, H., Boudry, P., McCombie, H. & Vernet, P. (1997) Flowering time in wild beet (*Beta vulgaris* ssp. maritima) along a latitudinal cline. *Acta Oecologica-International Journal of Ecology*, **18**, 47–60.

Walisch, T.J., Colling, G., Poncelet, M. & Matthies, D. (2012) Effects of inbreeding and interpopulation crosses on performance and plasticity of two generations of offspring of a declining grassland plant. *American Journal of Botany*, **99**, 1300–1313.

Weißhuhn, K., Prati, D., Fischer, M. & Auge, H. (2012) Regional adaptation improves the performance of grassland plant communities. *Basic and Applied Ecology*, **13**, 551–559.

Westphal, C., Steffan-Dewenter, I. & Tscharntke, T. (2003) Mass flowering crops enhance pollinator densities at a landscape scale. *Ecology Letters*, **6**, 961–965.

Received 9 October 2015; accepted 4 March 2016
Handling Editor: Shiqiang Wan

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Graphs to the models in Table 1.

Table S1. Climate in the common gardens.

Table S2. *P*-values indicating significances of the genetic differentiation of plant origins.

Table S3. *P*-values and confidence intervals of the models testing for better performance of local plants and the effect of geographical and climatic distance on fitness-related traits and phenology traits.

Table S4. Values of individual traits for each species and regional ecotypes.