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Genetic diversity and fitness in *Scabiosa columbaria* in the Swiss Jura in relation to population size

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Abstract

Due to changes in land use, remnants of unfertilised, nutrient-poor calcareous grassland in the Swiss Jura are small in area and are highly fragmented. We selected 89 seed families from eleven populations of various sizes of *Scabiosa columbaria* for a study of molecular diversity, and used the same material in a greenhouse experiment to measure variation in fitness-related traits and the ability of populations to cope with competition. Using RAPD-PCR we detected 71 RAPD-phenotypes among 87 genotypes. Molecular diversity within populations was variable and relatively high, with an expected heterozygosity H_e ranging from 0.09 to 0.24. H_e, the Shannon index (SI) and the percentage of polymorphic bands were not correlated with population size, but the smallest populations had the lowest molecular diversity (H_e, SI). Population differentiation was moderate with 12% of the molecular diversity among populations. Measures of fitness in the greenhouse differed among seed families ($P < 0.001$), but not among populations. Mean above-ground biomass was largely reduced when plants had to compete with *Bromus*. Mean fitness of populations decreased when molecular diversity (H_e) was low, but only when plants had to compete with *Bromus* ($P = 0.02$). Accordingly, the relative competition ability of *Scabiosa* plants decreased when molecular diversity (H_e) was low ($P = 0.01$). Our results suggest an increased risk of local extinction of *Scabiosa columbaria* in the Swiss Jura caused by a decreased viability and reduced phenotypic plasticity due to genetic erosion in small populations.

Introduction

Over the last decades nutrient-poor calcareous grassland has declined drastically in the Swiss Jura due to changes in agricultural land use (see Zoller et al. 1986). Only 20% of the area of nutrient-poor grassland (Mesobromion) mapped around 1950 by Zoller et al. (1986) was still intact 35 years later (Stöcklin et al. 2000). Today, many populations of species characteristic of nutrient-poor grasslands are small in numbers and are isolated from each other by large distances. Conservation efforts stopped the ongoing destruction of Mesobromion remnants, but small populations may still suffer from a decrease in genetic diversity due to inbreeding and reduced gene flow between populations (Lacy 1987; Frankham 1996). Their fitness and competition ability may be reduced (Fenster and Dudash 1994; Young et al. 1996), and their risk of local extinction may be increased. Fischer and Stöcklin (1997) and Stöcklin and Fischer (1999) found that local extinctions of small plant populations occurred frequently even in intact grassland remnants in the Swiss Jura.

While many studies of endangered plants have examined variation in molecular markers, a few have also considered the evidence for a decrease in fitness with population size. Fischer and Matthies (1998) observed a decreased fitness with smaller population size in the short-lived *Gentianella germanica*, and Kery et al. (2000) observed the same in *Primula veris* and *Gentiana lutea*. Several other studies have confirmed that small populations are frequently not only genetically less diverse but also less viable (Oostermeijer et al. 1994; Lammi et al. 1999; Buza

et al. 2000; Luijten et al. 2000; Schmidt and Jensen 2000; Mavraganis and Eckert 2001). However, levels of genetic diversity and fitness are not always related (Podolsky 2001; Reed and Frankham 2001), and molecular data by itself is not sufficient to judge if populations are endangered through genetic erosion. Data on both genetic diversity and population viability in fitness-related traits are necessary to determine whether populations are threatened. Furthermore, it is important to know whether populations of an endangered species are still capable of responding to changing environmental conditions. Plastic adaptations allow individual organisms to maintain function and fitness across a range of diverse environments (Schlichting 1986; Sultan 2000). Small or genetically less diverse populations in particular may have a reduced ability to buffer the effects of poor environmental conditions or competition. For instance Kery et al. (2000) observed that plants from small populations of *Primula veris* were less able to respond plastically to an increase in nutrient availability than plants from larger populations. A similar reduction in the phenotypic response ability to an increase of competition was observed in populations with low molecular diversity in *Ranunculus reptans* (Fischer et al. 2000). The ability to cope with competition may be particularly important for endangered species. In this study, we combined the evaluation of molecular diversity in populations of different sizes from grassland remnants with a greenhouse experiment using the same populations to measure fitness and the phenotypic response to competition as a measure of plasticity.

We selected *Scabiosa columbaria* for this study for two reasons. Firstly, this geographically widespread species is restricted, in the Swiss Jura, to nutrient-poor Mesobromion remnants, and populations vary in size from less than a hundred to several thousand individuals. Secondly, variation in molecular diversity and fitness-related traits in populations at the margins of the geographical range of this species have been studied in the Netherlands and in Sweden. In the Netherlands, genotypic and phenotypic variation correlated with population size, indicating that genetic erosion occurs in small populations (Bijlsma et al. 1991; Ouborg et al. 1991; Van Treuren et al. 1991; Van Treuren et al. 1993; Bijlsma et al. 1994; Van Treuren et al. 1994). In Sweden, a high phenotypic differentiation among populations was observed, but there was no correlation between genetic diversity and population size (Waldmann and Andersson 1998). Peripheral populations might be more prone to fitness losses due to genetic erosion than populations from central regions, because peripheral populations are more likely to occur in ecologically marginal habitats (Lesica and Allendorf 1995). In the Swiss Jura, *Scabiosa columbaria* is near the centre of its distribution and the results of the present study can be compared to the effects observed in the more marginal populations in the Netherlands.

