Molecular systematics of the genus *Senecio* L. I: Hybridization in a British polyploid complex

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A survey of chloroplast DNA and nuclear ribosomal DNA variation was conducted in the allohexaploid *Senecio cambrensis*, the putative introgressant *S. vulgaris* var. *hibernicus* and their parental taxa: *S. vulgaris* var. *vulgaris* and *S. squalidus*. Ribosomal DNA allowed the differentiation of the two parental taxa and supported the hybrid origin of *S. cambrensis* at three of the four sites sampled; at the fourth site, in Edinburgh, non-additive rDNA phenotypes were found. No evidence, for or against the introgressive origin of var. *hibernicus* was found. Chloroplast DNA analysis provided independent evidence for the dual origin of *S. cambrensis* in Wales and Scotland, although the two parental taxa could not be distinguished.

Keywords: allopolyploidy, Asteraceae, introgression, molecular variation, *Senecio cambrensis*, *S. vulgaris*.

Introduction

Senecio vulgaris L., the common groundsel, is one of the most widespread annual/ephemeral species in Britain. Two subspecies of *S. vulgaris* are formally recognized: ssp. *vulgaris* and ssp. *denticulatus* (O. F. Muell.) P. D. Sell. *Senecio vulgaris* ssp. *vulgaris* is the common weedy taxon, whilst ssp. *denticulatus* is rare in Britain, being confined to a few coastal sites. The work described in this report concerns ssp. *vulgaris* only, and attempts to assess the effects of interaction of this taxon with the introduced species, *S. squalidus* L.

Senecio squalidus, the Oxford Ragwort, was introduced into the Oxford Botanic Garden in the seventeenth century. Its subsequent escape and spread has been well documented (Druce, 1927; Kent, 1956; Crisp, 1972). It is now common in most areas south of the central valley of Scotland. *Senecio squalidus* establishes in open habitats and waste places, often growing in mixed populations with *S. vulgaris*, though it can persist into later stages of succession due to its perennial habit.

Senecio squalidus is diploid ($2n=20$) whereas *S. vulgaris* is tetraploid ($2n=40$). The two species can hybridize and produce either almost sterile triploid hybrids (Ingram *et al.*, 1980) or fertile tetraploid hybrids (Taylor, 1984), although this latter type has been recorded only once from an artificial cross.

Two evolutionary events have occurred in Britain consequent upon the introduction of *Senecio squalidus*.

The first of these is the formation of an inland radiate groundsel, which has been designated var. *hibernicus* Syme ($2n=40$). Evidence has accumulated that var. *hibernicus* is of introgressive origin. The suggestion was initially based on historical (Druce, 1927; Crisp, 1972), morphological (Monaghan & Hull, 1976) and cytological (Ingram *et al.*, 1980) evidence. The alternative hypothesis was that a single gene mutation gave rise to this form (Stace, 1977). Very recently Abbott *et al.* (1992) have shown that an aspartate aminotransferase allele *Aat-3c*, which is present at high frequency in British *S. squalidus*, is common in var. *hibernicus* but absent from British, Irish and mainland European monomorphic populations of var. *vulgaris*. This is strong evidence for an introgressive origin of var. *hibernicus*.

The second evolutionary event is the origin of an endemic hexaploid species, *Senecio cambrensis* Rosser ($2n=60$). In contrast to the origin of var. *hibernicus* the origin of *S. cambrensis* has never been in any real doubt. Following the discovery of *S. cambrensis* in Wales, Rosser suggested that it was an allohexaploid hybrid between *S. vulgaris* and *S. squalidus* (Rosser, 1955). Artificial crosses and morphological studies have since confirmed this interpretation of the origin of *S. cambrensis* (Taylor, 1984; Harland, 1954). More

recently a population of *S. cambrensis* was discovered in Edinburgh, Scotland (Abbott *et al.*, 1983). It was suggested that this population may represent either long distance dispersal of achenes ('seeds') or a second origin of the species in Scotland (Abbott *et al.*, 1983). Ashton & Abbott (1992) have used allozyme data to show that a dual origin of the species, in Wales and Scotland, has occurred.

The past decade has seen an increasing awareness of the potential of DNA to answer biosystematic questions (Palmer, 1987; Jorgensen & Cluster, 1988). Two DNA sequences have proved to be particularly useful, the nuclear ribosomal genes (rDNA) and the chloroplast genome (cpDNA). The structure and arrangement of nuclear rDNA genes is well understood (Jorgensen & Cluster, 1988). These genes are tandemly arranged and biparentally inherited.

