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Phenotypic plasticity in *Carlina vulgaris*: effects of geographical origin, population size, and population isolation

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Abstract If phenotypic plasticity is under genetic control, it may vary in amount and pattern on a geographical scale, e.g. among different regions of a species' distribution. It may also differ between large and small or between less and more isolated populations, due to differences in genetic diversity. In a 2-year common garden study, the responses of several traits to drought and fertilizer treatments were studied in the grassland herb *Carlina vulgaris*. Individuals originating from populations of different size and degree of isolation in six European countries, representing "central" and "marginal" regions, were compared. Fertilizing had a negative effect on early plant survival, as well as on flowering probability in surviving plants. However, in those plants that flowered, fertilizing strongly increased mean number of flowerheads, flowerhead area (a correlate of seed number), and seed mass. Drought had generally weaker effects but enhanced survivorship, indicating that this treatment was closer to optimal conditions than were non-drought conditions. For some traits there were significant interactions of region \times fertilizer, but the geographical pattern of reaction norms was inconsistent and lent no support to the hypothesis that central and marginal populations differ in overall plasticity. Population size and isolation had hardly any influence on treatment responses, but populations within regions differed in their mean response to fertilizing with regard to survival and flowering probabilities, as well as in their response to drought with regard to survival and total

flowerhead area. It is concluded that response to raised nutrient levels is highly variable within populations, ranging from death to strongly increased reproductive output, but also among populations irrespective of size or isolation. This also goes for the response to water supply, though this variation shows a more unclear pattern. There is no evidence that small or isolated/marginal populations are less plastic than large or non-isolated/central populations, and the explanation for differences in treatment responses among plant populations should be sought in other population characteristics.

Keywords Drought · Fertilizer · Fitness · Genetic diversity · Monocarpic perennial

Introduction

Phenotypic plasticity is the ability of an individual to respond physiologically and/or morphologically to changes in its environment. Plasticity is considered adaptive when it allows the individual to maintain viability and reproductive output across a range of environmental conditions. Although the adaptiveness of plasticity is not always clear, it is a highly important ecological and evolutionary property, especially for sessile organisms such as plants (Bradshaw 1965; Schlichting 1986; Sultan 1995, and references therein). It has been proposed that plasticity is an alternative strategy to genetic adaptation for coping with environmental heterogeneity in time and space, and that the relationship between genetic diversity and phenotypic plasticity should therefore be inverse (Marshall and Jain 1968). However, many empirical studies (Schlichting and Levin 1984; Macdonald and Chinnappa 1989; Vasseur and Aarssen 1992; Stewart and Nilsen 1995; Black-Samuelsson and Andersson 1997) have found no such relationship, and Schlichting (1986) has cautioned against

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generalized conclusions. Bradshaw (1965) and Schlichting (1986) have argued that plasticity is a trait itself and thus under genetic control. Hence, plasticity may differ in strength or direction not only among taxa but also among populations of the same taxon, since these may differ in genetic make-up due to drift or due to adaptations to different habitat conditions (Schlichting and Levin 1984, 1990; Schlichting 1986; Andersson and Widén 1993; Pigliucci and Schlichting 1995; Sultan 1995, 2001; Leiss and Müller-Schärer 2001; Verhoeven et al. 2004). It is also likely that differences in the mean expression of plasticity will increase with interpopulation distance, and be larger between populations in different regions than between populations within the same region (cf. Bradshaw 1965).

Many plant species that were formerly common in the agricultural landscape of Europe have declined during recent decades, due to destruction and fragmentation of their habitats following large-scale changes in land use. Semi-natural, unproductive, extensively managed grasslands, which are often very species-rich, are among the habitat types most affected (Willems et al. 1993; Eriksson et al. 1995; Eisto et al. 2000; Franzén and Eriksson 2003). Habitat fragmentation leads to smaller populations and greater interpopulation distances, which may in turn lead to reduced genetic diversity within populations due to restricted gene flow, drift, and inbreeding (e.g. Menges 1991; Ouborg et al. 1991; Fischer and Matthies 1998; Fischer et al. 2000, and references therein). Such “genetic erosion” is assumed to be especially strong in populations near the margin of a species’ distribution (Black-Samuelsson and Andersson 1997). If phenotypic plasticity is under genetic control, it is likely to be affected by increased inbreeding and homozygosity (Schlichting 1986; Paschke et al. 2003; but see Bradshaw 1965). This could place an additional threat to rare species or small and isolated populations, since a loss of plasticity reduces the potential to respond to environmental changes in a relevant way. However, the relationships between population size and isolation, genetic diversity, and phenotypic plasticity can be complicated (Schlichting 1986). Several studies have found a positive association between size and genetic diversity of plant populations (e.g. Ouborg et al. 1991; Lammi et al. 1999; Müller-Schärer and Fischer 2001; Vergeer et al. 2003), but indications of the same relationship between plasticity and population size or genetic diversity are scarce and not very strong (Fischer et al. 2000; Kéry et al. 2000; Paschke et al. 2003; Pluess and Stöcklin 2004).

In the present study, we examine variation in plasticity in the monocarpic perennial *Carlina vulgaris* in response to water and nutrients, two fundamental plant resources that may vary considerably in time and space. We compare individuals originating from populations in different European regions, including both central and marginal parts of the species’ distribution, and of different size and degree of isolation. We chose *C. vulgaris* as the study object because it is a short-lived plant of

dry, nutrient-poor grasslands, and thus endangered by the ongoing fragmentation of these habitats. Hence, it is important to assess whether small and isolated populations can maintain their ability to adjust to altered habitat conditions, especially since reduced population size and increased isolation often also implies a reduction in habitat quality and increased effects of environmental stochasticity (see e.g. Kéry et al. 2000). Moreover, since *C. vulgaris* is a relatively poorly dispersed species (Klinkhamer et al. 1996; Franzén and Eriksson 2003), low interpopulation gene flow and, consequently, population differentiation with regard to plasticity are likely.

