Molecular Ecology (2001) **10**, 17–28

Genetic structure of the annual weed *Senecio vulgaris* in relation to habitat type and population size

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Abstract

Throughout the world, the highly selfing annual common groundsel, *Senecio vulgaris* (Asteraceae) is a common weed. Recently, it has also colonized ecological compensation areas in agro-ecosystems. We investigated the genetic structure of *S. vulgaris* using random amplified polymorphic DNA (RAPD) profiles of 80 plants from nine populations representing three habitat types in two regions in Switzerland. RAPD variation among regions (19.8%), among populations within regions (19.2%) and within populations (61.1%) was highly significant (AMOVA; $P < 0.001$). Gene flow estimated from the observed differentiation among populations ($\Phi_{ST} = 0.382$) was low (assuming Wright's island model, $N_e m = 0.404$). Genetic distances between pairs of populations were significantly correlated with geographical distances (Mantel test; $r = 0.37$, $P < 0.03$). Molecular variance obtained with AMOVA was lowest in the small populations in compensation areas (1.13), intermediate in vineyard populations (2.49), all located in northern Switzerland and highest in the larger vegetable field populations from western Switzerland (3.41; $P < 0.05$). Overall, there was a positive correlation of molecular variance and population size ($P < 0.05$), as expected under genetic drift. However, molecular variance was negatively correlated with population size among populations in ecological compensation areas, suggesting that selection was also important. We also applied triazine herbicide to leaves of three offspring of each of the 80 plants. Plants from populations of compensation areas showed higher mean levels and reduced variation in the resistance to triazine herbicide than plants from vineyards and vegetable fields. This suggests that compensation areas were colonized from adjacent corn fields, in which there has been selection for herbicide resistance. We discuss the implications of our results for the biological control of *S. vulgaris*.

Keywords: biological control, genetic variation, population genetic structure, population size, RAPD–PCR, weed

Received 23 May 2000; revision received 7 August 2000; accepted 22 August 2000

Introduction

Most weed species grow over a broad range of environmental conditions and exhibit large variation in size and morphology (Barrett 1982). This variation may either be a consequence of phenotypic plasticity or result from genetic differentiation within and between populations (Bradshaw 1965; Schlichting 1986; Barrett 1988; Schmid 1992; Hermanutz & Weaver 1996). Genetic variation is important as a prerequisite for further evolution, for successful establishment after seed dispersal and as a major determinant of plant response to pathogens (Silvertown & Lovett Doust 1993). Studies of variation in flower morphology, isozymes (Warwick 1990), DNA markers (Cavan & Moss 1997; Dietz *et al*. 1999), physiology (Darmency & Aujas 1986), effects of herbicides (Gasquez 1991), or bioassays with pathogens (Burdon *et al*. 1983) show that most weeds are genetically variable, with the exception of those which reproduce exclusively asexually (Cousens & Croft 2000).

Senecio vulgaris L. (Asteraceae), common groundsel, is a very short-lived, predominantly selfing annual. While it most probably originated in southern Europe (Kadereit

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1984), it is now common throughout the world. Because of its short generation time, its capacity for prolific seed production and rapid germination throughout the year, *S. vulgaris* is a troublesome weed, especially in plant nurseries, orchards, vineyards and fields with frequent cultivation (Holm *et al*. 1997). *Senecio vulgaris* was the first species which developed herbicide resistance and nowadays triazine herbicide-resistant populations are common and widespread in Europe and North America (Holt & LeBaron 1990). While dunes probably constitute the natural habitat of *S. vulgaris*, it is mainly associated with disturbed habitats such as ruderal sites (gravel pits, waste grounds, roadsides) and frequently cultivated crop fields, where it grows in large populations. More recently, small populations of *S. vulgaris* have established in so-called areas of ecological compensation in agro-ecosystems. In Switzerland, a large number of compensation strips has been installed during the past 10 years to promote species diversity in arable and grassland systems, such as by sowing wild flower strips (*Buntbrachen*) or by establishing crop strips without input of fertilizers and herbicides (*Ackerschonstreifen*). Due to its potential as a weed, *S. vulgaris* has never been used in such seed mixtures of wild flowers.

Because of the problems associated with intensive use of herbicides, biological control of weeds is considered a valuable alternative, i.e. the deliberate use of natural enemies to reduce biomass or population density of a target weed (Müller-Schärer *et al*. 2000). *Puccinia lagenophorae* (Uredinales, Basidiomycetes), a naturalized rust fungus from Australasia, is presently the most common pathogen on *S. vulgaris* in central Europe. In Switzerland, it was found associated with *S. vulgaris* in various habitats, including crop fields and ecological compensation areas (Müller-Schärer & Wyss 1994). With *S. vulgaris* and *P. lagenophorae* as a model system, a 'system management approach' of biological weed control is being developed which aims at management of the weed-pathosystem in order to maximize the natural spread and disease severity of a pathogen (Müller-Schärer & Frantzen 1996; Frantzen & Hatcher 1997; Frantzen & Müller-Schärer 1998; Müller-Schärer & Rieger 1998). Knowledge of amount and distribution of phenotypic and genetic variation in *S. vulgaris* populations is important both to achieve this goal (Frantzen & Müller-Schärer 1998) and for measures to minimize the development of resistance to the pathogen (Wyss & Müller-Schärer 1999). It is of particular interest whether compensation area populations are genetically different from crop field populations and which proportion of genetic variation of *S. vulgaris* is harboured in ecological compensation areas, because genetic variation in compensation areas may affect the success of biological control of *S. vulgaris* not only there, but also through gene flow in adjacent crop fields.

