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Genetic diversity for esterases in the recently evolved stabilized introgressant, *Senecio vulgaris* L. var. *hibernicus* Syme, and its parental taxa *S. vulgaris* L. var. *vulgaris* L. and *S. squalidus* L.

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The level of genetic diversity in the recently evolved stabilized introgressant *Senecio vulgaris* var. *hibernicus* was compared to that within its parental taxa, *S. vulgaris* var. *vulgaris* and *S. squalidus*, over three esterase loci — *αEst-1*, *βEst-1* and *βEst-3*. It was established that var. *hibernicus* contained much less genetic diversity at the *αEst-1* and *βEst-3* loci than was present in var. *vulgaris*. This was evident irrespective of whether var. *vulgaris* was sampled from populations containing both var. *hibernicus* and var. *vulgaris* or populations monomorphic for var. *vulgaris*. Moreover, the *βEst-1* locus which was present in *S. squalidus*, but absent from var. *vulgaris*, was also absent from var. *hibernicus*, while the *βEst-3b* allele which occurred at high frequency (92 per cent) in *S. squalidus* was present at a low frequency (8 per cent) in var. *hibernicus*. It is concluded that var. *hibernicus* has gained no increased genetic diversity for esterases via introgression of germplasm from *S. squalidus*. The maintenance of a low level of genetic diversity for esterases in var. *hibernicus*, relative to var. *vulgaris* in populations containing both variants, was surprising in view of the level of intervariant crossing known to occur in such populations. The possibility of a post-mating breeding barrier existing between the two variants of *S. vulgaris* which might maintain genetic differences is briefly discussed.

Keywords: esterase diversity, introgression, population genetic structure, *Senecio squalidus*, *Senecio vulgaris*.

Introduction

Introgressive hybridization has long been recognized as a powerful mechanism for generating new genetic variation within plant species (Anderson, 1949, 1953; Stebbins, 1969; Grant, 1981). Only recently, however, has it been confirmed that certain products of the process may evolve into new variant forms of a species with distributions beyond the zone of active hybridization (Rieseberg *et al.*, 1990; Abbott *et al.*, 1992). In the genus *Senecio*, a survey of allozyme variation for aspartate aminotransferase (AAT) confirmed that the radiate variant of *S. vulgaris* L., var. *hibernicus* Syme, originated in Britain following introgression of germplasm from the introduced *S. squalidus* ($2n=20$) into the native non-radiate variant of *S. vulgaris* L., var. *vulgaris* L. ($2n=40$), (Abbott *et al.*, 1992). The radiate variant, which produces capitula with ray florets, in contrast to the non-radiate variant with capitula containing only disc florets, is believed to have originated during the early part of the 19th century (Abbott *et al.*, 1992), following the escape of *S. squalidus* from the Oxford Botanic Garden at the end of the 18th century (Druce, 1927). The variant is now widespread in Britain and occurs with the non-radiate variant as an early colonist of open disturbed ground in urban areas.

The survey of AAT variation conducted by Abbott *et al.* (1992) established that at one locus, *Aat-3*, which is duplicated in *S. vulgaris*, an allele which occurs at high frequency in British populations of *S. squalidus* was also common in radiate *S. vulgaris* var. *hibernicus*, but was rare among individuals of non-radiate *S. vulgaris* var. *vulgaris* which co-occurred with var. *hibernicus* and was absent from populations monomorphic for var. *vulgaris*. Apart from providing confirmation of the introgressive origin of var. *hibernicus*, the survey

established that var. *hibernicus* exhibited greater genetic diversity than var. *vulgaris* for *Aat-3*.

Assuming a single or limited number of origins, a recently evolved stabilized introgressant is expected to contain only a sample of the allelic diversity present within the ancestral non-introgressed taxon. In addition, only a small proportion of genes specific to or at high frequency in the donor species is likely to become incorporated into a stabilized introgressant. Selection is expected to remove those donor genes which reduce fitness, while repeated backcrossing to the 'recipient' species will reduce the frequency of donor genes which are selectively neutral. Founder effects and drift may act to oppose the loss of donor alleles (especially in a colonizing species such as *S. vulgaris*), as will mating which reduces backcrossing, e.g. positive assortative mating and/or self-fertilization. Eventually, however, those donor alleles which remain present in a stabilized introgressant are likely to have been favoured by selection or associated with loci at which such alleles occur.

To provide an estimate of genetic diversity in *S. vulgaris* var. *hibernicus*, relative to that within var. *vulgaris* and *S. squalidus* at loci other than the *Aat-3* locus, a survey of allozyme variation was conducted over 12 additional enzyme systems. Of these systems, only two (α- and β-esterase) proved to be polymorphic in British *S. vulgaris*. This paper, therefore, centres on the results of a survey of esterase variation in the three taxa of *Senecio* following a genetic analysis of the inheritance of esterase variants in *S. vulgaris*.

