Population biology
of *Carlina vulgaris* and *Hypochoeris radicata*
in fragmented European grasslands
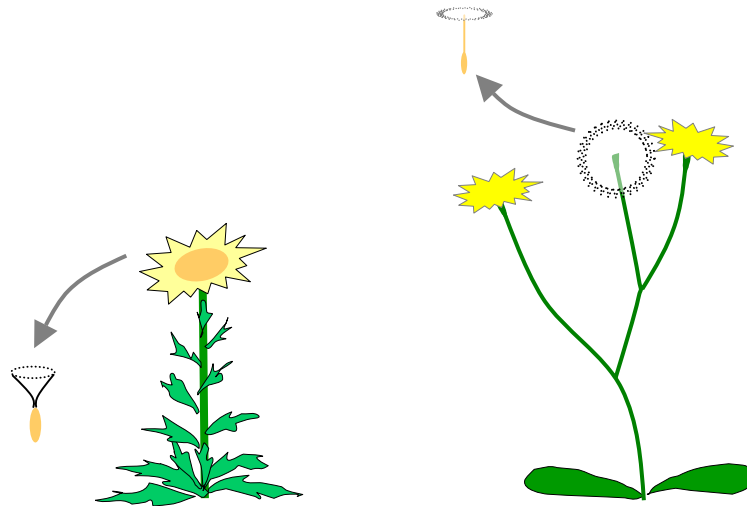
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The endless variety of organisms, in their beauty, complexity and diversity
gives to the biological sciences a fascination which is unrivalled by the physical world.

(D. Briggs & S. M.Walters 1997, Plant variation and evolution)

Contents

CHAPTER 1

General Introduction

In this thesis I study the effects of habitat fragmentation on individual fitness, quantitative genetic variation and local adaptation in the grassland species *Carlina vulgaris* L. and *Hypochoeris radicata* L. In this introductory chapter I describe the consequences of habitat fragmentation for plant populations and give a short theoretical background on the processes involved. I outline the possible importance of this study for purposes of species conservation and habitat restoration, and finally I give an overview of the contents of this thesis.

Causes and consequences of habitat fragmentation

The massive extinction of species has become a severe problem in species conservation during the last decades (Clarke & Young 2000, Davies *et al.* 2001). Worldwide 12.5% of all vascular plants are at risk of extinction (in Frankham *et al.* 2002), and also in European countries, a considerable number of plant species is threatened (e.g. Landolt 1991, Korneck *et al.* 1996, van Groenendael *et al.* 1998). The main causes for these extinction processes are changes in land use during the last decades, e.g. intensification of agricultural use and abandonment of extensively used farmland, and also building of new residential or industrial areas and roads. As a consequence many large natural or semi-natural habitats were destroyed or became fragmented. This process is much faster than the formerly natural fragmentation that was mainly caused by different environmental conditions (Saunders *et al.* 1991, Young *et al.* 1996, Clarke & Young 2000). Populations in fragmented habitats become smaller in size or even extinct, leading to increased isolation among populations. Small and isolated populations are more threatened by extinction than those that are large and well connected, because they are more susceptible to demographic and environmental stochasticity (Menges 1991a, 1992, Matthies *et al.* 2004). Moreover, genetic erosion due to random genetic drift and inbreeding is stronger in small populations and may negatively affect the fitness of individual plants and whole populations (e.g. Barrett & Kohn 1991, Oostermeijer *et al.* 1996, Young *et al.* 1996). The negative effects of small population size and isolation are called Allee effects (Allee *et al.* 1949, Groom 1998, Stephens & Sutherland 1999). However, some authors regard only the negative effects of reduced density within populations as Allee effects.

Genetic variation

Genetic variation is the sum of the genetic richness of all individuals of a species, between populations (population genetic variation) and among the individuals within one population. It is determined by four evolutionary processes: mutation, heterogeneous selection, random

genetic drift and gene flow (Barrett & Kohn 1991). Most studies of genetic variation have used molecular markers that are selectively neutral and have measured the allelic richness per locus or the proportion of heterozygous individuals, i.e. gene diversity (Waldmann & Andersson 1999). These markers thus provide insights into random evolutionary processes, i.e. genetic drift, pollination and dispersal processes, rather than into adaptive processes of ecological significance (Thompson 1999, but see Petit *et al*. 2001). Moreover, the variation in morphological traits (i.e. quantitative genetic variation) partly reflects genetic variation (Young *et al*. 1996). The quantitative genetic variation is a result of the reaction of plants to environmental conditions due to selection and is under polygenetic control (Young *et al*. 1996, Lynch *et al*. 1999). The study of quantitative genetic variation provides information about variation in fitness-related traits and can be useful for suggesting management strategies for species conservation (Storfer 1996, Knapp & Rice 1998, McKay *et al*. 2001, Frankham *et al*. 2002).

The importance of genetic diversity for the evolution of a species and thus for its persistence is manifold (Frankel & Soulé 1981, Gilpin & Soulé 1986, Frankham 1995). As example, the genetic diversity (i.e. heterozygosity) of an individual may positively influence its fitness, because it decreases the susceptibility to pathogens or increases plant size (Boyce 1992, Reed & Frankham 2003). Lower individual fitness in small populations in combination with reduced genetic variation has been found in a number of studies (e.g. Fischer & Matthies 1998a, Kéry *et al*. 2000, Paschke *et al*. 2002a, Hooftman *et al*. 2003, Vergeer *et al*. 2003a). Reduced genetic variation in small and isolated populations is the result of genetic drift, which is the random change of the allelic composition between generations, and may result in the loss of rare alleles (Lacy 1987, Hartl & Clark 1989, Barrett & Kohn 1991, Ellstrand & Elam 1993). Decreased genetic variability in small and isolated plant populations has been found in a number of studies based on molecular markers (e.g. Ouborg *et al*. 1991, Raijmann *et al*. 1994, Fischer & Matthies 1998b, Young *et al*. 1999), but little is known about the effects of population size and isolation on phenotypic variation of plant populations (Oostermeijer *et al*. 1994a, Ouborg & van Treuren 1995, Podolsky 2001).

Gene flow and local adaptation

The long-term ability of a population to react to changing environmental conditions depends on its genetic diversity (Frankel & Soulé 1981, Barrett & Kohn 1991, Eberhart *et al*. 1991, Mitton 1993, Helenurm 1998, Frankham 1999). The local adaptation to environmental

conditions is a process that drives population differentiation (Linhart & Grant 1996, Kassen 2002), but may be counteracted by gene flow between populations (Linhart & Grant 1996, Nagy & Rice 1997)

Plants with locally adapted genotypes may show a home-site advantage, i.e. they grow better in their site of origin than plants from other sites (McGraw & Antonovics 1983, van Andel 1998, Hufford & Mazer 2003, Kawecki & Ebert 2004). This home-site advantage is a consequence of selection and is supposed to increase with increasing environmental or genetic dissimilarity between populations (Frankham *et al.* 2002). In total these differences increase with increasing geographic distance and thus home-site advantages also might increase. However, local adaptation on large geographical scales has rarely been studied (Galloway & Fenster 2000, Montalvo & Ellstrand 2000, Joshi *et al.* 2001, Santamaria *et al.* 2003).

Local adaptation can be constrained by increased gene flow among populations (Futuyama 1998, Lenormand 2002). Furthermore, gene flow can counteract the loss of genetic variation (Slatkin 1987). Gene flow in plants may occur through pollen or seeds and is a force that is opposed to population differentiation (Dewey & Heywood 1988). The extent of gene flow decreases with increasing geographical distance and mainly depends on foraging distances of pollinators, on the breeding system of a species and on a species' ability to disperse pollen and seeds (Slatkin 1987, Ellstrand 1992, Ellstrand & Elam 1993, Rathcke & Jules 1993). Due to habitat fragmentation gene flow among populations may decrease, differentiation among populations increase and the variation within populations decrease (Hamrick *et al.* 1991).

Inbreeding

Inbreeding through selfing or crossings between close relatives (i.e. biparental inbreeding) may lead to decreased genetic variability of individuals due to a reduction in the number of alleles per locus or in the degree of heterozygosity. Both may result in the reduction of various components of plant fitness, i.e. inbreeding depression (e.g. Charlesworth & Charlesworth 1987, Barrett & Kohn 1991, Dudash & Fenster 2000). Two mechanisms which can occur simultaneously may lead to inbreeding depression: First, in the case of overdominance both types of homozygotes have lower fitness than the heterozygote, and thus inbreeding depression occurs if heterozygosity decreases. Second, in the case of partial dominance, inbreeding depression is the result of the expression of deleterious recessive

alleles at homozygous loci (Charlesworth & Charlesworth 1987, Lynch *et al*. 1995, Byers & Waller 1999, Dudash & Fenster 2000).

Variation in the expression of inbreeding depression has been found among different genotypes (Helenurm & Schaal 1996, Pico *et al*. 2004a) and populations (Johnston & Schoen 1996, Ferdy *et al*. 2001, Bram 2002), but little is known about large-scale geographical patterns of inbreeding depression (e.g. differences among geographical regions). In small and isolated populations the possibility that two individuals are related is higher than in large populations and thus the risk of inbreeding increases (Ellstrand & Elam 1993). Reduced fitness of plants in small compared to large populations due to increased inbreeding has been found in several species (e.g. Fischer & Matthies 1998a, Kéry *et al*. 2000, Luijten *et al*. 2000, Lienert *et al*. 2002a, Paschke *et al*. 2002a, Hooftman *et al*. 2003, Vergeer *et al*. 2003a).

Outbreeding

The mating between genetically distant individuals, i.e. mating between individuals from different populations (intraspecific hybridisation) or between distant individuals within one population, may lead to both a reduction and an increase in offspring fitness (e.g. Lynch 1991, Hufford & Mazer 2003). If populations suffer from decreased genetic variability intraspecific hybridisation can enhance genetic variation, and the fitness of offspring may increase relative to parental fitness, the so-called heterosis effect. Heterosis has been reported following crosses between populations (e.g. Oostermeijer *et al*. 1995, Byers 1998, Fenster & Galloway 2000). Furthermore, in self-incompatible species mating success may increase after inter-population crosses, because the chance of pollination with compatible pollen increases. However, crosses between populations may also lead to decreased offspring vigour (out-breeding depression), in particular if dispersal and exchange of pollen is limited and genetic differentiation between populations is high (Waser & Price 1989, Waser 1993). There are two mechanisms, which may be responsible for outbreeding depression. If populations have become adapted to different local conditions, interpopulation hybridisation may result in the dilution of adapted genotypes in the next generation. The other mechanism underlying outbreeding depression is due to new deleterious gene interactions by the breaking up of co-adapted gene-complexes by recombination (Fenster & Dudash 1994, Fenster & Galloway 2000, Edmands 2002, Hufford & Mazer 2003). This process mainly occurs in the second generation and is called hybrid breakdown (Hufford & Mazer 2003). Outbreeding depression is likely to increase with the geographical or genetic distance between individuals (Waser &

Price 1994, Montalvo & Ellstrand 2001). Few studies have investigated effects of outbreeding at large geographical scales (Fenster & Galloway 2000, Keller *et al*. 2000, Montalvo & Ellstrand 2001).

Effects of habitat fragmentation with respect to different life history traits

The life history traits of a species, e.g. the breeding system, longevity and dispersal ability, are important for a species' persistence at a given site, because they influence its suscep-tibility to negative effects of habitat fragmentation. In small and isolated populations pollen quantity and quality is decreased. Species that reproduce mainly by selfing are in general less sensitive to this pollinator limitation than outcrossers (Sih & Baltus 1987, Steffan-Dewenter & Tscharntke 2002). Furthermore, the breeding system strongly influences gene flow between populations and thus genetic variation and differentiation (Ellstrand & Elam 1993, Hamrick & Godt 1996a).

  Well dispersed species have a higher ability to reach and colonise new habitats and thus are able to compensate for local population extinction (Menges 1991a, Hanski & Gilpin 1997). Gene flow between populations is higher in good dispersers than in poorly dispersing species and may prevent genetic erosion. However, species with low dispersal ability may persist, because they are long-lived or are able to reproduce by selfing (Fischer & Stöcklin 1997). In long-lived species genetic variation is often higher compared to short-lived species (Hamrick *et al*. 1991). As a consequence negative effects of habitat fragmentation should be expressed earlier and more strongly in short-lived species and in those species with limited dispersal and colonising ability than in long-lived species with good dispersal and colonising ability (Young *et al*. 1996, Fischer & Stöcklin 1997). Moreover, it has to be taken into account, that irrespective of their life history traits, species that normally occur in large continuous populations are more sensitive to habitat fragmentation than those that are adapted to small population size and less connected habitats (Huenneke 1991).

Applications for landscape and species conservation management

The understanding of the effects of local adaptation, genetic variation and habitat fragmen-tation has become important for the restoration of habitats that have been damaged or destroyed (Wilkinson 2001). Restoration measures are tools of modern landscape manage-ment and may include the reintroduction of endangered plant species by seeds, juveniles or adults into areas where the target species is absent or where small populations should be

reinforced. Another measure for restoration, the introduction of foreign seeds to increase the diversity of plant species, e.g. in intensively managed farmlands, has increased in landscape management (Keller *et al*. 2000). Most recommendations of genetic management arise directly or indirectly from results of quantitative genetic studies (Frankham 1999), and they mostly suggest the use of local provenances for these introductions (Wilkinson 2001), because differentiation between populations is high. However, this may not be possible when only few or no local seed sources are available. Little is known about the genetic conse-quences of using non-local seeds or plants and thus there is a controversial discussion about the need of using local seed stock for reintroduction (Sackville Hamilton 2001, Wilkinson 2001). While Wilkinson (2001) states that the consideration of provenance is not important, because there is no general evidence for decreased hybrid vigour due to outbreeding depress-ion, many other authors have stressed the problems that may arise by introducing foreign plant material (Hodder & Bullock 1997, van Groenendael *et al*. 1998, Keller *et al*. 2000, Moore 2000, Sackville Hamilton 2001). In their review about genetic differentiation in the age of restoration, Hufford & Mazer (2003) conclude that further research is necessary to determine patterns of outbreeding depression that are relevant to restoration; see also Walker *et al*. (2004) for grasslands. Moreover, studies about the importance of local adaptation for the success of the reintroduction of plant species are required (van Andel 1998, van Groenendael *et al*. 1998, Hufford & Mazer 2003). If plants are adapted to specific conditions at their site of origin, their probability to fail in a new environment is high.

This thesis

Motivation of this thesis

This thesis is part of the large EU- project TRANSPLANT that studied the extinction risk and the reintroduction of plant species in a fragmented Europe. In contrast to most recent studies that only took small geographical regions into account, in this thesis I extended the study on the effects of habitat fragmentation to a large geographical scale to focus on regional differences, e.g. caused by climatic differences.

Study system

As study system I used species-rich grasslands that occur throughout Europe. These grass-lands are semi-natural habitats that require low-intensity management by grazing or mowing for their persistence; they belong to the most species-rich plant communities in Europe, and they contain a high number of endangered plant and animal species (Korneck *et al.* 1996, Ssymank *et al.* 1998, WallisDeFries *et al.* 2002). In the last decades the number and area of species-rich grasslands have decreased dramatically in many parts of Europe, e.g. in Germany and the Netherlands (WallisDeVries *et al.* 2002), Great Britain (Keymer & Leach 1990), Sweden (Cousins 2001, Cousins & Eriksson 2001) and Switzerland (Zoller & Wagner 1986, Landolt 1991). The main causes for this habitat loss are abandonment, afforestation or conversion of species-rich grasslands into fertile pastures, meadows or arable fields. They are thus an excellent model system to study effects of habitat fragmentation at a European scale (Fig. 1).

Study species

In this thesis I study the two Asteraceae, *Carlina vulgaris* L. and *Hypochoeris radicata* L., which occur in species-rich grasslands throughout Europe. Both are mainly outcrossing species (Grime *et al.* 1988), but differ in various life history traits and in their pattern of distribution. *C. vulgaris* is a monocarpic perennial whose age at flowering depends on the size of the rosette (Watt 1981, Klinkhamer *et al.* 1992, Rose *et al.* 2002). In contrast, *H. radicata* is a polycarpic perennial which produces side rosettes and thus is able to grow clonally (Aarssen 1981, Turkington & Aarssen 1983, de Kroon *et al.* 1987, de Kroon *et al.* 2000). Both species produce single-seeded fruits (achenes) with a pappus that enhances dispersal by wind. However, while *H. radicata* is well dispersed (Soons & Heil 2002), *C. vulgaris* is considered to be poorly dispersed (Franzén & Eriksson 2003).

C. vulgaris is restricted to dry, nutrient-poor, more or less open habitats (Grime *et al.* 1988, Meusel & Kästner 1994, Klinkhamer *et al.* 1996) and has been threatened by habitat deterioration and fragmentation during the last decades (Meusel & Kästner 1994, Korneck *et al.* 1996). In contrast, *H. radicata* has an almost cosmopolitan distribution (Turkington & Aarssen 1983) and is not considered to be threatened. The commonness of *H. radicata* is probably the result of the high frequency of suitable habitats and the species' high ability to reach and colonise new habitats (Grime *et al.* 1988). For further information on the species see the methods sections of the following chapters.

Outline of this thesis

This thesis consists of four studies:

In Chapter 2 I report on the effects of habitat fragmentation on the reproduction and performance of *Carlina vulgaris*. Components of fitness of plants in the field and of offspring grown in a common garden were studied in 74 populations of different size and degree of isolation from seven European regions (Fig. 1). Furthermore, environmental conditions (e.g. climate data and nutrient availability) and vegetation composition were recorded at each site. By comparing the performance of plants in a common garden and in natural populations and by taking environmental conditions into account it is possible to distinguish between genetic effects, i.e. inbreeding depression and genetic drift, and environmental effects on plant performance.
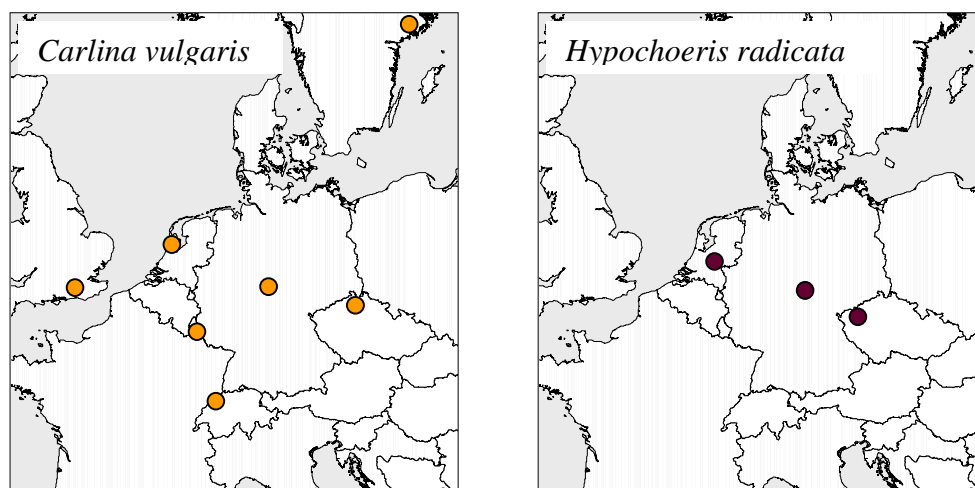

Figure 1. Map of the study regions for *Carlina vulgaris* and *Hypochoeris radicata*. For *C. vulgaris*: northwestern (NW-) Czechia, central (C-) Germany, southern (S-) Sweden, Luxemburg, northwestern (NW-) Switzerland, western (W-) Netherlands and southern (S-) England. For *H. radicata*: NW-Czechia, C-Germany and central (C-) Netherlands.

In Chapter 3 the partitioning of variation in quantitative genetic traits within populations, among populations within regions and among regions is investigated in both, *Hypochoeris radicata* and *Carlina vulgaris*. In each species, several populations of different size and degree of isolation from several European regions (Fig. 1) were studied. Offspring were grown in a common garden, and several morphological traits were measured during growth and flowering. The results were compared and analysed with respect to the different life histories of the two species. The quantitative genetic distances between each pair of

populations were correlated with geographical distances. Effects of habitat fragmentation were studied by relating the variation within populations to their size and their degree of isolation.

Results of reciprocal transplant experiments are presented in Chapter 4. In cooperation with colleagues from four European countries, I carried out transplantations of seedlings of *C. vulgaris* between and within several European regions (Fig. 1, except for southern Britain and western Netherlands) to investigate local adaptation on a large (between regions) and a small (within regions) geographical scale. For the within-region experiment, populations of different size from southern Sweden, central Germany and nothwestern Czechia were used to study possible differences in adaptive ability due to habitat fragmentation.

In Chapter 5 I report on the study of the effects of cross-proximity on reproduction and offspring fitness in populations of *H. radicata* originating from different geographical regions (Fig. 1). I raised plants from five populations each in central Germany, northwestern Czechia and the central Netherlands in a common garden and carried out hand pollinations (selfing, crosses between offspring of the same seed family, crosses between seed families, crosses between populations within regions and crosses between populations from different regions). The offspring were then grown until flowering, and fitness-related traits were recorded to study the effects of inbreeding and intraspecific hybridisation over large distances.

Plant size, fecundity and offspring performance in relation to habitat quality, population size and isolation in the monocarpic *Carlina vulgaris* in seven European regions

with Henrik Berg and Diethart Matthies

ABSTRACT

We studied the effects of habitat conditions and population size and isolation on the performance of *C. vulgaris* from 74 populations in seven European regions. To separate genetic from environmental effects, we studied fitness-related traits both in plants in natural populations and in offspring grown for two growing seasons in a common garden. In the common garden several measures of performance (e.g. germination, survival probability and a multiplicative fitness measure) of plants from large populations were higher than in plants from small populations, and plant size in both years decreased with increasing isolation of the populations, indicating genetic drift and inbreeding depression in small and isolated populations. In the field, only seed set was reduced in small populations which could be due to genetic effects or due to pollen limitation. In contrast, plant size in the field was not affected by population size or isolation. Environmental variables that varied among regions explained most of the variation between populations. In field populations plant size and seed set increased with decreasing latitude of the sites. In offspring in the common garden plant size also decreased with increasing latitude, whereas survival and total fitness increased. Species composition as indirect measure of habitat conditions explained parts of the variation among populations both in the field and in offspring in the common garden. The results suggest that reproduction and offspring performance may be reduced in small populations of *C. vulgaris* across Europe due to genetic deterioration. However, in field populations environmental conditions are more important for the performance of that species suggesting to maintain or establish regional conservation priorities.

INTRODUCTION

Changes in land use over the last decades have led to increased fragmentation of plant populations due to deterioration and destruction of habitats throughout Europe (Keymer & Leach 1990, Saunders *et al*. 1991, Poschlod & Schuhmacher 1998). As a consequence populations of a large number of plant species were reduced in size, and the distance between the remnant populations increased (Korneck *et al*. 1996, Fischer & Stöcklin 1997). Both small population size and isolation increase the risk of extinction of plant populations by increasing environmental, demographic and genetic stochasticity and disrupting metapopulation dynamics (Gilpin & Soulé 1986, Soulé 1986, Menges 1991a, Boyce 1992, Matthies *et al*. 2004).

Among these threatened habitat types are nutrient-poor grasslands. These species-rich grassland communities grow in semi-natural habitats and depend on extensive land use by grazing or mowing. Small patches in particular are often no longer managed which leads to accumulation of nutrients, to a higher productivity and to decreased species richness in those patches (Huber 1994). Deterioration of habitats may lead to decreased reproduction of plants (Widén 1993, Oostermeijer *et al*. 1994b, Eisto *et al*. 2000) and may strongly affect the viability and persistence of individual plants and plant populations (Roach & Wulff 1987, Dueck & Elderson 1992, Huber 1994, Pegtel 1998, Vergeer *et al*. 2003b). In particular those species restricted to specific habitat types or of a short life cycle, e.g. monocarpic species, run a high risk of local extinction (Fischer & Stöcklin 1997, but see Krauss *et al*. 2004).

Fragmentation of habitats may also cause a reduction in pollen quantity or quality leading to reduced reproduction in smaller populations. Pollen limitation in small populations might be the result of reduced activity of pollinators (Jennersten 1988, Steffan-Deventer & Tscharntke 1999), because pollinators are less attracted to small or low-density plant populations (Sih & Baltus 1987, Oleson & Jain 1994). Decreased pollen supply has been suggested as one reason for reduced fecundity in small populations (Lamont *et al*. 1993, Aizen & Feinsinger 1994), in particular in obligate outcrossers (Larson & Barrett 2000).

Another possible consequence of small population size is increased genetic drift and inbreeding, i.e. mating among close relatives, leading to decreased genetic variation and increased expression of deleterious alleles (Gilpin & Soulé 1986, Charlesworth & Charlesworth 1987, Lacy 1987, Barrett & Kohn 1991, Ellstrand & Elam 1993) which in turn may negatively affect individual fitness in small populations (Franklin 1980, Lande 1995). Decreased fitness in small compared to large populations has been found in a number of plant

species (Menges 1991b, Oostermeijer *et al*. 1994a, Young *et al*. 1996, Fischer & Matthies 1998a, Lienert *et al*. 2002a, b, Paschke *et al*. 2002a, b, Vergeer *et al*. 2003a), but not in all species that have been studied (Widén 1993, Ouborg & van Treuren 1995). Gene flow between populations increases with decreasing distance between populations (Berge *et al*. 1998, Richards 2000) and can counteract negative effects of small population size on genetic variability (Newmann & Tallmon 2001). Thus, negative effects of population size may be less severe in non-isolated compared to isolated populations. However, a few studies have investigated the combined effects of reduced population size and isolation on plant performance (Lienert *et al*. 2002a, b, Paschke *et al*. 2002a, Hooftman *et al*. 2003).

Nearly all studies have investigated the effects of habitat fragmentation in one small region. However, the threat due to habitat fragmentation might vary in different geographic regions (Hooftman *et al*. 2003, Vergeer *et al*. 2003a). Populations in naturally fragmented habitats or at the margin of a species' range might be adapted to fragmentation (Lammi *et al*. 1999), because gene flow among those populations has been limited for a long time. Thus, negative effects of small population size should have been expressed a long time ago, and it is likely that deleterious alleles have been purged (Byers & Waller 1999, Frankham *et al*. 2001).

We studied the effects of population size and isolation and of habitat characteristics on the performance of the monocarpic *Carlina vulgaris* in natural populations from seven European regions. In addition, we grew offspring from the same populations in a common environment to separate genetic from environmental effects. *C. vulgaris* used to be a common species, but has declined and is even threatened in some areas (Korneck *et al*. 1996), because it is mainly restricted to nutrient-poor dry grasslands, which are among the most threatened habitats in Europe (Willems 1990, WallisDeVries *et al*. 2002).

We address the following questions: (1) Which are the most important factors for the performance of *C. vulgaris* across a large geographical scale? (2) Do population size and population isolation influence performance of plants in the field? (3) Is the fitness of offspring from small and isolated populations reduced in a common environment?

MATERIAL AND METHODS

Study species

Carlina vulgaris is a monocarpic perennial. The probability of flowering increases with the size of the rosette, and the age of flowering plants varies between two and at least eleven years (Watt 1981, Klinkhamer *et al*. 1991, Rose *et al*. 2002). From June to September plants produce one to several flower heads each with up to 300 disc florets. The florets are protandrous and self-compatible, but mainly insect-pollinated (Greig-Smith & Sagar 1981, Grime *et al*. 1988). The most important pollinators are large insects (Apoidea), of which bumble bees (*Bombus*) are the most important (Meusel & Kästner 1994, personal observations). 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 & Sagar 1981, Grime *et al*. 1988, Franzén & Eriksson 2003). *C. vulgaris* grows in dry, nutrient-poor, more or less open habitats, predominantly in semi-natural calcareous grasslands, but also in quarries, coastal dunes and open pine forests (Verkaar & Schenkeveld 1984, Grime *et al*. 1988, Meusel & Jäger 1991, Meusel & Kästner 1994, Klinkhamer *et al*. 1996). In Europe the species is distributed throughout sub-oceanic to sub-Mediterranean regions from S-Italy to S-Sweden (62 °N). Because of habitat deterioration and fragmentation over the last decades, many populations are now small and isolated, particularly in the north-east of the range (Meusel & Kästner 1994, Korneck *et al*. 1996).

Characteristics of habitats and populations

In late summer 2000, we chose in each of seven European countries a region with a large number of populations of *C. vulgaris* and selected 74 populations that varied in size and distance to the nearest population (Table 1).

A population was defined as a group of plants that were at least 80 m from the nearest conspecific. The distance between a population and the nearest conspecific was used as measure for population isolation. The altitude, latitude and longitude of each population were determined by maps. The number of flowering individuals was counted in Germany and Sweden in 2000 and in all regions in 2002. Thus, the population sizes recorded in 2002 were used in the analyses. However, in Germany and Sweden correlations between population sizes recorded in the both years were very high (r > 0.94, p < 0.001). In each population, the largest mature fruit head of each of 20 randomly selected individuals, which grew at least 5 m apart from each other, was collected. In very small populations all mature individuals were

sampled. The fruit heads were sent to Marburg (Germany), where the seeds were removed from the fruit heads and partitioned into ripe and unripe seeds. The number of ripe and unripe seeds including not pollinated florets per fruit head was counted, and seed set was calculated. Ripe seeds were weighed and mean seed mass of each plant was calculated.

Table 1. Number, size, distance to nearest conspecific population (isolation) and location of populations of *Carlina vulgaris* studied in seven European regions. Values for population size, isolation, location and altitude are given as ranges per region.

Region	Number of populations	Population size	Isolation [m]	Longitude	Latitude	Altitude [m asl]
S-Sweden	12	11–1640	80–1000	13.148 E–17.585 E	58.251 N–59.001 N	5–200
C-Germany	21	1–1000	100–7000	9.767 E–9.933 E	51.187 N–51.456 N	210–520
W-Netherlands	6	50–700	80–500	4.491 E–4.746 E	52.569 N–53.050 N	10–15
S-Britain	6	20–400	80–400	0.262 W–0.054 E	50.842 N–51.260 N	52–134
NW-Czechia	6	30–1000	200–1000	14.417 E–15.998 E	50.100 N–50.333 N	190–320
Luxembourg	11	29–20000	100–1700	5.882 E–6.394 E	49.478 N–49.761 N	305–380
NW-Switzerland	12	15–1500	100– 500	6.744 E–7.483 E	47.321 N–47.807 N	481–651

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 indicator values for nitrogen, soil reaction, moisture and continentality were calculated per site (Persson 1981, Ellenberg *et al*. 1992). In addition, the maximum and mean height of the vegetation, the proportion of area covered by all plant species and the proportion of bare ground were recorded. Moreover, at each site the angle of the inclination of the slopes, deviation of the exposition from north (0-180 °, north = 0 °) and whether a site was managed or not (0 = management, 1 = abandonment) were recorded.

To estimate the relative nutrient availability at the study sites, we carried out a bioassay. In July and August 2002, in each population eight soil cores were randomly collected, mixed and air dried 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 x 9 x 9.5 cm) were filled with soil from each population and in each pot five seedlings of *Arrhenatherum elatius* were grown as phytometers in a glasshouse. After eight weeks all above-ground plant parts were harvested, dried for 24 hours at 80 °C and weighed. Total above-ground biomass per pot was used as an estimate of nutrient availability.

Plant performance in the field

In late summer 2002, 20 flowering and 20 vegetative plants of *C. vulgaris* were selected randomly in the centre of each study population and their performance was recorded. For flowering plants, we recorded plant height, number of fruit heads and (except for English populations) diameter of the largest and the smallest fruit head. Total fruit head area of a plant was estimated as the product of the number of fruit heads and the mean area of a fruit head which was calculated as: $0.5*[\pi*(\text{radius of largest fruit head})^2 + \pi*(\text{radius of smallest fruit head})^2]$. For non-flowering plants the diameter of the rosette was measured as an estimate of plant size.

Common garden study

At the end of March 2001, in Marburg, Germany, up to 40 seeds from each of 10 randomly chosen seed families per population were sown into plastic pots (9 x 9 x 9.5 cm) filled with commercial nutrient-poor garden soil (TKS 1, Floragard GmbH, Oldenburg, Germany) and placed at 15-20 °C in a greenhouse. After three weeks the number of seeds that had germinated was counted, and, in mid-May, five randomly chosen seedlings per family were transplanted individually into pots of the same size and filled with the same soil as used before. If five seeds or less had germinated we used all seedlings of a family. The seedlings were randomly placed into flowering beds in the Botanical Garden of the University of Marburg. In total, we transplanted 3105 seedlings of *C. vulgaris* from 631 seed families and 74 populations. Plants were grown for two growing seasons until they had either died or produced fruits.