Ribosomal DNA has not been used extensively as a marker for introgressive hybridization. A number of workers have suggested that rDNA, particularly the intergenic spacer subrepeats may be useful markers to follow introgression of nuclear DNA (Appels *et al.*, 1986; Tremousaygue *et al.*, 1988; McIntyre *et al.*, 1988; Springer *et al.*, 1989). The introgressive origin of a taxon might be expected to have one (or more) of three effects. (i) An increase in the level of rDNA diversity in the introgressed taxon compared to the non-introgressed taxon (Schaal *et al.*, 1987). (ii) The presence in the population of at least some individuals with additive restriction profiles between the two presumptive parents (Arnold *et al.*, 1990; Cordesse *et al.*, 1990; Rieseberg *et al.*, 1990). (iii) No effect at all due to the loss of rDNA loci from the introgressing taxon, in the introgressant.

Ribosomal RNA genes have been used to infer hybridity in a number of species (e.g. Doyle & Doyle, 1988; Talbert *et al.*, 1990; Doyle *et al.*, 1985). In each case the supposed hybrid had the additive rDNA patterns of the two putative parents.

The Angiosperm chloroplast genome (cpDNA) is a small circular molecule which is usually inherited uniparentally through the maternal parent (Palmer, 1987). These features make cpDNA a potentially useful molecule for studying allopolyploid and introgressive speciation in plants. A number of studies have now used cpDNA to study introgression (Reiseberg *et al.*, 1988; Reiseberg *et al.*, 1990) and allopolyploidy (Palmer *et al.*, 1983; Soltis & Soltis, 1989; Doyle *et al.*, 1990).

The aims of the present investigation were: (i) to attempt to confirm the introgressive origin of *Senecio vulgaris* ssp. *vulgaris* var. *hibernicus* using rDNA and cpDNA markers; and (ii) to attempt to confirm the independent hybrid origins of *S. cambrensis* in Wales and Scotland using rDNA and cpDNA markers.

Whereas the second of these two aims was achieved we were unable to confirm or deny the introgressive origin of var. *hibernicus*. This result demonstrates the need to consider many different approaches in an investigation of this nature. Interesting information has been obtained regarding the effect of hybridity on rDNA markers, which is being investigated further because it has implications for the interpretation of results from this type of study.

Materials and methods

Plant material

Achenes from single individuals of *Senecio cambrensis*, *S. squalidus*, *S. vulgaris* ssp. *vulgaris* var. *hibernicus* and *S. vulgaris* ssp. *vulgaris* var. *vulgaris*, representing 116 accessions (Table 1), were grown in 10 cm pots containing Arthur Bower universal compost. The plants were illuminated for 16 h per day using 400 W halogen or mercury lamps. Leaf material was harvested when the plants were 10–12 weeks old.

DNA extraction and molecular methods

Intact total DNA was extracted from leaf tissue according to the protocol of Hattori *et al.* (1987). Subsequently the protocol of Doyle & Doyle (1987) was found to be effective when only very small quantities of plant material were available. The DNA was used for both the rDNA and cpDNA analysis. For the rDNA analysis total DNA was extracted from individual plants, whereas in the cpDNA analysis total DNA was extracted from a pooled sample of 10 plants, each having the same female parent.

One-microgram aliquots of total DNA were digested with 5–10 units of restriction enzyme, using a universal buffer [TA, 33 mM Tris-acetate (pH 7.9), 66 mM potassium acetate, 10 mM magnesium acetate, 4 mM spermidine tetrachloride, 0.5 mM dithiothreitol]. Each individual was treated with the battery of restriction enzymes as indicated in Table 1. In the cpDNA analysis a total of 11 restriction enzymes were used; one tetranucleotide cutting enzyme (*HaeIII*), 10 hexanucleotide cutting enzymes (*BamHI*, *BglII*, *EcoRI*, *EcoRV*, *HinDIII*, *KpnI*, *PstI*, *SacI*, *XhoI*) and one heptanucleotide cutting enzyme (*BstEII*). In the rDNA survey only three enzymes were used (*BamHI*, *EcoRI*, *EcoRV*).

