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Does natural selection promote population divergence? A comparative analysis of population structure using amplified fragment length polymorphism markers and quantitative traits

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Abstract

Divergent natural selection is considered an important force in plant evolution leading to phenotypic differentiation between populations exploiting different environments. Extending an earlier greenhouse study of population differentiation in the selfing annual plant *Senecio vulgaris*, we estimated the degree of population divergence in several quantitative traits related to growth and life history and compared these estimates with those based on presumably neutral molecular markers (amplified fragment length polymorphisms; AFLPs). This approach allowed us to disentangle the effects of divergent selection from that of other evolutionary forces (e.g. genetic drift). Five populations were examined from each of two habitat types (ruderal and agricultural habitats). We found a high proportion of total genetic variance to be among populations, both for AFLP markers (ϕ_{ST} = 0.49) and for quantitative traits (range of Q_{ST}: 0.26–0.77). There was a strong correlation between molecular and quantitative genetic differentiation between pairs of populations (Mantel's r = 0.59). However, estimates of population differentiation in several quantitative traits exceeded the neutral expectation (estimated from AFLP data), suggesting that divergent selection contributed to phenotypic differentiation, especially between populations from ruderal and agricultural habitats. Estimates of within-population variation in AFLP markers and quantitative genetic were poorly correlated, indicating that molecular marker data may be of limited value to predict the evolutionary potential of populations of *S. vulgaris*.

Keywords: drift, genetic structure, molecular markers, phenotypic plasticity, population differentiation, selection

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Introduction

Evolutionary biologists have shown that natural selection is a powerful force shaping the phenotypes of organisms and leading to adaptations to local environmental conditions (Linhart & Grant 1996). The direction and strength of selection on phenotypic traits may vary considerably with geography. Given sufficient heritable variation in fitness-related traits, coarse-grained spatial heterogeneity in the selective regime is expected to lead to genetic divergence between populations. Ultimately, this process may lead to the evolution of reproductive barriers and the emergence of new species (Schluter 2000).

An analysis of population differentiation caused by divergent selection on phenotypic traits requires a quantitative genetic approach in order to separate genetic from environmental components of variation within and among populations. However, even when genetic differentiation among populations is detected, it remains unknown which process has led to the observed pattern. Indeed, evolutionary forces other than natural selection, such as mutation and random genetic drift, could also have caused populations to diverge in their phenotypes.

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The importance of genetic drift and gene flow as evolutionary forces in natural populations has been thoroughly addressed in studies using molecular markers such as allozymes, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and microsatellites. The association of these molecular traits with phenotypes is usually weak or absent, such that their distribution within and among populations is little influenced by natural selection. In theory, the effects of drift on polygenic quantitative traits are not expected to differ from that on single loci (Rogers 1986). In the absence of any selective forces, there should thus be a close correspondence in the population structure inferred from molecular markers and from quantitative traits. When selection is important and favours different phenotypes in different populations, we would expect among-population variation in quantitative traits (estimated using the Q_{ST} statistic; Spitze 1993) to exceed that of neutral molecular markers (estimated using the F_{ST} statistic). When selection favours similar phenotypes in different populations, estimates of Q_{ST} are expected to be lower than estimates of F_{ST}. A comparative analysis of population differentiation in neutral molecular markers and fitness-related quantitative traits should therefore allow us to disentangle the role of different long-term evolutionary forces in shaping present-day patterns of population genetic variation (Spitze 1993; Podolsky & Holtsford 1995; Bonnin *et al*. 1996; Lynch *et al*. 1999; McKay & Latta 2002). In addition, it can guide researchers to identify traits that may be involved in the local adaptation of populations.

In this study, we extend previous studies of phenotypic population differentiation and local adaptation in the weedy annual plant *Senecio vulgaris* (Leiss & Müller-Schärer 2001a,b). In particular, we focus on characterizing the degree of quantitative genetic differentiation in several growth and life history traits of *S. vulgaris* and relating the population structure expressed in these traits with that of AFLP markers. *S. vulgaris* has a predominantly self-fertilizing breeding system (Hull 1974; Campbell & Abbott 1976) and is thus expected to exhibit strong genetic differentiation among populations.

