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Low genetic differentiation among seasonal cohorts in *Senecio vulgaris* as revealed by amplified fragment length polymorphism analysis

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Abstract

Common groundsel, *Senecio vulgaris* (Asteraceae), is a highly selfing semelparous ephemeral weed that belongs to the few plant species in central Europe capable of growing, flowering and fruiting all year round. In temperate climates, flowering *S. vulgaris* cohorts were found to appear up to three times per year. Using amplified fragment length polymorphism (AFLP) molecular markers we examined temporal genetic differentiation among spring, summer and autumn cohorts at each of seven sites located in two regions in Switzerland. Strong genetic differentiation among cohorts may indicate the existence of seasonal races of *S. vulgaris*, reproductively isolated by nonoverlapping flowering phenologies. Analysis of molecular variance (amova) revealed that < 2.5% of the AFLP variation resided among cohorts within sites, whereas there was significant genetic differentiation among plants from different sites (15.6%) and among individuals within cohorts (81.9%). Significant genetic differentiation was also observed between the two regions. Isolation-by-distance was found on a regional scale, but not on a local scale. Gene flow was estimated to be ≈ 15-fold higher among cohorts within sites than among sites. We further found, on average, similar levels of genetic diversity within the three seasonal cohorts. The results of this study demonstrate that season of growth represents a weak barrier for genetic exchange among *S. vulgaris* populations and does not affect molecular variance. Therefore, there is no evidence for the existence of seasonally specialized races of *S. vulgaris*. We discuss some implications of the results for the biological control of *S. vulgaris* using a native rust fungus.

Keywords: AFLP, genetic variation, population genetic structure, *Senecio vulgaris*, temporal genetic variation, weed

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Introduction

While the vast majority of plant species in central Europe are either perennial or display an annual life cycle, a few species like *Senecio vulgaris*, *Capsella bursa-pastoris*, *Stellaria media*, *Poa annua* and *Veronica persica* can grow, flower and fruit all year round. In a number of species distinct seasonal cohorts have been described. For example, in populations of *Sinapis arvensis* two seasonal cohorts have been observed, an autumn cohort arising from newly shed nondormant seeds and a spring cohort arising from dormant seeds (Edwards 1980). Studies on the population dynamics of common groundsel, *S. vulgaris*, have demonstrated that in temperate climates population density shows important fluctuations over the year with densities being highest in late spring, mid-summer and late autumn (Vack 1992). In Switzerland, Leiss & Müller-Schärer (2001) observed three such distinct cohorts of flowering plants. It can be asked whether each cohort of *S. vulgaris* simply arises from seeds shed by the previous generation, or whether seasonal genetic differentiation may occur resulting from genetically based variation in the timing of germination. Heritable variation in dormancy within and among populations have been described for a number of species (see Baskin & Baskin 1998). For example,

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in *S. vulgaris* Ren & Abbot (1991) reported innate dormancy in seeds of Mediterranean origin, whereas seeds from the British Islands germinated almost immediately (see also Popay & Roberts 1970; Roberts & Feast 1972). Innate dormancy in Mediterranean seeds enable populations to adopt a winter-annual life cycle avoiding periods of high mortality during dry summers. In other species, seasonal ecotypes with distinct morphologies and life histories have been described, which may even overlap in their geographical range (Zopfi 1991). In *S. sylvaticus*, populations with either summer or winter annual life forms were found, with summer types having an endosperm-located dormancy, which prevented germination in the same growing season. Winter annual life forms lacked this dormancy and germinated almost immediately. The lack of dormancy in winter types was correlated with increased frost resistance enabling them to survive during winter (Ernst 1989). In a similar study, Vack (1992) compared summer and winter samples of seeds of *S. vulgaris* collected at each of two sites and found significant differentiation between seasons within sites in germination behaviour and life history traits. However, whether the observed differentiation has a genetic basis or is due to other influences (e.g. maternal environmental effects, differences in duration of seed storage) could not be distinguished.

We set out to test for seasonal genetic differentiation in *S. vulgaris* using the method of amplified fragment length polymophism (AFLP). We expected to find significant divergence in AFLP molecular markers if spring, summer and autumn cohorts exhibit a high degree of seasonal specialization and if there is essentially no gene flow between cohorts (scenario a in Fig. 1). By contrast, low differentiation is expected if cohorts overlap to some degree in their flowering periods (scenario b) or if they simply arise from seeds produced by the previous generation (scenario c).

S. vulgaris ssp. *vulgaris var. vulgaris* (Asteraceae), is a self-fertile, highly self-pollinating ephemeral (Campbell & Abbott 1976), that most likely originated in southern Europe (Kadereit 1984) and nowadays displays an almost worldwide distribution (Mitich 1995). Dunes probably represent the only natural habitat of *S. vulgaris*, but the plant typically occurs in ruderal habitats with anthropogenic disturbance, such as gravel pits, waste grounds and roadsides, as well as in agricultural habitats with frequent cultivation such as horticultural crops, where it is considered a troublesome weed (Holm *et al*. 1997). Because *S. vulgaris* is tolerant or resistant to a range of herbicides (Ryan 1970; Holt & LeBaron 1990) and because the intensive use of herbicides may eventually result in a series of problems, a biological control project, based on induced epidemics of the naturally occurring rust fungus *Puccinia lagenophorae* (Uredinales, Basidiomycetes) has been initiated (Frantzen & Hatcher 1997; Müller-Schärer & Rieger 1998; Wyss & Müller-Schärer 1999; Frantzen 2000; Frantzen *et al*. 2001). In this respect,