Digested DNA samples were run on 1 per cent agarose gels at 40 mA overnight in a SEB gel buffer (0.04 M Tris-HCl, 0.02 M sodium acetate, 1 mM disodium EDTA, pH 7.85), blotted on to Amersham Hybond C-banding using 20× SSC as the transfer buffer and baked for 2 h at 80°C. DNA probes were

labelled by the random primer method (Feinberg & Vogelstein, 1983) using ^{32}P -dATP (3000 Ci/mM, 10 μCi). Hybridization took place overnight at 65°C in Buffer III [0.6 M sodium chloride, 10 mM PIPES (pH

6.8), 1 mM disodium-EDTA (pH 8.5), 10 $\times$ Denhardt's solution]. Filters were washed for the cpDNA analysis according to Palmer (1986). The wash conditions for the rDNA analysis were: a brief rinse in 2 $\times$ SSC at

Table 1 Locations and numbers of individuals sampled in populations of *Senecio cambrensis*, *S. squalidus*, *S. vulgaris* ssp. *vulgaris* var. *vulgaris* and *S. vulgaris* ssp. *vulgaris* var. *hibernicus*. Figures in parentheses indicate the number of plants sampled. Total number of plants sampled is 116

Location	Grid reference	Analysis*
<i>S. cambrensis</i>		
Brymbo, Wales (3)	SJ296539	r(A), c(A)
Leith Docks, Edinburgh (1)	NT268765	r(A), c(0)
Mochdre, Wales (3)	SH822791	r(A), c(0)
Salamander Street, Edinburgh (7)	NT276763	r(A), c(1)
<i>S. squalidus</i>		
Brymbo, Wales (1)	SJ296539	r(A), c(A)
Cardiff, Wales (3)	ST173733	r(A), c(0)
Grangemouth, Scotland (3)	NS977814	r(A), c(0)
Leith Docks, Edinburgh (11)	NT268765	r(A), c(0)
Mochdre, Wales (3)	SH822781	r(A), c(0)
Lincoln (4)	SK971710	r(A), c(0)
Sheffield (2)	SK350870	r(A), c(1)
York (7)	SE590510	r(A), c(1)
Stoke (1)	SP360780	r(0), c(1)
Salamander Street, Edinburgh (1)	NT276763	r(0), c(1)
<i>S. vulgaris</i> var. <i>vulgaris</i> (M)		
Bo'Ness, Scotland (1)	NS990810	r(A), c(0)
Cambridge (4)	TL460580	r(A), c(0)
Lincoln (1)	SK970710	r(A), c(0)
Migvie, Scotland (1)	NJ437068	r(A), c(1)
Puffin Island, Wales (2)	SH653824	r(A), c(0)
St Andrews, Scotland (8)	NO484162	r(A), c(0)
York (4)	SE605508	r(A), c(1)
<i>S. vulgaris</i> var. <i>vulgaris</i> (P)		
Brymbo, Wales (3)	SJ296539	r(A), c(3)
Lincoln (1)	SK970710	r(A), c(0)
Grangemouth, Scotland (5)	NS977814	r(A), c(0)
Leith Docks, Edinburgh, (3)	NT268765	r(A), c(0)
Mochdre, Wales (2)	SH822791	r(A), c(1)
Salamander Street, Edinburgh (4)	NT276763	r(A), c(1)
<i>S. vulgaris</i> var. <i>hibernicus</i> (P)		
Lincoln (1)	SK970710	r(A), c(0)
Grangemouth, Scotland (5)	NS977814	r(A), c(0)
Leith Docks, Edinburgh (3)	NT268765	r(A), c(0)
Mochdre, Wales (1)	SH822791	r(A), c(1)
Newcraighall, Edinburgh (6)	NT270730	r(A), c(0)
Salamander Street, Edinburgh (7)	NT276763	r(A), c(1)
York ('RJA') (11)	SE590510	r(A), c(1)
York ('Warr') (3)	SE605508	r(A), c(0)
Brymbo, Wales (1)	SJ296539	r(A), c(1)

*'r' indicates rDNA analysis and 'c' indicates cpDNA analysis. The number in parentheses indicates the number of individuals analysed, while 'A' indicates all. Herbarium vouchers of all material have been deposited at StA.

room temperature followed by a 20-min wash in $1 \times \text{SSC} + 0.1$ per cent SDS at 65°C and a subsequent 25-min wash in $0.3 \times \text{SSC} + 0.1$ per cent SDS at 65°C .

Probe characteristics

Two types of chloroplast probes were used in this study; (i) cloned *Lactuca sativa* cpDNA fragments (Jansen & Palmer, 1987) and (ii) total *L. sativa* cpDNA. Cloned cpDNA probes were used either singly (C1, C2, C4, C6, C7, C9, C15) or as a mixture (C5a–C5c; C10–C11–C12; C13–C14). These probes sampled approximately 80 per cent of the *Senecio* chloroplast genome. The remainder of the genome was sampled using total cpDNA as a probe. Total cpDNA was prepared from lettuce, according to the method of Palmer (1986). The purified cpDNA was digested with *EcoRI* prior to labelling.

