Molecular systematics of the genus *Senecio* L. II: The origin of *S. vulgaris* L.

STEPHEN A. HARRIS & RUTH INGRAM

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

The origin of *Senecio vulgaris* L. and the relationship of its two subspecies, ssp. *vulgaris* and ssp. *denticulatus* (O. F. Muell.) P. D. Sell, are examined using nuclear ribosomal and chloroplast DNA analyses. No evidence was found to support either an allopolyploid or an autopolyploid origin of *S. vulgaris*, although it would appear that *S. vernalis* Waldst. & Kit. is not one of the progenitor taxa. Two results of particular interest were found: (i) the apparent identity of the chloroplast genomes of *S. vulgaris* ssp. *vulgaris* and *S. squalidus* L. and (ii) the divergence of the chloroplast genomes of ssp. *vulgaris* and Ainsdale ssp. *denticulatus* by at least eight site mutations. These results are discussed in the light of evidence derived from morphological, cytological and allozyme studies.

Keywords: Asteraceae, molecular variation, *Senecio vulgaris* ssp. *vulgaris*, *S. vulgaris* ssp. *denticulatus*.

Introduction

Senecio vulgaris L., the common groundsel, is one of the most widespread annual/ephemeral species in Britain. Taxonomically two subspecies are recognized, which differ ecologically. *Senecio vulgaris* ssp. *vulgaris* is the common weedy groundsel associated with disturbed sites, whilst ssp. *denticulatus* (O. F. Muell.) P. D. Sell is rare in Britain, being confined to a few coastal sites, where it grows on sand dunes (Lancashire and Channel Islands). This subspecies is a winter annual. Kadereit (1984a) discusses the life-history and germination pattern of ssp. *denticulatus* and states that this subspecies becomes a montane element in the Mediterranean. Allen (1967) lists the coastal sites in Europe where the subspecies occurs. Early records of ssp. *denticulatus* (Allen, 1967; Perring & Sell, 1968; Crisp, 1972) from other coastal sites in the British Isles (Devon, Cornwall, Cheshire and the Isle of Man) have not been confirmed in recent years (Ashton, 1990). This may be due to extinction of the subspecies at these sites or the early confusion surrounding the nomenclature of the intraspecific ranks of *S. vulgaris* (Allen, 1967).

Morphologically, *Senecio vulgaris* ssp. *denticulatus* is distinguished from ssp. *vulgaris* by the possession of short ray florets, a densely arachinoid indumentum and

characteristically spatulate leaf shape (Allen, 1967; Kadereit, 1984b).

Within *Senecio vulgaris* ssp. *vulgaris*, two varieties are recognized: var. *vulgaris*, the more frequent non-radiate variety and var. *hibernicus*, an inland radiate variety which has increased in frequency in recent years. Isozyme analysis indicates that the latter variety has originated through introgression from *S. squalidus*, following the introduction of *S. squalidus* in the seventeenth century and its more recent spread and naturalization (Abbott *et al.*, 1991).

There are thus three intraspecific taxa recognized in *Senecio vulgaris*, all of which are tetraploid ($2n=4x=40$). Kadereit (1984b) suggested that *S. vulgaris* ssp. *denticulatus* is an autopolyploid of fairly recent origin with *S. vernalis* ($2n=2x=20$) as its parent. Kadereit also suggested that ssp. *denticulatus* is an ancestral form of ssp. *vulgaris* through selection for an ephemeral life-history as an agricultural weed. An alternative suggestion, based on cytological studies of chromosome pairing in species–species hybrids was put forward by Weir & Ingram (1980). They suggested an allopolyploid origin for *S. vulgaris* between *S. squalidus*, or a taxon related to it, and a second unidentified taxon. These two suggestions need not be mutually exclusive because there can be no clear division between autopolyploidy and allopolyploidy,

and the nature of the control of chromosome pairing in polyploids of this group is complex (Ingram & Noltie, 1987, 1989).

Recently polyploid speciation has been analysed using molecular techniques. Both the nuclear and chloroplast genomes have been successfully used. The nuclear genome is inherited biparentally and tandemly arranged ribosomal sequences (rDNA) have proved very useful (Jorgensen & Cluster, 1988). Ribosomal RNA genes have proved useful in confirming hybridity in *Claytonia* (Doyle & Doyle, 1988). Similar examples are found in *Tripsacum andersonii* (Talbert *et al.*, 1990) and the Saxifragaceae (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 *et al.*, 1988; Harris & Ingram, 1991b). Chloroplast DNA has been used to address questions of both allopolyploid and autopolyploid speciation, although allopolyploid speciation is the most studied of these two modes. Studies of allopolyploid speciation using cpDNA markers have been made, for example, in *Papaver* (Milo *et al.*, 1988), *Senecio cambrensis* (Harris & Ingram, 1991a), *Tragopogon* (Soltis & Soltis, 1989) and *Oryza* (Dally & Second, 1990). Autopolyploid speciation has been successfully studied in *Heuchera grossulariifolia* (Wolf *et al.*, 1990) and *H. micranthera* (Soltis *et al.*, 1989).