Traits related to vegetative growth were recorded in the first year (2001), whereas reproductive traits were recorded in the second year of growth when most of the plants flowered. Length and width of the longest leaf were measured two weeks, six weeks and one year (size second year) after transplanting and estimates of leaf size were obtained as the product of the length and the width of the longest leaf. In 2001, the relative growth rates of leaf length and width were calculated as log (measurement at six weeks) - log (measurement at two weeks). In summer 2002, at the peak of flowering, the number of flowering stalks and of fruit heads was counted for each plant, and the diameter of all fruit heads, the height of the plants and the cumulative length of all branches were determined. To estimate plant fecundity, we calculated total fruit head area as the product of the number of fruit heads and the mean area of a fruit head (as described above). This procedure was identical to the one

used in the field and thus directly comparable. The estimates obtained were strongly correlated with the sum of all fruit head areas ($r = 0.99$, $p < 0.001$) calculated from the size of all individual fruit heads of a plant. Survival to flowering was recorded and all above-ground parts of flowering plants were harvested after they had set fruit and died, dried for 12 hours at 80 °C and weighed. As a multiplicative fitness function, we calculated the mean fruit head area produced per seed as the product of germination, survival to flowering and total fruit head area.

Data analysis

Differences in species composition among populations were investigated with detrended correspondence analysis (DCA). DCA-scores along the first two axes were used as variables reflecting environmental differences among habitats. To analyse the environmental gradients along the DCA-axis we calculated correlations between DCA-scores, environmental variables and mean indicator values.

Several of the performance traits of *C. vulgaris* were intercorrelated. We therefore used principal component analysis (PCA) to calculate uncorrelated linear combinations of the characters measured in the common garden. We performed PCA at the level of the individual plant ($n = 2060$). Percentage germination was not excluded in the PCA analysis, because it was recorded for each seed family. Therefore we present it as individual character. The relationship between the individual characters and the PCA-scores was studied by mean of rotated component loadings. PCA-scores obtained from varimax rotation of the extracted principal components were used for further analyses.

We performed backward multiple regression analyses ($p > 0.10$ to exclude) with population means of each performance trait as dependent variable to analyse the effects of habitat characters (including latitude and longitude), species composition, population size and distance to the nearest population on seed set and plant performance in the field and on offspring performance in the common garden. We did not include region in the analyses. In analyses of variance (SS-Type II) with region as dependent and habitat characters as independent variables 80% of the differences between regions were explained by effects of the DCA-scores, the longitude of the site, the cover of the vegetation and the altitude above sea level. Thus, by using habitat characteristics, we directly took the differences between regions into account and it was possible to specify the ecologically important traits which are responsible for the variation among *C. vulgaris* populations from different regions.

In the analyses of offspring performance the mass of the seeds produced by a mother plant was used as a covariate in the multiple regression analyses to adjust for maternal effects. Population size and isolation were log-transformed prior to analyses. Germination was square root-transformed prior to analyses to achieve homoscedasticity and normally distributed residuals.

All statistical analyses were performed with SPSS (release 11.0; SPSS Inc, Chicago, Illinois, USA) except for the detrended correspondence analysis that was performed with Canoco 4.5 (Ter Braak & Šmilauer 2002).

RESULTS

Habitat conditions and their relationship with population size

Most of the habitat conditions varied considerably among, but also within regions (Table 2). In Czechia and the Netherlands most of the sites were unmanaged, whereas in Sweden only 8% of the sites were not managed during the study years. Slopes were flattest in the Netherlands. There, *Carlina vulgaris* grows in dune valleys, whereas the typical habitats in the other regions are dry calcareous grasslands on slopes.

Table 2. Habitat conditions in 74 populations of *Carlina vulgaris*: mean values and standard errors in each region are shown.

Habitat conditions	Czechia	Germany	The Netherlands	Great Britain	Sweden	Switzerland	Luxembourg
Deviation from North [0-180°]	90.0 ± 20.9	136.0 ± 12.1	30.0 ± 22.6	123.3 ± 22.6	127.5 ± 16.0	126.5 ± 16.0	108.8 ± 17.5
Inclination [°]	11.6 ± 3.7	15.2 ± 2.1	4.2 ± 4.0	25.0 ± 4.0	17.3 ± 2.8	20.1 ± 2.8	15.1 ± 3.1
Unmanaged sites [%]	100.0	52.4	83.3	33.3	8.3	66.7	40.0
Biomass of phytometer [mg]	97.8 ± 25.3	72.0 ± 14.6	110.2 ± 25.3	136.9 ± 25.3	174.3 ± 19.3	71.3 ± 18.6	78.7 ± 21.2
Cover of vegetation [%]	81.4 ± 6.9	73.3 ± 4.0	86.9 ± 6.9	72.9 ± 6.9	94.0 ± 5.3	66.2 ± 5.1	89.3 ± 5.8
Cover of bare ground [%]	5.0 ± 7.2	12.9 ± 4.1	5.6 ± 7.2	4.1 ± 7.2	3.3 ± 5.5	22.4 ± 5.3	10.7 ± 6.0
Mean vegetation height [cm]	29.3 ± 3.2	12.4 ± 1.8	14.7 ± 3.4	7.8 ± 3.4	5.8 ± 2.4	14.2 ± 2.4	24.8 ± 2.6
Nitrogen indicator value	3.5 ± 0.2	3.4 ± 0.1	4.0 ± 0.2	3.4 ± 0.2	3.7 ± 0.1	3.8 ± 0.1	3.3 ± 0.1
Soil reaction indicator value	7.2 ± 0.1	7.2 ± 0.1	5.8 ± 0.1	7.2 ± 0.2	5.5 ± 0.1	7.1 ± 0.1	7.4 ± 0.1
Continentality indicator value	4.1 ± 0.1	3.8 ± 0.0	3.6 ± 0.1	3.4 ± 0.1	3.7 ± 0.0	3.7 ± 0.0	3.7 ± 0.1
Moisture indicator value	4.2 ± 0.1	4.0 ± 0.1	4.5 ± 0.1	4.3 ± 0.1	4.6 ± 0.1	4.2 ± 0.1	4.1 ± 0.1

The vegetation was tallest in Czechia and Luxembourg. Across all regions the maximum height of the vegetation ranged between 30 and 130 cm (mean 81.6 ± 25.9 cm). High vegetation cover and biomass of the phytometers indicated that nutrient availability was highest at the Swedish sites although the vegetation was lowest. Soil nutrient availability and vegetation cover were lowest at the Swiss and the German sites. Mean indicator values for soil reaction indicated neutral conditions in most regions, but slightly acidic soil conditions in Sweden and in the Netherlands. Mean indicator values for continentality were positively correlated to the mean longitude of the sites in each region ($r = 0.79$, $p = 0.03$, $n = 7$) indicating that the vegetation composition reflected the longitudinal position of the sites.
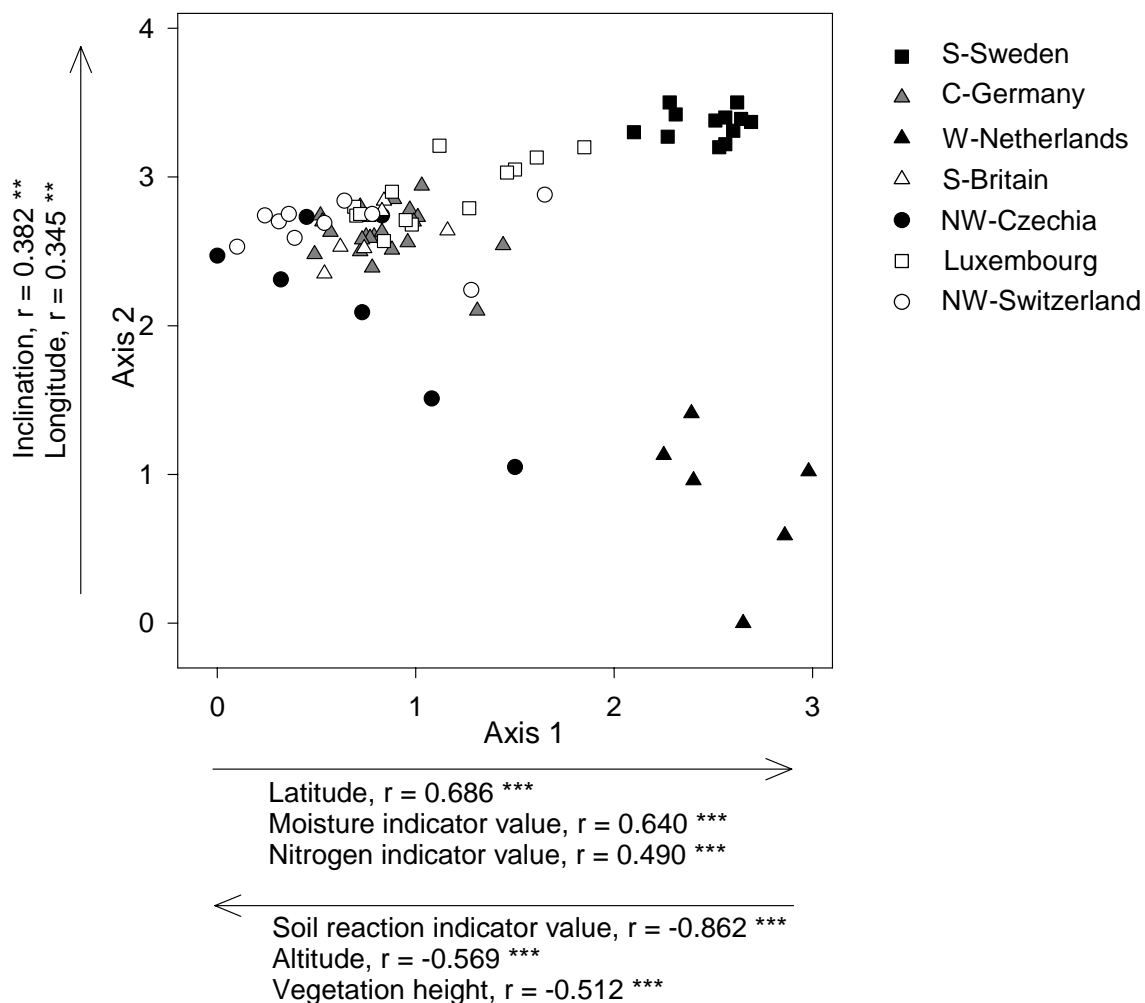

Figure 1. Site-scores resulting from DCA (detrended correspondence analysis) of the species composition in 74 populations of *Carlina vulgaris* in seven European regions. Correlation coefficients (r) indicate the relationship between DCA-scores and habitat characters that explained most of the variation in species composition. ** $p < 0.01$, *** $p < 0.001$.

Across all sites, the most frequent plant species associated with *C. vulgaris* were *Bromus erectus*, *Linum catharticum*, *Lotus corniculatus* and *Hieracium pilosella* which are characteristic species of nutrient-poor grasslands. The detrended correspondence analysis (DCA) indicated considerable differences in species composition among sites. The length of gradient of the first axis of the DCA was 3.25; the scores of the first two DCA-axes (eigenvalues: 0.435, 0.309) were used for further analyses and interpretation. Both the first and the second DCA-axes (Fig. 1) were correlated with the geographical position of the populations. The first axis was strongly correlated with the mean indicator values for soil reaction ($r = -0.86$), the latitude of the sites ($r = 0.69$), the mean indicator values for moisture ($r = 0.64$) and nitrogen ($r = 0.49$) and the dry mass of the phytometer ($r = 0.47$, all $p < 0.001$). Species with high scores on the first axis were *Luzula campestris*, *Agrostis tenuis* and *Potentilla erecta*; species with low scores were *Koeleria pyramidata*, *Brachypodium pinnatum* and *Leontodon hispidus*. The second axis was weakly related to the longitude of the sites ($r = 0.35$, $p = 0.003$, Fig. 1) and to the inclination of the slopes ($r = 0.38$, $p = 0.003$).

Table 3. Results of backward multiple regression analyses of the effects of various habitat characteristics, population size (log) and distance to the nearest population (log) on fitness-related characters of *C. vulgaris* plants in the field. Standardised partial regression coefficients are given. (n in 2002 = 74, n in 2000 = 67). $+ p < 0.1$, $* p < 0.05$, $** p < 0.01$, $*** p < 0.001$.

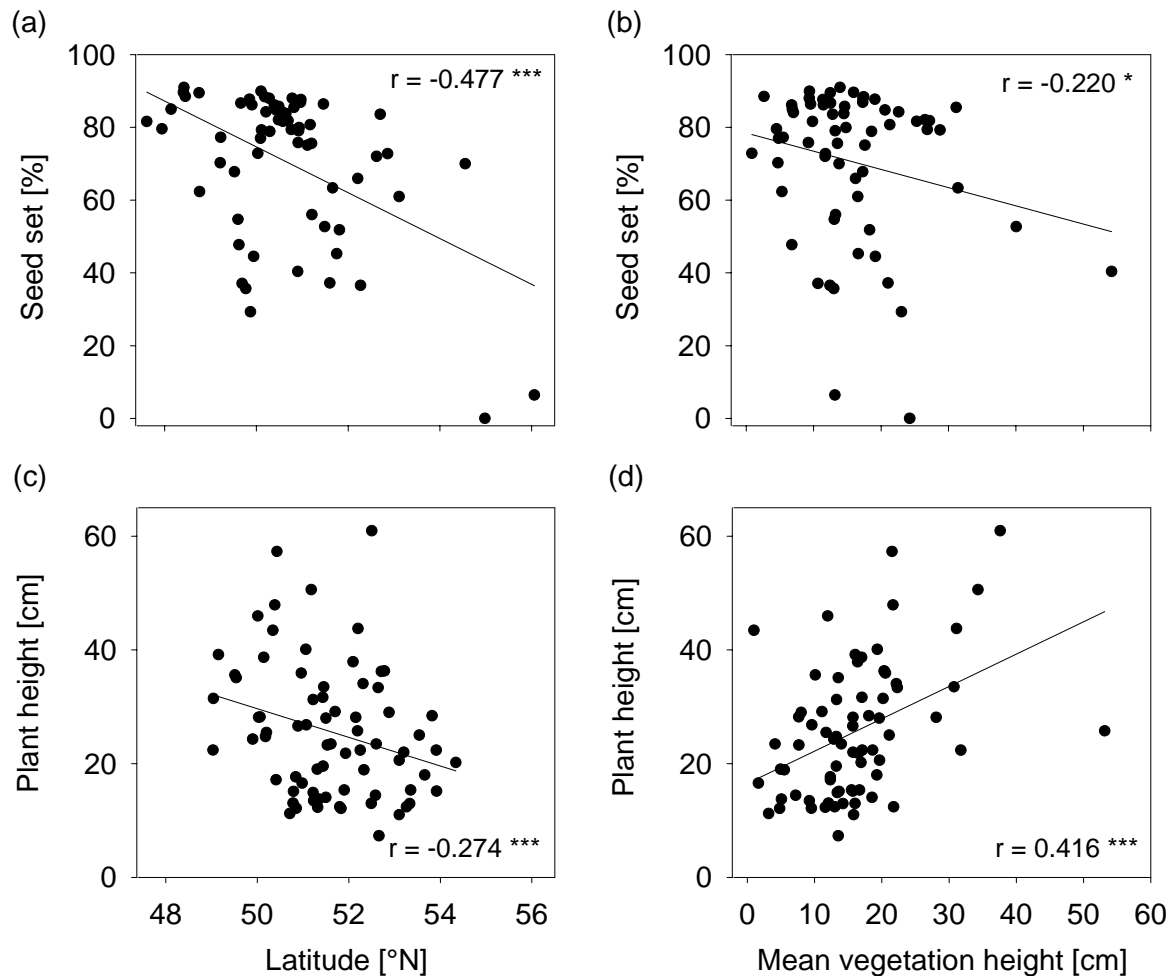| Model summary	Seed set in 2000 $r^2 = 0.48**$	Seed mass in 2000 $r^2 = 0.28***$	Rosette diameter in 2002 $r^2 = 0.24**$	Plant height in 2002 $r^2 = 0.61***$	Flower head area in 2002 $r^2 = 0.51***$
Latitude	-0.740***	-0.525***	0.360*	-0.803***	-
Longitude	-	-	-0.286*	0.360**	0.301**
Altitude	-0.257+	-	-	-0.523**	-
DCA-scores species, axis 1	-	-	-	0.358**	-
DCA-scores species, axis 2	-	0.234*	-	0.294*	-0.413**
Mean vegetation height	-0.300*	0.229*	0.365**	0.539***	0.389***
Management (yes =0, no =1)	0.233*	-	0.204+	0.256**	-
Cover of bare ground	-0.347**	-	-	-	-
Nutrient availability	-	-	0.212+	-	-
Inclination	-	-	-	-0.217*	-0.180+
Distance to nearest population	0.171+	-	-	-	-
Population size	0.337**	-	-	-	-

Figure 2. The influence of the latitude of the sites (a, c) and the mean height of the surrounding vegetation (b, d) on seed set in 2000 (a, b) and plant height (c, d) in 2002 in populations of *C. vulgaris*. Semi-partial plots, values of the independent variables were adjusted for all other significant explanatory variables in the multiple regression model (s. Table 3). * $p < 0.05$, *** $p < 0.001$.

Effects of population size, distance to the nearest neighbouring population and habitat characters on performance in the field

The performance of flowering plants in the field was strongly influenced by site characters (Table 3, Fig. 2), in particular by the latitude and the longitude of the sites, and by the mean height of the vegetation. Seed set, seed mass and plant height decreased with increasing latitude, whereas rosette size increased. Plant height and the area of the fruit heads (i.e. reproduction) increased with increasing longitude. The height of the vegetation positively in-fluenced seed mass, rosette size, plant height and fruit head area, but had a negative effect on seed set. Plants in managed sites had smaller rosettes and smaller stalks than those in unma-naged sites, whereas plants at higher altitudes had lower seed set and were smaller. Population size had a positive effect on seed set (Table 3, Fig. 3), but on none of the other traits.
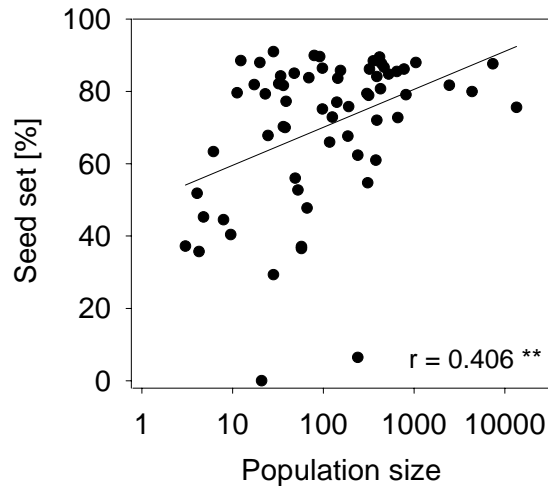
Figure 3. The influence of the size of the population (log) on seed set in the field in 2000 in 67 populations of *C. vulgaris*. Semi-partial plots: values of the independent variable were adjusted for all other significant explanatory variables in the multiple regression model (s. Table 3). ** p < 0.01.

Table 4. The relationship between fitness-related characters and principal component scores given as loadings of principal components after varimax rotation. Bold-faced component loadings show the highest correlations (r > 0.45) between measured values and principal component scores (n = 2060).

	Rotated component loadings	
	PC size year 2	PC size year 1
Above-ground biomass	**0.892**	0.231
Total fruit head area	**0.879**	-0.027
Number of flower heads	**0.760**	0.142
Cumulative length of branches	**0.728**	0.326
Mean fruit head area	**0.499**	-0.212
Leaf size at 1 year	**0.486**	0.085
Leaf size at 2 weeks	0.257	**0.892**
Relative growth rate of length of leaf	0.086	**-0.659**
Relative growth rate of width of leaf	0.066	**-0.652**
Leaf size at 6 weeks	0.359	**0.648**
% Variance explained	30.64	21.54

Performance in the common garden

PCA reduced the set of fitness-related characters to two main uncorrelated principal components (PCs) which together explained 52.2% of the total variation (Table 4). The first component (PC size year 2) was mainly correlated to characters that describe the size and fecundity of flowering plants, e.g. the above-ground biomass, the cumulative branch length, the number of flower heads and the total fruit head area. The second component (PC size year 1) was mainly correlated to characters that describe the size and growth of the rosettes in the first year.

Effects of the size of the population of origin, its distance to the nearest population and the habitat conditions on performance in the common garden

Germination was positively correlated with the cover of the vegetation and the population size at the site of origin, but was not affected by the location of the sites (Table 5). Locality of the sites together with DCA-scores and elevation, i.e. regional differences, were the main predictors for the proportion of plants that survived to flowering, for multiplicative fitness and for the PCs related to first and second year performance. However, the performance of surviving plants in both years decreased with increasing latitude of the population of origin, whereas variables that included survival increased due to a high probability for survival of Swedish plants (Table 5, Fig. 4). Seed mass of the mother plant had a positive effect on germination and on PC size year 1, but no effect on PC size year 2. Population size had a positive effect on germination, on the number of individuals that survived to flowering, on PC size in the second year and on multiplicative fitness (Table 5, Fig. 5). The performance in both study years decreased with increasing isolation (Fig. 6).

Table 5. Results of backward multiple regression analyses of the effects of habitat conditions (for mangement: 0 = no, 1 = yes), population size (log) and distance to the nearest population (log) on germination, survival to flowering, multiplicative fitness (germination * survival to flowering* area of flower heads), PC size year 1 and PC size year 2 in *C. vulgaris* grown in a common garden. Standardised partial regression coefficients are given (n = 74). + $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

|	Germination	PC size year 1	Survival to flowering	PC size year 2	Multiplicative fitness
Model summary	$r^2 = 0.23$***	$r^2 = 0.50$***	$r^2 = 0.46$***	$r^2 = 0.65$***	$r^2 = 0.44$***
Family seed mass	0.283 **	0.203 +	-	-	-
Latitude	-	-0.817 ***	0.588 **	-0.405 **	0.476 **
Longitude	-	0.529 ***	0.240 +	0.304 **	0.283 *
Altitude	-	-	0.429 **	-	0.489 **
DCA-scores species, axis 1	-	-0.219 +	-0.458 **	-0.219 +	-0.486 ***
DCA-scores species, axis 2	-	-	-	0.467 ***	-
Mean vegetation height	-	-	-	0.313 **	-
Cover of vegetation	0.345 **	-	-0.209 *	-	-
Management	-	-	-0.181 +	0.154 +	-
Inclination [°]	-	-	-	-0.194 *	-
Distance to nearest pop.	-	-0.258 **	0.183 +	-0.288 **	-
Population size	0.238 *	-	0.207 *	0.264 **	0.232 *
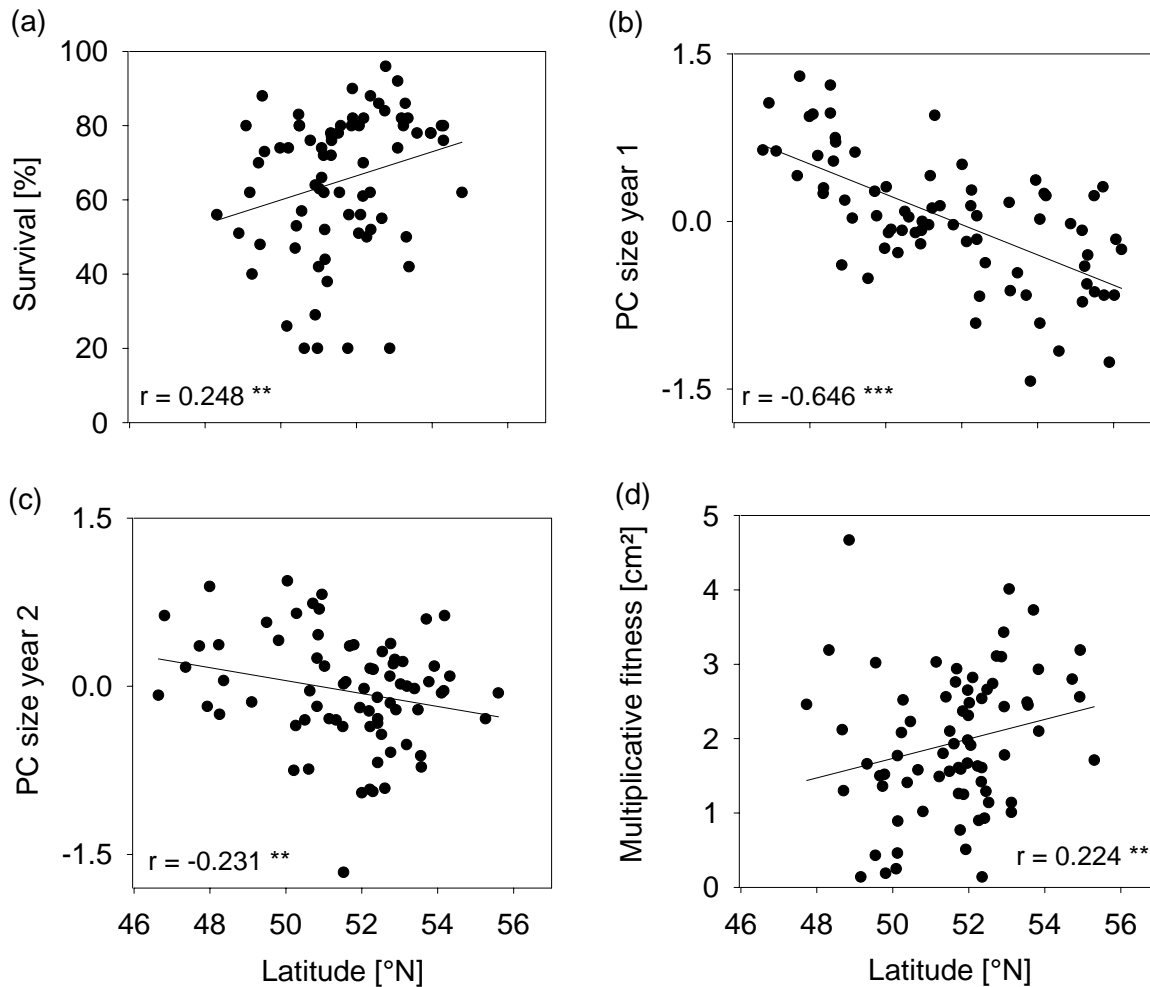
Figure 4. The influence of the latitude of the sites of the population of origin on (a) % survival to flowering, (b) principal components related to first year performance, (c) principal components related to second year performance and (d) multiplicative fitness in offspring of 74 populations of *Carlina vulgaris*. Semi-partial plots: values of the independent variable were adjusted for all other significant explanatory variables in the multiple regression model (s. Table 5). ** p < 0.01, *** p < 0.001.

Relationship between performance in the field and in the common garden

Plants in the field were on average 7 cm higher and produced 0.5 more fruit heads which were 0.74 cm² larger in total area than plants in the common garden indicating a slightly lower performance of cultivated plants. The size of the rosettes (length of longest leaf) and the height of flowering plants in the common garden were positively correlated to the respective traits measured in the field (Table 6). In particular, plant height in the field and in the common garden were strongly correlated. However, there was no relationship between reproductive characters measured in the garden and fitness-related traits in the field.
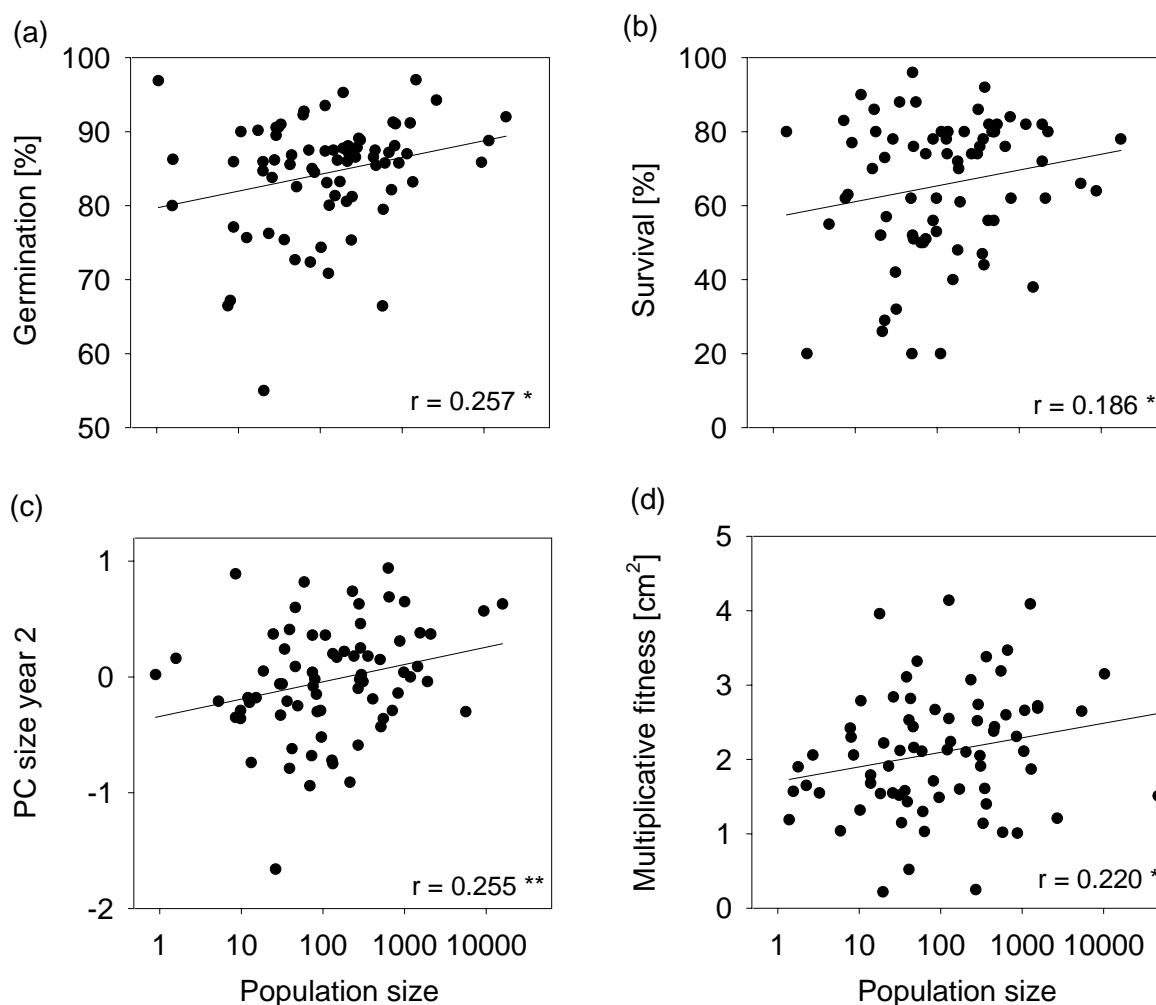
Figure 5. The influence of the size of the population of origin on (a) germination, (b) survival to flowering, (c) the principal component related to second year performance and (d) multiplicative fitness in offspring of 74 populations of *Carlina vulgaris*. Semi-partial plots: values of the independent variable were adjusted for all other significant variables in the multiple regression model (s. Table 5). * p < 0.05, ** p < 0.01.

Table 6. Correlation coefficients for the relationship between plant traits in the field and in the common garden at the population level. * p < 0.05, ** p < 0.01, *** p < 0.001.