Here, we studied genetic variation in plants representing nine populations of *S. vulgaris* from three different habitat types in Switzerland: ecological compensation areas (*Buntbrachen* and *Ackerschonstreifen*), vineyards and vegetable fields. The last two represent a perennial and an annual cropping system, respectively. In all three habitat types, *S. vulgaris* is a troublesome weed and biological control is a potential control measure. Because *S. vulgaris* is highly selfing and because its populations are separated by unsuitable habitat such as forests, grasslands and cereal fields, we expect pronounced differentiation among populations. Moreover, we hypothesize that genetic diversity may differ between populations of *S. vulgaris* in different habitat types. On the one hand, low management intensity and low levels of disturbance and herbicide input in areas of ecological compensation enable high plant diversity and high pollinator activity, which may increase the chance for successful seed-set by *S. vulgaris* and might favour outcrossing in this predominantly selfing species (Campbell & Abbott 1976), which in turn may increase genetic diversity. On the other hand, populations in ecological compensation areas may constitute sink populations from adjacent corn fields in which there has been strong selection for herbicide resistance. Occurrence of triazine herbicide resistance in populations from ecological compensation areas would provide support for this hypothesis. Colonization by a few (herbicide-resistant) founders may lead to lower genetic variability in *S. vulgaris* populations in compensation areas compared with populations in crop fields. The level of disturbance by management interventions is highest and the herbicide use most intensive in the vegetable fields, in which more than one crop is grown per year. Levels of agricultural interventions are intermediate in vineyards and lowest or negligible in the compensation areas.

We studied random amplified polymorphic DNA (RAPD; Williams *et al*. 1990). Once this method is established, RAPD–PCR (polymerase chain reaction) is quick and easy, requires little plant material and offers a high resolution (Steinger *et al*. 1996). We ask how molecular genetic (RAPD) variation is partitioned between two sampling regions, among populations within regions and within populations. Moreover, we estimate gene flow and ask whether genetic and geographical distances are correlated. Then we ask whether observed RAPD patterns reflect effects of selection, or effects of genetic drift. If drift is more important we do not expect genetic differences between populations to be related to the different habitat types, but we expect lower genetic variation in smaller populations than in larger ones. In contrast, if selection is more important, genetic differences between populations may be related to the different habitat types and genetic variation may be lower in larger populations than in smaller ones.

Inevitably, the interpretation of the RAPD structure of *S. vulgaris* is complicated by the fact, that different habitat types occur in different geographical regions and that populations in different habitat types differ in their sizes.

Therefore, to obtain further information on the importance of selection, we complement the molecular data with an experiment on levels and variation in triazine resistance, using offspring of the plants analysed with RAPDs. There we ask whether average levels and variation in triazine herbicide resistance of plants of *S. vulgaris* vary between habitat types and populations of different size. Then, we discuss the genetic structure of *S. vulgaris* populations in the three habitat types and evaluate possible implications for biological control of *S. vulgaris* with the naturalized rust fungus *P. lagenophorae*.

Materials and methods

Selection and size of study populations and sampling of plant material

Many ecological compensation areas, which have been established in large numbers in the Klettgau area of northeast Switzerland in the late 1980s, have been colonized by *Senecio vulgaris*. In the Klettgau area *S. vulgaris* is also a common weed in vineyards. As a weed in vegetable fields *S. vulgaris* can frequently be found in the canton of Fribourg, about 130 km southwest of the Klettgau. We studied nine populations of *S. vulgaris*, three in the canton of Fribourg and six in the Klettgau (Table 1). The three selected populations in vegetable fields in the canton of Fribourg were situated within a diameter of 6 km and the six populations in compensation areas and in vineyards in the Klettgau were randomly dispersed within a diameter of 14 km. Pollinator activity (measured on 23 July 1998 as number of potentially pollinating insects flying over five 4-m lines per population during 2 min per line) was significantly higher in compensation areas (19.9 insects/min) than in vineyards (6.4 insects/min; S. Rigamonti, unpublished data).

In early May 1997, we estimated population size of *S. vulgaris* by multiplying average densities with the area occupied by *S. vulgaris*. Such areas were either entire ecological compensation plots (strips 6–12 m wide) or homogeneously managed crop fields. To assess average density at a site, we counted all *S. vulgaris* plants in six 1 m^2 plots regularly placed in a 1-by-100 m transect. In each population, we collected seeds from 20 plants randomly selected within the transect (in vegetable fields in fall 1996 and in the Klettgau in early May 1997). Thus, plant samples of all nine populations represent areas of the same size. During October and November 1997 we grew maternal seed families from all sampled plants under homogeneous conditions in a heated greenhouse.

