Heredity **68** (1992) 425–435

Received 20 May 1991

Introgressive origin of the radiate groundsel, *Senecio vulgaris* L. var. *hibernicus* Syme: *Aat-3* evidence

RICHARD J. ABBOTT, PAUL A. ASHTON & DAVID G. FORBES

Department of Biology and Preclinical Medicine, Sir Harold Mitchell Building, University of St Andrews, St Andrews, Fife KY16 9TH, Scotland, U.K.

A survey of allelic variation at the *Aat-3* locus in *Senecio squalidus* and *S. vulgaris* revealed that the *Aat-3c* allele, which was present at high frequency in British populations of *S. squalidus*, was also common in British radiate groundsel (*S. vulgaris* var. *hibernicus*) but was rare among individuals of the non-radiate groundsel (*S. vulgaris* var. *vulgaris*) which co-occurred with var. *hibernicus* and was absent from British, Irish and mainland European populations monomorphic for var. *vulgaris*. This evidence is taken as confirmation of an introgressive origin of *S. vulgaris* var. *hibernicus* across a chromosome barrier following hybridization between *S. vulgaris* var. *vulgaris* ($2n = 40$) and radiate *S. squalidus* ($2n = 20$) and backcrossing to *S. vulgaris* var. *vulgaris*. Genetic analysis showed that the *Aat-3* locus, which is duplicated in *S. vulgaris* is not linked to the ray floret locus controlling capitulum type. It is suggested that the close association between the *Aat-3c* allele and the radiate allele in populations of *S. vulgaris* polymorphic for capitulum type may be maintained by selection favouring a co-adapted complex of genes introgressed from *S. squalidus*, although alternative explanations are not ruled out. The introgression of the *Aat-3c* allele and associated genetic material from *S. squalidus* into *S. vulgaris* is likely to have enhanced the level of genetic variation present within *S. vulgaris* and may have been a factor that has favoured the spread of *S. vulgaris* var. *hibernicus* in Britain following its origin last century.

Keywords: introgression, isozyme variation, population genetic structure, *Senecio vulgaris*, *Senecio squalidus*.

Introduction

Gene exchange between species via introgressive hybridization is a well known phenomenon among plants (Anderson, 1949; Stebbins, 1959, 1969; Grant, 1981; Briggs & Walters, 1984). Several studies have utilized allozymes to demonstrate the presence of backcrossed individuals in hybrid swarms of wild plants (Levin, 1975; Ellstrand *et al.*, 1985; Olivieri, 1985; Rollo *et al.*, 1985; Soltis & Soltis, 1986; Arnold *et al.*, 1990); however, there is little evidence that such individuals evolve into new taxa distributed beyond the zone of active hybridization (Heiser, 1973). One example of such an event in the genus Helianthus (Heiser, 1951), namely the origin of *Helianthus annuus* ssp. *texanus* Heiser, following introgression of genes from *H. debilis* ssp. *cucumerifolium* (Torrey and Gray) Heiser into *H. annuus* L., is supported by recent evidence from a study of Rieseberg *et al.* (1990) which used DNA markers. However, another example in the same genus, i.e. the origin of a Californian weedy race of *H. bolanderi* following introgression of genes from *H. annuus* into a serpentine race of *H. bolanderi* (Heiser, 1949), is disputed by the allozyme and molecular evidence (Rieseberg *et al.*, 1988). Other possible examples of the evolution of stabilized introgressants in wild plants (Grant, 1950; Levin, 1963; Ornduff, 1967; Bloom, 1976; Davis, 1985) have yet to be subjected to similar detailed analysis involving genetic markers.

Within the British flora a good example of the origin of a new taxon via introgression is considered to be that of the inland radiate morph of the groundsel, *Senecio vulgaris* L. var. *hibernicus* Syme, $2n = 40$ (Stace, 1987). This morph was first described in the British Isles in 1866 (Syme, 1875) from material which grew around Cork in Ireland; however, Crisp (1972) has since identified a specimen of the same variant collected from

Oxford in 1832 and deposited in the herbarium at Liverpool University. The variant is believed to have originated following introgression of genes from the introduced radiate species *Senecio squalidus* L. ($2n=20$) into the common and widespread non-radiate morph of groundsel, *Senecio vulgaris* L. var. *vulgaris* L. ($2n=40$). Initial evidence in support of this origin (reviewed by Stace, 1977) was based on: (i) the parallel spread of *S. squalidus* and *S. vulgaris* var. *hibernicus* in Britain over the past 150 years (Crisp, 1972), following the escape of *S. squalidus* from the Oxford Botanic Garden at the end of the 18th Century (Druce, 1927); and (ii) the presence of morphological characters in var. *hibernicus*, which are intermediate to those exhibited by *S. squalidus* and *S. vulgaris* var. *vulgaris*, e.g. leaf characters (Monaghan & Hull, 1976).

More compelling evidence for an introgressive origin of var. *hibernicus* was advanced by Ingram *et al.* (1980) who successfully synthesized several fertile radiate plants that were tetraploid ($2n=40$), and resembled var. *hibernicus*, by backcrossing the artificially synthesized triploid hybrid *S. vulgaris* var. *vulgaris* × *S. squalidus* ($2n=30$), to the female parent (var. *vulgaris*), and then selfing the products. The extreme low fertility of the triploid hybrid and the reduced fertility of the initial backcross products, led Ingram *et al.* (1980) to accept Stace's view that the origin of radiate var. *hibernicus* was a rare event and that factors other than frequent introgression must account for the rapid spread of this taxon in Britain in recent years.

Despite the historical, morphological and crossing evidence that favours an introgressive origin of var. *hibernicus*, it remains feasible that the variant originated following a single gene mutation in non-radiate var. *vulgaris*. Capitulum type in *S. vulgaris* (radiate v. non-radiate) is controlled by a single gene with two alleles showing incomplete dominance (Trow, 1912). As non-radiate mutants naturally occur at low frequency in some British populations of the related radiate *S. squalidus* L. (Ingram & Taylor, 1982) and *S. jacobea* L. (Harper & Wood, 1957), it is feasible that the reverse mutation might occur in *Senecio* as in certain other Compositae e.g. *Bidens cernua* L. (Stace, 1977).

The introgressive origin of var. *hibernicus* would be more certain if it were shown that along with the radiate allele, other genes specific to *S. squalidus* had been introduced into *S. vulgaris* and were now present at high frequency in var. *hibernicus*, but absent from non-radiate *S. vulgaris* material isolated from *S. squalidus*.

Here we report on a study of allozyme variation for aspartate aminotransferase (AAT) in *S. vulgaris* and *S. squalidus*, which provides evidence that genetic material in addition to the radiate allele has been introgressed from *S. squalidus* into *S. vulgaris*. Based on this and the previous evidence reviewed by Stace (1987), we propose that the radiate variant of *S. vulgaris* (var. *hibernicus*) is a classic example of a taxon which originated in recent times as a stabilized introgressant, and has since become widely distributed in Britain.