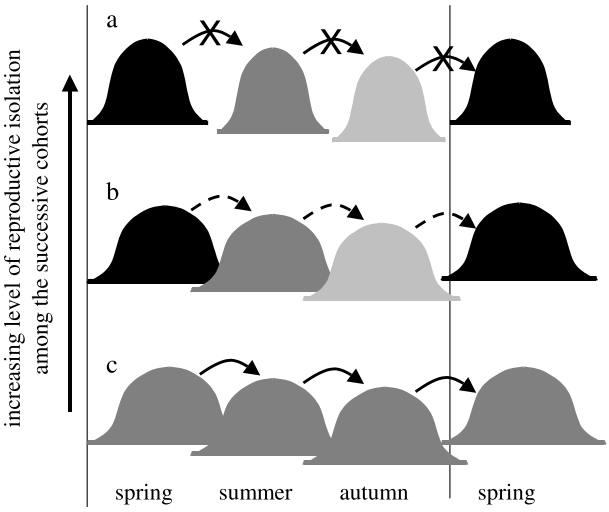

Fig. 1 Hypothetical pattern of temporal differentiation among seasonal cohorts in *Senecio vulgaris*; (a) annual life cycle with seed dormancy and strong seasonal specialization; the spring, summer and autumn cohorts are reproductively fully separated; (b) seasonal specialization with partially overlapping cohorts, resulting in some gene flow among the seasonal cohorts; (c) no seasonal differentiation, with seasonal cohorts simply arising from seeds produced in the previous generation (no dormancy); in addition, generations may partially overlap during the growing season. ⇸, ⇢ and → indicate no, little and strong gene flow among the seasonal cohorts respectively.

knowledge of the amount and distribution of genetic variation in *S. vulgaris* populations is important because it affects biocontrol success by influencing disease development and spread of the pathogen, on the one hand (short-term effect), and the development of resistance to the pathogen, on the other hand (long-term effect). Studies of the genetic structure of *S. vulgaris* populations have so far concentrated on the spatial aspect in relation to habitat type and population size (Leiss & Müller-Schärer 2001; Müller-Schärer & Fischer 2001; Steinger *et al*. 2002) whereas little attention has been given to temporal variation within sites.

In this study we used AFLP markers to examine temporal and spatial variation in the genetic structure of *S. vulgaris* populations. The study was conducted at seven sites located in two regions in Switzerland. We chose vineyards as experimental sites because of common cultivation practices with relatively low disturbance levels allowing *S. vulgaris* to naturally form three 'generations' per year. The specific questions addressed in this study were:

1 Is there significant differentiation in AFLP markers among spring, summer and autumn cohorts of *S. vulgaris* sampled at seven sites, and how does this compare with spatial genetic differentiation?
2 Does the genetic diversity within cohorts differ among the three seasonal cohorts?

Table 1 Study populations (cohorts) of *Senecio vulgaris*. *n* AFLP phenotypes denotes the number of different AFLP phenotypes to which examined plants belonged ($N = 18$, except W3 su with $N = 17$); molecular variance (AMOVA sum of squares divided by $N - 1$) is a measure of genetic variability (see Materials and methods); F and W denote cohorts from the Fribourg and Wallis regions, respectively; su, au and sp denote plants collected in summer, autumn and spring, respectively

Population	Site	Longitude (east)	Latitude (north)	Altitude (m)	*n* AFLP phenotypes	Molecular variance
Fribourg						
F1 su	Praz	7°05′721″	46°57′234″	430	16	6.21
F1 au					17	10.60
F1 sp					17	7.63
F2 su	Lugnorre	7°05′714″	46°56′938″	517	18	7.39
F2 au					17	8.28
F2 sp					18	7.03
F3 su	Vallamand	7°02′063″	46°55′651″	508	18	6.92
F3 au					18	6.24
F3 sp					18	6.42
F4 su	Mur	7°03′716″	46°56′580″	490	17	9.09
F4 au					17	10.45
F4 sp					18	9.02
Wallis						
W1 su	Leytron	7°13′191″	46°11′163″	514	18	7.85
W1 au					16	7.46
W1 sp					17	7.54
W2 su	Chamoson	7°13′384″	46°11′586″	580	18	9.22
W2 au					18	9.37
W2 sp					17	7.18
W3 su	Sensine	7°17′893″	46°14′357″	772	17	6.91
W3 au					12	4.81
W3 sp					16	6.97

Materials and methods

Plant material

We studied *Senecio vulgaris* populations occurring in seven vineyards (sites) located in two regions in Switzerland. Four sites were situated in the canton of Fribourg and three in the canton of Wallis at a straight distance of ≈ 90 km (Table 1). The two regions are separated by a high mountain range. The sites within both regions were situated within a diameter of ≈ 10 km. At each site samples were collected in summer 2000 (late July), autumn 2000 (late October) and spring 2001 (late May/early June) resulting in a total of 21 studied *S. vulgaris* cohorts. Plant material was collected by taking ≈ 2 cm² of young leaf tissue from 18 flowering plants selected every 3 m along a transect spanning the vineyard rows, resulting in 378 samples. At each site, the transect was delimited at exactly the same place for all of the three sampling dates and the sampling area was the same size for all sites. The collected plant material was carefully cleaned and rinsed with distilled water, placed in Eppendorf tubes and frozen on dry ice for the transfer to the laboratory. The samples were then frozen in liquid nitrogen, freeze-dried for 48 h and subsequently stored at –20 °C on silica gel.