S. vulgaris occurs in a range of habitats, including ruderal sites and agricultural fields, which vary greatly in soil fertility. Plants were therefore collected from both ruderal (waste land, roadsides, gravel pits) and agricultural (vineyards, apple orchards, vegetable crops) habitats, and progeny of maternal genotypes were grown under two levels of nutrient supply. This design allows us to not only test for population divergence at different hierarchical levels (among habitats, among populations), but also to examine divergence in trait averages across nutrient levels as well as in trait plasticities in response to nutrient supply. Population differentiation in phenotypic plasticity in response to major environmental factors has received relatively little attention in past studies, despite its important implications for adaptive evolution (Oyama 1994; Donohue *et al*. 2001). A recent greenhouse study on adaptation in *S. vulgaris* revealed phenotypic differentiation among populations from agricultural vs. ruderal habitats, and increased responsiveness of reproductive traits to nutrient fertilization in genotypes collected from agricultural habitats (Leiss & Müller-Schärer 2001a).

Here, in addition to among-population variation, we estimated within-population variation (broad-sense heritabilities) in trait averages and plasticities and compared these estimates with population estimates of molecular diversity. This analysis was intended to test whether molecular marker data, which are relatively easy to obtain, serve as a good indicator for quantitative genetic variation in ecologically important traits. Data about the general congruence of molecular and quantitative genetic variation will help to inform conservation biologists, who increasingly rely on molecular markers to assess the genetic diversity and evolutionary potential of endangered plant and animal populations. The following specific questions were addressed:

1 What is the pattern of among- and within-population variation in AFLP markers?
2 Are populations differentiated in quantitative traits associated with fitness, and how does population divergence of these traits compare with that of AFLP markers?
3 Is there a positive correlation between estimates of within-population variation in AFLP markers and in quantitative traits?

Materials and Methods

Species and population description

Senecio vulgaris L. (common groundsel, Asteraceae), is a short-lived annual herb found primarily at ruderal sites, along roadsides, and as a weed in various cropping systems. With the exception of the warmest regions of the world, *S. vulgaris* occurs as a weed in arable land throughout the globe causing high economic losses. In Central Europe, it can produce up to three generations per year. Population size can fluctuate largely both among and within years depending on disturbance regimes and climatic factors. The species is predominantly selfing and produces large numbers of seeds that disperse by wind. The seeds have a hairy pappus and can travel over long distances, which allows the plant to quickly colonize newly disturbed sites. Seed dormancy is rather limited and thus *S. vulgaris* does not build up a large and persistent seed bank (Popay & Roberts 1970; Roberts & Feast 1972).

The plants used in our study originated from 50 putative genotypes (individual plants) of *S. vulgaris* ssp. *vulgaris*

var. *vulgaris*. Seeds of these maternal genotypes were collected in 1996 from five ruderal (gravel pit, wasteland) and five agricultural (apple orchard, vegetable field, vineyard) populations of *S. vulgaris* in Switzerland (see also Leiss & Müller-Schärer 2001a). Seeds were collected in each population along a transect with a minimal distance between sampling points of 1 m. Ruderal and agricultural populations were interspersed. The distance between pairs of populations ranged from 3 to 86 km (median 17 km).