Materials and methods

Seeds (achenes) or whole plants of the radiate (var. *hibernicus*) and non-radiate (var. *vulgaris*) variants of *S. vulgaris* were collected from 13 British populations that were polymorphic for capitulum type. Similarly, seeds or plants were collected from 15 populations that were monomorphic for non-radiate var. *vulgaris* (seven British populations, one Irish and seven from mainland Europe), and also from 20 British populations of *S. squalidus*. The locations of populations and the size of samples collected are listed in Tables 3 and 4. The distribution of British populations sampled of *S. vulgaris* and *S. squalidus* is illustrated in Fig. 1. Populations of var. *vulgaris* from mainland Europe were isolated from the natural distribution of *S. squalidus* in Europe (Alexander, 1979). Nearly all other populations of *S. vulgaris* grew in areas where *S. squalidus* also occurred or was present nearby. In most instances, plants were sampled from populations which grew on open wasteground or along the edges of paths and pavements in urban areas; however, some samples of var. *vulgaris* came from more natural open habitats, e.g. sand dunes (at Aberffraw) and cliff tops (on Puffin Island). 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. On average, material from approximately 30 individuals per taxon was analysed electrophoretically per population.

Plants raised from seed, or transplanted from the field, were cultivated in a greenhouse in pots or trays containing a peat-based compost. Starch gel electrophoresis was conducted on crude protein extracts of leaf tissue. The extraction buffer was composed of 50 ml of gel buffer (see below); 37 mg KCl; 14 mg $MgCl_2$; 18 mg EDTA (tetrasodium); 2 ml mercaptoethanol; 0.5 ml Triton X-100; and 25 mg PVPP. Homogenates were applied to paper wicks, and enzymes were separated in 11 per cent (w/v) starch gels run at a constant voltage of 250 V. The electrode buffer was 0.192 M boric acid adjusted to pH 8.3 with lithium hydroxide (giving a final LiOH molarity of 0.03 M), while the gel buffer was composed of one part electrode buffer to nine parts of

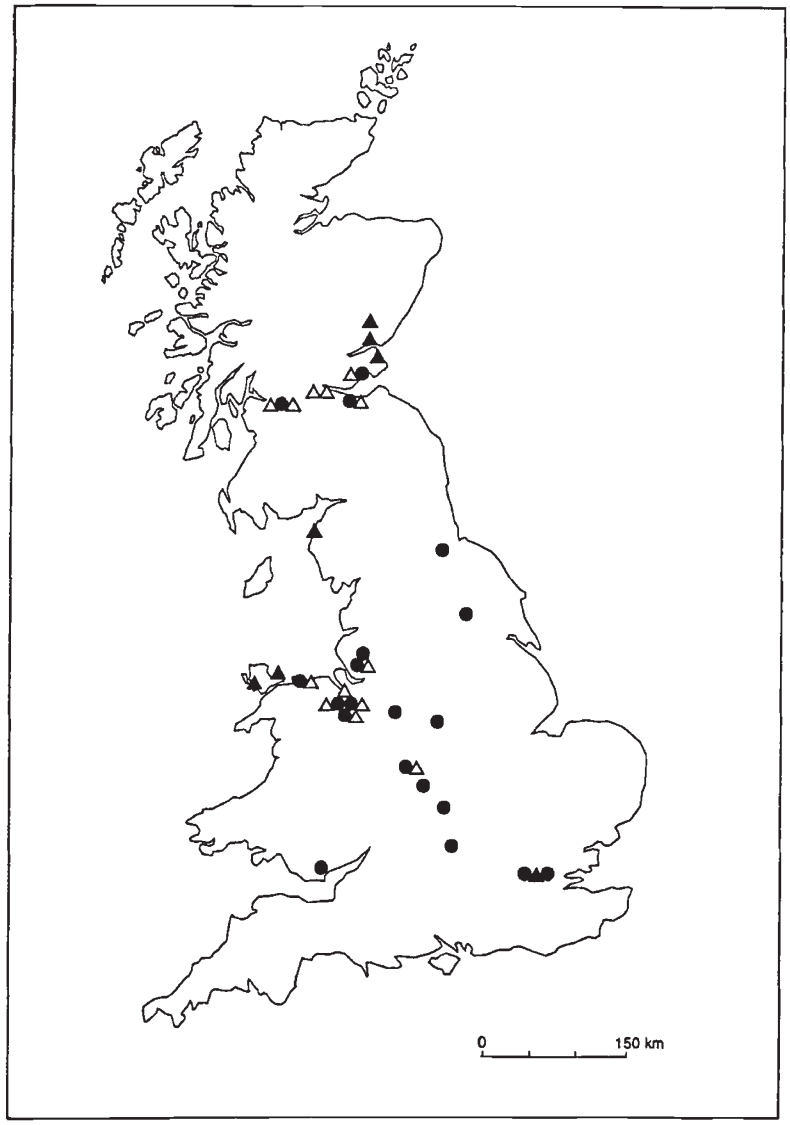

Fig. 1 Distribution of British populations of *Senecio* spp. surveyed for *Aat-3* variation: *S. squalidus* (●); *S. vulgaris* var. *vulgaris* and var. *hibernicus* (△); *S. vulgaris* var. *vulgaris* (▲).

0.05 M Tris 0.007 M citrate, pH 8.3. Gels were stained for AAT at 37°C. The stain solution was composed of 50 ml 0.1 M Tris-HCl (pH 8.5), 18 mg α-ketoglutaric acid, 65 mg aspartic acid, 250 mg PVP, 25 mg EDTA (tetrasodium), 710 mg Na_2HPO_4, 1 mg pyridoxal-5-phosphate, and 200 mg Fast Blue BB (after Gottlieb, 1973).

Genetic analysis of AAT variants

Three zones of activity were detected on gels stained for AAT in both *S. squalidus* and *S. vulgaris* (Ashton, 1990). Two zones, designated as *Aat-1* and *Aat-2* were located close to each other, approximately equidistant from the origin and the anodal front. Within each of these zones a band was either present or absent depending on the individual surveyed. Individuals were frequently double-banded producing both *Aat-1* and *Aat-2* isozymes, or single-banded for one or other isozymes. No individuals were found which lacked both bands. A genetic analysis (Ashton, 1990) failed to establish a simple model of inheritance for the presence/absence of *Aat-1* and *Aat-2* isozymes in *S. squalidus* and these isozymes were not investigated in the present study. In the third zone of activity, nearer to the origin (Fig.2), *S. squalidus* individuals exhibited either a single fast allozyme (*Aat-3b*), a single slow allozyme (*Aat-3c*), or were triple-banded possessing both allozymes and an additional intermediate allozyme. A genetic analysis (Ashton, 1990), confirmed that the *Aat-3* allozymes in *S. squalidus* are the products of a single diallelic gene with the triple-banded phenotype representing the heterozygote.

