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Local adaptation in the monocarpic perennial *Carlina vulgaris* at different spatial scales across Europe

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Abstract Spatial variation in environmental conditions can lead to local adaptation of plant populations, particularly if gene flow among populations is low. Many studies have investigated adaptation to contrasting environmental conditions, but little is known about the spatial scale of adaptive evolution. We studied population differentiation and local adaptation at two spatial scales in the monocarpic grassland perennial *Carlina vulgaris*. We reciprocally transplanted seedlings among five European regions (northwestern Czech Republic, central Germany, Luxembourg, southern Sweden and northwestern Switzerland) and among populations of different sizes within three of the regions. We recorded survival, growth and reproduction over three growing periods. At the regional scale, several performance traits and the individual fitness of *C. vulgaris* were highest if the plants were grown in their home region and they decreased with increasing transplant distance. The effect\s are likely due to climatic differences that increased with the geographical distance between regions. At the local scale, there were significant interactions between the effects of the population of origin and the transplant site, but these were not due to an enhanced performance of plants at their home site and they were not related to the geographical or environmental distance between the site of origin and the transplant site. The size of the population of origin did not influence the strength of local adaptation. The results of our study suggest that *C. vulgaris* consists of regionally adapted genotypes, and that distance is a good predictor of the extent of adaptive differentiation at large scales (> 200 km) but not at small scales. We conclude that patterns of local adaptation should be taken into account for the efficient preservation of genetic resources, when assessing the status of a plant species and during conservation planning.

Keywords Plasticity · Population differentiation · Population size · Reciprocal transplant experiment

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Introduction

Many plant species have a large geographical range, over which environmental conditions can vary considerably. There are two principal mechanisms that may explain why a species is able to grow under different conditions: phenotypic plasticity and evolutionary

adaptation. Phenotypic plasticity is the capacity of a genotype to express varying phenotypes depending on environmental conditions (Rice and Emery 2003). When phenotypic responses to the environment are adaptive, plasticity allows individual genotypes to maintain fitness under diverse environmental conditions (Sultan and Spencer 2002). A plant species may thus have a general-purpose genotype, which is very plastic and able to grow, survive and reproduce under different conditions.

A plant species may also consist of a number of different ecotypes that are adapted to the particular environmental conditions at different sites (Bradshaw 1984; Schlichting and Pigliucci 1998). The evolution of locally adapted genotypes requires consistent geographic variation in selection regimes that cause directional trait changes, as well as limited gene flow among populations. Differences in selection pressures that can result in locally specialised ecotypes can be due to heterogeneity in abiotic factors like climate (Joshi et al. 2001; McKay et al. 2001) and soil conditions (Snaydon and Davies 1982; Gauthier et al. 1998), and due to differences in biotic factors like competitors, parasites, pathogens or mutualists (Parker 1995; Linhart and Grant 1996; Prati and Schmid 2000; Gilbert 2002). Genetic differentiation in response to physical environments typically occurs in a comparatively simple, contiguous fashion, whereas differentiation in response to biotic factors frequently shows fine-scale mosaic patterns (Linhart and Grant 1996; Thompson and Cunningham 2002).

It has been suggested that phenotypic plasticity and genotypic variation are alternative means of adaptation to heterogeneous environments in plants, because phenotypic plasticity may reduce the effectiveness of selection in eliminating maladapted genotypes (Marshall and Jain 1968; Sultan and Spencer 2002; Rice and Emery 2003). However, phenotypic plasticity does not necessarily preclude local adaptation (Schlichting 1986; Hangelbroek et al. 2003). Because the costs of plasticity are often high (DeWitt et al. 1998), dispersal distances in plants are typically low and most species show large genetic variation, local genetic differentiation is common in plants, and most species probably consist of many specialised genotypes that are adapted to the particular conditions at a site or even within a specific site (van Tienderen 1990; Linhart and Grant 1996).

A useful approach to investigate local adaptation is provided by reciprocal transplant experiments (Nagy and Rice 1997; Kawecki and Ebert 2004). Under the environmental conditions at a transplant site, genetic differences between populations can be studied by quantifying the phenotypic differences among plants of different origins; moreover, reciprocal transplant experiments permit the responses to different environments to be examined (Linhart and Grant 1996; Briggs and Walters 1997). Most transplant studies have shown that genotypes grow better at their site of origin than at foreign sites (Smith and Bradshaw 1979; van Andel 1998; Hufford and Mazer 2003), indicating home-site advantages. However, most studies have focused on adaptation to contrasting environments, i.e. on ecotypic differentiation (e.g. van Tienderen and van der Toorn 1991; Nagy and Rice 1997; Gauthier et al. 1998), and have been carried out at small spatial scales (e.g. McGraw and Antonovics 1983; Waser and Price 1985). In contrast, little is known about patterns of adaptation at larger geographical scales (Schmidt and Levin 1985; Galloway and Fenster 2000; Santamaria et al. 2003). Because environmental differences are likely to increase and gene flow is likely to decrease with geographical distance, it may be expected that the extent of adaptive differentiation increases with the geographical distance between populations (Montalvo and Ellstrand 2000; Joshi et al. 2001). Understanding the geographical scale over which plant species are adapted is of fundamental interest to evolutionary biologists and biogeographists, and has recently become even more important because of concerns arising from ongoing restoration efforts (McKay et al. 2005).