DNA extraction and amplification

For RAPD analysis, we selected up to 10 maternal seed families per population (from some of the populations less than 10 seed families had germinated). Then, we randomly selected one plant per maternal seed family on 26 October 1997. Finally, we took one young leaf from each of 80 plants, representing 7–10 seed families of each of the nine populations (Table 1). The leaves were rinsed with distilled water, put into Eppendorf tubes, lyophilized and stored at –18 °C. DNA was extracted in November 1997 using a CTAB procedure (modified from Rogers & Bendich 1988; see Steinger *et al*. 1996). Amplification reactions

Table 1 Study populations of *Senecio vulgaris*

Population	Site	Longitude (east)	Latitude (north)	Colonized area (m^2)	Population size	*n* plants examined	*n* RAPD phenotypes	Molecular variance
Klettgau								
CA1	Klettgau 1	8°30′57″	47°41′59″	500	240	9	3	0.94
CA2	Klettgau 2	8°30′54″	47°42′01″	500	315	7	2	0.80
CA3	Rafz/Eglisau	8°30′52″	47°35′17″	200	68	8	3	1.66
VI1	Siblingen	8°31′44″	47°42′30″	1000	2500	10	5	2.21
VI2	Oberhallau	8°28′14″	47°42′45″	1000	320	10	7	2.62
VI3	Hallau	8°27′49″	47°42′26″	1000	600	9	8	2.63
Fribourg								
VE1	Sugiez	7°06′11″	46°57′15″	100	468	7	7	3.38
VE2	Ried	7°11′03″	46°56′59″	270	2376	10	7	3.44
VE3	Kerzers	7°10′51″	46°58′42″	205	3690	10	7	3.41

Colonized area, indicates the total area of the population of *S. vulgaris*; population size, denotes the number of flowering plants in May 1997; *n* plants examined, denotes the number of plants used for molecular analyses; *n* RAPD phenotypes, denotes the number of different RAPD phenotypes to which the examined plants belonged; molecular variance (AMOVA sum of squares divided by N-1) is a measure of genetic variability (see Methods); CA, denotes populations in ecological compensation areas; VE, those in vegetable crops; and VI, those in vineyards.

(25 μL) contained DyNAzyne™ II reaction buffer [10 mM Tris–HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100), 100 μM each dATP, dCTP, dGTP, dTTP, 0.2 μM primer (Operon Technologies Inc.), 0.5 units DyNazyme™ II polymerase (Finnzymes OY)] and approximately 20 ng DNA. Reactions were held in polycarbonate microtitre plates and overlaid with one drop of mineral oil. The samples were incubated in a DNA thermocycler (MJ Research Inc.; model PTC100–96 V) programmed with the folllowing parameters: 1 min at 93 °C followed by 45 cycles of 30 s at 92 °C, 30 s at 36 °C and 90 s at 72 °C. An incubation of 5 min at 72 °C was included as a final step. Amplified DNA fragments were separated by electrophoresis on 1.6% agarose gels and subsequently visualized by ethidium bromide staining. Gels were digitized on a UV table with a video camera-based imaging system. In a first series of amplifications 20 10-base primers (Kit A from Operon) were tested for reproducibility of the amplified fragment profile using a minimum of four replicates of a single DNA extract. The first 10 primers yielding reproducible patterns (primers A1, A2, A5, A8, A10, A11, A14, A15, A17 and A18) were selected for the RAPD analysis of all 80 sampled plants. The presence or absence of bands was scored for 74 band positions in the range of intermediate molecular weight (Stewart & Porter 1995).

Triazine herbicide resistance

On 21 October 1998, one year after the RAPD analyses, 7–10 seeds, resulting from selfing of the 80 plants used for the molecular analyses, were sown in seed trays and kept in the greenhouse of the Department of Biology of the University of Fribourg. Two weeks later, three seedlings per maternal plant were planted individually into 9-cm diameter plastic pots, which were filled with nutrient-amended peat (Floragard TKS2; Floragard). The pots were placed randomly on a tray in the greenhouse and kept at a 16-h day (with a light intensity of c. 250 μmol m^{-2} s^{-1} at 23 °C and 60% humidity) and an 8-h night (at 16 °C and 80% humidity). On 17 November 1998, when plants had grown on average eight true leaves, they were treated with triazine (Gesaprim; 50% atrazine, Novartis Agro AG), using a concentration corresponding to a normal field application rate of 1.5 kg active ingredient ha^{-1}. For the treatment, plants were arranged on a surface of 0.62 m^2 and sprayed for 31 s with an aerospray [Aero-Spray 2000; Birchmeier & Cie; standard nozzle (0.16) with 1 mL/s at 200 kPa and a spray distance of 30 cm]. This corresponds to a herbicide dosage of 500 L/ha. Effects of the herbicide were assessed 15 days after application. Phytotoxic symptoms of all plants were individually ranked according to the European Weed Research Society (EWRS) classification scheme for plant tolerance (scaling from 1 to 9; with 1 indicating no symptoms/healthy plant and 7–9 representing heavy damage to death; Anonymous 1992). This measurement is a good indicator of the level of triazine resistance (Frey *et al*. 1999). Plants in our study showed phytotoxic symptoms in the range of 1–8 at 15 days after herbicide application, when the experiment was stopped. According to our previous studies (Frey *et al*. 1999), plants with values above 3 will die prior to seed set.