In *S. vulgaris*, individuals are typically triple banded within the *Aat-3* zone of activity (Ashton, 1990), although a rare six-banded phenotype also occurs (Fig.

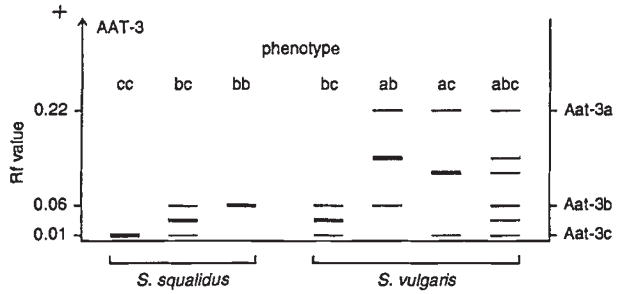

Fig. 2 Electrophoretic phenotypes resolved for *Aat-3* in *Senecio squalidus* and *S. vulgaris*. Staining intensity of bands varied in the six-banded *Aat-3* phenotype observed in *S. vulgaris* reflecting gene dosage.

2). Three different triple-banded phenotypes have been resolved in *S. vulgaris* and are designated as *Aat-3ab, Aat-3ac* and *Aat-3bc* (Fig. 2). To establish the genetic basis of this variation, and also the possibility of linkage between alleles encoding *Aat-3* allozymes and those at the ray floret locus controlling capitulum type in *S. vulgaris*, a genetic analysis was conducted which involved examination of selfed offspring, and F_1 and F_2 families produced from reciprocal crosses between available radiate and non-radiate inbred lines that differed in *Aat-3* phenotype (Tables 1 and 2). Crosses were made after emasculating capitula of maternal plants using the procedure described by Ornduff

Table 1 χ^2 test for segregation ratio of genotypes for capitulum and *Aat-3* type in the F_2 s of reciprocal crosses between radiate (RR) and non-radiate (NN) *S. vulgaris* lines. Designated *Aat-3* genotypes of parents are enclosed in brackets

Parents	F_2 genotype									
Capitulum type	*RR*	*RN*	*NN*							
L26-RR,(cc,bb) × M2-NN,(aa,bb)	43	100	92							25.65***
M2-NN,(aa,bb) × L26-RR,(cc,bb)	43	103	50							1.01
L15-RR,(aa,bb) × S2-NN,(cc,bb)	73	117	58							2.60
S2-NN,(cc,bb) × L15-RR,(aa,bb)	72	113	55							3.22
F6-RR,(cc,aa) × B7-NN,(cc,bb)	43	81	34							1.13
B7-NN,(cc,bb) × F6-RR,(cc,aa)	81	127	57							4.80
M12-RR,(cc,aa) × M28-NN,(aa,bb)	45	90	50							0.40
M28-NN,(aa,bb) × M12-RR,(cc,aa)	60	92	48							2.72
M22-RR,(cc,aa) × M29-NN,(aa,bb)	41	65	43							2.48
M29-NN,(aa,bb) × M22-RR,(cc,aa)	12	24	21							4.26
L13-RR,(cc,aa) × M24-NN,(aa,bb)	27	73	35							1.84
M24-NN,(aa,bb) × L13-RR,(cc,aa)	13	21	11							0.38
F5-RR,(cc,aa) × S7-NN,(aa,bb)	17	31	14							0.29
S7-NN,(aa,bb) × F5-RR,(cc,aa)	72	92	33							16.30**
Aat-3 type	*aa,bb*	*ac,bb*	*cc,bb*							
L26-RR,(cc,bb) × M2-NN,(aa,bb)	60	127	56							0.63
M2-NN,(aa,bb) × L26-RR,(cc,bb)	47	111	47							1.41
L15-RR,(aa,bb) × S2-NN,(cc,bb)	62	132	58							0.70
S2-NN,(cc,bb) × L15-RR,(aa,bb)	57	135	56							1.96
	cc,aa	*cc,ab*	*cc,bb*							
F6-RR,(cc,aa) × B7-NN,(cc,bb)	42	95	31							4.32
B7-NN,(cc,bb) × F6-RR,(cc,aa)	72	167	57							6.40*
	cc,aa	*cc,ab*	*aa,ab*	*aa,cc*	*aa,ac*	*bb,cc*	*aa,bc*	*ac,bb*	*ab,cc*	$\chi^2_{(8)}$‡
M12-RR,(cc,aa) × M28-NN,(aa,bb)	13	19	18	14	32	21	11	17	12	11.92
M28-NN,(aa,bb) × M12-RR,(cc,aa)	14	22	18	21	51	32	5	19	13	11.70
M22-RR,(cc,aa) × M29-NN,(aa,bb)	10	23	10	14	32	19	9	19	5	4.87
M29-NN,(aa,bb) × M22-RR,(cc,aa)	3	14	3	11	9	7	4	5	3	11.17
L13-RR,(cc,aa) × M24-NN,(aa,bb)	10	20	8	18	41	26	5	19	11	5.11
M24-NN,(aa,bb) × L13-RR,(cc,aa)	3	7	2	5	16	6	3	8	3	1.94
F5-RR,(cc,aa) × S7-NN,(aa,bb)	3	10	6	10	16	11	4	9	2	3.37
S7-NN,(aa,bb) × F5-RR,(cc,aa)	22	22	13	30	64	18	19	29	2	23.30**

†Test of 1:2:1 ratio.

‡Test of 1:2:1:2:4:2:1:2:1 ratio (i.e. due to disomic digenic inheritance).

Derivation of material: B (Brymbo), F (Ffrith), L (Leith), M (Methil), S (Southsea).

NB: average percentage of seed sown per F_2 family producing individuals scored was 74.7 per cent for capitulum type and 76.4 per cent for *Aat-3* type.