Habitat fragmentation, which results in decreased population size and increased isolation of populations, may influence the extent of local adaptation. However, the effects of this can be difficult to predict. On the one hand, fragmentation might be expected to increase local adaptation, because gene flow (which could dilute local adaptations) is lower among isolated populations. On the other hand, the effects of random genetic drift in small populations could become more important than those of selection and thus reduce or eliminate existing local adaptations (Frankham et al. 2002) and reduce the ability to adapt to future changes in local environmental conditions (Barrett and Kohn 1991; Helenurm 1998; Frankham 1999). However, little is known about the effects of population size and isolation on local adaptation (Helenurm 1998; Hooftman et al. 2003).

Improving our understanding of the extent of local adaptation and its spatial scale has become an increasingly important task (van Andel 1998; van Groenendael et al. 1998; Hufford and Mazer 2003; McKay et al. 2005), because the introduction of foreign seed material to restore populations and to increase the biodiversity in intensively managed farmlands has become a frequent practice in modern landscape management (Keller et al. 2000). Moreover, the

reintroduction of endangered plants into sites where they have become extinct and the reinforcement of small populations are increasingly being discussed as potential conservation measures. The right choice of seed or plant material is crucial to the success of such projects, because if the plants are adapted to specific conditions at their site of origin they may fail in a new environment.

We studied population differentiation, plastic responses and local adaptation of the declining monocarpic perennial *Carlina vulgaris* L. over three growing seasons at two spatial scales. We chose *C. vulgaris* as a model species, because it is a monocarpic plant with a short generation time, it occurs in different parts of Europe in similar types of habitat, is poorly dispersed, and it is declining due to habitat destruction and fragmentation. We reciprocally transplanted seedlings among five European regions (in north-western Czech Republic, central Germany, Luxembourg, southern Sweden and northwestern Switzerland) and among several populations of different sizes within three of these regions. The regions chosen encompassed strong gradients in climatic conditions and the geographical distances between sites of origin and transplant sites varied widely among the pairs of populations. We could thus examine whether the fitness of plants decreased with increasing distance to the site of origin in addition to testing home-site advantages.

To obtain estimates of life-time fitness, we studied the whole life cycle of the plants and used a matrix model approach to estimate individual fitness (McGraw and Caswell 1996). We address the following questions. (1) Do individuals perform differently at different transplant sites? (2) Is there genetic differentiation among the populations? (3) Do plants perform better at their home sites than at foreign sites, and does plant fitness decrease with increasing distance to the site of origin? (4) Do home-site advantages differ among populations of different sizes?

Materials and methods

Study species

Carlina vulgaris is a monocarpic perennial of dry, nutrient-poor, more or less open habitats. Most populations grow in semi-natural calcareous grasslands, but the plant also occurs in quarries, coastal dunes and open pine forests (Verkaar and Schenkeveld 1984; Grime et al. 1988; Meusel and Kästner 1994). The probability of flowering increases with the size of the rosette (Klinkhamer et al. 1991, 1992), and the age of flowering plants varies between 2 and at least 11 years (Watt 1981; Klinkhamer et al. 1996; Rose et al. 2002). From the end of June to September, reproducing plants produce one to several flower heads, each with up to 300 violet or yellow florets. In most plants, the first flower head produced is the largest one. The florets are protandrous and self-compatible, but mainly insect-pollinated. Seed set starts in September, and it may take several months until all seeds are dispersed. Dispersal is limited although the achenes have a pappus (Greig-Smith and Sagar 1981; Franzén and Eriksson 2003). In Europe, the species is distributed in (sub-)oceanic to sub-Mediterranean regions from southern Italy (39 N) to southern Sweden (62 N). Because of habitat deterioration and fragmentation in the last decades, many populations are now small and isolated, particularly in the north-east of the distribution area (Meusel and Kästner 1994).

Design of the reciprocal transplant experiments

Reciprocal transplant experiments were carried out at two different scales, referred to as ‘‘regional scale’’ and ‘‘local scale’’ in the following.

Regional scale

In late summer 2000, two large populations in nutrient-poor grasslands with similar vegetation were chosen in each of five European regions (northwestern Czech Republic, central Germany, Luxembourg, southern Sweden and northwestern Switzerland, see Appendix 1 in the Electronic Supplementary Material (ESM)). Geographical distance between populations ranged from 237 to 1,439 km (median 620 km, Appendix 2 in the ESM). In each population one complete mature fruit head from each of 20 randomly chosen individuals was collected and sent to Germany. Seeds from the two populations of each region were mixed, divided randomly into five batches and send to the collaborators in the four transplant regions or kept in Germany, respectively. We used seeds from two populations per region to obtain a more representative sample of genotypes. In March 2001, seeds were germinated in nutrient-poor gardening soil in each study region. Three weeks after germination seedlings were transplanted individually into small pots (3 cm diameter) and kept in glasshouses.

In mid-May, juveniles from all study regions were transplanted into one site at each region. In each region one of the two populations of origin was chosen at random as a transplant site. At each transplant site, five plots (3.2×0.6 m each) were established at random

and marked with iron rods. In each plot, five rows set 15 cm apart were defined and the vegetation was cut within 5 cm-wide strips on both sides of the rows to minimise competition for the transplants during the early stages. Juveniles were planted 15 cm apart along the rows in random order, and the number of leaves and length of the longest leaf were recorded for each plant in order to estimate initial sizes. Twenty replicate plants from each region of origin, i.e. 100 juveniles overall, were planted in each plot. After transplanting, the plants were watered for two weeks in order to facilitate establishment. Two weeks after transplanting, the juveniles that had died were replaced, because we assumed that the plants had died due to the transplanting procedure. If no juvenile from the same region of origin was available, the dead plant was not replaced and it was removed from further analyses.