Materials and methods

Seeds or whole plants of *S. vulgaris* var. *hibernicus* and var. *vulgaris* were collected from 18 British populations that were polymorphic for capitulum type. In addition, seeds were collected from seven populations monomorphic for var. *vulgaris*, and from 23 British populations of *S. squalidus*. Site descriptions are given in Ashton (1990) and Irwin (1990). The seed of each parent plant sampled was germinated to provide one offspring per mother plant for electrophoresis. Alternatively, material from individuals collected directly from the field was used for electrophoresis.

Starch gel electrophoresis was conducted on crude protein extracts of young leaves or flower buds. The following enzymes were assayed: acid phosphatase, α-esterase (αEST), β-esterase (βEST), glucose-6-phosphate dehydrogenase, glutamate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, malate dehydrogenase, malic enzyme, peroxidase, 6-phosphogluconate dehydrogenase, phosphoglucomutase, and phosphoglucose isomerase. Details of electrophoretic and staining procedure are given in Ashton (1990) and Irwin (1990). British material of *S. vulgaris* (var. *vulgaris* and var. *hibernicus*) was found to be monomorphic for all systems assayed except α- and β-esterase.

Genetic analysis of esterase variants

A preliminary survey of esterase variation in a sample of *S. vulgaris* from Edinburgh (Nat. Grid Ref. NT261762) revealed polymorphism within one zone of activity on gels stained for α-esterase, and in two zones of activity on gels stained for β-esterase (Fig. 1). Two esterase bands, fast and slow, occurred in both the *αEst-1* and *βEst-2* zones of activity, while three bands — fast, intermediate and slow — were resolved in the *βEst-3* zone of activity. The most anodally migrating (fast) isozyme within each zone was designated *a*, the next *b*, and so on. The esterase variation found in the Edinburgh sample encompassed the range of variants detected subsequently in other populations of *S. vulgaris*. Crosses were made between true breeding individuals which exhibited different single banded phenotypes in one or more zones of esterase activity (Table 1). Details of the crossing procedure are given in Irwin (1990) and Abbott *et al.* (1992). F_1 offspring, and F_2 families (produced after selfing F_1), were grown on and screened for esterase phenotype. In cases where parents were of alternative capitulum type, plants were raised to flowering and recorded for capitulum type.

In *S. squalidus*, a preliminary survey of material sampled from Edinburgh (Nat. Grid Ref. NT268765) revealed the same esterase variants within the *βEst-2* and *βEst-3* zones of activity as found in *S. vulgaris*. It was assumed that the inheritance of variants within the *βEst-3* zone was the same as in *S. vulgaris* (see below). *S. squalidus*, in contrast to *S. vulgaris*, did not exhibit any α-esterase activity, but produced a third zone of β-esterase activity (*βEst-1*) with individuals showing monomorphism for a single band (*βEst-1a*). Lack of expression of *βEst-1* in *S. vulgaris* and *αEst-1* in *S. squalidus* seems to result from gene absence rather than suppression, as both genes are expressed in the allohexaploid *S. cambrensis* which combines the genomes of both species (Ashton & Abbott, 1992; and personal observation).

Results

Inheritance of esterase variation in S. vulgaris

For *αEst-1* and *βEst-3*, crosses between parents which differed in single-banded phenotype produced F_1 offspring exhibiting a double-banded phenotype. The F_2 families segregation fast-, double- and slow-banded

phenotypes in a ratio not significantly different from 1:2:1 (Table 1). It is concluded that variation for each of these enzymes is controlled by a single locus with two co-dominant alleles occurring at the *αEst-1* locus, and three co-dominant alleles at the *βEst-3* locus.

For *βEst-2*, F_1 offspring produced a single fast-banded phenotype (*βEst-2a*), while in the F_2, fast and slow (*βEst-2b*) banded phenotypes segregated in a ratio not significantly different from 3:1. A possible explanation for the absence of the expected double banded 'heterozygous' phenotype in the F_1 and F_2 generations, is that variation for *βEst-2* is due to polymorphism at a 'modifier' locus which has an effect on the electrophoretic properties of the esterase produced by the *βEst-2* locus. The occurrence of modifier genes which affect the mobility of particular proteins in plants and animals is well known (Manwell & Baker, 1970; Weeden & Wendel, 1989), and can cause one electrophoretic variant to appear to be dominant to another. In the two crosses which involved parents with alternative capitulum types, the F_2 plant's segregated radiate, intermediate and non-radiate phenotypes in a ratio not significantly different from 1:2:1 (Table 1). This confirmed the disomic monogenic inheritance of capitulum type in *S. vulgaris* previously reported by Trow (1912), Hull (1974) and Abbott *et al.* (1992).