Table 2 Results of χ^2 contingency tests for joint segregation of alleles at ray floret locus and first *Aat-3* locus; and ray floret locus and second *Aat-3* locus in *S. vulgaris*. Designated *Aat-3* genotypes of parents are enclosed in brackets

Parents	$\chi^2_{(4)}$
First *Aat-3* locus	
L26-RR,(cc,bb) × M2-NN,(aa,bb)	7.62
M2-NN,(aa,bb) × L26-RR,(cc,bb)	1.02
L15-RR,(aa,bb) × S2-NN,(cc,bb)	1.66
S2-NN,(cc,bb) × L15-RR,(aa,bb)	3.08
M12-RR,(cc,aa) × M28-NN,(aa,bb)	0.58
M28-NN,(aa,bb) × M12-RR,(cc,aa)	5.97
M22-RR,(cc,aa) × M29-RR,(aa,bb)	0.85
M29-RR,(aa,bb) × M22-NN,(cc,aa)	2.86
L13-RR,(cc,aa) × M24-NN,(aa,bb)	0.98
M24-NN,(aa,bb) × L13-RR,(cc,aa)	0.77
F5-RR,(cc,aa) × S7-NN,(aa,bb)	5.50
S7-NN,(aa,bb) × F5-RR,(cc,aa)	4.37
Second *Aat-3* locus	
F6-RR,(cc,aa) × B7-NN,(cc,bb)	0.08
B7-NN,(cc,bb) × F6-RR,(cc,aa)	4.40
M12-RR,(cc,aa) × M28-NN,(aa,bb)	14.03**
M28-NN,(aa,bb) × M12-RR,(cc,aa)	1.80
M22-RR,(cc,aa) × M29-NN,(aa,bb)	6.97
M29-NN,(aa,bb) × M22-RR,(cc,aa)	2.79
L13-RR,(cc,aa) × M24-NN,(aa,bb)	3.00
M24-NN,(aa,bb) × L13-RR,(cc,aa)	3.19
F5-RR,(cc,aa) × S7-NN,(aa,bb)	6.71
S7-NN,(aa,bb) × F5-RR,(cc,aa)	2.91

NB: $\chi^2_{(4)} = 9.49$ when $P = 0.05$.

(1964). Selfed and crossed capitula were enclosed in bags made of lens tissue before and following treatment until seed was set.

Results

Genetic analysis of Aat-3 *variation in* S. vulgaris

Selfed offspring of *S. vulgaris* lines bred true for capitulum type and *Aat-3* phenotype. Failure of each triple-banded phenotype to segregrate on selfing confirmed that the *Aat-3* locus is duplicated in *S. vulgaris* with each locus homozygous for different alleles. The F_1 offspring of all crosses bore capitula with short ray florets and exhibited a six-banded *Aat-3abc* phenotype in which the staining intensity of bands reflected gene dosage and therefore *Aat-3* genotype. A six-banded F_1 phenotype is expected for a tetraploid individual which is heterozygous for three alleles encoding a dimeric enzyme, due to the production of three intragenic heterodimers and three homodimeric allozymes.

In regard to capitulum type (Table 1), a significantly distorted ratio from the expected 1:2:1 ratio was observed in two out of 14 F_2 families examined; however, in each case the ratio produced by the reciprocal cross was not significantly different from expected. The occurrence of distorted ratios is frequently observed in material of hybrid origin (Weeden & Wendel, 1989) and this may account for the distortions recorded in the present analysis. That said, no irregularities in the normal pattern of disomic monogenic inheritance have been recorded in previous genetic analyses of capitulum type in *S. vulgaris* (Trow, 1912; Hull, 1974; R. J. Abbott, unpublished observations).

All four crosses for *Aat-3*, between the *Aat-3ab* and *Aat-3bc* phenotypes, produced F_2 families in which the segregation ratio was not significantly different from 1:2:1 (Table 1). This ratio would be expected following crosses between parental genotypes designated as *Aat-3aa,bb* and *Aat-3cc,bb* respectively. The cross between the *Aat-3ac* and *Aat-3bc* phenotypes also segregated 1:2:1 in one family, while in the F_2 of the reciprocal the expected three phenotypes were produced despite a distorted segregation ratio. A 1:2:1 segregation ratio in the F_2 of this cross would occur with parental genotypes designated as *Aat-3cc,aa* and *Aat-3cc,bb* respectively. Finally, seven of the eight crosses between the *Aat-3ac* and *Aat-3ab* phenotypes segregated nine genotypes in the F_2 in a 1:2:1:2:4:2:1:2:1 ratio as expected with parental genotypes designated as *Aat-3cc,aa* and *Aat-3aa,bb* respectively (assuming independent segregation of the two *Aat-3* loci). In the remain ing F_2 family, the ratio was significantly distorted although all nine genotypes were recovered. Taken overall, the results confirm that there are two unlinked *Aat-3* loci present in *S. vulgaris*, and that the *Aat-3a* allele occurs at each locus.

With two unlinked *Aat-3* loci present in *S. vulgaris*, measurement of linkage to the ray floret locus requires consideration of each *Aat-3* locus in turn. Crosses between radiate plants of *Aat-3cc,bb* genotype and non-radiate plants of *Aat-3aa,bb* genotype measure linkage between the ray floret locus and the first *Aat-3* locus, while crosses between radiate individuals of *Aat-3cc,aa* genotype and non-radiate plants of *Aat-3cc,bb* genotype estimate linkage between the ray floret locus and the second *Aat-3* locus. F_2 data from crosses between radiate plants of *Aat-3cc,aa* genotype and non-radiate plants of *Aat-3aa,bb* genotype provide estimates of linkage between the ray floret locus and each *Aat-3* locus taken in turn. Chi-squared contingency tests of the joint segregation of capitulum type and *Aat-3* type revealed no evidence of linkage between the ray floret locus and either *Aat-3* locus in all except one cross examined (Table 2). In the single

cross which showed significant joint segregation of capitulum type and *Aat-3* type at the second *Aat-3* locus analysed, a recombination fraction and its standard error were calculated using the maximum likelihood procedures of Allard (1956). This was performed by means of the LINKAGE-1 program of Suiter *et al.*, (1983). The recombination fraction between the pair of loci remained high, $r = 0.44 \pm 0.04$. Given the absence of significant joint segregation of the two loci in the reciprocal cross, and the results for the other crosses examined, it is concluded that the ray floret locus is not linked to either *Aat-3* locus found in *S. vulgaris*.

