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POPULATION SIZE AND IDENTITY INFLUENCE THE REACTION NORM OF THE RARE, ENDEMIC PLANT *COCHLEARIA BAVARICA* ACROSS A GRADIENT OF ENVIRONMENTAL STRESS

MELANIE PASCHKE,[1] GIORGINA BERNASCONI, AND BERNHARD SCHMID
Institute of Environmental Sciences, Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland
[1]*E-mail: paschke@uwinst.unizh.ch*

Abstract.—Habitat degradation and loss can result in population decline and genetic erosion, limiting the ability of organisms to cope with environmental change, whether this is through evolutionary genetic response (requiring genetic variation) or through phenotypic plasticity (i.e., the ability of a given genotype to express a variable phenotype across environments). Here we address the question whether plants from small populations are less plastic or more susceptible to environmental stress than plants from large populations. We collected seed families from small (<100) versus large natural populations (>1000 flowering plants) of the rare, endemic plant *Cochlearia bavarica* (Brassicaceae). We exposed the seedlings to a range of environments, created by manipulating water supply and light intensity in a 2 × 2 factorial design in the greenhouse. We monitored plant growth and survival for 300 days. Significant effects of offspring environment on offspring characters demonstrated that there is phenotypic plasticity in the responses to environmental stress in this species. Significant effects of population size group, but mainly of population identity within the population size groups, and of maternal plant identity within populations indicated variation due to genetic (plus potentially maternal) variation for offspring traits. The environment × maternal plant identity interaction was rarely significant, providing little evidence for genetically- (plus potentially maternally-) based variation in plasticity within populations. However, significant environment × population-size-group and environment × population-identity interactions suggested that populations differed in the amount of plasticity, the mean amount being smaller in small populations than in large populations. Whereas on day 210 the differences between small and large populations were largest in the environment in which plants grew biggest (i.e., under benign conditions), on day 270 the difference was largest in stressful environments. These results show that population size and population identity can affect growth and survival differently across environmental stress gradients. Moreover, these effects can themselves be modified by time-dependent variation in the interaction between plants and their environment.

Key words.—Endemic plant species, environmental stress, genetic variation, phenotypic plasticity, population size, reaction norm.

Recent habitat fragmentation or habitat change lead to widespread reductions in population sizes of plant and animal species (Vitousek 1994). In the resulting small populations, genetic variation is often reduced (Lacy 1987; Raijmann et al. 1994; Fischer and Matthies 1998), presumably due to bottlenecks, inbreeding, or genetic drift (Templeton and Read 1994; Falconer and Mackay 1996). This may lower the persistence of such populations under present conditions and the evolutionary potential to adapt to new conditions. Lowered persistence may, for example, be caused by the higher pathogen susceptibility of genetically homogeneous compared to heterogeneous populations (Schmid 1994) or by the lower vitality of homozygous compared to heterozygous individuals (Oostermeijer et al. 1996; Paschke et al. 2002a). Adaptation to new conditions may be hampered because genetic variation neutral in the present but adaptive in a novel environment is lost by genetic drift (Lynch and Lande 1993; van Tienderen and de Jong 1994).

However, there is debate over the importance of genetic factors relative to environmental and demographic factors in conservation biology (Schemske et al. 1994). Furthermore, widespread colonizing species can also have low genetic variation within their typically small populations, but may have evolved high levels of phenotypic plasticity (i.e., the ability of a given genotype to express a variable phenotype across environments) to cope with environmental heterogeneity (Marshall and Jain 1968; Schmid 1985). A plastic genotype may show a more positive response to a benign or a less negative response to a stressful environment than a less plastic genotype does. Despite the relative fitness advantage of the more plastic genotype over the less plastic one in both cases of environmental change (Schlichting and Pigliucci 1998), there are also limits and costs to plasticity (DeWitt 1998; van Kleunen et al. 2000), and under a longer-term perspective adaptation to new conditions by genetic change will become inevitable (Schmid et al. 1996).

The problem for rare or even endemic species as compared to widespread colonizing species is that plasticity itself can be controlled by genes (Scheiner and Lyman 1991; Schlichting and Pigliucci 1995; Via et al. 1995). If a rare species and its populations only grow under a few, narrow environmental conditions, in which plasticity genes are selectively neutral or even slightly deleterious, these genes will be rapidly lost leading to a reduction of phenotypic plasticity and of the possibility to react to novel environmental conditions by plasticity. Plasticity may also be reduced, in those species lacking an evolutionary history of small population sizes, through increased levels of inbreeding and homozygosity. That plasticity itself has a genetic basis, and that genetic variation in plasticity can be lost by inbreeding or drift, casts doubts on the reliability of plasticity to ensure the ability to cope with novel environments in endangered species. Indeed, species lacking sufficient plasticity to maintain growth and reproduction in degraded environments may be at a particular risk of extinction (Sultan 2000).

There is little empirical evidence for a positive relation in

TABLE 1. Habitat types of *Cochlearia bavarica* following Abs (1999), and number of populations sampled for each population size group.

Features	Habitat type			
	I	II	III	IV
	woodland springs, tufaceous limestone or gravel	woodland springs, fine soil, rich in organic material	calcareous fens	river banks and ditches
Water supply	high and constant	high and constant	high and constant	high and constant
Light availability	seasonally variable	seasonally variable	constant	constant
Nutrient supply	unbalanced; low K and P	sufficient	high	high
Populations sampled	3 large and 4 small	1 large and 1 small	1 large and 2 small	1 small

rare species between population size and the amount of, or genetic variation in, plasticity. Some experiments assessed the effect of small population size on the response to environmental variation (Widén and Andersson 1993). In one case the positive response to benign conditions was reduced (Kéry et al. 2000); in another case the negative response to stressful conditions increased (Fischer et al. 2000) for small compared to large populations. It remains unclear whether phenotypic differences between small and large populations of endangered plants should be more pronounced (and therefore assessed, or populations be preserved) under (1) stressful versus benign conditions (Hoffmann and Parson 1991; Hoffmann and Merilä 1999), (2) novel or rare versus common conditions (Pigliucci et al. 1995), or (3) variable versus constant conditions (Schmid 1985; Sasaki and Ellner 1995).

We used *Cochlearia bavarica* (Brassicaceae; Vogt 1985) as a model species to test whether plants from smaller populations are less plastic in their response to different environments, and whether this leads to lower performance of these plants compared to plants from larger populations under both stressful and benign conditions. We grew seed families from eight small and five large natural populations in a range of experimentally manipulated environments. Population sizes at the time of sampling ranged from 10 to more than 3000 flowering plants but had been declining since the late 1980s (Matthias Berg, Bayerisches Landesamt für Umweltschutz, Munich, Germany, pers. comm. 1995). We knew from previous studies (Paschke et al. 2002a) that the small populations were less variable in their allozyme pattern than the large populations. The benign test environments were similar to the natural ones, whereas the stressful test environments represented less frequent or even novel conditions for the species. Plants were followed from seedling to vegetative adult stages of the life cycle to address changes in reaction norms over time (Schmid 1992). According to the developmental reaction norm concept of Pigliucci (1998), the timing of plant development, including plastic responses to the environment, can itself be plastic. The ability to alter the developmental trajectory in response to the environment therefore may be limited to specific time windows. Furthermore, the timing of responses may vary among genotypes and populations (Sultan 2000).