DNA extraction and AFLP analysis

Genomic DNA was extracted from freeze-dried leaf tissue (≈ 20 mg) using a modified Rogers & Bendich (1988) CTAB procedure (see Steinger *et al*. 1996). One of the summer cohort samples from Sensine was lost during the extraction procedure. For AFLP analysis (Vos *et al*. 1995), PCR amplification of DNA was carried out essentially as described elsewhere (Schwarz *et al*. 2000). Briefly, 0.5 µg DNA was digested with *Mse*I and *Eco*RI restriction enzymes and two adaptors were ligated to the sticky ends of the fragments. After dilution (20-fold), the restriction–ligation products were selectively pre-amplified by PCR with adaptor-homologous primers that each comprised one additional nucleotide. PCR amplifications were performed in a thermal cycler (MJ Research Inc.; model PTC100-96V) programmed with the following thermal profile: 2 min at 72 °C followed by 20 cycles of 20 s DNA denaturation at 94 °C, 30 s annealing at 56 °C and 2 min extension at 72 °C. The last cycle was followed by 30 min at 60 °C before holding at 4 °C.

Products of the preselective amplification step were diluted 20-fold and subject to a second round of amplification using primers containing three selective nucleotides. The *Eco*RI primer (E-AGG) was labelled with 5-FAM; the *Mse*I primers (M-CAA; M-CAG; M-CAC; M-CTT) remained

unlabelled. The thermal cycler was programmed as follows: 2 min denaturation at 94 °C followed by 30 cycles of 30 s denaturation at 94 °C, 30 s annealing (see below) and 2 min extension at 72 °C. In the first cycle the annealing temperature was set at 66 °C. It was subsequently reduced by 1 °C each cycle for the next 9 cycles and thereafter maintained at 56 °C for the remaining 20 cycles. The last cycle was followed by 30 min at 60 °C before holding at 4 °C.

For fluorescence fragment analysis 2 μL of the 5-FAM-labelled PCR products were mixed with 24.6 μL deionized formamide and 0.4 μL of a labelled internal length standard (GeneScan-500 TAMRA; Applied Biosystems). The labelled PCR products were then denaturated for 5 min at 95 °C, quickly chilled on ice and subsequently analysed by capillary gel electrophoresis using an ABI Prism™ 310 automated genetic analysis system equipped with GENESCAN Version 3.1 (Applied Biosystems). Multilocus profiles were scored for the presence (1) or absence (0) of fragments between 50 and 500 bp and assembled in a binary data matrix table comprising scores at 111 polymorphic band positions for each of the 377 individuals. Only AFLP markers that could be scored unambiguously by eye were retained for analysis.

Statistical analyses

Variation in AFLP pattern was statistically analysed with analysis of molecular variance (AMOVA) using ARLEQUIN Version 2.000 (Schneider *et al*. 2000). AMOVA analyses are based on the pairwise squared Euclidean distances between AFLP phenotypes. Because AFLP markers can only take the values 0 and 1, these Euclidean distances correspond to the number of markers in which pairs of AFLP phenotypes differ. Using AMOVA, we calculated variance components and their level of significance for variation among sites, among cohorts within sites and among individuals within cohorts. We also tested whether there is genetic differentiation between plants from the two regions. We computed ϕ-statistics, which are analogs of *F*-statistics, to estimate pairwise genetic distances among the 21 *S. vulgaris* cohorts. To obtain an insight into the spatial and temporal patterns of gene flow, we estimated the average number of individuals exchanged among cohorts using the formula $N_em = (1/4)[(1/F_{ST}) - 1]$ (Wright 1951; see however, Whitlock & McCauley 1999). We used a Mantel permutation test (Mantel 1967) implemented in ARLEQUIN to test whether genetic distances between pairs of cohorts were significantly correlated with the corresponding geographical distances.

Genetic variability within each 21 cohorts was estimated by calculating molecular variance, i.e. within-cohort sum of squares divided by $N - 1$ (see Fischer & Matthies 1998). The sum of squares were obtained from AMOVA Version 1.55 (Excoffier *et al*. 1992; Steward & Excoffier 1996). Bartlett tests implemented in the same program were used to test homogeneity of molecular variance between pairs of cohorts. Because the AMOVA program is limited in the number of samples it can process for the Bartlett test, we had to conduct this test using a subset of only 13 (randomly selected) instead of the 18 plant samples collected in each of the 21 *S. vulgaris* cohorts. The dendrogram, generated using POPGENE Version 1.3.1 (Yeh *et al*. 1997), was based on Nei's (1978) genetic distance between populations using the unweighted pair group method with arithmetic averaging (UPGMA) modified from the NEIGHBOR procedure of PHYLIP Version 3.5.

Table 2 AFLP markers generated among 377 *Senecio vulgaris* individuals representing 21 cohorts from 7 sites located in 2 regions in Switzerland using 4 *Eco*RI + AGG:*Mse*I + 3 nucleotides primer pair combinations

Primer pair	Total no. of markers	No. polymorphic markers	% polymorphic markers
*Eco*RI + AGG:*Mse*I + 3			
CAA	107	31	29.0
CAC	84	44	52.4
CAG	56	19	33.9
CTT	81	17	21.0
Total	328	111	33.8

Results

AFLP polymorphism

The four primer pair combinations used for AFLP analysis generated a total of 328 AFLP markers of which 111 were polymorphic (34%) across the 377 *Senecio vulgaris* individuals analysed. Primer pairs varied greatly not only in the number of markers generated (56–107), but also in their degree of polymorphism (21–52%) (Table 2).