Local scale

Within each of three regions (in northwestern Czech Republic, central Germany, southern Sweden), four populations of different size were chosen (Appendix 1 in the ESM), seeds were sampled, and seedlings were raised as described above. The geographical distance between populations varied from 4 to 103 km (median 23 km, Appendix 3 in the ESM). In mid-May, juveniles from all populations within each region were transplanted into each site within that region, including their site of origin, in the same way as described for the regional transplants. However, only five replicates per population of origin were transplanted into five plots (45×60 cm each), resulting in 20 juveniles per plot. We used a smaller number of replicates in the local experiment than in the regional experiment, because there were fewer juveniles available from the small populations.

Measurement of plant performance

Growth, survival and reproduction of each transplant were recorded during three growth periods from spring 2001 to autumn 2003. Plant size and survival were recorded each autumn. The number of rosettes, the number of leaves and the length of the longest leaf were recorded for non-flowering plants. The product of the number of leaves and the length of the longest leaf was calculated as an estimate of rosette size. The number of inflorescences and the diameter of each inflorescence were recorded for flowering plants. Some plants that had flowered already in 2002 and their above-ground parts were harvested immediately after the seeds had matured; the above-ground parts of all other plants were harvested in autumn 2003. All plant material was air-dried, sent to Germany, dried for 12 h at 80°C and weighed. We used the biomass of flowering plants irrespective of the year of flowering as a measure of final plant size because there were no differences in above-ground biomass of flowering plants among the years (regional scale: $F = 0.07$, $P = 0.79$, $n = 521$; local scale: $F = 1.26$, $P = 0.26$, $n = 201$). To obtain an estimate for the number of seeds (s) produced by the transplants, a regression of the number of seeds per plant versus the total area of the fruit heads was used ($r = 0.91$, $P < 0.001$, $n = 30$). From recruitment experiments carried out in Germany, we calculated a mean germination probability (g) that was assumed to be the same for all origins at all transplant sites, because we had no data on site or origin by site interactions at the germination stage. Fecundity (F) of each individual was calculated as $F = s \times g$. To obtain an estimate of individual fitness, age-structured Leslie matrices that incorporated time of reproduction were constructed for each individual using the survival and fecundity data, and dominant eigenvalues (finite rates of growth, λ) were calculated (McGraw and Caswell 1996). The matrices contained the transitions between the following age classes: first-year plants (four months old), second-year plants (16 months), third-year plants (28 months) and fourth-year plants (40 months). In this model, the fecundity of the monocarpic plants in the first year was zero, and in the second and third years it was either zero or the value obtained. Survival to the second and third year was either zero or one (McGraw and Caswell 1996). Individuals that died prior to flowering had zero fitness. For those plants that did not flower, but were still alive at the end of the experiment after 28 months (19.8% of the individuals planted), we estimated the combined probability of survival and reproduction and the fecundity in the following year from regression equations. We first analysed the relationship between the combined probability of survival and reproduction in the third year as dependent variable and rosette size at the end of the second year, transplant site, plot within transplant site, population of origin and their interactions as independent variables. Using the logistic regression equations obtained, we predicted the probability of survival and reproduction in the fourth year from the rosette size at the end of the third year. Similarly, fecundity of plants in the fourth year was predicted using regression equations for the relationship between fecundity and rosette size of the year before flowering (regional scale: $r = 0.55$, $P < 0.001$, $n = 512$; local scale: $r = 0.51$, $P < 0.001$, $n = 306$). Mean individual fitness values (λ_i) were calculated for plants from each origin

at each site. This model implicitly assumed that all remaining plants would die after four years, either because they flowered, or because they would not survive until the fifth year. It is likely that some plants would have flowered in later years, but simulations and elasticity analyses indicated that the possible error due to the fact that we could not follow the fates of all plants until they flowered and died is small, because the proportion of plants that were expected to survive for longer than four years was very small, and the longer reproduction is delayed, the lower the individual fitness of a plant. As an alternative measure of fitness, the number of seeds produced per seedling planted was calculated. This measure does not take into account the effects of variation in the age at reproduction on fitness.

Characterisation of habitat conditions

To characterise habitat conditions, the composition of the vegetation at each study site was recorded by estimating the cover of each plant species. From these data, mean Ellenberg indicator values for nitrogen, soil reaction, moisture and continentality of climate were calculated for each site (Persson 1981; Ellenberg et al. 1992). In addition, the maximum and mean heights of the vegetation were recorded. To characterise climatic conditions, we obtained data for mean summer and winter temperatures and summer and winter precipitations over the last 30–40 years from weather stations within each study region.

We carried out a bioassay to estimate the relative nutrient availability at the study sites. In July and August 2002, we sampled soil from eight random cores in each population, mixed them and then air-dried them spread-out on a laboratory bench. At the study sites, the upper soil layers frequently dry out completely, and this treatment thus mimics a natural process. In November 2002, three plastic pots ($9 \times 9 \times 9.5$ cm) were filled with soil from each population and five seedlings of *Arrhenatherum elatius* were grown as phytometers in each pot in a glasshouse. After eight weeks, all above-ground plant parts were harvested, dried for 24 h at 80°C and weighed. Total above-ground biomass per pot was used as an estimate of nutrient availability.