MATERIALS AND METHODS

Study Species

Cochlearia bavarica Vogt (Brassicaceae) is a perennial herbaceous plant species with specific habitat requirements and a narrow distribution range. It is endemic to Bavaria (Central Europe, Germany), has an allohexaploid karyotype ($2n = 36$) and presumably originated from hybridization between *C. pyrenaica* and *C. officinalis* (Koch et al. 1996, 1998). *Cochlearia bavarica* flowers from May to June and mature seeds are dispersed from July to August. In the field most seeds germinate within the same growing season, and after overwintering the new plants normally remain at the vegetative stage for a second year and flower after their second winter (Abs 1999; under fertilized conditions in the common garden plants mostly flower after their first winter, Paschke et al. 2002b). *Cochlearia bavarica* is usually monocarpic; that is, a high percentage of plants (50–75%) die after first reproduction (Abs 1999).

The typical habitats of *C. bavarica* are calcareous springs, small rivers, and drainage ditches (Table 1; Abs 1999). Water flows through the soil continuously over the entire year, thus leading to a relatively constant and low soil temperature allowing the plants to grow even in winter (Abs et al. 2001). By contrast, light and nutrient supply can vary among the four different habitat types described for the species (Table 1; Abs 1999). In woodland-spring habitats light availability can be low during late spring and summer, due to shading by deciduous trees. Woodland springs occur on two habitat types: (I) tufaceous limestone, formed by the precipitation of calcium carbonate on mosses, or gravel. These habitats are characterized by an unbalanced nutrient supply with low availability of potassium and phosphate. Woodland-spring habitats of type II occur on fine soil, rich in organic material, offering a sufficient nutrient supply. Further, the species can occur in calcareous fens (III) and on the banks of small rivers and drainage ditches (IV), which are characterized by high nutrient supply and seasonally more or less constant light availability. Reproductive and vegetative traits can vary between habitat types. Abs (1999) found the highest reproduction in habitat types I and III, and the lowest reproduction in habitat type II (see also Abs et al. 2001). Importantly, both small and large populations occur in all habitat types and it seems unlikely that small and large populations have adapted to different environmental conditions. Indeed, differences in reproduction between small and large populations could not be attributed to differences in environmental conditions in their native habitats in a previous study (Paschke et al. 2002a).

The known distribution of *C. bavarica* encompasses two regions, west and southeast of Munich (Germany), with a total of 21 sites and 30 populations (Paschke et al. 2002a).

Therefore one site can harbor more than one distinct population. Sites within regions are separated by distances of 500–3000 m, and populations within sites are separated by distances of 50–150 m (Paschke et al. 2002a).

Field Collection of Seed Families in Small and Large Populations

In May 1998, we surveyed 22 of the 30 known populations and recorded the number of flowering plants as an estimate of population size. We selected 13 populations of contrasting size: five large (>1000 flowering plants), and eight small populations (<100 flowering plants). Over half of the populations used in our experiment originated from tufaceous limestone woodland springs (Table 1).

Within each population, we randomly selected 10 maternal plants, and from each plant, we collected five to 20 fruits at maturity in June 1998. We counted the number of inflorescences on each plant. This measure adequately describes maternal plant size (Paschke et al. 2002a). From the collected fruits we obtained 87 ± 47 seeds (mean ± SE) per maternal plant. The offspring (obtained from seeds) from individual maternal plants are called seed families (N = 130 seed families) and probably often consist of maternal half-sibs due to pollination by multiple pollen parents (Paschke et al. 2002b). Seeds were dried and then allowed to germinate on wet filter paper in petri dishes at a 16°C/14 h light and 10°C/10 h dark day-night regime. After 15 days we assessed germination rate. The percentage of seeds that germinated varied among families (range = 2–100%) and between population size groups. Significantly fewer seeds per family germinated in the small than in the large population size group (see Results). In total, seeds from 125 of 130 seed families (96%) germinated (94%; i.e., 75 of 80 families from small populations and 100%; i.e., 50 of 50 families from large populations). In some families fewer seeds germinated than would have been needed for replicates over all environments. These families were excluded leading to a final sample size of 119 seed families: 71 of 80 seed families (89%) for the eight small, and of 48 of 50 seed families (96%) for the five large populations. Moreover, the percentage of seeds that germinated for each family included in the experiment was used as a covariate in the initial model for the analysis of the growth responses of plants across experimental environments, to correct for the potential bias of among-family and among-population variation in germination success.

Thirty days after transferring seeds to petri dishes (one seed family per dish), we measured the height (length of the longest leaf to the nearest mm) of three randomly chosen seedlings within each petri dish (initial seedling height). After an additional 21 days, the seedlings were transferred to the greenhouse and exposed to experimental conditions (see below). The day on which they were transferred to the greenhouse is referred to in the following as day 1 of the experiment.

Greenhouse Experiment with Different Test Environments

Plants were exposed to a 2 × 2 factorial design in which water supply and light intensity were manipulated to create different stress levels (Fig. 1). In the high-water group, we watered the plants daily for 5 min, and the soil was always wet. In the low-water group, we watered the plants every seven days for 10 min, so that the soil had enough time to become completely saturated but dried out between waterings. Because *C. bavarica* is restricted to habitats with continuous and high water supply, we considered the high-water condition as the natural one and the low-water condition as the novel and presumably more stressful environment for the plants. Within each water treatment, plants were either under natural, and hence seasonally variable, daylight (high light), or light intensity was reduced by 53% (low light) using a plastic box (Wavibox, 31620A, GVZ-Boltec, Zurich, Switzerland). Under natural conditions light availability is heterogeneous among habitat types (Table 1). At deciduous woodland-spring habitats (types I and II) shading by ash and alder can reduce light availability, except from autumn to spring. In the open habitats (types III and IV) shading only occurs by other herbaceous species and light availability is therefore more constant throughout the year. Based on this a priori knowledge, we assumed that stress should be highest when water was scarce, increasing in the order high water/high light < high water/low light < low water/high light < low water/low light. Nutrient supply also varies under natural conditions but was kept at a constant, presumably not stressful level in the experiment (standardized substrate with balanced nutrients, 1/3 sand and 2/3 soil; BF4, Tref Substrate, Coevorden, The Netherlands).

Whenever possible we planted 12 seedlings of each seed family, three to each of the four experimental environments described above. In 16 of the 119 families we had only enough material to plant one or two seedlings in some environments. Thus, in total there were n = 1406 plants at day 1 of the greenhouse experiment (Fig. 1). We took care to plant seedlings of the same family in different quick-pot trays. There were 10 or 11 trays with 54 pots per tray for each treatment level. Within trays, families were assigned randomly to pots. Plants were transplanted to larger-sized quick-pot trays (35 pots per tray) on day 210, when their position between and within the trays was re-randomized. Trays were distributed over two tables and were re-randomized between the tables but within the same environment every four weeks. Half of each table was used for the high- and the other half for the low-water treatment, and trays with high and low light were physically interspersed (Fig. 1A). The re-randomization procedures described above ensured that confounding of water-treatment with table effects was virtually excluded.