Generally, one can expect *C. vulgaris* to be able to maintain viability and reproductive output under experimental drought stress, through plastic adjustments of e.g., root/shoot ratio and flowering phenology (cf. Taiz and Zeiger 1991; Sultan and Bazzaz 1993a; Bell and Sultan 1999; Rajakaruna et al. 2003). This ability may be more pronounced in individuals from the continental, drier parts of the species’ distribution. As for nutrient enrichment, the response may be absent or even negative in this species, since it is adapted to relatively nutrient-poor conditions (cf. Chapin 1980; Marschner 1995). Thus, the increasing eutrophication of the European agricultural landscape (Vergeer et al. 2003) may be a threat to this species beside the effects of actual habitat fragmentation. However, this general pattern of plasticity could also be modified by the size and degree of isolation of the population of origin, to the extent that these factors determine genetic diversity and individual performance in populations; appropriate responses may be weaker or absent in the smallest and in the most isolated or marginal populations. We address the following questions: (1) Do *C. vulgaris* offspring originating from different parts of the species’ distribution, in particular central versus marginal parts, exhibit different amounts or patterns of plasticity? (2) Are offspring from small populations less plastic than offspring from large populations? (3) Are offspring from more isolated populations less plastic than offspring from less isolated populations?

Materials and methods

Study species

Carlina vulgaris L. (Asteraceae) is an herbaceous, perennial, monocarpic thistle with a tap root and a leaf rosette. At the earliest, in their second year plants produce one or more erect, usually branched flower stems. Reproduction is strongly dependent on plant size. One to several flowerheads, usually two to five, are produced in a corymb. The insect-pollinated, mainly outcrossed florets are produced from June to October. Seeds (achenes) have a small pappus and are presumably dispersed

over rather short distances, and there seems to be no persistent seed bank (Verkaar and Schenkeveld 1984; Hegi 1987; Klinkhamer et al. 1991, 1996; Rose et al. 2002; Franzén and Eriksson 2003).

Carlina vulgaris is confined to more or less open, dry, and nutrient-poor habitats, and typically occurs in calcareous grasslands but also in coastal dunes (Körner and Meusel 1986; Hegi 1987; Klinkhamer et al. 1996). It is distributed throughout Siberia, the Caucasus, Asia Minor, and most of Europe northwards to ca. 62°N, though populations in parts of eastern Europe, the Alps, and Siberia are sometimes regarded as belonging to a different species, *Carlina biebersteinii* Bernh. ex Hornem. (Körner and Meusel 1986). Seed recruitment and population persistence in *C. vulgaris*, as in many other monocarpic perennials, is largely dependent on vegetation disturbance through grazing or trampling (Verkaar and Schenkeveld 1984; Sebald et al. 1996; Löfgren et al. 2000; Rose et al. 2002; Franzén and Eriksson 2003), and in open habitats it appears to be sensitive to strong drought (van der Meijden et al. 1992). The species has declined due to the cessation of traditional grassland management in large parts of central Europe (Korneck et al. 1996; Sebald et al. 1996).

Populations and plant material

In autumn 2000, seeds of *C. vulgaris* were collected in six populations in western Czech Republic, 15 in central Germany, nine in Switzerland, 11 in Luxemburg, seven in The Netherlands, and 11 in central Sweden (Table 1). Population size ranged from 6 to ca. 20,000 flowering individuals. Distance to the closest *C. vulgaris* population (a measure of population isolation) ranged from 100 m to 7 km, and the different countries ("regions" from here on) also represent different degrees of "centrality" within the distribution of *C. vulgaris*: Czech populations were considered the most central and Swedish populations the most marginal. Population size and isolation were not correlated ($r = -0.073$, $n = 57$, $P > 0.1$), and regions did not differ in mean size or isolation of populations ($F_{5,53} = 1.47$ and 0.98 , respectively; $P > 0.1$). In each population, seeds of 15–20 plants (when available) were collected and kept separated by family. All seeds were sent to the Department of Biology of the University of Marburg, Germany, where they were weighed family-wise and then stored in a refrigerator at +5°C. In March 2001, seeds were sown, separated by family, into pots and placed in a greenhouse.

Seeds germinated in April 2001. During 2 May to 14 May, seedlings (mostly two) from each family were transplanted individually into plastic pots (9×9×9.5 cm) filled with a nutrient-poor commercial soil mixed with sand, and placed into flower beds in the Botanical Garden of the University of Marburg. In total, there were 1,202 seedlings originating from 517 mother plants in 59 populations (Table 1).

Table 1 Overview of the *Carlina vulgaris* populations sampled in Czech Republic (CZ), Germany (D), Switzerland (CH), Luxemburg (LU), The Netherlands (NL), and Sweden (SE), with original numbers of seed families and seedlings used in the common garden experiment

Population	Region	Size	Isolation	Families	Seedlings
1	CZ	200	1,000	10	20
2	CZ	30	400	8	20
3	CZ	90	1,000	10	20
4	CZ	180	1,000	10	20
5	CZ	1,000	100	10	20
6	CZ	700	700	10	20
7	D	150	600	10	21
8	D	6	600	5	20
9	D	1,000	110	10	20
10	D	20	110	10	21
11	D	250	300	10	20
12	D	100	150	10	20
13	D	12	150	6	20
14	D	950	100	10	20
15	D	10	1,200	10	19
16	D	43	400	10	21
17	D	6	100	5	21
18	D	32	7,000	10	21
19	D	400	600	10	20
20	D	500	600	10	21
21	D	500	700	10	20
22	CH	180	500	10	20
23	CH	40	500	5	20
24	CH	500	200	10	20
25	CH	15	100	10	20
26	CH	36	200	8	20
27	CH	1,500	400	3	20
28	CH	125	100	3	20
29	CH	200	200	10	21
30	CH	40	200	7	20
31	LU	2,800	300	10	20
32	LU	96	200	10	20
33	LU	37	200	10	21
34	LU	244	150	10	20
35	LU	28	150	10	20
36	LU	9,300	1,400	10	20
37	LU	29	1,700	10	20
38	LU	123	500	10	20
39	LU	1,200	1,600	10	20
40	LU	11,000	100	10	20
41	LU	20,000	100	10	20
42	NL	500	No data	10	20
43	NL	150	200	10	21
44	NL	350	200	10	20
45	NL	125	No data	10	21
46	NL	700	500	5	21
47	NL	200	300	10	20
48	NL	50	100	7	23
49	SE	11	100	6	20
50	SE	11	580	6	20
51	SE	200	1,000	7	20
52	SE	258	100	9	20
53	SE	300	130	10	20
54	SE	312	150	7	21
55	SE	1,630	100	5	21
56	SE	27	150	10	26
57	SE	1,200	200	10	21
58	SE	330	130	10	20
59	SE	30	200	5	20

