Hybrid origin of the Oxford Ragwort, *Senecio squalidus* L.

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ABSTRACT

A survey of morphometric and allozyme variation was conducted to test the hypothesis that Oxford ragwort, *Senecio squalidus* L. (Asteraceae) is a diploid hybrid species which originated in cultivation from hybrid material between *S. aethnensis* Jan. ex DC. and *S. chrysanthemifolius* Poiret that was introduced to the Oxford Botanic Garden from Mt Etna, Sicily, in the 17th century. Morphometric analysis of material raised under the same glasshouse conditions showed that *S. squalidus* closely resembled material derived from hybrid swarms between *S. aethnensis* and *S. chrysanthemifolius* on Mt Etna, and produced a phenotype that was normally intermediate in leaf shape and capitulum size to that of its two putative parent species. Allozyme analysis further showed that populations of *S. squalidus* were very similar genetically to Sicilian material with some populations clustering tightly with hybrid material and with *S. chrysanthemifolius*. Most notably, *S. squalidus* and hybrid material were polymorphic and exhibited almost identical frequencies for two alleles (*a* and *b*) that distinguish pure populations of the two parent species at the *Acp-2* locus. Thus, in combination with previous documentary evidence, the morphometric and allozyme results obtained from the present study provide strong support for the hypothesis that *S. squalidus* is a new diploid hybrid species which originated allopatrically through stabilization of hybrid material of *S. aethnensis* and *S. chrysanthemifolius* introduced to the British Isles from the hybrid zone on Mt Etna, Sicily. Because allozyme diversity within *S. squalidus* was found to be much reduced to that present within Sicilian material, it is likely that only a small sample of hybrid material (and allelic diversity) was introduced to the British Isles from Sicily.

KEYWORDS: hybrid speciation, morphometrics, allozymes, introduced plants, Asteraceae.

INTRODUCTION

In a series of articles published between 1955 and 1966, Douglas Kent chronicled the introduction, escape from cultivation, and subsequent spread of the Oxford ragwort, *Senecio squalidus* L. (Asteraceae), in the British Isles (Kent, 1955, 1956, 1957, 1960, 1963, 1964a,b,c, 1966). The information contained in these reports is of considerable value for two reasons. First, there are very few examples of an introduced invasive species for which such details of introduction and spread have been rigorously documented. Second, a detailed knowledge of the spread of the Oxford ragwort in the British Isles has been of crucial importance to the timing of two notable evolutionary events that followed hybridization of the species with the native Groundsel, *Senecio vulgaris* L., viz. the origin of the new allopolyploid species, *S. cambrensis* Rosser, independently in Wales and Scotland, during the 20th century (Ashton & Abbott 1992a; Lowe & Abbott 1996), and the origin of the radiate form of *S. vulgaris* (var. *hibernicus* Syme), a stabilised introgressant, during the 19th century (Abbott 1992; Abbott *et al.* 1992a). Although Kent (1956) provided information on the source of *S. squalidus* material introduced to the British Isles, no details were available on how this material might have become modified during cultivation before it escaped and became invasive. In this paper, we provide evidence that the material originally introduced to the British Isles was hybrid in nature and that *S. squalidus*, as recognised in these islands, arose as a derivative of this material most probably during the period of cultivation.

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According to Kent (1956) and Druce (1927), *S. squalidus* (2n = 20) was collected from Mt Etna, Sicily, and introduced to the Oxford Botanic Garden, England, soon after the Garden opened in 1621. The exact date of its introduction is unknown as early records from the Garden were lost in a fire. Present records show that it has been in cultivation at Oxford since at least 1690 (Kent 1956). Linnaeus described the species in 1753, from plants raised by him possibly from seed sent from Oxford (Walker 1833), although evidence for the latter is disputed (Kent 1956). In 1792, the species was reported growing on walls in the Oxford area (Druce 1927) and was described as a garden escape. The subsequent spread of the species away from Oxford was aided initially by exchange of seeds between botanic gardens but, in the late 19th century, following the establishment of the railway system in the British Isles, *S. squalidus* began spreading rapidly from Oxford via the railway network. More recently, the species has spread along motorway verges in Britain, and is now ubiquitous on wasteground especially in urban areas. Records show that the species began to spread northwards from Oxford in the late 19th century and reached different parts of the north of England between the early to mid-part of the 20th century (Kent 1964b,c). The species became established in the wild in the Central Belt of Scotland in the mid-1950s (Kent 1955, 1966), and continues to spread northwards into Fife and north of the River Tay at the present time (Abbott 1992, and pers. obs.).