We counted the leaves and measured plant height as the distance between the soil and the uppermost leaf-tip of the plant (pulling the leaf up along the measuring ruler) for each plant on day 60, 120, 210, and 270. As an overall estimate of plant performance, we used total plant size defined as the product of the number of leaves and plant height (Fig. 1B). This was considered the best nondestructive measure of total plant size for *C. bavarica* in previous work (Paschke et al. 2002a). Considering the generally strong allometries between plant size and reproduction, further growth, and clonal propagation (see e.g. Harper 1977; Schmid and Weiner 1993; Schmid et al. 1995), we used this size measure as an estimate of plant performance and lifetime fitness. On day 120 and

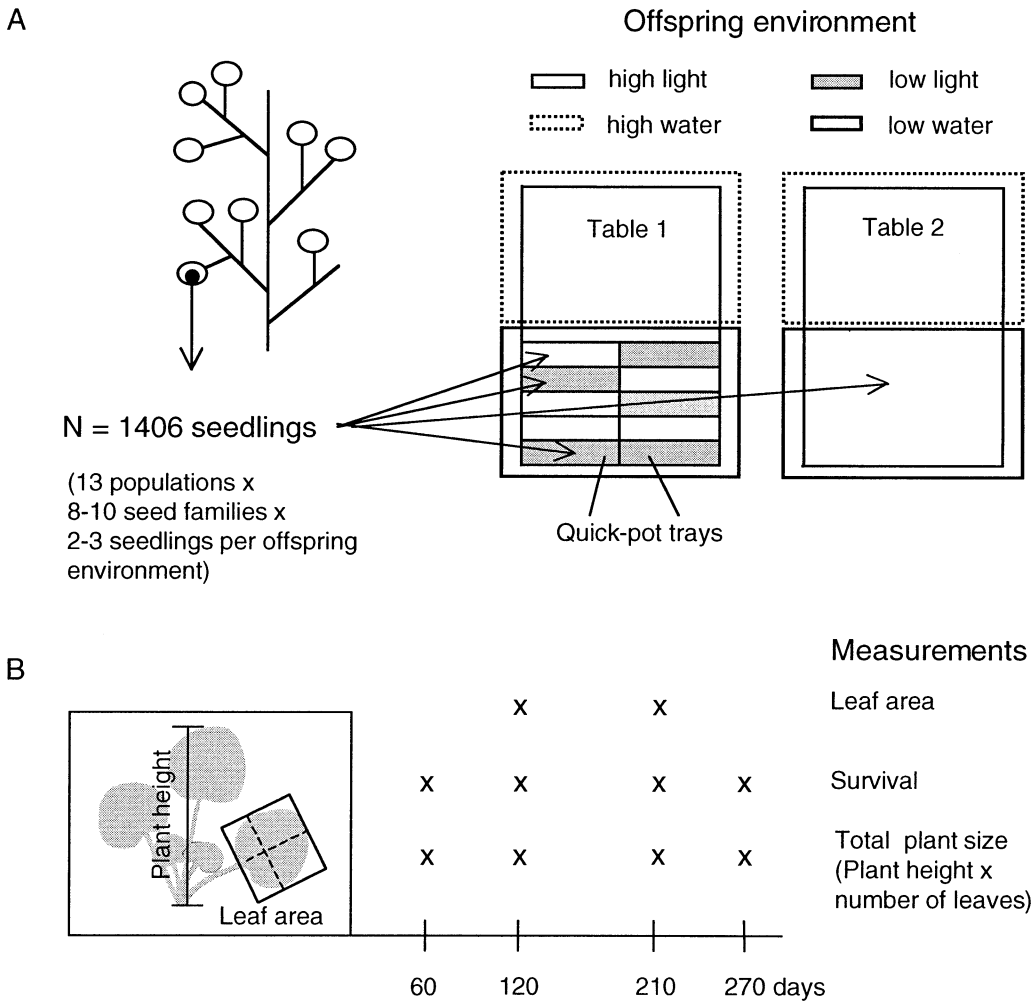

FIG. 1. (A) Experimental design: 1406 seedlings from 125 seed families (classified by maternal plant) from five large and eight small populations were grown on two tables in a greenhouse. Two factorially crossed two-level (high/low) treatments were applied: water (applied to half-tables) and light (applied to trays). Three (rarely two) seedlings per family were randomly assigned to different trays within each test environment. Trays were re-randomized within and between tables (see Materials and Methods). (B) Definition and schedule of variables measured.

day 270, we also estimated leaf area for one randomly selected leaf per plant (leaf length × leaf width, Fig. 1B). We recorded plant survival on census days 60, 120, 210, and 270. A plant that was brown and dry was considered dead. Plant survival was very high during the first 270 days. On day 270, the water supply was interrupted for a week. Thereafter plants started to wilt and mortality was so high that we harvested the aboveground parts of all plants on day 300, before they had started to produce inflorescences. We dried the plants (120 h/65°C) to determine their dry mass.

Statistical Analysis

We analyzed the effect of environmental manipulation and of population size group and population identity on total plant size and leaf area with repeated-measures analysis of variance (ANOVA) using a split-plot approach with averaged *F*-tests for within-subject effects (Hand and Taylor 1991; as implemented in SPSS 10.0; SPSS 2000). Degrees of freedom were adjusted with Greenhouse-Geisser Epsilon (SPSS 10.0; SPSS 2000).

In addition, all offspring traits (i.e. survival rate and dry mass measured one single time, and each repeated measure of plant size and leaf area) were analyzed in separate ANOVAs to gain detailed insight into treatment effects at the different measurement times. In repeated-measures and separate ANOVAs, appropriate *F*-values were calculated as described in Table 2. Offspring environment was tested against the random effect of environment-by-population identity in the repeated-measures ANOVA, but against the random effect of tray in the separate ANOVAs (tray was not available as a term in the repeated-measures analyses because of replanting to new trays between measurements). We fitted the linear effect of offspring environment quality (as measured by the mean performance of all plants in each environment; see stability analysis, Bell et al. 2000), the deviation from this linear contrast, and all corresponding interactions. Mean performance per environment was different for each mea-

TABLE 2. Dummy analysis of variance table for field-collected seed families (maternal plant identity) of *Cochlearia bavarica*. We fitted the terms sequentially and calculated appropriate *F*-values. Maternal-plant-identity interactions were not included in the model and are pooled with the residual term. Square brackets indicate nesting. See text for explanation of terms.