Statistical analysis

Variation in RAPD patterns was analysed with analysis of molecular variance (program AMOVA, version 1.55, see Excoffier *et al*. 1992; Stewart & Excoffier 1996). AMOVA analyses are based on the pairwise squared Euclidean distances between RAPD phenotypes. Because band states can only take the values 0 and 1, these Euclidean distances equal the number of band states in which pairs of RAPD phenotypes differ. Because sums of squares in a conventional analysis of variance (ANOVA) can be written as sums of squared pairwise differences, AMOVA is closely related to ANOVA. AMOVA allowed us to calculate variance components and their significance levels for variation among regions, among populations within regions and within populations. We also used AMOVA to test for differences between vineyard and compensation area populations within the Klettgau region. Because significance tests in AMOVA are based on permutation procedures, they are essentially distribution free (Excoffier *et al*. 1992). The program also extracts analogues of *F*-statistics, so-called Φ-statistics. Thus, pairwise genetic distances (Φ_{ST}) among the nine populations and their levels of significance were also obtained from the AMOVA. Under the assumptions of Wright's island model, gene flow (N_em) can be approximated as $N_em = [1/(F_{ST} - 1)]/4$ (Wright 1951; but see Whitlock & McCauley 1999). Homogeneity of molecular variance in pairs of populations was tested with Bartlett's test, which is also implemented in the program AMOVA 1.55.

We used a Mantel permutation test (Mantel 1967) to test whether genetic distances between pairs of populations were significantly correlated with corresponding geographical distances [program MANTEL of the 'R' package (release 3.0) for multivariate analysis; Legendre & Vaudor 1991].

We analysed molecular variance with ANOVA, fitting the (log-transformed) covariate population size, the factor habitat type and their interaction. To consider covariate and factor before the highly significant two–way interaction we used sequential sums-of-squares (Payne *et al*. 1993). The levels of significance of habitat type and population size did not depend on their sequence in the model.

To analyse phytotoxicity levels after herbicide application, we used a hierarchical ANOVA with populations nested within habitat types and seed families nested within populations. Because at the time of herbicide application the number of leaves did not vary among plants from the

three habitat types, developmental stage was not considered in this analysis. To quantify variation in the phytotoxicity levels among plants, we used the Berger-Parker Dominance index, which indicates the proportion of the most frequent phytotoxicity phenotype in a population (Southwood 1978). Higher values of this index indicate lower levels of variation.

Results

Partitioning of RAPD variation and gene flow

The final presence–absence matrix contained scores of 80 individuals at each of the 74 bands (primer A1, eight bands; A2, nine bands; A5, six bands; A8, three bands; A10, nine bands; A11, ten bands; A14, three bands; A15, eight bands; A17, 11 bands; A18, seven bands). The 80 plants of *Senecio vulgaris* belonged to 42 different RAPD phenotypes. Twenty-six of the 74 scored bands were polymorphic (35%). None of the nine populations was monomorphic. For each population sample of 6–10 plants, we detected between two and eight different RAPD phenotypes (Table 1). The pair of RAPD phenotypes with the largest Euclidean distance differed in 15 of the 26 polymorphic bands.

Table 2 Summary of hierarchical analysis of molecular variance (AMOVA)

		Variance components		
Level of variation	d.f.	Absolute	%	*P*
Among regions	1	0.786	19.77	<0.001
Among populations	7	0.762	19.18	<0.001
Within populations	71	2.428	61.05	<0.001
Total	79			

Plants represented nine populations of *Senecio* vulgaris from two regions, canton of Friboug (three populations) and Klettgau (six populations). The analysis is based on RAPD phenotypes consisting of scores at 26 polymorphic band positions. Levels of significance are based on 1000 iteration steps.

Variation in RAPD banding pattern among the two regions, among populations within regions and within populations was highly significant ($P < 0.001$) at each level, indicating genetic divergence both among regions and among populations (Table 2). Φ_{ST}, the correlation among random RAPD phenotypes within populations relative to the correlation of random pairs drawn from the whole sample was 0.382. Φ_{CT}, the correlation among random phenotypes within regions relative to the correlation of random pairs drawn from the whole sample was 0.210 and Φ_{SC}, the correlation of random phenotypes within populations, relative to that of random pairs drawn from the region, was 0.219. Gene flow, i.e. the average number of individuals exchanged among populations per generation (N_em), was low (0.404). To estimate gene flow among populations within regions, we used Φ_{SC} instead of Φ_{ST} and obtained N_em as 0.892. Within the Klettgau region, there was a significant difference between *S. vulgaris* populations in vineyards and in ecological compensation areas (AMOVA, 21% of the variation between habitat types; $P < 0.001$.

Twenty-eight of the 36 pairwise genetic distances (Φ_{ST}) between populations were significant (Table 3). The three populations from ecological compensation areas did not significantly differ from each other, although one was more than 12 km away from the others.

Correlation between genetic and geographical distances between pairs of populations

Pairwise genetic distances were positively correlated with geographical distances (nine populations; $r = 0.366$; $P = 0.028$; Mantel-test). When we excluded the three populations from vegetable fields in the canton of Fribourg and only

Table 3 Pairwise genetic distances (Φ_{ST}) among nine populations of *Senecio vulgaris*

	Population							
	CA1	CA2	CA3	VI1	VI2	VI3	VE1	VE2
CA1	—							
CA2	0.05	—						
CA3	0.01	0.07	—					
VI1	0.02	0.03	0.03	—				
VI2	0.41***	0.40***	0.39***	0.21*	—			
VI3	0.54***	0.50***	0.48***	0.36***	0.23***	—		
VE1	0.41***	0.36***	0.32***	0.21*	0.15*	0.21*	—	
VE2	0.50***	0.49***	0.44***	0.36***	0.18***	0.34***	0.06	—
VE3	0.50***	0.49***	0.43***	0.32***	0.20***	0.36***	0.12*	0.12

*$P < 0.05$; ***$P < 0.001$. *P*-values indicate the probability that a random genetic distance Φ_{ST} is larger than the observed distance and are based on 1000 iterations. Populations are denoted as in Table 1.