Data analysis

Differences in species composition among sites were investigated by the ordination technique known as *detrended correspondence analysis* (DCA; Hill and Gauch 1980) in order to detect the main gradients in species composition. Cover values were log-transformed prior to analysis and down-weighting of rare species was carried out (Ter Braak and Šmilauer 2002). DCA scores along the first two axes were used as variables reflecting environmental differences among sites. At the regional scale, absolute differences between each pair of populations were calculated for mean summer and winter temperatures, summer and winter precipitations, DCA scores, Ellenberg indicator values for nitrogen, soil reaction and moisture, the mean height of the vegetation and the biomass of the phytometer. At the local scale, the same pairwise differences were calculated except for climatic variables, because no climate data were available for the individual sites. Pearson correlations were used to assess the relationship between plant performance and differences in environmental variables.

We used general linear models to analyse continuous variables (rosette size, biomass, individual fitness), and analyses of deviance for survival and flowering data (Table 1). Mean deviances due to a factor were divided by their appropriate error mean deviances to obtain quasi-*F*-values, analogous to the calculation of *F*-values in ordinary analysis of variance (Francis et al. 1993).

The size of the rosettes at the time of transplanting (number of leaves × length of longest leaf = initial size) was used as a covariate to adjust for maternal effects and effects of different growing conditions before transplanting. However, including the effects of the covariate rarely changed the results qualitatively and so the results are presented without the covariate.

To test specific hypotheses about the interaction between the effects of transplant site and origin, two contrasts were calculated independently: A ‘‘home versus away’’ contrast, and a linear contrast testing the effect of the geographical distances between the sites of origin and the transplant sites. The effects of the site of origin, the interaction between the effects of site of origin and the transplant site, the home versus away contrast and the distance contrast were tested against the interaction between the effects of site of origin and plot. At the local scale, the effects of region and size of population of origin were also fitted (Table 1b). Above-ground biomass was log-transformed prior to analysis to obtain normally distributed residuals and homoscedasticity. General linear models were fitted with the statistical package SPSS 11.0 (SPSS Inc., Chicago, IL, USA). Analyses of deviance were calculated with the statistical package R, Version 1.9.1 (The R Development Core Team 2004). Leslie matrices were analysed with MATLAB (Student Edition, Version 5.0). DCA were carried out with Canoco 4.5 (Ter Braak and Šmilauer 2002).

Table 1 Skeleton analyses of variance (or deviance) for (a) the regional and (b) the local transplant experiments

Source of variation	*df*	Error term
(a) Regional transplant experiment		
[Initial size	1	Residual]
Site	4	Plot
Plot	20	Residual
Origin	4	Origin × plot
Origin × site	16	Origin × plot
Home versus away	1	Origin × plot
Distance	1	Origin × plot
Origin × plot	63–80	Residual
Residual	363–2,335	
(b) Local transplant experiment		
[Initial size	1	Residual]
Region	2	Site
Site	6–9	Plot
Plot	34–48	Residual
Population size	1	Origin
Origin	7–8	Origin × plot
Origin × site	18–27	Origin × plot
Home versus away	1	Origin × plot
Distance	1	Origin × plot
Origin × plot	55–144	Residual
Residual	179–1,225	

The range in degrees of freedom is given if they varied depending on the traits studied

Results

Regional transplant experiment

The site at which the transplants grew had overall effects on several measures of plant performance, but not on individual fitness (Table 2). After 16 months, plants at the Swedish site were much smaller than in the other regions (Fig. 1a). Because of their small size, none of the plants at the Swedish site flowered during the second growing period, whereas 9–26% of the plants flowered (Fig. 1b). The identity of the plots within a site had highly significant effects on all characters, indicating differences in the environmental conditions among the plots within a site. The origin of the plants influenced nearly all measures of performance, indicating genetic differentiation among origins (Table 2). All measures of performance were lower for plants from Sweden than for plants from the other regions (Fig. 2).

Several traits were influenced by interacting effects of origin and transplant site (Table 2). Most of these interactions were related to the geographical distance between the site of origin and the site to which the plants had been transplanted. After 16 months, the size of the plants was already influenced by distance. The further away from their home site the plants were growing, the smaller they were and the less likely they were to flower (Table 2, Fig. 3a,b). At the end of the experiment after 28 months, survival (Fig. 3c) and the size of the flowering plants decreased with increasing distance between the home and the transplant site (Table 2). Due to the effects on survival and flowering probability, individual fitness also decreased with the distance between the home and the transplant site (Fig. 3d). For early flowering probability, survival and individual fitness, the performances of plants grown at their home site were higher than those at away sites (Fig. 3).