Source of variation	Mean square	Variance ratio
Maternal plant size (covariate)	MS_M	MS_M/MS_F
Germination rate (covariate)	MS_G	MS_G/MS_F
Initial seedling height (covariate)	MS_S	MS_S/MS_F
Offspring environment (linear)	$MS_{E(lin)}$	$MS_{E(lin)}/MS_T$
Offspring environment (deviation)	$MS_{E(dev)}$	$MS_{E(dev)}/MS_T$
Tray [offspring environment]	MS_T	MS_T/MS_R
Population size group	MS_{PS}	MS_{PS}/MS_P
Population identity [population size group]	MS_P	MS_P/MS_F
Maternal plant identity [population identity]	MS_F	MS_F/MS_R
Offspring environment (linear) × population size group	$MS_{E(lin)\times PS}$	$MS_{E(lin)\times PS}/MS_{E(lin)\times P}$
Offspring environment (deviation) × population size group	$MS_{E(dev)\times PS}$	$MS_{E(dev)\times PS}/MS_{E(dev)\times P}$
Offspring environment (linear) × population identity [population size group]	$MS_{E(lin)\times P}$	$MS_{E(lin)\times P}/MS_{E(lin)\times F}$
Offspring environment (deviation) × population identity [population size group]	$MS_{E(dev)\times P}$	$MS_{E(dev)\times P}/MS_R$
Offspring environment (linear) × maternal plant identity [population identity]	$MS_{E(lin)\times F}$	$MS_{E(lin)\times F}/MS_R$
Residual	MS_R	

surement period and therefore this linear effect was not considered for the repeated-measures ANOVAs. Maternal plant size, mean germination rate, and mean initial seedling height (means for the seeds from each maternal plant) were used as covariates. Maternal plant size and germination rate deviated significantly from a normal distribution. They were therefore square-root and arcsine-square-root transformed, respectively.

For interpretation, there are several terms of variance in ANOVA that reveal major sources of observed phenotypic variation (described e.g. in Schmid and Dolt 1994; Pigliucci et al. 1995). Environmental effects point to phenotypic plasticity (adaptive and nonadaptive). Population-size-group, population-identity and maternal-plant-identity effects reveal genetic (plus potentially maternal) variation for the across-environment offspring character means. Finally, significant environment × population size and environment × population-identity interactions indicate genetic (plus potentially maternal) variation among the populations for plastic response. These interaction terms characterize variation in the mean reaction norm of the population size groups and the populations. Maternal-plant-identity × environment interactions characterize variation among seed families within populations in reaction norms.

RESULTS

Effects of Time of Measurement on Offspring Traits

The time of measurement had a highly significant effect on plant size and leaf area (Table 3), reflecting seasonal variation in the growth patterns of plants. Overall, total plant size increased from day 1 (August 1998) to day 120 (December 1998; Fig. 2). From day 120 to day 210 (March 1999), plant size decreased as a consequence of leaves wilting and being replaced by new ones. Then, from day 210 to day 270 (May 1999), plant size increased again. The same pattern was found for leaf area, which increased until day 120 and decreased afterwards. The number of leaves increased steadily over time, also during winter, as is typical for *C. bavarica* in the field (Abs et al. 2001): from 6.20 ± 0.04 leaves (mean ± SE) on day 60 to 9.06 ± 0.08 on day 120, 9.55 ± 0.08 on day 210, and 16.8 ± 0.28 on day 270. In contrast, the length of the longest leaf increased from day 60 to day 120, and decreased from day 120 to day 270. Average plant height changed from 4.67 ± 0.04 cm on day 60 to 5.04 ± 0.05 cm on day 120, 3.43 ± 0.04 cm on day 210, and 3.18 ± 0.04 cm on day 270.

Effects of the Environmental Treatment on Offspring Traits

We found highly significant effects of offspring environment on all measured traits of offspring performance (Table 3). In the separate ANOVAs, the linear contrast of offspring environment was always significant but also the deviation from linearity was often significant (Table 4). A significant time × environment interaction in the repeated-measures analysis (Table 3) indicated that the effects of the environmental treatments on the plants were not consistent over time. Our assumption was that stress was highest when water was scarce, increasing in the order high water/high light < high water/low light < low water/high light < low water/low light. However, we found a more complex pattern and the relative rank of the environments changed with time and thus with plant development. We found that the low-water treatments were, on average, more stressful than the high-water treatments for the period 60–210 days (Fig. 2) and that the low-water/high-light treatment was overall the most stressful for the plants. However, on day 270, the relative ranking of environments as assessed through plant performance changed: low-light treatments were in the mean more stressful than high-light treatments. This indicates that environmental factors did not affect plants in the same way at all growth stages, and suggests that plant sensitivity to water or light stress was particularly acute during some specific time windows.

Effects of Population Size on Covariates and Effects of Covariates on Offspring Traits

Germination rate of the seeds of each maternal plant was significantly lower in the small than in the large population size group (0.62 ± 0.03% vs. 0.75 ± 0.04%, mean ± SE; Table 5). Initial seedling height (mean per maternal plant) was not significantly affected by population size group (small

TABLE 3. Repeated-measures analysis of variance for plant size (leaf number × leaf height) from day 60 to day 270 and leaf area (leaf length × leaf width) from day 120 to day 210. Calculations of *F*-values follow Table 2. Degrees of freedom are adjusted by Greenhouse-Geisser Epsilon for plant size. For leaf area no correction was necessary because there were only two measuring dates.

Source of variation	Total plant size day 60–270				Leaf area day 120–270			
	df	MS	*F*	*P*	df	MS	*F*	*P*
Between-subject effects								
Maternal plant size	1	1388.58	4.93	<0.05	1	46.02	18.50	<0.005
Germination rate	1	132.60	0.47		1	7.69	3.09	
Initial seedling height	1	15656.53	55.58	<0.005	1	54.90	22.06	<0.005
Offspring environment	3	9086.52	54.23	<0.005	3	67.59	38.65	<0.005
Population size group	1	10960.74	2.24		1	12.69	0.50	
Population identity	11	4895.57	17.38	<0.005	11	25.47	10.24	<0.005
Maternal plant identity	106	281.69	1.68	<0.005	106	2.49	1.42	<0.01
Offspring environment × population size group	3	975.25	2.23		3	3.01	0.81	
Offspring environment × population identity	33	437.91	2.61	<0.005	33	3.72	2.13	<0.005
Offspring environment × maternal plant identity	300	167.55	2.17	<0.005	301	1.75	1.01	
Between-subject residual	826	77.12			832	1.74		
Within-subject effects								
Time	2.2	156325.91	49.46	<0.005	1	1389.53	227.57	<0.005
Time × maternal plant size	2.2	1606.83	3.06	<0.05	1	3.55	2.13	
Time × germination rate	2.2	3628.46	6.90	<0.005	1	14.38	8.63	<0.005
Time × initial seedling height	2.2	3160.96	6.01	<0.005	1	6.11	3.67	<0.05
Time × offspring environment	6.7	17370.69	55.65	<0.005	3	256.39	185.52	<0.005
						1.85		
Time × population size group	2.2	3789.16	2.08		1	7.97	0.23	
Time × population identity	24.5	1825.78	3.47	<0.005	11	1.67	4.79	<0.005
Time × maternal plant identity	236.3	525.73	1.68	<0.005	106	2.31	1.20	<0.10
Time × offspring environment × population size group	6.7	722.43	1.19		3	2.66	0.87	
Time × offspring environment × population identity	73.6	606.28	1.94	<0.005	33	1.38	1.92	<0.005
Time × offspring environment × maternal plant identity	668.9	312.13	0.80		301	1.38	0.82	
Within-subject residual	1841.7	389.75			832	1.68		