Greenhouse experiment

The design of the quantitative genetic experiment was described previously by Leiss & Müller-Schärer (2001a). In short, plants were first grown from seeds for one generation under uniform conditions in the greenhouse to minimize environmentally induced maternal effects that could potentially lead to biased estimates of genetic variance. After selfing, 24 seeds of each of the 5 maternal genotypes per population were germinated in 6 pots (9 cm diameter) filled with peat substrate and the first emerging seedling of each pot was used for the experiment, while the others were removed. Three seedlings per genotype were randomly assigned to two fertilization treatments: no fertilizer addition and addition of slow-release NPK-fertilizer (Tardit Top©) to the peat substrate during potting. Each replicate of a genotype and treatment was grown until seed set in one of three greenhouse benches (blocks) in a randomized complete block design. In this study, we considered only plants from the uninfected control group, and omitted plants that were experimentally inoculated with a rust fungus (see Leiss & Müller-Schärer 2001a). Thus, the experimental design can be summarized as follows: 2 habitats × 5 populations × 5 genotypes × 2 nutrient treatments × 3 replicates, yielding a total of 300 plants. Only 10 plants died during the experiment, independent of the fertilization treatment or the genotype, so the experiment was relatively balanced.

Plants were harvested when the first flower head set seeds and the following characters were measured: average area of the third and fourth leaf (hereafter leaf area), dry mass of the vegetative parts of the shoot (vegetative biomass), time to the expansion of the cotyledons (time to emergence), time to first seed set, number of seeds in the first maturing flower head (seeds per flower head), total number of flower heads, dry mass of the reproductive parts of the plant (reproductive biomass). Reproductive allocation was calculated as the proportion of total shoot biomass accounted for by the biomass of reproductive parts.

AFLP analysis

DNA samples were obtained by germinating seeds of each of the 50 genotypes in separate pots. Two fresh, green leaves of each genotype were collected and immediately frozen in liquid nitrogen. Leaves were lyophilized for 24 h and ground to a fine powder with the aid of a shaking mill. Genomic DNA was extracted using a standard CTAB procedure as described by Steinger *et al*. (1996), and subsequently quantified using agarose gels and ethidium bromide staining. AFLP analysis (Vos *et al*. 1995) was carried out as described by Kollmann *et al*. (2000). In brief, 0.5 mg DNA was digested with *Mse*I and *Eco*RI restriction enzymes, and synthetic adapters were ligated to the fragments using T4 DNA ligase. After preselective polymerase chain reaction (PCR) amplification, fragments were amplified in a second PCR run using eight primer combinations each with three selective bases (M-CAA, M-CAG, M-CAC, M-CAT, M-CTA, M-CTG, M-CTC, M-CTT). The *Eco*RI primer (E-AGG) was labelled with a fluorescent dye (5-FAM). Preliminary tests showed that the primers chosen produce highly reproducible AFLP patterns. Amplified fragments were separated by capillary electrophoresis with an ABI Prism™ 310 Genetic Analyser (Applied Biosystems, Rotkreuz, Switzerland) using GeneScan-500 (TAMRA) (Applied Biosystems) as an internal standard. Electropherograms were scored visually for the presence (1) or absence (0) of fragments in the range 50–500 bp using GENESCAN software (Version 3.1, Applied Biosystems).

Statistical analysis

The 0/1 matrix obtained from scoring the AFLP markers was subject to analysis of molecular variance (AMOVA) using the software package ARLEQUIN (Version 2.0; Schneider *et al*. 2000). AMOVA is based on pairwise squared Euclidean distances among AFLP phenotypes, and allows the molecular variance to be partitioned among several hierarchical levels, analogous to conventional analysis of variance (Excoffier *et al*. 1992). The significance of variance components at each hierarchical level is tested with a permutation procedure. From the variance components, a ϕ_{ST}-statistic can be calculated, which is a good estimator of F_{ST}. ϕ_{ST} is defined as the proportion of total molecular variance that is due to differences among the units of a given level. Here, we partitioned the total variance into components attributable to differences among habitat types (i.e. ruderal vs. agricultural), among populations within habitats, and among individuals within populations.