Genetic diversity

S. squalidus. In all populations of *S. squalidus*, the *Aat-3c* allele was more common that the alternative *Aat-3b* allele (Table 3), and over all individuals surveyed its frequency was estimated to be 74 per cent. Measures of gene diversity (Nei, 1973) at the *Aat-3* locus showed that within-population diversity ($Hs = 0.3643$) accounted for most of the total gene diversity ($Ht = 0.3916$), between-population diversity being low ($Dst = 0.0273$). This pattern of diversity is expected in species that reproduce by predominant outcrossing (Hamrick & Godt, 1990). *F*-statistics (Wright, 1951), computed using the method of Hartl (1981), revealed that the total inbreeding coefficient ($Fit = 0.2723$) was greater than expected with panmixia, due largely to a deficiency of heterozygotes from expected ($Fis = 0.2174$), rather than genetic subdivision between populations ($Fst = 0.0701$). However, only in three of the 20 populations surveyed were the observed genotype frequencies significantly different from those

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

Population	National Grid Reference	Sample size	Allele frequency		H_0	F_0
			b	*c*		
England						
Banbury	SP463404	50	0.34	0.66	0.360	+0.198
Birmingham	SP092876	21	0.31	0.69	0.333	+0.220
Darlington	NZ296146	15	0.33	0.67	0.000*	+1.000
Dartford	TQ555743	25	0.32	0.68	0.400	+0.081
Derby	SK362356	44	0.30	0.70	0.455	−0.094
Kingston-u-Thames	TQ191691	48	0.16	0.84	0.312	−0.185
Oxford	SP505065	46	0.33	0.67	0.434	+0.011
St Helens	SJ524926	46	0.22	0.78	0.304	+0.108
Stoke-on-Trent	SJ886407	49	0.09	0.91	0.184	−0.102
Warwick	SP286655	44	0.48	0.52	0.500	−0.010
Wigan	SD594034	36	0.18	0.82	0.250	+0.155
York	SE612518	26	0.40	0.60	0.500	−0.037
Scotland						
Glasgow	NS534671	34	0.19	0.81	0.206	+0.334
Edinburgh	NT268765	44	0.15	0.85	0.252	+0.008
Methil	NT376995	26	0.35	0.65	0.453	−0.020
Wales						
Cardiff	ST173733	22	0.32	0.68	0.000*	+1.000
Mochdre	SH813774	26	0.42	0.58	0.308	+0.370
Wrexham						
Brymbo	SJ296539	33	0.15	0.85	0.182	+0.293
Rhostyllen	SJ312492	27	0.31	0.69	0.259*	+0.399
Southsea	SJ306515	25	0.00	1.00	0.000	+1.000
Total		687	0.26	0.74	0.303	+0.213

Allele frequencies have been rounded up to two decimal places.
*Observed genotypic frequencies significantly different from expectation under Hardy–Weinberg equilibrium ($P < 0.05$).

expected at Hardy–Weinberg equilibrium. If the *Fis* value of 0.2174 was due entirely to selfing (s), then an average selfing rate of approximately 36 per cent would be postulated for *S.squalidus* in the wild

$$\left(s = 1 - \frac{1-F}{1+F}\right).$$

This value is unexpectedly high given the very low automatic selfing ability previously reported for *S. squalidus* (Gibbs *et al.*, 1975). Spatial substructuring of populations or assortative mating are alternative factors which might lead to a deficiency of heterozygotes in *S. squalidus* populations; these factors, together with the direct estimation of outcrossing rates in the wild, will require investigation in future.

S. vulgaris. Because the *Aat-3a* allele appears to occur at both *Aat-3* loci in *S. vulgaris* it is not possible to determine the exact genotype of an individual containing this allele from its isozyme phenotype. The analysis of *Aat-3* diversity in *S. vulgaris* is restricted, therefore, to an examination of phenotype frequencies. All populations monomorphic for the non-radiate morph (var. *vulgaris*) were monomorphic for the *Aat-3ab* phenotype (Table 4). This phenotype was also predominant (frequency 0.97) among non-radiate plants in populations polymorphic for capitulum type, being fixed in 8 of 13 polymorphic populations surveyed. In contrast, var. *hibernicus* was monomorphic for the *Aat-3ac* phenotype in one population (at Methil), and in nine of the remaining 12 populations surveyed, was polymorphic for *Aat-3* phenotype. None of the populations surveyed contained individuals which produced a single-banded phenotype, i.e. were homozygous for the same allele at each duplicated locus. Taken over all individuals surveyed, it was apparent that whereas the *Aat-3c* allele was common in var. *hibernicus*, it was very rare among individuals of var. *vulgaris* which co-occurred with var. *hibernicus*, and was absent from British, Irish and mainland European populations monomorphic for var. *vulgaris*. In populations polymorphic for capitulum type, both *S. vulgaris* variants exhibited a marked deficiency of observed free heterozygotes, i.e. six-banded phenotypes at the *Aat-3* locus (Table 4), indicating a high level of inbreeding.

Discussion

The results of the survey of allozyme variation for AAT in *S. squalidus* and *S. vulgaris* has provided strong evidence that the radiate variant of *S. vulgaris* var. *hibernicus* ($2n = 40$) originated following introgression of genetic material across a chromosome barrier from *S. squalidus* ($2n = 20$) into the non-radiate *S. vulgaris* ($2n = 40$). The *Aat-3c* allele which occured at high frequency in *S. squalidus* (74 per cent of 687 plants surveyed), was also common in *S. vulgaris* var. *hibernicus* (present in 47 per cent of 351 plants tested), but was rare among individuals of var. *vulgaris* which co-occurred with var. *hibernicus* (3 per cent of 384 plants examined), and was absent from populations monomorphic for var. *vulgaris* (428 plants tested). Taken together with the historical, morphological, and crossing evidence previously reported (Stace, 1977; Ingram *et al.*, 1980), this genetic evidence can be taken as confirmation of an introgressive origin of *S. vulgaris* var. *hibernicus*.

Introgression of the *Aat-3c* allele from *S. squalidus* into *S. vulgaris* could have proceeded along two pathways. Either via backcrossing following the formation of a triploid F_1 hybrid ($2n = 30$), as postulated and demonstrated previously for the introgression of the ray floret allele into *S. vulgaris* (Ingram, 1978; Ingram *et al.*, 1980), or alternatively via backcrossing following the formation of a tetraploid F_1 hybrid ($2n = 40$). Fusion of an unreduced *S. squalidus* gamete with a normal gamete of *S. vulgaris* var. *vulgaris* would produce a tetraploid F_1 which is likely to exhibit higher pollen fertility than a triploid F_1 and thus facilitate introgression more easily. Currently it is not possible to state with certainty along which of these two pathways introgression has proceeded, and it is feasible that both pathways were involved in the transfer of the *Aat-3c* allele from *S. squalidus*. The fact that the *Aat-3a* allele has remained absent from *S. squalidus* in Britain, although common in *S. vulgaris*, is evidence against introgression proceeding from *S. vulgaris* into *S. squalidus*.