Size: rough number of reproductive plants. Isolation: distance to closest *C. vulgaris* population in metres

Treatments and data collection

Each plant was assigned to one of four treatments: control (C); drought (D); fertilizer (F); and the combination of drought and fertilizer (DF). The number of plants was the same for all four treatments. Since most of the seed families were only represented by two plants, there were in most cases two different treatments per family. F and C plants were kept constantly moist during the entire growing season (May–September), whereas D and DF plants twice each growing season (2001 and 2002) were subjected to a drought treatment, i.e. they received no water for a period of 2–4 days, until they started to wilt. The drought treatment was applied during dry and hot periods in July and August. Before and after each application, the drought plants were watered like the other plants. The plants assigned to the F and DF treatments received 60 ml of an 8:8:6 NPK fertilizer (Wuxal Super, Aglukon, Spezialdünger GmbH Sseldorf, Germany), diluted 1:200; this was equivalent to ca. 0.025 g N and P per pot. This treatment was applied three times each growing season. All plants were randomized three times during each growing season.

A number of traits was recorded in each plant, all of which have shown plastic responses to variation in both water and nutrient availability (Schlichting and Levin 1988, 1990; Klinkhamer et al. 1991; Sultan and Bazzaz 1993a, b; Leiss and Müller-Schärer 2001; Paschke et al. 2003; Rajakaruna et al. 2003; Dorken and Barrett 2004; Verhoeven et al. 2004). Length and width of the longest rosette leaf were measured twice in 2001, before and after treatments (early June and mid September, respectively), and relative growth rates of leaf length and width were calculated. The flowering period lasted from mid June to mid November, but most plants produced their first florets between late July and early August. Twenty-seven plants (2% of total) initiated flower stems already in the first year, whereas in the second year 901 plants (87% of the remaining) flowered. Seventeen of the plants that had flowered in 2001 (but only two that had set seed) survived until the next year, and out of these 14 flowered again in 2002. In flowering plants, the following traits were recorded: height of flower stem; date of first floret opening; number of flowerheads; and diameter of each flowerhead, from which flowerhead area was calculated. Total flowerhead area was then calculated as the sum of the areas of all flowerheads; since we could not make a reliable count of seeds but found that flowerhead area is correlated with seed number in *C. vulgaris* ($r = 0.430$, $n = 1,067$, $P < 0.001$), we used this as a measure of total reproductive output. Since so few plants flowered in 2001, reproductive traits were only analysed for 2002; however, in the analysis of total flowerhead area those plants that flowered in 2001 were also included, since we wanted to analyse reproductive output for all flowering plants during the 2-year period.

All plants dying during the study period were registered, and survival until the second growing season was

taken as the probability of surviving to reproduction, since *C. vulgaris* does not reproduce before this age under natural conditions (Klinkhamer et al. 1991, 1996). Thus, the ten plants that had flowered and died already in 2001 were not included in the analysis of survival until the second growing season, since that would not make sense for a monocarpic species.

By the autumn of 2002 all plants had flowered and died, or died without flowering (except for 16 plants, evenly distributed among treatments, that had neither flowered nor died), and the aboveground parts were harvested. The harvested material was dried at 80°C for 12 h, and biomass and mean seed mass (of ten seeds per plant) were determined.

Data analysis

The effects of region, population within region, population size and isolation, treatments, and the interactive effects of these factors on each continuous trait were examined using a nested factorial ANOVA model (GLM function; Table 2) with each plant as an observation. Region and treatments were treated as fixed factors and population as a random factor, and population size and isolation (both \log_{10} -transformed) were included as covariates. The effects of region, population size, and isolation were tested against the residual variation among populations, whereas those of drought, fertilizer, and drought \times fertilizer, as well as those of their interactions with region, population size, and isolation, were tested against the corresponding interactions with population. Maternal seed mass was included as a covariate to adjust for maternal effects (Roach and Wulff 1987). The effects on the binary variables survival until the second growing season and flowering (initiation of flower stem; yes or no) were analysed with a similar model, but by analysis of deviance (logistic regression function; Sokal and Rohlf 1995), instead of ANOVA. This procedure produces approximate *F*-values, which are analogous to *F*-values (Francis et al. 1993). Due to the low number of seedlings per seed family (Table 1) and because very few families were subjected to all treatments, family was not included in any of these analyses.

Significant effects of plant origin (region, population identity/size/isolation) were considered indications of genetically based differences in trait means, effects of treatment would indicate plasticity, and interactions between origin and treatment would suggest genetic differences in plasticity due to plant origin (Schlichting 1986). In preliminary analyses, other population characteristics than size and isolation that might have an influence on reaction norms were also tested: mean and maximum vegetation height, vegetation cover, and presence or absence of management. However, these had no effect on the reaction to treatment of any of the traits. To test whether effects of population size or isolation on treatment responses varied among regions, the interac-