The aims of the present investigation were: (i) to determine the possible origin of *Senecio vulgaris* s.l. using cpDNA and rDNA markers; and (ii) to analyse the relationship between *S. vulgaris* ssp. *vulgaris* and ssp. *denticulatus* using rDNA and cpDNA markers. In pursuit of these aims the rDNA and cpDNA genomes of representatives from all subspecific taxa of *S. vulgaris*, and of *S. squalidus* and *S. vernalis* have been analysed. As a control *S. cambrensis* ($2n=6x=60$), the allohexaploid hybrid of *S. vulgaris* and *S. squalidus*, has been included and the genomes of *S. jacobaea* and *S. paludosus* have been used as outgroups.

Materials and methods

Plant material

Achenes from single individuals of *Senecio cambrensis* Rosser, *S. jacobaea* L., *S. paludosus* L., *S. squalidus* L., *S. vernalis* Waldst. & Kit., *S. vulgaris* L. ssp. *denticulatus* (O. F. Muell.) P. D. Sell, *S. vulgaris* L. ssp. *vulgaris* var. *hibernicus* Syme and *S. vulgaris* L. ssp. *vulgaris* var. *vulgaris*, representing 19 accessions (Table 1) were grown as stated in Harris & Ingram (1991a). All

of the taxa, except *S. paludosus* (Section *Doria*) have been variously placed in either Section *Jacobaea* or Section *Senecio* (Chater & Walters, 1976; Alexander, 1979).

DNA extraction and molecular methods

DNA extraction and molecular methods are given in Harris & Ingram (1991a). 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*).

Probe characteristics

Cloned *Lactuca sativa* cpDNA fragments (Jansen & Palmer, 1987) 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.

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). In addition two rDNA clones from *Taraxacum* were used (King & Schaal, 1990). pTEE3 is a 3.9 kb *EcoRI* fragment which contains the coding region of the rDNA repeat. pTEE5 is a 5.3 kb *EcoRI* fragment which contains the majority of the intergenic spacer and part of the 18S rDNA gene.

Data analysis

Sequence divergence estimates for cpDNA were obtained by a maximum likelihood method (Nei, 1987; eq. 5.50 and 5.51) using only the mutations which could positively be inferred as site mutations. Other mutations were ignored in these estimates, which therefore underestimates the degree of divergence. Phylogenetic analysis was conducted only on site mutations using the branch and bound Wagner parsimony program PENNY in the package PHYLIP (Felsenstein, 1985). The branch and bound option is guaranteed to find all the most parsimonious trees. Restriction site mutations were polarized using the outgroup *S. paludosus* (Section *Doria*).

The data from the rDNA analysis were treated rather differently due to the large degree of intertaxon variation and the difficulty of assigning length or site

Table 1 Locations of the *Senecio* taxa studied

Taxon	Location	Grid reference	Number of individuals	Source	Code
Section <i>Senecio</i>					
<i>S. vulgaris</i> ssp. <i>vulgaris</i> var. <i>vulgaris</i>	Migvie, Aberdeenshire (M)	NJ437068	1	RJA	V1
	York (P)	SE590510	1	PA	V2
	Mochdre, Wales (P)	SH822781	1	PA	V3
	Salamander Street, Edinburgh (P)	NT276763	1	PA	V4
<i>S. vulgaris</i> ssp. <i>vulgaris</i> var. <i>hibernicus</i>	Mochdre, Wales	SH822781	1	PA	H1
	Brymbo, Wales	SJ296539	1	PA	H2
	Salamander Street, Edinburgh	NT276763	1	PA	H3
	York	SE590510	1	RJA	H4
<i>S. vulgaris</i> ssp. <i>denticulatus</i>	Ainsdale, Lancashire	SD295124	1	PA	D1
<i>S. squalidus</i>	Salamander Street, Edinburgh	NT276763	1	PA	S1
	Brymbo, Wales	SJ296539	1	PA	S2
	Stoke	SP360780	1	PA	S3
	York	SE590510	1	PA	S4
	Sheffield	SK350870	1	PA	S5
<i>S. cambrensis</i>	Salamander Street, Edinburgh	NT276763	1	SAH	C1
	Brymbo, Wales	SJ296539	1	PA	C2
<i>S. vernalis</i>	Schlusserlacker Weide, Eppelheim near Heildelberg, Germany	—	1	PA	Ve1
<i>S. jacobaea</i>	Tentsmuir Forest, Fife	NO499241	1	SAH	J1
Section <i>Doria</i>					
<i>S. paludosus</i>	British material [SMW 232-72W]	—	1	CaBG	P1

Monomorphic (M) and polymorphic (P) *S. vulgaris* ssp. *vulgaris* populations.