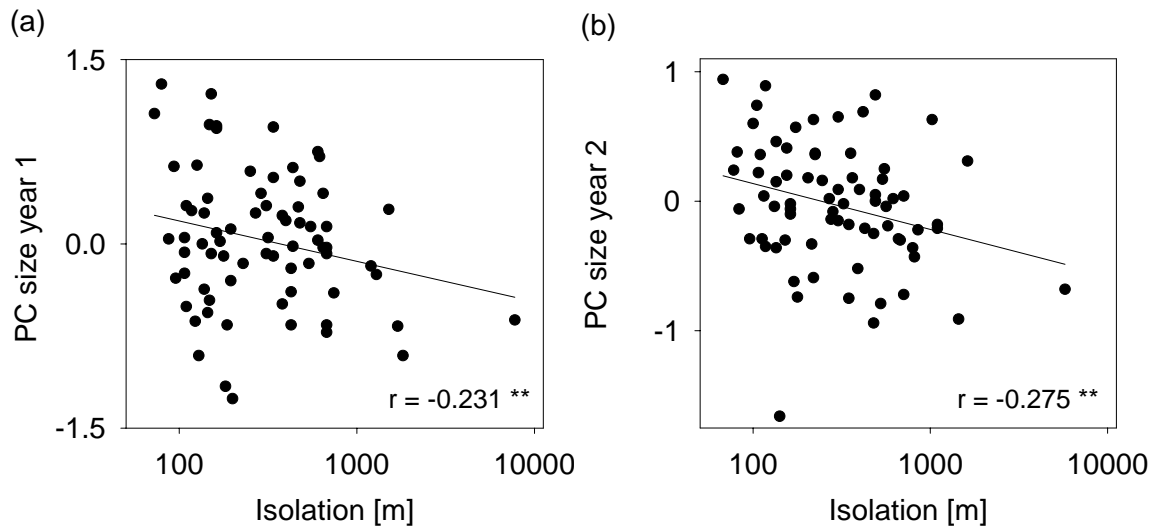|		Field			
	n	Rosette size	Plant height	Number of flower heads	Fruit head area
Common garden					
Length of longest leaf	74	0.331*	0.590***	0.325**	0.289*
Plant height	74	0.175	0.766***	0.401***	0.462***
Number of flower heads	74	-0.162	0.110	0.084	0.096
Fruit head area	66	-0.096	0.053	0.090	0.114

Figure 6. The influence of the isolation of the population of origin on (a) the principal component (PC) related to first year performance and (b) the PC related to second year performance in offspring of 74 populations of *C. vulgaris*. Semi-partial plots: values of the independent variable were adjusted for all other significant explanatory variables in the multiple regression model (s. Table 5). ** $p < 0.01$.

DISCUSSION

There was considerable variation among populations in fitness-related traits, both in the field and in the common garden. Variation among populations in plants that were grown in a common environment suggests genetic differentiation among populations (see also Chapter 3). In both parts of this study environmental conditions, in particular those that are related to regional differences, mainly influenced plant performance. Moreover, in the common garden fitness-related characters increased with increasing size of the populations and decreased with increasing isolation, but population size and isolation did not affect most measures of performance of populations in the field, except for seed set that increased with population size.

Lower seed set and offspring performance in small compared to large field populations could be an effect of lower habitat quality, inbreeding depression and decreased genetic variability due to genetic drift (Barrett & Kohn 1991, Widén 1993, Ågren 1996, Fischer & Matthies 1998a, b, Kéry *et al.* 2000). In addition to these effects a decrease in seed set is often caused by pollen limitation in small populations (e.g. Jennersten & Nilson 1993, Byers 1995, Gigord *et al.* 1999, Hendrix & Kyhl 2000, Kéry *et al.* 2000). However, few studies have shown these effects experimentally (Ågren 1996, Groom 1998). Pollen limitation mainly affects outcrossing species which depend on pollen supply from genetically distant individuals (Larson & Barrett 2000). One would expect a negative relationship between distance to the nearest population and seed set, because isolated populations should suffer

more strongly from pollen limitation (Rathcke & Jules 1993, Aizen & Feinsinger 1994, Groom 2001). However, in field populations of *C. vulgaris* seed set even slightly increased with distance to the nearest population. We suggest that the effectiveness of pollination was higher in isolated populations, because pollinators were forced to forage more intensely in a smaller area. Moreover, species which are, as *C. vulgaris*, mainly pollinated by insects with large body size (e.g. bumble bees) are less strongly affected by increasing isolation, because the foraging distance increases with the body size of the pollinators (Gathmann *et al*. 1994, Steffan-Deventer & Tscharntke 1999). Moreover, monocarpic and short-lived species are less sensitive towards pollen limitation, because pollen limitation strongly reduces the life-time reproductive fitness of those plants and selection should be stronger on traits minimising pollen limitation (Larson & Barrett 2000).

The decrease of offspring fitness in smaller populations of *C. vulgaris* cannot directly be explained by effects of pollinators. However, pollinators could behave differently in small populations in terms of foraging more intensely on a lower number of plant individuals. As a consequence inbreeding in these small populations would increase and lead to a reduction in plant fitness. Moreover, plant fitness is supposed to be positively associated with genetic variation (Boyce 1992, Ellstrand & Elam 1993, Reed & Frankham 2003). Decreased genetic variation in individuals of small populations due to increased genetic erosion or increased inbreeding has been found in a number of studies (e.g. van Treuren *et al*. 1991, Luijten *et al*. 2000, Vergeer *et al*. 2003a, Pluess & Stöcklin 2004). In *C. vulgaris* we suggest that genetic effects are responsible for the reduced fitness of plants from small populations, because plants were grown in a common environment. A positive relationship between population size and offspring performance in a common environment has been found for a number of rare species (Oostermeijer *et al*. 1994a, Heschel & Paige 1995, Fischer & Matthies 1998a, Kéry *et al*. 2000, Fischer *et al*. 2003). In common species decreased fitness was found in small populations of *Carex davalliana* (Hooftman *et al*. 2003), *Succisa pratensis* (Vergeer *et al*. 2003a) and *Pimpinella saxifraga* (Berg, Becker & Matthies in prep.).

A second effect of habitat fragmentation on plant populations is an increased isolation of the remnant populations. Isolated populations might be more sensitive towards genetic erosion, because gene flow that may enhance genetic variation within populations is lower (Hamrick *et al*. 1991, Ellstrand & Elam 1993). However, little is known about the effects of population isolation on plant performance independent from the effects of population size (Lienert *et al*. 2002a, b, Paschke *et al*. 2002a, Hooftman *et al*. 2003). Lienert *et al*. (2002a) found a decrease in several performance traits with increasing isolation of the populations of

Swertia perennis in the field. However, the authors investigated those effects indirectly by relating population isolation to heterozygosity and then heterozygosity to offspring fitness. In *Carex davalliana* isolation negatively influenced plant performance, but population size had more severe effects (Hooftman *et al.* 2003). These results indicate that gene flow between populations is not able to counteract the negative effects of small population size and is generally low in this species. In *Cochlearia bavarica* (Paschke *et al.* 2002a) performance in the field was reduced in small, but not in isolated populations. Our results are in line with these studies suggesting that effects of population size are more important than effects of isolation, because population size had positive effects on all fitness-related traits except for plant size in the second year, whereas isolation had negative effects on performance in the first and in the second year, but not on survival or overall fitness. Altogether both, positive effects of population size and negative effects of population isolation on plant performance suggest that genetic effects reduce performance of plants from fragmented populations of *C. vulgaris*.

Maternal carry-over effects could influence offspring fitness in the common garden (Schaal 1980, Roach & Wulff 1987, Schmitt *et al.* 1992, Schmid & Dolt 1994, Ouborg & van Treuren 1995). In *C. vulgaris* the size of plants in the common garden was positively related to plant size and reproduction in the field which could be caused by both genetic or maternal carry-over effects. Moreover, the strong relationship between environmental conditions in the field and performance of plants in the common garden could be due to carry-over effects. Maternal effects are often mediated through seed size. However, mean initial seed mass in particular influenced the early traits germination and leaf size of juveniles in the common garden, but not plant height, survival and multiplicative fitness suggesting that maternal effects mainly influenced the growth of offspring in the first life stages (see Roach & Wulff 1987, Ouborg *et al.* 1991, Montalvo & Shaw 1994). The correlation between traits of adult plants (e.g. plant height) in the common garden and those in the field thus cannot be explained by maternal carry-over, but indicates population differentiation.

The best predictors for both the performance in the field and in the common garden were the geographical position of the population of origin indicating regional differences and also the height and the composition of the vegetation which are influenced by nutrient availability. The size of the plants in the field decreased with increasing latitude of the sites reflecting adaptation to harsher environmental conditions in the northern range of *C. vulgaris*. Increasing latitude also negatively influenced plant size in the common garden. Similar latitudinal patterns in plant size, growth or sexual reproduction have been observed in a number of common garden studies (Li *et al.* 1998, Weber & Schmid 1998, Dorken & Eckert

2001, Olsson & Ågren 2002, Kollmann & Bañuelos 2004). Survival and as a consequence multiplicative fitness were lower in southern than in northern populations of *C. vulgaris* indicating a trade-off between survival probability and plant size. Similarly, Olsson & Ågren (2002) suggested that in *Lythrum salicaria* increased allocation to the production of winter buds is responsible for the higher survival of plants from northern populations. In *C. vulgaris* smaller above-ground size could be compensated by increased growth of roots in northern populations that could be responsible for higher survival of those plants.

The height of the vegetation positively influenced the performance of *C. vulgaris* plants in the field, while the management of the sites had negative effects on seed set, plant height and rosette size in natural populations. However, increased size of plants in unmanaged sites that might reflect higher nutrient availability due to accumulation of nutrients, but not necessarily increased overall individual fitness or fitness of plant populations, because population growth rate is also influenced by recruitment and survival. Recruitment in *C. vulgaris* is higher in disturbed patches with open vegetation than in densely covered patches (Klinkhamer *et al.* 1996, Löfgren *et al.* 2000). Moreover, survival to flowering might be lower in unmanaged than in managed sites due to increased competition by other plants. Decreased survival with increasing productivity or competition has been found for a number of grassland species (Křenová & Lepš 1996, Colling *et al.* 2002, Jurjavicic *et al.* 2002, Vergeer *et al.* 2003b). Thus, the population growth rate and as a consequence population persistence might be lower in unmanaged and more productive sites than in managed sites, although individual plants are larger and produce more seeds. A larger size of plants, but higher mortality in response to fertilisation were found in a study that investigated the phenotypic plasticity of *C. vulgaris* using a subset of our populations (Berg *et al.* 2005).

In contrast to effects found in the common garden, the fitness of plants in the field was not lower in small than in large populations and no indications of negative effects of inbreeding or genetic drift were found in natural populations. In the field populations, influences of the habitats might have masked effects of inbreeding that in the common garden were visible as lower seed set and reduced offspring fitness. Moreover, in the natural environment, inbred plants that were smaller and might have produced fewer seeds may have died prior to flowering. However, some studies have found even stronger effects of inbreeding in a natural environment than under good conditions in a common garden (Dudash & Fenster 2000).

Reduced seed set in small populations due to genetic erosion, but no reductions in ramet size in the field were also found in *Arnica montana* (Kahmen & Poschlod 2000). These results are

in line with those of Ouborg & van Treuren (1995) who found lower genetic diversity in small populations of *Salvia pratensis*, but no effect of population size on plant fitness in a common garden experiment. They suggested that environmental conditions are of crucial importance for the fitness of those populations although they did not study specific environmental characters. Similar to our study, Vergeer *et al.* (2003a) found that overall, effects of soil conditions on the performance of *Succisa pratensis,* both in the field and in offspring in a common garden were stronger than genetic effects. Eisto *et al.* (2000) found no decrease of germination and seedling growth in small populations of *Campanula cervicaria*, but reductions in population size due to closing of the vegetation.

Conclusions

With respect to our initial questions we may conclude that plant performance in populations of *Carlina vulgaris* is mainly influenced by habitat conditions indicating local adaptation to environmental conditions. This underlines the importance of preventing habitat destruction and to maintain the habitat conditions as has already been suggested for other species (e.g. Oostermeijer *et al.* 1994b, 1998 , Eisto *et al.* 2000).

In addition to environmental conditions, inbreeding depression, genetic drift and pollen limitation in small populations due to habitat fragmentation may affect the performance of individual plants and the persistence of plant populations. For the monocarpic *C. vulgaris* which only reproduces once and has no seed bank, a reduced production of seeds might be particularly important for the persistence of plant populations rather than inbreeding depression or environmental or demographic stochasticity (see also Verkaar & Schenkeveld 1984, Stöcklin & Fischer 1999). Consequently, for conservation purposes it would be more important to maintain large compared to small populations, because in large populations reproduction is higher. However, population isolation should be taken into account. Small populations might still be pollinated by pollen from connected populations or they function as connecting elements that maintain gene flow between large populations.

Because *C. vulgaris* is affected by consequences of habitat fragmentation across a large European scale, active population management is important not only in regions where the species is rare, but also in those where it is still common.

Quantitative genetic variation at different geographical

scales – a comparison of two Asteraceae

with different life history traits

with Henrik Berg and Diethart Matthies

ABSTRACT

Variation in quantitative characters is the basis for the adaptive evolution of populations and thus important for their long-term survival. We studied variation in several quantitative genetic traits in 74 populations of the short-lived, monocarpic *Carlina vulgaris* from seven geographical regions (southern Sweden, western Netherlands, central Germany, southern Britain, northwestern Czechia, Luxembourg, northwestern Switzerland) and 32 populations of the widespread perennial *Hypochoeris radicata* from three geographical regions (the central Netherlands, central Germany, northwestern Czechia). In both species populations of different size and degree of isolation were chosen from each region. Seeds were collected from several mother plants per population, and the offspring were grown in a common garden until flowering. In both species most traits varied considerably among seed families within populations, among populations within regions and among populations between regions. A higher overall proportion of phenotypic genetic variation among populations in *C. vulgaris* (75.8%) than in *H. radicata* (50.7%) and a stronger population differentiation as estimated by Q_{ST} (0.17 in *C. vulgaris, vs.* 0.11 in *H. radicata*) suggests lower gene flow between populations in *C. vulgaris*. Pairwise genetic distances for morphological traits between populations within regions were not related to geographical distances in both species. However, in both species discriminant function analyses of morphological traits at the population level reflected the large differences among regions. These results suggest that at the large scale selective forces which are closely related to geographical distances are most important for population differentiation, whereas at the small scale genetic drift or environmental differences that are not related to geographical distances drive population differentiation. In *C. vulgaris* genetic variation within populations as measured by coefficients of variation among seed families (CVs) was not influenced by population size or isolation. In contrast, in *H. radicata* the CVs for some traits increased with distance to the nearest population indicating instability of quantitative traits in isolated populations.

INTRODUCTION

Plant populations become divergent as a result of mutation, heterogeneous selection, migration or genetic drift (Endler 1977, Levin 1979, Loveless & Hamrick 1984, Barrett & Kohn 1991). The variation among phenotypes and genotypes is the most important source for the evolution of species (Hamrick *et al*. 1991, Storfer 1996, Frankham 1999) and thus very important for species conservation (Frankel & Soulé 1981, Falconer & Mackay 1996, Reed & Frankham 2003).

Many studies have quantified the differentiation among and within populations based on molecular markers which are assumed to be selectively neutral (reviewed in Hamrick *et al*. 1991, Hamrick & Godt 1996b, Linhart & Grant 1996). However, comparisons of the differentiation between populations based on molecular markers (F_{ST}) with those based on quantitative traits (Q_{ST}) have shown that values of Q_{ST} frequently exceed those of F_{ST} (e.g. Spitze 1993, Podolsky & Holtsford 1995, Yang *et al*. 1996, Waldmann & Andersson 1998, reviewed in Merilä & Crnokrak 2001, McKay & Latta 2002). The variation in quantitative traits is under polygenetic control and selection (Falconer & McKay 1996, Widén & Schiemann 2003) and therefore reveals ecologically important differentiation processes (Storfer 1996, Reed & Frankham 2001). In contrast, molecular markers miss important variation that is caused by environmental heterogeneity (Merilä & Crnokrak 2001, McKay & Latta 2002). The association between genotypes and phenotypes is typically weaker in quantitative traits that are under strong selection, i.e. fitness-related traits, than in those under weak selection, i.e. morphological traits (Reed & Frankham 2001). Additive genetic variation is reduced due to selection in fitness-related traits leading to lower heritability (Houlé 1992, Falconer & Mackay 1996, Merilä & Sheldon 1999, but see Podolsky & Holtsford 1995).

Populations at the margin of a species' range are often smaller and more isolated than those in the centre of its range. As a consequence genetic variability and fitness may be reduced in marginal populations due to increased genetic drift and inbreeding (Garcia *et al*. 2000, Dorken & Eckert 2001, Jump & Woodward 2003, Santamaria *et al*. 2003, but see Smith *et al*. 1997, Kark *et al*. 1999) and differentiation among populations may be higher (Ellstrand & Elam 1993, Lesica & Allendorf 1995). However, divergence in quantitative characters could also be enhanced in isolated populations at a species' range margin (Lesica & Allendorf 1995). Little is known about differences in the variation of quantitative traits between central and marginal populations of a species and about the distribution of quantitative genetic variation in different regions.

Gene flow among populations reduces population differentiation (Slatkin 1987, Dewey & Heywood 1988, Lynch 1988, Hamrick *et al.* 1991, Linhart & Grant 1996), because it enhances the genetic similarity of populations (Holt & Gomulkiewicz 1997). Specific life history traits of species, e.g. obligate outbreeding, longevity and good dispersal ability enhance gene flow among populations. As a consequence populations of outbreeding, long-lived and well-dispersed species are less differentiated than those of selfing, short-lived and poorly dispersed species (Loveless & Hamrick 1984, Charlesworth & Charlesworth 1995, Hamrick & Godt 1996a, Linhart & Grant 1996). Short-lived species are especially susceptible to genetic drift which does lead to lower variation within populations than in long-lived species. Gene flow between populations is assumed to decrease with increasing distance between populations. Thus, by relating genetic to geographical distances one could estimate the relative influence of gene flow and genetic drift on population differentiation (e.g. Hutchison & Templeton 1999).

The genetic variation within and between populations is also influenced by the size and the isolation of populations. Fragmentation of habitats reduces the size of plant populations and increases the isolation of the remnant populations. As a consequence increased genetic differentiation between populations and lower genetic variation within populations due to genetic erosion and inbreeding in small populations are expected (Lacy 1987, Ellstrand & Elam 1993, Young *et al.* 1996). Most studies of the effects of fragmentation on the genetic structure have used neutral molecular markers. These studies found positive effects of population size on genetic variability (Raijmann *et al.* 1994, Godt *et al.* 1996, Sun 1996, Young *et al.* 1996, Fischer & Matthies 1998b, Luitjen *et al.* 2000, Lienert *et al.* 2002a, Paschke *et al.* 2002a). However, little is known about effects of population size on quantitative traits (Ouborg *et al.* 1991, Oostermeijer *et al.* 1994a, Ouborg & van Treuren 1995).

We studied the variation in quantitative and qualitative morphological traits of progeny originating from populations of different size and degree of isolation in several European regions in two Asteraceae with contrasting life history, *Carlina vulgaris* L. and *Hypochoeris radicata* L. *C. vulgaris* is a short-lived monocarpic perennial which is considered to be poorly dispersed, whereas *H. radicata* is a long-lived perennial that is well dispersed. We grew offspring of both species from several seed families of each population and of several populations from several regions in a common garden to partition the phenotypic variation among genotypes within populations, among populations within regions and among regions. We related phenotypic variation between populations to geographical distance to estimate the amount of gene flow between populations.

We address the following questions: (1) Is the phenotypic differentiation among populations lower in the common, well-dispersed plant *Hypochoeris radicata* than in the rarer poorly dispersed *Carlina vulgaris*? (2) Does the phenotypic differentiation among populations within regions increase with geographical distance? (3) Does the quantitative genetic variation within populations increase with population size and decrease with population isolation?

MATERIAL AND METHODS

Study species

Carlina vulgaris is a monocarpic perennial which produces rosettes consisting of several incised and spiny leaves. The probability of flowering increases with the size of the rosette. Plants flower between the age of two and at least eleven years (Watt 1981, Klinkhamer *et al.* 1991, Rose *et al.* 2002) and produce one to several flower heads of which the first one is usually the largest. Each flower head contains up to 300 violet or yellow disc florets which open from late June to September and are protandrous and self-compatible, but mainly insect-pollinated (Grime *et al.* 1988). Dispersal of seeds is limited although the achenes have a pappus, because the seeds are large (Greig-Smith & Sagar 1981, Grime *et al.* 1988, Franzén & Eriksson 2003). *C. vulgaris* grows in dry, nutrient-poor, more or less open habitats, predominantly in semi-natural calcareous grasslands, but also in quarries, coastal dunes and open pine forests (Verkaar & Schenkeveld 1984, Grime *et al.* 1988, Meusel & Jäger 1991, Meusel & Kästner 1994, Klinkhamer *et al.* 1996). In Europe, the species is distributed in sub-oceanic to sub-Mediterranean regions from S-Italy (38 °N)to S-Sweden (62 °N). In particular in the north east of the range, the number and size of the populations have declined due to habitat deterioration and fragmentation in the last decades (Meusel & Kästner 1994, Korneck *et al.* 1996).

Hypochoeris radicata is a polycarpic perennial. Plants form rosettes of lobed leaves and are able to grow clonally by means of side rosettes (Turkington & Aarssen 1983, de Kroon *et al.* 1987, 2000). Each plant produces several branched stalks with one flower head at each end. A flower head consists of a large number of self-incompatible yellow flowers. The single seeded fruits (achenes) have a pappus and are well dispersed (Soons & Heil 2002). The main flowering season is from early June to September (Grime *et al.* 1988). *H. radicata* grows in pastures, but also in lawns and prefers nutrient-poor, slightly acidic soils (Grime *et al.* 1988). It is native to Europe up to 62 degrees North, but today has an almost cosmopolitan distribution; the species is a good coloniser and is even considered a weed in other continents

(Turkington & Aarssen 1983). In contrast to *C. vulgaris, H. radicata* is widespread and common in many parts of Europe.

Sampling and data collection

In summer 2000, populations that varied in size and distance to the nearest population were chosen in seven (*C. vulgaris*) respectively three (*H. radicata*) European countries (Table 1, Fig. 1). In *H. radicata* all study regions are in the centre of the distribution area; in *C. vulgaris* the Swedish populations are considered as marginal and Czech and German populations are considered as central. We defined a population as a group of plants that was at least 80 m from the nearest conspecific.
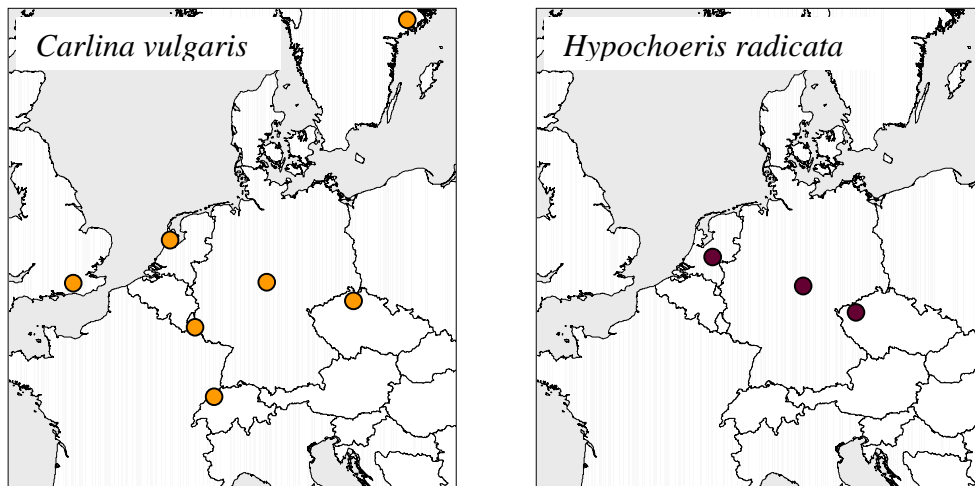

Figure 1. Regions in which populations of *Carlina vulgaris* and *Hypochoeris radicata* were sampled.

In each population, one mature fruit head was collected from each of 20 randomly selected individuals which grew at least 5 m apart from each other, except for very small populations in which all mature individuals were sampled. The fruit heads were sent to Marburg (Germany), where the seeds were removed from the fruit heads and partitioned into ripe and unripe seeds. The number of ripe seeds per fruit head was counted, the seeds were weighed and mean seed mass per family was calculated.

At the end of March 2001, up to 40 seeds from each of ten randomly chosen seed families per population were sown into plastic pots (9 x 9 x 9.5 cm) filled with commercial nutrient-poor garden soil (TKS 1, Floragard GmbH, Oldenburg, Germany) and placed at 15-20 °C in a greenhouse. At the end of April, five randomly chosen seedlings per seed family were transplanted individually into pots of the same size and filled with the same soil

as used before. If five or fewer seeds had germinated we used all seedlings of a family. The transplanted seedlings were randomly placed into flowering beds in the Botanical Garden of the University of Marburg. In total, we transplanted 3105 seedlings of *C. vulgaris* from 631 seed families and 74 populations, and 1173 seedlings of *H. radicata* from 243 families and 32 populations.

Table 1. Number, size, isolation, location and altitude of the populations of *Carlina vulgaris* and *Hypochoeris radicata* studied in each region, and mean distance between the populations. The mean distance between populations was calculated as the mean of all pairwise distances between the populations within one region.

Region	Number of populations	Population size	Distance to nearest pop. [m]	Mean distance [km]	Longitude [°]	Latitude [°]
Carlina vulgaris						
S-Sweden	12	11–1640	80–1000	132.63	13.148 E–17.585 E	58.251 N–59.001 N
C-Germany	21	1–1000	100–7000	10.49	9.767 E–9.933 E	51.187 N–51.456 N
W-Netherlands	6	50–700	80–500	34.89	4.491 E–4.746 E	50.843 N–53.050 N
S-Britain	6	20–400	80–400	27.74	0.262 W–0.054 E	50.842 N–51.260 N
NW-Czechia	6	30–1000	200–1000	61.33	14.417 E–15.998 E	50.100 N–50.333 N
Luxembourg	11	29–20000	100–1700	15.22	5.882 E–6.394 E	49.478 N–49.761 N
NW-Switzerland	12	15–1500	100–500	25.25	6.744 E–7.483 E	47.321 N–47.807 N
Hypochoeris radicata						
NW-Czechia	5	100–1500	80–200	103.68	12.817 E–15.867 E	50.037 N–50.167 N
C-Germany	22	9–30000	80–1700	9.58	9.725 E–9.884 E	51.164 N–51.366 N
C-Netherlands	5	150–2000	100–750	13.51	5.958 E–6.110 E	52.332 N–52.536 N

In both species we recorded a number of morphological and fitness-related traits (Table 2). In *H. radicata* all characters were recorded in the first growing season when most of the individuals flowered. In *C. vulgaris* all characters related to the size and shape of leaves were recorded in the summer of the first year (2001), whereas reproductive traits were recorded in summer 2002, when all plants had either flowered or died. In *H. radicata* we measured reproduction in the first year of flowering.

In both species the length and width of the longest rosette leaf were measured two weeks, six weeks and one year (*C. vulgaris* only) after transplanting. As an estimate of leaf size the length times width of the leaf was calculated. Relative growth rate (RGR) of leaves in 2001 was calculated as log (leaf size at six weeks) - log (leaf size at two weeks). The ratio of leaf length to width was calculated six weeks after transplanting. To characterise the depth of the incision of leaves, the widest and the smallest width of a lobe at the widest part of a fully developed leaf was measured and the ratio of the smallest to the widest part was calculated. In

H. radicata, in addition, the number of the lobes per leaf and the leaf length were recorded to calculate the number of lobes per cm of leaf length. The hairiness of the leaves on the upper and on the lower surface of one mature leaf of both species was estimated using a three point scale (1 = few hairs, 2 = moderate number of hairs, 3 = many hairs). In *C. vulgaris* some plants had black leaf margins, whereas most of the plants had green leaf margins. The colour of the leaf margin was recorded as a binomial variable (0 = green, 1 = black). In *C. vulgaris* the length of one mature leaf and the number of spines at the margin of one complete half of this leaf was recorded, and the number of spines per cm of leaf length was calculated. In both species one mature leaf per plant was harvested, dried between sheets of paper to keep it flat, weighed, scanned and its area was determined with the image processing system WinFOLIA 5.01 (Régent Instruments Inc., Québec, Canada) to calculate specific leaf weight.

To study the phenology of the plants, they were checked every second or third day whether they had started to flower. We expressed flowering time for each plant as the number of days after the first individual of the species had started to flower. In *C. vulgaris* the diameter of the main flowering stalk was measured 1 cm above ground and the colour of the flowering stalk (0 = green, 1 = violet) was recorded. Moreover, the number of nodes per cm of stalk length was determined. In both species the hairiness of the flowering stalks was estimated. We used a four point scale for the hairiness at the middle of the flowering stalks in *C. vulgaris* and a five point scale for the hairiness at the base of the stalks in *H. radicata.*

In summer 2002, at the peak of flowering, the number of flowering stalks and fruit heads, the diameter of all fruit heads, the height of the plants and the cumulative length of all branches were determined for each plant. The length of the longest stalk (plant height) and the length of all branches (cumulative branch length) were measured, and in *C. vulgaris* in addition the length of the part of the thickest stalk without branches was measured. The relative number of flower heads per stalk was calculated by dividing the number of flower heads of each plant by the number of flowering stalks. In *C. vulgaris* the proportion of the flowering stalk that was branched was calculated as (1 − length of the stalk part without branches / plant height). In *C. vulgaris* the area of each fruit head was calculated from the diameter (d) at the time of fruiting as $((d/2)^2 * \pi)$, and the mean fruit head area was calculated as (total fruit head area / number of fruit heads); in *H. radicata* the diameter of three flower heads was measured when flower heads were closed and the mean was calculated.

Table 2. Quantitative traits measured and included in principal component analyses in *Carlina vulgaris* and *Hypochoeris radicata*. Transformations performed to achieve normality for single traits are given in parenthesis. Characters with a factor loading of at least 0.6 (boldface) on one of the extracted principal components were selected for single trait analyses.

Carlina vulgaris	*Hypochoeris radicata*
Fitness-related traits	
Vegetative traits	
Leaf size (at 2 weeks, at 6 weeks, at 1 year); (log)	**Leaf size (at 2 weeks, at 6 weeks)**
RGR (relative growth rate) of leaf size	**RGR (relative growth rate) of leaf size**
	Dry mass of rosette leaves (vegetative components)
	Number of side rosettes
Reproductive traits	
Number of flowering stalks (log)	**Number of flowering stalks (sqrt)**
Plant height (log)	**Plant height**
Cumulative branch length (log)	
Number of fruit heads (sqrt)	**Number of flower heads (log)**
Total fruit head area (log + 1)	Dry mass of flowering stalks (rep. components); (sqrt)
Above-ground biomass: total (log)	**Above-ground biomass: total (log)**
Seed mass	**Seed mass**
Diameter at stalk base	**Number of seeds in one flower head**
	Number of seeds per plant (sqrt)
Morphological traits	
Vegetative traits	
Relative depth of incision of leaves	**Relative depth of incision of leaves**
Colour of leaf margin (0 = green, 1 = black)	**Number of incisions per cm leaf (log)**
Ratio of leaf length to leaf width (at 6 weeks)	**Ratio of leaf length to leaf width (at 6 weeks) (log)**
Hairiness upper leaf surface (3 point scale)	**Hairiness upper leaf surface (3 point scale)**
Hairiness lower leaf surface (3 point scale)	**Hairiness lower leaf surface (3 point scale)**
Number of spines per cm leaf	
Specific leaf weight	**Specific leaf weight**
Reproductive traits	
Day of first flower from June 18th, 2nd year	**Day of first flower, from June 28th, 1st year (sqrt)**
	Day of first flower, from May 21st, 2nd year (log)
Mean fruit head area	**Mean flower head area**
Proportion of stalk length branched	**Relative number of flower heads per stalk**
Hairiness of stalks (4 point scale)	Hairiness of stalks at stalk base (5 point scale)
Colour of flowering stalk (0 = green, 1 = violet)	
Number of internodes per cm stalk	**Ratio of reproductive to vegetative dry mass components (log)**
Length of longest outer bract	
Length of ray florets	

In *C. vulgaris* the above-ground parts of fruiting plants were harvested after the fruits had matured and the plant had died, dried for 12 hours at 80 °C and weighed. Ten seeds at the margin of the largest fruit head were weighed and mean seed mass was calculated. In *H. radicata* the above-ground biomass of plants was harvested in autumn 2001 after most seeds were ripe and partitioned into a vegetative (rosette leaves) and a reproductive part (flowering stalks + base of fruit heads). One complete mature fruit head was harvested between June and August 2001, and for each plant the date of harvest was recorded. All seeds of a head were counted and weighed to calculate mean seed mass. Total seed production was calculated as the product of the number of fruit heads per plant and the number of seeds of the harvested fruit head. In *C. vulgaris* the total fruit head area of each plant was used as an estimate of total seed production.