We selected plants from eleven populations from grassland remnants, containing c. 90 up to c. 2000 flowering individuals, for a molecular study using RAPD-PCR. We used the same material for a greenhouse experiment, investigating differences in fitness-related traits and plasticity among these populations in response to competition.

We used the molecular approach to address two questions: (1) How large is the molecular diversity in *Scabiosa columbaria* from grassland remnants in the Swiss Jura, and how is this diversity partitioned within and among populations? (2) Is there a difference in molecular diversity due to population size? The greenhouse study was designed to address two additional questions: (3) Do populations differ in fitness-related traits, and if so can these differences be related to population size and to molecular diversity measured with RAPD-PCR? (4) Are such effects dependent on environmental conditions, such as the presence or absence of *Bromus erectus* as a competitor?

Methods

Study species, sites and seed material

Scabiosa columbaria L. (Dipsacaceae) is a perennial species with a maximum life span of ten years (Grime et al. 1988). Flowering rosettes have stems 20 to 80 cm in height, with one to a few flower heads (Hegi 1918). Each flower head produces up to 100 achaena (Grime et al. 1988). The flowers are mainly pollinated by Aphidae (Hymenoptera), Syrphidae (Diptera) and Rhopalocerae (Lepidoptera) (Knuth 1898). *S. columbaria* is predominantly an outcrossing species (Van Treuren et al. 1994).

The distribution of *S. columbaria* extends throughout Eurasia and NW Africa (Hegi 1918) on nutrient-poor soils (Lauber and Wagner 1996). In the Swiss Jura *S. columbaria* occurs in nutrient-poor grassland (Mesobromion) remnants. In Central Europe such extensively used grassland probably reached its greatest extension in the 19th century when arable

fields of low productivity were replaced by meadows (Zoller 1954; Behre and Jacomet 1991; Pott 1995). After the Second World War the area of such grassland declined drastically due to changes in land use (e.g. abandonment, fertilisation), restricting species which had previously been widely distributed for several centuries to small and isolated habitats.

For this study seed material of a total of 89 individuals of *S. columbaria* from eleven populations of different sizes were collected in autumn 1997. In general, seeds of nine mother plants (seed families; Table 1) per population were randomly chosen with a spacing of several meters along a transect through the population. The mean number of flowering individuals during the years 1996 to 2000 was used as a measure of population size, with the exception of two populations for which data from only one year were available (Table 1). The selected populations were distributed over an area of 37 × 11 km.

RAPD-PCR

One individual from each of the 89 seed families was chosen for the molecular analysis. Leaf material was sampled from plants raised in the greenhouse (see below) and was shock frozen in liquid nitrogen (−98 °C). After freeze drying (Unicryo MC 4L, Uniequip GmbH, Martinsried, Germany) and grinding of the leaf material (Retsch MM2, Retsch GmbH and Co KG, Haan, Germany), DNA was extracted with a DNeasy Plant Mini Kit (QIAGEN GmbH, Hilden, Germany). From sixty primers ten base pairs long, the ones with the highest C/G nucleotide contents (i.e. those with higher binding stability due to three hydrogen bonds compared to two in A/T nucleotides) were screened and the band patterns were checked for repeatability. The first six primers with reproducible, polymorphic banding patterns were selected for the screening of all 89 sampled plants (see Appendix 1 for primer sequences).

Amplifications were carried out in 25 μL volumes, containing 5 μl of template DNA (5 ng DNA/μl); 11.3 μl ddH_2O; 0.5 μl MgCl (25 mM); 0.5 μl dNTP's (5 mM); 2.5 μl TaqPolymerase Buffer (10X; amersham pharmacia biotech); 5 μl Primer (5 μM); and 0.2 μl Taq DNA Polymerase (5000 units/ml; amersham pharmacia biotech). Polymerase chain reactions (PCR) were performed in a thermal cycler (PTC-100, MJ Research, Inc., Watertown, Mass., USA) with denaturing of the DNA at 93 °C for 1 min followed by 34 cycles of: 30 s at 92 °C; 30 s at 37 °C; and 1.5 min at 72 °C. Following the 34 cycles, a final step of 5 min at 72 °C was carried out. Samples were kept at 4 °C until analysis. PCR products were separated on 1.6% agarose gels (Sea Kem LE agarose) in 1X TAE (Tris/Acetat/EDTA) buffer in an electrical field (170 mV, c. 1.5 h). The banding pattern was made visible with ethidium bromide in an UV-light source. The presence or absence of bands was scored for 16 good visible bands with a length of between 500 and 2000 base pairs. Two samples were not analysed with all six primers due to low quantity of the DNA extraction. Therefore the final presence-absence matrix contained 87 individuals scored for 16 bands.