The source and nature of *S. squalidus* material now established in the British Isles has been questioned in recent times from two standpoints. First, Walters (1963) emphasized the morphological similarity between *S. squalidus* in the British Isles and *S. rupestris* Waldst. and Kit. (2n = 20), a closely related species that occurs on mountains in central and southern Europe. This raised the possibility that *S. rupestris* was an alternative source of some, if not all, material of *S. squalidus* established in Britain. Because of this morphological similarity, Alexander (1979) in a taxonomic revision of Mediterranean *Senecio*, section *Senecio*, subsumed *S. rupestris* under *S. squalidus*, although he considered British plants to be atypical of the taxon. Second, Crisp (1972), in a biosystematic analysis of *Senecio* material growing on Mt Etna, was unable to recognise the taxon *S. squalidus* found in Britain, and argued instead that British *S. squalidus* is a product of hybridization between the diploid Sicilian species *S. aethnensis* Jan. ex DC. (2n = 20) and *S. chrysanthemifolius* Poiret (2n = 20), and that there is no evidence of material from elsewhere in Europe having contributed to the British taxon. According to Crisp (1972), both Sicilian species occur on Mt Etna with *S. chrysanthemifolius* present up to approximately 1000m altitude and *S. aethnensis* from approximately 1600 to 2600 m altitude. Between these two species at 1300 m +/- 300 m, a series of hybrid swarms are present within which are found certain plants that bear a close morphological resemblance to British *S. squalidus*. Crisp (1972) advanced the view that material introduced to the British Isles most likely was collected from these hybrid populations, and following a century of cultivation in the Oxford Botanic Garden, stabilized derivatives of this hybrid material escaped at the end of the 18th Century and have since spread rapidly to many parts of the British Isles. He concluded that "British *S. squalidus* is of hybrid origin..." and "...can be treated as a separate species as it is both geographically isolated from the parent species, and it has evolved .... over many generations to a stage where it is morphologically distinct from either of them, although still polymorphic and in general intermediate between them." For the purposes of this paper, and in keeping with the views of Crisp (1972), we treat as separate species *S. squalidus* found in the British Isles and *S. rupestris* from central and southern Europe .

A survey of chloroplast (cp) DNA variation in *S. squalidus, S. aethnensis, S. chrysanthemifolius* and *S. rupestris* (Abbott *et al.* 1995) showed that all material of *S. squalidus* examined from the British Isles (21 accessions from 20 populations) possessed the same cpDNA haplotype (haplotype B) as found in material of *S. chrysanthemifolius* surveyed (11 accessions from seven populations in Sicily). Haplotype B also occurred in three of five individuals of *S. aethnensis* examined, and five of seven individuals surveyed from the hybrid swarms on Mt Etna. Other individuals from these populations possessed haplotype A, which differed by a single restriction site mutation from haplotype B. In contrast, only one accession surveyed of *S. rupestris* (from Abruzzi, central Italy) possessed haplotype B, while other accessions of this species contained either haplotype A (from the Italian Alps, Romania, N Bulgaria, and introductions to Germany), or haplotype C (from S Bulgaria and Greece). Haplotype C differed from haplotype A by a length mutation and three restriction site mutations. The occurrence of haplotype B in all samples of *S. squalidus* from the British Isles and in the two Sicilian species, *S. aethnensis* and *S. chrysanthemifolius*, and in their

hybrid swarms, supported the hypothesis that *S. squalidus* is derived from hybrid material introduced from Sicily. However, because haplotype B is also present in *S. rupestris*, albeit at low frequency, it can not be ruled out that the British population of *S. squalidus* might be derived either in part or entirely from introductions of *S. rupestris*.

Here we report the results of a comparison of morphometric and allozyme variation within material of *S. squalidus* from the British Isles with that within *Senecio* material sampled from Mt Etna, Sicily, namely *S. aethnensis, S. chrysanthemifolius*, and hybrid populations between these two species. These results provide further support for Crisp's (1972) hypothesis of a hybrid origin of *S. squalidus* in the British Isles.

MATERIALS AND METHODS

PLANT MATERIAL

Seed was collected separately from approximately 30 plants from each of the populations listed in Table 1 and Fig. 1. This entailed sampling three populations of each of *S. aethnensis, S. chrysanthemifolius*, and three of their hybrid swarms occurring on Mt Etna, Sicily, plus nine populations of *S. squalidus* from the British Isles, and a single population of *S. vernalis* Waldst. and Kit. from Germany. This material was analysed for allozyme variation, while subsamples were used in a survey of morphometric variation.