The 377 individuals belonged to 348 different AFLP phenotypes. Between 16 and 18 different AFLP phenotypes were detected within each examined cohort with the exception of the autumn cohort from Sensine (W3 au) in which 12 phenotypes were identified (Table 1). Plants from the region of Fribourg and Wallis did not share any common AFLP phenotype. In the region of Fribourg, only two individuals [one from the autumn cohort from Praz (F1 au) and one from the spring cohort from the same site (F1 sp)] shared an identical AFLP phenotype among the 12 studied cohorts. In the region of Wallis, one AFLP phenotype appeared in at least one individual in seven of the nine studied cohorts. A pairwise comparison among the cohorts from Wallis revealed that 7 of the 23 pairs of cohorts shared 1 or 2 (3 pairs) identical AFLP phenotypes comprising cohorts from the same site but growing in different seasons.

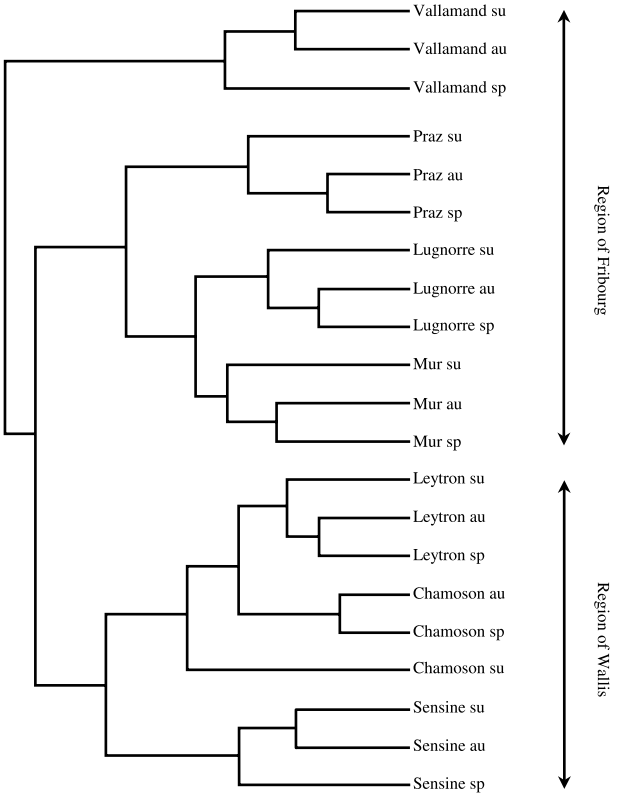

Fig. 2 UPGMA dendrogram based on Nei's genetic distance, modified from the NEIGHBOR procedure of PHYLIP Version 3.5. One hundred and eleven loci of 377 *Senecio vulgaris* individuals were grouped as 21 cohorts for analysis with POPGENE. Distance metrics among populations were based on Nei's unbiased measures of genetic identity and genetic distance. su, au and sp denote summer, autumn and spring cohorts, respectively.

Genetic distances, variance partitioning and gene flow

The dendrogram resulting from cluster analysis shows that the 21 *S. vulgaris* cohorts clustered almost perfectly into 7 groups representing the 7 sites with each group comprising its summer, autumn and spring cohort (Fig. 2). The sites also clustered into the two regions with the exception of the three cohorts from Vallamand which clearly formed a discrete group.

AMOVA performed over all 21 cohorts for partitioning of AFLP variation among sites, among cohorts within sites (among collection dates within sites) and among individuals within cohorts revealed highly significant ($P < 0.001$) effects at each hierarchical level (Table 3). We found 15.6 and 81.9% of the variance arising from variation among sites and among individuals within cohorts, respectively. In contrast, collection date (season) accounted for only 2.5% of the variance indicating that this factor contributed only marginally to genetic differentiation among the *S. vulgaris* cohorts. Conducting the same analysis for both regions separately essentially led to the same results with 3.4% ($P < 0.001$) and 1.3% ($P < 0.077$) of AFLP variation arising among cohorts within sites in Fribourg and Wallis, respectively. Φ_{ST}, the correlation among random AFLP phenotypes within cohorts relative to the correlation of random pairs drawn from the whole sample was 0.181. Φ_{CT}, the correlation among random phenotypes within sites relative to the correlation of random pairs drawn from the whole sample was 0.156 and Φ_{SC}, the correlation of random phenotypes within cohorts relative to that of random pairs drawn from the site was 0.029. Gene flow, i.e. the average number of individuals exchanged among sites per generation (N_em) was 1.13. To estimate gene flow among cohorts within sites we used Φ_{SC} instead of Φ_{ST} (cf Statistical analyses) and obtained an N_em of 16.74, indicating that season of growth represented a weak barrier for genetic exchange, if at all any.

Table 3 Summary of hierarchical analysis of molecular variance (AMOVA) for 377 *Senecio vulgaris* individuals representing 21 cohorts from seven sites located in two regions in Switzerland, canton of Fribourg (four sites) and Wallis (three sites). At each of seven sites plants were collected in summer, autumn and spring. Data were grouped according to sites of collection. The analysis is based on 111 polymorphic AFLP markers. Levels of significance are based on 1000 iteration steps.