Statistical analysis of RAPD banding patterns were based on four assumptions: (i) RAPD fragments behave as diploid, dominant markers with alleles being either present (amplified) or absent (nonamplified); (ii) comigrating fragments represent homologous loci; (iii) polymorphic loci are inherited in a nuclear (Mendelian) fashion (Arafeh et al. 2002); and (iv) populations are in Hardy-Weinberg equilibrium (HWE; F_{is} = 0). This last assumption is supported by results from Van Treuren et al. (1993, 1994). *S. columbaria* is highly susceptible to inbreeding and in their study with allozymes the inbreeding coefficient (F_{is}) in natural populations did not differ from zero. Assuming that the populations were in HWE, allele frequencies were estimated based on the square root of the frequency of the null (recessive) allele.

The **molecular diversity within populations** was quantified in three different ways: (i) Nei's expected heterozygosity (H_e), (ii) the percentage of polymorphic bands out of all polymorphic bands (%P) and (iii) the Shannon index (SI). The percentages of poylmorphic bands were corrected for unequal sample sizes by rarefaction (Heck et al. 1975). By rarefaction the mean percentage of polymporphic bands in a population was calculated using the percentage of polymorphic bands of all possible combinations of four individuals (the lowest n) out of all sampled individuals per population. For all calculations only the polymorphic markers were used. Moreover, we checked the whole data set for private polymorphism (unique to a given population). To quantify the variation of molecular diversity among populations we calculated the coefficient of variation (CV) for H_e, SI and %P. H_e and SI were calculated with POPGENE (Version 1.21; Yeh et al. 1997).

The **molecular differentiation among populations** was calculated by Nei's (1973) estimator G_{ST}, the fixation index. G_{ST} values are based on Wright's

Table 1. Population size, location, habitat area, land use, the number of studied seed families and the molecular diversity (H_e) in eleven populations of *Scabiosa columbaria* from the Swiss Jura from an area of c. 400 km^2. Population size was measured as the mean number of flowering individuals during the years 1996–2000

Site	Population size	Longitude [m][1]	Latitude [m][1]	Altitude [m]	Habitat area [ha]	Land use in 1996	No. of studied seed families	Molecular diversity (H_e)
SLB	93	591375	251520	805	3.39	Pasture	4	0.089
OLT	102	598800	251250	590	0.94	Fallow	7	0.137
HEL	131	623240	244785	860	3.46	Pasture	9	0.175
HAS	158*	596500	249100	405	0.8	Pasture	9	0.158
LIW	248	599500	251200	670	2.99	Pasture	9	0.237
CDM	500	586500	250550	755	2.93	Pasture	9	0.239
NEW	544	608975	255825	520	7.9	Pasture	8	0.195
RIT	603	604750	254075	440	8.4	Pasture	9	0.183
LCO	901*	599180	246175	640	8.09	Pasture	7	0.202
RDS	920	595800	250850	520	0.08	Hay meadow	9	0.197
BEU	2003	605150	246125	625	5.18	Pasture	9	0.165

[1]Longitude and latitude according to the Swiss topographical maps (Bundesamt für Landestopographie, Wabern, Switzerland).
*Number of flowering plants was only available for the year 1996.

F-statistics and are identical to F_{ST} values if a locus consists of two alleles as applicable in RAPD marker analysis (Nybom and Bartish 2000). The F-statistic was calculated across all bands by using POPGENE.

Genetic distances between each population pair were quantified with Nei's original pair-wise genetic distance (TFPGA Program, Version 1.3; Miller 1997) and the pair-wise genetic distance F_{ST} (Arlequin, Version 1.1; Schneider et al. 1997). A Mantel test was used to test whether the matrix of Nei's original pair-wise genetic distance correlates with the matrix of pair-wise geographic distances (10.000 permuations; TFPGA Program).

Experimental design of the greenhouse study

In the greenhouse a total of 720 plants from 89 seed families (8 replicates per seed family) taken from eleven populations were exposed to two competition treatments (with and without competition with *Bromus erectus*). To eliminate the possible effects on the experiment of small pot size, we planted eight *S. columbaria* plants of different populations into rectangular pots of 16.7×26.6×21.5 cm (in the statistical analysis these pots were considered as subplots). In the treatment which included interspecific competition seeds of *Bromus erectus* were added. Thereby we were able to measure the response to interspecific competition while holding intraspecific competition constant in both treatments (Reynolds 1999).

Around 30 seeds per *S. columbaria* seed family were germinated separately in pot soil. After four weeks, eight seedlings per seed family (four in each competition treatment) were chosen randomly and planted into pots. The pots had a hole at the bottom of each wall and a drainage mat at the base, and were filled with 14.5 cm of marl, followed by a 5.5 cm layer of a 1:1 mixture of marl and natural soil from calcareous grassland and finally covered with 1.5 cm of the same natural soil. In the pots including interspecific competition c. 200 seeds of *Bromus erectus* were added. After germination the *Bromus* seedlings were reduced to 20 individuals per pot. The resulting 90 pots (45 per treatment) were randomly placed on 5 mobile greenhouse tables. Pots on the tables as well as the tables within the greenhouse were randomly repositioned every 4 weeks.