To characterise habitat conditions, the composition of the vegetation at each study site was recorded by estimating the cover of each plant species. In addition, the maximum and mean height of the vegetation, the proportion of area covered by all plant species and the proportion of bare ground were recorded. Moreover, at each site, the inclination of the slope and the deviation of the exposition from north (0-180°, north = 0°) was recorded. To estimate the relative nutrient availability at the study sites we carried out a bioassay. In July and August 2002, in each population eight soil cores were randomly collected, mixed and air dried 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 x 9 x 9.5 cm) were filled with soil from each population, and in each pot five seedlings of *Arrhenatherum elatius* were grown as phytometers in a glasshouse. After eight weeks all above-ground plant parts were harvested, dried for 24 hours at 80 °C and weighed. Total above-ground biomass per pot was used as an estimate of nutrient availability.

Data analysis

Effects of region, population and family on each trait were analysed by General Linear Models using a mixed model with region as fixed, and population and family as random factors. The effect of region was tested against the variation among populations within regions, the effect of population was tested against the variation among seed families within populations and the effect of seed family was tested against the residual variation among individual plants. To achieve homoscedasticity and normally distributed residuals several traits were log- or square root-transformed prior to analysis (Table 2). We used multiple discri-

minant function analyses (DFA, Quinn & Keough 2002) at the population level with regions as predefined groups to test the extent of morphological separation between the regions.

Some of the traits were inter-correlated. We thus used principal component analysis (PCA) to identify uncorrelated linear combinations of the investigated traits. Principal components with eigenvalues > 1 were extracted after varimax rotation. For *Carlina vulgaris* we excluded the variables day of first flower and seed mass from the PCA, because the number of replicates was smaller than half of the total number of individuals planted due to high mortality before flowering. Variance components were calculated to partition the variation in single traits and in the principal components (PCs) into variation among regions (V_{Reg}), variation among populations within regions (V_{Pop}), variation between families within populations (V_{Fam}) and residual variation (V_E), which mainly represents environmental variation within families. We used the Restricted Maximum Likelihood (REML) method to estimate variance components, because the design was slightly unbalanced due to smaller sample sizes in small populations and because several individuals died during the experiment. Confidence intervals (95%) based on bootstrapping (1000 bootstraps), were used to test whether variance components differed from zero. An estimate of the population differentiation in quantitative traits (Q_{ST}), which is the analogue of F_{ST} based on molecular markers was obtained for single traits and principal components as $Q_{ST} = V_{Pop} / V_{Pop} + 8V_{Fam,}$ assuming that offspring from a seed family in both species are half-sibs (following Yang *et al.* 1996). The genetic variability within each population was estimated by calculating narrow sense heritabilities as $h^2 = 4V_{Fam} / 4V_{Fam} + V_E$. Narrow sense heritabilities account for the part of the total variance that can be explained by additive genetic differences.

According to Houlé (1992) coefficients of variation rather than heritability calculations reflect the evolutionary potential of a population, because adaptability of a trait depends on the total variation of a trait and not on the ratio of additive genetic variation to total phenotypic variation. We therefore in addition calculated coefficients of variation (CV) among plants within seed families (CV_{Fam}), among seed families within populations (CV_{Pop}) and among populations within regions (CV_{Reg}) for each trait. Differences among regions in these CVs and in the mean CV of all traits were studied by analyses of variance. The variables population size and isolation (distance to the nearest conspecific population) were used as covariates to test their effects on the variability in quantitative traits.

Differences in species composition among populations were investigated by detrended correspondence analysis (DCA, Canoco 4.5, Ter Braak & Šmilauer 2002). Environmental distances between populations within regions were calculated as Euclidian distances between

the DCA-scores along the first two axes based on species composition (E1) and characters that describe vegetation structure and nutrient availability at the sites (E2) in the Cluster analysis procedure in SPSS (11.0).

Pairwise Q_{ST}-values that measure the genetic distance were calculated for each pair of populations within each region and related to pairwise geographical distances and environmental distances by Mantel tests (Mantel 1967). The Euclidian Distances (E1 and E2) between populations based on environmental variables had no effect on the genetic distance between populations. The results are therefore not presented.

Mantel tests were performed with zt-win (Vers. 1.0, Bonnet & Van de Peer 2001). All other statistical analyses were performed with SPSS (11.0; Chicago, Illinois, USA 2001).

RESULTS

Covariation among traits and morphological variation

Carlina vulgaris

Almost all investigated traits in *C. vulgaris* varied considerably among regions, among populations within regions and among seed families within populations (Tables 3 and 4). Plants from the Czech region were largest with respect to several size-related traits and Dutch and Swedish plants were smallest. Plant size decreased with increasing latitude of origin, although due to small sample sizes not significantly (e.g. plant height: r = 0.64, p = 0.12, n = 7). Dutch and English plants had the lowest fruit head area, indicating the lowest reproduction and thus fitness of a plant. The pattern of lowest and highest values varied for morphological traits much more among regions than for fitness-related traits. English plants produced leaves with a higher specific leaf weight and deeper incisions than leaves originating from other regions. Dutch plants started on average 15 to 20 days later to flower than plants originating from the other regions, whereas Swedish plants flowered earliest.

In discriminant analyses at the population level with regions as predefined groups the combined morphological traits discriminated better among the regions than the fitness-related traits (Fig. 2). The first two discriminant functions explained 75% of the variation among populations in morphological traits, 72% of that in fitness-related traits and 67% of that in all traits together. With respect to their morphological traits, populations from Sweden, the Netherlands, and Britain were clearly separated from populations from the other regions, whereas the Central European populations (from Germany, Switzerland, Luxembourg and Czechia) were less well separated from each other and formed a large cluster (Fig. 2c).

Table 3. Means of traits (± 1 SE) of *Carlina vulgaris* for each region based on population means. Maximum values are in boldface, minimum values are underlined. Different letters indicate significant differences (p < 0.05) between regions according to Tukey's post hoc test. For calculation of trait means see text.

	NW-Czechia	C-Germany	W-Netherlands	S-Britain	S-Sweden	NW-Switzerland	Luxembourg
Fitness-related traits							
Leaf size at 2 weeks [cm²]	18.27±1.33 [cd]	11.95±0.45 [ab]	8.84±1.20 [a]	10.97±0.94 [ab]	9.21±0.67 [a]	**22.18±1.51** [d]	14.27±0.73 [bc]
Leaf size at 6 weeks [cm²]	20.11±1.18 [e]	13.81±0.54 [c]	11.30±1.13 [a]	12.62±0.88 [b]	11.18±0.53 [a]	**24.24±1.48** [f]	17.70±0.55 [d]
RGR of leaf size	0.05±0.01 [ab]	0.08±0.00 [c]	**0.12±0.02** [e]	0.08±0.01 [bc]	0.11±0.02 [de]	0.05±0.01 [ab]	**0.12±0.02** [e]
Number of flowering stalks	**1.35±0.09** [c]	1.30±0.05 [c]	1.12±0.06 [a]	1.23±0.07 [ab]	1.06±0.03 [a]	1.27±0.07 [b]	1.29±0.07 [bc]
Plant height [cm]	**28.82±2.41** [e]	15.23±0.84 [bc]	18.46±2.11 [c]	16.06±2.01 [b]	12.32±1.47 [a]	22.10±2.02 [d]	24.22±1.54 [d]
Cumulative branch length [cm]	**48.38±4.15** [f]	27.68±1.74 [c]	22.25±2.57 [b]	26.42±3.70 [b]	18.33±2.04 [a]	38.13±3.01 [d]	43.70±2.73 [e]
Number of fruit heads	**3.07±0.25** [d]	2.88±0.09 [d]	1.22±0.05 [a]	2.11±0.18 [b]	1.87±0.14 [b]	2.61±0.12 [bc]	2.77±0.13 [b]
Total fruit head area [cm²]	**3.86±0.39** [e]	3.42±0.19 [de]	1.30±0.17 [a]	2.19±0.30 [b]	2.84±0.32 [cd]	3.09±0.17 [c]	3.08±0.20 [c]
Above-ground biomass [g]	**2.46±0.25** [c]	1.73±0.07 [b]	1.18±0.15 [a]	1.66±0.17 [b]	1.74±0.07 [b]	2.43±0.16 [c]	2.29±0.12 [c]
Seed mass [mg]	0.56±0.05 [a]	0.58±0.02 [a]	0.70±0.04 [b]	0.66±0.04 [b]	**0.78±0.04** [c]	0.64±0.03 [b]	0.68±0.03 [b]
Diameter at stalk base [mm]	**42.31±2.26** [b]	36.63±0.86 [a]	34.04±2.89 [a]	35.83±2.28 [a]	41.72±1.52 [b]	42.57±2.01 [b]	35.43±0.71 [a]
Morphological traits							
Relative depth of incision of leaves [%]	18.32±1.15 [b]	24.68±0.62 [e]	20.31±1.00 [c]	**36.84±1.80** [g]	15.88±0.83 [a]	23.31±1.17 [d]	29.21±0.96 [f]
Colour of leaf margin (0 = green, 1 = black)	0.01±0.01 [a]	0.01±0.00 [a]	0.00±0.00 [a]	0.03±0.01 [a]	0.00±0.00 [a]	**0.31±0.12** [c]	0.06±0.04 [b]
Ratio of leaf length to leaf width	**3.35±0.19** [d]	2.91±0.07 [b]	2.98±0.14 [b]	2.97±0.11 [b]	2.41±0.08 [a]	3.04±0.12 [c]	3.02±0.06 [b]
Hairiness upper leaf surface [3 p. scale]	1.93±0.03 [ab]	**1.99±0.01** [c]	1.94±0.02 [bc]	1.93±0.03 [abc]	1.91±0.04 [ab]	1.88±0.04 [ab]	1.87±0.04 [a]
Hairiness lower leaf surface [3 p. scale]	2.53±0.10 [a]	2.96±0.01 [c]	2.92±0.05 [c]	**2.98±0.00** [c]	2.72±0.06 [b]	**2.98±0.01** [c]	**2.98±0.01** [c]
Number of spines per cm leaf	4.03±0.36 [a]	5.55±0.18 [c]	4.98±0.19 [b]	**6.81±0.30** [d]	6.13±0.20 [d]	5.02±0.15 [b]	5.41±0.15 [c]
Specific leaf weight [mg/cm²]	10.16±0.14 [bc]	10.49±0.19 [c]	9.02±0.27 [a]	**12.09±0.29** [e]	9.75±0.17 [b]	11.08±0.24 [d]	11.45±0.23 [d]
Day of first flower, from June 18th, 2nd year	50.22±2.35 [c]	45.81±1.57 [b]	**65.03±0.81** [d]	48.92±3.29 [b]	45.04±4.31 [a]	47.03±1.21 [b]	47.87±0.93 [bc]
Mean fruit head area [cm²]	1.21±0.08 [e]	1.16±0.06 [de]	1.01±0.12 [a]	1.02±0.08 [b]	**1.52±0.13** [cd]	1.10±0.04 [c]	1.05±0.04 [c]
Proportion of stalk length branched [%]	64.14±2.64 [de]	53.29±1.41 [b]	**69.40±3.34** [e]	55.49±3.69 [bc]	43.61±3.97 [a]	56.80±4.10 [d]	56.76±2.00 [c]
Hairiness of stalks [4 p. scale]	1.74±0.11 [a]	**2.19±0.06** [d]	1.94±0.06 [c]	1.85±0.08 [abc]	1.92±0.12 [bc]	1.78±0.08 [abc]	1.80±0.08 [ab]
Proportion of plants with violet stalk [%]	56.53±7.43 [a]	72.56±3.64 [bc]	**82.20±1.68** [c]	81.92±3.27 [c]	71.81±8.25 [bc]	61.22±8.64 [ab]	76.12±4.89 [bc]
Number of internodes per cm stalk	**1.15±0.04** [e]	0.66±0.04 [b]	0.70±0.05 [b]	0.81±0.07 [c]	0.51±0.05 [a]	1.01±0.13 [d]	0.91±0.05 [cd]
Length of longest outer bract [mm]	16.30±0.38 [a]	16.57±0.31 [a]	16.72±0.48 [a]	**19.33±0.29** [b]	18.91±0.58 [b]	16.06±0.36 [a]	16.37±0.28 [a]
Length of ray florets [mm]	**11.72±0.28** [d]	10.89±0.14 [ab]	11.29±0.20 [ab]	11.55±0.11 [cd]	11.32±0.13 [bcd]	10.85±0.20 [a]	11.23±0.17 [abc]

Table 4. Results of hierarchical ANOVAs and analyses of deviance (colour of flowering stalk) for the effect of regions, populations within regions, and families within populations on nine fitness-related and 13 morphological traits of *Carlina vulgaris*. F-values, respectively Quasi-F-values are shown. Degrees of freedom were 6 (region), 65-68 (population) and 403-556 (seed family). Seed mass was used as a covariate to adjust all fitness-related traits for maternal effects. ** $p < 0.01$, *** $p < 0.001$.

|	Region F or Quasi-F	Population F or Quasi-F	Family F or Quasi-F	Error df
Fitness-related traits				
# Flowering stalks	4.35**	1.66**	1.24**	1910
Plant height	17.49***	7.21***	1.78***	1331
# Fruit heads	19.74***	2.80***	1.13**	1879
Total fruit head area	5.69***	3.46***	1.19**	1333
Above-ground biomass	9.38***	2.74***	1.23**	1369
Seed mass	11.96***	2.69***	1.13	441
Diameter at stalk base	7.32***	2.45***	1.34***	1873
Morphological traits				
Rel. depth of incision of leaves	47.96***	2.65***	1.64***	2426
Ratio of leaf length to width	8.26***	6.94***	2.04***	2429
Hairiness upper leaf surface	2.74**	2.70***	1.71***	2431
Hairiness lower leaf surface	16.57***	5.99***	1.34***	2431
# Spines per cm leaf	9.76***	6.83***	1.84***	2423
Specific leaf weight	18.67***	3.45***	1.58***	2403
Day of first flower	4.46**	11.57***	1.71***	1094
Mean fruit head area	4.45***	3.51***	1.23**	1369
Proportion of stalk branched	9.12***	5.42***	1.45***	1115
Hairiness of flowering stalks	4.48**	5.80***	1.13*	1351
Colour of flowering stalks	1.31	4.41***	1.68***	1868
# Internodes per cm stalk	16.76***	7.42***	1.85***	1327
Length of ray florets	3.36**	2.32***	1.13*	1344
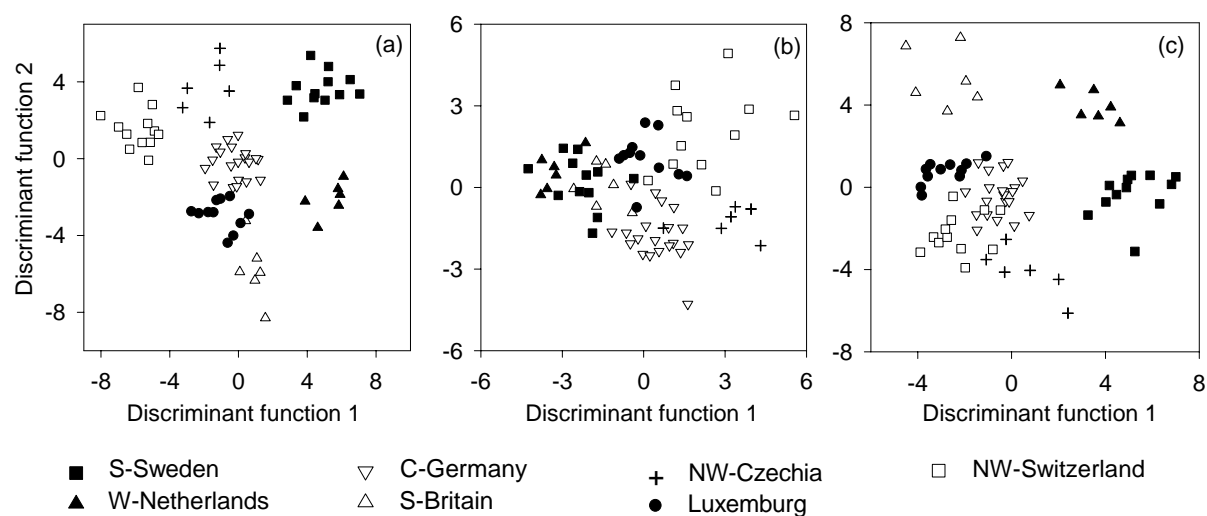
Figure 2. Among region variation in *Carlina vulgaris*. The first two discriminant functions are shown for (a) all investigated traits, (b) fitness-related traits and (c) morphological traits.

This pattern thus corresponds to the geographical pattern of the populations. Variables with the highest coefficients for the first discriminant function were relative incision of leaves (-0.47), hairiness of the stalks (-0.27) and of the lower leaf surface (-0.34), and for the second function these were relative incision of leaves (0.42), proportion of branched stalks (-0.31) and hairiness of the upper leaf surface (0.22). However, all significant discriminant functions together predicted group membership correctly for 100% of all populations if only the morphological traits or all traits were included, whereas group membership was only 90% correctly predicted if only fitness-related traits were included, indicating that regional differences were smaller in fitness-related traits (Fig. 2b).

Table 5. Loadings of principal components (PCs) for characters studied in *Carlina vulgaris* after varimax rotation. Highest correlations (> 0.40) between original characters and PCs are in boldface. Components are named according to the traits with which they are highly correlated: STALK = PC stalk, SIZE = PC plant size, REPRO = PC reproduction, FSIZE = PC flower size, LSHAP = PC leaf shape, LSTRU = PC leaf structure, STAP = PC stalk appearance, HAIR = PC hairiness. Only characters with loadings > 0.30 are shown. N = 1643.

	Rotated component loadings							
	STALK	SIZE	REPRO	FSIZE	LSHAP	LSTRU	STAP	HAIR
# Internodes per cm stalk	**0.80**	0.15	0.10	0.03	0.27	-0.01	-0.05	-0.12
Plant height	**0.77**	**0.42**	0.18	0.07	0.25	0.01	-0.04	-0.09
Proportion of stalk branched	**0.67**	-0.08	-0.16	-0.17	0.06	-0.13	-0.11	0.12
Colour of leaf margin	**0.40**	0.09	0.02	-0.05	-0.03	0.05	0.33	0.24
Diameter of stalk base	-0.08	**0.77**	0.05	0.14	-0.04	-0.09	0.03	-0.05
Above-ground biomass	0.19	**0.66**	**0.53**	0.34	0.05	0.07	0.03	0.01
Leaf size at 1 year	0.25	**0.59**	-0.10	0.07	-0.06	0.15	0.05	-0.00
Leaf size at 6 weeks	0.29	**0.50**	0.17	-0.06	0.27	0.20	-0.21	0.17
# Flower heads	0.05	0.29	**0.82**	0.18	-0.04	-0.02	0.07	0.02
# Flowering stalks	-0.05	-0.29	**0.73**	-0.12	0.09	0.14	-0.07	0.01
Cumulative branch length	**0.53**	**0.45**	**0.57**	0.06	0.20	0.09	0.01	-0.05
Mean flower head area	-0.18	0.21	-0.07	**0.80**	-0.03	-0.09	0.01	0.00
Length of ray florets	0.17	-0.09	0.12	**0.73**	-0.07	-0.05	0.01	0.05
Total fruit head area	-0.07	**0.39**	**0.47**	**0.68**	-0.05	-0.06	0.04	0.03
Length of longest outer bract	-0.35	0.17	-0.17	**0.48**	0.16	0.24	-0.01	-0.15
Ratio: leaf length to leaf width	0.19	-0.03	0.02	0.01	**0.79**	0.15	0.00	0.07
# Spines per cm leaf length	-0.15	-0.03	-0.05	0.09	**-0.76**	0.31	0.04	0.03
Specific leaf weight	-0.06	0.26	0.03	-0.01	-0.15	**0.73**	-0.07	0.11
Rel. depth of incision of leaves	-0.01	-0.09	0.09	-0.06	0.05	**0.71**	0.15	0.02
Stalk colour violet	0.07	-0.07	0.00	0.15	-0.01	0.15	**0.79**	-0.13
Hairiness of flowering stalks	-0.35	0.11	0.01	-0.16	-0.02	-0.09	**0.65**	0.24
Hairiness upper leaf surface	-0.06	0.06	0.06	0.07	0.07	-0.09	-0.08	**0.79**
Hairiness lower leaf surface	0.10	-0.09	-0.06	-0.04	-0.02	0.26	0.15	**0.62**
% Variance explained	11.69	11.08	9.55	9.29	6.71	6.38	5.59	5.40

PCA reduced the characters studied to eight principal components (PCs) with $\lambda > 1$, which together explained 65.7% of the total variation (Table 5): Of these, PC stalk (STALK), PC plant size (SIZE), PC reproduction (REPRO) and PC flower size (FSIZE) were mainly related to fitness-related traits, whereas PC leaf shape (LSHAP), PC leaf structure (LSTRU), PC stalk appearance (STAP) and PC hairiness (HAIR) were mainly related to morphological traits. The four principal components that combined mainly fitness-related traits explained together 41.6% of the variation, those that combined mainly morphological traits explained 24.1%.

Table 6. Trait means (± 1 SE) of *Hypochoeris radicata* for each region based on population means. Maximum values are in boldface, minimum values are underlined. Different letters indicate significant differences ($\alpha = 0.05$) between regions according to Tukey's post hoc test. For calculation of trait means see text.

	NW-Czechia	C-Germany	C-Netherlands
Fitness-related traits			
Leaf size at 2 weeks [cm²]	27.18 ± 1.38 [a]	**29.80 ± 1.56** [a]	_23.17 ± 1.34_ [a]
Leaf size at 6 weeks [cm²]	28.85 ± 1.55 [c]	**29.15 ± 1.40** [b]	_22.75 ± 1.18_ [a]
RGR of leaf size	**0.03 ± 0.01** [b]	0.00 ± 0.01 [a]	_-0.01 ± 0.01_ [a]
Number of side rosettes	**1.64 ± 0.45** [a]	_1.14 ± 0.09_ [a]	1.17 ± 0.22 [a]
Number of flowering stalks	_4.50 ± 0.37_ [a]	**6.09 ± 0.14** [b]	5.82 ± 0.44 [b]
Dry mass of rosettes leaves [g]	1.10 ± 0.09 [b]	**1.11 ± 0.05** [c]	_0.90 ± 0.07_ [a]
Plant height [cm]	**29.60 ± 1.86** [b]	29.41 ± 1.02 [b]	_20.31 ± 1.33_ [a]
Number of flower heads	_8.15 ± 0.64_ [a]	**10.65 ± 0.35** [c]	8.64 ± 0.85 [b]
Dry mass of flowering stalks [g]	0.64 ± 0.08 [b]	**0.92 ± 0.05** [c]	_0.49 ± 0.09_ [a]
Above-ground biomass: total [g]	1.74 ± 0.17 [b]	**2.08 ± 0.10** [c]	_1.38 ± 0.14_ [a]
Seed mass [mg]	_0.53 ± 0.03_ [b]	**0.61 ± 0.01** [b]	0.57 ± 0.05 [a]
Number of seeds in one flower head	68.40 ± 3.58 [b]	**74.87 ± 2.02** [b]	_46.74 ± 3.15_ [a]
Number of seeds per plant	563.5 ± 68.0 [b]	**816.4 ± 35.5** [c]	_420.6 ± 68.7_ [a]
Morphological traits			
Relative depth of incision of leaves	_47.39 ± 1.66_ [a]	51.36 ± 0.90 [b]	54.60 ± 1.62 [b]
Ratio of leaf perimeter to area	3.88 ± 0.18 [a]	3.82 ± 0.09 [a]	**4.01 ± 0.23** [a]
Number of incisions per cm leaf	_0.94 ± 0.04_ [a]	0.98 ± 0.02 [a]	**1.05 ± 0.02** [b]
Ratio of leaf length to leaf width	2.82 ± 0.05 [b]	**2.84 ± 0.03** [b]	_2.62 ± 0.04_ [a]
Hairiness upper leaf surface [3 p. scale]	1.59 ± 0.12 [a]	_1.58 ± 0.03_ [a]	**1.60 ± 0.05** [a]
Hairiness lower leaf surface [3 p. scale]	_1.13 ± 0.13_ [a]	1.37 ± 0.03 [ab]	**1.39 ± 0.03** [b]
Specific leaf weight [mg/cm²]	12.49 ± 0.25 [a]	_12.23 ± 0.15_ [a]	**12.57 ± 0.33** [a]
Hairiness at stalk base [5 p. scale]	_0.21 ± 0.08_ [a]	**0.94 ± 0.09** [b]	0.16 ± 0.07 [a]
Day of first flower, from June 28th, 1st y.	28.23 ± 1.22 [b]	_23.02 ± 1.38_ [a]	**33.46 ± 1.95** [c]
Day of first flower, from May 21th, 2nd y.	_40.33 ± 2.74_ [a]	50.41 ± 1.55 [a]	**56.45 ± 3.55** [b]
Mean flower head area [mm²]	**91.36 ± 1.39** [c]	87.84 ± 0.94 [b]	_84.32 ± 1.77_ [a]
Rel. no. flower heads per stalk	**1.92 ± 0.05** [b]	1.78 ± 0.04 [b]	_1.47 ± 0.04_ [a]
Ratio: rep. to veg. dry mass components	0.66 ± 0.08 [a]	**0.85 ± 0.05** [b]	_0.55 ± 0.06_ [a]

Hypochoeris radicata

There was significant variation among regions, among populations within regions, and among seed families within populations in most of the investigated traits in *H. radicata* (Tables 6 and 7). However, several traits did not differ among regions: leaf size at 2 weeks, relative growth rate of leaves, number of side rosettes, mass of rosette leaves, hairiness of upper and lower leaf surface and specific leaf weight.

Table 7. Results of hierachical ANOVAs for the effect of regions, populations within regions, and families within populations on 12 fitness-related and 10 morphological traits of *Hypochoeris radicata*. F-values are shown. Degrees of freedom were 2 (region), 29-30 (population) and 204-210 (seed family). Covariates (cov.) were used to adjust for maternal or sampling effects, if significant: 1 = seed mass of mother plant, 2 = harvest date of biomass, 3 = harvest date of fruit head, 4 = length of longest stalk. + p < 0.10, * p < 0.05, ** p < 0.01, *** p < 0.001.

	Region F	Population F	Family F	Error df	Cov.
Fitness-related traits					
Leaf size at 2 weeks	2.50	6.94***	3.40***	901	
Leaf size at 6 weeks	2.84+	6.44***	2.83***	891	1
RGR of leaf size	1.46	1.48+	2.00***	890	1
# Side rosettes	0.23	3.05***	1.78***	898	1
Dry mass of rosette leaves.	2.34	5.39***	2.76***	898	1
# Flowering stalks	10.38***	3.39***	2.25***	873	1
Plant height	11.04***	5.95***	2.28***	873	1
# Flower heads	7.59**	4.15***	2.52***	872	1
Above-ground biomass: total	4.16*	5.67***	3.13***	897	1,2
# Seeds in one flower head	9.33**	2.21**	1.23*	783	1,3
# Seeds per plant	15.16***	3.41***	1.87***	783	1
Seed mass	3.32+	1.88**	1.40**	772	1,3
Morphological traits					
Relative depth of incision of leaf	2.91+	1.92**	1.52***	899	
# Incisions per cm leaf	3.74*	3.17***	1.44***	906	
Ratio of leaf length to width	5.57**	2.88***	1.69***	899	
Hairiness upper leaf surface	1.49	1.45+	1.60***	897	
Hairiness lower leaf surface	1.91	1.17	1.77***	905	
Specific leaf weight	1.08	1.42+	1.54***	879	
Day of first flower, 1st year	7.33**	9.24***	2.38***	853	1
Day of first flower, 2nd year	2.72+	5.69***	1.14	609	1
Rel. number of flower heads per stalk	4.56*	2.08**	1.54***	879	4
Ratio: rep. to veg. dry mass components	9.00***	5.20***	2.27***	906	

Most of the fitness-related traits, which are mainly related to reproduction, had the highest values for plants originating from the German region and lowest for those from the Dutch region. For example, in Dutch plants the number of seeds per plant was 25% lower than in Czech and 84% lower than in German plants. As in *C. vulgaris,* the regional pattern of largest and smallest values was more consistent for fitness-related traits than for morphological traits. In both years plants from the Netherlands flowered latest.

In discriminant function analyses at the population level with regions as predefined groups all traits combined, the fitness-related traits combined and the morphological traits combined indicated strong regional differentiation (Fig. 3). Two discriminant functions explained 100% of the variation. Group membership was explained correctly for 100% of all populations if only the morphological traits or all traits were included, and for 97% if only fitness-related traits were included. As in *C. vulgaris,* the combined morphological traits discriminated slightly better among the regions than the fitness-related traits. The traits with the highest coefficients for the first discriminant function were the number of seeds per flower head (0.49), the day of first flower (-0.29), the ratio of length to width of the leaf (0.25) and the ratio of reproductive to vegetative components (0.24), and for the second function these were the hairiness of the lower leaf surface (0.45), the relative incision of leaves (0.37) and the number of incisions per cm leaf length (0.21).

Populations of *H. radicata* from the Netherlands, Germany and Czechia were separated from each other in the same way with respect to all traits, combined fitness-related traits or combined morphological traits.
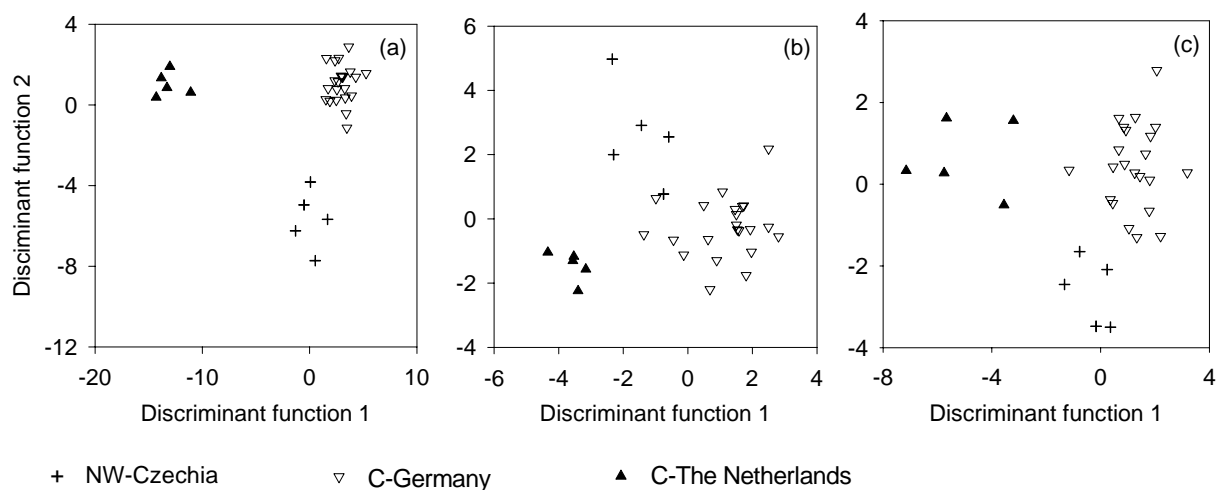

Figure 3. Among region variation in *Hypochoeris radicata*. The first two axes of discriminant functions are shown for (a) all investigated traits, (b) fitness-related traits and (c) morphological traits.

Eight main principal components resulting from PCA explained together 68.0% of the total variation in 24 traits (Table 8). PC rosette size (SIZE), PC reproduction 1 (REP1), PC reproduction 2 (REP2) and PC seeds (SEED) were mainly related to fitness-related traits and explained most of the variation (45.9%). PC hairiness (HAIR), PC leaf shape (LSHAP) and PC specific leaf weight (SLW) were mainly related to morphological traits and explained 16.5% of the variation. The PC relative growth rate (RGR) could not be classified clearly as morphological or fitness-related, because it was positively related to relative growth rate and negatively to the ratio of the length to the width of the longest leaf.

Table 8. Loadings of principal components (PC) for the studied characters in *H. radicata* after varimax rotation. Highest correlations (> 0.40) between original characters and PCs are in boldface. Components are named according to the traits with which they were highly correlated: SIZE = PC vegetative fitness, REP1 = PC reproductive fitness 1, REP2 = PC reproductive fitness 2, SEED = PC seeds, HAIR = PC hairiness of leaves, RGR = relative growth rate of leaves, LSHAP = PC leaf shape, SLW = specific leaf weight. N= 926.