A cloned nuclear ribosomal DNA repeat from *Triticum aestivum* 'Chinese Spring' was used to locate ribosomal sequences in the *Senecio* nuclear genome. The probe, pTA71, is a complete rDNA repeat of 9.1

kb cloned into an *EcoRI* site of pUC19 (Gerlach & Bedbrook, 1979).

Results

Molecular variation in *Senecio vulgaris* ssp. *vulgaris* s.l.

Intraspecific DNA variation was identified in both the nuclear (rDNA) and chloroplast genomes (Fig. 1, Tables 2–3) of *Senecio vulgaris* var. *vulgaris* and var. *hibernicus*.

Variation in the rDNA was identified with *EcoRV* (2 phenotypes; V-1 and V-4) and *BamHI* (3 phenotypes; B-1, B-2, B-3). No variation was found with *EcoRI*. Shannon–Weaver diversity estimates were calculated (Table 2) for the polymorphic enzymes and *t*-tests conducted according to Hucheson (1970). No significant differences ($P=0.05$) were found between monomorphic and polymorphic populations of *S. vulgaris* var. *vulgaris* (*EcoRV*, $t=0.43$, d.f. = 34; *BamHI*, $t=0.15$, d.f. = 33), between *S. vulgaris* var. *vulgaris* s.l.

Fig. 1 Range of rDNA phenotypes identified in various *Senecio* taxa when total DNA, digested with either (a) *BamHI*, (b) *EcoRI* or (c) *EcoRV*, is probed with pTA71.

Table 2 Frequencies of rDNA phenotypes found in various *Senecio* species and identified with (a) *EcoRI*, (b) *EcoRV* and (c) *BamHI*. Shannon–Weaver diversity estimates [Is] and variances [$V(Is)$] are given

Taxon*	Number of populations	Number of individuals	Phenotype number							
			1	2	3	4	5	6	Is	V(Is)
<i>EcoRI</i>										
<i>S. cambrensis</i>	4	13	0.39	—	—	—	0.62	—	0.96	0.22
<i>S. squalidus</i>	8	26	—	0.50	0.39	0.12	—	—	1.39	0.06
<i>S. vulgaris</i>										
var. <i>vulgaris</i> (M)	7	11	1.00	—	—	—	—	—	0.00	0.00
var. <i>vulgaris</i> (P)	6	14	1.00	—	—	—	—	—	0.00	0.00
var. <i>vulgaris</i> s.l.	13	25	1.00	—	—	—	—	—	0.00	0.00
var. <i>hibernicus</i>	9	29	1.00	—	—	—	—	—	0.00	0.00
<i>EcoRV</i>										
<i>S. cambrensis</i>	4	13	0.39	—	—	—	0.62	—	0.96	0.05
<i>S. squalidus</i>	8	22	—	0.68	0.32	—	—	—	0.90	0.03
<i>S. vulgaris</i>										
var. <i>vulgaris</i> (M)	7	19	0.90	—	—	0.11	—	—	0.49	0.06
var. <i>vulgaris</i> (P)	6	16	0.94	—	—	0.06	—	—	0.34	0.06
var. <i>vulgaris</i> s.l.	13	35	0.91	—	—	0.09	—	—	0.42	0.03
var. <i>hibernicus</i>	9	31	0.81	—	—	0.19	—	—	0.71	0.03
<i>BamHI</i>										
<i>S. cambrensis</i>	4	12	0.50	—	—	—	0.08	0.42	1.32	0.17
<i>S. squalidus</i>	8	30	—	—	—	1.00	—	—	0.00	0.00
<i>S. vulgaris</i>										
var. <i>vulgaris</i> (M)	7	16	0.81	0.13	0.06	—	—	—	0.87	0.13
var. <i>vulgaris</i> (P)	6	18	0.78	0.17	0.06	—	—	—	0.94	0.10
var. <i>vulgaris</i> s.l.	13	34	0.76	0.15	0.09	—	—	—	1.01	0.03
var. <i>hibernicus</i>	9	33	0.54	0.45	0.01	—	—	—	1.02	0.05

*M = Monomorphic populations, P = Polymorphic populations.

and var. *hibernicus* (*EcoRV*, $t = 1.18$, d.f. = 66; *BamHI*, $t = 0.02$, d.f. = 62) and between monomorphic var. *vulgaris* and var. *hibernicus* populations (*EcoRV*, $t = 0.77$, d.f. = 38; *BamHI*, $t = 0.35$, d.f. = 29).