CaBG — Cambridge University Botanic Gardens, PA — Paul Ashton, RJA — Richard Abbott, SAH — Stephen Harris.

mutations to the differences. Shared fragment patterns were scored and a measure of phenotype similarity calculated (Nei, 1987; eq. 5.53) before the values were clustered using an Unweighted Pair Mean Group Analysis (UPGMA; Sneath & Sokal, 1973).

Results

Ribosomal DNA variation

Twenty-two rDNA phenotypes were found using three enzymes (*Bam*HI, *Eco*RI, *Eco*RV) within and between *Senecio vulgaris* ssp. *vulgaris* s.l., *S. vulgaris* ssp. *denticulatus*, *S. squalidus*, *S. cambrensis*, and *S. vernalis*. The rDNA variation encountered within *Senecio vulgaris* ssp. *vulgaris* s.l., *S. squalidus* and *S. cambrensis* has been described in Harris & Ingram (1991a).

Analysis of the patterns on the basis of shared fragments, followed by clustering using UPGMA (Fig. 1) clearly shows that all of the taxa could be distinguished with two or more of the enzymes used. The nature of

this variation, either as the result of length or site mutations, could not be determined. However, it is clear that the rDNA phenotypes found in *S. vulgaris* ssp. *denticulatus* are quite different from any of the intraspecific variants which were encountered in *S. vulgaris* ssp. *vulgaris* s.l. (Fig. 2).

Chloroplast DNA variation

An average of 387 restriction fragments were produced by the 11 enzymes used in this study, which sampled approximately 2211 bp or 1.5 per cent of the *Senecio* chloroplast genome (assuming that the cpDNA is 150 kb in length; Jansen & Palmer, 1987). Sixty-four site mutations and one length mutation were inferred from the data (Table 2). In addition a further 35 mutations were found which could not be explained as either length or site mutations (Table 2), these mutations were excluded from further data analysis. Two additional site mutations were also excluded from the dataset for further analysis because they were either

Fig. 1 UPGMA phenogram of the rDNA phenotypes identified in the genus *Senecio*. (a) *Bam*HI, (b) *Eco*RI and (c) *Eco*RV.

correlated with herbicide resistance in *Senecio* (mutation No. 58; Bleyden, 1988) or unique to one *S. squalidus* accession (mutation No. 49).

Sequence divergence estimates calculated from the site mutation data (Table 3, Fig. 3) show that between-species divergence estimates range from 0.000 per cent (between *S. squalidus* and *S. vulgaris* ssp. *vulgaris*) to 1.352 per cent (between *S. vulgaris* ssp. *vulgaris* and *S. paludosus*). These are minimum estimates because several mutations were excluded. In addition intra-specific variation was encountered between the accessions of *S. squalidus*, *S. vulgaris* ssp. *vulgaris* s.l. and *S. cambrensis*. The only length mutation was encountered in a Welsh population of *S. cambrensis* (Harris & Ingram, 1991a).

Fig. 2 Autoradiogram of *Senecio vulgaris* ssp. *vulgaris* (lanes 1, 3, 5) and *S. vulgaris* ssp. *denticulatus* (lanes 2, 4, 6) probed with *Taraxacum* ribosomal DNA probe pTEE5. A. *Bam*HI, B. *Eco*RI and C. *Eco*RV.

Thirty-four of the 62 restriction sites used in the phylogenetic analysis were autapomorphic and 28 were synapomorphic, with respect to the *S. paludosus* outgroup. Wagner parsimony analysis resulted in three most parsimonious trees (each of 62 steps) to explain the data (Fig. 4). The difference between the trees was due to the non-resolution of the trichotomy between the 'composite' taxon (*S. vulgaris* ssp. *vulgaris*, *S. cambrensis* and *S. squalidus*), *S. vulgaris* ssp. *denticulatus* and *S. vernalis*. This trichotomy is supported by 15 synapomorphies.

Table 2 Restriction fragment changes observed between taxa of *Senecio*. Restriction site mutations, length mutations and unidentified mutations. The outgroup, *S. paludosus* has characters states in column 0. Figures in parentheses indicate fragments which were not detected, but hypothesized to be present. Taxa coding as Table 1