		Variance components		
Level of variation	d.f.	Absolute	%	*P*
Among sites	6	1.477	15.61	< 0.001
Among cohorts within sites	14	0.234	2.47	< 0.001
Within cohorts	356	7.751	81.92	< 0.001
Total	376		100	

AMOVA performed on data grouped according to geographical location revealed that there was significant genetic variation between the individuals from the two regions ranging from 5.2 to 9.5% depending on season (Table 4). Genetic variation among sites within regions was about double that high ranging from 11.6 to 15.3%.

Of the 210 pairwise genetic distances (pairwise Φ_{ST}) between pairs of cohorts, 195 were significant and 182 distances were significant at the 0.1% level (Table 5). Pairwise genetic distances calculated among the summer, autumn and spring cohorts within sites were generally smaller than those observed among sites and 12 of the 21 distances were statistically not significant at $P < 0.05$. Moreover, only four of the nine remaining distances displayed a level of significance superior to the 1% level and appeared in Lugnorre (F2) and Vallamand (F3). Lugnorre and Vallamand were also the two sites where pairwise genetic distances among collection dates were the most pronounced with Φ_{ST} being however, not larger than 0.10. Three of the four pairwise genetic distances among the autumn and spring

Table 4 Summary of hierarchical analysis of molecular variance (AMOVA) for 377 *Senecio vulgaris* individuals representing 21 cohorts from 7 sites located in 2 regions in Switzerland, canton of Fribourg (four sites) and Wallis (three sites). At each seven sites samples were collected in summer, autumn and spring. Data were grouped according to region of collection. The analysis is based on 111 polymorphic AFLP markers and was performed for each collection date separately. Levels of significance are based on 1000 iteration steps.

		Variance components			
Source of variation	Collection date	d.f.	Absolute	%	*P*
Among regions	Summer	1	0.508	5.23	< 0.031
	Autumn	1	0.787	7.73	< 0.001
	Spring	1	0.886	9.46	< 0.030
Among sites within regions	Summer	5	1.478	15.23	< 0.001
	Autumn	5	1.258	12.36	< 0.001
	Spring	5	1.085	11.58	< 0.001
Within sites	Summer	118	7.720	79.54	< 0.001
	Autumn	119	8.134	79.91	< 0.001
	Spring	119	7.398	78.97	< 0.001

cohorts from Leytron (W1) and Chamoson (W2) were not statistically significant at $P < 0.05$.

Correlation between geographical and genetic distances

The matrix of pairwise genetic distances (Φ_{ST}) among the seven sites, obtained after pooling the individuals of the three seasonal cohorts within each site, strongly correlated with the matrix of corresponding geographical distances (Mantel test; $r = 0.61$, $P < 0.005$). There was, however, no correlation between the two matrices when the analysis of distances among sites was performed separately for the region of Fribourg (four sites) and the region of Wallis (three sites), indicating that genetic and geographical distances were significantly correlated at the regional scale, but not at the local scale. When analysing distances among the seven sites separately for each collection date, we found a strong correlation between the matrix of pairwise genetic distances (Φ_{ST}) and the corresponding matrix of geographical distances for the autumn (Mantel test; $r = 0.52$; $P < 0.01$) and spring cohorts ($r = 0.57$; $P < 0.01$), but only a weak correlation for the summer cohort ($r = 0.24$; $P = 0.089$).

Table 5 Pairwise genetic distances (Φ_{ST}, lower diagonal of the matrix) among 21 *Senecio vulgaris* cohorts. Levels of significance are given in the upper diagonal of the matrix: **P* < 0.05; ***P* < 0.01; ****P* < 0.001; ns, not significant at *P* < 0.05. *P*-values indicate the probability that a random genetic distance (Φ_{ST}) is larger than the observed distance and are based on 1000 iterations steps. Frames highlight pairwise genetic distances among cohorts within sites and their level of significance. Cohorts as described in Table 1.