Eight polymorphic cpDNA probe–enzyme combinations (PECs) were found in ssp. *vulgaris* s.l.. These were found in both varieties and none were found which were unique to var. *hibernicus*.

The absence of additive rDNA patterns between *Senecio vulgaris* var. *vulgaris* and *S. squalidus* in var. *hibernicus* and the lack of an increase in the rDNA diversity of var. *hibernicus* compared to var. *vulgaris* will be discussed in the light of the results obtained from *S. cambrensis*.

Molecular variation in *Senecio squalidus*

Intraspecific DNA variation was identified in both the nuclear (rDNA) and chloroplast genomes (Fig. 1, Tables 2–3) of *Senecio squalidus*. Variation in the rDNA was identified with *EcoRI* (three phenotypes;

I-2, I-3, I-4) and *EcoRV* (two phenotypes; V-2, V-3). No variation was found with *BamHI*.

Nine polymorphic cpDNA PECs were found in *Senecio squalidus*. Of these, three were found to be unique to a single line of *S. squalidus* (Sheffield).

Molecular variation in *Senecio cambrensis*

The hybrid nature of *Senecio cambrensis* from Wales (Mochdre and Brymbo) and Scotland (Leith Docks) was confirmed by the presence of additive rDNA restriction profiles for *BamHI*, *EcoRI* and *EcoRV* (B-5, B-6, I-5, V-5, Fig. 1, Table 2). The hybrid nature of *S. cambrensis* from Salamander Street (Scotland) could not be confirmed as additive *EcoRI* and *EcoRV* patterns were found for only one out of six individuals. In all cases the *S. vulgaris* s.l. phenotype was found for *EcoRI* (I-1) and *EcoRV* (V-1). All individuals from this population showed *S. vulgaris* s.l. type *BamHI* phenotypes (B-1) including the individual which was additive for *EcoRI* and *EcoRV*.

Table 3 Polymorphic cpDNA probe-enzyme combinations in *Senecio cambrensis* (c), *S. squalidus* (s), *S. vulgaris* ssp. *vulgaris* var. *vulgaris* (v), *S. vulgaris* ssp. *vulgaris* var. *hibernicus* (h)

Enzyme	Probe	State†		Taxa*			
		A	B	c	h	s	v
<i>Bam</i> HI	C6	—	5.70				*
<i>Bam</i> HI	C6	5.38	5.70	*			
<i>Bgl</i> III	C6	2.95	3.31	*			
<i>Bst</i> EII	C4	2.95	3.31	*		*	
<i>Eco</i> RI	C4	—	3.00, 2.00, 1.70			*	
<i>Eco</i> RI	C4	—	3.00, 1.70				*
<i>Eco</i> RI	C6	1.2	—	*	*		
<i>Eco</i> RI	C6	—	2.50, 2.10			*	
<i>Eco</i> RI	C6	—	1.40			*	
<i>Eco</i> RI	C7	—	1.40, 0.77			*	*
<i>Hae</i> III	C6	2.3	2.10 + (0.20)			*	
<i>Hae</i> III	C15	1.4	—	*		*	
<i>Eco</i> RV	C6	3.30	—		*	*	*
<i>Hin</i> DIII	C10-C12	11.30	6.00 + 4.80	*	*		*
<i>Sac</i> I	C6	—	4.80	*		*	*
<i>Sac</i> I	C6	3.11	3.50	*			
<i>Xho</i> I	C7	—	2.30		*		*

†Indicates fragment differences. Where 'A' and 'B' are the changes which occur within the taxa indicated. '—' fragment absent and fragments in parentheses are hypothesized fragments beyond the resolution limit of assay system.

*Distribution of polymorphic states ('A' and 'B') among *Senecio* taxa. c = *S. cambrensis*, h = *S. vulgaris* ssp. *vulgaris* var. *hibernicus* s = *S. squalidus* and v = *S. vulgaris* ssp. *vulgaris* var. *vulgaris*.

Results of experimental reciprocal crosses between *Senecio squalidus* and *S. vernalis* Waldst. & Kit. have shown that cpDNA in *Senecio* is inherited through the maternal parent (Harris, 1990). The lack of variation between the cpDNAs of *S. vulgaris* ssp. *vulgaris* s.l. and *S. squalidus* made it impossible to identify the maternal parent of the allohexaploid hybrid, with the probe-enzyme combinations used in this study. Some insight may, however, be obtained from the observation that the C10/11/12-*Hin*DIII polymorphism was found in *S. vulgaris* ssp. *vulgaris* s.l. but was absent from *S. squalidus*. The presence of this polymorphism in Scottish *S. cambrensis* suggests that, at least in this location, the maternal parent was *S. vulgaris* ssp. *vulgaris* s.l.