The experiment started on the 1st of June 1999, and the final harvest was seven months later, at the beginning of January 2000. Greenhouse conditions were similar during the whole experiment (14 h day length, 1 kw-lamps were activated if outdoor light was lower than 180 μmol m^{-2} s^{-1}; day temperature was maintained at 25 °C, night temperature at 10 °C (using additional heating from November to January)). All plants were watered every two days and received the same amount of fertiliser (a full fertiliser, equivalent to 4.7 kg N ha^{-1} was applied in the first six weeks of the experiment in six equal portions).

Fitness measurements, competition ability and statistical analysis

After 210 days (Jan. 2001) the plants were harvested, subdivided into above- and below-ground material, and dried at 80 °C for 48 h. Above-ground biomass was used as a fitness measure. In *S. columbaria*, as in other species, above-ground biomass correlates well with reproductive output (Van Treuren et al. 1993). To test for treatment effects and biomass differences among populations, and seed families within populations, a nested multi-factorial mixed model ANOVA type III was used.

We used the phenotypic response to competition as a measure of plasticity. The relative ability of a population to cope with competition was calculated as the proportional reduction in plant size in the treatment with *Bromus* compared with the plant size in the pure stand (Snaydon 1991). Following Snaydon (1991), the relative severity of competition was calculated using the population mean of within seed family reduction of biomass due to competition as the logarithm of the above-ground biomass per plant grown in a pure stand minus the logarithm of the above-ground biomass per plant grown in competition with *Bromus*. The measure for the relative severity of competition was subtracted from 1 to provide a measure of the ability to cope with competition: the lower the relative competition ability, the more reduced the plasticity of a population.

As a measure of fitness variation, means per population of within seed family coefficients of variation (CV) for above-ground biomass were calculated. The CV was corrected for different sampling size after Sokal and Rohlf (1995): $CV^* = [1 + (1/4n)] \times [SD \times 100/mean]$.

Because of the low sample size we calculated non parametric Spearman's Rho (r_s) correlations. JMP (Version 3.1; 1995, SAS Institute, Cary, N.C., USA) was used for all analyses. Data sets were transformed if necessary to meet the requirements of ANOVA.

Results

RAPD-phenotypes and polymorphism

The eleven populations of *Scabiosa columbaria* differed greatly in the composition of their RAPD-phenotypes. The 87 plants comprised 71 different RAPD-phenotypes. Seven RAPD-phenotypes were found in more than one population; only one phenotype was found twice in the same population. In each individual population, between 5 and 12 of the 16 reproducible RAPD-bands were polymorphic, with a mean of 8.9 (SE = 0.61). No private bands (unique to one population) were found.

Molecular diversity within populations and population size

The molecular diversity of individual populations calculated from polymorphic RAPD-bands was relatively high: Nei's expected heterozygosity H_e ranged from 0.089 to 0.239 (CV = 23.4%) with a mean of 0.18 (SE = 0.01). The Shannon index ranged from 0.14 to 0.36 (CV = 23.6%) with a mean of 0.27 (SE = 0.02) and the percentage of polymorphic bands within populations after rarefaction ranged from 31.3% to 59.0% (CV = 17.6%) with a mean of 44.0% (SE = 2.3).

There was no significant relationship between the molecular diversity within population (H_e) and logarithmic population size ($r_s = 0.49$, $P = 0.13$), neither when the Shannon index ($r_s = 0.46$, $P = 0.15$) nor when the percentage of polymorphic bands ($r_s = 0.41$, $P = 0.21$) was used as a measure of molecular diversity. However, the two smallest populations showed the lowest molecular diversity ($H_e = 0.14$ and 0.09 in OLT and SLB, respectively; SI = 0.21 and 0.14 in OLT and SLB, respectively). Furthermore, OLT was the only population in which a RAPD-phenotype was repeated, and SLB had the lowest percentage of polymorphic bands.

Molecular differentiation among populations

The G_{ST} value indicated a moderate genetic differentiation among populations. 12% of the RAPD-band variation was among and 88% within populations ($G_{ST} = 0.12$ (SD = 0.07); $H_t = 0.21$ (SD = 0.03); $H_s = 0.18$ (SD = 0.02)).

Genetic and geographic distances

The 55 pair-wise genetic distances among the eleven populations were generally small. Nei's original pair-wise genetic distances calculated from RAPD-band frequencies varied between 0.01 and 0.09. Genetic distances from pair-wise combinations of populations were significant in nine cases (F_{ST} statistics calculated with the program Arlequin, see methods). The pair-wise genetic distances among populations did not correlate with the corresponding geographic distances ($r = -0.1$, $P = 0.65$; Mantel-test).