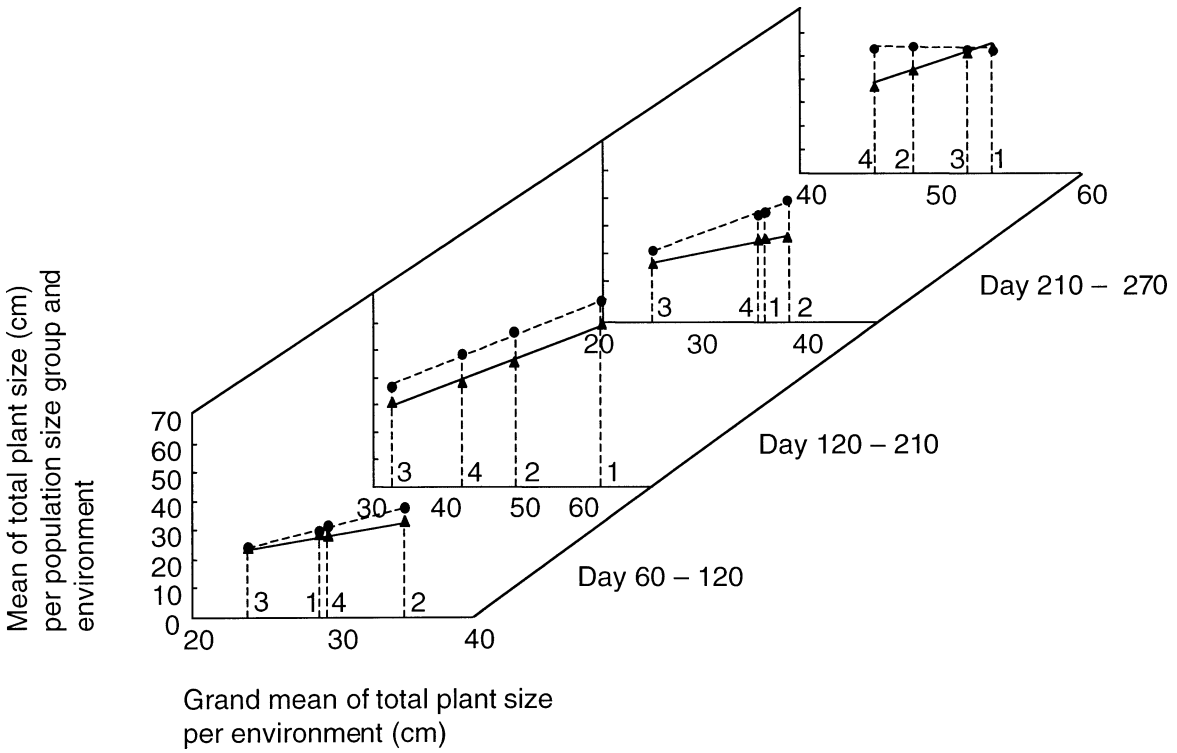

FIG. 2. Growth response (total plant size in cm) of *Cochlearia bavarica* offspring derived from maternal plants of large (circles) versus small (triangles) populations in four test environments: high water/high light (1), high water/low light (2), low water/high light (3), low water/low light (4); x-axis: mean total plant size for each environment in ascending order. The rank order of environments, in terms of offspring performance, changed through time.

populations: 0.34 ± 0.34 cm, large populations: 0.38 ± 0.38 cm; Table 5). However, the effect of population size group was tested against the highly significant effect of population identity (Table 5).

Effects of the covariates maternal plant size, germination rate, and initial seedling height were often significant for offspring characters in the separate ANOVAs (Table 4) and in the repeated-measures ANOVA (Table 3). Maternal plant size was significantly positively correlated with leaf area on day 120 and marginally on day 270 (Table 4). Germination rate and initial seedling height were positively correlated with total plant size, leaf area, and dry mass on day 300 (Table 4). Germination rate was positively correlated with initial seedling height (Table 5). Maternal plant size was not significantly correlated with germination rate and initial seedling height.

Effects of Population Size, Population Identity, and Maternal Plant Identity on Offspring Traits

In ANOVA, significant effects of population size group, population identity, and maternal plant identity reveal genetic variation (plus potentially maternal variation; see e.g. Schmid and Dolt 1994) for trait mean values across environments. Variation among maternal plants; that is, seed families, within populations was large and significant (Table 3; Table 4) for most offspring traits, except leaf area on day 120 and survival on day 300. Because the species is self-incompatible (Hock 2000), each maternal plant probably represented a different genotype, explaining the large variation among seed families, despite the fact that seed families are probably half-sibs which will increase within family variation as well.

There was substantial and significant variation among populations within population size groups for all offspring traits (Table 3; Table 4). Most likely due to this large within-component of variance, the differences between the two population size groups, averaged across environments, at best reached only marginal significance (Table 4; see also the low-performing large population no. 23, labeled in Figs. 3–4). Nevertheless, seed families derived from large populations usually did show higher plant performance than seed families derived from small populations. Offspring from the large population size group had greater total plant size (60–270

TABLE 4. Analysis of variance for total plant size, leaf area, dry mass, and plant survival (% offspring per maternal plant family) shown separately for each time of measurement. *F*-values were calculated according to Table 1.

	Total plant size after 60 days				Total plant size after 120 days			
Source of variation	df	MS	*F*	*P*	df	MS	*F*	*P*
Maternal plant size	1	217.8	1.23		1	1214.0	2.21	
Germination rate	1	316.6	1.79		1	3477.7	6.33	<0.05
Initial seedling height	1	3420.9	19.39	<0.005	1	24994.5	45.52	<0.05
Offspring environment (linear)	1	20492.6	265.46	<0.005	1	82445.7	182.98	<0.005
Offspring environment (deviation)	2	420.0	5.44	<0.05	2	38457.7	85.35	<0.005
Tray	37	77.2	0.88		37	450.6	1.52	<0.05
Population size group	1	1950.4	0.83		1	13499.5	1.90	
Population identity	10	2338.6	13.26	<0.005	11	7090.9	12.91	<0.005
Maternal plant identity	106	176.4	2.01	<0.005	106	549.1	1.85	<0.005
Offspring environment (lin.) × population size group	1	294.2	0.36		1	879.7	1.17	
Offspring environment (dev.) × population size group	2	252.3	0.50		2	148.1	0.28	
Offspring environment (lin.) × population identity	11	819.3	6.89	<0.005	11	753.6	2.36	<0.05
Offspring environment (dev.) × population identity	22	504.0	5.75	<0.005	22	523.7	1.77	<0.005
Offspring environment (lin.) × maternal plant identity	104	118.8	1.36	<0.05	104	319.1	1.08	
Residual	1071	87.6			1063	296.6		

TABLE 4. Continued.