Table 2 Analysis of the effects of drought (D) and fertilizer (F) treatments, and interactions of these treatments with region (R), population size (PS), population isolation (PI), and population identity (P), on various traits in offspring of *C. vulgaris*

df	RGR leaf length		RGR leaf width		Survival		Flowering		Stem height		Days until flowering		Flowerheads		Mean flowerhead area		Total flowerhead area		Biomass		Seed mass	
	F	F	F	F	Approx.	F	Approx.	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
Drought	1	0.23	0.50	5.84**↑	2.08	1	0.04	4.32*↑	0.04	0.40	1	0.77	0.52	0.53								
Fertilizer	1	96.45***↑	13.58**↑	82.66***↓	54.86***↓	1	1.85	1.49.86***↓	171.05***↑	83.29***↑	1	351.90***↑	367.10***↑	56.29***↑								
D × F	1	3.41	1.90	1.69	7.92**	1	7.68**	0.09	0.00	3.03	1	2.96	10.07**	2.17								
Region × D	5	0.65	0.96	0.82	1.57	5	2.25	0.24	0.85	0.92	5	0.19	0.42	0.61								
R × F	5	3.77**	1.01	4.34**	1.23	5	3.98**	1.01	1.01	1.68	5	1.53	4.33**	1.14								
R × D × F	5	0.71	0.27	2.00	0.99	5	1.82	1.91	0.68	2.52*	5	0.36	0.93	2.23								
Population	1	1.45	3.60	1.00	1.24	1	0.42	0.09	0.09	0.25	1	0.36	0.06	1.19								
size × D																						
PS × F	1	0.09	0.00	2.28	2.02	1	0.10	4.12*	0.00	1.00	1	0.02	1.03	0.25								
PS × D × F	1	0.79	0.05	3.65	0.00	1	0.01	1.65	0.01	0.05	1	0.24	0.43	0.16								
Population	1	4.92*	6.31*	0.11	0.18	1	3.60	0.48	0.03	0.78	1	0.01	0.68	2.40								
isolation × D																						
PI × F	1	0.27	1.21	0.26	0.30	1	0.16	1.84	0.45	0.12	1	0.00	0.00	0.00								
PI × D × F	1	2.12	0.03	0.39	1.61	1	3.71	3.97	0.25	0.04	1	0.16	0.51	0.07								
Population × D	49	1.22	1.11	1.80**	1.13	49	1.13	1.05	1.05	1.03	49	1.54*	1.23	0.98								
P × F	49	0.79	1.20	2.03***	1.46*	49	0.87	1.12	1.02	0.78	49	0.95	0.93	0.84								
P × D × F	49	1.04	1.12	1.03	0.56	49	0.87	0.90	1.27	1.19	49	1.08	0.88	1.64								
Residual	901-757					630					700-185											

Arrows indicate the direction of main treatment effects. Survival until the second growing season and flowering probability were analysed by analysis of deviance while all other traits were analysed by ANOVA. Total flowerhead area also includes plants flowering in 2001. The changes in df of the residual is due to mortality prior to the flowering period, to reproductive traits only being recorded in flowering plants, and to many flowering plants failing to produce ripe seed

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

tions of region \times size/isolation, as well as the interactions of region \times size/isolation \times drought/fertilizer, were also included into the model described above and tested against the model residual. These interactions had no significant effect on any of the traits, so they were omitted in the final analyses.

To be able to interpret the origin \times treatment interactions found in the analyses described above, we quantified the magnitude of plasticity of each trait, i.e. the strength of its response to treatment, as the difference between treatment (drought or fertilizer) mean and control (non-drought or non-fertilizer, respectively) mean for each population (cf. Andersson and Widén 1993; Pluess and Stöcklin 2004). This measure also gives the direction of the response. Among-region differences in response of each trait (population means) were then examined with post hoc (Tukey) tests. Effects of population size and isolation on trait responses were examined with correlation analyses and curve estimations.

To meet assumptions of normality, the response variable number of flowerheads was square root-transformed for analysis, whereas height of flower stem, mean and total flowerhead area, and biomass were \log_{10} -transformed. All analyses were carried out with SPSS 11.0 (Brosius 2002).

Results

Although included in the analyses, the effects on the different traits of maternal seed mass, region, and identity, size, and isolation of populations *per se* will not be considered further in this paper; these effects are treated specifically in another study including more regions and populations (U. Becker, H. Berg, and D. Matthies, in preparation), and are therefore omitted in Table 2. For the present study, we are only interested in the effects of treatments and, above all, in the interactions between plant origin and treatments.

Effects of drought

The drought treatment had a positive effect on the probability of survival until the second growing season, especially in fertilized plants (Tables 2, 3; Fig. 1), but slightly increased mean number of days until flowering (Tables 2, 3). No other significant main effects of

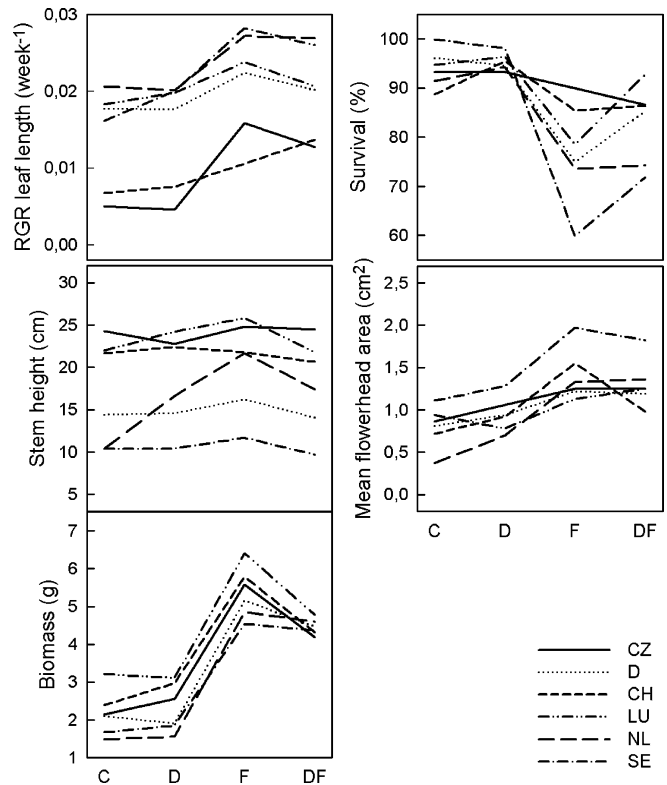


Fig. 1 Norms of reaction to different treatments (*C* control; *D* drought; *F* fertilizer; *DF* drought + fertilizer) of various traits in offspring of *C. vulgaris* originating from Czech Republic (*CZ*), Germany (*D*), Switzerland (*CH*), Luxemburg (*LU*), The Netherlands (*NL*), and Sweden (*SE*). The graphs for height, flowerhead area, and biomass show back-transformed data