Table 4 Pairwise tests of heteroscedasticity of molecular variance among nine populations of *Senecio vulgaris*. Bartlett's B is given for each pair of populations

	Population							
	CA1	CA2	CA3	VI1	VI2	VI3	VE1	VE2
CA1	—							
CA2	1.27	—						
CA3	1.55	2.02	—					
VI1	2.46	2.48	1.34	—				
VI2	7.85***	6.36***	5.56***	3.19*	—			
VI3	10.63***	8.01***	7.19***	5.40***	3.43***	—		
VE1	7.47***	5.88***	4.34**	3.00*	2.20*	2.77**	—	
VE2	10.69***	8.61***	7.09***	5.71***	2.89***	5.11***	1.35	—
VE3	10.61***	8.62***	6.87***	5.16***	3.18***	5.48***	1.86	2.09*

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. *P*-values indicate the probability that a random B is larger than the observed B and are based on 1000 iterations. Populations are denoted as in Table 1.

Table 5 Summary of analysis of variance of the relationship of habitat type and population size (after log-transformation) with molecular variance (a measure of RAPD variability within populations, see Methods) of nine Swiss populations of *Senecio vulgaris*

Source of variation	d.f.	Sum of squares	*F*	*P*
Habitat type	2	7.89	954.4	0.0001
Log (pop. size)*	1	0.237	57.3	0.0047
Habitat type-by-log (pop. size)	2	0.292	35.3	0.0081
Error	3	0.0124		

*pop. size, population size.

analysed distances among the six Klettgau populations, the resulting positive correlation of genetic and geographical distances was weak and not statistically significant (six populations; $r = 0.0075$; $P = 0.40$; Mantel-test).

Molecular variation

Molecular variance per population sample (which represented similar areas in all populations) differed significantly among populations of different regions, of different habitat types and among populations within habitat types ($P < 0.001$; Bartlett test). Twenty-eight of 36 pairwise Bartlett tests indicated significant differences in population homogeneity of molecular variation (Table 4).

Molecular variance was highest in populations in vegetable fields (3.41), intermediate in vineyard populations (2.49) and lowest in populations in ecological compensation areas (1.13; ANOVA and Scheffé *F*-test; $P < 0.001$ for all comparisons). Within habitat types, molecular variance was negatively correlated with population size (ANOVA, $P < 0.05$, Table 5). This was pronounced in the ecological compensation areas, but not in the crop populations (as indicated by the habitat type-by–population size interaction). Overall, the correlation between population size and molecular variance was significantly positive (Spearman's rank-correlation coefficient $r_s = 0.72$, $P < 0.05$; Fig. 1a).

Mean and variation in the level of herbicide resistance

Plants from populations of different habitat types differed in their level of phytotoxicity 15 days after application of atrazine to their leaves (ANOVA; $P < 0.03$). Mean levels of phytotoxicity were lowest (i.e. the level of resistance was highest) in plants from populations in ecological compensation areas (mean score 2.9). Levels of phytotoxicity were intermediate in plants from vineyard populations (mean score 4.3) and they were highest in plants from vegetable field populations (mean score 6.1). While the mean level observed for plants from compensation areas indicates resistance, mean levels of plants from vegetable fields and vineyards are so high, that most plants would have died before reproduction. Levels of phytotoxicity differed significantly among seed families within populations ($P < 0.01$), suggesting genetic variation in resistance also within populations (Table 6). Within habitat types, phytotoxicity levels were independent of population size. However, overall phytotoxicity levels were higher in larger populations (Spearman's rank-correlation coefficient $r_s = 0.56$, $P < 0.04$) and in populations with higher molecular variance ($r_s = 0.72$, $P < 0.01$; Fig. 1b).

The Berger–Parker Dominance index, which indicates the proportion of the most frequent phytotoxicity phenotype (i.e. higher values of this index indicate lower variation), tended to be higher in populations in compensation areas (0.65) than in those in vineyards (0.47) or in vegetable fields (0.35). Within habitat types, this index was independent of population size. However, overall the correlations of the Berger–Parker Dominance index with population size (Spearman's rank-correlation coefficient $r_s = -0.74$, $P < 0.03$) and with molecular variance ($r_s = -0.44$, $P < 0.001$;