Enzyme	Probe	Character states		Taxa
		0	1	
Restriction site mutations				
1. <i>Bam</i> HI	C2	2.4 + 0.7	3.0	All except J1
2. <i>Bam</i> HI	C4	24.8	19.1 + 4.3	All except J1
3. <i>Bam</i> HI	C6	6.3	5.4 + (0.7)	All
4. <i>Bam</i> HI	C6	5.4 + (0.6)	6.0	Ve1
5. <i>Bam</i> HI	C6	5.4	5.2 + (0.2)	D1
6. <i>Bam</i> HI	C6	0.95	0.91 + (0.04)	All
7. <i>Bam</i> HI	C6	0.91	0.88 + (0.03)	J1
8. <i>Bam</i> HI	C9	2.4	2.3 + (0.1)	All
9. <i>Bam</i> HI	C9	7.4	4.8 + 2.3	J1
10. <i>Bam</i> HI	C10–C12	19.3	11.8 + 8.5	All
11. <i>Bgl</i> III	C2	5.4	3.8 + 1.2	All except J1
12. <i>Bgl</i> III	C4	7.6 + (0.4)	8.0	All except J1
13. <i>Bgl</i> III	C5ac	8.4	8.2 + (0.2)	All except J1
14. <i>Bgl</i> III	C5ac	7.4 + (0.6)	8.0	All except J1
15. <i>Bgl</i> III	C6	1.2 + (0.1)	1.3	J1
16. <i>Bgl</i> III	C6	3.0	2.9 + (0.1)	J1
17. <i>Bgl</i> III	C6	3.0	2.8 + (0.2)	D1
18. <i>Bgl</i> III	C6	3.1 + (0.4)	3.5	Ve1
19. <i>Bgl</i> III	C6	3.0 + (0.3)	3.3	Ve1
20. <i>Bgl</i> III	C6	3.3	3.0 + (0.3)	All
21. <i>Bgl</i> III	C7	1.6 + (0.1)	1.7	D1
22. <i>Bgl</i> III	C7	3.0 + (0.4)	3.4	Ve1
23. <i>Bgl</i> III	C7	2.8	1.6 + 1.3	All except J1
24. <i>Bgl</i> III	C10–C12	2.1	1.8 + (0.3)	J1
25. <i>Bst</i> EII	C1	1.6	1.1 + (0.5)	J1
26. <i>Bst</i> EII	C4	16.2 + (1.3)	17.5	All
27. <i>Eco</i> RI	C4	2.1 + (0.1)	2.2	J1
28. <i>Eco</i> RI	C6	2.0 + 1.7	3.7	All except J1
29. <i>Eco</i> RI	C7	0.35 + (0.06)	0.41	J1
30. <i>Eco</i> RI	C9	5.7	5.4 + (0.3)	All
31. <i>Eco</i> RI	C10–12	3.0 + (0.1)	3.1	D1
32. <i>Eco</i> RI	C10–12	4.2	3.9 + (0.3)	J1
33. <i>Eco</i> RI	C10–12	2.0	1.9 + (0.1)	J1
34. <i>Eco</i> RI	C15	0.4	0.37 + (0.03)	J1
35. <i>Eco</i> RI	C15	2.6 + (0.5)	3.2	J1
36. <i>Eco</i> RI	C15	2.0	1.4 + (0.6)	J1
37. <i>Eco</i> RV	C2	3.8 + (0.3)	4.1	J1
38. <i>Eco</i> RV	C5ac	19.0 + (3.4)	22.4	All
39. <i>Eco</i> RV	C6	1.6	1.4 + (0.2)	J1
40. <i>Eco</i> RV	C6	7.8 + (0.9)	8.7	Ve1
41. <i>Eco</i> RV	C7	4.6 + (0.1)	4.7	D1
42. <i>Eco</i> RV	C7	1.5	1.4 + (0.1)	J1
43. <i>Eco</i> RV	C10–C12	9.3 + (1.0)	10.3	All
44. <i>Hae</i> III	C1	10.9 + (0.4)	11.3	All
45. <i>Hae</i> III	C5ac	2.9 + (0.1)	3.0	All
46. <i>Hae</i> III	C5ac	2.4	2.1 + (0.3)	All except J1
47. <i>Hae</i> III	C6	2.6	2.4 + (0.2)	D1
48. <i>Hae</i> III	C6	2.6	2.1 + (0.5)	J1