Table 3 Mean \pm SE of various traits in offspring of *C. vulgaris* under different treatments

	Control	Drought	Fertilizer	D + F
RGR leaf length (week ⁻¹)	0.013 \pm 0.001 ^b	0.015 \pm 0.001 ^b	0.021 \pm 0.001 ^a	0.020 \pm 0.001 ^a
RGR leaf width (week ⁻¹)	0.002 \pm 0.001 ^b	0.003 \pm 0.001 ^b	0.007 \pm 0.001 ^a	0.005 \pm 0.001 ^{ab}
Survival (%)	94.6 \pm 1.9 ^a	95.6 \pm 1.9 ^a	75.8 \pm 1.9 ^c	82.9 \pm 1.9 ^b
Flowering (%)	91.5 \pm 2.3 ^a	89.2 \pm 2.3 ^{ab}	81.5 \pm 2.3 ^b	86.2 \pm 2.3 ^{ab}
height of flower stem (cm)	16.22 \pm 0.02 ^b	17.74 \pm 0.02 ^{ab}	19.63 \pm 0.02 ^a	17.22 \pm 0.02 ^b
Days until flowering	50.80 \pm 1.99 ^a	50.37 \pm 1.08 ^a	40.31 \pm 1.03 ^b	41.14 \pm 0.92 ^b
Number of flowerheads	3.18 \pm 0.04 ^b	3.25 \pm 0.04 ^b	8.00 \pm 0.05 ^a	7.30 \pm 0.05 ^a
Mean flowerhead area (cm ²)	0.77 \pm 0.03 ^b	0.93 \pm 0.02 ^b	1.38 \pm 0.02 ^a	1.29 \pm 0.02 ^a
Total flowerhead area (cm ²)	3.45 \pm 0.02 ^b	3.41 \pm 0.02 ^b	12.52 \pm 0.02 ^a	9.91 \pm 0.02 ^a
Aboveground biomass (g)	2.10 \pm 0.02 ^c	2.25 \pm 0.02 ^c	5.36 \pm 0.02 ^a	4.45 \pm 0.02 ^b
Mean seed mass (mg)	0.63 \pm 0.06 ^{bc}	0.56 \pm 0.04 ^c	0.75 \pm 0.03 ^{ab}	0.80 \pm 0.03 ^a

Survival is until the second growing season (2002)

Values of reproductive traits are for 2002 only, except for total flowerhead area, which also includes plants flowering in 2001. The count of days until flowering starts with the earliest-flowering plant on day 1. Values of stem height, flowerhead number, flowerhead area, and biomass are back-transformed

Different letters indicate significant differences ($\alpha=0.05$) between treatments according to post hoc (Tukey) tests. For survival and flowering, post hoc tests were carried out on population percentages; for all other traits they were carried out with the value for each individual as an observation

drought were found, but an effect of the interaction of population identity \times drought on survival was observed as follows (Table 2): in 13 of the 59 populations, survivorship was lower in drought plants than in non-drought plants (Fig. 2). Survivorship per population ranged from 60% to 100% (median 86%) for non-drought plants, and from 64% to 100% (median 90%) for drought plants. There was also an interaction of population \times drought for total flowerhead area, though this trait was not affected by drought in itself (Table 2), but the population pattern was different from that for survival (Fig. 2). Twenty-four populations had larger mean total flowerhead area under drought, the rest smaller. The response of total flowerhead area to drought, quantified as treatment (D + DF) mean – control (C + F) mean for each population, was correlated with the responses of flowerhead number ($r=0.521$, $n=59$, $P<0.001$) and mean flowerhead area ($r=0.717$, $n=58$, $P<0.001$), which were, however, not correlated with each other. This indicates that the negative or positive effect of drought on total flowerhead area reflected its effect on number and/or mean area of flowerheads, depending on population identity.

Interactions of population isolation \times drought for RGR of leaf length and width were found (Table 2): isolation was negatively correlated to the response to drought of the two growth measures ($r = -0.331$ and -0.329 , respectively; $n=57$, $P<0.05$). RGR of leaf length and width were strongly intercorrelated

($r=0.768$, $n=1,124$, $P<0.001$). There was no interaction of region \times drought, or population size \times drought, for any trait (Table 2).

Effects of fertilizer

The fertilizer treatment had by far the strongest influence on plant performance. In the first year, it enhanced RGR of leaf length and width (Tables 2, 3; Fig. 1). In contrast, it had a strong negative effect on survival until the second growing season (Tables 2, 3; Fig. 1). This effect was consistent across populations: out of all 59 populations, only three had higher survivorship with fertilizer than without (Fig. 3). Survivorship per population ranged from 80% to 100% (median 100%) for unfertilized plants, and from 40% to 100% (median 80%) for fertilized plants. Flowering probability of the surviving plants in 2002 was also negatively affected by fertilizing (Tables 2, 3), and only eight populations had higher flowering percentage with fertilizer than without (Fig. 3). Flowering per population ranged from 56% to 100% (median 90%) for unfertilized plants, and from 40% to 100% (median 86%) for fertilized plants. There were also interactions of population \times fertilizer for survival and flowering (Table 2). The responses (population percentage of F + FD – percentage of C + D) of survival and flowering were positively intercorrelated ($r=0.597$, $n=59$, $P<0.001$), that is, for populations