	Leaf area after 120 days				Leaf area after 270 days			
Source of variation	df	MS	*F*	*P*	df	MS	*F*	*P*
Maternal plant size	1	10.93	5.72	<0.05	1	7.83	3.30	<0.10
Germination rate	1	0.31	0.16		1	24.10	10.17	<0.005
Initial seedling height	1	60.18	31.51	<0.005	1	14.07	5.94	<0.05
Offspring environment (lin.)	1	23.24	18.02	<0.005	1	21.69	8.03	<0.01
Offspring environment (dev.)	2	351.13	272.19	<0.005	2	140.32	51.97	<0.005
Tray	37	1.29	0.81		61	2.70	1.60	<0.0005
Population size group	1	4.66	0.23		1	12.70	0.86	
Population identity	11	19.91	10.42	<0.005	11	14.70	6.20	<0.005
Maternal plant identity	106	1.91	1.19		106	2.37	1.40	<0.01
Offspring environment (lin.) × population size group	1	0.10	0.03		1	1.25	0.34	
Offspring environment (dev.) × population size group	2	2.19	0.58		2	2.47	1.03	
Offspring environment (lin.) × population identity	11	3.22	2.24	<0.005	11	3.71	2.10	<0.05
Offspring environment (dev.) × population identity	22	3.75	2.34	<0.005	22	2.40	1.42	<0.10
Offspring environment (lin.) × maternal plant identity	104	1.44	0.90		104	1.76	1.04	
Residual	1066	1.60			955	1.69		

days; Fig. 2), final dry mass (Fig. 4C), and survival than did offspring from the small population size group.

Interactions

Significant environment × population-size and environment × population-identity interactions indicate genetic variation among the populations in their phenotypic response to the different environments; that is, different population reaction norms or plasticities. Maternal-plant-identity × environment interactions characterize variation in the reaction norms among seed families within populations; that is, genetic (plus potentially maternal) variation in plasticity within populations.

The interaction between the linear contrast (increasing stress) of offspring environment and population size was significant for total plant size on day 210 and marginally so on day 270 (Table 4). For survival up to 300 days, the interaction between the deviation from the linear contrast of offspring environment and population size was also marginally significant (Table 4). On day 210, positive size differences between plants derived from large versus small populations were most pronounced in benign environments (i.e., environments with high mean plant performance). In contrast, on day 270 positive size differences between plants derived from large versus small populations were most pronounced in stressful environments (i.e., environments with low mean plant performance, at this time the environment with low-water/low-light conditions; Fig. 2). These results indicate that plants derived from small populations on average express lower amounts of adaptive plasticity (Schmid 1992; Fischer et al. 2000), but at some times this makes them perform worse in benign, at other times perform worse in stressful, environments than plants derived from large populations. Nevertheless, the three-way interaction time × environment × population size was not significant in the repeated-measures analysis (Table 3), again due to large variation among plants derived from different populations within each population size group, especially one low-performing large population (no. 23, labeled in Figs. 3 and 4).

This variation among plants from different populations was reflected in large and highly significant environment × population-identity interactions (Table 3; Table 4), or time ×

TABLE 4. Extended.

Source of variation	Total plant size after 210 days				Total plant size after 270 days			
	df	MS	*F*	*P*	df	MS	*F*	*P*
Maternal plant size	1	87.3	0.20		1	1031.1	1.06	
Germination rate	1	1180.4	2.74		1	3092.2	3.16	<0.10
Initial seedling height	1	27760.6	64.54	<0.005	1	18838.1	19.28	<0.005
Offspring environment (linear)	1	37988.1	108.79	<0.005	1	17041.5	11.73	<0.005
Offspring environment (deviation)	2	127.4	0.36		2	386.9	0.27	
Tray	37	349.2	1.63	<0.01	61	1452.9	2.44	<0.005
Population size group	1	28104.4	4.46	<0.10	1	10952.5	1.49	
Population identity	11	6302.6	14.65	<0.005	11	7354.9	7.53	<0.005
Maternal plant identity	106	430.1	2.01	<0.005	106	977.2	1.64	<0.005
Offspring environment (lin.) × population size group	1	2867.5	5.19	<0.05	1	10887.0	4.47	<0.10
Offspring environment (dev.) × population size group	2	473.7	1.17		2	77.0	0.05	
Offspring environment (lin.) × population identity	11	552.9	2.80	<0.01	11	2436.8	4.29	<0.005
Offspring environment (dev.) × population identity	22	404.5	1.9	<0.01	22	1502.9	2.52	<0.005
Offspring environment (lin.) × maternal plant identity	104	197.2	0.92		104	568.4	0.95	
Residual	1057	213.6			953	595.6		

TABLE 4. Continued, extended.

Source of variation	Dry mass after 300 days				Survival after 300 days			
	df	MS	*F*	*P*	df	MS	*F*	*P*
Maternal plant size	1	0.012	1.00		1	0.007	0.17	
Germination rate	1	0.001	0.08		1	0.202	4.85	<0.05
Initial seedling height	1	0.254	21.16	<0.005	1	0.023	0.56	
Offspring environment (lin.)	1	4.250	472.22	<0.005	1	39.351	531.60	<0.005
Offspring environment (dev.)	2	0.018	2.00		2	0.048	0.64	
Tray	61	0.009	1.00		55	0.074	1.45	<0.05
Population size group	1	0.192	2.87		1	0.365	1.86	
Population identity	11	0.067	5.58	<0.005	11	0.196	4.72	<0.005
Maternal plant identity	106	0.012	1.33	<0.05	106	0.042	0.81	
Offspring environment (lin.) × population size group	1	0.002	0.29		1	0.069	0.83	
Offspring environment (dev.) × population size group	2	0.010	1.11		2	0.081	2.76	<0.10
Offspring environment (lin.) × population identity	11	0.007	0.78		11	0.084	1.91	<0.05
Offspring environment (dev.) × population identity	22	0.009	1.00		22	0.029	0.57	
Offspring environment (lin.) × maternal plant identity	104	0.009	1.00		104	0.044	0.85	
Residual	953	0.009			127	0.051		

TABLE 5. Analysis of variance for germination rate and initial seedling height (maternal plant means).

Source of variation	Germination rate				Initial seedling height			
	df	MS	*F*	*P*	df	MS	*F*	*P*
Maternal plant size	1	0.05	1.27		1	0.004	0.92	
Germination rate	—	—	—		1	0.018	4.13	<0.05
Population size group	1	0.54	3.93	<0.10	1	0.041	2.11	
Population identity	11	0.152	4.11	<0.005	11	0.019	4.34	<0.005
Residual (maternal plant identity)	111	0.04			110	0.004		

environment × population-identity interactions (Table 3), on total plant size (Fig. 3), leaf area (Fig. 4A, B), dry mass (Fig. 4C), and survival of plants on day 300. This reinforces the observation already made for populations of different size; that is, that populations of different identity not only show differential offspring performance across environmental stress gradients, but also that the different population reaction norms are themselves modified by time-dependent variation in the interaction between plants and particular environments.

In contrast, and despite the huge statistical power, the offspring environment × maternal-plant-identity interaction was rarely significant (total plant size on day 60 and in repeated-measures analysis; Tables 3 and 4). The significant cases may reflect maternal carryover effects influencing early characters (Roach and Wulff 1987; Schmid and Dolt 1994).