Table 2 continued

Enzyme	Probe	Character states		Taxa
		0	1	
49. <i>HaeIII</i>	C6	2.3	2.1 + (0.2)	S5
50. <i>HaeIII</i>	C7	0.8	0.6 + (0.2)	J1
51. <i>HaeIII</i>	C9	1.4 + (0.1)	1.5	All except J1
52. <i>HaeIII</i>	C9	0.6 + (0.2)	0.8	All except J1
53. <i>HaeIII</i>	C13–C14	2.0 + 1.1	3.1	All except J1
54. <i>HaeIII</i>	C13–C14	2.7	1.6 + 1.1	All except J1
55. <i>HaeIII</i>	C15	1.6 + (0.1)	1.7	All except J1
56. <i>HaeIII</i>	C15	0.9	0.86 + (0.04)	All except J1
57. <i>HinDIII</i>	C9	3.2 + (0.3)	3.5	J1
58. <i>HinDIII</i>	C10–C12	11.3	6.0 + 4.8	All except C1, H2, V4
59. <i>KpnI</i>	C9	5.5 + (0.2)	5.7	D1
60. <i>PstI</i>	C1	2.9	2.8 + (0.1)	All
61. <i>SacI</i>	C9	4.0	3.6 + (0.4)	All
62. <i>SacI</i>	C9	3.6 + (1.0)	4.6	J1
63. <i>XhoI</i>	C7	3.2 + (0.2)	3.4	D1
64. <i>XhoI</i>	C7	3.2 + (0.1)	3.3	J1
Length mutation				
65. <i>BamHI</i>	C6	5.38	5.70	C2
66. <i>BglIII</i>	C6	2.95	3.31	C2
67. <i>SacI</i>	C6	3.11	3.50	C2
Unidentified mutations				
68. <i>BamHI</i>	C5ac	25.3	33.6	J1
69. <i>BamHI</i>	C6	—	5.7	V1
70. <i>BglIII</i>	C7	3.0	—	J1, Ve1
71. <i>BstEII</i>	C4	—	3.6	All except C1, S5
72. <i>BstEII</i>	C4	5.5	—	All
73. <i>EcoRI</i>	C4	—	3.0, 2.0, 1.7	S5
74. <i>EcoRI</i>	C4	3.0, 1.7	—	All except V2
75. <i>EcoRI</i>	C6	1.2	—	C1, H2, J1, Ve1
76. <i>EcoRI</i>	C6	—	2.5, 2.1	S5
77. <i>EcoRI</i>	C6	2.5, 2.1	2.0, 1.7	All
78. <i>EcoRI</i>	C6	2.0, 1.7	2.5	J1
79. <i>EcoRI</i>	C7	—	1.4, 0.77	S3
80. <i>EcoRI</i>	C7	—	1.4	H1
81. <i>EcoRV</i>	C6	3.3	—	D1, H1, H2 H4, S2, S3, V4
82. <i>HaeIII</i>	C4	3.2, 1.7, 1.3	3.0, 1.4	All
83. <i>HaeIII</i>	C4	3.0, 1.4	3.4, 3.1	J1
84. <i>HaeIII</i>	C5ac	—	2.6	J1
85. <i>HaeIII</i>	C6	2.1, 1.5	2.3	All
86. <i>HaeIII</i>	C6	1.3	—	J1
87. <i>HaeIII</i>	C10–C12	2.7	—	D1, Ve1
88. <i>HaeIII</i>	C15	—	1.4	All except C1, J1, S5
89. <i>HinDIII</i>	C5ac	6.8, 3.3	10.3, 4.8, 3.7	All
90. <i>HinDIII</i>	C5ac	—	6.7	J1
91. <i>HinDIII</i>	C4	3.3	—	All
92. <i>HinDIII</i>	C6	18.3, 16.1, 8.1, 7.4	17.1, 7.6	All

Table 2 continued

Enzyme	Probe	Character states		Taxa
		0	1	
93. <i>Hin</i> DIII	C9	9.2, 8.5, 3.3	3.2	All
94. <i>Hin</i> DIII	C10–C12	11.9, 9.2, 5.4, 3.2	11.3, 7.1	All
95. <i>Sac</i> I	C4	1.4	—	All except D1, Ve1
96. <i>Sac</i> I	C6	4.8	—	All except C1, J1, S1 S5, V1
97. <i>Sac</i> I	C6	—	2.6	S5
98. <i>Sac</i> I	C6	5.5, 3.3, 1.9, 1.8	1.5, 3.1	All
99. <i>Sac</i> I	C6	3.1, 1.4	3.1, 2.6	J1
100. <i>Sac</i> I	C10–C12	8.7	6.9	J1
101. <i>Xho</i> I	C7	5.6	—	All
102. <i>Xho</i> I	C7	—	2.3	All except H3, V2

Table 3 Chloroplast DNA sequence divergence estimates (above diagonal) and standard errors (below diagonal) for various species of *Senecio*. The chloroplast DNAs of *S. vulgaris* ssp. *vulgaris* and *S. squalidus* are apparently identical and, therefore have a sequence divergence estimate of 0.000

	<i>vulgaris</i>	<i>paludosus</i>	<i>jacobaea</i>	<i>vernalis</i>	<i>denticulatus</i>
<i>vulgaris</i>	—	0.00820	0.0107	0.0023	0.0021
<i>paludosus</i>	0.0032	—	0.0103	0.0114	0.0135
<i>jacobaea</i>	0.0016	0.0008	—	0.0105	0.0115
<i>vernalis</i>	0.0007	0.0017	0.0016	—	0.0038
<i>denticulatus</i>	0.0007	0.0019	0.0014	0.0009	—

This tree highlights two observations which are of particular interest: (i) the apparent identity of ssp. *vulgaris* and *S. squalidus* cpDNAs and (ii) the separation of the cpDNAs of ssp. *vulgaris* and Ainsdale ssp. *denticulatus* by at least eight site mutations.