	Rotated component loadings							
	SIZE	REP1	REP2	SEED	HAIR	RGR	LSHAP	SLW
Dry mass of rosette leaves	**0.80**	0.06	-0.01	0.09	-0.09	-0.06	0.20	-0.09
Leaf size at 2 weeks	**0.83**	0.16	0.20	0.08	-0.01	-0.20	-0.14	0.05
Leaf size at 6 weeks	**0.81**	0.15	0.17	0.07	-0.03	0.24	-0.10	0.06
Above-ground biomass: total	**0.79**	0.31	0.32	0.17	-0.08	-0.08	0.15	-0.05
# Capitula	0.28	**0.89**	0.00	0.08	0.01	-0.07	0.05	-0.07
# Flowering stalks	0.08	**0.81**	0.04	-0.10	0.02	-0.07	0.06	-0.09
# Seeds per plant	0.32	**0.70**	0.14	**0.50**	-0.06	0.01	0.08	-0.07
Day of first flower, 1st year	-0.11	**-0.45**	**-0.42**	-0.26	-0.07	0.14	0.25	-0.03
Rel. no. of flower heads per stalk	-0.17	0.11	**-0.77**	0.02	0.03	0.03	-0.08	0.09
Plant height	**0.47**	-0.01	**0.74**	0.19	-0.06	-0.06	0.11	-0.07
Ratio: veg. to rep. biomass comp.	-0.11	**0.49**	**0.70**	0.17	0.01	0.01	-0.05	0.01
Dry mass of flowering stalks	**0.52**	**0.47**	**0.55**	0.21	-0.05	-0.07	0.07	-0.01
Mean flower head area	0.31	-0.23	**0.38**	0.16	0.02	0.10	0.25	0.27
Hairiness at stalk base	0.11	0.25	**0.36**	0.04	-0.22	0.03	0.03	0.15
Seed mass	0.06	0.05	0.04	**0.70**	0.10	-0.10	-0.03	0.05
# Seeds in one flower head	0.26	0.14	0.24	**0.70**	-0.10	0.08	0.11	-0.04
Hairiness lower leaf surface	-0.03	0.03	-0.01	0.02	**0.86**	-0.02	-0.02	0.00
Hairiness upper leaf surface	-0.08	-0.02	-0.10	0.04	**0.86**	0.03	0.08	-0.05
RGR of leaf size	-0.18	-0.05	-0.03	0.00	-0.05	**0.86**	0.10	-0.03
Ratio of leaf length to leaf width	-0.12	0.06	0.02	0.12	-0.06	**-0.69**	0.28	-0.08
# Incisions per cm leaf length	-0.15	0.06	-0.09	0.03	-0.10	-0.01	**-0.70**	0.08
Relative depth of incision of leaves	-0.08	0.16	0.02	0.03	-0.03	-0.13	**0.64**	0.09
# Side rosettes	0.20	0.07	-0.15	0.02	-0.04	0.00	0.11	**-0.80**
Specific leaf weight	0.23	-0.07	-0.29	0.01	-0.15	0.05	0.15	**0.62**
% Variance explained	15.26	12.53	11.08	6.98	6.52	5.67	5.16	4.83

Partitioning of variation in Carlina vulgaris and Hypochoeris radicata

In *C. vulgaris*, most of the quantitative genetic variation (V_G) was among regions (mean and sd of PCs: 40.2% ± 22.6%; mean and sd of single traits: 40.3% ± 20.6%) and among populations within regions (PCs: 38.5% ± 19.5%, single traits: 35.5% ± 14.5%; Table 9, Fig. 4). The means for Q_{ST} and heritability estimates were lower for fitness-related traits ($Q_{ST} = 0.15 ± 0.07$, $h^2 = 0.19 ± 0.09$) than for morphological traits ($Q_{ST} = 0.19 ± 0.09$, $h^2 = 0.35 ± 0.14$), but only the differences in heritability were significant ($F_{1,18} = 5.52$, $p = 0.03$). Most of the total phenotypic variation (V_P) was among plants within seed families (Table 9).

Table 9. Variance component estimates for seven fitness-related and 13 morphological traits of *Carlina vulgaris* at the region (V_{Reg}), population (V_{Pop}) and seed family (V_{Fam}) level analysed by REML and related to the total phenotypic variation ($V_P = V_{Reg} + V_{Pop} + V_{Fam} + V_E$) and the total genetic variation ($V_G = V_{Reg} + V_{Pop} + V_{Fam}$). V_E represents the mainly environmental residual variation among plants. Maximum values for the proportion of total genetic variation are in boldface. Differentiation among populations (Q_{ST}) and narrow sense heritability (h^2) values are also given.

	Proportion of total phenotypic variance [%]				Proportion of total genetic variance [%]				
	V_{Reg}	V_{Pop}	V_{Fam}	V_E	V_{Reg}	V_{Pop}	V_{Fam}	Q_{ST}	h^2
Fitness-related traits									
# Flowering stalks	2.03	1.99	3.95	92.03	25.39	25.00	**49.60**	0.059	0.147
Plant height	30.03	19.71	8.62	41.64	**51.45**	33.78	14.77	0.222	0.453
# Fruit heads	17.59	4.54	22.12	75.13	**70.72**	18.25	11.03	0.171	0.127
Total fruit head area	7.24	3.52	2.67	86.56	**53.86**	26.24	19.90	0.142	0.110
Above-ground biomass	13.62	7.11	4.84	74.44	**53.28**	27.92	18.92	0.138	0.156
Seed mass	12.61	11.62	4.40	71.36	**44.03**	40.59	15.37	0.248	0.198
Diameter at stalk base	4.80	3.79	7.72	83.70	29.43	23.24	**47.34**	0.058	0.269
Morphological traits									
Rel. depth of incision of leaves	32.16	3.84	8.02	55.98	**73.06**	8.72	18.23	0.056	0.364
Ratio of leaf length to width	12.93	18.51	12.18	56.38	29.64	**42.44**	27.92	0.160	0.463
Hairiness upper leaf surface	1.12	6.12	11.51	81.25	4.65	35.29	**60.06**	0.073	0.362
Hairiness lower leaf surface	24.30	9.76	4.33	61.62	**63.30**	25.42	11.29	0.220	0.220
# Spines per cm leaf	21.95	15.39	8.34	54.32	**48.05**	33.69	18.26	0.187	0.362
Specific leaf weight	21.61	5.99	6.13	66.27	**64.06**	17.77	18.17	0.109	0.270
Day of first flower	22.55	33.28	7.91	36.26	35.38	**52.21**	12.42	0.344	0.466
Mean fruit head area	5.97	12.74	6.78	74.51	23.43	**49.97**	26.60	0.190	0.267
Proportion of stalk branched	18.30	17.58	6.21	57.91	**43.48**	41.77	14.75	0.261	0.214
Hairiness of stalks	4.24	15.09	3.86	76.81	18.29	**65.06**	16.65	0.328	0.167
Colour of flowering stalk	1.39	13.73	8.72	76.16	5.85	**57.59**	36.56	0.164	0.314
# Internodes per cm stalk	31.01	20.28	7.99	40.72	**52.31**	34.21	13.48	0.241	0.440
Length of ray florets	1.80	5.33	3.57	89.31	16.81	**49.81**	33.38	0.157	0.667
Mean of all traits	14.36	11.50	7.49	67.62	40.32	35.45	24.24	0.176	0.302
(Standard deviation)	(10.55)	(7.88)	(4.30)	(16.45)	(20.59)	(14.53)	(14.11)	(0.084)	(0.145)

The proportion of total genetic variation among regions was highest for the relative depth of incision of leaves, the number of fruit heads per plant, the specific leaf weight and the hairiness of the lower leaf surface. The proportion of variation among populations within regions was highest for the hairiness of stalks, the colour of stalks, the day of first flower and the mean fruit head area.
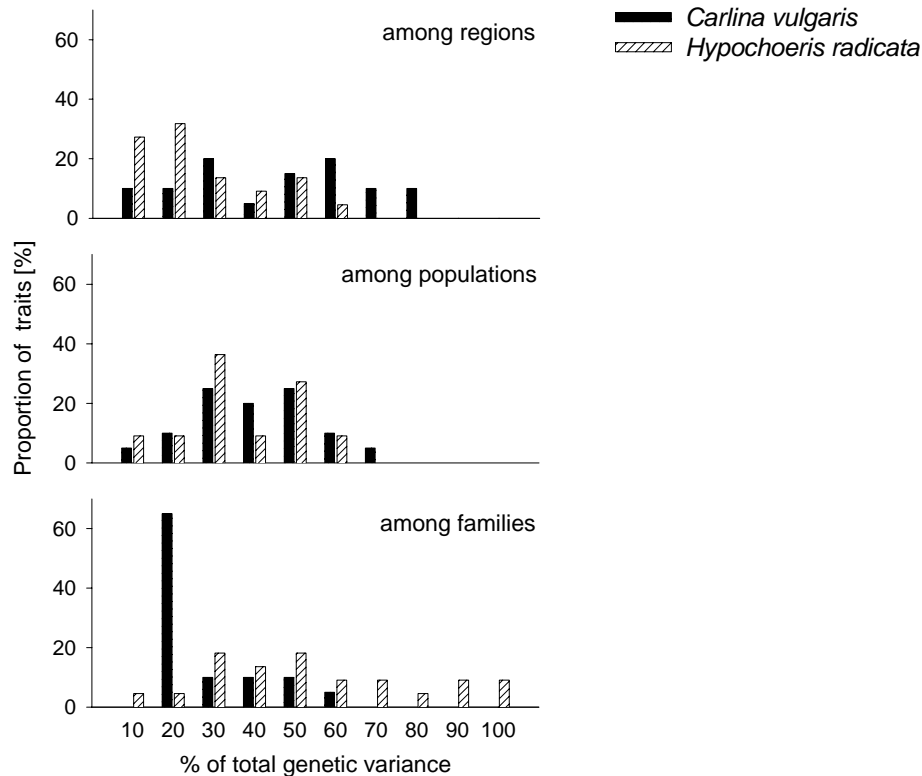
Figure 4. Frequency histograms of the proportion of traits of *Carlina vulgaris* and *Hypochoeris radicata* as a function of the proportion of total genetic variance explained by each variance component. 20 traits (s. Table 9) were studied in *C. vulgaris* and 22 (s.Table 10) in *H. radicata.*

In *Hypochoeris radicata,* the partitioning of variation was different from that in *C. vulgaris* (Table 10, Fig. 4). The proportion of genetic variation was largest among families within populations (mean and sd of PCs: 49.9 ± 18.1%, mean and sd of single traits: 49.3 ± 25.8%) and was much lower among regions (PCs: 17.2 ± 19.0%, single traits: 20.8 ± 16.6%). For two out of 22 traits, Q_{ST}-values did not differ from zero (Table 10).

As in *C. vulgaris* the highest proportion of total phenotypic variation was among plants within seed families (68.78 ± 16.6%). Population differentiation in traits of *H. radicata* was lower ($Q_{ST} = 0.11 ± 0.11$) than in *C. vulgaris* ($F_{1,40} = 5.25$, p = 0.03).

Table 10. Variance component estimates for 12 fitness-related and 10 morphological traits of *Hypochoeris radicata* at the region (V_{Reg}), population (V_{Pop}) and seed family (V_{Fam}) level analysed by REML and related to the total phenotypic variation ($V_P = V_{Reg} + V_{Pop} + V_{Fam} + V_E$) and the total genetic variation ($V_G = V_{Reg} + V_{Pop} + V_{Fam}$). V_E represents the mainly environmental residual variation among plants. Maximum values for the proportion of total genetic variation are in boldface. Differentiation among populations (Q_{ST}) and narrow sense heritability (h^2) values are also given.

	Proportion of total phenotypic variance [%]				Proportion of total genetic variance [%]				
	V_{Reg}	V_{Pop}	V_{Fam}	V_E	V_{Reg}	V_{Pop}	V_{Fam}	Q_{ST}	h^2
Fitness-related traits									
Leaf size at 2 weeks	6.32	**27.17**	23.13	43.38	11.16	47.99	40.85	0.402	0.132
Leaf size at 6 weeks	6.28	**24.28**	19.36	50.07	12.59	48.63	38.78	0.136	0.607
RGR of leaf size	0[a]	4.08	**18.03**	77.89	0[a]	20.20	79.80	0.028	0.481
# Side rosettes	0[a]	0[a]	**16.67**	83.33	0[a]	0[a]	100.00	0[a]	0.414
Dry mass of rosette leaves	3.52	**20.86**	20.63	54.95	7.82	46.34	45.84	0.112	0.600
# Flowering stalks	**20.00**	10.00	15.00	55.00	44.44	22.22	33.33	0.077	0.522
Plant height	**26.39**	17.04	12.41	57.87	47.26	30.51	22.23	0.082	0.462
# Flower heads (log)	10.08	13.81	**18.67**	57.44	23.69	32.45	43.86	0.085	0.565
# Seeds in one flower head	**10.07**	4.19	4.56	81.18	53.53	22.27	24.20	0.103	0.183
# Seeds per plant	**20.88**	8.84	12.41	57.87	49.57	20.98	29.45	0.082	0.462
Above-ground biomass: tot.	8.18	**22.19**	21.66	47.97	15.72	42.64	41.64	0.113	0.644
Seed mass	2.76	3.56	**7.98**	85.70	19.30	24.90	55.80	0.072	0.290
Morphological traits									
Rel. depth of leaf incision	2.60[a]	3.21	**9.27**	84.85	17.15	21.24	61.61	0.041	0.304
# incisions per cm leaf	3.80[a]	7.56	**6.93**	81.72	20.78	41.34	37.88	0.120	0.253
Ratio of leaf length to width	4.35	4.35	**13.04**	78.26	20.00	20.00	60.00	0.040	0.400
Hairiness upper leaf surface	0.18	1.95	**10.61**	87.26	1.39	15.32	83.29	0.022	0.327
Hairiness lower leaf surface	0.74	0.56	**13.38**	85.32	5.04	3.82	91.14	0.005	0.385
Specific leaf weight	1.00[a]	2.01[a]	**9.28**	88.61	0.88	17.68	81.44	0.026[a]	0.295
Day of first flower, 1st year	22.98	**26.17**	11.85	39.07	31.44	57.81	10.75	0.216	0.548
Day of first flower, 2nd year	8.54	**15.77**	2.43	73.26	31.92	58.98	9.10	0.402	0.132
Rel. no. ower heads p. stalk	2.07	2.85	**9.00**	86.08	14.87	20.48	64.66	0.038	0.295
Ratio: veg. to repr. dry mass	12.50	**18.75**	12.50	56.25	28.57	42.86	28.57	0.158	0.471
Mean of all traits	8.84	10.87	**13.13**	68.79	20.78	29.94	49.29	0.107	0.399
(Standard deviation)	(8.25)	(9.12)	**(5.55)**	(16.61)	(16.56)	(16.26)	(25.76)	(0.109)	(0.152)

In both species, large standard deviations for mean variance components at all levels indicate that the pattern of variation varied among traits. Differences in variability measured as coefficients of variation (CVs) of family means and of population means were also considerable. The CVs of the traits at different spatial levels (within seed families, among seed families within populations, and among populations within regions) were positively correlated in both species (Fig. 5 and 6). In *C. vulgaris* all correlations were highly significant

(Fig. 5), whereas in *H. radicata* the relationship between the CV within seed families and among seed families was not significant (Fig. 6). In *C. vulgaris* mean CVs across the three spatial levels were highest for the above-ground biomass (32.8%), the number of flower heads (34.1%) and the total fruit head area (44.6%) and lowest for the length of the ray florets (9.3 %), and the hairiness on upper and on lower leaf surface (7.8%, 4.4%). In *H. radicata* mean CVs were highest for the number of seeds per head (25.1%), biomass of the leaves (27.1%) the hairiness of the lower leaf surface (27.4%) and the number of side rosettes (104.9%), and lowest for the start of flowering in 2001 and 2002 (9.8%, 8.6%) and the ratio of the length to the width of the longest leaf (8.8%). In *H. radicata* the CVs for the number of side rosettes by far exceeded all other values and their inclusion in the correlation analysis would result in strong, but biased correlations.

Figure 5. The relationship between the variability measured as coefficients of variation (CV) calculated at different levels in *Carlina vulgaris*: Within seed families (a, b), among seed families within populations (a, c) and among means of populations within regions (b, c). Each dot represents a trait. *** p < 0.001.
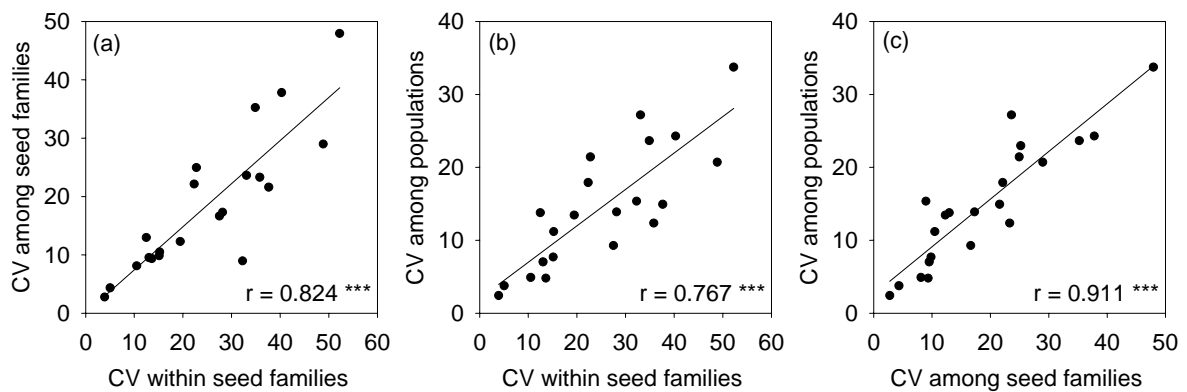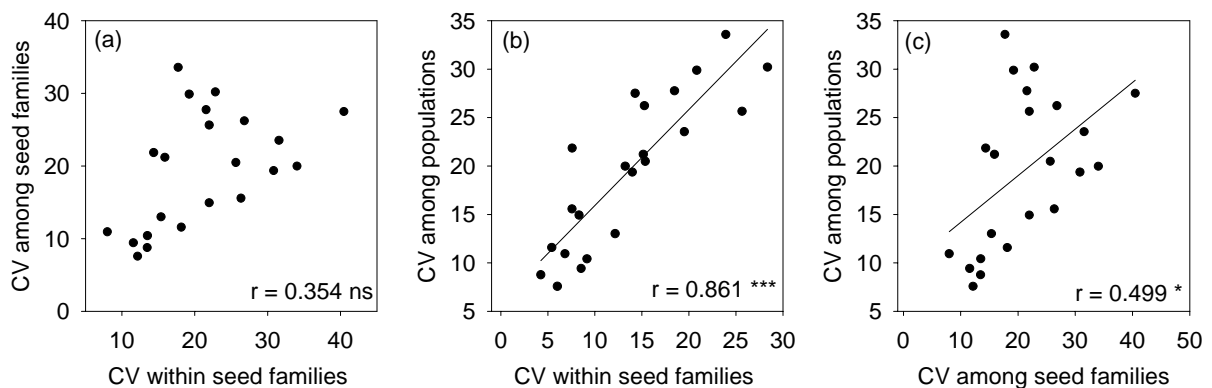

Figure 6. The relationship between the variability measured as coefficients of variation (CV) calculated at different levels in *Hypochoeris radicata*: Within seed families (a, b), among seed families within populations (a, c) and among means of populations within regions (b, c). Each dot represents a trait. ns not significant, * p < 0.05, *** p < 0.001.

Relationship among quantitative genetic and geographical distances

Pairwise quantitative genetic and geographical distances between populations within regions were only very rarely positively related in both species. This was true for both single traits and groups of traits (PCs). In *C. vulgaris*, pairwise Q_{ST}-values for means of all PCs and geographical distances were weakly positively correlated only in Switzerland (Table 11). Against expectation there were even negative relationships between geographical distance and Q_{ST} in Czechia (*C. vulgaris*) and Germany (*C. vulgaris* and *H. radicata*).

Table 11. Results of Mantel tests of the relationship between mean pairwise Q_{ST}-values of 8 principal components and geographical distances for each pair of populations in each region in (a) *Carlina vulgaris* and (b) *Hypochoeris radicata*. Significant correlations (one-tailed) are bold-faced.

a)

	r	p
NW-Switzerland	**0.213**	**0.046**
NW-Czechia	-0.479	0.031
S-Great Britain	0.180	0.251
C-Germany	-0.331	<0.001
Luxembourg	-0.121	0.206
S-Sweden	-0.005	0.484
W-Netherlands	0.149	0.350

b)

	r	p
NW-Czechia	0.213	0.264
C-Germany	-0.174	0.005
C-Netherlands	-0.079	0.431

Effects of population size and distance to the next population on quantitative genetic variation

In *C. vulgaris*, population size or distance to the nearest population had no effects on variability within populations expressed as coefficients of variation of single traits (Table 12) and of eight principal components (p > 0.05) with the exception of the CV of the length to width ratio of the longest leaf which increased slightly with population size.

In contrast, in *H. radicata*, the variability among seed families within populations increased with increasing distance to the nearest population in some traits (size of leaves, number of flower heads, plant height, total above-ground biomass and ratio of the length to the width of the longest leaf) and marginally in the mean of all traits (Table 13). Population size had a negative effect on the variability of total above-ground biomass. Distance to the nearest population or population size had no effect on CVs of principal components (p > 0.05).

Table 12. F-values for the effect of regions on coefficients of variation within seed families (CV_{Fam}) and for effects of region, population size (log-scale) and isolation (log-scale) on coefficients of variation among seed families within populations (CV_{Pop}) in *Carlina vulgaris*. Arrows indicate the direction of the effects ($\uparrow$, positive effects; $\downarrow$, negative effects).+ $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Region CV_{Fam}	Region CV_{Pop}	Population size	Isolation
Fitness-related traits				
# Flowering stalks	7.63***	8.06***	1.10	0.01
Plant height	0.53	1.18	2.78	2.09
# Fruit heads	13.36***	5.58***	0.01	2.00
Total fruit head area	1.32	4.72**	0.07	1.46
Above-ground biomass	2.01+	1.01	0.08	0.16
Seed mass	1.57	2.03+	1.48	0.03
Diameter at stalk base	0.83	1.05	0.01	0.28
Morphological traits				
Rel. depth of incision of leaves	5.72***	2.83*	3.22$\downarrow$+	2.07
Ratio of leaf length to leaf width	2.98*	2.33*	4.38$\uparrow$*	2.65
Hairiness upper leaf surface	1.61	0.62	2.23	1.15
Hairiness lower leaf surface	16.16***	8.10***	0.29	3.40$\uparrow$+
# Spines per cm leaf	5.21***	4.85***	0.04	0.05
Specific leaf weight	2.63*	1.85	0.10	0.13
Day of first flower	3.81*	1.43	2.53	0.64
Mean fruit head area	3.78**	3.81**	0.38	1.38$\uparrow$+
Prop. of stalk length branched	2.73*	5.07***	0.39	0.27
Hairiness of stalks	1.73	2.63*	1.83	0.50
Colour of flowering stalk	1.84	1.07	0.01	3.10$\downarrow$+
# Internodes per cm stalk	3.10**	1.15	2.13	0.02
Length of ray florets	0.78	1.26	1.31	3.87$\uparrow$+
Mean of all traits	2.89**	3.39**	1.35	1.82

DISCUSSION

Genetic differentiation and partitioning of variation

Strong intraspecific variation in quantitative traits has been found in a number of plant species (e.g. Meagher *et al*. 1978, Prentice 1984, Andersson 1991, Widén & Andersson 1993, Bonnin *et al*. 1996, BlackSamuelsson *et al*. 1997). Such divergence among and within populations is a result of mutation, heterogeneous selection, migration and genetic drift (Loveless & Hamrick 1984, Barrett & Kohn 1991). In contrast, gene flow tends to reduce the differentiation among populations (Loveless & Hamrick 1984, Hamrick *et al*. 1991, Linhart & Grant 1996).

Table 13. F-values obtained from ANOVA for the effects of region on coefficients of variation (CVs) within seed families (CV_{Fam}) and effects of region, population size (log-scale) and isolation (log-scale) on the CVs among seed families within populations (CV_{Pop}) in *Hypochoeris radicata* resulted. Arrows indicate the direction of the effects (↑, positive effects; ↓, negative effects). $+ p < 0.10$, $* p < 0.05$, $** p < 0.01$, $*** p < 0.001$.

	Region CV_{Fam}	Region CV_{Pop}	Population size	Isolation
Fitness-related traits				
Leaf size at 2 weeks	0.36	0.26	2.57	4.68↑*
Leaf size at 6 weeks	1.01	0.50	2.44	4.73↑*
RGR of leaf size	1.04	0.23	1.19	0.50
# Side rosettes	0.35	0.94	0.02	0.51
Dry mass of rosette leaves	0.06	1.41	0.90	1.54
# Flowering stalks	9.75***	1.30	0.73	1.02
Plant height	5.37*	1.37	0.45	8.10↑**
# Flower heads (log)	1.26	0.07	2.60	4.25↑*
Dry weight total (sqrt)	0.55	0.25	4.99↓*	5.25↑*
Seed mass (Covariable)	7.90**	5.65**	0.43	0.37
# Seeds in one flower head	15.55***	1.16	0.13	5.94
# Seeds per plant	3.90*	0.43	3.73↓+	3.09↑+
Morphological traits				
Rel. depth of incision of leaf	1.18	1.14	1.63	0.00
# Incisions per cm leaf (log)	1.09	2.72	3.84↓+	0.54
Ratio of leaf length to width	1.83	1.15	0.14	4.60↑*
Hairiness upper leaf surface	3.35*	4.60*	0.34	0.56
Hairiness lower leaf surface	31.36***	12.40***	1.40	0.88
Specific leaf weight	0.70	0.37	0.98	0.00
Day of first flower, 1st year	4.55*	1.96	0.02	0.17
Day of first flower, 2nd year	2.66+	2.51	1.81	0.95
Rel. no. flower heads p. stalk	2.09	2.43	1.00	0.27
Ratio: dry mass of leaves to fl. stalk	3.46*	1.66	1.53	1.60
Mean of all traits	0.70	0.84	2.33↓+	2.52↑+

In our study on *C. vulgaris* and *H. radicata*, both the results of the nested ANOVAs and the variance component estimates indicate substantial morphological variation within populations, among populations within regions and among regions. However, the degree of variation differed between the two species and among different traits. The positive relationships found for different traits between the variation within seed families and among seed families within populations, and between the variation within seed families and among populations within regions, indicate that certain traits (e.g. the number of fruit heads and the total fruit head area in *C. vulgaris*, and the number of side rosettes, the hairiness of the lower leaf surface and the vegetative biomass in *H. radicata*) show high variation at all spatial levels.

Genetic diversity increases the ability of a species to react to changing environmental conditions (Barrett & Kohn 1991, Mitton 1993, Frankham 1999). Therefore quantitative traits with high genetic variability might be under strongest selection.

The two study species varied in the partitioning of variation at different hierarchical levels. In *C. vulgaris* the mean proportion of variation in a trait that was among populations (75.8%) was much higher than in *H. radicata* (50.7%). We suggest that the higher probability of gene exchange due to higher dispersal and weaker fragmentation in *H. radicata* leads to lower differentiation among populations. The partitioning of variation in *H. radicata* is similar to that found for reproductive traits in the very long-lived *Lathyrus vernus*, that was investigated in populations from three regions (C-Sweden, S-Sweden, C-Europe, Widén & Schiemann 2003). In *L. vernus*, 57.6% of the total variation was among individuals within populations, 29% among populations within regions and 13.4% among regions. The higher proportion of variation among populations within and between regions in *C. vulgaris* than in *H. radicata* and *Lathyrus vernus* suggests stronger divergence due to genetic drift or selection in this short-lived, monocarpic and poorly dispersed plant.

Recently, it has been suggested that Q_{ST} as an analogue to F_{ST} is a good measure of population differentiation in quantitative traits. The low values for Q_{ST} for *H. radicata* are similar to those recorded for *Salix torminalis*, *Silene diclinis* and *Pinus contorta* (Merilä & Crnokrak 2001, all < 0.1). All these plants are long-lived, well-dispersed and mainly wind-pollinated species. Therefore, gene flow between populations that prevents population differentiation due to genetic drift or heterogeneous selection is high. The Q_{ST}-values for *Carlina vulgaris* are in-between those recorded for long-lived or well-dispersed species and those for short-lived and selfing species (Merilä & Crnokrak 2001).

A higher divergence between and lower divergence within populations is expected in species that are rare or restricted to specific habitats (e.g. *C. vulgaris*) (Hamrick & Godt 1996a), but this can not be generalised (Hamrick *et al*. 1991). For instance, Waldmann & Andersson (1998) found lower population differentiation for eight fitness-related traits in the rare *Scabiosa canescens* than in the common *S. columbaria*. The authors suggest a higher adaptive potential in the more widespread species as the reason for the higher population divergence.

A number of studies have compared the divergence in quantitative traits with that in molecular markers (e.g. Bonnin *et al*. 1996, Waldmann & Andersson 1998, Merilä & Crnokrak 2001, Petit *et al*. 2001, McKay & Latta 2002, Steinger *et al*. 2002). In these studies values for Q_{ST} exceeded those for F_{ST}. It has been suggested that heterogeneous selection is

responsible for the stronger differentiation in quantitative traits. For *H. radicata,* values of F_{ST} (0.04) which resulted from the analysis of microsatellites in several populations in the Netherlands (Carolin Mix, personal communication) were also lower than the values of Q_{ST} (0.11) that resulted from this study indicating heterogeneous selection. However, these measures of differentiation are not directly comparable, because the values for Q_{ST} and F_{ST} were obtained from different populations.

If different traits are compared, a fitness component usually shows lower heritability and higher differentiation among populations than a trait that is only weakly related to fitness (Andersson 1991, Platenkamp & Shaw 1992, Falconer & McKay 1996), because the environmental influence is higher on traits which are under strong selection. In *C. vulgaris,* heritability was significantly lower for fitness components than for traits that were not directly related to fitness. The higher population differentiation in quantitative traits than in molecular markers for *H. radicata* and the lower heritability for fitness-related traits of *C. vulgaris* suggest that selection is an important factor for the population differentiation in both species. However, because in *H. radicata* there were no differences in the heritability between fitness-related and morphological traits and because values of Q_{ST} were low, the effects of selection are probably counterbalanced by other factors, e.g. gene flow.

In both species, populations from different study regions were clearly genetically differentiated from each other. Similarly, in *Lathyrus vernus* differences in leaf shape were also positively associated with geographical distances across three European regions (Widén & Schiemann 2003). At the large scale, heterogeneous selection which is related to climatic gradients might result in population differentiation (Clausen *et al.* 1940, Weber & Schmid 1998, Joshi *et al.* 2001). Variation in quantitative traits therefore is a useful tool to analyse large scale geographical patterns of population differentiation. However, within regions the genetic distance between populations was not related to the geographical distance in our study species. Previous studies found that allozyme variation on average shows stronger association to geographical distances than morphological variation (Lönn & Prentice 1995, Allen *et al.* 1996, Widén & Schiemann 2003, but see Podolsky & Holtsford 1995, Oostermeijer & de Knegt 2004). On smaller scales climatic differences are small and are not able to drive geographical patterns of population differentiation. Here, environmental conditions other than climate are the main selective forces (Schmid 1985, Bell *et al.* 2000, Joshi *et al.* 2001). However, in *H. radicata* and *C. vulgaris* we found no relationship between the distances in traits that reflect environmental conditions (nutrient availability, vegetation structure, vegetation composition) and quantitative genetic distances. Factors that were not recorded in this

study might have been responsible for the population differentiation within regions (e.g. competitors, herbivores, parasites and pathogens, mutualists). Alternatively, random genetic drift might have been more important than selection for the genetic divergence within regions. In this case we would expect a stronger association between genetic distances based on neutral molecular markers and geographical distances than between quantitative genetic and geographical distances.

Geographical distribution of variation

Due to increased genetic drift and inbreeding in small and isolated populations at the margin of a species' range, peripheral populations may have lower genetic variation than central populations (e.g. Durka 1999, Jiminez *et al.* 1999, Lammi *et al.* 1999, Tyler 2002, Jump *et al.* 2003, Kapralov 2004, but see Lesica & Allendorf 1995, Schiemann *et al.* 2000). For instance, increasing genetic variation in allozymes (allelic richness, heterozygosity, inbreeding coefficient F_{IS}) with decreasing latitude has been found in *Silene nutans* in Northern Europe (van Rossum & Prentice 2004) and in *Erythronium montanum* in North America (Allen *et al.* 1996). However, there was no effect of latitude on the variation in morphological traits in the latter species. Morphological variation between populations is expected to be higher in isolated populations at the margin of a species' range than in interconnected central populations because divergence in quantitative characters is expected to increase (Lesica & Allendorf 1995). In *C. vulgaris*, variation among and within regions was high, but was not related to the position of a region with regard to the species' range margin.