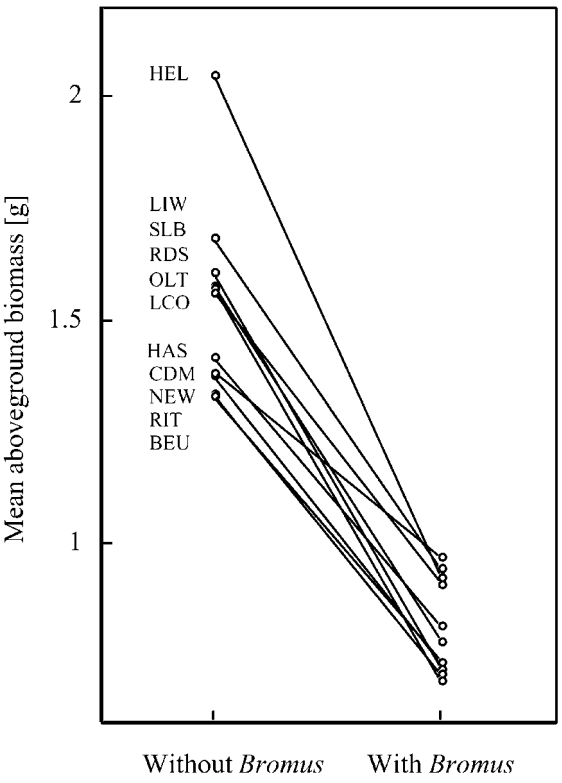

Figure 1. Mean above-ground biomass of eleven populations of *Scabiosa columbaria* from a common garden experiment in two treatments (including *Bromus erectus* as a competitor or not). See Table 1 for the acronyms of populations.

Fitness measurements in the greenhouse

The competition treatment had a significant effect on the growth of *S. columbaria*. Mean above-ground biomass of individuals was reduced by 57% (0.59 g ± 0.03 instead of 1.03 g ± 0.03 without *Bromus*, $P < 0.001$; Figure 1). Root biomass was even more reduced (by 77%, $P < 0.001$) when plants had to compete with *Bromus erectus*. Populations and seed families within populations did not differ in their response to competition ($P = 0.17$, $P = 0.18$ for the interaction of treatment with populations or seed families, respectively; Table 2). But mean above-ground biomass differed significantly among seed families within populations ($P < 0.001$, Table 2). Differences among seed families within populations explained 12.2%, and random variation among individuals explained 85.3% of the total variation in biomass.

Table 2. Nested, mixed-model ANOVA type III for above-ground biomass of seed families from eleven *Scabiosa columbaria* populations grown in the greenhouse for 210 days. Plants were grown with or without competition from *Bromus*

	df	SS	MS	Variance component		*P*
				Absolute	In %	
Competition	1	17.04	17.04			***
Competition∗population	10	1.11	0.11			0.17
Competition∗seed family	78	7.05	0.09			0.18
Plot	4	0.93	0.23	0.00148	1.62	*
Subplots within plot	80	5.72	0.07	0	0	0.67
Among populations	10	2.09	0.21	0.00080	**0.86**	0.25
Seed families within populations	78	12.11	0.16	0.01114	**12.21**	***
Residuals	457	35.58	0.08	0.07787	**85.31**	
Total	718				100.00	

$^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

Fitness, relative competition ability and population size

Among fitness within populations (i.e. above-ground biomass means for seed families within populations) and logarithmic population size we found no significant relationship ($r_s = -0.25$, $P = 0.47$); neither for the treatment without competition ($r_s = -0.56$, $P = 0.07$), nor when *Bromus* was added as a competitor ($r_s = 0.10$, $P = 0.77$).

The relative competition ability was also not correlated with logarithmic population size ($r_s = 0.38$, $P = 0.25$; Figure 2). However, the three smallest populations showed the highest reduction in biomass indicating a larger loss of fitness due to competition with *Bromus*.

Fitness, relative competition ability and molecular diversity

Fitness within populations (i.e. above-ground biomass means for seed families within populations) increased with molecular diversity when *S. columbaria* had to compete with *Bromus* (H_e: $r_s = 0.69$, $P = 0.02$, Figure 3A; SI: $r_s = 0.67$, $P = 0.02$; %P: $r_s = 0.71$, $P = 0.01$). However, when plants were grown without competition with *Bromus*, fitness was not correlated with molecular diversity (H_e: $r_s = -0.05$, $P = 0.89$, Figure 3B; SI: $r_s = -0.08$, $P = 0.81$; %P: $r_s = -0.03$, $P = 0.94$).

The relative competition ability also increased with molecular diversity within populations (H_e: $r_s = 0.65$, $P = 0.03$, Figure 4; SI: $r_s = 0.68$, $P = 0.02$; %P: $r_s = 0.61$, $P = 0.047$). Thus, competi-

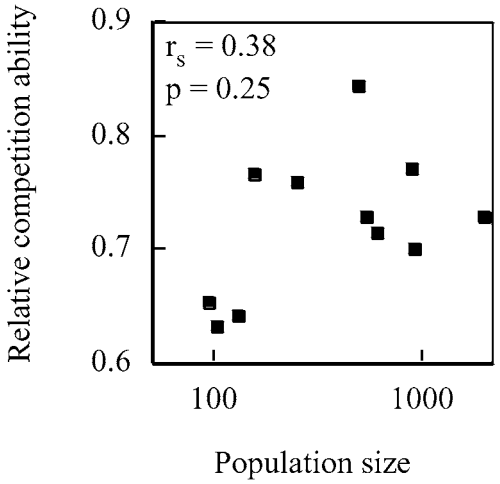

Figure 2. Spearman's rank correlation of the relative competition ability (a measure of phenotypic plasticity) with log population size for eleven *Scabiosa columbaria* populations in a common environment (the relative competition ability is calculated as the proportional reduction in plant size of *S. columbaria* in a mixture with *Bromus* compared with the plant size in the pure stand, see methods for details).

tion had a larger negative effect on populations with a low molecular diversity, i.e. populations with a low molecular diversity were less able to resist to competition.