The effects of origin, site and the distance between site of origin and transplant site on an alternative

Table 2 Regional scale. Effects of transplant site and population of origin on life-history traits of transplanted individuals of *Carlina vulgaris*

	Rosette size, 16 months	Flowering, 16 months	Survival	Flowering, 28 months	Biomass, veg. pl., 28 months	Biomass, flowering plants	Individual fitness
	F	Quasi-*F*	Quasi-*F*	Quasi-*F*	*F*	*F*	*F*
Site	3.34*	5.45**	2.26†	5.03**	2.35†	*2.67*†	1.40
Plot	19.56***	11.60***	7.44***	5.52***	6.33***	28.84***	12.82***
Origin	12.75***	17.30***	14.93***	28.63***	0.82	19.36***	14.71***
Origin × site	1.71†	0.90	1.74†	3.24***	3.19**	5.30***	1.37
Home	0.38	3.21↑†	9.58↑**	2.69	0.62	0.45	7.24↑**
Distance	6.81↓***	6.80↓*	15.90↓***	0.15	1.45	8.39↓***	12.20↓***
Origin × plot	1.73***	1.61***	1.19	0.64	0.89	0.64	1.53**

Seedlings were transplanted reciprocally among five European regions. *F*-values (continuous characters) and quasi-*F*-values (survival to flowering or to the end of the experiment, flowering) resulted from analyses of variance and deviance, respectively (see Methods). Arrows indicate the direction of significant home (↑, home site advantage) or distance effects (↓, negative effect of distance)

† $P < 0.1$

*$P < 0.05$

**$P < 0.01$

***$P < 0.001$

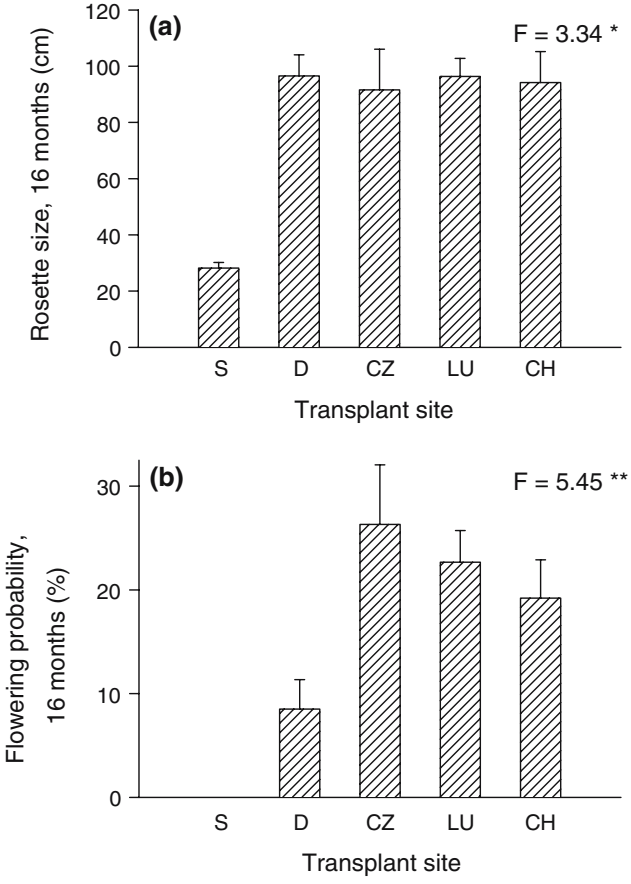

Fig. 1a–b Regional scale. Effect of transplant site (*S*: southern Sweden, *D*: central Germany, *CZ*: northwestern Czech Republic, *LU*: Luxembourg, *CH*: northwestern Switzerland) on **a** rosette size (length of longest leaf × number of leaves) 16 months after transplanting, and **b** the proportion of plants flowering in the second year (16 months after transplanting). $*P < 0.05$, $**P < 0.01$. *Vertical bars* denote 1 SE

measure of fitness, the reproduction per seedling planted, were qualitatively the same (results not shown). The size of the plants at the time of planting had significant effects on most of the traits measured, but including initial size as a covariate in the analysis did not change the results qualitatively.

Differences in several environmental traits were correlated with geographical distance. The differences in mean winter temperature, indicator value for soil reaction and the DCA scores along the first axis increased with geographical distance ($r = 0.94$, 0.89 and 0.80; all $P < 0.01$, $n = 10$). Consequently, individual fitness was negatively related to the difference in mean winter temperature, indicator value for soil reaction and the DCA scores along the first axis between the home and the transplant site ($r = –0.59$, –0.63 and –0.59; all $P < 0.01$, $n = 25$), as well as to mean summer temperature ($r = –0.52$, $P < 0.01$, $n = 25$).

Fig. 2a–d Regional scale. Effect of population of origin (*S*: southern Sweden, *D*: central Germany, *CZ*: northwestern Czech Republic, *LU*: Luxembourg, *CH*: northwestern Switzerland) on **a** rosette size (length of longest leaf × number of leaves) 16 months after transplanting and **b** proportion of plants flowering 16 months after transplanting, **c** survival until flowering or until the end of the third growing season, and **d** individual fitness in *Carlina. vulgaris*. $***P < 0.001$. *Vertical bars* denote 1 SE ▶

Local transplant experiment

The region from which the plants originated and into which they were transplanted, the site within the region, and the plot within the site influenced several measures of performance, indicating effects of spatial environmental variation at various levels (Table 3). All measures of plant performance were lowest for the study populations in Sweden. Plants from different populations of origin within the regions differed strongly in their performance, indicating genetic differentiation, but these differences were mostly not attributable to the size of the original population.

Regression coefficients for the effect of population size on plant size (rosette size after 16 months, biomass of flowering plants and individual fitness) were positive, and population size explained 15–42% of the variation in the continuous traits studied, but due to low statistical power (only 12 populations) only the effect of population size on rosette size after 16 months was significant (Table 3, Fig. 4).