	Cohorts																				
	F1			F2			F3			F4			W1			W2			W3		
	su	au	sp	su	au	sp	su	au	sp	su	au	sp	su	au	sp	su	au	sp	su	au	sp
F1 su		*	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
F1 au	0.05		ns	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
F1 sp	0.03	–0.01		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
F2 su	0.28	0.15	0.18		**	***	***	***	***	***	***	*	***	***	***	***	***	***	***	***	***
F2 au	0.24	0.13	0.17	0.06		*	***	***	***	*	***	*	***	***	***	***	***	***	***	***	***
F2 sp	0.22	0.11	0.15	0.07	0.03		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
F3 su	0.28	0.16	0.19	0.19	0.20	0.21		ns	***	***	***	***	***	***	***	***	***	***	***	***	***
F3 au	0.30	0.19	0.21	0.19	0.20	0.20	0.01		***	***	***	***	***	***	***	***	***	***	***	***	***
F3 sp	0.33	0.20	0.25	0.22	0.21	0.23	0.09	0.10		***	***	***	***	***	***	***	***	***	***	***	***
F4 su	0.17	0.08	0.11	0.14	0.09	0.05	0.17	0.17	0.21		*	*	***	***	***	***	***	***	***	***	***
F4 au	0.14	0.04	0.08	0.09	0.08	0.08	0.13	0.14	0.19	0.04		ns	***	***	***	***	***	***	***	***	***
F4 sp	0.17	0.06	0.07	0.08	0.04	0.04	0.14	0.16	0.18	0.03	0.01		***	***	***	***	***	***	***	***	***
W1 su	0.25	0.17	0.22	0.23	0.23	0.23	0.17	0.21	0.26	0.18	0.15	0.18		ns	ns	***	***	***	***	***	***
W1 au	0.25	0.15	0.20	0.22	0.22	0.23	0.16	0.20	0.22	0.19	0.16	0.17	< 0.01		ns	***	***	ns	*	*	**
W1 sp	0.26	0.15	0.20	0.22	0.20	0.23	0.17	0.22	0.24	0.18	0.15	0.15	< 0.01	< 0.01		***	ns	ns	***	***	**
W2 su	0.23	0.12	0.14	0.19	0.19	0.20	0.17	0.21	0.26	0.14	0.11	0.13	0.10	0.12	0.08		ns	*	***	***	***
W2 au	0.23	0.10	0.14	0.20	0.17	0.19	0.18	0.22	0.25	0.15	0.12	0.12	0.09	0.08	0.03	0.01		ns	***	***	***
W2 sp	0.22	0.11	0.15	0.21	0.18	0.19	0.18	0.24	0.25	0.15	0.13	0.13	0.09	0.09	0.03	0.04	0.02		***	***	***
W3 su	0.28	0.17	0.22	0.22	0.22	0.22	0.17	0.20	0.26	0.17	0.15	0.17	0.05	0.05	0.06	0.11	0.11	0.09		ns	ns
W3 au	0.39	0.28	0.34	0.34	0.35	0.35	0.26	0.27	0.35	0.27	0.24	0.29	0.12	0.10	0.15	0.25	0.23	0.22	0.03		ns
W3 sp	0.30	0.19	0.24	0.24	0.25	0.25	0.19	0.21	0.27	0.19	0.17	0.18	0.09	0.08	0.09	0.16	0.15	0.13	< 0.01	0.04	

Table 6 Pairwise tests of heteroscedasticity of molecular variance among 21 *Senecio vulgaris* cohorts. Bartlett's *B* is given for each pair of cohorts in the lower diagonal of the matrix. Levels of significance are given in the upper diagonal of the matrix: **P* < 0.05; ***P* < 0.01; ****P* < 0.001; ns, not significant at *P* < 0.05. *P*-values indicate the probability that a random *B* is larger than the observed *B* and are based on 1000 iterations steps. Frames highlight pairwise tests among cohorts within sites and their level of significance. Cohorts as described in Table 1.

	Cohorts																				
	F1			F2			F3			F4			W1			W2			W3		
	su	au	sp	su	au	sp	su	au	sp	su	au	sp	su	au	sp	su	au	sp	su	au	sp
F1 su		ns	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
F1 au	1.62		ns	***	***	***	***	***	***	***	*	*	***	***	***	***	***	***	***	***	***
F1 sp	1.10	0.77		***	***	***	***	***	***	***	*	*	***	***	***	***	***	***	***	***	***
F2 su	4.60	3.08	3.23		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
F2 au	3.74	2.40	2.82	2.08		ns	***	***	***	***	*	*	***	***	***	***	***	***	***	***	***
F2 sp	3.16	2.34	2.48	1.82	1.16		***	***	***	*	*	*	***	***	***	***	***	***	***	***	***
F3 su	4.92	3.59	3.83	3.56	4.02	3.93		ns	*	***	***	***	***	***	***	***	***	***	***	***	***
F3 au	4.39	3.55	3.63	2.98	3.52	3.06	1.10		***	***	***	***	***	***	***	***	***	***	***	***	***
F3 sp	5.43	3.96	4.30	4.06	3.72	3.89	2.01	1.84		***	***	***	***	***	***	***	***	***	***	***	***
F4 su	2.83	1.90	1.99	2.92	1.84	1.52	3.50	2.93	3.62		ns	ns	***	***	***	***	***	***	***	***	***
F4 au	2.72	1.36	1.88	2.44	1.69	1.72	3.43	3.16	4.07	1.35		ns	***	***	***	***	***	***	***	***	***
F4 sp	2.96	1.57	1.45	2.23	1.75	1.64	3.18	3.08	3.64	1.32	0.88		***	***	***	***	***	***	***	***	***
W1 su	4.11	2.79	3.67	4.04	3.93	4.08	3.35	3.47	4.27	3.02	2.79	3.05		ns	ns	***	***	ns	***	***	***
W1 au	4.30	2.94	3.84	4.35	4.30	4.39	3.67	3.57	4.25	3.51	3.18	3.43	1.04		ns	***	***	ns	ns	***	***
W1 sp	4.39	2.85	3.61	4.25	3.94	4.27	3.60	3.82	4.47	3.45	2.99	3.16	0.78	0.78		*	*	ns	***	***	***
W2 su	4.39	2.57	2.92	3.97	3.97	3.98	3.99	4.23	5.29	3.23	2.75	2.70	2.16	3.07	2.21		ns	ns	***	***	***
W2 au	3.96	2.04	2.57	3.95	3.34	3.76	3.75	4.10	4.79	2.99	2.26	2.27	1.92	2.40	1.59	1.03		ns	***	***	***
W2 sp	3.59	2.37	2.74	3.86	3.35	3.60	3.64	3.91	4.46	3.02	2.68	2.59	1.74	2.36	1.38	1.22	1.21		***	***	***
W3 su	4.74	3.76	4.07	4.02	4.16	4.12	3.74	3.63	4.66	3.37	3.47	3.45	2.04	2.23	2.32	2.97	3.15	2.38		ns	ns
W3 au	7.22	6.55	7.09	6.97	7.33	7.00	5.72	4.75	6.72	5.73	6.12	6.54	3.30	2.57	3.09	5.95	5.75	4.72	2.21		ns
W3 sp	5.12	3.90	4.23	4.34	4.60	4.61	4.21	3.79	4.86	3.56	3.82	3.79	2.48	2.58	2.43	3.77	3.57	2.72	0.87	2.15	