Biomass variation and molecular diversity

Populations with low molecular diversity had higher mean coefficient of biomass variation (CV*) among seed families within populations (H_e: $r_s = -0.86$, $P = 0.0006$; SI: $r_s = -0.84$, $P = 0.001$; %P: $r_s = -0.60$, $P = 0.05$). The effect was also present, when the treatment including competition with *Bromus* was considered separately (H_e: $r_s = -0.68$, $P = 0.02$; SI: $r_s = -0.67$, $P = 0.02$; %P: $r_s = -0.65$, $P = 0.03$), but was not apparent in the treatment without competition with *Bromus* (H_e: $r_s = -0.31$, $P = 0.34$; SI: $r_s = -0.23$, $P = 0.48$; %P: $r_s = -0.02$, $P = 0.96$). This result indicates higher fitness variation in populations with lower molecular diversity, particularly in the treatment including interspecific competition.

Discussion

With RAPD-PCR we found a relatively high molecular diversity in the eleven populations of *Scabiosa columbaria* from grassland remnants in the Swiss Jura, weak indications of a lower molecular

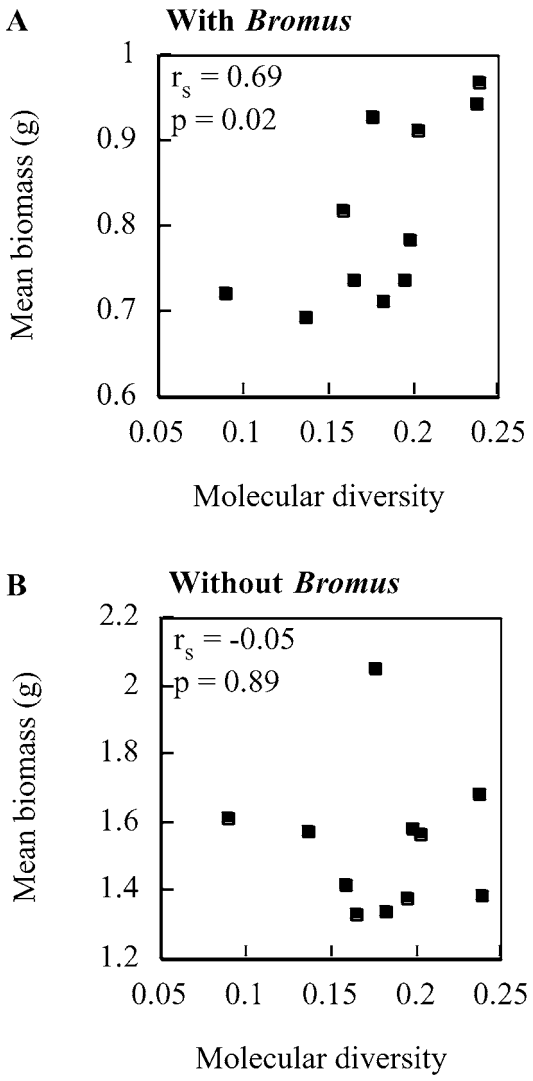

Figure 3. Spearman's rank correlation of mean above-ground biomass of seed families in eleven populations of *Scabiosa columbaria* with the corresponding molecular diversity (H_e) from RAPD-PCR. Plants were grown in a common environment: (A) plants had to compete or (B) had not to compete with *Bromus*.

diversity in small populations, and evidence for reduced competition ability in populations with decreased molecular diversity in the greenhouse experiment. In particular, we observed lower population viability with decreasing molecular diversity when plants had to compete with *Bromus*. The large differences in levels of molecular diversity observed across populations may explain why we found this correlation in spite of the small sample size per population in our study. Our results suggest that populations of *Scabiosa* with a low molecular diversity

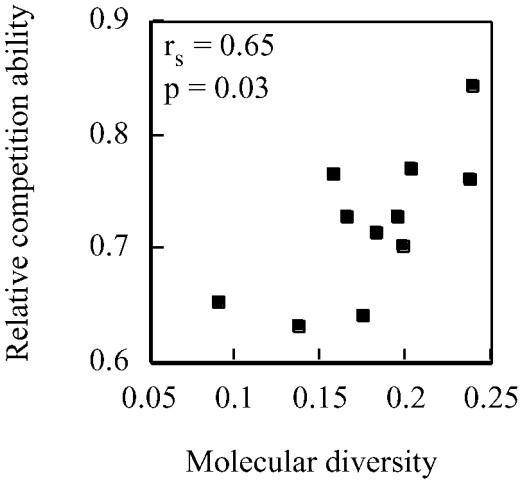

Figure 4. Spearman's rank correlation of the relative competition ability (a measure of phenotypic plasticity) in a common environment of eleven *Scabiosa columbaria* populations with the corresponding molecular diversity (H_e) from RAPD-PCR (the relative competition ability is calculated as the proportional reduction in plant size of *S. columbaria* in a mixture with *Bromus* compared with the plant size in the pure stand, see methods for details).

in the Swiss Jura are at risk of local extinction. However, there was only weak indirect evidence for a poorer plant performance in small populations, indicating that population size is not always the best indicator for population viability.