Although the maternal parentage of *Senecio cambrensis* could not be identified in both populations, the two populations analysed for cpDNA variation could be distinguished on the basis of the only length mutation identified in this study (Fig. 2). The length mutation, approximately 350 bp, was identified with the probe C6 and the restriction enzymes *Bam*HI, *Bgl*III

and *Sac*I. The length mutation was found in Welsh *S. cambrensis* (Brymbo) but was absent from Scottish *S. cambrensis* (Salamander Street) and all of the geographically separated populations of the two parental species which were analysed. The occurrence of this mutation is an important marker in *S. cambrensis* because it strongly suggests that the Welsh and Scottish populations had separate origins.

Differentiation of the taxa

A total of 16 phenotypes were identified with the three restriction enzymes used in the rDNA analysis (Fig. 1). *Senecio vulgaris* s.l. and *S. squalidus* could readily be differentiated on the basis of their rDNA phenotypes with each of the enzymes used (Table 2). Thus rDNA phenotypes produced by *Bam*HI, *Eco*RI and *Eco*RV are useful markers to distinguish the DNA of *S. vulgaris* s.l. Analysis of the rDNA using the single cutting enzyme *Xba*I has shown that a single rDNA repeat length of approximately 15 kb is found in *S. vulgaris* s.l., while in *S. squalidus* two repeat lengths are found, one of approximately 14.1 kb and the other of approximately 12.8 kb (Harris, 1990). In both cases no evidence of intraspecific variation in repeat length was found. Intraspecific variation was identified with *Bam*HI and *Eco*RV in *S. vulgaris* ssp. *vulgaris* s.l. and with *Eco*RI and *Eco*RV in *S. squalidus*. The intraspecific variation which we have encountered is probably a result of site mutations and, therefore, each enzyme may be treated as identifying an independent change.

In contrast to the rRNA genes it proved impossible to differentiate *Senecio vulgaris* s.l. and *S. squalidus* on the basis of their respective chloroplast genomes, even when up to 11 enzymes were used (cloned cpDNA probes, 11 enzymes; total cpDNA, nine enzymes). Intraspecific cpDNA variation was encountered (Table 4) although the range of variation found was encompassed by both taxa. At least one of these polymorphisms has previously been reported. Bleyden (1988) reports that a 10.2 kb (11.3 kb in this study) *Hin*DIII fragment is associated with triazine-susceptible *S. vulgaris* biotypes while a single site mutation producing two fragments, 5.6 kb and 4.3 kb (6.0 and 4.8 kb this study) respectively, is associated with a triazine-resistant biotype. This polymorphism was absent in all of the *S. squalidus* populations which were examined.

Discussion

The origin of var. *hibernicus*

The rDNA restriction pattern phenotypes of *Senecio vulgaris* s.l. and *S. squalidus* differ and therefore the

Fig. 2 *Bam*HI-digested total *Senecio* DNA probed with *Lactuca sativa* cpDNA probe C6. Lanes 1, 8, 9 — Welsh *S. cambrensis*; lanes 2, 3 — *S. vulgaris* ssp. *vulgaris* s.l.; lanes 4, 5 — *S. squalidus*; 6, 7 — Scottish *S. cambrensis*, 'L' — position of the 350 bp length mutation.

potential exists for tracing introgression between these two species. In the introduction it was pointed out that the effect of such introgression would depend on circumstances.

The first possibility would be an increase in rDNA diversity in var. *hibernicus* compared to var. *vulgaris*. Diversity estimates have shown that no such increase can be traced in the present case.

The second possibility would be the presence of at least some individuals of var. *hibernicus* with additive rDNA phenotypes. Again no such additive phenotypes were traced, although we have demonstrated that additive rDNA phenotypes are not always identified, even when known hybrid genomes are analysed. This point is discussed below.

The third possibility is that the rDNA from one of the parental taxa is lost in var. *hibernicus* due to random events so that introgression is again not detectable.

In the present example, isozyme data provide very good evidence of an introgressive origin of var. *hibernicus* (Abbott *et al.*, 1992). Therefore the conclusion must be that rDNA analysis is less suitable than isozyme analysis for tracing introgression in this particular case, even though the two parental taxa have easily distinguishable rDNA phenotypes.