Discussion

Two major observations may be made from the nuclear rDNA and cpDNA data in this study: (i) the rDNAs of *Senecio squalidus* and *S. vulgaris* ssp. *vulgaris* s.l. are clearly very different, but their cpDNAs are apparently inseparable, and (ii) *S. vulgaris* ssp. *vulgaris* and Ainsdale ssp. *denticulatus* have very divergent cpDNAs and rDNAs.

The range of minimum cpDNA sequence divergence estimates within the genus *Senecio*, 0.000–1.352 per cent (mean 0.842) are towards the upper end of the

range found in other studies: e.g. *Clarkia* Section *Peripetasma*, 0.17–1.50 per cent (Sytsma & Gottlieb, 1986); *Oncidium*, 0.14–2.77 per cent (Chase & Palmer, 1989); *Pisum*, 0.10–0.81 per cent (Palmer *et al.*, 1985). These represent minimum estimates for the genus because not all of the mutations scored in this study were used and secondly, the sample represents a very limited selection from this large and very diverse genus (Jeffrey *et al.*, 1977; Jeffrey, 1978).

The origin of Senecio vulgaris s.l.

Kadereit (1984b) proposed that *Senecio vulgaris* ssp. *vulgaris* was the autopolyploid derivative of *S. vernalis* via *S. vulgaris* ssp. *denticulatus*. If these events were of recent occurrence then one would expect that the nuclear and organelle genomes of all three taxa would be very similar. All of the enzymes studied, except

Fig. 3 UPGMA phenogram of chloroplast DNA divergence estimates (100 p) based on those changes which could be ascribed to site mutations.

EcoRI, allowed the nuclear rDNA of all three taxa to be distinguished. In the case of *EcoRI*, this enzyme does not distinguish *S. vernalis* and *S. vulgaris* ssp. *vulgaris* rDNA. Similarly the cpDNA data allows each of the taxa to be distinguished, although the absence of synapomorphies between any two of the three taxa did not allow resolution of the three taxon trichotomy. Clearly the species are closely related compared to either *S. jacobaea* or *S. paludosus* in the cpDNA analysis and *S. squalidus* in the rDNA analysis.

Weir & Ingram (1980) have proposed an allopolyploid origin of *S. vulgaris*, with half the tetraploid genome homeologous to *S. squalidus*. In this case one would expect *S. vulgaris* ssp. *vulgaris* s.l. to carry the cpDNA of the maternal parent and have an additive rDNA phenotype between the *S. squalidus* and an unknown *Senecio* species. Comparison of the rDNA phenotypes reveal no *S. squalidus* fragments in *S. vulgaris* ssp. *vulgaris*, other than the fragments common to all taxa, while the chloroplast DNA analysis reveals that *S. vulgaris* ssp. *vulgaris* and *S. squalidus* have identical cpDNA profiles. One interpretation of this data is that *S. squalidus* is the maternal parent of *S. vulgaris* ssp. *vulgaris*, but this is unlikely given the evidence from the nuclear genome which does not indicate additive rDNA profiles.

This analysis of the nuclear rDNA and cpDNA does not provide unequivocal support for either an autopolyploid or an allopolyploid origin of *Senecio vulgaris* ssp. *vulgaris*. However, the distinct nuclear and organelle genomes of *S. vernalis* do not support the suggestion that this taxon is a recent ancestor of *S. vulgaris* ssp. *vulgaris*. Thus we suggest that the progenitor(s) of *S. vulgaris* must be searched for among other species, particularly those which occur in the Mediterranean region (Kadereit, 1984b).

Fig. 4 Wagner parsimony tree of chloroplast DNA site mutations. The terminal branch referred to as 'composite' is *Senecio vulgaris* ssp. *vulgaris*, *S. squalidus* and *S. cambrensis*.

The status of *Senecio vulgaris* ssp. *denticulatus*

This study raises some questions about the status of Ainsdale *Senecio vulgaris* ssp. *denticulatus* as a subspecies of *S. vulgaris*. Both the nuclear and the cpDNA data indicate that ssp. *vulgaris* and ssp. *denticulatus* are different. The rDNA differences cannot be explained on the basis of simple length mutations in the intergenic spacer, rather, multiple mutations must be responsible for the pattern. Similarly the cpDNAs of ssp. *vulgaris* and Ainsdale ssp. *denticulatus* differ by at least eight site mutations. We would like to propose three possible explanations for the observed data: (i) localized hybridization and gene exchange has occurred, or is occurring, at the Ainsdale site between *S. vulgaris* s.l. and an unidentified *Senecio* species; (ii) multiple reciprocal allopolyploid origins of *S. vulgaris* may have occurred in the past leading to two lineages with differing cpDNAs; and (iii) the subspecific taxonomy of *S. vulgaris* is incorrect.