We used random effects ANOVA as implemented in the SAS software package (SAS Institute, 1989) to estimate components of variance in quantitative traits attributable to genetic and environmental sources. Two-level hierarchical models with population and genotype (nested within population), and three-level hierarchical models with habitat type, population (within habitat), and genotype (within

population) as model terms were run in SAS using the NESTED procedure (to extract variance components) and the GLM procedure (for significance testing). Analysis was carried out with the average trait values across the two fertilization levels, and with the plasticities of the traits in response to fertilization. Trait plasticities were calculated for each genotype as the difference in trait values between fertilization levels within each block (Ebert *et al*. 1993). Block main effects were fitted prior to analysis to reduce unwanted environmental variation, and residuals were saved for subsequent analyses. The degree of population differentiation in phenotypic traits was represented by Q_{ST}, the proportion of total genetic variance that is among populations. For a completely selfing species $Q_{ST} = \sigma^2_{pop}/(\sigma^2_{pop} + \sigma^2_{genotype})$, where σ^2_{pop} is the among-population variance component and $\sigma^2_{genotype}$ is the among-genotype (within-population) variance component (Bonnin *et al*. 1996). We estimated *within*-population variation of phenotypic traits from their broad-sense heritabilities (h^2), calculated as $h^2 = \sigma^2_{genotype}/(\sigma^2_{genotype} + \sigma^2_{residual})$ Falconer & Mackay 1996). Variance components used for heritability calculations were extracted from the two-level hierarchical ANOVA models described above. Heritabilities were considered to be significantly larger than zero when the genotype term in ANOVA was significant. For comparative purposes we also report coefficients of genetic variance ($CV_{genetic}$) calculated as the square-root of genotypic variance divided by trait mean (Houle 1992).

Results

Among-population variation in AFLP markers

We scored a total of 589 AFLP markers of which 183 (31%) were polymorphic. Among the 50 individuals analysed, a total of 48 genotypes were distinguished. In one population (Rosé) three of the five individuals had the same genotype, whereas the other nine populations were composed of different genotypes.

Populations exhibited strong spatial structure as indicated by the large Pop-ϕ_{ST} value of 0.49 ($P < 0.001$) estimated with AMOVA. Genetic distances (ϕ_{ST}) between pairs of populations are presented in Table 1. Genetic distance between population pairs was only weakly associated with geographical distance, as indicated by the small and non-significant Mantel correlation between the two distance matrices (Mantel's $r = 0.18$, $P = 0.23$). We performed a second AMOVA grouping populations according to whether they occurred in ruderal or agricultural habitats and including habitat type as an additional factor in the model. Habitat type explained a small but significant fraction of the total molecular variance (Hab-$\phi_{ST} = 0.07$, $P < 0.001$).

Among-population variation in quantitative traits

Multivariate analysis of variance on trait averages (across nutrient levels) and plasticities (in response to nutrients) revealed a highly significant population effect (for trait averages: Wilks' $\lambda = 0.0096$, $F_{72,208} = 3.31$, $P < 0.0001$; for trait plasticities: Wilks' $\lambda = 0.0646$, $F_{72,208} = 1.65$, $P < 0.004$). Univariate ANOVA detected significant or marginally significant ($P < 0.06$) among-population variation in the averages of all eight traits (Table 2). Estimates of Pop-Q_{ST}, indicating the proportion of genetic variation that is among populations, were relatively high ranging from 0.26 to 0.77. The highest Pop-Q_{ST} estimates were found for leaf area, reproductive mass and number of flower heads. Pop-Q_{ST} estimates of these traits were considerably higher than the Pop-ϕ_{ST} estimate based on AFLP markers. Significant among-population variation was also observed in the plasticity of two traits: number of flower heads and vegetative biomass (Table 2). A comparison of Pop-Q_{ST} (average over eight traits) and Pop-ϕ_{ST} between pairs of populations revealed a highly significant correlation

Table 1 Degree of genetic differentiation for AFLP markers (ϕ_{ST}, below diagonal) and quantitative traits (Q_{ST}, above diagonal) between pairs of populations of *Senecio vulgaris*. Q_{ST} estimates are averages over eight quantitative traits. R denotes populations from ruderal habitats, A denotes populations from agricultural habitats