One possible hypothesis is that the rDNA of the *Senecio squalidus* genome was lost early in introgression. The initial hybrid between *S. squalidus* and *S. vulgaris* ssp. *vulgaris* is likely to have been

triploid ($2n = 3x = 30$), with two arrays of *S. vulgaris* rRNA and one array of *S. squalidus* genes. Ingram *et al.* (1980) demonstrated that rare functional gametes from the largely sterile hybrid were more or less diploid because the backcross offspring from the triploid hybrid were approximately tetraploid. If at this stage the two *S. vulgaris* arrays were included in the functional gamete with either the exclusion of the whole *S. squalidus* chromosome or the loss of the whole *S. squalidus* rDNA array, then neither an additive pattern nor an increase in rDNA diversity would be expected in var. *hibernicus*. This scenario is best explained if var. *hibernicus* is the result of a single or few introgression events.

The origin of Senecio cambrensis

The rDNA evidence supports a hybrid origin for *Senecio cambrensis* between *S. vulgaris* ssp. *vulgaris* and *S. squalidus*; a similar conclusion has been reached from studies based on cytology (Rosser, 1955), morphology (Taylor, 1984) and allozymes (Ashton & Abbott, 1992).

This investigation of *Senecio cambrensis* has, however, generated a very interesting result for a population at Salamander Street in Edinburgh. In this population only one out of six accessions showed an additive rDNA phenotype for *Eco*RI and *Eco*RV. The other five accessions (and the *Bam*HI phenotype of the above accession) showed profiles which were

compatible with *S. vulgaris* ssp. *vulgaris* only (i.e. I-1:V-1:B-1). This result was unexpected and suggests that either the taxon described as *S. cambrensis* at this site is not a hybrid between the two putative parents or that the *S. squalidus* genome (at least the rDNA) in these plants is effectively absent. All the evidence that is available (e.g. Ashton, 1990; Taylor, 1984) supports the view that this taxon is a hybrid. Moreover, one of the plants at this site did have additive *EcoRI* and *EcoRV* phenotypes (although the *BamHI* phenotype was non-additive). Cytological evidence also supports the apparent normality of the Salamander Street population. The plants are hexaploid ($2n = 6x = 60$), as would be expected in the polyploid hybrid between *S. vulgaris* and *S. squalidus*. Favourable preparations show six NORs which would also be expected because *S. vulgaris* has four NORs and *S. squalidus* two NORs (R. Ingram, unpublished data).

A similar result to this has been reported by Zimmer *et al.* (1988) for an artificial cross between *Zea mays* and *Z. luxurians*. The F_1 progeny of this cross were screened with the restriction enzymes *EcoRI* and *SstI*. All of the plants had additive *EcoRI* phenotypes but four out of the 12 individuals tested had only the *Z. mays* *SstI* phenotype. That is, some of the F_1 hybrids apparently lacked the rRNA genes from *Z. luxurians* when tested with one enzyme (*SstI*) but not the other (*EcoRI*). No explanation for this phenomenon was suggested other than that there was a need to know more about the inheritance of rDNA. Fabijanski *et al.* (1990) report the presence of non-additive phenotypes in hexaploid *Avena* species using a random repeat sequence probe.

In *Senecio cambrensis* non-additive phenotypes have been obtained for most individuals of one population unlike the situation in *Zea*, where some additive phenotypes were found. There is, however, no evidence to suggest that if more enzymes were used, additive phenotypes would not be obtained. If the hybrid nature of *S. cambrensis* from Salamander Street is accepted, then two possible suggestions may be put forward. (i) Some *Senecio squalidus* plants at the Salamander Street site possessed *S. vulgaris* type rDNA phenotypes (either as a rare rDNA phenotype or to the exclusion of other phenotypes). Following the hybridization event, rare rDNA phenotypes were amplified in some lines but not in others. (ii) The *S. squalidus* rDNA is heavily methylated and therefore not available for digestion by methylation-sensitive enzymes (Flavell *et al.*, 1986), all of the enzymes which were used in the rDNA study were methylation sensitive.

Nothing is known regarding the rDNA phenotypes of Salamander Street *Senecio squalidus*. It is, however, difficult to believe that accessions from this site would

have radically different rDNA phenotypes from the other *S. squalidus* accessions examined. The presence of a rare rDNA with a similar phenotype to that of *S. vulgaris* may be possible, but one would have to envisage a rapid amplification and fixation of an originally rare *vulgaris*-type *S. squalidus* rDNA variant (in a period of approximately 20 years; Abbott *et al.*, 1983).