Taking each of the proposals separately. Localized gene exchange between ssp. *denticulatus* and a related species of *Senecio* may explain the result but the lack of ssp. *vulgaris* rDNA fragments in Ainsdale ssp. *denticulatus* would tend to indicate that this is not the case,

especially given that the rDNA fragment pattern changes between *ssp. vulgaris* and *ssp. denticulatus* are not of a simple nature. Support for the difference between *ssp. Ainsdale denticulatus* and *ssp. denticulatus* from other sites was found using β -Est loci (Ashton, 1990). In Ashton's study *ssp. denticulatus* from Jersey had an identical phenotype to *ssp. vulgaris*, whereas Ainsdale *ssp. denticulatus* possessed unique alleles at the β -EST-1 and β -EST-2 loci.

Multiple reciprocal allopolyploid origins of *S. vulgaris* between two diploid progenitors with different cpDNAs is possible but would require that the products of the two different crosses gave rise to two lineages (Murai & Tsunewaki, 1984); either *ssp. vulgaris* or *ssp. denticulatus*. The difficulty with this hypothesis is that one would expect similar rDNA phenotypes.

Incorrect classification of *ssp. denticulatus* confounded by morphological convergence may explain the data. However, a change in status of *ssp. denticulatus* should not be made until more accessions from the entire range of *ssp. denticulatus* have been studied as the result found here may be peculiar to Ainsdale *ssp. denticulatus*.

An unexpected result, given the degree of cpDNA divergence between apparently conspecific taxa, was the similarity between the chloroplast genomes of *S. squalidus* and *S. vulgaris ssp. vulgaris*, although they could be distinguished on their rDNA phenotypes. This may imply a hybridization event following the introduction of *S. squalidus* to the British Isles, but further work is in progress on this problem.

These results reported in this paper add more data to the observations that molecular evidence may not always be concordant with evidence from other data sources. The results of such studies are of interest as they may provide evidence of interesting evolutionary pathways which are not observed when more traditional datasets are used. Some of the possible explanations for the lack of concordance between molecular data and other data sources have been reviewed by Sytsma (1990). Clearly one such result has been obtained in *S. vulgaris s.l.*, but further evidence is required before more definite conclusions about the causes of the differences can be made.

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References

- ALEXANDER, J. C. M. 1979. The Mediterranean species of *Senecio* Sections *Senecio* and *Delphinifolius*. *Notes. Roy. Bot. Gard. Edinb.* **37**, 387–428.
- ALLEN, D. E. 1967. The taxonomy and nomenclature of the radiate variants of *Senecio vulgaris* L. *Watsonia*, **6**, 280–282.
- ABBOTT, R. J., ASHTON, P. A. AND FORBES, D. G. 1992. Introgressive origin of the radiate groundsel, *Senecio vulgaris* var. *hibernicus* Syme: Aat-3 evidence. *Heredity*, **68**, 425–435.
- ASHTON, P. A. 1990. *Multiple origins of Senecio cambrensis Rosser, and related evolutionary studies in British Senecio*. PhD Thesis, University of St Andrews.
- CHASE, M. W. AND PALMER, J. D. 1989. Chloroplast DNA sequences of Lilioid monocots: Resources, feasibility and an example from the Orchidaceae. *Am. J. Bot.* **76**, 1720–1730.
- CHATER, A. O. AND WALTERS, S. M. 1976. *Senecio* L. In: (eds Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. and Webb, D. A. (eds) *Flora Europaea* Vol. 4. Cambridge University Press: Cambridge. pp. 191–205.
- CRISP, P. 1972. *Cytotaxonomic studies in the section Annui of Senecio*. PhD Thesis, University of London.
- DALLY, A. M. AND SECOND, G. 1990. Chloroplast DNA diversity in wild and cultivated species of rice (Genus *Oryza*, Section *Oryza*). Cladistic mutation and genetic distance analysis. *Theor. Appl. Genet.*, **80**, 209–222.
- DOYLE, J. J. AND DOYLE, J. L. 1988. Natural interspecific hybridization in Eastern North American *Claytonia*. *Am. J. Bot.*, **75**, 1238–1246.
- DOYLE, J. J., SOLTIS, D. E. AND SOLTIS, P. S. 1985. An intergeneric hybrid in the Saxifragaceae: evidence from ribosomal RNA genes. *Am. J. Bot.*, **72**, 1388–1391.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.
- GERLACH, W. L. AND BEDBROOK, J. R. 1979. Cloning and characterisation of ribosomal RNA genes from wheat and barley. *Nucl. Acids. Res.*, **7**, 1869–1885.
- HARRIS, S. A. AND INGRAM, R. 1991a. Molecular systematics of the genus *Senecio* L. I: Hybridization in a British polyploid complex. *Heredity*, **69**, 1–10.
- HARRIS, S. A. AND INGRAM, R. 1991b. Chloroplast DNA and bio-systematics: The effects of intraspecific diversity and plastid transmission. *Taxon*, **40**, 393–412.
- INGRAM, R. AND NOLTIE, H. J. 1987. The control of chiasma frequency within a polyploid series of the genus *Senecio* (Compositae). *Genetica*, **72**, 37–41.
- INGRAM, R. AND NOLTIE, H. J. 1989. Early adjustment of patterns of metaphase association in the evolution of a polyploid species. *Genetica*, **78**, 21–25.