Chi-squared contingency tests of the joint segregation of pairs of loci revealed no evidence of linkage between the locus controlling capitulum type (ray floret locus) and the three esterase loci (*αEst-1*, *βEst-2* and *βEst-3*) (Table 2). There was also no evidence of linkage between the *βEst-2* locus (or the modifier locus which may produce polymorphism for *βEst-2*) and the two other esterase loci. Only between the *αEst-1* and *βEst-3* loci was linkage established. The recombination fraction between this pair of loci was calculated for each of four different crosses using the maximum likelihood procedures of Allard (1956). This was conducted by means of the LINKAGE-1 program of Suiter *et al.* (1983). For three of the crosses the recombination fraction ranged between 0.32 and 0.38, however in the fourth cross the value dropped to 0.22.

Genetic diversity

In view of the inability to resolve a 'heterozygous' double-banded phenotype at the *βEst-2* locus, and also because *βEst-2* frequently showed weak activity

Table 1 χ^2 tests for segregation ratio of phenotypes for capitulum type and *αEst-1* *βEst-2* and *βEst-3* type in the F_2 families of crosses between different lines of *S. vulgaris*. Enclosed in brackets are the designated genotypes of parents at the *αEst-1*, *βEst-2* and *βEst-3* loci respectively

Parents	F_2 phenotype			χ^2
(a) Capitulum type	*RR*	*RN*	*NN*	
E37-*RR* (*aa, aa, cc*) × E45-*NN* (*bb, bb, cc*)	16	43	19	1.05
E40-*RR* (*aa, bb, cc*) × E49-*NN* (*bb, bb, aa*)	16	42	18	0.95
(b) *αEst-1* type	*aa*	*ab*	*bb*	
E37-*RR* (*aa, aa, cc*) × E45-*NN* (*bb, bb, cc*)	22	50	25	0.36
E40-*RR* (*aa, bb, cc*) × E49-*NN* (*bb, bb, aa*)	21	48	27	0.75
E22-*NN* (*aa, aa, cc*) × E49-*NN* (*bb, bb, aa*)	49	73	47	3.18
E45-*NN* (*bb, bb, cc*) × E46-*NR* (*aa, bb, bb*)	21	43	31	2.96
E49-*NN* (*bb, bb, bb*) × E46-*NR* (*aa, bb, bb*)	31	54	19	2.92
(c) *βEst-2* type	*aa*		*bb*	
E37-*RR* (*aa, aa, cc*) × E45-*NN* (*bb, bb, cc*)	66		31	2.47
E22-*NN* (*aa, aa, cc*) × E49-*NN* (*bb, bb, aa*)	128		41	0.05
E46-*NR* (*aa, bb, bb*) × E31-*NN* (*aa, aa, cc*)	72		28	0.48
E22-*NN* (*aa, aa, cc*) × E4-*NN* (*aa, bb, aa*)	79		21	0.85
(d) *βEst-3* type	*aa*	*ac*	*cc*	
E22-*NN* (*aa, aa, cc*) × E49-*NN* (*bb, bb, aa*)	45	90	34	1.43
E22-*NN* (*aa, aa, cc*) × E4-*NN* (*aa, bb, aa*)	16	60	24	5.28
E40-*RR* (*aa, bb, cc*) × E49-*NN* (*bb, bb, aa*)	28	44	24	1.00
	bb	*bc*	*cc*	
E46-*NR* (*aa, bb, bb*) × E31-*NN* (*aa, aa, cc*)	17	58	25	3.84
E45-*NN* (*bb, bb, cc*) × E46-*NR* (*aa, bb, bb*)	22	46	27	0.62
	aa	*ab*	*bb*	
E49-*NN* (*bb, bb, aa*) × E46-*NR* (*aa, bb, bb*)	25	59	20	2.36

Table 2 Results of χ^2 contingency tests for joint segregation of alleles at the ray floret and esterase loci

Parents	d.f.	χ^2	$r \pm$-S.E.
(i) Ray floret: *αEst-1*			
E37-*RR* (*aa, aa, cc*) × E45-*NN* (*bb, bb, cc*)	4	5.26	
E40-*RR* (*aa, bb, cc*) × E49-*NN* (*bb, bb, aa*)	4	3.48	
(ii) Ray floret: *βEst-2*			
E37-*RR* (*aa, aa, cc*) × E45-*NN* (*bb, bb, cc*)	2	0.50	
(iii) Ray floret: *βEst-3*			
E40-*RR* (*aa, bb, cc*) × E49-*NN* (*bb, bb, aa*)	4	4.65	
(iv) *αEst-1*: *βEst-2*			
E37-*RR* (*aa, aa, cc*) × E45-*NN* (*bb, bb, cc*)	2	1.79	
E22-*NN* (*aa, aa, cc*) × E49-*NN* (*bb, bb, aa*)	2	0.37	
(v) *αEst-1*: *βEst-3*			
E40-*RR* (*aa, bb, cc*) × E49-*NN* (*bb, bb, aa*)	4	15.29	0.32 ± 0.04
E22-*NN* (*aa, aa, cc*) × E49-*NN* (*bb, bb, aa*)	4	21.11	0.35 ± 0.03
E45-*NN* (*bb, bb, cc*) × E46-*NR* (*aa, bb, bb*)	4	44.28	0.22 ± 0.03
E49-*NN* (*bb, bb, bb*) × E46-*NR* (*aa, bb, bb*)	4	6.98	0.38 ± 0.04
(vi) *βEst-2*: *βEst-3*			
E22-*NN* (*aa, aa, cc*) × E49-*NN* (*bb, bb, aa*)	2	1.79	
E22-*NN* (*aa, aa, cc*) × E4-*NN* (*aa, bb, aa*)	2	3.15	
E46-*NR* (*aa, bb, bb*) × E31-*NN* (*aa, aa, cc*)	2	1.70	