The biomasses of non-flowering plants in the third year and the individual fitnesses of plants at the specific sites varied depending on their origin (significant origin by site interactions in Table 3), i.e. plants from a certain population did not perform equally well at all sites. The interaction effect on individual fitness was considerably stronger when initial plant size was included as a covariate in the model ($F = 1.91$, $P < 0.01$). This variation among plants was not related to the geographical distance between home and transplant site, and plants did not grow better at their home site than at other (away) sites. There was thus no evidence for local adaptation. Moreover, the different performances of plants could not be explained by differences in environmental conditions between the home and transplant site. None of the differences in the various indicator values, in the axis scores of the DCA analysis, in the height of the vegetation and in nutrient availability as estimated by the phytometer significantly correlated with plant performance (all $r < 0.24$, $P > 0.10$, $n = 48$). There was thus no evidence for ecotypic differentiation at the local scale. The performances of plants at specific sites did not depend

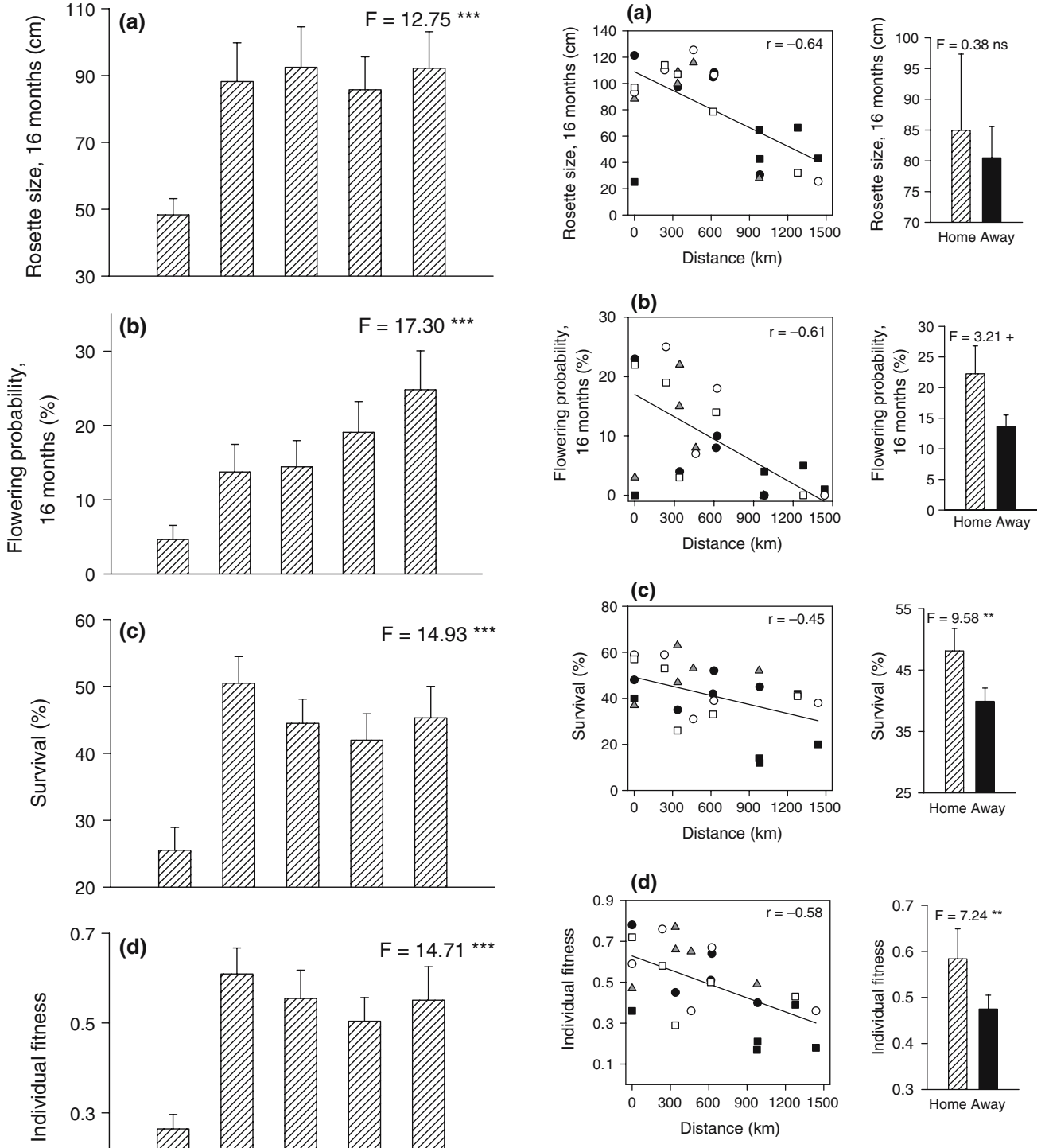

Fig. 3a–d Regional scale. Effect of distance from the site of origin to the transplant site and home versus away effects on **a** rosette size (length of longest leaf × number of leaves) 16 months after transplanting, **b** proportion of flowering plants 16 months after transplanting, **c** probability of survival until flowering or until the end of the third growing season, and **d** individual fitness in *Carlina vulgaris*. Populations of origin: *filled circles* northwestern Czech Republic, *filled triangles* central Germany, *filled squares* southern Sweden, *squares* Luxembourg, *circles* northwestern Switzerland. *ns* not significant, $+P < 0.10$, $^{**}P < 0.01$. *Vertical bars* denote 1 SE

on the size of the original population (no significant interaction between population size and transplant site; all $F < 1.52$, all $P > 0.22$).