Molecular variation within cohorts

Molecular variance within cohorts (which were sampled from equal areas in all populations) was significantly different among the 21 cohorts ($P < 0.001$; Bartlett test). Of the 210 pairwise Bartlett tests of homogeneity of molecular variation, 189 were statistically significant (Table 6). However, 17 of the 21 pairwise Bartlett tests performed among the summer, autumn and spring cohorts within sites were not statistically significant at $P < 0.05$. Mean molecular variance differed significantly among sites (ANOVA; $P < 0.05$), but was similar across the three collection dates averaged across all sites (7.66 ± 1.06 SD in summer; 8.17 ± 2.00 in autumn; 7.40 ± 0.76 in spring). There was also no difference in mean molecular variance within cohorts between the regions of Fribourg (7.94 ± 1.48 SD) and Wallis (7.48 ± 1.27 SD).

Discussion

Analysis of genetic variation

Because of the rapidity and ease with which reliable and high-resolution markers can be generated, AFLP molecular markers have become a major tool in assessing genetic differences among individuals, populations and independently evolving lineages, such as species (see Mueller & Wolfenbarger 1999). In our study, AFLP molecular markers proved to be a powerful method for the detection of both temporal and spatial genetic variation. With four primer pair combinations we obtained 111 polymorphic markers (Table 2) and could differentiate 348 AFLP phenotypes among the 377 *Senecio vulgaris* individuals analysed in our study. Furthermore, the few plants displaying identical AFLP phenotypes were found mostly either within the same cohort or at the same site, and these may well represent identical genotypes.

The AMOVA revealed significant genetic differentiation among *Senecio vulgaris* cohorts. However, the estimation of population genetic parameters from data obtained from random amplified polymorphic DNA (RAPD; Williams *et al*. 1990) or AFLP molecular markers is still a matter of debate because the inference of F_{ST} estimates from dominant markers is based on two assumptions, namely that bands are homologous and that populations are in Hardy–Weinberg equilibrium (Lynch & Milligan 1994; Ayres & Ryan 1999). The generally high levels of significance in our

study suggest, however, that our results are robust to deviations from these assumptions. Nevertheless, deviations from Hardy–Weinberg equilibrium due to the high degrees of selfing in *S. vulgaris* (Campbell & Abbott 1976) may inflate AFLP-based F_{ST} estimates. However, our estimate of F_{ST} for *S. vulgaris* was far smaller than the average observed for highly selfing annuals (Hamrick & Godt 1990; Bussell 1999; see Discussion below).

Genetic differentiation in space and time

Cluster analysis of AFLP data using the UPGMA algorithm revealed that there was significant genetic differentiation among sites and between regions, while cohorts within sites were much less differentiated, if at all (Fig. 2). AMOVA confirmed these results obtained from cluster analysis. Depending on season, we found 5.2–9.5% of AFLP variation among regions, compared with 11.6–15.2% and 79–80% among and within sites, respectively (Table 4). Moreover, we found 82% of AFLP variation within cohorts, compared with 16% among sites and only 2% among cohorts within sites (Table 3).

Because *S. vulgaris* is an ephemeral that is predominantly self-pollinating (Hull 1974; Campbell & Abbot 1976), strong genetic differentiation among sites was expected (Hamrick *et al*. 1991; Hamrick & Godt 1996). In their review of more than 400 plant species, Hamrick & Godt (1990) used G_{ST} values to indicate the proportion of isozyme diversity residing among populations. They reported an average G_{ST} of 22% for perennial herbs compared with 36% for annuals and an average G_{ST} of 20% for animal-pollinating outcrossers compared with 51% for selfers. In a comprehensive review, Bussell (1999) assembled reported RAPD-based G_{ST} values for 35 plant species and calculated averages of 19.3% for 29 outbreeding species and 62.5% for 6 inbreeding species. Compared with these values the *S. vulgaris* cohorts of our study appear to be far less differentiated than expected for a highly selfing ephemeral. Our value of genetic differentiation among sites would rather correspond to that observed in mixed mating or outcrossing species. In their study using RAPD markers to analyse the genetic structure of *S. vulgaris* populations, Müller-Schärer & Fischer (2001) detected 39% of RAPD variation among sites and suggested that reduced genetic differentiation among populations might originate from a higher degree of outcrossing in *S. vulgaris* than assumed previously. However, using AFLP markers to analyse genetic variation of *S. vulgaris* populations occurring in a range of habitats, we found 49% of AFLP variation residing among sites (Steinger *et al*. 2002), which corresponds well to values reported for selfers. The comparatively low genetic differentiation observed among our studied vineyard sites suggests relatively high levels of gene flow by pollen and/or seeds. Indeed, in both regions, but mainly in the Wallis, the whole area comprising our sites is characterized by many adjacent vineyards with well-synchronized phenologies of their accompanying flora including *S. vulgaris*, thus without effective barriers to gene flow like grasslands and forests. This characteristic of the landscape is likely to have facilitated genetic exchange among *S. vulgaris* populations from different sites, which within both regions were situated within a diameter of ≈ 10 km. In the Wallis region, where genetic differentiation among sites was found to be particularly small (Table 5), gene flow by seed is likely to have been promoted by the strong winds characteristic of this region (André Ancey, pers. commun.). Indeed, *S. vulgaris* has small seeds with a large pappus that may be wind dispersed over considerable distances (Holm *et al*. 1997). Human activity related to the cultivation of vineyards may have further favoured exchange of seeds among sites.