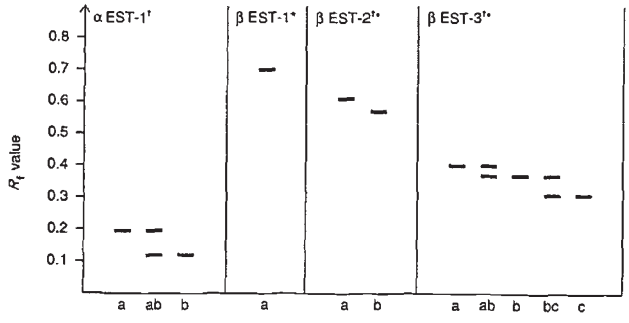

Fig. 1 Electrophoretic phenotypes resolved for esterases in *Senecio vulgaris*† and *S. squalidus**. (The *βEst-3b, bc* and *c* phenotypes correspond to the *βEst-3a, ab* and *b* phenotypes in Ashton & Abbott, 1992).

making identification of the phenotype difficult in some samples, the survey of esterase diversity was restricted to the *βEst-3* locus in *S. squalidus* and the *αEst-1* and *βEst-3* loci in *S. vulgaris*. The most common allele at the *βEst-3* locus in all populations of *S. squalidus* was the *βEst-3b* allele (Table 3). This allele was fixed in five out of 23 populations surveyed, and occurred at a frequency of 92 per cent over all individuals examined. It follows that the total gene diversity (H_T) estimated according to Nei (1973), i.e.

$$H_T = 1 - \sum_{i=1}^{K} \bar{x}_i^2$$

where $\bar{x}_i^2$ is the mean frequency of the ith of K alleles for the populations surveyed, was low in *S. squalidus* at the *βEst-3* locus (Table 4), and resulted largely from diversity within populations (H_S) rather than between them (D_{ST}). F statistics (Wright, 1951) computed using the computer package BIOSYS-1 (Swofford & Selander, 1981), showed that the overall correlation between uniting gametes (F_{IT}) was greater than expected with panmixia, due largely to a deficiency in heterozygotes from expected within populations (F_{IS}) rather than genetic subdivision between populations (F_{ST}) (Table 5). Nevertheless, positive fixation index values (F_0) were computed for only half of the 18 polymorphic populations examined (Table 3) showing that there was no deficiency in heterozygotes from expected in many of the populations surveyed.

In contrast to the situation in *S. squalidus*, the most common allele at the *βEst-3* locus in *S. vulgaris* was the *βEst-3c* allele (Table 6). This allele occurred at a frequency of 93 per cent in var. *hibernicus*, 82 per cent in var. *vulgaris* from populations polymorphic for capitulum type, and 83 per cent among individuals from populations monomorphic for var. *vulgaris*. At the *αEst-1* locus, one allele (*αEst-1a*) was again present at very high frequency (92 per cent) in var. *hibernicus*, while in var. *vulgaris* from populations polymorphic and monomorphic for capitulum type, the allele occurred at 79 and 52 per cent, respectively.

Table 3 Allele frequencies, observed heterozygosity (H_0) and Wright's Fixation Index (F_0) at the *βEst-3* locus in populations of *Senecio squalidus*