Genetic diversity and population differentiation

The amplification of randomly selected gene loci (RAPD-PCR-technique) is usually a more sensitive method to detect genetic variation in plant species compared to gene product level methods (e.g. isozymes), and is now well established as a sensitive method for detecting genetic structure among populations (Nybom and Bartish 2000). We found 71 different RAPD-phenotypes among the 87 *Scabiosa* plants. None of the sampled populations was monomorphic, and measures of molecular diversity (Nei's expected heterozygosity) revealed a variable and relatively high level of genetic diversity across the eleven populations. The within-population gene diversity ranged from 0.089 to 0.239 (0.18 (SD = 0.04)), comparable to the mean of 0.214 (SD = 0.117) reported for 41 RAPD-marker studies reviewed by Nybom and Bartish (2000). However, the proportion of among-population genetic diversity (G_{ST}) was relatively low in our study (only 12%). Similar values based on RAPD-markers have been reported by Vazquez et al. (1999) for *Sideritis pusilla* (11.3%) and Papa et al. (1998) for *Hordeum vulgare* (11%). In the endangered *Gentianella germanica* from the Swiss Jura, Fischer and Matthies (1998) observed a much higher coefficient for RAPD (37%), and the mean value reported for 31 RAPD studies by Nybom and Bartish (2000) was 29%. In their review, these authors demonstrated that with RAPD-data among-population diversity increases with the geographic distribution of sampled populations. The comparably low among-population diversity in *Scabiosa* may in part reflect our restricted study area. Our results indicate some genetic differentiation among the populations of *Scabiosa*, but this is probably not very pronounced.

A lack of a relationship between genetic and geographic distances was found in other studies as well, such as in Grünbauer et al. (1999). Gene flow among populations of characteristic species from grassland remnants is probably low because changes in agricultural land use strongly enhanced their isolation (Stöcklin et al. 2000). Seed dispersal over several kilometres is unlikely in *Scabiosa* because of the generally short dispersal distances of seeds (Cain et al. 2000). Gene flow due to pollen transfer might be more important. *Scabiosa columbaria* is visited by honeybees (Müller 1873) which forage in a diameter of up to 6 km around their beehive. Migrating butterfly species might occasionally visit inflorescences of *Scabiosa* (Müller 1873), but since butterflies are not effective pollinators (A. Erhardt, personal communication) this probably does not matter for gene flow. The lack of any significant relationship between genetic and geographic distances, and the lack of a spatial pattern in our molecular data in general, suggests that the observed population differentiation might result from random genetic processes, and that migration between remnants is possibly too low to compensate for this.

Population size, molecular diversity and population viability

Genetic erosion is a likely outcome of small population size especially in species which were formerly more common. Genetic drift is insignificant in large populations but becomes important when population size crashes to a small number following dramatic range size reduction and fragmentation (Srikwan and Woodruff 2000). In small populations drift as well as inbreeding can lead to a loss of less frequent alleles and thereby the level of homozygosity may

increase. As a consequence, genetic diversity tends to be reduced and the chance that harmful alleles are expressed increases (Ellstrand and Elam 1993; Young et al. 1996). We expected to find genetic effects of small population size in *Scabiosa columbaria*, because strong inbreeding depression in experimental progenies from small and large populations of *S. columbaria* in the Netherlands suggests that this species is highly susceptible to inbreeding (Van Treuren et al. 1993). Nevertheless, no clear relationship between population size and the level of inbreeding was observed in the Netherlands indicating that the plants studied had not yet suffered severely from inbreeding because otherwise genetic load, causing fitness reductions, would have substantially reduced inbreeding depression in smaller populations.

In our molecular study we found only weak indications for a lower genetic diversity in small populations of *Scabiosa*. The smallest populations showed the lowest molecular diversity (H_e and SI). The greenhouse experiment provided additional evidence for a genetic deterioration in the studied populations. Offspring from populations with low molecular diversity were phenotypically less plastic, making them less able to cope with interspecific competition with *Bromus erectus* (Figure 3); this is because they had a lower viability in the treatment including *Bromus* (Figure 4). We cannot completely exclude the possibility that the negative effects on offspring fitness could be caused by maternal carry-over effects (Oostermeijer et al. 1994). However, a reduced performance of offspring is cautiously considered as an indication of genetic effects (Fischer and Matthies 1998; Kery et al. 2000). In our study, plant performance correlated with genetic diversity and thus genetic erosion is at least in part responsible for the effect. Reduced performance is often linked with smaller population size (Fischer and Matthies 1998; Fischer et al. 2000; Kery et al. 2000), but in our study there was only weak indirect evidence that decreasing plant performance was due to decreasing population size. This may either be due to the difficulty of accurately measuring population size in perennial species, or because population size is only one of several possible reasons for a low genetic diversity (Vergeer et al. 2003). When evidence of reduced genetic diversity in small populations had been demonstrated, differences in the phenotypic performance of plants were not always correlated with population size. For instance, in the Netherlands variations in fitness traits were not related to population size in populations of *Salvia pratensis* or of *Scabiosa columbaria* (Ouborg et al. 1991; Van Treuren et al. 1993; Ouborg and Van Treuren 1995). Interestingly, in our study, plants from populations with a low molecular diversity were not only more affected by competition but they were as well phenotypically more variable, i.e. biomass variation of individuals around the seed family-mean was larger. This indicates that seed families in populations with low molecular diversity were also developmentally less stable, what might be an explanation for their reduced competition ability.