Population	Coordinates	Arco.	Court.	Rosé	Sion	Sugiez	Praz	Conth.	Couss.	Corj.	Ches.
Arconciel (R)	46°44′52″/7°7′25″	—	0.410	0.295	0.194	0.082	0.558	0.132	0.355	0.338	0.144
Courtepin (R)	46°51′58″/7°7′30″	0.313	—	0.454	0.425	0.587	0.356	0.380	0.604	0.470	0.381
Rosé (R)	46°47′3″/7°3′48″	0.388	0.726	—	0.698	0.541	0.607	0.370	0.645	0.530	0.364
Sion (R)	46°14′5″/7°21′42″	0.542	0.758	0.942	—	0.501	0.676	0.269	0.447	0.360	0.143
Sugiez (R)	46°57′56″/7°6′34″	0.226	0.501	0.581	0.576	—	0.692	0.206	0.473	0.463	0.011
Praz (A)	46°57′18″/7°5′59″	0.431	0.620	0.786	0.797	0.534	—	0.188	0.673	0.610	0.503
Conthey (A)	46°13′31″/7°18′10″	0.223	0.461	0.619	0.632	0.359	0.418	—	0.319	0.202	0.085
Coussiberle (A)	46°53′16″/7°6′47″	0.329	0.503	0.663	0.676	0.369	0.577	0.385	—	0.381	0.187
Corjolens (A)	46°47′13″/7°2′44″	0.216	0.450	0.551	0.606	0.308	0.462	0.208	0.320	—	0.141
Chesopelloz (A)	46°48′29″/7°4′47″	0.295	0.500	0.650	0.669	0.346	0.553	0.294	0.229	0.299	—

Table 2 Estimates of within- and among-population variation in quantitative genetic traits. Coefficients of genetic variation ($CV_{genetic}$, square-root of genotype variance divided by trait mean), broad-sense heritabilities (h^2), and Q_{ST}-values were calculated from variance components estimated with hierarchical ANOVA models (see Materials and Methods). Pop-Q_{ST} and Hab-Q_{ST} refer to estimates of quantitative-genetic differentiation at the level of populations and habitats, respectively. Adjusted Pop-Q_{ST} refers to Pop-Q_{ST} estimates from which the among-habitat variance has been statistically removed. Corresponding estimates of population differentiation for AFLP markers are given at the bottom of the table.

	$CV_{genetic}$	h^2	Pop-Q_{ST}	Hab-Q_{ST}	Adjusted Pop-Q_{ST}
Trait averages					
Time to emergence	0.134	0.18*	0.37†	0	0.37
Time to seed set	0.049	0.16*	0.49*	0	0.49
Seeds per flower head	0.212	0.41***	0.27*	0	0.27
Number of flower heads	0.136	0.29**	0.62***	0.42*	0.47
Leaf area	0.114	0.18*	0.77***	0.31	0.71
Reproductive mass	0.095	0.15†	0.70***	0.50*	0.54
Vegetative mass	0.148	0.34***	0.26*	0	0.26
Reproductive allocation	0.079	0.27**	0.56***	0.35†	0.42
Average over traits:	0.121	0.25	0.51	0.20	0.44
Trait plasticities					
Time to emergence	0	0	0	0	0
Time to seed set	0	0	0	1*	0
Seeds per flower head	0.089	0.06	0	0	0
Number of flower heads	0	0	1**	0.60	1
Leaf area	0	0	1	0	1
Reproductive mass	0	0	1	0.67	1
Vegetative mass	0.047	0.02	0.85*	0	0.85
Reproductive allocation	0	0	1	0	1
Average over trait plasticities:	0.017	0.01	0.61	0.28	0.61
			Pop-ϕ_{ST}	Hab-ϕ_{ST}	adj. Pop-ϕ_{ST}
AFLP markers			0.49	0.07	0.46

†$P < 0.06$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

between the two distance metrics (Mantel's $r = 0.59$, $P = 0.008$). Similar to that found for AFLP markers, genetic distance in phenotypic traits (Q_{ST}) between pairs of populations was not significantly correlated with geographical distance (Mantel's $r = -0.19$, $P = 0.19$).