Population	National grid reference	Sample size	*a*	*b*	*c*	H_0	F_0
England							
Banbury	SP463404	50	0.01	0.95	0.04	0.060	+0.375
Birmingham	SP092876	21	0.02	0.93	0.05	0.095	+0.294
Chesterfield	SK383713	50	0.06	0.77	0.17	0.340	+0.092
Darlington	NZ296146	15	0.13	0.84	0.03	0.067*	+0.767
Dartford	TQ555743	25	0.04	0.96	—	0.000*	+1.000
Derby	SK362356	44	—	1.00	—	0.000	—
Kingston	TQ191691	48	—	1.00	—	0.000	—
Oxford	SP505065	46	0.01	0.88	0.11	0.130*	+0.387
Sheffield	SK395845	50	—	0.79	0.21	0.220*	+0.337
St Helens	SJ524926	46	—	0.93	0.07	0.130	−0.070
Stoke-on-Trent	SJ886407	49	—	0.76	0.24	0.367	+0.007
Warwick	SP286655	44	—	0.98	0.02	0.045	−0.023
Wigan	SD594034	36	—	1.00	—	0.000	—
York	SE612518	26	—	1.00	—	0.000	—
Scotland							
Edinburgh	NT268765	44	0.01	0.91	0.08	0.182	−0.088
Glasgow	NS534671	34	0.07	0.93	—	0.147	−0.079
Grangemouth	NS945825	35	—	0.89	0.11	0.000*	+1.000
Methil	NT376995	26	—	0.96	0.04	0.077	−0.040
Wales							
Cardiff	ST173733	22	—	0.98	0.02	0.045	−0.023
Mochdre	SH813774	26	—	0.90	0.10	0.192	−0.106
Wrexham (Brymbo)	SJ296539	33	—	0.92	0.08	0.152	−0.082
Wrexham (Rhostyllen)	SJ312492	27	—	1.00	—	0.000	—
Wrexham (Southsea)	SJ306515	25	—	0.98	0.02	0.040	−0.020
Total		822	0.01	0.92	0.07	0.113	0.268

*Observed genotypic frequencies significantly different from expected ($P < 0.05$) based on a test using exact probabilities.

The lower level of genetic diversity in var. *hibernicus* relative to var. *vulgaris* based on allelic frequencies was reflected in estimates of total gene diversity (H_T) (Table 4). It was evident that over both esterase loci var. *vulgaris* contained approximately 3–3.5 times the amount of gene diversity present in var. *hibernicus*. For populations monomorphic for var. *vulgaris*, gene diversity stemmed mainly from allelic diversity between populations, i.e.

$G_{ST} = 0.827$, where $G_{ST} = D_{ST/HT}$,

whereas for populations polymorphic for capitulum type, diversity resulted largely from variation within populations (var. *vulgaris*, $G_{ST} = 0.292$; var. *hibernicus* $G_{ST} = 0.337$). [Note that G_{ST} is equivalent to F_{ST} (Swofford & Selander, 1981) as is evident from the values for these two statistics presented in Tables 4 and 5]. Low G_{ST} values are unexpected in colonizing and predominantly self-fertilizing species such as *S. vulgaris*. Much more common are high G_{ST} values, i.e. of the type found for populations monomorphic for var. *vulgaris* (Hamrick & Godt, 1990). In this regard, the accuracy of the low G_{ST} ($= F_{ST}$) values calculated for both variants of *S. vulgaris* in polymorphic populations should be treated with some caution given the large sampling errors associated with these statistics when certain alleles occur at low frequency.

As expected for a predominantly selfing species, estimates of F_{IT} were high in *S. vulgaris*, with the highest values exhibited by populations monomorphic for var. *vulgaris* (Table 5). These populations displayed a marked deficiency of heterozygotes from that expected with panmixia within populations (high F_{IS} values) in addition to the considerable genetic subdivision between populations already mentioned (high F_{ST} and G_{ST} values). The high F_{IT} values exhibited by var. *vulgaris* and var. *hibernicus* from populations polymorphic for capitulum type resulted largely from heterozygote deficiency within populations.

The genetic similarity between populations within

Table 4 Estimates of gene diversity in *S. squalidus*, *S. vulgaris* var. *vulgaris* and var. *hibernicus*. *S. vulgaris* taxa from populations polymorphic and monomorphic for capitulum type are indicated by (p) and (m) respectively

Taxon	Locus	H_T	H_S	D_{ST}	G_{ST}
S. squalidus	*βEst-3*	0.143	0.132	0.011	0.077
S. vulgaris var. *vulgaris* (p)	*βEst-3*	0.292	0.194	0.098	0.336
	αEst-1	0.313	0.235	0.078	0.249
	Mean	0.302	0.214	0.088	0.292
S. vulgaris var. *hibernicus* (p)	*βEst-3*	0.112	0.070	0.042	0.375
	αEst-1	0.103	0.074	0.029	0.284
	Mean	0.109	0.072	0.037	0.337
S. vulgaris var. *vulgaris* (m)	*βEst-3*	0.287	0.050	0.237	0.826
	αEst-1	0.486	0.084	0.402	0.827
	Mean	0.386	0.067	0.319	0.827

Table 5 *F* statistics estimated for *S. squalidus*, *S. vulgaris* var. *vulgaris* and var. *hibernicus*. *S. vulgaris* taxa from populations polymorphic and monomorphic for capitulum type are indicated by (p) and (m) respectively