It is noteworthy that in our study fitness of *S. columbaria* only correlated with molecular diversity when plants suffered from additional competition with *Bromus*. Firstly, this indicates that the effect of harmful alleles is more severe under stressful conditions, and illustrates how important it is to include environmental variation in any study of plant fitness. Experiments have so far only been done to test the effect of stress on the magnitude of inbreeding depression (Wright 1977; Charlesworth and Charlesworth 1987; Reed et al. 2002); nevertheless Cheptou et al. (2000) concluded from results of a recent study that the effect of inbreeding depression in natural populations is more severe when competition occurs. Secondly, the reduced ability to resist competition suggests that a lower genetic diversity is likely to reduce the tolerance to changes in environmental conditions (Huenneke 1991). Similarly, Kery et al. (2000) observed that plants from smaller populations of the grassland species *Primula veris* were less able to respond to an increase in nutrient availability. Since phenotypic plasticity itself has a genetic basis, such a decrease in plasticity can result from inbreeding (Schlichting 1986). In isolated grassland remnants a reduced plasticity will strongly affect fitness in the long term, since such remnants are frequently subjected to changes in land use or gradual changes in nutrient availability from eutrophication.

Conclusions for conservation

Scabiosa columbaria is still relatively common in the Swiss Jura, and populations of this species were found in 36 out of 58 investigated remnants of calcareous grasslands (Ryf 1997). Nevertheless, our study shows that populations of this species may be endangered due to decreased plant viability caused by a reduced genetic diversity. Most notably, the decrease in phenotypic plasticity observed in the greenhouse study is likely to have negative consequences in the field at

least in the long term, because this effect is an indication that populations with lower molecular diversity have a reduced potential for adaptation to future changes in habitat conditions. It has been argued that demographic factors are more important than genetics for the short term fate of local populations (Lande 1988), but it is now accepted that in small populations genetic effects may interact with demographic variability to produce an "extinction vortex", if population size is substantially reduced by stochastic events (Gilpin and Soulé 1986). Such effects are not unlikely in *Scabiosa columbaria*, because this plant is a short-lived perennial with a maximum life span of only a few years. In grassland remnants, considerable fluctuations in the number of flowering individuals have been observed from year to year, with several populations constantly below a hundred individuals (J. Stöcklin, unpublished). If a population in an isolated habitat becomes locally extinct, re-colonisation is uncertain because *Scabiosa* is considered to be a bad coloniser (Grime et al. 1988).

The results of the present study are in line with what has been found in the Netherlands and do not indicate that populations from the centre of a species distribution area are less prone to genetic erosion than peripheral populations. In their study with allozymes Van Treuren et al. (1991) observed a significant correlation between the size of populations and the level of polymorphism in *S. columbaria*, and concluded that small populations were genetically less variable and more differentiated. Together with the observation that *S. columbaria* is highly susceptible to inbreeding (Van Treuren et al. 1993), the decreased level of genetic and phenotypic variation in smaller populations from the Netherlands was considered as a clear indication of a significant increase in the extinction probability of small populations (Bijlsma et al. 1994). Since different types of molecular markers have been used, direct comparisons of the observed levels of diversity between the study in the Netherlands and our results are difficult. Population differentiation is probably stronger in the Netherlands compared to what we found in the Swiss Jura, but this may be an effect of our small study area. However, both the study from the Netherlands and our own results suggest that in this species populations may genetically suffer from small size and isolation and that this may have consequences for their long-term survival. Restoration efforts should therefore not only tend towards increasing local population sizes, but should also consider genetic diversity taking into account the possible interactions between genetic and non-genetic effects on population viability.

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Appendix 1

Primer sequences used for RAPD-PCR and the number of well reproducible polymorphic bands per primer.

	Primer sequence	No. of polymorphic bands
FMI, Basel, Switzerland	CACCTTTCCC	1
FMI, Basel, Switzerland	CACAGGCGGA	4
FMI, Basel, Switzerland	GGGCCACTCA	2
FMI, Basel, Switzerland	CCCTACCGAC	3
Microsynth, Balgach, Switzerland	GTGACGTAGG	4
Microsynth, Balgach, Switzerland	ACCCATGCGG	2
Total		16

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