Grouping the populations in ANOVA according to habitat type revealed that habitat explained a significant proportion of the among-population variance for the averages of several traits including number of flower heads, reproductive mass and reproductive allocation ($P = 0.0503$). Populations from agricultural habitats exhibited higher values for these traits than ruderal populations (Leiss & Müller-Schärer 2001a). Hab-Q_{ST} estimates for these traits ranged from 0.35 to 0.50, which is considerably larger than the Hab-ϕ_{ST} estimate for AFLP markers (Hab-$\phi_{ST} = 0.07$). Once the effect of habitat was accounted for in ANOVA, most Pop-Q_{ST} values were close to the Pop-ϕ_{ST} estimate (Table 2). An exception was the Pop-Q_{ST} for leaf area, which remained high (0.71). There was also significant among-habitat variation in the plasticity in time to seed set, and a marginally significant habitat effect ($P = 0.07$) for the plasticity in number of flower heads. Plasticity in these traits was higher in agricultural than in ruderal populations (Leiss & Müller-Schärer 2001a).

Within-population variation in quantitative traits and AFLP markers

Broad-sense heritabilities of trait averages were significantly or marginally significantly > 0 in all cases, ranging from 0.15 for reproductive biomass to 0.41 for seeds per flower head (Table 2). In contrast, heritabilities of trait plasticities were low in all cases. We tested for an association between genetic variation within populations and the degree of population differentiation by correlating the heritabilities of trait averages with the Q_{ST}-values of these traits (see Lynch *et al*. 1999). The correlation was negative ($r = -0.63$) but not significant ($P = 0.09$). Figure 1 depicts the relation between within-population variance in AFLP markers and average heritability over the eight quantitative traits. The correlation between the two population measures of variability was not significant ($r = 0.20$, $P = 0.57$). In addition, all separate regressions of heritabilities for the individual traits on population estimates of molecular variance were nonsignificant. Overall, variation in quantitative traits tended to be larger in populations from agricultural habitats than in those from ruderal habitats (*t*-test, $P < 0.07$; Fig. 1), but no such effect was found for AFLP markers.

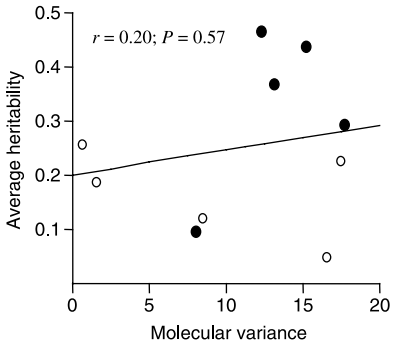

Fig. 1 Relationship between estimates of average heritability over eight quantitative traits and molecular variance (a measure of within-population diversity of AFLP markers; see Materials and Methods) within ruderal (○) and agricultural (●) populations.

Discussion

Among-population variation

Our study revealed strong population differentiation in both molecular markers (AFLP) and in several fitness-related phenotypic traits of the weedy annual *Senecio vulgaris*, the latter confirming previous findings (Leiss & Müller-Schärer 2001a). Given that *S. vulgaris* is predominantly a selfing species (Hull 1974; Campbell & Abbott 1976), we expected to find a large proportion of the total genetic variation to reside among populations. Our estimate of the degree of population differentiation based on AFLP markers (ϕ_{ST} = 0.49) corresponds closely with the average G_{ST}-value reported for widespread selfers (G_{ST} = 0.446; Hamrick & Godt 1996), but is slightly higher than the ϕ_{ST}-value of 0.38 reported for RAPD markers in a similar study with *S. vulgaris* (Müller-Schärer & Fischer 2001). Although our estimates of population differentiation may be upwardly biased owing to high levels of inbreeding, the results nevertheless suggest, that gene flow among populations of *S. vulgaris* is very restricted. This is surprising given that the species has small seeds with a large pappus that can potentially disperse by wind over large distances. Geographic distance, however, had little predictive power in explaining genetic differentiation among populations, similar to what was found with RAPDs for a different set of populations of *S. vulgaris* (Müller-Schärer & Fischer 2001). This suggests that an isolation-by-distance model may be of limited value in this species, although the statistical power of our test was relatively weak.