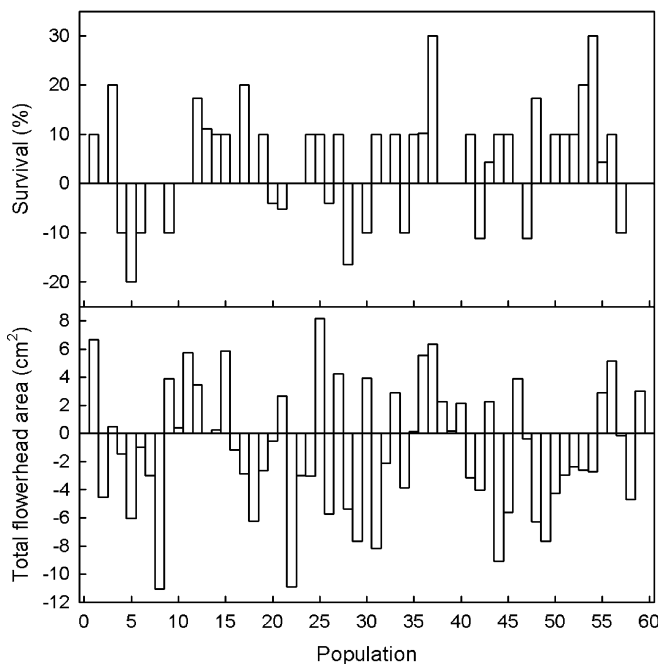


Fig. 2 Response to drought treatment of percentage survival until the second growing season and mean total flowerhead area in offspring of *C. vulgaris* originating from 59 populations. Response is presented as the difference in percentage/mean between plants that were drought-treated (D + DF) and those that were not (C + F). For descriptions of populations, see Table 1

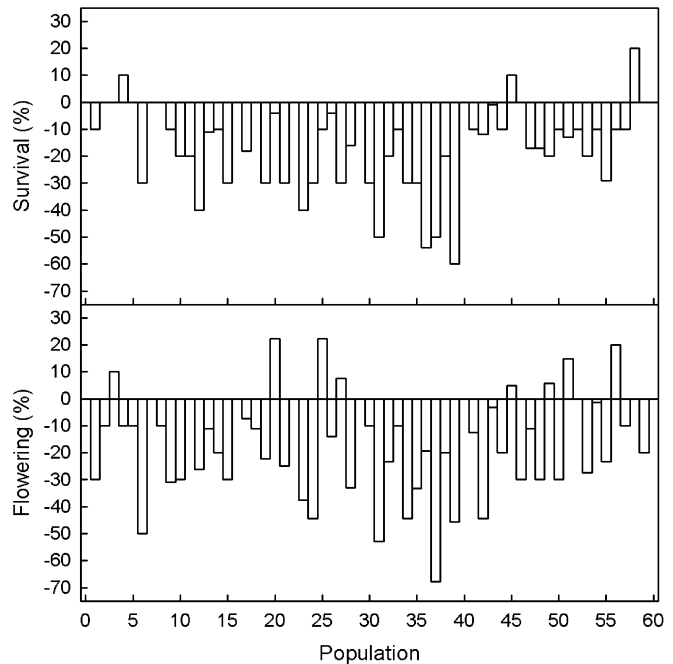


Fig. 3 Response to fertilizer treatment of percentage survival until the second growing season and flowering percentage in offspring of *C. vulgaris* originating from 59 populations. Response is presented as the difference in percentage between plants that were fertilized (F + DF) and those that were not (C + D). For descriptions of populations, see Table 1

with low survivorship following fertilizing, flowering probability of surviving plants was in general also lower (Fig. 3).

In flowering plants, fertilizing reduced the mean time until flowering by ca. 8 days (Tables 2, 3). Mean number of flowerheads was strongly increased by fertilizing (Tables 2, 3; range 1–20 flowerheads for unfertilized plants, 1–36 for fertilized plants), an effect very constant across populations; only one population had slightly lower flowerhead number in fertilized than in unfertilized plants. This positive effect of fertilizing was perhaps partly due to the reduction in time until flowering; there was a negative correlation between number of days until flowering and flowerhead number ($r = -0.224$, $n = 543$, $P < 0.001$). Fertilizing also had a positive effect on mean area of flowerheads (Fig. 1), and hence a strong effect on total flowerhead area (Tables 2, 3). Fertilized plants had higher aboveground biomass than unfertilized plants (Tables 2, 3; Fig. 1), which was expected since biomass was strongly correlated with total flowerhead area ($r = 0.728$, $n = 711$, $P < 0.001$; cf. Körner and Meusel 1986). Mean seed mass was also enhanced by fertilizing (Tables 2, 3).

For RGR of leaf length, survival, height of flower stem, and biomass, significant interactions were found between the effects of region and fertilizer, indicating geographical variation in plasticity (Table 2; Fig. 1). As for RGR, posthoc tests showed that Czech and Swedish populations exhibited the strongest positive response to fertilizing, followed in descending order by Dutch, Swiss, German, and Luxemburg populations. Populations from the two latter regions responded significantly weaker than Swedish populations. Swedish populations had the highest mortality following fertilizing, significantly higher than that of populations from all other regions except for The Netherlands, whereas Czech and Swiss populations were hardly affected. Dutch plants responded to fertilizing with considerably taller flower stems, while plants from the other regions did not react. Dutch plants also showed the strongest response of biomass to fertilizing, partly due to the enhanced stem growth; stem height and biomass were positively correlated ($r = 0.511$, $n = 864$, $P < 0.001$). However, for the response of this trait, the actual differences among regions were small and not significant according to post hoc tests.

There was an interaction of population size \times fertilizer for time until flowering (Table 2); however, no significant linear or nonlinear relationship was found between population size and response to fertilizing ($r = -0.055$, $n = 56$, $P > 0.1$). No interaction of population isolation \times fertilizer was found (Table 2).

Interactions between drought and fertilizer

For flowering, there was an interaction of drought \times fertilizer: both D and DF plants had higher flowering probability than F plants (Tables 2, 3). Height

of flower stem was not significantly affected by drought or fertilizer in themselves, but there was an interaction between the two: F plants had taller stems than DF plants (Tables 2, 3; Fig. 1). There was also an interaction effect on biomass, corresponding to that for stem height (Tables 2, 3; Fig. 1). An interaction of region \times drought \times fertilizer for mean flowerhead area was also found, implying that Swiss plants, unlike plants from the other regions, had considerably smaller flowerheads under the DF than under the F treatment (Table 2; Fig. 1). Many other traits showed a tendency of weaker effects of fertilizer in DF plants (Table 3; Fig. 1).