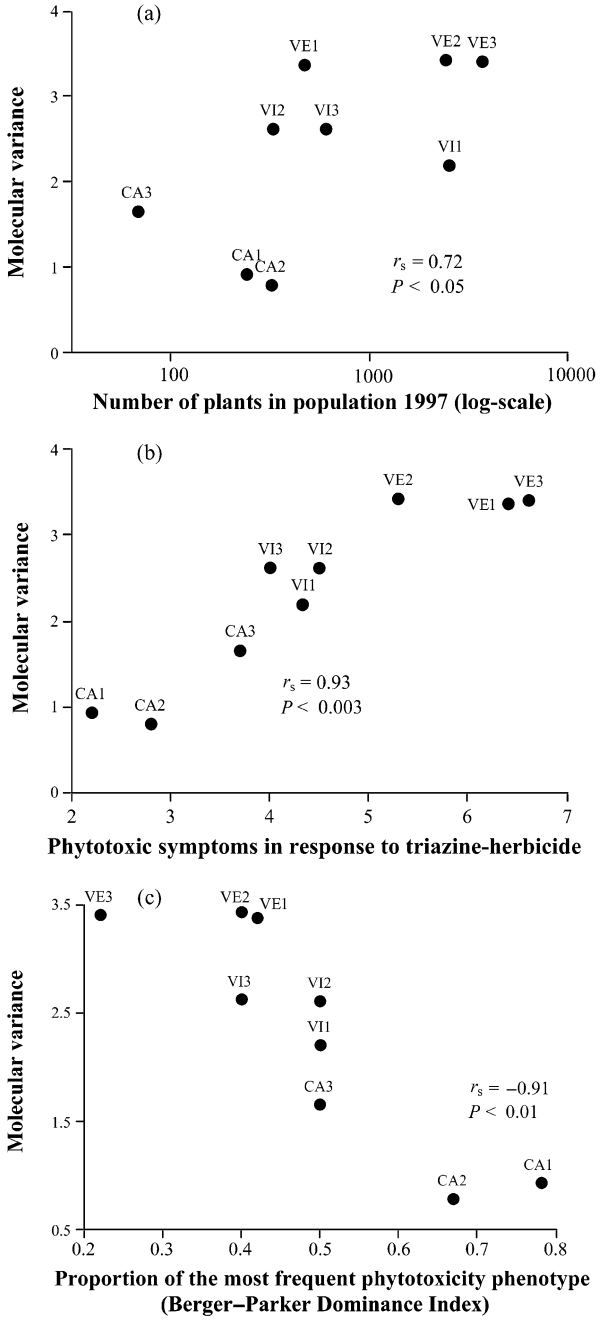

Fig. 1 The relationship between molecular variance (a measure of RAPD variability within populations of *Senecio vulgaris*, which is based on the same sampling area in all nine study populations, see Methods) and (a) population size (number of flowering plants per population in May 1997); (b) level of mean herbicide resistance (where higher values of the phytotoxic symptoms indicate lower resistance); and (c) Berger–Parker Dominance index (which indicates the proportion of the most frequent phenotype in terms of herbicide resistance, see Methods; higher values of this index indicate lower variation). Measurements of phytotoxicity of triazine herbicide were made on selfed offspring of the 80 plants used for RAPD–PCR analyses. Population symbols are labelled according to habitat types: CA denotes populations in ecological compensation areas; VE, those in vegetable crops; and VI, those in vineyards.

Table 6 Summary of hierarchical analysis of variance of phytotoxic symptoms (level of resistance against triazine herbicides) of plants from nine Swiss populations of *Senecio vulgaris* from different habitat types assessed 15 days after herbicide application

Level of variation	d.f.	Sum of squares	*F*	*P*
Habitat type	2	65.56	5.88	0.039
Populations within habitat types	6	33.47	2.01	0.076
Seed families within populations	71	197.31	1.78	0.002
Error	160	250.00		

Fig. 1c) were negative, indicating smaller variation in the response to triazine herbicide among plants from smaller populations with smaller molecular variance.

Discussion

Analysis of genetic variation with RAPD

Since the development of appropriate statistical procedures (see Excoffier *et al*. 1992; Lynch & Milligan 1994; Stewart & Excoffier 1996) RAPD (Williams *et al*. 1990) has been widely used for the analysis of population genetic structures (Bussell 1999). This is of advantage because DNA polymorphisms, such as those detected by RAPD, are assumed to be less biased estimators of genetic variation than variation at the level of gene products such as allozymes (Stewart & Excoffier 1996). In our study, RAPD proved to be a high-resolution method for the detection of genetic variation in *Senecio vulgaris* and indicated pronounced genetic differentiation among populations. However, the estimation of population genetic parameters from RAPD data is under debate because the inference of F_{ST} from generally dominant RAPD data is based on the two assumptions of homologous null bands and of Hardy–Weinberg equilibrium (Lynch & Milligan 1994; Ayres & Ryan 1999). The high levels of significance in the AMOVA (Table 2) suggest that our results are robust to deviations from the two assumptions. Nevertheless, deviations from Hardy–Weinberg equilibrium due to the high degrees of selfing in *S. vulgaris* (Campbell & Abbott 1976) may inflate RAPD-based F_{ST} estimates. However, our estimate of F_{ST} for *S. vulgaris* was smaller than the average observed for highly selfing annuals (Hamrick & Godt 1990; Bussell 1999; as discussed below).

In our study we used 74 markers of which 26 were polymorphic (RAPDs). Because the variance of population genetic estimates hardly decreases any more in this range of marker numbers (Aagard *et al*. 1998), we consider the number of markers in our population genetic study as sufficient. Because RAPD-based estimates of differentiation might be biased towards higher values compared with

estimates based on other markers, it was suggested to use 100 plants per population and to restrict data sets according to the '3/*N*-criterion', i.e. to exclude markers with very low frequencies of null alleles (Lynch & Milligan 1994; see Isabel *et al*. 1999 for an application). However, genetic distances obtained from a restricted data set were consistent with those obtained from the complete set in a study on 11 populations of *Hippophae rhamnoides* represented by about 10 plants each (Bartish *et al*. 1999). In addition, these authors conclude that the 3/*N*-criterion may only be appropriate in analyses with larger population samples while it may even underestimate genetic differentiation among populations in small samples of closely related populations (Bartish *et al*. 1999).