Effects of habitat fragmentation

Population genetic theory predicts that in small and isolated populations genetic variation will be reduced due to genetic drift (Gilpin & Soulé 1986, Lacy 1987, Barrett & Kohn 1991, Ellstrand & Elam 1993), and a positive relationship between plant population size and molecular genetic variation has been found in a number of studies (Raijmann *et al.* 1994, Godt *et al.* 1996, Sun 1996, Fischer & Matthies 1998b, Luitjen *et al.* 2000, Lienert *et al.* 2002a, Paschke *et al.* 2002a). However, the relationship between quantitative genetic variation and population size is less clear. Positive effects of population size on the variability in morphological traits have been found in *Salvia pratensis* and *Scabiosa columbaria* (Ouborg *et al.* 1991). However, other studies found no effect of population size on the variation in

quantitative traits (Widén & Andersson 1993, Lönn & Prentice 1995) or even negative effects for a considerable proportion of the studied traits (Oostermeijer *et al*. 1994a, Podolsky 2001).

In our study, the variation in 7 out of 22 quantitative traits increased with distance to the nearest population in *H. radicata,* whereas isolation had much weaker effects that were not consistent in direction in *C. vulgaris*. In both study species population size did not have any consistent effects on the variation in quantitative traits, except for three traits in *H. radicata* and one trait in *C. vulgaris* where the variation tended to decrease with increasing population size. Negative effects of population size and positive effects of distance to the nearest conspecific population, as we found in *H. radicata*, at first glance suggest a higher evolutionary potential in small or isolated populations. However, single rare alleles which might not be able to cause responses to environmental changes may be responsible for the high phenotypic variability in small or isolated populations (Holsinger 1999). Thus, higher variability in small and isolated populations might rather indicate instability due to increased inbreeding that leads to increased homozygosity (Oostermeijer *et al*. 1994a). We suggest that in *H. radicata* a sudden reduction of gene flow might have stronger consequences than in *C. vulgaris,* because the species depends on gene flow among populations. In contrast, *C. vulgaris* might be used to live in more or less fragmented habitats. In summary, severe negative consequences of habitat fragmentation on the morphological variation in small and isolated populations were not found for these two common species. However, in a related study we found a decrease in several fitness components in offspring of *C. vulgaris* from small populations (Chapter 2).

Conclusions

Our results suggest that lower dispersal ability and a shorter generation time in *Carlina vulgaris* lead to higher population differentiation among and within regions than in *Hypochoeris radicata*. Strong regional differentiation in both species suggests that geographical differences are important for evolutionary processes. In both species, pairwise genetic distances among populations within regions were not correlated with the geographical distances between populations. We suggest that random genetic drift and environmental conditions that are not related to geographical patterns play an important role for the differentiation on the smaller scale. Thus, each geographical region, irrespective whether it is at the margin or in the centre of a species' range, has to make efforts to preserve the specific genotypes to conserve the evolutionary potential of a species.

Local adaptation

at different spatial scales across Europe in the

fragmented monocarpic perennial

Carlina vulgaris

with Guy Colling, Petr Dostal, Anna Jakobsson and Diethart Matthies, submitted

ABSTRACT

1. Spatial variation in environmental conditions can lead to local adaptation of plant populations, in particular if gene flow among populations is low. To study population differentiation and local adaptation in the monocarpic grassland perennial *Carlina vulgaris*, we carried out reciprocal transplant experiments among (regional scale) and within (local scale) five respectively three European regions and recorded survival, growth and reproduction over three growing periods.

2. At the regional scale, the individual fitness of *C. vulgaris* was highest if plants grew in their home region and performance of plants decreased with increasing transplant distance. The effects could be due to climatic differences or differences in edaphic conditions that increased with the geographical distance between regions.

3. 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 a higher performance of plants at their home site and not related to geographical or environmental distance between the population of origin and the transplant site. The size of the population of origin did not influence the strength of local adaptation, but several fitness-related traits increased with population size.

4. 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 (> 200 km), but not at small scales. Patterns of local adaptation should be taken into account for the efficient preservation of genetic resources, the assessment of the status of plant species and in conservation planning.

INTRODUCTION

Many plant species have a large geographical range over which environmental conditions vary considerably. There are two principal mechanisms that may explain why a species is able to grow under different conditions. A plant species may have a general-purpose genotype which is very plastic and able to grow, survive and reproduce under different conditions, or a plant species may consist of a number of different ecotypes that are adapted to particular environmental conditions (Bradshaw 1984, Schlichting & Pigliucci 1998). Because the costs of plasticity are high (DeWitt *et al*. 1998) and most species show large genetic variation, most plant populations probably consist of many specialised genotypes, which are adapted to particular conditions even within a specific site (van Tienderen 1990, Linhart & Grant 1996).

Reciprocal transplant experiments are a useful approach to investigate local adaptation (Nagy & Rice 1997, Kawecki & 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 (Linhart & Grant 1996, Briggs & Walters 1997); moreover, reciprocal transplant experiments permit examination of responses to different environments (Briggs & Walters 1997). Indeed, many studies have shown that genotypes grow better at their site of origin than at foreign sites (Clausen *et al*. 1940, 1948, Smith & Bradshaw 1979, McGraw & Antonovics 1983, van Andel 1998, Hufford & Mazer 2003), indicating home-site advantages. However, most studies have focussed on adaptation to contrasting environments, i.e. on ecotypic differentiation (e.g. van Tienderen & van der Toorn 1991, Kindell *et al*. 1996, Nagy & Rice 1997, Gauthier *et al*. 1998), and were carried out at small spatial scales (e.g. McGraw & Antonovics 1983, Waser & Price 1985). In contrast, little is known about patterns of adaptation at larger geographical scales (Schmidt & Levin 1985, Galloway & Fenster 2000, Santamaria *et al*. 2003). Because environmental differences are likely to increase with geographical distance, it is to be expected that the extent of adaptive differentiation increases with the geographical distance between populations (Montalvo & Ellstrand 2000, Joshi *et al*. 2001).

Due to habitat deterioration and fragmentation in the last decades, the populations of many plants in Europe have decreased in size and become more isolated (Saunders *et al*. 1991, Jennersten *et al*. 1992). In small and isolated populations genetic diversity is expected to decrease due to random genetic drift (e.g. van Treuren et al. 1991, Raijmann *et al*. 1994, Young *et al*. 1996, Fischer & Matthies 1998b) and inbreeding is expected to increase (Barrett & Kohn 1991). This can have negative effects on plant fitness in the short term (e.g. Menges

1991, Oostermeijer *et al.* 1994, Fischer & Matthies 1998a, Kéry *et al.* 2000, Vergeer *et al.* 2003, Hooftman *et al.* 2003). Moreover, fragmentation may also reduce the ability of populations to adapt to environmental conditions (Barrett & Kohn 1991, Eberhart *et al.* 1991, Mitton 1993, Helenurm 1998, Frankham 1999), because genetic variability is lower and selection might be less effective in small than in large populations (Frankham *et al.* 2002). However, there are hardly any studies on the effects of population size and isolation on local adaptation (Helenurm 1998, Hooftman *et al.* 2003).

A better understanding of the extent of local adaptation and its spatial scale has become increasingly important (van Andel 1998, van Groenendael *et al.* 1998, Hufford & Mazer 2003), 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). Furthermore, 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 for the success of such projects. If plants are adapted to specific conditions at their site of origin they may fail in a new environment. An understanding of the genetic differentiation among populations and the extent of local adaptation is also important for assessing local or regional extinctions. The extinction of a species in parts of its range would be of less concern if the species had a general purpose genotype than if it represented the loss of a regionally adapted genotype.

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 reciprocally transplanted seedlings among different European regions (in north-western Czechia, central Germany, Luxembourg, southern Sweden and northwestern Switzerland) and among several populations of different size within each region. The geographical distances between transplant sites and sites of origin varied 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 & 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?

MATERIAL 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 & Schenkeveld 1984, Grime *et al*. 1988, Meusel & Kästner 1994, Klinkhamer *et al*. 1996). The probability of flowering increases with the size of the rosette (Klinkhamer *et al*. 1991, 1992), and the age of flowering plants varies between two and at least eleven 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 (Greig-Smith & Sagar 1981, Grime *et al*. 1988). 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 & Sagar 1981, Grime *et al*. 1988, Franzén & Eriksson 2003). In Europe, the species is distributed in (sub-)oceanic to sub-Mediterranean regions from S-Italy (39 °N) to S-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 & Kästner 1994, Korneck *et al*. 1996).

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 in each of five European regions were chosen (Table 1). Geographical distance between populations ranged from 237 to 1439 km (median 620 km, Table 2). In each population one complete mature fruit head of each of 20 randomly chosen individuals was collected and sent to Germany. The diameter of each fruit head was measured and its number of seeds was counted. 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. 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

transplant site. At each transplant site five plots (3.2 x 0.6 m each) were established at random and marked with iron rods. In each plot five rows 15 cm apart from each other were defined and the vegetation was cut within 5 cm wide strips at both sides along the rows to minimise competition for the transplants during the early stages. Juveniles were planted 15 cm apart from each other along the rows in random order and their number of leaves and the length of their longest leaf were recorded to estimate their initial size. In each plot 20 replicate plants from each region of origin, i.e. 100 juveniles overall, were planted. After transplanting plants were watered for two weeks in order to facilitate establishment. Two weeks after transplanting 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 removed from further analyses.

Table 1. Populations of *Carlina vulgaris* used in the reciprocal transplant experiments. All populations used in the local transplant experiments (LT) functioned both as source and target populations. In the regional transplant experiments, seeds from two source populations (RS) in each region were pooled and transplanted to one target site (RT) in each region.

Region	No.	Population	Transplant type	Coordinates		Population size	Altitude [m.a.s.l.]
				N	E		
NW-Czechia	1	Cervena Piska, Cent. Bohemia	LT, RS, RT	50.29	14.53	1000	190
	2	Kopec, Cent. Bohemia	LT	50.25	14.42	100	200
	3	Piletice, E Bohemia	LT, RS	50.25	15.87	120	260
	4	Podloucky, E Bohemia	LT	50.60	15.20	50	350
C-Germany	1	Abterode, Cent. Germany	LT, RS, RT	51.22	9.93	500	290
	2	Rommerode, Cent. Germany	LT, RS	51.22	9.77	950	420
	3	Epterode, Cent. Germany	LT	51.24	9.80	43	490
	4	Groß Schneen, Cent. Germany	LT	51.43	9.93	30	250
S-Sweden	1	Langmären, Sormland	LT	58.84	17.39	11	15
	2	Hunga, Sormland	LT	58.92	17.51	11	15
	3	Lindsbacke, Sormland	LT, RS	58.74	16.99	200	20
	4	Studsvik, Sormland	LT	58.76	17.39	27	5
	5	Tuntorp, Sormland	RS, RT	59.00	17.12	50	35
NW-Switzerland	1	Bonfol, Jura	RS, RT	47.48	7.14	1500	440
	2	Soyieres, Jura	RS	47.52	7.46	650	450
Luxembourg	1	Kayl-Leiffrächen	RS, RT	49.48	6.02	11000	350
	2	Geyersknapp	RS	49.86	6.39	1200	310

Local scale. Within each of three regions, four populations of different size were chosen (Table 1), seeds were sampled and seedlings were raised as described above. Geographical distance between populations varied from 4 to 103 km (median 23 km, Table 3). 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 x 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.

Table 2. Geographical distances [km] between sites used in the *regional* transplant experiments.

Transplant site	Population of origin				
	S-Sweden	C-Germany	NW-Czechia	Luxembourg	NW-Switzerland
S-Sweden	0	977	982	1277	1439
C-Germany	-	0	339	338	462
NW-Czechia	-	-	0	616	624
Luxembourg	-	-	-	0	237
NW-Switzerland	-	-	-	-	0

Table 3. Geographical distances [km] between sites used in the *local* transplant experiments.

Transplant site		NW-Czechia (CZ)					C-Germany (D)					S-Sweden (S)			
		1	2	3	4		1	2	3	4		1	2	3	4
1	CZ	0	61.3	58.2	67.9	D	0	11.3	9.1	23.5	S	0	11.4	25.8	8.9
2		-	0	94.9	103.1		-	0	3.9	26.5		-	0	36.3	19.2
3		-	-	0	9.8		-	-	0	22.7		-	-	0	23.2
4		-	-	-	0		-	-	-	0		-	-	-	0

Growth, survival and reproduction of each transplant was recorded during three growth periods from spring 2001 until autumn 2003. Plant size and survival was recorded each autumn. For non-flowering plants the number of rosettes, the number of leaves and the length of the longest leaf was recorded. As an estimate of rosette size the product of the number of leaves and the length of the longest leaf was calculated. For flowering plants the number of inflorescences and the diameter of each inflorescence was recorded. Some plants flowered already in 2002 and their above-ground parts were harvested immediately after seeds had matured; above-ground parts of all other plants were harvested in autumn 2003. All plant material was air-dried, sent to Germany, dried for 12 hours 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: $F = 1.26$, $p = 0.26$, $n = 201$). For each plant the mass of ten seeds from the margin of the largest fruit head was determined. To obtain an estimate for the number of seeds (s) produced by the transplants a

regression of the number of seeds in a fruit head on the area of the fruit heads obtained in 2000 was used (r = 0.51, p < 0.001, n = 281). 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. Fecundity (F) of each individual was calculated as F = s * g. To obtain an estimate of individual fitness, Leslie matrices that incorporated time of reproduction were constructed using the survival and the fecundity data and dominant eigenvalues (finite rate of growth) were calculated for each individual (McGraw & 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 we assumed that surviving plants would flower in the following year and estimated survival and fecundity for the following year from regression equations. We first analysed the relationship between survival to 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 equation obtained, we predicted the probability of survival to 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 of 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). For plants from each origin at each site mean individual fitness values (λ_i) were calculated.

A relative measure of fitness (selection coefficient, McGraw & Antonovics 1983) was used to estimate the fitness of each origin (λ_i) relative to that of plants from the origin with the highest individual fitness (λ_{max}) at each particular site. Selection coefficients (s) were calculated as follows: s = 1 − (λ_i/λ_{max}). Low values for the selection coefficient indicate that there is low selection against a specific origin whereas high values (max = 1) indicate that those origins perform much worse than those with the best performance at a site.

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 height of the vegetation were recorded. To characterise climatic conditions, we obtained data for mean summer and winter temperature and summer and winter precipitation over the last 30 – 40 years from weather stations within each study region. To estimate the relative nutrient availability at the study sites, we carried out a bioassay. In July and August 2002, we sampled soil from eight random cores in each population, mixed and 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 x 9 x 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 hours at 80 °C and weighed. Total above-ground biomass per pot was used as estimate of nutrient availability.

Data analysis

Differences in species composition were investigated with detrended correspondence analysis (DCA). DCA-scores along the first two axes were used as variables reflecting environmental differences among habitats. At the regional scale absolute differences between each pair of populations were calculated for mean summer and winter temperature, summer and winter precipitation, 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 climate data were only available for the regions.

Table 4. Skeleton analysis of variance (or deviance) for (a) the regional and (b) the local transplant experiment. The range of the degrees of freedom is given if they varied depending on the traits studied.

(a)

Source of variation	df	Error term
Initial size	1	Residual
Site	4	Plot
Plot	20	Residual
Origin	4	Origin x plot
Origin x site	13–16	Origin x plot
Home vs. away	1	Origin x plot
Distance	1	Origin x plot
Residual effect	11–14	Origin x plot
Origin x plot	63–80	Residual
Residual	428–2332	

(b)

Source of variation	df	Error term
Initial size	1	Residual
Region	2	Site
Site	6–9	Plot
Plot	34–48	Residual
Population size	1	Origin
Origin	7–8	Origin x plot
Origin x site	18–29	Origin x plot
Home vs. away	1	Origin x plot
Distance	1	Origin x plot
Residual effect	15–26	Origin x plot
Origin x plot	54–144	Residual
Residual	302–1462	

We used general linear models to analyse continuous variables, and analyses of deviance for survival and flowering data (Table 4). Mean deviances due to a factor were divided by their appropriate error mean deviances, analogous for 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 x length of longest leaf = initial size) was used as a covariate to adjust for maternal effects and effects of different growing conditions before transplanting. The interaction between the effects of transplant site and origin was decomposed into a 'home vs. away' contrast (referred to as local vs. foreign contrast in Kawecki & Ebert (2004)) and linear contrasts consisting of the geographical distances between the site of origin and the transplant site. The effects of the site of origin, the interaction between the effects of site of origin and transplant site, the home vs. away contrast and the distances were tested against the interaction between the site of origin and plot. At the local scale, the effects of region and size of the population of origin were fitted in addition (Table 4b). The ANOVA models for the effects on the selection coefficients were similar to those described above, but the origin by transplant interaction was the residual, because we calculated one selection coefficient for each population of origin at each transplant site.

Rosette size and above-ground biomass were log-transformed and individual fitness (local experiment) was square root-transformed prior to analysis to obtain normally distributed residuals and homoscedasticity. Population size was log-transformed prior to analysis. Analyses of variance were performed with the statistical package SPSS 11.0 (release 11.0; SPSS Inc, Chicago, Illinois, USA). Analyses of deviance were calculated with the statistical package R, version 1.9.1. Leslie matrices were analysed with Matlab (student edition version 5.0, 1996). Detrended correspondence analyses were carried out with Canoco 4.5 (Ter Braak & Šmilauer 2002).

RESULTS

Regional transplant experiment

The size of the juveniles of *C. vulgaris* at the time of transplantation to the field strongly influenced their later growth and also overall individual fitness (Table 5). Effects of the studied factors on plant performance were therefore corrected for the effects of initial size.

The site at which the transplants grew had overall effects on plant performance, but these effects were transient. After 16 months, plants at the Swedish site were much smaller than in the other regions (Fig. 1). Because of their small size, none of the plants at the Swedish site flowered during the second growing period, whereas in the other regions already 10–16% of the plants flowered. In contrast, the identity of the plots within a site affected most characters, indicating differences in the environmental conditions among the plots within a site.

Table 5. *Regional scale*. Effects of transplant site and population of origin on life-history traits of transplanted individuals of *Carlina vulgaris*. Seedlings were transplanted reciprocally among five European regions. The interactions among transplant site and population of origin were partitioned into a home vs. away contrast and an effect of geographical distance. 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 analyses of deviance, respectively. Also included in the model is the effect of the initial size of the seedlings at the time of transplanting. Arrows indicate the direction of significant home ($\uparrow$, home-site advantage) or distance effects ($\downarrow$, negative effect of distance). $+ \ p < 0.1$, $* \ p < 0.05$, $** \ p < 0.01$, $*** \ p < 0.001$.

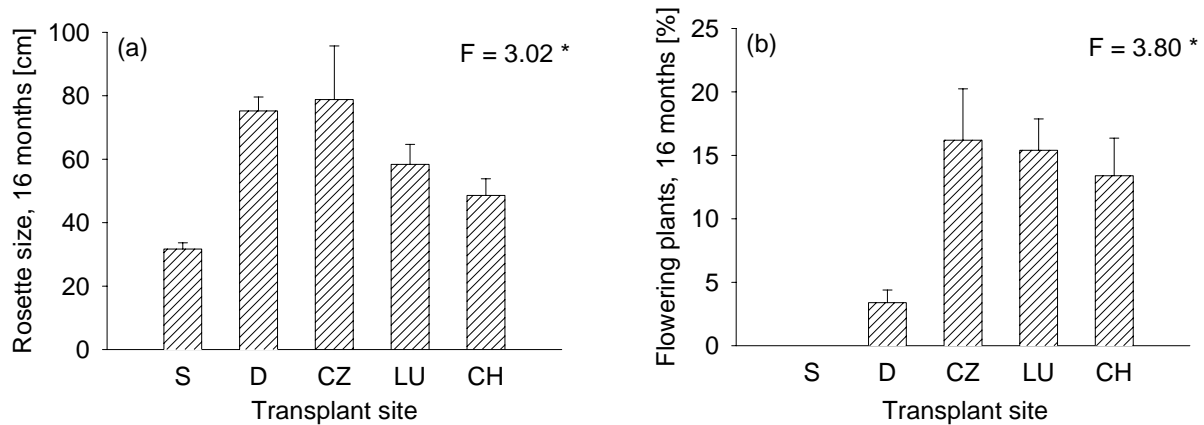|	Rosette size, 16 months	Flowering, 16 months	Survival	Flowering, 28 months	Biomass, veg. pl., 28 months	Biomass, flowering plants	Individual fitness
	F	Quasi-F	Quasi-F	Quasi-F	F	F	F
Initial size	327.94 ***	1.38	1.79	9.27 **	24.99 ***	10.47 **	42.95 ***
Site	3.02 *	3.80 *	1.89	1.15	1.35	2.47 +	1.13
Plot	20.22 ***	2.68 ***	2.19 **	2.33 **	6.35 ***	12.47 ***	12.94 ***
Origin	12.95 ***	19.59 ***	13.33 ***	25.00 ***	0.84	2.91 *	13.60 ***
Origin x site	1.90 *	1.85 *	1.76+	1.53	3.38 ***	2.23 *	1.52
Home	0.15	11.00 $\uparrow$**	8.72 $\uparrow$**	2.60	0.60	0.10	6.80 $\uparrow$*
Distance	15.00 $\downarrow$***	7.80 $\downarrow$**	5.74 $\downarrow$*	2.79 $\downarrow$+	0.94	1.61	4.37 $\downarrow$*
Residual effect	1.09	0.77+	0.85	1.36	3.75 ***	2.44 **	0.94
Origin x plot	1.34 *	80.76 ***	55.14 ***	53.84 ***	0.86	1.24	1.48 **

Figure 1. *Regional scale*. Effect of transplant site (S: S-Sweden, D: C-Germany, CZ: NW-Czechia, LU: Luxembourg, CH: NW-Switzerland) on (a) rosette size 16 months after transplanting and (b) proportion of flowering plants in the second year (= 16 months after transplanting). Dependent variables in (a) were adjusted for the effects of initial size of the rosettes. * $p < 0.05$.

The origin of the plants influenced most measures of performance, indicating genetic differentiation among origins (Table 5). All measures of performance were lower for plants from Sweden than for plants from the other regions, except for the biomass of flowering plants that was lowest for plants originating from Germany (Fig. 2).

Several traits were influenced by interacting effects of origin and transplant site (Table 5). 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 plants were growing, the smaller they were and the less likely it was that they flowered (Table 5, Fig. 3a, b). At the end of the experiment after 28 months, both survival (Fig. 3c) and marginally the probability of flowering decreased with increasing distance between the home and the transplant site (Table 5). As a consequence of the effects on survival and flowering probability in the second and third growing periods, individual fitness also decreased with distance between the home and the transplant site (Fig. 3d).

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 ($r = 0.94$; $r = 0.89$, $r = 0.80$; 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 ($r = -0.59$, $r = -0.63$, $r = -0.59$; all $p < 0.01$, $n = 25$), but also to mean summer temperature ($r = -0.52$, $p < 0.01$, $n = 25$).
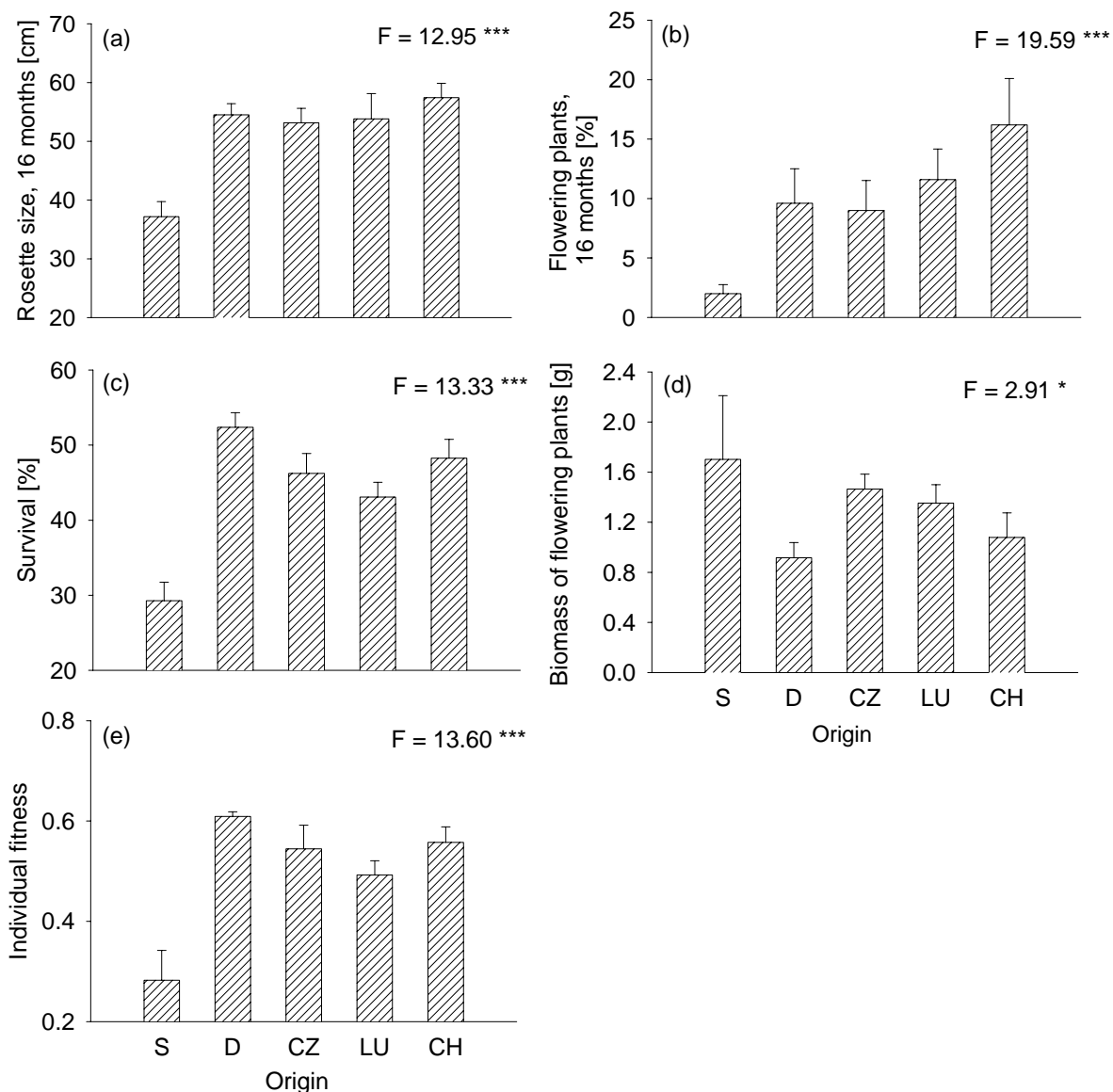
Figure 2. *Regional scale*. Effect of population of origin (S: S-Sweden, D: C-Germany, CZ: NW-Czechia, LU: Luxembourg, CH: NW-Switzerland) on (a) rosette size and (b) proportion of flowering plants 16 months after transplanting, (c) survival until flowering or until the end of the third growing season, (d) biomass of flowering plants, (e) mean individual fitness in *C. vulgaris*. Dependent variables in (a), (d) and (e) were adjusted for the effects of initial size of the rosettes, the target sites and for the plots within target sites. * p < 0.05, *** p < 0.001.

The data for the individual fitness of plants from different origins at the different sites were used to calculate selection coefficients. Selection against different origins was different ($F_{1,15} = 21.80$, p < 0.001, Tables 6 and 10). On average selection was strongest against plants originating from Sweden (mean: 0.57) and strongest in Czechia and Germany (mean: 0.28, 0.26). In contrast selection was weakest against plants originating from Germany and weakest in Luxembourg and Sweden. However, there was also a significant interaction between the effects of origin and mean selection strength ($F_{4,14} = 4.24$, p = 0.04, Fig. 4) per target.

Table 6. *Regional scale*. Selection coefficients of individual fitness for *Carlina vulgaris* from five European regions that were reciprocally transplanted among the regions. Selection coefficients potentially range between 0 and 1. A value of 0 indicates that plants of the respective origin had the highest individual fitness at a transplant site. Values in boldface indicate that plants from that origin performed best at their home site.

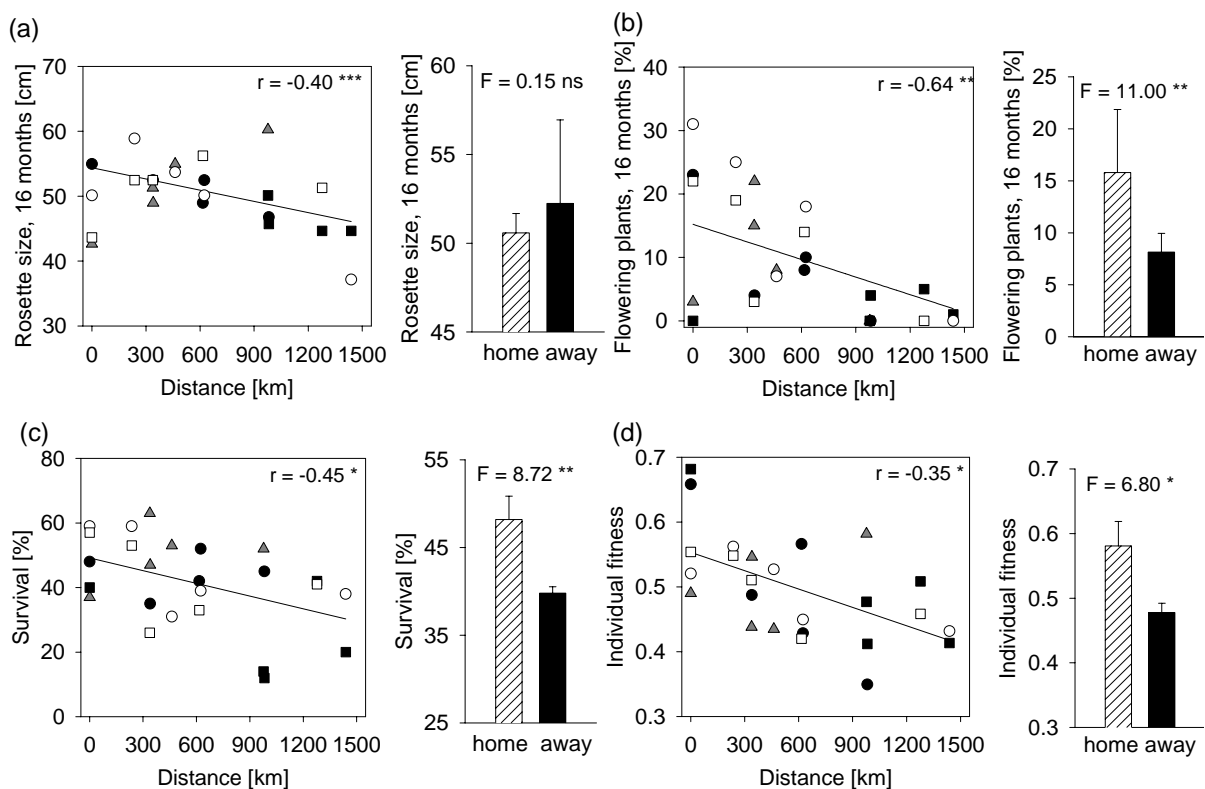| Target region	Region of origin					
	S-Sweden	C-Germany	NW-Czechia	Luxembourg	NW-Switzerland	Mean
S-Sweden	0.27	0	0.18	0.12	0.27	0.17
C-Germany	0.64	**0**	0.04	0.38	0.23	0.26
NW-Czechia	0.73	0.15	**0**	0.36	0.14	0.28
Luxembourg	0.73	0	0.22	0.11	0.11	0.15
NW-Switzerland	0.49	0	0.17	0.04	0.01	0.24
Mean	0.57	0.03	0.12	0.21	0.15	

Figure 3. *Regional scale*. Effect of distance from the site of origin to the transplant site and home vs. away effects on (a) rosette size, 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) mean individual fitness at a site in *C. vulgaris*. Dependent variables in (a) and (c) were adjusted for the effects of initial size of the rosettes, for the region of the transplant site, for the transplant site and for the site of origin. Populations of origin: ● = NW-Czechia, ▲ = C-Germany, ■ = S-Sweden, □ = NW-Switzerland, ○ = Luxembourg. ns not significant, * p < 0.05, ** p < 0.01, *** p < 0.001.
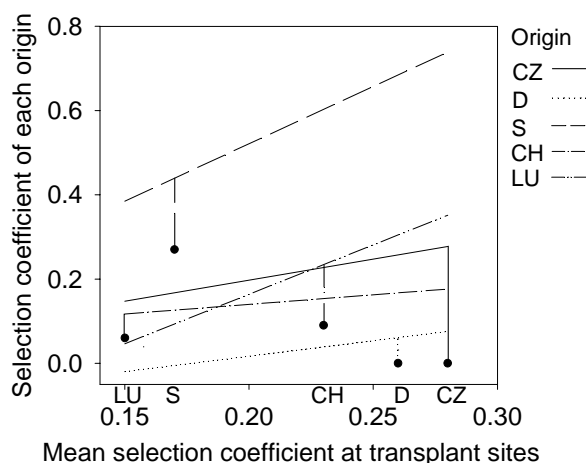
Figure 4. Stability of different origins of *Carlina vulgaris* across five European sites. Lines result from regressions of the selection coefficient of each origin against the mean selection coefficient in each target site. Target sites with a selection coefficient of 0 would indicate that all origins perform equally and that selection does not act. The higher the selection coefficient in a target, the more differently do the different origins perform within that specific target site.