Taxon	Locus	F_{IT}	F_{ST}	F_{IS}
S. squalidus	*βEst-3*	0.302	0.078	0.243
S. vulgaris var. *vulgaris* (p)	*βEst-3*	0.916	0.355	0.874
	αEst-1	0.849	0.252	0.799
	Mean	0.882	0.292	0.833
S. vulgaris var. *hibernicus* (p)	*βEst-3*	0.789	0.371	0.665
	αEst-1	0.867	0.287	0.813
	Mean	0.826	0.331	0.740
S. vulgaris var. *vulgaris* (m)	*βEst-3*	0.983	0.825	0.906
	αEst-1	0.960	0.827	0.769
	Mean	0.969	0.826	0.820

taxa, and also between taxa, was further quantified by computing Nei's genetic identity, *I*, (Nei, 1972) for each pair of populations taken in turn

$$I = \frac{\Sigma x_i y_i}{\sqrt{\Sigma x_i^2 \Sigma y_i^2}}$$

where x_i and y_i are the frequencies of the *i*th allele in populations *X* and *Y* respectively). This was conducted using BIOSYS-1, after which a mean genetic identity was calculated for each taxon or pair of taxa investigated (Table 7). Mean genetic identity averaged over both esterase loci was greater among populations of var. *hibernicus* than populations of var. *vulgaris*, with populations monomorphic for var. *vulgaris* exhibiting the lowest gene identity. Comparisons between taxa, revealed that the genetic identity between var. *vulgaris* and var. *hibernicus*, sampled from populations polymorphic for the two variants, was considerably greater than the identities computed between populations of these taxa and populations monomorphic for var. *vulgaris*.

Analysis at the *βEst-3* locus showed that genetic identities between *S. vulgaris* taxa and *S. squalidus* were very low, as expected given the level of divergence in allelic frequencies between these two species. Not surprisingly, a very high value for *I* was computed for *S. squalidus* populations at the *βEst-3* locus emphasizing the lack of allelic diversity between populations in this species.

Estimates of gametic phase disequilibrium ($\hat{D}$) at the *αEst-1* and *βEst-3* pair of loci were calculated for the few populations of *S. vulgaris* in which both loci were polymorphic (Table 8). Values of gametic phase disequilibrium ($\hat{D}$) were estimated from genotypic frequencies following the procedure of Weir *et al.* (1972) and tested for significance using the statistic

$$Q = n\hat{D}^2/(p_1p_2q_1q_2)$$

Table 6 Allele frequencies, observed heterozygosity (H_0) and Wright's Fixation Index (F_0) at the *αEst-1* and *βEst-3* loci in *Senecio vulgaris*-var. *vulgaris* (V) and var. *hibernicus* (H)