DISCUSSION

In this study, we exposed seed families from small and large natural populations of the rare, endemic plant *Cochlearia bavarica* to a range of experimentally manipulated environmental stress conditions and followed their growth and survival over 300 days. In the following, we will discuss (1) how the plants responded to the different environments over time, (2) the nature of variation among and within populations in mean offspring performance across environments, and (3) interactions between population size, population identity, and seed family, and effects of the different environments over time.

What Are Stressful Environments for Cochlearia bavarica?

For interpretation of the results of this experimental study it is necessary to know whether the environments simulated in the greenhouse were indeed stressful to a different degree for *C. bavarica*. Stress can be empirically estimated through phenotypic responses of the plant, either because it directly lowers plant performance via negative effects on growth and survival (e.g. Aronson et al. 1992; Tang and Turner 1999), or because it induces compensatory changes to restore normal

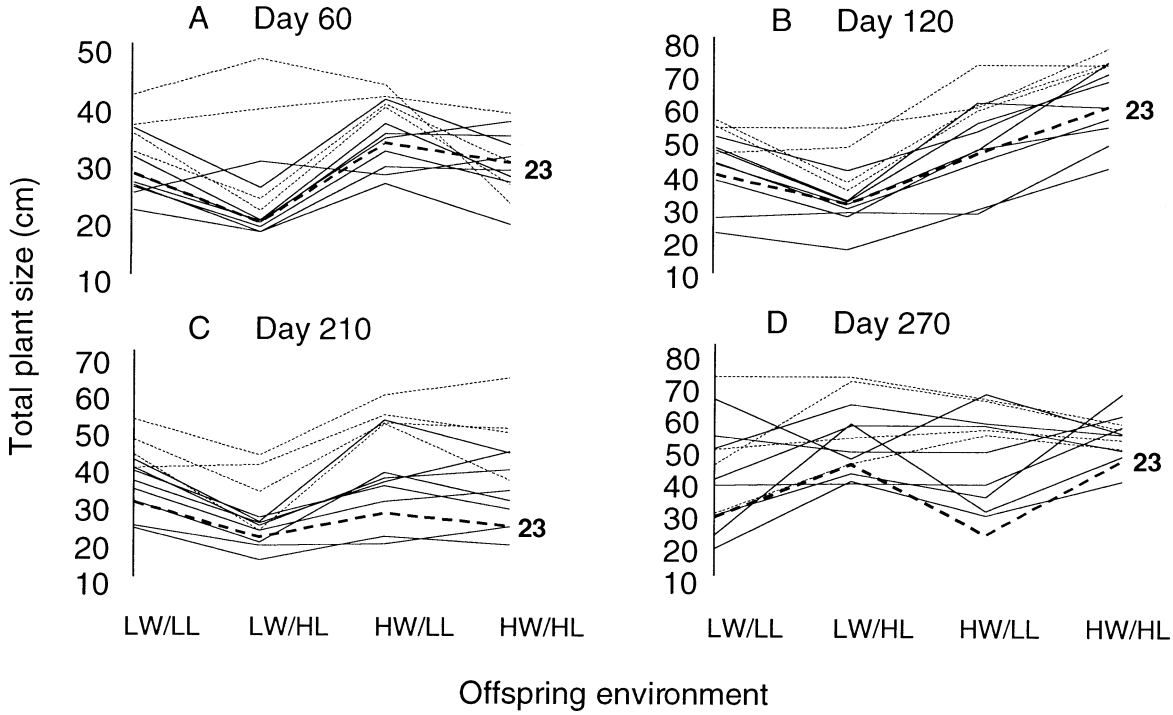

FIG. 3. Reaction norms for total offspring plant size after (A) 60, (B) 120, (C) 210, and (D) 270 days of five large (dashed lines) and eight small populations (solid lines). One low-performing large population (no. 23) is marked specifically. Environments on the x-axis are ordered in the sequence of presumably decreasing stressfulness: LW/LL, low water/low light; LW/HL, low water/high light; HW/LL, high water/low light; HW/HL, high water/high light.

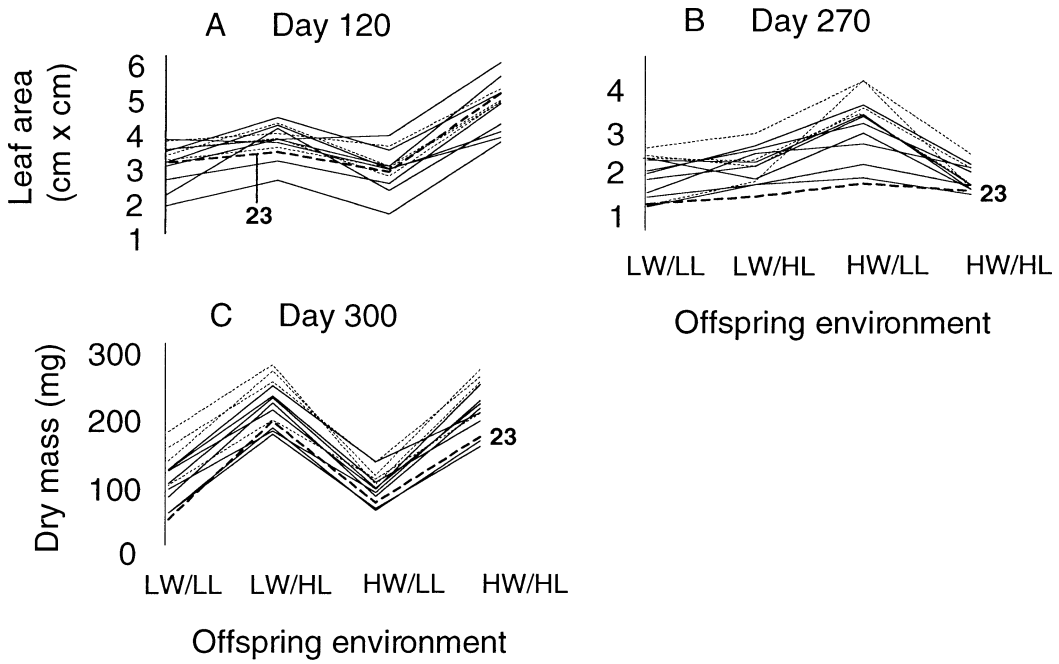

FIG. 4. Reaction norms for leaf area after (A) 120, (B) 270 days, and for (C) dry mass after 300 days, for five large (dashed lines) and eight small (solid lines) populations. One low-performing large population (no. 23) is marked specifically. Environments on the x-axis are ordered in the sequence of presumably decreasing stressfulness (see Fig. 3).

plant growth and survival (e.g., induced changes in allocation patterns; Bell and Sultan 1999). We used differences in total plant size and leaf area as indicators of reduced plant performance to evaluate which environments were more stressful than others.