The main objective of this study was to explore the role of divergent selection in determining population differentiation in quantitative traits. The appropriate null hypothesis for such a test is not a complete absence of population subdivision, because random genetic drift and mutation are equally likely to cause populations to diverge as is selection. This is especially true for selfing species with strongly fluctuating population sizes such as *S. vulgaris*. Because random drift will affect all loci to an equal degree, we used AFLP markers to distinguish the influence of drift on population divergence in quantitative traits from that of selection. This test assumes that AFLP loci are selectively neutral, which is a reasonable, although unproven, assumption. The studied populations of *S. vulgaris* exhibited strong population differentiation in most phenotypic traits measured. Population divergence in several traits (number of flower heads, reproductive biomass, leaf area) considerably exceeded that of AFLP markers. This indicates that divergent directional selection likely played an important role in the phenotypic differentiation of populations for these traits.

Our sampling of populations from two different types of habitat allowed us to examine population differentiation at a higher hierarchical level, that is between ruderal and agricultural populations. While the degree of divergence at the level of habitat types was relatively small for the AFLP markers (Hab-ϕ_{ST} = 0.07), it was considerably higher for several quantitative traits (range of Hab-Q_{ST}: 0–0.50). Once the effect of habitat type on the quantitative traits was statistically removed, estimates of population divergence did not deviate much from the neutral expectation inferred from the AFLP data. Together these findings indicate that divergent selection most strongly led to phenotypic divergence between populations from different habitat types, but may have played a minor role in contributing to population structure within habitat types. It must be noted, however, that a close correspondence between molecular and quantitative measures of divergence does not imply an absence of selection, because there are many ways in which selection can lead to a given level of population differentiation.

Our procedure to disentangle selection from other forces promoting population divergence rests on the assumption that molecular markers are selectively neutral and unlinked to loci under selection. However, computer simulations have shown that in highly selfing organisms variation at neutral loci can be greatly influenced by selection acting on linked and even unlinked loci via genetic hitchhiking (Hedrick 1980). Charlesworth *et al*. (1997) demonstrated that, for subdivided populations, heterogeneous selection enhances F_{ST} values even at neutral loci distant to a selected locus. A similar increase in F_{ST} was also observed when selection eliminates linked deleterious mutations from the population (background selection). These theoretical considerations suggest that for a highly inbreeding species, such as *S. vulgaris*, a test for divergent selection driving phenotypic differentiation, which is based on the comparison between F_{ST} and Q_{ST}, may be rather conservative.

In light of these considerations, it was surprising to find only weak population differentiation at the habitat level for AFLP markers (Hab-ϕ_{ST} = 0.07), but strong differentiation for several quantitative traits (Hab-Q_{ST} range: 0–0.51). This finding speaks against an important role of divergent

selection shaping the distribution of AFLP markers among the studied populations. This is in line with a recent simulation study demonstrating that, for quantitative traits affected by many loci, pronounced differentiation is possible with only minor differentiation of allele frequencies at the underlying quantitative trait loci (Latta 1998). It is also possible that the rate of outcrossing in *S. vulgaris* is higher than thought previously and that this may have reduced linkage disequilibrium among loci. Hull (1974) estimated outcrossing in this species to be usually 1%, but frequencies up to 10 and 15% were found in some populations. Campbell & Abbott (1976) using a morphological marker (radiate flowers) to estimate outcrossing rates observed an average frequency of outcrossing of 22%, but this could be an overestimate of natural rates because of the experimental procedure used in their study. Nevertheless, outcrossing events may occur regularly in *S. vulgaris*. This may also explain why we found such a high number of multilocus genotypes in the studied populations.