The radiate variant of *S. vulgaris* differs markedly from the non-radiate form in mating system. In populations polymorphic for capitulum type, non-radiate var. *vulgaris* normally outcrosses at a rate of $\leqslant 1$ per cent, whereas radiate var. *hibernicus* shows between 3 and 35 per cent outcrossing (Marshall & Abbott, 1982, 1984a). This difference has been attributed, in part, to (i) the pistillate ray florets of radiate plants outcrossing at higher frequencies than the hermaphroditic disc florets of which non-radiate capitula are entirely composed (Marshall & Abbott, 1984b); and (ii) greater relative attractiveness of radiate capitula to pollinators due to the presence of ray florets (Abbott & Irwin, 1988). Because of its greater outcrossing rate, var. *hibernicus* is subject to a 'cost of outcrossing' in populations polymorphic for capitulum type (Fisher, 1941; Maynard-Smith, 1978; Lloyd, 1979); despite this disadvantage, however it has spread widely in Britain since its origin in the 19th century (Stace,

1977). Elsewhere, Abbott (1986) and Abbott & Horrill (1991) have suggested that the success of the radiate variant is likely to stem from the beneficial effects of other genes introgressed into *S. vulgaris* along with the radiate allele, and now stabilized in var. *hibernicus*, rather than any direct benefits of increased outcrossing *per se*, e.g. a reduction in inbreeding depression (Charlesworth & Charlesworth, 1987). The occurrence of the *Aat-3c* allele in var. *hibernicus*, provides evidence that a specific gene other than the radiate allele has been introgressed into the taxon at one locus and, as a result, the variant shows an increased level of allelic variation at this locus.

A surprising result to emerge from the genetic analysis of *Aat-3* variation in *S. vulgaris* was the presence of the '*a*' allele at both *Aat-3* loci within the species. It would follow that some individuals homozygous for the '*a*' allele at each locus and producing therefore a single-banded phenotype would be expected to have been detected in the survey of *Aat-3* variation in wild material. In no instance, however, were such individuals found (Table 4). If the *Aat-3ab* phenotype is produced only by the *Aat-3aa,bb* genotype while the *Aat-3ac* phenotype is produced only by the *Aat-3cc,aa* genotype [as indicated by the genetic analysis (Table 1)], then segregation of the *Aat-3aa,aa* genotype would not occur in populations of non-radiate *S. vulgaris* var. *vulgaris* (all of which are monomorphic for the *Aat-3ab* phenotype), but should occur in those populations polymorphic for capitulum type which contain the *Aat-*

Table 4 *Aat-3* phenotype frequencies in *S. vulgaris* var. *vulgaris* (V) and var. *hibernicus* (H)

Population	National Grid Reference	Variant	Sample size	Phenotype frequency			
				ab	*ac*	*bc*	*abc*
Polymorphic for var. *vulgaris* and var. *hibernicus*							
England							
Birmingham	SP045835	V	50	1.00	—	—	—
		H	50	1.00	—	—	—
St Helens	SJ524944	V	40	1.00	—	—	—
		H	40	0.85	0.12	0.03	—
Scotland							
Glasgow (i)	NS578664	V	25	1.00	—	—	—
		H	25	1.00	—	—	—
Glasgow (ii)	NS534671	V	25	1.00	—	—	—
		H	25	0.44	0.48	0.08	—
Grangemouth (i)	NS913823	V	25	0.96	—	0.04	—
		H	25	0.32	0.68	—	—
Grangemouth (ii)	NS977814	V	25	1.00	—	—	—
		H	25	1.00	—	—	—
Edinburgh	NT268765	V	27	0.92	0.04	0.04	—
		H	25	0.28	0.08	0.64	—
Methil	NT376995	V	32	1.00	—	—	—
		H	36	—	1.00	—	—
Wales							
Mochdre	SH822781	V	20	1.00	—	—	—
		H	24	0.08	0.88	0.04	—
Wrexham							
Brymbo	SJ296539	V	38	0.87	0.03	0.03	0.07
		H	21	0.38	0.33	0.29	—
Ffrith	SJ286556	V	10	1.00	—	—	—
		H	16	—	0.87	0.13	—
Rhostyllen	SJ312492	V	23	0.96	—	0.04	—
		H	15	0.73	—	0.27	—
Southsea	SJ306515	V	44	0.96	—	0.02	0.02
		H	24	0.25	0.17	0.50	0.08
Total		V	384	0.971	0.005	0.013	0.011
		H	351	0.533	0.336	0.125	0.006

Table 4 *Continued*

Population	National Grid Reference	Variant	Sample size	Phenotype frequency *ab*	*ac*	*bc*	*abc*
Monomorphic for var. *vulgaris*							
Britain							
England							
London	TQ349807	V	45	1.00	—	—	—
Workington	NX994295	V	40	1.00	—	—	—
Scotland							
Dundee	NO394295	V	45	1.00	—	—	—
Kirriemuir	NO388533	V	27	1.00	—	—	—
St Andrews	NO510165	V	100	1.00	—	—	—
Wales							
Aberffraw	SH366656	V	10	1.00	—	—	—
Puffin Island	SH653824	V	10	1.00	—	—	—
Ireland							
Glengariff		V	26	1.00	—	—	—
Mainland Europe							
France							
Pont Croix (Brittany)		V	21	1.00	—	—	—
Poland							
Warsaw		V	29	1.00	—	—	—
Spain							
Bejar		V	19	1.00	—	—	—
Matalascanas		V	37	1.00	—	—	—
Switzerland							
Basel		V	11	1.00	—	—	—
Grindelwald		V	4	1.00	—	—	—
Interlaken		V	4	1.00	—	—	—
Total		V	428	1.00	—	—	—

3ab and *Aat-3ac* phenotypes. The absence of the *Aat-3aa,aa* genotype from these latter populations remains a puzzle, unless the genotype exhibits low relative fitness or there is a lack of crossing between the *Aat-3ab* and *3ac* phenotypes in the wild.

Assuming that individuals of *S. vulgaris* var. *vulgaris* in populations which are monomorphic for non-radiate capitulum type are all of *Aat-3aa,bb* genotype, then it is of interest to speculate on how the *Aat-3a* allele became established at the second *Aat-3* locus as in the *Aat-3cc,aa* genotype. Although mutation cannot be ruled out, the most likely explanation is that during the introgressive origin of var. *hibernicus*, recombination between homoeologous chromosomes occurred and resulted in the transfer of the '*a*' allele between the two *Aat-3* loci in *S. vulgaris*. In this respect, it is of interest that multivalent formation, which provides opportunities for recombination to occur between homoeologous chromosomes, has been recorded by Ingram (1977, 1978) at meiosis in the artificially synthesized triploid hybrid between *S. vulgaris* and *S. squalidus* and also in the backcross progeny to *S. vulgaris*.

Finally, in view of the close association recorded between the *Aat-3c* allele and the radiate allele in populations of *S. vulgaris* polymorphic for capitulum type, it is somewhat surprising that neither *Aat-3* locus was found to be linked to the ray floret locus. Because the maternal outcrossing rate of the radiate morph resulting from intermorph crosses can reach 35 per cent in some polymorphic populations, it may have been anticipated that the *Aat-3c* allele would be more common in non-radiate plants (i.e. those which co-occur with the radiate morph) than was recorded. It is possible that the strong association which is present between the *Aat-3c* and ray floret alleles is maintained by selection favouring a co-adapted complex of genes that has been introgressed from *S. squalidus*. Alternatively, or in addition, there may be positive assortative mating between genotypes at the *Aat-3* locus which would greatly reduce the rate of transfer of the *Aat-3c* allele from var. *hibernicus* to var. *vulgaris*. The rarity with which six-banded heterozygotes at the *Aat-3* locus were recorded in wild polymorphic populations (Table 4) lends some support to this second possibility.