Local transplant experiment

As in the regional transplant experiment, the performance of plants was strongly influenced by their size at the time of transplanting and effects were therefore corrected for initial plant size (Table 8). The region into which the plants 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 the various levels.

Table 7. *Local scale*. Means and standard errors of characters related to plant size in each region. Means were adjusted for the effects of initial size of the rosettes.

	S-Sweden	C-Germany	NW-Czechia
Rosette size [cm]	40.73 ± 1.87	59.58 ± 2.31	109.29 ± 6.40
Number of seeds per plant	14.99 ± 3.24	21.17 ± 1.10	74.62 ± 6.06
Biomass, flowering plants [g]	0.48 ± 0.13	0.82 ± 0.04	2.79 ± 0.25

Most measures of plant size were lowest for plants from Sweden, but in contrast to the regional experiment these effects remained during the whole period of the experiment (Tables 7 and 8). Plants from different populations of origin differed strongly in their performance, indicating genetic effects, but these differences were mostly not attributable to the size of the population of origin.

Table 8. *Local scale*. Effects of transplant region, transplant site within region, population of origin, size of the population of origin and interactions among transplant site and population of origin (subdivided into a home vs. away contrast and a contrast of geographical distance) on life-history traits of *Carlina vulgaris*. 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. Also included in the model is the effect of the initial size of the seedlings at the time of transplanting. Arrows indicate the direction of (marginally) significant effects ($\uparrow$, positive effect). + $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	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
Initial size	552.10 ***	202.40 ***	2.25	174.93 ***	50.02	14.26 ***	0.25
Region	23.32 ***	1.66	1.17	1.53	7.65*	80.85 ***	0.36
Site	1.12	7.20 ***	8.66***	9.66 ***	2.12+	0.37	6.02 ***
Plot	4.88 ***	0.46	2.56***	3.07 ***	1.87**	4.28 ***	4.45 ***
Population size (log)	4.76 $\uparrow$+	0.35	0.48	0.01	0.77	0.93	1.52
Origin	2.73 **	1.04	1.60	4.09 ***	3.44**	10.95 ***	3.39 **
Origin * site	0.81	0.12	1.31	0.68	2.57***	0.47	1.91 **
Home vs. away	1.31	0.13	0.65	0.32	0.17	0.16	1.22
Distance	0.44	0.08	0.03	0.74	0.08	0.01	0.95
Res. effect	0.70	0.12	1.15	0.66	2.55***	0.52	2.04 **
Origin x plot	1.12	10.26 ***	0.87	1.07	1.12	1.26	0.80

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 12–37% of the variation in continuous traits, but due to low statistical power (only 12 populations) only the effect of population size on rosette size after 16 months was marginally significant (Table 8, Fig. 5a).
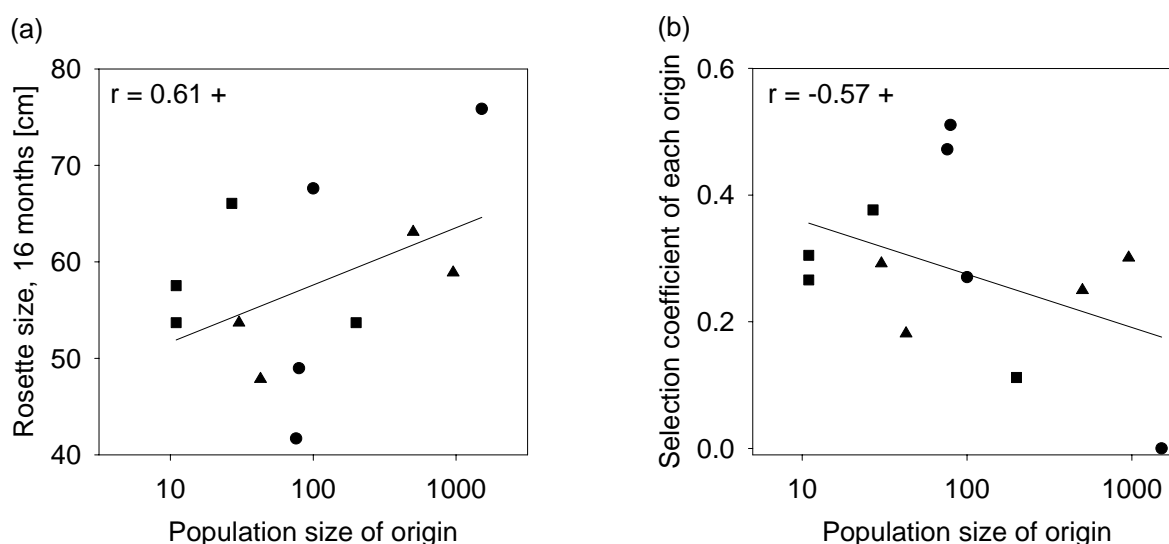

Figure 5. *Local scale*. Effect of the size of the population of origin (a) on rosette size, 16 months after transplanting and (b) on the selection coefficient of individual fitness at a site in *C. vulgaris*. Dependent variable in (a) was adjusted for the effects of initial size of the rosettes, for the transplant site and plot within site; dependent variable in (b) was adjusted for the effect of the mean selection coefficient at each target. ● = NW-Czechia, ▲ = C-Germany, ■ = S-Sweden. + p < 0.10.

The biomass of non flowering plants in the third year and the individual fitness of plants at the specific sites varied depending on their origin (significant origin by site interaction in Table 8). These differences among plants were not due to the geographical distance between home and transplant site, and plants did not grow better at their home site than at other (away) sites. Moreover, the different performance of plants could also 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 did significantly correlate with plant performance (all $r < 0.24$, $p > 0.10$, $n = 48$). Neither did the response of plants to the conditions at different sites depend on the size of the population of origin (no significant interaction between population size and transplant site; $F_{9,17} < 1.52$, $p > 0.22$).

Table 9. *Local scale*. Selection coefficients of individual fitness for *Carlina vulgaris* that were reciprocally transplanted between sites within three regions in Europe. Selection coefficients potentially range between 0 and 1. A value of 0 indicates that plants from the respective origin had the highest individual fitness at a site. Values in boldface indicate that plants from that origin performed best at their home site.

Transplant site			Site of origin			
		1	2	3	4	Mean
NW-Czechia	1	**0**	0.70	0.49	0.17	0.34
	2	0	0.61	0.71	0.36	0.42
	3	0	0.56	0.76	0.50	0.45
	4	0	0.52	0.28	0.39	0.23
	Mean	0	0.60	0.56	0.36	
C-Germany	1	0.03	0.45	0	0.45	0.21
	2	0	0.24	0.05	0.25	0.13
	3	0.24	0.16	0.30	0	0.18
	4	0.37	0	0.03	0.21	0.15
	Mean	0.16	0.21	0.09	0.20	
S-Sweden	1	0.08	0.10	0	0.48	0.17
	2	0.76	0.63	0	0.62	0.50
	3	0.38	0.25	0.17	0	0.20
	4	0	0.08	0.29	0.41	0.20
	Mean	0.31	0.27	0.11	0.38	

Strength of selection varied among regions and was strongest in Czechia (mean: 0.36) and weakest in Germany (mean: 0.16), indicating that mean differences in fitness among plants from different origins at a site were largest in Czechia (Tables 9 and 10). There, plants originating from one particular population (pop. 1) performed best at all sites. Selection against plants from large populations was weaker than against those from small populations (Fig. 5b). However, in contrast to the regional scale there was no significant interaction between the effects of origin and mean selection strength ($F_{11,24} = 1.34$, $p > 0.05$).

DISCUSSION

Our results show that *Carlina vulgaris* plants from all regions of origin may grow over a wide range of latitudes and longitudes within Europe, because plants from no origin failed completely at any of the other sites. In the regional transplant experiment, growth of plants at the northernmost, i.e. the Swedish site, was up to the second growth period lower than at the other sites and plants flowered later, but there were no differences among the sites in overall plant fitness after three years.

With respect to early growth and time to flowering our results were thus similar to those of other reciprocal transplant experiments that have found decreased growth with increasing latitude in the aquatic *Potamogeton pectinatus* (Santamaria *et al*. 2003), and delayed reproduction at northern sites in the monocarpic *Daucus carota* (Lacey 1988). Overall, however, our results indicate that there were no consistent differences among regions in habitat quality, but that plant performance was strongly affected by interactions between the effects of the region of origin and the transplant site.

Table 10. *Regional and local scale*. Effects of the transplant region, the mean selection coefficient at a transplant site (i.e. habitat quality), the site of origin, the size of the population of origin and the interactions among habitat quality and site of origin on selection coefficients in *Carlina vulgaris*. Juveniles were reciprocally transplanted across different geographical scales (regional and local). + $p < 0.1$, * $p < 0.05$, *** $p < 0.001$. The arrow indicates the direction of the effect of population size ($\uparrow$, positive effect).

	Regional scale		Local scale	
	df	F	df	F
Region			2	6.95**
Mean selection coefficient per target	1	6.41**	1	14.19**
Population size			1	3.88$\uparrow$+
Origin	4	21.80***	8	3.54**
Origin x mean selection coefficient per target	4	3.24*	11	1.34
Error	15		24	

In the regional transplant experiment at the European scale there was strong evidence for adaptive genetic differentiation in *C. vulgaris*. Plant performance in the home region was higher than in the other regions, and several components of fitness and individual fitness 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 is to be expected, because with increasing distance both the genetic isolation of populations and environmental differences between sites are likely to increase (Galloway & Fenster 2000). However, very few studies have investigated the relationship between transplant distance and plant fitness. Similarly to our results, performance of the widespread forage plants *Trifolium pratense*, *Dactylis glomerata* and *Plantago lanceolata* decreased continuously with distance to 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 (1000 and 2000 km; Galloway & Fenster 2000). In the Californian shrub *Lotus scoparius*, geographic distance between populations was only

weakly correlated with genetic distance and had little value in predicting plant fitness (Montalvo & 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 a 1000 km suggests that differences in climatic conditions are most likely 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.

The median distance between populations in the local transplant experiment 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 transplantation 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 of the two experiments suggest that local adaptation increases with the geographical distance between populations, but that in the local transplant experiment the geographical distances were too small 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 as in the local transplant experiment with *C. vulgaris* (e.g. references in Linhart & Grant 1996, Nagy & Rice 1997, Gauthier *et al*. 1998, Petit *et al*. 2001; but see Schemske 1984, Rice & Mack 1991, Rapson & Wilson 1988, Platenkamp 1990, Helenurm 1998), but most studies have compared plant performance in specific contrasting environments (Galloway & 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 & Mack 1991).

The large plot effects 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.

C. vulgaris occurs in fragmented populations that are frequently small and isolated, and gene flow by pollen and seed dispersal is probably very restricted. Fragmentation could potentially affect both the performance of *C. vulgaris* and the adaptive differentiation among populations. The fitness of plants from small and isolated populations is often reduced due to increased drift and inbreeding, and reduced genetic diversity (e.g. Fischer & Matthies 1998a, b, Kéry *et al.* 2000, Hooftman *et al.* 2003, Vergeer *et al.* 2003). In *C. vulgaris*, the performance of plants originating from small populations was lower than that of plants from large populations, although due to low statistical power for most traits this effect was not significant.

In small populations, the effects of drift or inbreeding 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 population of origin and transplant site would indicate that populations of different size differ in their degree of local adaptation. This has rarely been studied, but in *Arabis fecunda* local adaptation occurred despite very small effective population sizes (McKay *et al.* 2001). In *C. vulgaris* we found no evidence that population size influenced the degree of local adaptation.

Patterns of genetic differentiation and local adaptation have been found to be fairly consistent across fitness components in some studies (Nagy & Rice 1997, Gauthier *et al.* 1998, Galloway & Fenster 2000), whereas in others local adaptation varied among traits (McGraw & Antonovics 1983, van Groenendael 1985, van Tienderen & van der Toorn 1991) or among years (Rice & 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 already in 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 *Plantago lanceolata*, differences between populations in the survival of adults were more pronounced than differences in the juvenile phase (van Groenendael 1985, van Tienderen & 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 & Primack 1982, van Tienderen & 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 parts 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. Korneck *et al.* 1996, Landolt 1991), 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 (> 200 km), but not at the smaller spatial scale. 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. 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 & Mazer 2003, Vergeer *et al.* 2004). 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.

Effects of pollination distance on reproduction and

offspring performance in the widespread perennial

Hypochoeris radicata: Experiments with plants

from three European regions

with Tanja Reinhold and Diethart Matthies, submitted

ABSTRACT

Variation in the expression of inbreeding and outbreeding depression has been found among different genotypes and among populations, but little is known about large scale geographical patterns (e.g. differences among geographical regions) in the effects of in- and outbreeding within species. We studied the effects of cross-proximity on seed production and offspring performance in the perennial *Hypochoeris radicata* (Asteraceae) from three European regions (in Germany, Czechia and the Netherlands). Crosses were carried out within the same plant (selfing), and between plants from the same seed family, from the same population, from different populations of the same region and from different regions. Independent of the region of origin of the mother plant, seed set and germination after inbreeding and crosses between populations were lower than after within population crosses, indicating inbreeding and outbreeding depression. However, crosses between regions resulted in higher seed set than within population crosses and similar germination. For late traits, the effects of inbreeding and interpopulation crosses differed among regions. For offspring from Czech mother plants survival, flowering and multiplicative fitness were highest after within population crosses. In contrast, for German mother plants offspring resulting from interpopulation crosses had the highest fitness, indicating heterosis, and for Dutch plants there were no clear effects of the pollination treatments. Our results suggest that outbreeding depression does not necessarily increase with interpopulation distance and that the sensitivity of populations to introgression may vary among regions.

INTRODUCTION

Inbreeding through selfing or crossings between close relatives (i.e. biparental inbreeding) may strongly reduce the fitness of plants, i.e. result in inbreeding depression (e.g. Charlesworth & Charlesworth 1987, Barrett & Kohn 1991, Dudash & Fenster 2000). Negative effects of inbreeding have been found on various components of plant fitness, e.g. seed quantity and quality, seed germination, plant growth and survival, flowering and seed production (Levin 1984, Holtsford & Ellstrand 1990, Hauser & Loeschke 1995, Dudash & Fenster 2001, Liu & Koptur 2003). Two mechanisms which may occur simultaneously are thought to be responsible for inbreeding depression. In the case of overdominance heterozygotes have a higher fitness than both types of homozygotes, whereas in the case of partial dominance inbreeding depression is the result of the expression of deleterious recessive alleles at homozygous loci (Charlesworth & Charlesworth 1987, Lynch *et al.* 1995, Byers & Waller 1999, Dudash & Fenster 2000).

Hybridisation between populations may contribute to a recovery from genetic drift or inbreeding depression (Lynch 1991, Hufford & Mazer 2003) and may enhance mating success, in particular in self-incompatible species. Relative to parental fitness, increased offspring vigour (heterosis) after crosses between populations has been reported in a number of studies (Oostermeijer *et al.* 1995, Byers 1998, Fenster & Galloway 2000). It has therefore been suggested to artificially increase gene flow among isolated populations of rare plants, e.g. by introduction of individuals or pollen from different populations (van Treuren *et al.* 1993, Oostermeijer *et al.* 1995, Sheridan & Karowe 2000). However, crosses among populations can also lead to decreased offspring vigour (outbreeding depression), in particular if dispersal is limited and genetic differentiation between populations is high (Waser & Price 1989, Waser 1993). Two mechanisms may cause outbreeding depression. If populations have become adapted to different local conditions by selection, interpopulation hybridisation may result in the dilution of adapted genotypes in the next generation. The other mechanism is the break-up of co-adapted gene-complexes by recombination that enhances the chance of new deleterious gene interactions (Fenster & Dudash 1994, Fenster & Galloway 2000, Hufford & Mazer 2003). This process mainly occurs in the second generation. Outbreeding depression is likely to increase with the geographical or genetic distance between individuals (Waser & Price 1994, Montalvo & Ellstrand 2001) and is higher in interspecific crosses than in intraspecific crosses (Hufford & Mazer 2003). Assuming that both inbreeding and out-breeding depression occur, one could expect an optimal distance for crosses between two

individuals that results in the highest progeny fitness (Waser & Price 1989, 1994, Paschke *et al*. 2002; but see Trame *et al*. 1995). Variation in the expression of inbreeding and outbreeding depression has been found among different genotypes (Helenurm & Schaal 1996, Pico *et al*. 2004a) and among populations (Charlesworth & Charlesworth 1987, Johnston & Schoen 1996, Ferdy *et al*. 2001, Bram 2002), but little is known about large scale geographical patterns (e.g. differences among geographical regions) in the effects of in- and outbreeding within species.

An understanding of the relative magnitudes of inbreeding and outbreeding depression has become increasingly important, because habitats are becoming more and more fragmented and there is large-scale introduction of seed material from other countries in landscape management (Keller *et al*. 2000). Habitat fragmentation leads to reduced size and increased isolation of plant populations. In small populations plant performance may be reduced (inbreeding depression) due to increased mating among relatives (e.g. Ellstrand & Elam 1993, Young *et al*. 1996, Fischer & Matthies 1998a, Paschke *et al*. 2002b). On the other hand, crossings among genetically distant plants are also becoming more frequent, because foreign seed material is used to increase the biodiversity in intensively managed farmlands (Keller *et al*. 2000). Genetic introgression may then result in outbreeding depression, because the offspring is not adapted to the local conditions or because of hybrid breakdown (Hufford & Mazer 2003). For instance, negative effects of crosses between plants from Switzerland and Germany (but not from two other countries) were found for *Agrostemma githago* and *Papaver rhoeas* (Keller *et al*. 2000). The possibility of outbreeding depression has also to be considered when seeds or plants from different populations are used to reinforce populations of endangered species (Hodder & Bullock 1997, van Andel 1998, van Groenendael *et al*. 1998). However, few studies have investigated the effects of large-scale interpopulation hybridisation on seed set and offspring performance (Fenster & Galloway 2000, Montalvo & Ellstrand 2001; see reviews by Edmands 2002, Hufford & Mazer 2003).

The strength of in- and outbreeding depression depends on particular life-history traits of each species which influence the gene flow among populations, e.g. the breeding system, dispersal ability and longevity (Hamrick *et al*. 1979, Montalvo *et al*. 1997). Outbreeding species are more prone to inbreeding depression than mainly selfing species (Husband & Schemske 1996), because high selfing rates result in a higher efficiency of selection against recessive deleterious alleles. Common species are more sensitive than naturally rare species, because rare species might have a temporal advantage of adaptation to low gene flow among populations (Huenneke 1991). Highly selfing plants and those with low interpopulation gene

flow are expected to be more sensitive against outbreeding depression than outbreeders and well-dispersed species (Waser 1993, Dudash & Fenster 2000).

We studied the effects of crossing distance on seed production and offspring fitness in the widespread *Hypochoeris radicata* L. (Asteraceae) from several populations from three European regions. *H. radicata* is considered to be largely self-incompatible and strongly reduced seed set after selfing has been found in two populations from the Netherlands (Pico *et al*. 2004b). We raised plants from several populations from Germany, Czechia and the Netherlands in a common garden, hand-pollinated them and analysed effects of crossing distance on seed set, seed germination and offspring survival, growth and flowering. Crosses were carried out within plants (selfing), within seed families, within populations, between populations within regions (mean crossing distance 43 km) and between regions (mean crossing distance 445 km).

We addressed the following questions: (1) Do inbreeding and interpopulation crosses reduce seed set and offspring performance in *H. radicata*? (2) Do plants that originate from different geographical regions vary in their response to different pollination treatments?

MATERIAL AND METHODS

Study species

Hypochoeris radicata is a polycarpic perennial that is able to grow clonally by means of side rosettes (Turkington & Aarssen 1983, de Kroon *et al*. 1987). A flower head consists of 50 to 100 single yellow florets that are considered to be self-incompatible (Pico *et al*. 2004b). The main flowering season is from the beginning of June until September (Grime *et al*. 1988). Main pollinators are bees (Aphidae), hoverflies (Syrphidae) and bumblebees (Bombus). The flower heads produce achenes (from here on called seeds) that have a pappus and that are well dispersed (Soons & Heil 2002). *H. radicata* is native to Europe up to 62 degree North, but today has an almost cosmopolitan distribution; the species is a good colonizer and is considered a weed in America (Turkington & Aarssen 1983). *H. radicata* mainly grows in pastures, but also in lawns and prefers nutrient-poor, slightly acidic soils (Grime *et al*. 1988).

Pollination experiments

In summer 2000, seeds of *H. radicata* were collected in three European regions: Western Bohemia in Czechia (12.817-15.867 E, 50.037-50.167 N), Northern Hesse in Germany

(9.725-9.884 E, 51.164-51.366 N) and Salland in the Netherlands (5.958-6.110 E, 52.332-52.536 N). From five large populations (> 200 flowering plants) in each region one fruit head of each of 20 plants was sampled. The sampled plants were growing at least 5 m apart from each other. In March 2001, seeds were germinated and the plants were grown in the Botanical Garden of the University of Marburg, Germany.

In July 2001, just before the first florets opened, we covered one flower head per plant with a bag (size: 10 x 10 cm) of fine-mesh nylon (mesh size < 0.5 mm) to exclude pollinators. Each bag was fastened to a bamboo stick and the plants were checked daily for flowering. Once florets were open, hand pollinations were carried out by carefully rubbing two flower heads against each other. We used two heads of the same plant for self-pollinations and two heads of different plants for cross-pollinations. Thus, florets from each bagged flower head served both as pollen donors and as pollen acceptors. Because not all florets within one flower head open simultaneously, we repeated the pollination treatment with each specific pair of flower heads at least once, usually twice, one to three days after the previous treatment depending on weather conditions to saturate the stigmas with pollen.

We investigated the effects of five different crossing distances: (1) Self-pollination (selfing) was carried out using two flower heads from the same individual. Two individuals from different families were used in each population. (2) Within family crosses (WFC) were carried out with two different individuals originating from the same seed family (half sibs). From each population one to three seed families were used. (3) Within population crosses (WPC) were carried out using five pairs of individuals from different seed families within a population. (4) Between population crosses (BPC) were carried out using plants from two different populations within each region. Four different individuals from each population were pollinated with pollen from one individual from each of the other four populations within each region. The mean distance among populations within regions was 43 km. (5) Between country crosses (BCC) were carried out between plants of different regions with a mean distance of 445 km. Altogether we carried out 106 pollinations using 187 individuals. Flower heads remained bagged until the mature seeds were harvested in August 2001. The ripe fruit heads were dried at room temperature and the number of ripe dark seeds was counted. Seed set was calculated as the proportion of florets that developed ripe seeds. Seeds were then stored at 8 °C.

Offspring performance

At the end of April 2002, from each flower head 30 randomly selected ripe seeds, or all seeds, if less had been produced, were sown into commercial nutrient-poor garden soil (TKS 1, Floragard GmbH, Oldenburg, Germany, mixed with 10% sand) in plastic pots (9 x 9 x 9.5 cm); the pots were then kept in a greenhouse at 15-20 °C. Germination was recorded four weeks after sowing and at the same time the pots were placed into flower beds in the Botanical Garden of the University of Marburg. At the end of June two juveniles per pot were randomly chosen and transplanted individually into pots filled with the same type of soil. The resulting 297 plants were watered and their position randomised regularly throughout the summer. In September 2001, survival, flowering and the number of flower heads were recorded for each plant. The above-ground parts were harvested, dried for 12 hours at 80 °C and weighed.

Data Analysis

A multiplicative fitness function was calculated as the product of the proportion of seeds that germinated per flower head, the probability of survival of the seedlings of a flower head until five months after transplanting, and the mean above-ground biomass of the progeny of a flower head after five months. It thus represents the mean biomass produced per seed.

Continuous traits, except for seed set and germination were analysed by general linear models. Because of the hierarchical design, Type I sums of squares were used. The region of origin and the pollination distance were treated as fixed factors, whereas the population was treated as a random factor. According to the rules for the analysis of mixed models (Zar 1996), the effect of the region of origin of the maternal plants was tested against the variation among populations. The effects of pollination distance and their interactions with the region of origin were tested against the population by treatment interaction. The effects of population and the treatment by population interaction were tested against the variation among the plant pairs used for the pollination treatments. To analyse the form of the response of the studied traits to pollination distance, effects were partitioned into linear, quadratic and cubic contrasts.

Binomial variables like survival and flowering and seed set and germination were analysed by analyses of deviance. Mean deviances due to a factor were divided by their appropriate error mean deviances, analogous to the calculation of F-values in ordinary analysis of variance (Francis *et al*. 1993). To adjust for maternal effects, the biomass of the

plants that were pollen receptors was used as a covariate in the analyses of seed set and seed mass and the mean seed mass per seed family was used as a covariate in the analysis of offspring performance. In preliminary analyses the number of pollinations carried out with each plant pair was in no case significant and results are therefore presented without this covariate. Data were analysed with SPSS 11.0 (release 11.0; SPSS Chicago, Illinois, USA).

RESULTS

Effects on early traits

Most of the plants (77.5%) produced seeds. Although *H. radicata* is considered to be self-incompatible, the proportion of plants that produced at least one seed by selfing was considerable (69.3%) and not significantly lower than that by outcrossing (78.8%, $Chi^2 = 1.12$, $p > 0.05$). Crossing distance strongly influenced the early traits seed set, seed mass and germination. Particularly strong was the difference between selfed and outcrossed plants.

Table 1. Results of analyses of deviance (seed set, germination) or variance (seed mass) of the effects of region and population of origin of the mother plants, pollination treatment and their interactions on seed production, seed mass and germination of offspring. To analyse the form of the relationship between pollination distance and the response variables, the treatment effects were partitioned into polynomial contrasts. + $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Seed set			Seed mass			Germination		
Source of variation	df	MD	Quasi-F	df	MS	F	df	MD	Quasi-F
Biomass of mother plant	1	25.12	0.54	1	0.53	6.39*			
Seed mass of mother plant							1	2088.13	29.56***
Region of mother plant	2	37.81	1.41	2	0.07	0.38	2	569.19	2.85+
Population of mother plant	12	26.87	0.69	12	0.18	0.78	12	199.69	3.21**
Pollination distance	4	283.09	9.79***	4	0.26	2.86*	4	310.39	8.34***
Linear term	1	495.08	17.11***	1	0.59	6.39*	1	244.93	6.58*
Quadratic term	1	274.08	9.47**	1	0.38	4.14*	1	138.20	3.71+
Cubic term	1	351.04	12.13**	1	0.04	0.48	1	449.58	12.08**
Region * poll. distance	8	32.04	1.07	8	0.10	0.88	8	21.49	0.58
Region * linear term	2	35.71	1.23	2	0.30	3.32*	2	12.31	0.33
Region * quadratic term	2	53.43	1.85	2	0.06	0.64	2	42.70	1.15
Region * cubic term	2	21.80	0.75	2	0.04	0.47	2	30.40	0.82
Population * poll. distance	43	28.93	0.75	37	0.09	0.88	35	37.21	0.60
Parental plant pair	53	38.81	0.83	37	0.10	1.22	37	62.18	1.33
Residual	31	46.53		19	0.09		18	70.63	

Seed set after selfing was 91% lower than after within population crosses, and germination was 90% lower after selfing (Table 1, Fig. 1). In contrast, the mass of seeds resulting from selfing was higher than that resulting from outcrossing, but this was due to the fact that individual seed mass decreased with the number of seeds per flower head (n = 125, r = -0.30, p = 0.001), indicating a trade-off between seed mass and seed number within flower heads.
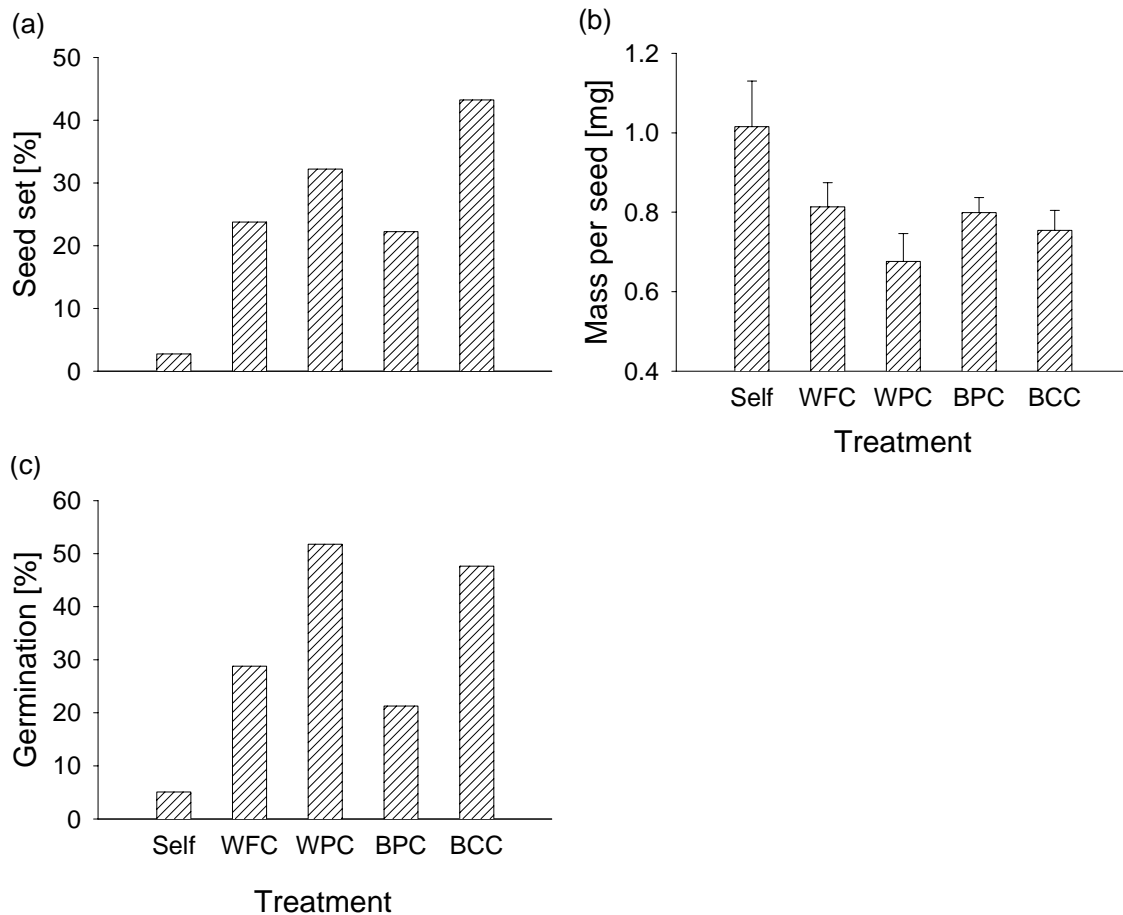

Figure 1. (a) Seed set, (b) seed mass and (c) germination after different pollination treatments (self: selfing, WFC: within family crosses, WPC: within population crosses, BPC: between population crosses, BCC: between country crosses).