- JANSEN, R. K. AND PALMER, J. D. 1987. Chloroplast DNA from lettuce and *Barnadesia* (Asteraceae): structure, gene localisation and characterisation of a large inversion. *Curr. Genet.*, **11**, 553–564.
- JEFFREY, C. 1978. Generic and Sectional limits in *Senecio* (Compositae). II. Evaluation of some recent studies. *Kew. Bull.*, **34**, 49–58.
- JEFFREY, C., HALLIDAY, P., WILMOT-DEAR, M. AND JANES, S. W. 1977. Generic and Sectional limits in *Senecio* (Compositae). I. Progress report. *Kew. Bull.*, **32**, 47–67.
- JORGENSEN, R. A. AND CLUSTER, P. A. 1988. Modes and tempos in the evolution of nuclear ribosomal DNA: new characters for evolutionary studies and new markers for genetic and population studies. *Ann. Miss. Bot. Gard.*, **75**, 1238–1247.
- KADEREIT, J. W. 1984a. Studies on the biology of *Senecio vulgaris* L. ssp. *denticulatus* (O. F. Muell.) P. D. Sell. *New Phytol.*, **97**, 681–689.
- KADEREIT, J. W. 1984b. The origin of *Senecio vulgaris* (Asteraceae). *Pl. Syst. Evol.*, **145**, 135–153.
- KING, L. M. AND SCHAAL, B. A. 1990. Genotypic variation within asexual lineages of *Taraxacum officinale*. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 998–1002.
- MILO, J., LEVY, A., LADIZINSKY, G. AND PALEVITCH, D. 1988. Phylogenetics and genetic studies in *Papaver* Section *Oxytona*: cytogenetics, isozyme analysis and chloroplast DNA variation. *Theor. Appl. Genet.*, **75**, 795–802.
- MURAI, K. AND TSUNEWAKI, K. 1984. Intraspecific variation of chloroplast DNAs (ctDNAs) in *Aegilops triuncialis* and geographical distribution of cytoplasmic types. *Jpn. J. Breed. (Suppl.)*, **34**, 280–281.
- NEI, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- PALMER, J. D., JANSEN, R. K., MICHAELS, H. J., CHASE, M. W. AND MANHART, J. R. 1988. Chloroplast DNA variation and plant phylogeny. *Ann. Miss. Bot. Gard.*, **75**, 1180–1218.
- PALMER, J. D., JORGENSEN, R. A. AND THOMPSON, W. F. 1985. Chloroplast DNA variation and evolution in *Pisum*: Patterns of change and phylogenetic analysis. *Genetics*, **109**, 195–213.
- PERRING, F. H. AND SELL, P. D. 1968. *Critical Supplement to the Atlas of the British Flora*. Nelson and Sons, London.
- SNEATH, P. H. A. AND SOKAL, R. R. 1973. *Numerical Taxonomy*. Freeman, San Francisco.
- SOLTIS, D. E. AND SOLTIS, P. S. 1989. Allopolyploid speciation in *Tragopogon*: insights from chloroplast DNA. *Am. J. Bot.*, **76**, 1119–1124.
- SOLTIS, D. E., SOLTIS, P. S. AND NESS, B. D. 1989. Chloroplast DNA variation and multiple origins of autopolyploidy in *Heuchera micranthera*. *Evol.*, **43**, 650–656.
- SYTSMA, K. J. 1990. DNA and morphology: Inference of plant phylogeny. *Trend. Ecol. Evol.*, **5**, 104–110.
- SYTSMA, K. J. AND GOTTLIEB, L. D. 1986. Chloroplast DNA evolution and phylogenetic relationships in *Clarkia* Section *Peripetasma* (Onagraceae). *Evolution*, **40**, 1248–1261.
- TALBERT, L. E., DOEBLEY, J. F., LARSON, S. AND CHANDLER, V. L. 1990. *Tripsacum andersonii* is a natural hybrid involving *Zea* and *Tripsacum*: Molecular evidence. *Am. J. Bot.*, **77**, 722–726.
- WEIR, J. AND INGRAM, R. 1980. Ray morphology and cytological investigations of *Senecio cambrensis* Rosser. *New. Phytol.*, **86**, 237–241.
- WOLF, P. G., SOLTIS, D. E. AND SOLTIS, P. S. 1990. Chloroplast DNA and allozymic variation in diploid and autotetraploid *Heuchera grossulariifolia* (Saxifragaceae). *Am. J. Bot.*, **77**, 232–244.