Population	National grid reference	Variant	Sample size	*αEst-1*				*βEst-3*				
				a	*b*	H_0	F_0	*a*	*b*	*c*	H_0	F_0
Polymorphic for var. *vulgaris* and var. *hibernicus*												
England												
Birmingham	SP092876	V	50	1.00	—	0.000	—	—	0.02	0.98	0.000	1.000
		H	50	1.00	—	0.000	—	—	0.07	0.93	0.100	0.232
St Helens	SJ524926	V	40	1.00	—	0.000	—	0.05	0.49	0.46	0.275	0.496
		H	40	1.00	—	0.000	—	—	—	1.00	0.000	—
Scotland												
Edinburgh (i)	NT261762	V	44	0.84	0.16	0.045	0.830	—	0.13	0.87	0.023	0.896
		H	40	0.95	0.05	0.000	1.000	—	0.05	0.95	0.000	1.000
(ii)	NT276763	V	43	0.33	0.67	0.279	0.365	—	0.45	0.55	0.023	0.953
		H	45	0.84	0.16	0.090	0.662	—	0.10	0.90	0.200	−0.111
(iii)	NT268765	V	50	0.64	0.36	0.040	0.913	—	0.20	0.80	0.000	1.000
		H	70	0.48	0.52	0.060	0.857	—	0.02	0.98	0.070	−0.022
(iv)	NT335708	V	17	0.79	0.21	0.294	0.101	—	0.29	0.71	0.000	1.000
		H	13	0.92	0.08	0.000	1.000	—	—	1.00	0.000	—
(v)	NT337725	V	50	0.98	0.02	0.000	1.000	—	0.46	0.54	0.120	0.758
		H	50	1.00	—	0.000	—	—	0.07	0.93	0.020	0.846
Glasgow (i)	NS578664	V	25	1.00	—	0.000	—	—	0.28	0.72	0.000	1.000
		H	25	1.00	—	0.000	—	—	—	1.00	0.000	—
(ii)	NS534671	V	25	1.00	—	0.000	—	—	0.04	0.96	0.000	1.000
		H	25	1.00	—	0.000	—	—	—	1.00	0.000	—
Grangemouth (i)	NS977814	V	25	0.84	0.16	0.000	1.000	—	—	1.00	0.000	—
		H	25	0.98	0.02	0.040	−0.020	—	—	1.00	0.000	—
(ii)	NS913823	V	25	0.58	0.42	0.040	0.918	—	—	1.00	0.000	—
		H	25	0.96	0.04	0.000	1.000	—	—	1.00	0.000	—
Methil	NT376995	V	32	0.77	0.23	0.094	0.739	—	0.03	0.97	0.000	1.000
		H	36	1.00	—	0.000	—	—	—	1.00	0.000	—
Wales												
Cardiff	ST273733	V	33	0.94	0.06	0.000	1.000	—	0.73	0.27	0.000	1.000
		H	27	1.00	—	0.000	—	—	0.09	0.91	0.037	0.780
Mochdre	SH822781	V	45	0.62	0.38	0.000	1.000	—	—	1.00	0.000	—
		H	40	1.00	—	0.000	—	—	0.64	0.36	0.025	0.946
Wrexham (Brymbo)	SJ296539	V	38	0.70	0.30	0.026	0.938	—	—	1.00	0.000	—
		H	21	0.88	0.12	0.048	0.773	—	—	1.00	0.000	—
Wrexham (Ffrith)	SJ286556	V	37	1.00	—	0.000	—	—	—	1.00	0.000	—
		H	16	1.00	—	0.000	—	—	—	1.00	0.000	—
Wrexham (Rhostyllen)	SJ312492	V	28	0.97	0.03	0.000	1.000	—	—	1.00	0.000	—
		H	14	1.00	—	0.000	—	—	—	1.00	0.000	—
Wrexham (Southsea)	SJ306515	V	65	0.51	0.49	0.031	0.938	—	0.02	0.98	0.000	1.000
		H	32	1.00	—	0.000	—	—	0.03	0.97	0.000	1.000
Total		V	672	0.79	0.21	0.042	0.876	(0.003)	0.18	0.82	0.028	0.904
		H	594	0.92	0.08	0.019	0.881	—	0.07	0.93	0.034	0.755
Monomorphic for var. *vulgaris*												
England												
London	TQ349807	V	45	0.01	0.99	0.023	−0.011	—	—	1.00	0.000	—
Scotland												
Dundee	NO373295	V	30	0.95	0.05	0.033	0.649	—	0.02	0.98	0.033	−0.017
Methil	NT377998	V	25	—	1.00	0.000	—	—	—	1.00	0.000	—
Wales												
Aberffraw	SH366656	V	25	0.94	0.06	0.040	0.645	—	1.00	—	0.000	—
Puffin Island	SH653824	V	25	0.98	0.02	0.039	−0.020	—	0.20	0.80	0.000	1.000
Spain												
Matalascañas		V	20	0.20	0.80	0.000	1.000	—	—	1.00	0.000	—
Switzerland												
Basel		V	15	1.00	—	0.000	—	—	—	1.00	0.000	—
Total		V	185	0.52	0.48	0.022	0.957	—	0.17	0.83	0.005	0.980

The sample from the Edinburgh (i) population was different from that subjected to a preliminary survey of esterase variation in *S. vulgaris* (see Materials and Methods), and did not contain the *βEst-3a* allele found in the earlier sample.

Table 7 Mean genetic identities, I, (Nei, 1972) within and between taxa. Standard errors are in brackets. Populations of *S. vulgaris* polymorphic and monomorphic for capitulum type are indicated by (p) and (m) respectively

	Both esterase loci			
	S. vulgaris			
	var. *vulgaris* (m)	var. *vulgaris* (p)	var. *hibernicus*	*βEst-3* locus *S. squalidus*
S. vulgaris				
var. *vulgaris* (m)	0.640 (0.306)	0.675 (0.307)	0.671 (0.335)	0.231 (0.332)
var. *vulgaris* (p)		0.882 (0.119)	0.917 (0.104)	0.295 (0.297)
var. *hibernicus* (p)			0.958 (0.080)	0.097 (0.095)
S. squalidus				0.991 (0.012)

Table 8 Gametic disequilibrium ($\hat{D}$) values for the *αEst-1* and *βEst-3* pair of loci in populations of *S. vulgaris* var. *vulgaris* and var. *hibernicus*

	Polymorphic for capitulum type		Monomorphic for capitulum type	
Population	var. *vulgaris*	var. *hibernicus*	Population	var. *vulgaris*
Edinburgh				
(i)	+0.0027	−0.0225*	Dundee	+0.0008
(ii)	+0.0619†	+0.0005		
(iii)	+0.0120	+0.0112		
(iv)	+0.0311	—		
(v)	−0.0108	—		
Cardiff	+0.0443*	—		
Wrexham (Southsea)	−0.0001	—		
Methil	+0.0072†	—		

*$P < 0.05$; †$P < 0.01$.

where n is the number of individuals scored and p_1, p_2 and q_1, q_2 are the allele frequencies at the *αEst-1* and *βEst-3* loci respectively. Q is approximately distributed as χ^2 with one degree of freedom (Hill, 1974). In populations polymorphic for capitulum type, significant gametic phase disequilibrium was present in var. *vulgaris* at one site, and in var. *hibernicus* at a second site. Furthermore, at two other sites, $\hat{D}$ was close to being significant ($P < 10$ per cent) in var. *vulgaris*. Clearly, there is evidence of non-random associations occurring in allelic state between the linked *αEst-1* and *βEst-3* loci within var. *vulgaris* or var. *hibernicus* in at least some populations which are polymorphic for the two morphs. Furthermore, it is of interest that in such populations, one morph may exhibit significant gametic phase disequilibrium while the other does not.