In conclusion, it is worth considering whether var. *hibernicus* may have originated via introgression more than once in Britain. In the wild, the triploid F_1 hybrid is produced at very low frequencies in areas where *S. squalidus* and *S. vulgaris* co-occur (Marshall & Abbott, 1980); nevertheless such hybrids, verified by cytological analysis, have been reported from several locations throughout Britain (Stace, 1977; Brettell & Leslie, 1978; Marshall & Abbott, 1980; Taylor, 1984). Records of the putative tetraploid F_1 hybrid in Britain are less frequent and have been reported only twice (Crisp, 1972; Taylor, 1984). Early backcross products are likely to resemble *S. vulgaris* var. *hibernicus* quite closely (at least when the triploid hybrid is involved), but with reduced fertility and perhaps a more intermediate and less stable phenotype (Ingram *et al.*, 1980). Consequently, they are likely to be overlooked in natural populations. The only detailed survey, to date, of the possible occurrence of early backcross individuals in the wild, is that of Crisp (1972) on herbarium material collected in the British Isles from the mid-19th century to 1930. Crisp (1972) identified what appeared to be early backcrossed products at four locations: i.e. around Oxford, at Cork (Ireland), the Bristol/Cardiff area and the north-east Wales/Cheshire area. Within each of these areas, var. *hibernicus* was present before 1900, and it is feasible that in each case a separate origin of the taxon had occurred. Based on Crisp's findings, therefore, it would seem that var. *hibernicus* may have originated independently via introgression at several different locations in Britain during the past 150 years or so, following local hybridization between *S. vulgaris* var. *vulgaris* and *S. squalidus*. More precise evidence is required on this point, however, before such a conclusion is readily accepted.

Acknowledgements

Part of this research was conducted while PAA was in receipt of a postgraduate research studentship funded by the NERC. We are grateful to Christine Wilson for providing seed of *S. vulgaris* from Warsaw. We also wish to thank an anonymous reviewer whose constructive comments greatly improved the original manuscript.

References

ABBOTT, R. J. 1986. Life history variation associated with the polymorphism for capitulum type and outcrossing rate in *S. vulgaris*. *Heredity*, **56**, 381–392.

ABBOTT, R. J. AND HORRILL, J. C. 1991. Survivorship and fecundity of the radiate and non-radiate morphs of groundsel, *Senecio vulgaris* L., raised in pure stand and mixture. *J. Evol. Biol.*, **4**, 241–257.

ABBOTT, R. J. AND IRWIN, J. A. 1988. Pollinator movements and the polymorphism for outcrossing rate in Groundsel, *Senecio vulgaris* L. *Heredity*, **60**, 295–298.

ALEXANDER, J. C. M. 1979. The Mediterranean species of *Senecio* sections *Senecio* and *Delphinifolius*. *Notes. Roy. Bot. Gard. Edin.*, **27**, 387–428.

ALLARD, R. W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. *Hilgardia*, **24**, 235–278.

ANDERSON, E. 1949. *Introgressive Hybridization*. John Wiley, New York.

ARNOLD, M. L., HAMRICK, J. L. AND BENNETT, B. D. 1990. Allozyme variation in Louisiana irises: a test for introgression and hybrid speciation. *Heredity*, **65**, 297–306.

ASHTON, P. A. 1990. *Multiple Origins of* Senecio cambrensis *Rosser, and Related Evolutionary Studies in British Senecio*. Unpublished Ph.D. Thesis, University of St Andrews.

BLOOM, W. L. 1976. Multivariate analysis of the introgressive replacement of *Clarkia nitens* by *Clarkia speciosa polyantha*. *Evolution*, **30**, 412–424.

BRETTELL, R. I. S. AND LESLIE, A. C. 1978. *Senecio squalidus* L. × *S. vulgaris* L. in Cambridgeshire. *Watsonia*, **12**, 155.

BRIGGS, D. AND WALTERS, S. M. 1984. *Plant Variation and Evolution*. 2nd edn, Cambridge University Press, Cambridge.

CHARLESWORTH, D. AND CHARLESWORTH, B. 1987. Inbreeding depression and its evolutionary consequences. *Ann. Rev. Ecol. Syst.*, **18**, 237–268.

CRISP, P. C. 1972. *Cytotaxonomic studies in the section Annui of Senecio*. Unpublished Ph.D. Thesis, University of London.

DAVIS, J. L. 1985. Introgression in Central American *Phytolacca* (Phytolaccaceae). *Am. J. Bot.*, **72**, 1944–1953.

DRUCE, G. C. 1927. *The Flora of Oxfordshire*. 2nd edn, Clarendon Press, Oxford.

ELLSTRAND, N. C., LEE, J. H., KEELEY, J. E. AND KEELEY, S. C. 1987. Ecological isolation and introgression: biochemical confirmation of introgression in an *Arctostaphylos* (Ericaceae) population. *Acta. Oecol.*, **8**,299–308.

FISHER, R. A. 1941. Average excess and average effect of a gene substitution. *Ann. Eugen.*, **11**, 53–63.

GIBBS, P. E., MILNE, C. AND VARGAS CARRILLO, M. 1975. Correlation between the breeding system and recombination index in five species of *Senecio*. *New Phytol.*, **75**, 619–626.

GOTTLIEB, L. D. 1973. Genetic control of glutamate oxaloacetate transaminase isozymes in the diploid plant *Stephanomeria exigua* and its allopolyploid derivatives. *Biochem. Genet.*, **9**, 97–107.

GRANT, V. 1950. Genetic and taxonomic studies in Gilia. *Aliso*, **2**, 239–316.

GRANT, V. 1981. *Plant Speciation*. 2nd edn, Columbia University Press, New York.

HAMRICK, J. L. AND GODT, M. J. W. 1990. Allozyme diversity in plant species, pp. 43–63. In: Brown, A. H. D., Clegg, M. T., Kahler, A. L. and Weir, B. S. (eds) *Plant Population Genetics, Breeding, and Genetic Resources*. Sinauer, MA, pp. 43–63.

HARPER, J. L. AND WOOD, W. A. 1957. Biological Flora of the British Isles. *Senecio jacobaea* L. *J. Ecol.*, **45**, 617–637.

HARTL, D. L. 1981. *A Primer of Population Genetics.* Sinauer, MA.