The digests of *Senecio cambrensis* from Salamander Street revealed a smear of high molecular weight DNA at the top of the autoradiograph. This probably indicates sequences that share sequence similarity with the rDNA probe but are not cut due to methylation. It is not possible to distinguish those rDNA sequences which come from *S. vulgaris* and those which come from *S. squalidus*, without a more specific probe. It would seem likely that the explanation for non-additive rDNA phenotypes in Salamander Street *S. cambrensis* is the result of variable site methylation between the two genomes. However, this does not explain why variable site methylation should occur at this location and not at others.

This study provides an additional example of the failure to detect the rDNA of a taxon, which other data suggests ought to be present.

'RJA' is a collection of var. *hibernicus* from York which has been shown isozymically and morphometrically to be more 'squalidus-like' than other var. *hibernicus* populations (Irwin, 1990). Even this population, which presumably has arisen in the recent past ('Seed' collected in 1979), shows no evidence of *S. squalidus* rDNA being present, which is in contrast to the isozyme and morphological data. If as Irwin (1990) suggests, 'RJA' is the result of fusion between an unreduced *S. squalidus* gamete and a haploid *S. vulgaris* gamete one would expect an additive rDNA restriction pattern.

The absence of any differentiation between the cpDNAs of *Senecio vulgaris* and *S. squalidus* means that the maternal parent of *S. cambrensis* could not be determined. However the presence of a unique 350 bp length mutation in the chloroplast genome of Welsh *S. cambrensis* has proved to be of considerable interest.

This length mutation present in Welsh *Senecio cambrensis* could have arisen in two ways, either it occurred during (or shortly after) the initial hybridization event or it was present in the parental material.

Crisp (1972) left the question of the date of origin of *Senecio cambrensis* open. However, Abbott *et al.* (1983) have suggested that *S. cambrensis* originated in Wales between 1910 and 1925. If the length mutation occurred during or shortly after the initial hybridization event then it must have become fixed in the population within 80 years. Fixation of cpDNA mutations is

likely to be a very rare event, as a result of the large number of cpDNA molecules per cell and the low mutation rate (Birky *et al.*, 1983; Klekowski, 1988). Whether the mutation occurred in the initial triploid hybrid between *S. squalidus* and *S. vulgaris* ssp. *vulgaris* s.l. or following chromosome doubling cannot be identified. It could perhaps, be postulated that the presence of a partially foreign nucleus in a native cytoplasm could create some instability in the native cpDNA. Frankel *et al.* (1979) have reported changes in the cpDNA during two interspecific cytoplasm introgressions in *Nicotiana*. However, Galau & Wilkins (1989) have conducted a study into the effects of cpDNA of transferring plastids from *Gossypium harknessii* into *G. hirsutum* and *G. barbadense* nuclear backgrounds. After 13 successive backcross generations there were no changes in the 136 restriction fragments examined.

Tragapogon contains two examples of recent allopolyploid speciation, *T. mirus* and *T. miscellus*. These species probably originated about 50 years ago in North America. In each case the cytoplasm of the allopolyploid species were identical to one of the parental species (Soltis & Soltis, 1989). Even over much longer time scales the cpDNA of allopolyploids appears to be very stable (*Aegilops triuncialis*; Murai and Tsunewaki, 1984). However, in the case of the amphidiploid *Brassica napus*, the cpDNA was found to be different to the putative maternal parent, *B. oleracea* (Palmer *et al.*, 1983).

Length mutations are rare events (Palmer, 1987). Due to the potential rarity of the initial mutation event and its fixation in a population in a short period of time, the most likely explanation is that one of the parental taxa carries a length mutation which has not yet been located due to the limited size of the survey. To address this fascinating problem it will be necessary to undertake more extensive sampling of *Senecio cambrensis* and the progenitor taxa in Wales.

Whatever the cause of the length mutation in the Welsh *Senecio cambrensis* it provides independent evidence for the dual origin of *S. cambrensis* in Wales and Scotland (Ashton & Abbott, 1992) because in *S. cambrensis* from Scotland this mutation is absent. If the Scottish *S. cambrensis* had been the result of long distance fruit dispersal from Wales (Abbott *et al.*, 1983) then the cpDNA ought to be identical to that of Welsh *S. cambrensis*. Of particular interest would be a survey of *S. cambrensis* from Mochdre, Wales because Ashton & Abbott (1992) have suggested that this may represent a third site of origin of *S. cambrensis*.

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