Seed set and germination were lower after WFC (26 and 44%, respectively) and BPC (31 and 59%, respectively) than after WPC. After BCC seed set increased 34% compared to WPC, whereas germination was slightly reduced (8%). Moreover, germination differed significantly among seeds of mother plants from the different regions (Table 1). Seeds from German mother plants had the highest, those from Czech intermediate and those from Dutch mother plants the lowest germination (47% > 38% > 15%) pooled over all five pollination treatments. Those few Dutch seeds that resulted from selfing did not germinate at all. Germination

strongly increased with seed size (r = 0.45, p < 0.001, n = 118) which in turn was influenced by the size of the mother plant, suggesting maternal effects.

There was no interaction between the effects of pollination distance and region on the early traits (Table 1), indicating that the effects of the pollination treatments were similar for mother plants from different regions of origin.

Effects on late traits

In contrast to the effects of early traits, there were no consistent effects of the pollination treatments on late traits; instead the treatment effects depended on the region of origin of the mother plant (see significant interactions among effects of pollination distance and region, Table 2, Figs. 2 and 3). For offspring from Czech mother plants most measures of performance were highest for intermediate crossing distances. Survival, flowering probability and total biomass were highest for offspring from WPC, whereas reproduction was highest for offspring from WFC. For offspring from mother plants that originated from Germany or the Netherlands there was no consistent pattern for the reaction of different traits to the pollination treatments. However, the survival, flowering probability and biomass of offspring from German mother plants was highest for offspring resulting from between country crosses (Fig. 2). Surprisingly, the survival, reproduction and biomass of offspring from selfed German plants was similar to that of offspring from within population crosses.

The various life-history traits were combined to obtain a measure of multiplicative fitness. Offspring from the three countries varied in the pattern of their response to the pollination treatments (Fig. 3). For offspring from Czech mother plants, multiplicative fitness was reduced by 82% after selfing and 45% after WFC compared to WPC, and that of offspring from interpopulation crosses by 78% after BPC and 80% after BCC, indicating both inbreeding and outbreeding depression. In contrast, for offspring from German mother plants, plants resulting from interpopulation crosses had the highest fitness, indicating heterosis effects. For offspring from Dutch plants, there were no clear effects of the pollination treatments. Of the late traits, only flowering probability and total biomass were still positively influenced by seed mass.

Table 2. Results of analyses of variance (continuous traits) or deviance (survival, flowering) of the effects of region and population of origin, and distance between crossing partners on fitness-related traits of offspring grown for five months in a common garden. To analyse the form of the relationship between pollination distance and the response variables, the treatment effects were partitioned into polynomial contrasts. $+\ p < 0.10$, $*\ p < 0.05$, $**\ p < 0.01$.

| Source of variation	Survival until autumn			Flowering probability			Number of flower heads			Total biomass			Biomass produced per seed		
	df	MD	Quasi-F	df	MD	Quasi-F	df	MS	F	df	MS	F	df	MS	F
Seed mass of mother plant	1	0.33	0.16	1	4.74	4.56+	1	8.10	0.31	1	557.1	6.55*	1	313.14	12.80**
Region of mother plant	2	0.44	0.32	2	6.83	7.61**	2	43.76	1.70	2	2146.2	3.26+	2	431.81	6.56*
Population of mother plant	12	1.36	0.88	12	0.90	0.97	12	25.76	2.04+	12	659.2	1.49	12	65.85	0.97
Pollination distance	4	0.28	0.18	4	0.42	0.51	4	10.56	0.74	4	75.6	0.18	4	94.46	1.46
Linear term	1	0.16	0.11	1	0.36	0.44	1	14.92	1.03	1	127.3	0.35	1	144.60	2.23
Quadratic term	1	0.34	0.22	1	0.84	1.03	1	0.01	0.00	1	24.2	0.07	1	8.53	0.13
Cubic term	1	0.28	0.18	1	0.12	0.14	1	24.59	1.70	1	96.6	0.27	1	63.05	0.97
Region * pollination distance	7	3.29	2.13*	7	1.97	2.40	7	63.92	4.43**	7	1311.6	3.65**	8	129.62	2.00+
Region * linear term	2	7.77	5.04**	2	4.14	5.06*	2	59.12	4.09*	2	1807.8	5.03*	2	304.10	4.70*
Region * quadratic term	2	3.28	2.13	2	2.51	3.07+	2	32.53	2.25	2	979.2	2.72+	2	137.81	2.13
Region * cubic term	2	0.47	0.30	2	0.24	0.29	2	112.10	7.76**	2	1748.2	4.86*	2	34.07	0.53
Population * poll. distance	22	1.54	0.95	22	0.82	0.88	21	14.44	1.15	21	359.6	0.81	33	64.73	0.95
Parental plant pair	18	1.55	0.75	18	0.93	0.89	15	12.61	0.48	15	442.7	5.21*	31	68.07	2.78*
Residual	8	2.07		8	1.04		6	26.09		6	85.0		14	24.45	
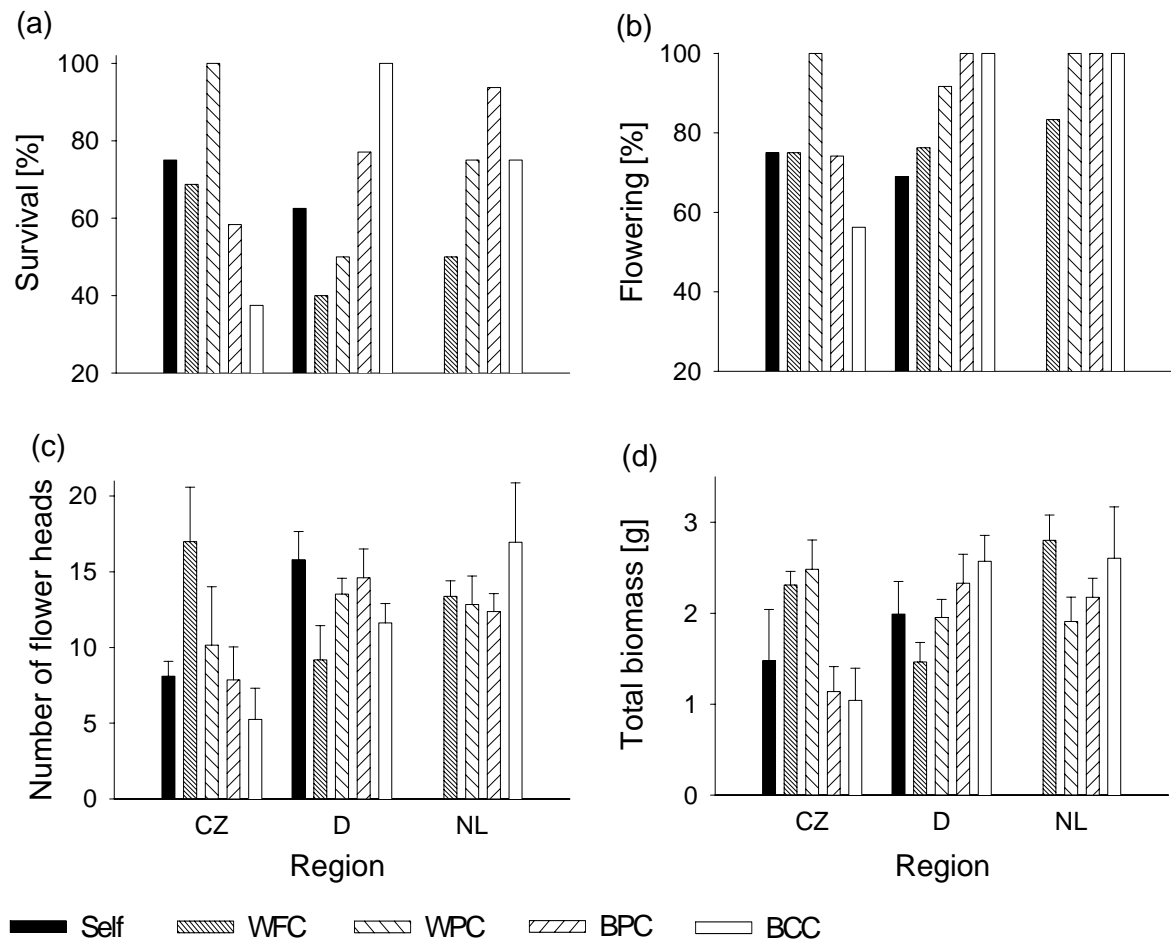
Figure 2. Performance of offspring resulting from different pollination treatments (self: selfing, WFC: within family crosses, WPC: within population crosses, BPC: between population crosses, BCC: between country cross). The mother plants originated from different European regions (CZ = Czechia, D = Germany, NL = The Netherlands): (a) percentage survival, (b) percentage of flowering plants, (c) number of flower heads, (d) total biomass. Dutch seedlings resulting from selfing did not germinate.
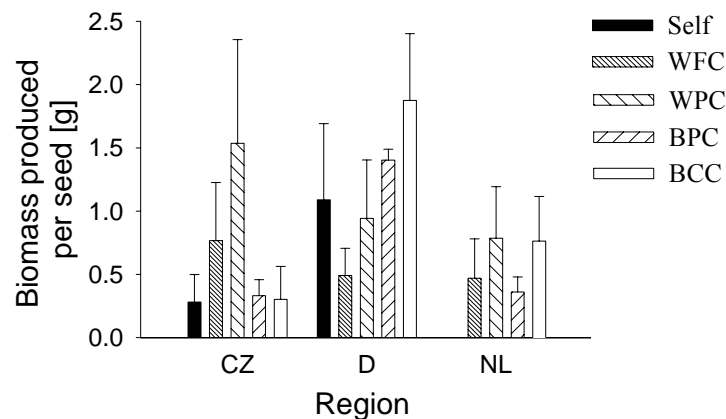

Figure 3. The effects of different pollination treatments on biomass produced per seed (self: selfing, WFC: within family crosses, WPC: within population crosses, BPC: between population crosses, BCC: between country cross). The mother plants originated from different European regions (CZ = Czechia, D = Germany, NL = The Netherlands).

DISCUSSION

The current study was designed to investigate the effects of both strong inbreeding and out-breeding (interpopulation crosses) on reproduction and offspring performance in *H. radicata.* Pollination distance strongly affected early traits like seed set, seed mass and germination irrespective of the region of origin of the mother plant. In contrast, the effects of pollination distance on late traits varied among plants from the three regions of origin.

Effects of inbreeding

Seed set was 91% lower after selfing than after crossings within populations. The very low seed set of selfed flowers is probably the result of self-incompatibility, because it is likely that *H. radicata,* as other Asteraceae (Richards 1997), has a multiallelic sporophytic self-incompatibility system. However, increased seed abortion as a result of inbreeding may also have contributed to the very low seed set (Pico *et al*. 2004b). Self-incompatibility was not strict, because 69% of selfed plants produced at least one seed. A partial breakdown of the self-incompatibility system has been found in other plant species, in particular Asteraceae (e.g. Reinartz & Les 1994, Byers 1995, Stephenson *et al*. 2000, Colling & Matthies 2004) and also in two Dutch populations of *H. radicata* (Pico *et al*. 2004b). The sporophytic self-incompatibility system of the Asteraceae may not only prevent seed production by selfing, but also by crosses between closely related individuals (Richards 1997). However, this was not the case in *H. radicata,* because crosses between plants from the same seed family resulted in much higher seed set than selfing.

Effects of inbreeding on germination were similar to those on seed set. Germination of seeds resulting from within family crosses and particularly from selfing was much lower than that of seeds resulting from within population crosses (-26% and -90%, respectively), indicating strong negative effects of inbreeding. In contrast, Pico *et al*. (2004b) found no effects of selfing on seed germination in plants from two populations from the Netherlands. Inbreeding depression may vary among populations (Johnston & Schoen 1996, Bram 2002), which might explain the contrasting results of our study and that of Pico *et al*. (2004b). The results of other studies on the strength of inbreeding depression at the stage of germination have varied. Among 31 outcrossing species, the mean inbreeding depression at the germination stage was only 12% (Husband & Schemske 1996). Several later studies also found no effect of inbreeding on germination (e.g. Groom & Preuninger 2000, Galloway *et al*. 2003, Pico *et al*. 2003, Colling & Matthies 2004), whereas others found relatively weak effects (e.g. Dudash & Fenster 2001, Liu & Koptur 2003, Pico *et al*. 2004a). The magnitude

of early inbreeding depression observed in our study was thus much higher than in other studies. This could be due to the incompatibility system of *H. radicata* that normally prevents inbreeding, but also due to effective purging of deleterious alleles.

For later traits, however, there was no overall effect of inbreeding in *H. radicata*. Instead, effects of inbreeding varied among plants from the three regions. In most outbreeding species, inbreeding depression is high for both early and late traits (Husband & Schemske 1996, Pico *et al*. 2004a). In our study a similar pattern was found only for Czech plants. Variation in inbreeding depression has previously been reported for different genotypes (Helenurm & Schaal 1996, Bram 2002, Pico *et al*. 2004a) and different populations (Johnston & Schoen 1996, Ferdy *et al*. 2001, Bram 2002; but see Groom & Preuninger 2000), but variation at a larger geographical scale has not been studied.

Inbreeding depression in early traits is thought to be caused by recessive deleterious alleles (Holtsford & Ellstrand 1990, Carr *et al*. 1997), whereas late acting inbreeding depression is due to the accumulation of mildly deleterious mutations (Husband & Schemske 1996). Our results would therefore suggest that deleterious recessive alleles are of similar frequency in the three study regions, whereas the accumulation of deleterious mutations varies among the regions.

Many studies have found maternal effects on the early development of offspring (Roach & Wulff 1987, Schmitt *et al*. 1992, Schmid & Dolt 1994, Galloway 2001, Pico *et al*. 2003). However, these maternal effects usually decrease as development proceeds. In *H. radicata,* the biomass of the mother plant influenced mean seed size and there were significant maternal effects mediated through seed size not only on early traits like germination, but also on biomass at time of flowering and multiplicative fitness. This could be due to the very high variation of seed mass in our study (0.5-2.5 mg).

Effects of interpopulation crosses

After crossings between populations both a reduction (outbreeding depression) and an increase in plant fitness (heterosis) have been observed. Outbreeding depression after interpopulation crosses has been found in several species (Lynch 1991, Waser 1993, Waser & Price 1994, Fischer & Matthies 1997, Keller *et al*. 2000, Paschke *et al*. 2002b), whereas in other species an increase of progeny fitness in the first generation has been observed (Oostermeijer *et al*. 1995, Fenster & Galloway 2000, Sheridan & Karowe 2000, Luijten *et al*. 2002). In *H. radicata,* the effect of interpopulation crosses depended on the distance between the populations. Compared to crosses within populations, seed set was 34% higher after

crosses between populations from different regions indicating heterosis, whereas it was 31% lower after crosses between populations within regions indicating outbreeding depression. Outbreeding depression was also found for germination after crosses between populations within regions (-59%). For late traits, similar to the effects of inbreeding, effects of inter-population crosses differed among regions. In offspring from Czech mother plants most fitness traits were lower after between than within population crosses, whereas in offspring from German mother plants fitness of offspring resulting from both types of interpopulation crosses was higher, indicating heterosis.

Outbreeding depression can be the result of the dilution of locally adapted genotypes or hybrid breakdown (Fenster & Dudash 1994, Fenster & Galloway 2000, Hufford & Mazer 2003). Because we studied offspring performance of *H*. *radicata* in a common garden, out-breeding depression was most likely the result of the disruption of coadapted gene complexes that performed well in the internal genetic environment.

Heterosis is often observed in the first generation after interpopulation crosses, but may be followed by hybrid breakdown, i.e. a decrease in offspring fitness, in the second generation (Keller *et al*. 2000, Fenster & Galloway 2000). The observed heterosis effects in *H*. *radicata* might therefore be transient. From our study we cannot predict the long-term consequences of large-distance outcrossing, because we only investigated the F1 generation. Heterosis and outbreeding depression even may occur simultaneously when the masking of deleterious re-cessive alleles and a disruption of coadapted gene complexes occur simultaneously (Lynch 1991, Fenster & Galloway 2000).

Previous studies that investigated the effects of interpopulation crosses over a range of distances have found increasing outbreeding depression with increasing genetic and environ-mental distance between populations (Montalvo & Ellstrand 2001) or outbreeding depression at very large crossing distances (Fenster & Galloway 2000). Similar effects of crossing distance were found in *H*. *radicata* only for late traits in offspring from Czech mother plants. This suggests that the extent of differentiation among populations differs between the three study regions and is highest in Czechia.

Conclusions

We found both inbreeding and outbreeding depression in a widespread, well-dispersed, common plant. This suggests that increased crossings between close relatives, e.g. due to fragmentation, may reduce offspring performance, and that introgression from introduced seed material could potentially have negative consequences for native plant populations.

However, in *H. radicata* there was no consistent pattern of the effects of geographical distance between crossed populations. Our results suggest that crosses between populations which are furthest away from each other do not necessarily result in the strongest outbreeding depression, and that the sensitivity of populations to introgression may vary between regions.

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SUMMARY

ZUSAMMENFASSUNG

The ongoing anthropogenic impact on our landscape, e.g. by intensification of agricultural use and abandonment of extensively used farmland, strongly affects the persistence of many plant and animal species and populations. Many formerly connected habitats were destroyed or became fragmented. As a consequence many populations became extinct and remnant populations became smaller in size and more isolated. These small and isolated populations are at a higher risk of extinction because they are more sensitive to demographic, environmental and genetic stochasticity. Genetic stochasticity, i.e. genetic drift, and inbreeding in small and isolated populations might lead to reduced individual fitness in the short term. In the long term the ability to react to changing environmental conditions might decrease. Moreover, the environmental conditions in remnant habitats might be of lower quality, e.g. due to increasing edge effects.

As species become restricted to remnant habitats, effective management for long-term conservation requires a quantitative understanding of the effects of habitat fragmentation on population viability. Most recent studies of the effects of habitat fragmentation were carried out at relatively small geographical scales. In this thesis I present four studies of the effects of habitat fragmentation on plant performance that investigate offspring performance, quantitative genetic variation and local adaptation in populations at a large geographical scale. Populations were studied in several European regions (in Sweden, The Netherlands, Germany, Britain, Czech Republic, Luxembourg and Switzerland). The target species *Carlina vulgaris* L. and *Hypochoeris radicata* L. have contrasting life histories with respect to dispersal ability and the frequency of flowering, but both species are widespread in species-rich semi-natural grasslands throughout Europe. Species-rich grasslands have dramatically declined in number and size in the last decades and are thus an excellent system to study effects of habitat fragmentation.

Individuals in small and isolated populations may have a lower performance than plants from large and non-isolated populations and they may be exposed to more unfavourable habitat conditions. In Chapter 2 I study the effects of habitat conditions and population size and isolation on the performance of *C. vulgaris* from 74 populations in seven European regions, both in natural populations and in a common garden. In the common garden several measures of performance were reduced in plants from small populations and plant size decreased with increasing isolation of the populations, indicating genetic drift and inbreeding depression in small and isolated populations. In the field, only seed set was reduced in small populations. Environmental variables (i.e. geographical position and species composition as indirect measures of habitat conditions) explained most of the variation in plant size among

populations in the field. The results suggest that reproduction and offspring performance may be reduced in small populations of *C. vulgaris* across Europe due to genetic deterioration, and because pollen quality and quantity might be reduced. However, in field populations environmental conditions have a stronger effect on the performance of the plant.

Variation in quantitative characters is the basis for the adaptive evolution of populations and thus important for their long-term survival. In Chapter 3 I analyse the variation in several quantitative genetic traits in offspring grown in a common garden from 74 populations of *Carlina vulgaris* from seven geographical regions and 32 populations of *Hypochoeris radicata* from three geographical regions. In both species, most traits varied considerably among seed families within populations, among populations within regions and among regions. The overall proportion of phenotypic genetic variation among populations was higher in *C. vulgaris* (75.8 %) than in *H. radicata* (50.7 %), suggesting less gene flow between populations in the poorly dispersed *C. vulgaris*. In both species genetic distances for quantitative traits were not related to geographical distances between populations within regions. However, the variation in morphological traits at the population level reflected the differences among regions. The results suggest that at the small scale genetic drift or environmental differences that are not related to geographical distances drive population differentiation, whereas at the large scale selective forces closely related to geographical distances are most important for population differentiation. In *H. radicata*, but not in *C. vulgaris*, genetic variation within populations as measured by coefficients of variation among seed families increased with distance to the nearest population for some traits indicating instability of quantitative traits in isolated populations.

Variation in environmental conditions can lead to local adaptation. In Chapter 4 population differentiation and local adaptation in *Carlina vulgaris* is studied at the European scale. Reciprocal transplant experiments were carried out among (regional scale) and within (local scale) five European regions and several performance measures were recorded over three growing periods. Only at the regional scale, the individual fitness of *C. vulgaris* and several other measures of the plant performance were highest if plants grew in their home region. Furthermore, performance of plants decreased with increasing transplant distance that in turn was positively correlated with climatic differences or differences in edaphic conditions. At the local scale, genotype x environment interactions were far less pronounced and were not related to geographical distance or environmental distance between the population of origin and the transplant site. 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 (> 200 km), but not at small scales. Thus, patterns of local adaptation have to be taken into account for the efficient preservation of genetic resources.

Variation in the expression of inbreeding and outbreeding depression has been found in several studies, but little is known about large scale geographical patterns (e.g. differences among geographical regions) in the effects of in- and outbreeding within species. In Chapter 5 I present a study on the effects of cross-proximity on seed production and offspring performance of *Hypochoeris radicata* from Germany, Czechia and The Netherlands. Crosses were carried out within plants (selfing), and between plants from the same seed family, from the same population, from different populations of the same region and from different regions. Seed set and germination after inbreeding and crosses between populations were lower than after within population crosses, indicating inbreeding and outbreeding depression. However, crosses between regions resulted in higher seed set than within population crosses. For late traits, there was regional variation in the effects of inbreeding and interpopulation crosses. For offspring from Czech mother plants in- and outbreeding depression was found in several fitness-related traits. In contrast, for German mother plants, offspring resulting from interpopulation crosses had the highest fitness, indicating heterosis, and for Dutch plants the pollination treatments had no clear effects. Our results suggest that outbreeding depression does not necessarily increase with interpopulation distance and that the sensitivity of populations to introgression may vary among regions.

In den letzten Jahrzehnten wurden viele Lebensräume durch Intensivierung der Landnutzung und auch durch Nutzungsaufgabe zerstört, die verbliebenen Resthabitate verkleinert und isoliert. Pflanzenpopulationen in solchen fragmentierten Habitaten sind einem erhöhten Aussterberisiko ausgesetzt, weil zufällig schwankende Umweltbedingungen sowie demographische und genetische Zufallsereignisse (genetische Drift) einen größeren Einfluss haben als in großen, nicht fragmentierten Populationen. Zudem gibt es in kleinen Populationen eine höhere Wahrscheinlichkeit von Inzucht. Starke genetische Drift und Inzucht können zu verminderter Variabilität einer Population und durch die Expression schädlicher rezessiver Allele zu einem Verlust an Fitness der Individuen führen. Langfristig kann eine verminderte genetische Variabilität, die das Evolutionspotenzial einer Population bildet, dazu führen, dass sich eine Population nicht mehr ausreichend an veränderte Umweltbedingungen anpassen kann. Gerade in fragmentierten Habitaten sind die Lebensbedingungen aber oftmals verändert, meist verschlechtert, z. B. durch den Eintrag vom Bioziden und Nährstoffen aus umliegenden Nutzflächen.

Für den dauerhaften Schutz von kleinen Populationen in Resthabitaten müssen zunächst die Auswirkungen der Habitatfragmentierung quantifiziert werden. Die meisten der insgesamt wenigen Studien zu diesem Thema fanden auf relativ kleinräumiger Skala statt. In dieser Arbeit stelle ich vier Studien vor, die den Einfluss von Habitatfragmentierung auf Pflanzen auf großräumiger Skala untersuchen, und zwar hinsichtlich der Entwicklung der Nachkommen, ihrer quantitativ-genetischen Variation und ihrer lokalen Anpassung. Dazu wurden Populationen von *Carlina vulgaris* und *Hypochoeris radicata* aus verschiedenen europäischen Regionen (*Carlina*: in Schweden, Holland, Deutschland, Großbritannien, Tschechien, Luxemburg, Schweiz; *Hypochoeris*: in Holland, Deutschland, Tschechien) untersucht. Beide Arten sind über ganz Europa verbreitet und wachsen in artenreichen, halbnatürlichen Grasländern, die in den letzten Jahrzehnten stark dezimiert wurden und daher ein gut geeignetes System zur Untersuchung von Effekten der Habitatfragmentierung sind. Die beiden untersuchten Arten unterscheiden sich in wichtigen biologisch-ökologischen Eigenschaften, nämlich dem Potenzial ihrer Samenausbreitung und in der Anzahl der Blühereignisse pro Individuum.

Pflanzen in kleinen und isolierten Populationen können im Vergleich zu solchen aus großen, nicht isolierten Populationen in ihrer Entwicklung und Reproduktion beeinträchtigt sein. Außerdem leben sie oftmals in schlechteren Habitaten. In Kapitel 2 untersuche ich Effekte von Habitatbedingungen, Populationsgröße und Isolation auf die Reproduktion und Entwicklung von Pflanzen aus 74 *Carlina*-Populationen aus sieben europäischen Regionen. Diese Studie wurde sowohl in den natürlichen Populationen im Feld als auch mit

Nachkommen im Versuchsgarten durchgeführt. Im Versuchsgarten war das Wachstum der Pflanzen aus kleinen Populationen im Vergleich zu solchen aus großen vermindert, und Pflanzen aus stärker isolierten Populationen waren kleiner als solche aus weniger stark isolierten. Diese Ergebnisse deuten darauf hin, dass in kleinen und isolierten Populationen genetische Drift und Inzuchtdepression existiert. Am natürlichen Wuchsort war in kleinen Populationen allerdings „nur" der Samenansatz vermindert. Die unterschiedliche Größe der Pflanzen verschiedener Populationen im Feld wurde zu einen großen Teil durch Variablen erklärt, die bestimmte Umweltbedingungen widerspiegeln, z. B. die geographische Lage der Flächen, die für Klimaunterschiede steht, oder die Artenzusammensetzung der umgebenden Vegetation als ein Maß für die Nährstoff- und Wasserversorgung. Diese Ergebnisse deuten darauf hin, dass die Reproduktion und auch die Entwicklung der Nachkommen von kleinen *C. vulgaris*-Populationen in Gesamteuropa durch genetische Erosion und/oder verminderte Pollenqualität und/oder -quantität herabgesetzt sind. Insgesamt waren jedoch die abiotischen Umweltbedingungen in den natürlichen Populationen für die Entwicklung der Pflanzen wichtiger als die Größe oder der Isolationsgrad der Populationen.

　　　Die Variation von quantitativen Merkmalen ist eine Grundlage der adaptiven Evolution von Populationen und ist deshalb wichtig für deren langfristiges Überleben. In Kapitel 3 untersuche ich die Variation verschiedener quantitativ-genetischer Merkmale an Nachkommen von 74 Populationen von *Carlina vulgaris* aus sieben europäischen Regionen und 32 Populationen von *Hypochoeris radicata* aus drei europäischen Regionen (siehe jeweils oben) im Versuchsgarten der Universität Marburg. Bei beiden Arten variierten die meisten Merkmale sowohl zwischen Samenfamilien innerhalb von Populationen, zwischen Populationen innerhalb von Regionen als auch zwischen Populationen zwischen Regionen erheblich. Die quantitativ-genetische Variation zwischen den Populationen (sowohl innerhalb als auch zwischen Regionen) lag jedoch bei *Carlina vulgaris* mit 75,8% (von der Gesamtvariation) deutlich höher als bei *Hypochoeris radicata* mit 50,7%. Dieser Unterschied deutet bei der schwach ausbreitenden *C. vulgaris* auf geringeren Genfluss zwischen Populationen als bei *H. radicata* hin. Allerdings standen die quantitativ-genetischen Distanzen bei beiden Arten in keiner Beziehung zu den geographischen Distanzen zwischen den Populationen innerhalb einer Region. Die Variation der morphologischen Merkmale auf dem Level der Populationen spiegelte jedoch geographische Unterschiede zwischen den Regionen wider. Damit deuten die Ergebnisse des 2. Kapitels darauf hin, dass auf kleiner räumlicher Skala genetische Drift oder Habitatunterschiede für die Differenzierung der Populationen verantwortlich waren. Auf großräumiger Skala waren dagegen für die

Differenzierung der Populationen selektiv wirksame Umweltbedingungen mit engem Bezug zu den geographischen Distanzen am wichtigsten. Bei *Hypochoeris radicata*, jedoch nicht bei *Carlina vulgaris*, nahm die Variation verschiedener Merkmale zwischen Samenfamilien innerhalb von Populationen mit der Distanz zur nächsten Population zu. Dieser Befund deutet auf Instabilität der quantitativen Merkmale in isolierten Populationen hin.

In Kapitel 4 untersuche ich die Differenzierung und lokale Anpassung von Populationen von *Carlina vulgaris*. Dazu wurden reziproke Verpflanzungsexperimente durchgeführt, und zwar zwischen (regionale Skala) und innerhalb (lokale Skala) von fünf europäischen Regionen (in Schweden, Deutschland, Tschechien, Luxemburg und der Schweiz). Die Entwicklung der verpflanzten Individuen wurde über drei Wachstumsperioden verfolgt. Auf regionaler Skala waren die individuelle Fitness sowie andere Maßzahlen für die Entwicklung der Pflanzen von *C. vulgaris* war am höchsten, wenn die Pflanzen in ihrer Ursprungsregion wuchsen und nahmen mit zunehmender Entfernung zwischen Ursprungsort und Pflanzort ab. Die geographische Distanz wiederum war positiv mit den klimatischen und edaphischen Distanzen zwischen den jeweiligen Flächen korreliert. Auf lokaler Skala waren dagegen Effekte lokaler Anpassung kaum ausgeprägt und die Entwicklung der Pflanzen stand in keiner Beziehung zur geographischen oder Umweltdistanz zwischen Ursprungsort und Pflanzort. Insgesamt lassen die Ergebnisse darauf schließen, dass *Carlina vulgaris* aus regional ange-passten Genotypen besteht und dass die geographische Distanz ein gutes Instrument ist, um das Ausmaß von adaptiver Differenzierung vorherzusagen, und zwar auf großer (> 200 km) nicht jedoch auf kleinräumiger Skala. Strategien zum Schutz der genetischen Ressourcen von *Carlina vulgaris*, müssen daher das räumliche Muster lokaler Anpassung berücksichtigen.

Ein unterschiedliches Ausmaß von Inzucht- und Auszuchtdepression wurde in verschie-denen Studien gefunden, aber über großräumige geographische Muster innerhalb einer Art ist kaum etwas bekannt. In Kapitel 5 präsentiere ich eine Studie über Effekte von Kreuzung innerhalb und zwischen verschiedenen europäischen Regionen (in Holland, Deutschland, Tschechien) auf die Ausbildung der Samen und die Entwicklung der Nachkommen von *Hypochoeris radicata*. Im einzelnen wurden Bestäubungen (i) innerhalb einer Pflanze, (ii) zwischen Pflanzen einer Samenfamilie, (iii) einer Population, (iv) verschiedener Populationen einer Region und (v) zwischen Populationen verschiedener Regionen durchgeführt. Der Samenansatz und die Keimrate der Samen waren sowohl nach Inzucht als auch nach Kreuzung zwischen Populationen innerhalb von Regionen geringer als nach Kreuzung innerhalb von Populationen. Diese Ergebnisse deuten auf Inzucht- und Auszuchtdepression hin. Allerdings erbrachten die Kreuzungen zwischen Regionen einen höheren Samenansatz

als Kreuzungen zwischen Populationen innerhalb einer Region. Für Merkmale eines späten Entwicklungsstadiums waren die Auswirkungen der Kreuzungsdistanz in den verschiedenen Regionen unterschiedlich. Für Nachkommen von tschechischen Mutterpflanzen wurde beides, Inzucht- und Auszuchtdepression für verschiedene fitness-relevante Merkmale beobachtet. Im Gegensatz dazu zeigten die Nachkommen von Kreuzung deutscher Populationen die höchste Fitness, was auf Heterosis hin deutet. Bestäubungen mit Nachkommen niederländischer Mutterpflanzen zeigten hingegen keinen klaren Effekt der Kreuzungsdistanz auf Merkmale des späten Entwicklungsstadiums. Insgesamt zeigen die Ergebnisse des 4. Kapitels, dass Auszuchtdepression nicht unbedingt mit der räumlichen Entfernung zunehmen muss, und dass die Empfindlichkeit von Populationen gegen Introgression variiert.

Dank

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