Table 3 Local scale. Effects of study region, transplant site within region, population of origin, size of the population of origin and interactions among transplant site and population of origin on life-history traits of *Carlina vulgaris*

	Rosette size, 16 months	Flowering, 16 months	Survival	Flowering, 28 months	Biomass, veg. pl., 28 months	Biomass, flowering plants	Individual fitness
	F	Quasi-*F*	Quasi-*F*	Quasi-*F*	*F*	*F*	*F*
Region	38.00***	9.52**	1.36	8.15**	6.84*	92.71***	0.36
Site	1.86†	2.82***	7.38**	4.46***	3.48**	0.35	2.85**
Plot	5.42***	4.12***	*3.02***	*5.44***	1.64**	5.30***	8.80***
Population size (log)	5.85*	< 0.1	0.95	0.40	0.07	1.20	3.18
Origin	3.44**	11.25	1.90†	6.36***	4.02**	10.90***	4.25**
Origin × site	0.76	1.13	1.34	0.73	2.10**	0.46	1.49†
Home versus away	1.84	1.61	0.70	0.19	0.26	0.13	0.36
Distance	1.03	0.18	0.70	0.36	0.21	0.21	0.76
Origin × plot	1.11	1.10	1.10	1.46***	1.21	1.27	1.52***

Juveniles were reciprocally transplanted among four transplant sites in each of three European regions. *F*-values (continuous characters) and quasi-*F*-values (survival until flowering or until the end of the experiment, flowering) resulted from analyses of variance and analyses of deviance, respectively (see Methods)

† $P < 0.1$

*$P < 0.05$

**$P < 0.01$

***$P < 0.001$

Discussion

Phenotypic plastic responses

Our results show that *C. vulgaris* plants from all regions of origin can grow over a wide range of latitudes and longitudes within Europe, because none of the transplants failed completely. In the regional transplant experiment, the growth of plants at the northernmost site, i.e. the Swedish site, was lower than at the other sites and the plants flowered later, but there were no differences among the sites in overall plant fitness after five years. With respect to growth and time to flowering, our results were thus similar to those from other reciprocal transplant experiments, which found decreased growth with increasing latitude for the aquatic species *Potamogeton pectinatus* (Santamaria et al. 2003), and delayed reproduction at northern sites for the monocarpic species *Daucus carota* (Lacey 1988) and *Verbascum thapsus* (Reinartz 1984).

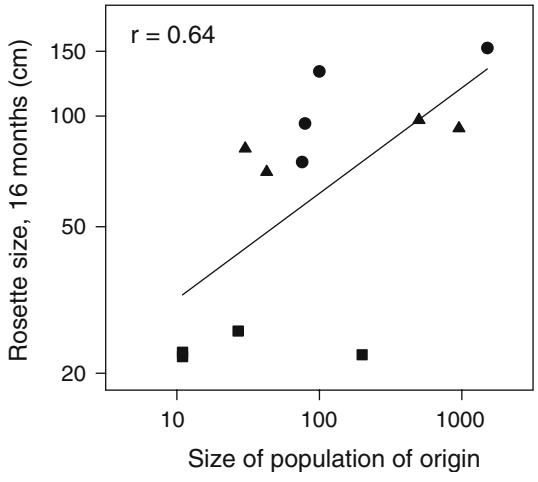

Fig. 4 Local scale. Effect of the size of the original population on rosette size (length of longest leaf × number of leaves) 16 months after transplanting. *Filled circles* northwestern Czech Republic, *filled triangles* central Germany, *filled square* southern Sweden

Effects of plant origin and local adaptation

Genetic differentiation among the plants from the five European regions was strong, as indicated by the significant effects of origin, and there was strong evidence that this differentiation was adaptive. Several components of fitness and overall individual fitness were higher in the home region than in the other regions and decreased with increasing distance between the home and the transplant region, indicating strong adaptation of *C. vulgaris* to conditions in the home region. An increase in the expression of local adaptation with transplant distance may be expected, because with increasing distance both the genetic isolation of populations and environmental differences between sites are likely to increase (Galloway and Fenster 2000). However, very few studies have investigated the relationship between transplant distance and plant fitness. Similarly to our results, the performances of the

widespread forage plants *Trifolium pratense*, *Dactylis glomerata* and *Plantago lanceolata* decreased continuously with distance from the home site (Joshi et al. 2001), whereas in the annual legume *Chamaecrista fasciculata* there was evidence for local adaptation only at the largest spatial scales (1,000 and 2,000 km; Galloway and Fenster 2000). In the Californian shrub *Lotus scoparius*, however, geographic distance between populations was only weakly correlated with genetic distance and had little value when predicting plant fitness (Montalvo and Ellstrand 2000).

Possible selective factors that may result in local adaptation include climatic and edaphic conditions as well as biotic conditions (competitors, herbivores, parasites and pathogens, mutualists). In *C. vulgaris*, the continuous decrease of plant fitness with transplant distance over a range of more than 1,000 km suggests that differences in climatic conditions, in particular winter temperatures, are responsible for the observed effects. However, differences both in temperature and in soil reaction increased with geographical distance between study sites and possible climatic and edaphic effects were therefore confounded.