Discussion

Variation in responses within and among populations

The application of fertilizer had strong but ambiguous effects on performance of *C. vulgaris* in the present study. The increased mortality and reduced flowering probability in surviving plants following fertilizing (contrary to the findings of Klinkhamer et al. 1991) suggest that these nutrient levels were in themselves harmful to many individuals, a situation common in species of infertile habitats (Chapin 1980; Marschner 1995; Vergeer et al. 2003). In contrast, those of the fertilized plants that did flower had a much higher mean reproductive output, approximated as total flowerhead area, than unfertilized plants. Fertilizer-tolerant individuals were able to utilize the raise in nutrients to increase not only growth and reproduction but also mean seed mass (a trait considered relatively stable; Bradshaw 1965), which may in turn further offspring establishment. This would seem an appropriate response for a monocarpic species (van der Meijden et al. 1992; Sultan 2001), and an ability untypical of plants adapted to nutrient-poor soils (Chapin 1980; Marschner 1995; cf. Chapin and Shaver 1985; Stewart and Nilsen 1995). This also goes for the increase in leaf area, which is a plastic response to increased resource availability considered characteristic of species of more competitive habitats (Dorken and Barrett 2004).

The reaction to the increase in nutrients thus differed fundamentally among individuals within our study populations, some exhibiting an increase and some a decrease in vigour (cf. Sultan and Bazzaz 1993a; Sultan 1995). Our design did not allow us to examine whether this was due to genetic differences among seed families. Some studies (Sultan and Bazzaz 1993a; Black-Samuelsson and Andersson 1997; Leiss and Müller-Schärer 2001) have detected variation in plasticity among genotypes/families within populations, while others (Andersson and Widén 1993; Pigliucci and Kolodynska 2002; Paschke et al. 2003) have not. However, the population \times fertilizer interactions for survival and flowering indicate that populations differ in their average tolerance to fertilizing, i.e. some populations contain higher

proportions of fertilizer-tolerant individuals than others (Fig. 3). This may be due to adaptation to local nutrient regimes (cf. Schlichting 1986; Leiss and Müller-Schärer 2001; Verhoeven et al. 2004). It is uncertain whether our results are applicable to field conditions, where *C. vulgaris* would probably be outcompeted by species with higher capacity to take advantage of increased nutrient levels (see Klinkhamer et al. 1996). However, Eriksson et al. (1995) did not find any significant relationship between occurrence of *C. vulgaris* and nutrient influence in Swedish pastures, so it is possible that high nutrient levels can have both favourable and adverse effects also in natural populations of this species.

On a larger spatial scale, the region \times fertilizer interactions suggest that variation in responses is partly due to geographical origin, but the pattern differs among traits. Plants from the “central” Czech Republic and the “marginal” Sweden showed the strongest mean increase in RGR of leaf length in response to fertilizing. In contrast, Swedish plants suffered the highest mortality following fertilizing, whereas, in particular, plants from the more central Czech Republic and Switzerland were more tolerant. Plants from The Netherlands, at the margin of the species’ continental distribution, increased their flower stem growth following fertilizing, and consequently attained the strongest increase in aboveground biomass. This increase may have come across through reduced allocation to root biomass (cf. Schlichting and Levin 1988, 1990; Sultan and Bazzaz 1993b), which was not measured in this study. A tall flower stem should be favourable in a nutrient-enriched grassland environment, where surrounding vegetation is also likely to grow tall, but only Dutch plants showed plasticity for this trait. The regional pattern for stem height is also quite different from that for RGR of leaf length, which can be viewed as another example of plastic response to increased competition under high-nutrient conditions (Dorken and Barrett 2004). The interaction of region \times drought \times fertilizer for mean flowerhead area merely meant that Swiss plants differed in their reaction to the DF treatment from plants from the other regions. These were the only traits for which plants from different regions differed significantly in strength or direction of response to treatments. No clear geographical pattern of overall plasticity, neither as tolerance to fertilizer nor as possible responses to increased competition, emerged in the present study (Fig. 1), which is perhaps to be expected under the presumption that plasticity is both trait-specific and dependent on habitat, treatment, and/or developmental stage (Bradshaw 1965; Schlichting and Levin 1984, 1990; Sultan 1995; Paschke et al. 2003).

Other studies (Vasseur and Aarssen 1992; Pigliucci and Schlichting 1995; Stewart and Nilsen 1995) also did not find consistent geographical patterns of phenotypic plasticity in response to nutrient supply, when comparing genotypes from areas wide apart. Stewart and Nilsen (1995) attributed this to low genetic divergence among populations; however, in our case this seems less likely, considering the constant among-region phenotypic dif-

ferences observed in *C. vulgaris* offspring grown in a common environment (U. Becker, H. Berg, and D. Matthies, in preparation). Schlichting and Levin (1988, 1990) argued that a lack of among-population divergence in plasticity can be expected in traits strongly linked to the fitness of the organism, since the response of such traits should be evolutionary conservative. This may be the case in the present study, where there were almost no origin \times treatment interactions for purely reproductive traits.

Drought had weaker but generally favourable effects on plant fitness. This indicates that this treatment was closer to optimal conditions for *C. vulgaris*, a plant adapted to dry—mesic habitats (Körner and Meusel 1986), than were non-drought conditions. Water-saturation of the soil can cause decreased aeration of roots (Sultan and Bazzaz 1993a; Pigliucci and Kolodnynska 2002), which may be an explanation for the higher mortality in our C and F plants. We did not measure soil moisture in the pots, but took care that the soil was never waterlogged. The later onset of flowering is contrary to expectations for plants growing in dry habitats (Rajakaruna et al. 2003); alternatively, if the non-drought treatment was the more stressful environment, earlier flowering could be an appropriate response to this. However, the actual difference between drought and non-drought plants with regard to this trait was very small and mainly accounted for by DF versus F plants (Table 3). The drought \times fertilizer interactions for flowering and stem height also suggest that drought may weaken the effects of fertilizing, a tendency seen in many other traits including survival (Table 3; Fig. 1; cf. Chapin and Shaver 1985; Marschner 1995).