Discussion

It is evident from the results of the present survey that the recently evolved radiate stabilized introgressant of *S. vulgaris*, var. *hibernicus*, contains much less genetic diversity at two esterase encoding loci than the ancestral, non-radiate taxon *S. vulgaris* var. *vulgaris*. This was borne out from comparisons of allelic frequency, total gene diversity (H_T), and genetic identity (I) within taxa, and was evident irrespective of whether var. *vulgaris* was sampled from populations containing both var. *hibernicus* and var. *vulgaris*, or populations monomorphic for var. *vulgaris*. With respect to total gene diversity, the values obtained for var. *vulgaris* were 3–3.5 times greater than that for var. *hibernicus*.

The lower level of genetic diversity in var. *hibernicus* relative to var. *vulgaris*, based on esterase variation, accords with the findings of a previous study which investigated variation in the two morphs for a range of life-history traits (Abbott, 1986). For most characters investigated, var. *vulgaris* exhibited more genetic diversity within populations than was present in var. *hibernicus*. In contrast, a survey of variation for aspartate aminotransferase (AAT) in *S. vulgaris* (Abbott *et al.*, 1992) has shown that var. *hibernicus* contains more

allelic diversity for *Aat-3* than does var. *vulgaris* due to the introgression of the *Aat-3c* allele from *S. squalidus*.

A reduced level of genetic diversity in a recently evolved stabilized introgressant, relative to that in the species as a whole, is not unexpected given that only a small sample of the allelic diversity in the parental (recipient) species is likely to be incorporated in the introgressant. Only at loci where new alleles have been introgressed from the donor species is diversity likely to be increased. In the case of var. *hibernicus*, it is evident that the introgressant taxon has gained no increased genetic diversity for esterases from the donor species *S. squalidus*, despite the fact that *S. squalidus* possesses one esterase locus, *βEst-1*, which is not present in *S. vulgaris* var. *vulgaris*, and also contains an allele at high frequency at the *βEst-3* locus which is at low frequency in var. *vulgaris*. Presumably the effects of either recurrent backcrossing, selection or random factors acting independently or in combination have prevented the *βEst-1* locus from being incorporated into var. *hibernicus*. Similarly, one or more of these factors may have acted to prevent the maintenance of the *βEst-3b* allele in var. *hibernicus* at a frequency greater than in var. *vulgaris*, assuming, that is, that the *βEst-3b* allele was present in the *S. squalidus* plant(s) which first crossed with *S. vulgaris* var. *vulgaris* at the initial stage of the introgression process.

Although a reduced level of allelic diversity at the *αEst-1* and *βEst-3* loci in var. *hibernicus* relative to var. *vulgaris* may not be unexpected for comparisons involving populations monomorphic for var. *vulgaris*, it is surprising that this varietal difference is maintained in populations containing both variants. The outcrossing rate between the two variants in populations polymorphic for capitulum type is known to reach 35 per cent on occasion (Marshall & Abbott, 1984) and it would be expected, therefore, for differences in allelic frequency between the variants to disappear quickly given that neither esterase locus is linked to the ray floret locus. The fact that differences in allelic frequency are maintained at esterase loci might indicate that a breeding barrier exists between the two variants of *S. vulgaris* which is not reflected by the intermorph outcrossing rate. This is further suggested by the results of an analysis of gametic disequilibrium ($\hat{D}$) in each morph. In two of the three populations where it was possible to compute $\hat{D}$ for each morph, it was established that when one morph exhibited significant gametic disequilibrium the other did not. A reduction in gene flow between the two variants would occur if the products of intermorph crossing were of reduced fitness relative to intramorph offspring. Some evidence in support of this hypothesis comes from the work of Marshall (1982) who found that in all populations of *S. vulgaris*, investigated, which contained both var. *hibernicus* and var. *vulgaris*, the observed fixation index at the ray floret locus was greater than expected based on the recorded outcrossing rates of morphs in these populations. One explanation for this would be that the products of intermorph crossing were of reduced fitness. Further study is required, however, to substantiate this hypothesis.

If some form of breeding barrier is present between var. *hibernicus* and var. *vulgaris* in populations containing both variants, this might act not only to maintain existing genetic differences between the variants but also to promote further divergence between the two variants in the future.

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