In the local transplant experiment, the median distance between populations was only 23 km. Nevertheless, there was strong genetic differentiation among local populations, indicated by significant differences among populations in overall performance and significant origin by site interactions. However, in contrast to the regional transplant experiment, in the local transplant experiment the performance of plants was not consistently higher at their home site, and it was not related to the geographical or environmental distance between the site of origin and the transplant site. This could indicate either adaptation of populations to factors that were not recorded by us, e.g., to the presence of certain pathogens or mutualists, or non-adaptive differentiation among populations in the response to site conditions due to genetic drift. The combined results from the two experiments suggest that local adaptation increases with the geographical distance between populations, but that the geographical distances were too small in the local transplant experiment to result in a significant relationship between transplant distance and plant fitness.

Local adaptation has been found in many plant species at similar and even smaller scales to those covered in the local transplant experiment with *C. vulgaris* (e.g., references in Linhart and Grant 1996; Nagy and Rice 1997; Gauthier et al. 1998; Petit et al. 2001; but see Schemske 1984; Rapson and Wilson 1988; Platenkamp 1990), but most studies have compared plant performance in specific contrasting environments (Galloway and Fenster 2000). In contrast, our study sites were all situated in similar dry grassland habitats and environmental differences between sites in the local experiment may have been too small to result in the expression of local adaptation (cf. Rice and Mack 1991).

The large effects of plot on almost all traits indicate that the effects of local environmental heterogeneity within sites on the growth and survival of *C. vulgaris* were strong. Such small-scale patchiness has been assumed to favour the evolution of phenotypic plasticity over genetic differentiation (Bradshaw 1965; Platenkamp 1990). However, in *C. vulgaris* isolation and differences in selection regimes among sites within regions have apparently been strong enough to allow strong genetic differentiation among populations, although plasticity is strong (Berg et al. 2005).

Effects of population size

Carlina vulgaris occurs in fragmented populations that are frequently small and isolated, and gene flow by pollen and seed dispersal is probably very restricted. In small populations, the effects of drift could be stronger than those of selection and thus prevent adaptation to local conditions. In a reciprocal transplant experiment, significant interactions between the effects of the size of the original population and the transplant site would indicate that populations of different size differ in their degree of local adaptation. This has only rarely been studied, but in *Arabis fecunda* local adaptation occurred despite very small effective population sizes (McKay et al. 2001). Similarly, in *C. vulgaris* we found no evidence that population size influenced the degree of local adaptation, although one fitness-related trait increased with population size. This is in contrast to the results of Jacobsson and Dinnetz (2005), who found that local adaptation with respect to relative performance at the rosette stage increased with population size.

Differences in local adaptation among traits

Patterns of genetic differentiation and local adaptation have been found to be fairly consistent across fitness components in some studies (Nagy and Rice 1997; Gauthier et al. 1998; Galloway and Fenster 2000), whereas in others local adaptation varied among traits (McGraw and Antonovics 1983; van Groenendael 1985; van Tienderen and van der Toorn 1991) or among years (Rice and Mack 1991). In *C. vulgaris*, effects of local adaptation were stronger and more consistent across components of fitness and were

expressed earlier during the life cycle in the regional than in the local transplant experiment. In the regional study, local adaptation was expressed by the second year, whereas in the local transplant experiment only effects on traits in the third year were significant. Other studies have also found local adaptation to be more pronounced at later life stages. In *P. lanceolata*, differences between populations in the survival of adults were more pronounced than differences in the juvenile phase (van Groenendael 1985; van Tienderen and van der Toorn 1991). It has been suggested that early traits are strongly influenced by environmental conditions at a site that may overwhelm local adaptations (Antonovics and Primack 1982; van Tienderen and van der Toorn 1991). In the present study, the strong within-site environmental heterogeneity may have masked the expression of local adaptation in early traits in the local experiment, in which overall effects were less strong than in the regional experiment.

Conclusions

In conclusion, the results of our study suggest that *C. vulgaris* consists of regionally adapted genotypes throughout its European range. Individual regions therefore harbour only fractions of the total genetic variability of the species. To preserve the genetic variability of *C. vulgaris*, a declining plant in some parts of Europe (e.g. Landolt 1991; Korneck et al. 1996), it is therefore important to conserve viable populations in the different regions. This could be true for other grassland plants in Europe, because the strong genetic differentiation and local adaptation found in *C. vulgaris* may be typical for grassland species (cf. Joshi et al. 2001).

In our experiments there was evidence for local adaptation at the larger scale (> 200 km), but not at the smaller spatial scale (cf. Jacobsson and Dinnetz 2005). This suggests that the environmental heterogeneity experienced by *C. vulgaris* at the local scale is not comparable in magnitude to that at the regional scale, or that genotypic differences are not so strong at this scale and plastic responses prevail. An understanding of the spatial scale of adaptive evolution is of practical relevance for the selection of seed material used in restoration projects. Because of the possibility of ecotypic variation, it has been suggested that the introduction of genotypes from other regions should be avoided when reinforcing populations of rare or declining plants or restoring habitats (van Andel 1998; van Groenendael et al. 1998; Hufford and Mazer 2003; Vergeer et al. 2004; McKay et al. 2005). Our results support this view, but only for long-distance translocation of genotypes. Within regions, transplant distance is not important for the performance of plants, and the properties of potential source populations (e.g. size, genetic variability) are probably more important for the long-term success of restoration measures.

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