We found significant genetic divergence between the plants from the Fribourg region and those from the Wallis region (Table 4). 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 (Müller-Schärer & Fischer 2001). Hence, geographical and genetic distances are generally not correlated among populations in selfing species (Nevo *et al*. 1998; Fahima *et al*. 1999) unless these populations are sampled at large distances from one another (Tollefsrud *et al*. 1998). The observation that in our *S. vulgaris* study genetic and geographical distances were significantly correlated at the regional scale, but not at the local scale is in agreement with this general rule and corroborates the results obtained for the same plant species in an earlier study (Müller-Schärer & Fischer 2001).

Seasonal genetic differentiation?

The finding that only ≈ 2.5% of AFLP variation resided among cohorts within sites clearly indicates that season of growth represents a weak barrier to gene flow. We also found very similar levels of molecular diversity across cohorts at each site (Table 6). These results therefore provide evidence against the occurrence of seasonal races in *S. vulgaris* which are reproductively isolated due to nonoverlapping flowering phenologies. Seasonal genetic differentiation would have been expected if there is heritable variation in the timing of germination leading to asynchronous periods of flowering among seasonal races and therefore very low levels of gene flow. For example, in *S. vulgaris*, some seeds germinate in autumn and overwinter as juvenile plants, whereas others germinate in spring (Leiss & Müller-Schärer 2001). Autumn cohorts may have a head start and flower already in early spring, while spring cohorts may delay flowering until summer. Because the environmental

conditions experienced by autumn and spring cohorts are likely to be quite different, divergent selection might be expected to occur leading to phenotypic differentiation between seasonal races. Indeed, Vack (1992) found significant divergence between seeds of *S. vulgaris* collected at different times of the year for the characters germination speed and fecundity, although nongenetic causes for the observed differences could not be entirely ruled out. Our test with genetic markers could not confirm the existence of such seasonal differentiation. However, it is recognized that differentiation in phenotypic characters that are exposed to selection may often be larger than that of neutral molecular markers (McKay & Latta 2002; Steinger *et al*. 2002).

The low level of genetic differentiation among cohorts found in this study indicates that seasonal populations may simply arise from seeds shed in the previous generation (cf. Fig. 1c). However, it is also possible that heritable variation in the timing of germination does exist but that seasonal cohorts are to some degree overlapping such that gene exchange does occur (cf. Fig. 1b). These two scenarios are difficult to distinguish with molecular marker data as already low levels of gene flow among seasonal cohorts are expected to constrain genetic divergence resulting from drift (Hedrick 2000).

Assuming that seasonal specialization of cohorts is absent, we expect selection to favour high phenotypic plasticity enabling plants to survive and reproduce under a wide range of environmental conditions (van Tienderen 1991), including dry and warm conditions in summer and wet and cool conditions in spring and autumn. Indeed, in an earlier study with *S. vulgaris* we found that populations from agricultural habitats, which are characterized by temporally variable inputs of nutrients due to fertilizer application, exhibited higher plasticity in response to fertilization compared with populations from ruderal habitats, in which nutrient supply is more stable over time (Leiss & Müller-Schärer 2001; Steinger *et al*. 2002).

To date, few studies have investigated temporal variation in the genetic structure of plant populations (van der Vegte 1978; Ernst 1989; Zopfi 1991; Hossaert-McKey *et al*. 1996; Mengistu *et al*. 2000; Jaradat 2001). In a study with *Poa annua*, another species capable of forming more than one generation per year, Mengistu *et al*. (2000) observed significant genetic differentiation in RAPD markers among the autumn, winter and spring cohorts at only one experimental site, whereas at ten other sites average RAPD variation among seasonal cohorts within sites was found to be only 2%, which is very close to the result obtained in our study with *S. vulgaris*.

Implications for weed–pathogen interactions and biological control

Low levels or an absence of genetic variation in crop plants often lead to high levels of disease incidence and severity, which may result in devastating epidemics (Schmid 1994; Finck & Wolfe 1997). In contrast, many natural plant populations are genetically diverse and polymorphic for disease resistance (e.g. Burdon 1987; Fritz & Simms 1992; Cousens & Croft 2000). In our AFLP study, we found relatively high levels of genetic variation within *S. vulgaris* cohorts. As far as AFLP variation also reflects levels of genetic variation in resistance and/or tolerance traits to a biocontrol agent, our study suggests reduced biocontrol efficiency. However, the great genetic similarity among successive cohorts, as well as the high densities encountered in our study sites, might well counter this effect and favour biocontrol if the agent can persist in local populations. In our case, efficient inoculum of the rust pathogen *Puccinia lagenophorae* in one generation may also be efficient in the subsequent generation, which in the long-term may speed up local adaptation of the pathogen and thus biocontrol success. To test these hypotheses, complementary studies on the long-term dynamics of this weed–pathogen system are presently underway.

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This work forms 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 HMS is to develop environmentally sound strategies and techniques for the biological control of weeds, using both insects and pathogens.