An alternative procedure to test for divergent natural selection as the major cause of phenotypic differentiation would be to reciprocally transplant individuals from different populations or habitat types. Such transplant expeiments often show striking examples of a home-site advantage in major fitness components (survival and reproductive success) indicating that natural selection resulted in local adaptation (reviewed by Schluter 2000). Surprisingly, however, a transplant experiment with *S. vulgaris*, in which seeds were reciprocally sown between ruderal and agricultural habitats, showed no evidence for better home-site performance (Leiss & Müller-Schärer 2001b), possibly due to the occurrence of extreme environmental events (drought, high temperature) during the experiment.

The question of what ecological factors may be responsible for the inferred differences in selection regimes between agricultural and ruderal habitats is difficult to answer, as the two habitat types differ in innumerable ways. Agricultural sites in the study area certainly exhibit much higher concentrations of soil nutrients than ruderal habitats (K. Leiss & H. Müller-Schärer, unpublished data). A large body of comparative studies has shown that species from fertile sites are generally more responsive to nutrients than species from poor sites (e.g. Fichtner & Schulze 1992; Grime 1994). Based on these findings, we expected to observe population divergence in trait plasticity in response to nutrients, with populations from agricultural habitats displaying higher plasticity than ruderal populations. This prediction was partly confirmed. Agricultural populations were more plastic in time to first seed set (this study), and in number and mass of flower heads (Leiss & Müller-Schärer 2001a). Significant among-population variation was also found for plasticity in vegetative biomass, but differentiation in this trait occurred only at the level of populations and not between habitat types.

A number of authors have recently summarized published plant and animal studies comparing population differentiation in molecular markers (mostly isozymes) and quantitative genetic traits (Lynch *et al*. 1999; Schluter 2000; Merilä & Crnokrak 2001; McKay & Latta 2002). They found that in a majority of studies estimates of Q_{ST} averaged across traits were higher than those of F_{ST} or related statistics and that the magnitude of the difference decreases with increasing levels of molecular differentiation. The latter observation, however, may well be a statistical artefact, because measures of divergence cannot exceed the value of 1. Based on the available evidence to date, we can conclude that molecular markers may often provide conservative estimates of the degree of population differentiation in quantitative traits, although counter-examples do exist (Petit *et al*. 2001). Thus, directional natural selection seems to be a major force causing phenotypic divergence of natural populations, even at relatively small spatial scales (a few kilometres in this study).

Within-population variation

A remarkable outcome of our study was the finding that there was virtually no correspondence between the within-population variance of AFLP markers and quantitative traits. Some populations with high molecular diversity had low heritability in quantitative traits and vice versa. Furthermore, heritable variation in quantitative traits tended to be larger in agricultural populations than in ruderal populations, suggesting that the pace of adaptive evolution may proceed faster in agricultural habitats. Clearly, this important result would have been missed if only molecular marker data had been available. A recent meta-analysis that compared molecular and quantitative measures of genetic variation in both plants and animals also found a weak correspondence between the two types of genetic variation (Reed & Frankham 2001), suggesting that our result may be quite general. This result has important implications for conservation biologists who increasingly make use of molecular markers to assess the genetic diversity of endangered populations (articles in Smith & Wayne 1997). The aim of conservation efforts is usually not to preserve neutral genetic variation but to maintain a population's ability to respond to selection and adapt to changing environmental conditions. Estimates of heritability in important life history traits, although more difficult to obtain, are better predictors of the short-term evolutionary potential of populations (Lynch 1996; Reed & Frankham 2001), and can not easily be substituted by molecular markers.

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This study is part of a series of studies aimed at understanding the population biology and evolution of *Senecio vulgaris* in ruderal and agricultural habitats. The primary interest of the research group of H. Müller-Schärer is to develop environmentally sound techniques for the biological control of weeds, using both insects and pathogens.