HEISER, C. B. 1949. Study in the evolution of the sunflower species *Helianthus annuus* and *H. bolanderi. Univ. Calif. Publ. Bot.*, **23**, 157–196.

HEISER, C. B. 1951. Hybridization in the annual sunflowers *Helianthus annuus* × *H. debilis* var. *cucumerifolius. Evolution*, **5**, 42–51.

HEISER, C. B. 1973. Introgression re-examined. *Bot. Rev.*, **39**, 347–366.

HULL, P. 1974. Self-fertilisation and the distribution of the radiate form of *Senecio vulgaris* L. in Central Scotland. *Watsonia*, **10**, 69–75.

INGRAM, R. 1977. Synthesis of the hybrid *Senecio squalidus* L. × *S. vulgaris* L. F. *radiatus* Hegi. *Heredity*, **39**, 171–173.

INGRAM, R. 1978. The genomic relationship of *Senecio squalidus* L. and *S. vulgaris* L. and the significance of genomic balance in their hybrid, *S.* × *baxteri* Druce. *Heredity*, **40**, 459–462.

INGRAM, R. AND TAYLOR, L. 1982. The genetic control of a non-radiate condition in *Senecio squalidus* L. and some observations on the role of ray florets in the Compositae. *New Phytol.*, **91**, 749–756.

INGRAM, R., WEIR, J. A. AND ABBOTT, R. J. 1980. New evidence concerning the origin of inland radiate groundsel *Senecio vulgaris* L. var. *hibernicus* Syme. *New Phytol.*, **84**, 543–546.

LEVIN, D. A. 1963. Natural hybridization between *Phlox maculata* and *P. glaberrima* and its evolutionary significance. *Am. J. Bot.*, **50**, 714–719.

LEVIN, D. A. 1975. Interspecific hybridization, heterozygosity and gene exchange in *Phlox. Evolution*, **29**, 37–51.

LLOYD, D. G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. *Am. Nat.*, **113**, 67–79.

MARSHALL, D. F. AND ABBOTT, R. J. 1980. On the frequency of introgression of the radiate (T_r) allele from *Senecio squalidus* L. into *Senecio vulgaris* L. *Heredity*, **45**, 133–135.

MARSHALL, D. F. AND ABBOTT, R. J. 1982. Polymorphism for outcrossing frequency at the ray floret locus in *Senecio vulgaris* L. I. Evidence. *Heredity*, **48**, 227–235.

MARSHALL, D. F. AND ABBOTT, R. J. 1984a. Polymorphism for outcrossing frequency at the ray floret locus in *Senecio vulgaris* L. II. Confirmation. *Heredity*, **52**, 331–336.

MARSHALL, D. F. AND ABBOTT, R. J. 1984b. Polymorphism for outcrossing frequency at the ray floret locus in *Senecio vulgaris* L. III. Causes. *Heredity*, **53**, 145–149.

MAYNARD SMITH, J. 1978. The ecology of sex. In: J. R. Krebs and N. B. Davies (eds) *Behavioural Ecology: An Evolutionary Approach.* Blackwell Scientific Publications Ltd, Oxford, pp. 159–179.

MONAGHAN, J. AND HULL, P. 1976. Differences in vegetative characteristics among four populations of *Senecio vulgaris* L. possibly due to interspecific hybridization. *Ann. Bot.*, **40**, 125–128.

NEI, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Nat. Acad. Sci. U.S.A.*, **70**, 3321–3323.

OLIVIERI, I. 1985. Comparative electrophoretic studies of *Carduus pynochephalus* L., *C. tenuiflorus* Curt. (Asteraceae) and their hybrids. *Am. J. Bot.*, **72**, 715–718.

ORNDUFF, R. 1964. Evoltionary pathways of the *Senecio lautus* alliance in New Zealand and Australia. *Evolution*, **18**, 349–360.

ORNDUFF, R. 1967. Hybridization and regional variation in Pacific Northwestern Impatiens (Balsaminaceae). *Brittonia*, **19**, 122–128.

RIESEBERG, L. H., BECKSTROM-STERNBERG, S. AND DOAN, K. 1990. *Helianthus annuus* ssp. *texanus* has chloroplast DNA and nuclear ribosomal RNA genes of *Helianthus debilis* ssp. *cucumerifolius. Proc. Natl. Acad. Sci. U.S.A.*, **87**, 593–597.

RIESEBERG, L. H., SOLTIS, D. E. AND PALMER, J. D. 1988. A molecular re-examination of introgression between *Helainthus annuus* and *H. bolanderi* (Compositae). *Evolution*, **42**, 227–238.

ROLLO, C. D., MACFARLANE, J. D. AND SMITH, B. S. 1985. Electrophoretic and allometric variation in burdock (*Arctium* spp): hybridization and its ecological implications. *Can. J. Bot.*, **63**, 1255–1261.

STACE, C. A. 1977. The origin of radiate *Senecio vulgaris* L. *Heredity*, **39**, 383–388.

STACE, C. A. 1987. Hybridization and the Plant Species. In: Urbanska, K. M. (ed.) *Differentiation Patterns in Higher Plants.* Academic Press, London, pp. 115–127.

STEBBINS, G. L. 1959. The role of hybridization in evolution. *Proc. Am. Phil. Soc.*, **103**, 231–251.

STEBBINS, G. L. 1969. The significance of hybridization for plant taxonomy and evolution. *Taxon*, **18**, 26–35.

SOLTIS, D. E. AND SOLTIS, P. S. 1986. Intergeneric hybridization between *Conimitella williamsii* and *Mitella stauropetala* (Saxifragaceae). *Syst. Bot.*, **11**, 293–297.

SUITER, K. A., WENDEL, J. F. AND CASE, J. S. 1983. LINKAGE-1: a PASCAL computer program for the detection and analysis of genetic linkage. *J. Hered.*, **74**, 203–204.

SYME, J. T. B. 1875. *Senecio vulgaris* L. var. *hibernica mihi. Botl. Exch. Club, Rep. Curators*, **1872–74**, 27–28.

TAYLOR, L. 1984. *The potential for introgression in a British polyploid complex.* Unpublished Ph.D. Thesis, University of St. Andrews.

TROW, A. H. 1912. On the inheritance of certain characters in the Common Groundsel, *Senecio vulgaris* L., and its segregates. *J. Genet.*, **2**, 239–276.

WEEDEN, N. F. AND WENDEL, J. F. 1989. Genetics of plant isozymes. In: Soltis, D. E. and Soltis, P. S. (eds) *Isozymes in Plant Biology.* Chapman and Hall, London, pp. 46–72.

WRIGHT, S. 1951. The genetical structure of populations. *Ann. Eugen.*, **15**, 323–354.