Differentiation among populations

Senecio vulgaris is a short-lived annual and reported to be predominantly inbreeding (Campbell & Abbott 1976). Hence, genetic differentiation among populations may be expected (Hamrick *et al*. 1991; Hamrick & Godt 1996). Indeed, we detected 39% of RAPD variation among populations (Table 2, pooled over both regions). In their review comprising more than 400 plant species Hamrick & Godt (1990) used G_{ST} values to indicate the proportion of isozyme diversity residing among populations. They report an average G_{ST} of 22% for perennial herbs compared with 36% for annuals and an average G_{ST} of 20% for animal-pollinated outcrossers compared with 51% for selfers. To date, RAPD-based G_{ST} values are available for 35 plant species, with averages of 19.3% for 29 outbreeding species and 62.5% for six inbreeding species (Bussell 1999). Compared with these values the populations of *S. vulgaris* appear to be less differentiated than expected for a highly selfing annual. This may suggest that the degree of outcrossing in *S. vulgaris* is higher than previously assumed.

Pronounced genetic differentiation observed among populations of *S. vulgaris* suggests low gene flow and high degrees of population isolation. Indeed, cereal fields, grasslands and forests, which separate populations of *S. vulgaris*, constitute barriers against dispersal of seed and pollen.

Genetic and geographical interpopulation distances were significantly positively correlated in some plant species (Godt & Hamrick 1993; Godt *et al*. 1995; Berge *et al*. 1998; Ayres & Ryan 1999), but not in others (Berge *et al*. 1998; Fischer & Matthies 1998; Fischer *et al*. 2000; Schmidt & Jensen 2000). Close correspondence of geographical and genetic distances is only likely if there is at least some outcrossing in a species, if gene flow is a simple function of geographical distance and if such an effect of gene flow is not compensated by genetic drift or selection. In our study, genetic and geographical distances were significantly correlated at the regional scale including all study populations of *S. vulgaris*. Indeed, the large geographical distance between the populations in the canton of Fribourg and those in Klettgau may well explain the observed strong genetic differentiation among these populations (Tables 2 and 3) and the correspondingly low estimate of gene flow. At the more local scale, among Klettgau populations only, the positive relationship between geographical and genetic distances was weak and not statistically significant. Populations CA1, CA2 and VI1 (see Table 1), which were located less than 2 km from each other, were not genetically distinct (Table 3). This might reflect high gene flow over distances of a few kilometres (but see preceding paragraph). Moreover, it could be due to reduced statistical power. On the other hand, the significant genetic differentiation between the interspersed populations in vineyards and those in ecological compensation areas in the Klettgau region suggests that the observed pattern of differentiation among populations was at least in part also due to selection in different habitats. Similarly, selection may have contributed to the differences between the populations in the Klettgau region and those in vegetable fields in the canton of Fribourg.

Genetic variation in relation to habitat type and population size

Because the three compensation areas in our study have been established only recently, between 1992 and 1996, founder effects are a likely explanation for their small genetic variability. In contrast, recruitment of populations of *S. vulgaris* from a persistent seed bank, which has established before sites were transformed from crop fields into ecological compensation areas, is unlikely because most seedlings of *S. vulgaris* emerge in the first year and few seeds retain their viability for up to five years (Roberts 1964; Roberts & Feast 1972).

In corn fields single herbicide types such as triazines are repeatedly applied over decades, whereas *S. vulgaris* populations in vegetable crops are subjected to mixed herbicide and cultivation strategies which makes the development of one particular form of herbicide resistance and the associated reduction of genetic variation in resistance less likely (Moodie *et al*. 1997). It is well established that *S. vulgaris* populations in corn fields are highly triazine-resistant (Ammon & Beuret 1984; Holt & LeBaron 1990; Kees 1991). Nevertheless, it would be interesting to compare means and variations of the level of triazine resistance of *S. vulgaris* in Swiss corn fields with those in the habitat types of our study.

In our study, *S. vulgaris* populations more resistant to triazine herbicides (i.e. populations in compensation areas adjacent to corn fields) were found to be less genetically polymorphic than susceptible populations (in vineyards and vegetable fields), indicated by smaller molecular

variance and by reduced variation in the response to the triazine herbicide. This corresponds well with similar results in other species (Warwick & Black 1993). Levels of isozyme polymorphism in garden populations of *Chenopodium album* were higher than in field populations, which was attributed to the repeated use of triazine herbicides in the field populations (Al Mouemar & Gasquez 1983). In a similar study, DNA fingerprinting showed that herbicide-resistant populations of the primarily selfing *Avena fatua* and *A. ludoviciana* were genetically less diverse than those of sensitive plants, whereas resistant and susceptible patches of the outbreeding *Alopecurus myosuroides* were equally diverse (Cavan & Moss 1997). Low levels of genetic variability in triazine-resistant populations of *S. vulgaris* may well have been caused by bottleneck effects which were especially severe because of the low frequency of the resistance alleles and because the predominantly selfing mating system may hamper a quick recovery from reduced genetic variation (Moodie *et al*. 1997). In principle, conclusions drawn from variations in selectively neutral markers, such as isozymes or DNA polymorphisms, on quantitative genetic variation in fitness relevant traits must be considered with caution. However, in our study molecular variance of populations was closely related with their variability in response to triazine herbicide (Fig. 1c). We consider severe founder effects in *S. vulgaris* in the compensation areas of our study as very likely, because compensation areas were situated next to corn fields regularly treated with triazine herbicide. Colonization by resistant *S. vulgaris* genotypes from such fields may well have caused reduced genetic variation of populations in compensation areas, despite the higher activity of potential pollinators reported from these diverse and flower-rich habitats.