Populations differed in their average response to drought, with regard to survivorship (as was also found in *Cochlearia bavarica*; Paschke et al. 2003) and total flowerhead area. Some populations had lower survivorship under drought, i.e., as in the case of fertilizing, they had higher proportions of drought-sensitive (or excess-water-tolerant) individuals. However, these populations were not generally the same as those showing a negative response to drought of total flowerhead area (via reduced number and/or mean area of flowerheads), which does not support the notion of populations adapted to contrasting local water regimes (Fig. 2; cf. Paschke et al. 2003). We also did not find any large-scale geographical pattern of plasticity for drought, e.g. Czech populations showing a more positive response than Dutch and Swedish populations because of adaptation to drier conditions during the growing season (cf. Rajakaruna et al. 2003). Perhaps, in natural populations of *C. vulgaris*, the water levels of our non-drought pots are too infrequent, or of too short duration, for adaptive plasticity for excess of water to evolve (cf. Pigliucci and Kolodnynska 2002). Nevertheless, under drought as well as under non-drought conditions, the majority of plants in most populations managed to survive and reproduce, which can also be regarded as an indication of high plasticity (Sultan and Bazzaz 1993a, b; Sultan 1995).

Effects of population size and isolation on responses

Few studies (Fischer et al. 2000; Kéry et al. 2000; Paschke et al. 2003; Pluess and Stöcklin 2004) have treated effects of population size on phenotypic plasticity. These studies found positive, though rather weak, relationships between population size and strength of response to treatments, which they attributed to genetic erosion in small populations. In the present study, there was a significant population size \times fertilizer interaction for time until flowering, but this could not be explained by any linear, or nonlinear, relationship between population size and strength of response. U. Becker, H. Berg, and D. Matthies (in preparation) found reduced fitness in *C. vulgaris* offspring from small populations; if this is due to genetic erosion, it is not reflected in patterns of plasticity. This may not be surprising, if variation in the mean and in the plasticity of a trait is controlled by independent gene systems (Schlichting 1986; Schlichting and Levin 1990).

We found interactions of population isolation \times drought for RGR of leaf length and width, and negative correlations between isolation and response of the two growth measures. The correlation analyses indicated that plants from less isolated populations responded to drought with a slightly smaller decrease in leaf growth than plants from more isolated populations, though drought in itself had no significant effect on means of RGR (Table 2). This should not simply be interpreted as higher adaptive plasticity, or higher drought-tolerance, in less isolated populations (cf. Paschke et al. 2003); a reduced leaf area may be an adjustment to reduce water loss under dry conditions (Taiz and Zeiger 1991; Sultan and Bazzaz 1993a), and thus plants from the more isolated populations may respond more appropriately in this case. However, we found no correlation between drought response of RGR and any trait at later life stages, so this effect of water supply was probably of little importance for plant fitness. Thus, virtually no indication of an effect of population size or isolation on adaptive phenotypic plasticity was found in this study.

Conclusions

Referring to our initial questions, we may conclude that: (1) *C. vulgaris* offspring originating from different parts of the species' distribution differ in their response to nutrient supply for some traits, but there is no clear geographical pattern of overall plasticity or tolerance to fertilizing; (2) population size has no apparent effect on responses; (3) the negative response to drought of leaf growth increases slightly with increasing population isolation; otherwise, degree of isolation has no apparent effect on responses.

Despite the large geographical area from which the study plants originated, the reaction norms of most traits were similar among regions. The few

region \times treatment interactions found were due to differences in strength rather than direction of response, most apparent in the reaction norm of survival, which shows that Swedish populations are the most sensitive and Czech populations the least sensitive to the harmful effects of fertilizing. For other traits, the patterns of among-region variation were different and less clear. Perhaps it is more meaningful to interpret variation in plasticity on a smaller spatial scale. Individuals within populations exhibited strongly divergent reactions to fertilizing (death, failure to reproduce, or increased reproductive output), and populations within regions differed with regard to the type of reaction dominating. This also goes, however less pronounced, for the reactions of populations to water supply.

The variation in treatment responses among populations could not be attributed to their size or degree of isolation, again despite the wide range of these factors. This does not support the hypothesis that small and isolated populations have lower potential of responding in an appropriate way to environmental changes, although environmental stochasticity in itself is always a stronger threat to such populations (e.g. Kéry et al. 2000; Vergeer et al. 2003). Even if large and non-isolated populations in general have higher genetic diversity, there is no obvious reason why they should show overall stronger or more adaptive responses than less diverse populations (cf. Müller-Schärer and Fischer 2001; Vergeer et al. 2003), just as large and non-isolated populations of high genetic diversity do not necessarily have higher mean fitness than small and isolated populations of low diversity (Ouborg et al. 1991; Lammi et al. 1999; Eisto et al. 2000). If selection operates on plasticity (Bradshaw 1965; Schlichting 1986), its strength and direction may be more dependent on fitness-relevant habitat factors than on population size or isolation, and in a spatially heterogeneous habitat, genotypes with different response patterns will be able to coexist (Sultan and Bazzaz 1993a; Sultan 1995; Leiss and Müller-Schärer 2001). This may partly explain the divergence in reactions to fertilizing within our study populations, where spatial genetic structuring is likely due to short dispersal distances (Klinkhamer et al. 1996; Franzén and Eriksson 2003; cf. Andersson and Widén 1993). The difference between populations of high and low genetic diversity may thus lie in the amount of among-individual or among-family variation in plasticity rather than in the actual mean response (cf. Müller-Schärer and Fischer 2001), which could be a reason for the paucity of strong associations between population size and amount of plasticity found in the literature. The relationships between within-population genetic variation, variation in treatment responses, and heterogeneity of habitat factors could be the focus of future research on the adaptive significance of phenotypic plasticity in plants.

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