The significant effects of offspring environment on offspring characters showed that there is phenotypic plasticity in the response of *C. bavarica* to environmental variation, and the differences imply that some experimental environments are more stressful for the plants. Based on the a priori knowledge that *C. bavarica* only occurs in natural habitats with continuous water supply, we assumed that stress should be highest when water is scarce, increasing in the order high water/high light $<$ high water/low light $<$ low water/high light $<$ low water/low light. This prediction was crudely correct, but including the combination with light, which can vary over the year at natural habitats, the pattern became more complex. Indeed, the relative ranking of the environments according to stress changed with time (see different x-axes in Fig. 2) and thus with plant development. In the period from day 60 to day 210, high light, in particular with low water, was more stressful than low light (contrary to prediction); thereafter, low light was more stressful than high light (as predicted). This effect cannot be ascribed to mutual shading of the plants, because the distance among plants was sufficient throughout the experiment to prevent mutual shading. Rather, these results suggest that light conditions had different biological relevance during the first phase of the experiment from late summer through winter than during the second phase; that is, the following spring. Plants of different age or developmental stage may interact differently with experimental environments, experiencing them as more or less stressful over time. Thus, when compared between census days, the four experimental environments may best be treated as distinct sets of environments, rather than representing one invariant set. To describe such changes in plant reaction norms with time, Pigliucci (1998) introduced the concept of developmental reaction norm. Clearly, the expression of the same quantitative trait in a plant may be ''caused'' by different, more or less correlated, gene actions in different environments and at different stages of size and development.

Is There Genetic Variation for Offspring Traits in Cochlearia bavarica?

Germination rate and seedling height (means for seed families) were positively correlated with subsequent offspring performance. Seeds in families with high germination rate yielded larger seedlings, which developed into larger plants in the greenhouse. This may indicate genetic variation among maternal plants within populations or effects of common maternal or germination environment of individuals within seed families (all members of the same seed family germinated in the same petri dish). The significant variation among populations in germination rate and initial seedling height could not be explained by possible confounding effects of common germination environment (because families of the different populations were randomly mixed during germination), so that this variation presumably reflected genetic differences or common maternal environment at the level of entire populations.

During the greenhouse experiment, all plants were randomized and effects of common maternal and germination environment were accounted for by fitting the covariates ma-

ternal plant size, germination rate, and initial seedling height prior to any other factors in the ANOVAs (Roach and Wulff 1987). Thus, the subsequently fitted significant effects of population size group, of population identity within population size group, and of maternal plant identity within population on offspring traits measured in the greenhouse, except perhaps the earliest traits, probably reflected positive genetic components of variance (Schmid and Dolt 1994). The most significant of these components of variance for offspring traits were those due to population identity, followed closely by those due to maternal plant identity (see Tables 2 and 3). From this it can be concluded that there is both genetic differentiation among populations as well as heritability within populations for trait mean values across the four experimental environments, which is consistent with earlier observations about the distribution of allozyme variation among and within the same populations (Paschke et al. 2002a).

In view of the large variation among populations within population size groups, effects between population size groups were rarely significant. Nevertheless, offspring from the eight smaller populations had generally lower performance than offspring from at least four of the five larger populations (see Figs. 3 and 4). If environmental variables were correlated with population size, they could, through maternal carryover effects that were not eliminated by including the three above-mentioned covariates in the analysis, explain the differences between large and small populations (Oostermeijer et al. 1994). However, in the field, small and large populations occurred at all habitat types and this was taken into account for the sampling of populations (see Materials and Methods). Therefore we conclude that the lower mean performance of offspring from small populations also reflected mainly genetic differences.

Differences among and within Populations in the Response to Environmental Stress

Significant interactions of environment with population size group (on day 210 and marginally on day 270) and population identity indicated differences in the mean reaction norms between small versus large populations and among populations within size groups. However, among seed families within populations there was almost no indication for variation in the response to environmental stress. This indicates that within-population variation in plasticity had no genetic basis; that is, zero heritability. This is in strong contrast to the large within-population variation and presumed heritability in mean trait values (see previous section) and suggests that heritable variation for plasticity may have been eliminated from both large and small populations of *C. bavarica* by other or additional means than the suggested genetic drift in small populations. An experimental selection study using *Ranunculus reptans*, another endangered plant species in central Europe, recently made a similar observation for heritable variation in trait means but none in trait plasticities (van Kleunen et al. 2002). However, in the present study the variation in plasticities was only absent within populations. The large variation in plasticities among populations, which was in part due to differences in population size, lends support to the hypothesis that the potential to respond to environmental variation and stress was differently developed in these populations.

One explanation for differences in mean reaction norms among populations is the ''ecological hypothesis'' (Schlichting and Levin 1984; Donohue et al. 2001). This hypothesis predicts that if each population has adapted to the environmental variations characterizing its natural site, the pattern of selection on plasticity differs from one site to another and this in turn accentuates the differences in reaction norms among rather than within populations. However, in the case of the endemic *Cochlearia bavarica*, the known habitats of the different populations were characterized by low and similar environmental variation and for all populations the experimental environments with low water were least natural and most stressful (see Materials and Methods). Thus, divergent selection on plasticity among sites does not seem to be the most likely cause for our results. Alternatively, in endemic species with a history of bottlenecks, isolation and genetic drift among populations may better explain variations in mean population reaction norms; that is, variation in plasticity among populations (see introduction and e.g. Karron 1987). Although the genetic variation affected by genetic drift is thought to be neutral or under weak selection under normal environmental conditions, the loss of this neutral genetic variation may alter the ability of a population to react to novel environmental conditions through phenotypic plasticity (vanTienderen and de Jong 1994). On average, the mean reaction norms were lower for small than large populations for most offspring traits. Only one large population, number 23, showed a reaction norm similar to that of most small populations (see Figs. 3 and 4). Unfortunately, no data are available on the history (bottlenecks, expansions) of this particular population. That small populations have lower amounts of plasticity than larger ones is in accordance with our hypothesis. Genetic erosion may indeed affect the potential of the endemic *C. bavarica* to cope with environmental stress and degradation.

Interestingly, the variation among populations in mean reaction norms changed over time (see e.g. Sultan 2000). On day 210, maximum differences between small and large populations were recorded in the environment in which plants grew largest (thus the least stressful environment), whereas on day 270 differences were largest in the environment in which plants grew poorly (thus the most stressful environment). There is indeed uncertainty when to predict maximum differences: although some studies predict that differences in reaction norms will be largest in the most stressful environments, due to higher rates of evolution (Hoffmann and Merilä 1999), others found the largest differences in the most favorable environment, as expected if an optimal response is only possible under benign conditions (Gebhardt-Henrich and van Noordwijk 1991; Kéry et al. 2000). For *C. bavarica*, with its inconsistent pattern, we must conclude that small populations are not only endangered under the most stressful conditions, but also under favorable conditions. Moreover, this result implies that we need to consider the whole life cycle to get a complete assessment of environmental stress and the potential for plants to respond to it by adaptive plasticity (Schmid 1992; Pigliucci 1998; Sultan 2000). The time component of phenotypic response, with different ages and de-

velopmental stages varying in their response to environmental stress, would remain undetected in studies limited to one time of measurement.

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