In *S. vulgaris* populations in crop fields, especially in vegetable fields with their high level of disturbance and herbicide input, we observed the highest levels of per area genetic variation. In vineyards, with little herbicide input and an intermediate level of disturbance through rotational cultivation, both population size and genetic variation were found to be intermediate compared to populations from vegetable fields and compensation areas. Comparably high RAPD variation found in untreated populations of *Sinapis arvensis* and in populations from crop fields with rotational cropping and herbicide use could be explained if seedlings may escape herbicide treatment, show natural resistance, or germinate from the seed bank at a time when herbicide pressure is low (Moodie *et al*. 1997). An alternative explanation could be different patterns of genetic variation between selectively neutral and fitness-related markers. With regard to the present study, limited seed dispersal or pollen transfer also might have taken place into the vegetable field populations from *S. vulgaris* populations of adjacent crops with a different management regime, or from neighbouring ruderal sites or fallow land, where *S. vulgaris* often grows at high densities and over several years. Such gene flow through seed dispersal might have contributed to the relatively high genetic variation found in vegetable field populations.

On the other hand, at least in part, the high genetic variability of populations of *S. vulgaris* in crop fields may be due to their large size, which reduces the effects of genetic drift. Overall, the correlation between molecular genetic variation and population size in *S. vulgaris* was positive (Fig. 1a). Similarly, reduced genetic variation in smaller populations has been reported for 11 of 16 plant species mentioned in a review (Frankham 1996) and in six of seven species studied since then (Berge *et al*. 1998; Fischer & Matthies 1998; Persson *et al*. 1998; Prober *et al*. 1998; Fischer *et al*. 2000). These relationships were interpreted as results of genetic drift (Barrett & Kohn 1991; Ellstrand & Elam 1993; Young *et al*. 1998).

In contrast, we found molecular variance to decrease with increasing population size in the compensation areas of our study, whereas no such correlation was observed in crop fields (Fig. 1a). This suggests that effects of selection are also important during and after the foundation of populations in compensation areas. Such effects, which arise if some founding genotypes leave more descendants than others, may possibly have been promoted by the lack of disturbance in compensation areas.

Implications for weed–pathogen interactions and biocontrol

Low levels or absence of genetic variation in response to disease often lead to high levels of disease incidence (Schmid 1994) and severity in crops, which may result in devastating epidemics (Finckh & Wolfe 1997). In contrast, many natural plant populations are genetically diverse and polymorphic for disease resistance (Dinoor & Eshed 1984; Burdon 1987; Harry & Clarke 1987; Fritz & Simms 1992; Cousens & Croft 2000).

In our study of RAPD and of resistance to triazine herbicide we found high levels of genetic variation of large *S. vulgaris* populations in crop fields and low levels in smaller sink populations in ecological compensation areas. As far as RAPD variation and variation in response to the triazine herbicide may also reflect the genetic variation in response to a biocontrol agent, our study suggests that the chance of successful biological control of *S. vulgaris* using *Puccina lagenophorae* will at present not be impaired in ecological compensation areas as compared to the studied crop habitats. Nevertheless, it might be worthwhile to repeat this study of genetic variation in *S. vulgaris* populations at a later date, when the compensation areas are older than only a few years. The observed high densities of *S. vulgaris* populations in the two studied crop habitat

types could favour biocontrol and further build-up the speed of an epidemic. However, observed high levels of genetic variability of *S. vulgaris* populations in these habitats might counter this effect. To test such hypotheses, and to study the effects of selection due to *P. lagenophorae*, complementary studies on the dynamics of the *S. vulgaris* and *P. lagenophorae* populations, on the genetic composition of fungal rust populations in the studied habitats and on genetic variation in the response of *S. vulgaris* to the pathogen are presently underway.

Conclusion

The interpretation of the RAPD structure of *Senecio vulgaris* was complicated by the facts that different types of habitat occur in different geographical regions and that populations representing different habitat types differ in their sizes. For many species, such a situation may be the rule rather than the exception. Complementing the RAPD data with quantitative data on resistance phenotypes allowed us to conclude that the low genetic variability and small size of the studied compensation area populations are most probably due to founder effects by herbicide-resistant genotypes. In this way, our study exemplifies the usefulness of combining molecular data with quantitative data.

Acknowledgements

Many thanks to Serena Rigamonti for providing data on phytotoxicity, to Kirsten Leiss for collecting seed material in the canton of Fribourg, to Marius Käser for RAPD analyses and to Kirsten Leiss, Jos Frantzen, Thomas Steinger and Mark van Kleunen for comments on earlier drafts. We acknowledge financial support by the Swiss Priority Programme Environment/Biodiversity (NF 5001–50250/1.97) and the Swiss National Science Foundation (NF 31–46821.96).

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