MORPHOMETRIC ANALYSIS

Five plants (one offspring per mother plant) of each of *S. aethnensis, S. chrysanthemifolius*, and also of hybrid material from Mt Etna (two plants from population 7, and three plants from population 8), were raised with five plants from each of two populations of *S. squalidus* (from Oxford and Edinburgh) (Table 1). The 25 plants were grown from seed to maturity as single individuals in pots of 13 cm diameter containing compost. Pots were fully randomized within a greenhouse. Illumination was provided by natural daylight supplemented by 400-W mercury vapour lights to give a photoperiod of 16 h, while temperature was maintained at 20 °C +/- 2 °C . On the day of full anthesis of the apical capitulum, each plant was harvested and measured for 32 characters. The character set recorded (Table 2) was modified from that used by Irwin and Abbott (1992). Fifteen of the characters were descriptors of the capitulum and another 15 described leaf size and shape. The remaining two characters were plant height and inflorescence length. Details of measurement are given in James (1995). Data were subjected to univariate one-way ANOVA and Duncan's multiple range tests using the software package SPSS Base (version 7.5, SPSS Inc.

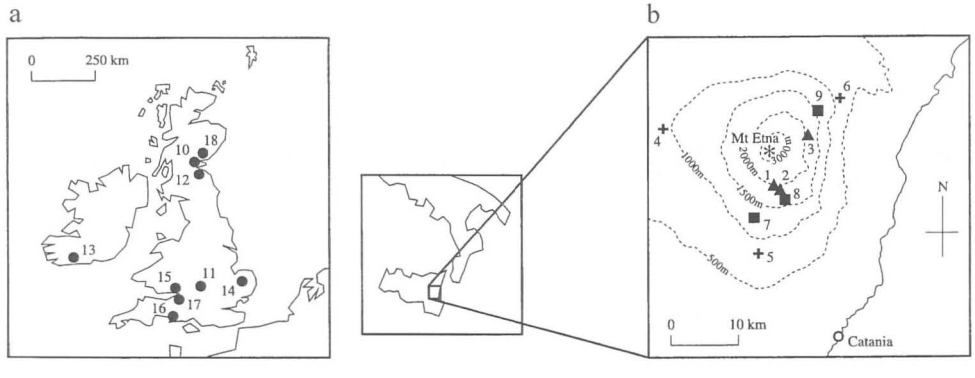

FIGURE 1. (a) Locations of populations of *Senecio squalidus* (●) sampled in the British Isles; (b) Locations of populations of *S. aethnensis* (▲), *S. chrysanthemifolius* (✚), and *S. aethnensis* × *S. chrysanthemifolius* (■) sampled from Mt Etna, Sicily. Key to population numbers is given in Table 1.

TABLE 1. GEOGRAPHIC LOCATIONS, SITE DESCRIPTIONS, COORDINATES, AND ALTITUDE OF SAMPLED POPULATIONS OF *SENECIO*

Species	No.	Locality	Lat.	Long.	Alt. (m)	Collector (date)
S. aethnensis	1	Italy, Sicily, Mt Etna, 2 km E Rifugio Sapienza, lava by roadside	37·42 N	15·02 E	1890	RJA (13.06.88)
	2	Italy, Sicily, Mt Etna, 3 km E Rif. Sapienza, volcanic ash	37·41 N	15·03 E	1650	RJA (13.06.88)
	3*	Italy, Sicily, Mt Etna, Rif. Citelli, volcanic ash	37·47 N	15·04 E	1750	RJA (16.06.88)
S. chrysanthemifolius	4*	Italy, Sicily, Mt Etna, Bronte, waste ground	37·45 N	14·50 E	760	RJA (11.06.88)
	5	Italy, Sicily, Mt Etna, 5 km W of Nicolosi, roadside	37·35 N	14·59 E	800	RJA (12.06.88)
	6	Italy, Sicily, Mt Etna, 4 km SE of Linguaglossa, roadside	37·50 N	15·07 E	750	RJA (16.06.88)
S. aet. × *S. chrys.*	7*	Italy, Sicily, Mt Etna, 4 km NNE of Ragalna Est, new lava	37·37 N	14·59 E	1195	RJA (12.06.88)
	8*	Italy, Sicily, Mt Etna, Paso Cannelli, N of Pedara, lava	37·40 N	15·04 E	1500	RJA (13.06.88)
	9	Italy, Sicily, Mt Etna, Villagio Turistico Mareneve, roadside	37·49 N	15·04 E	1400	RJA (16.06.88)
S. squalidus	10	British Isles, Scotland, Kirkaldy, car park	56·08 N	03·10 W	<50	RJA (10.09.91)
	11*	British Isles, England, Oxford, waste ground, Osney street	51·45 N	01·09 W	<50	RJA (22.05.91)
	12*	British Isles, Scotland, Edinburgh, Leith, Mill Lane, waste ground	55·58 N	03·10 W	<50	RJA & JAI (01.06.90)
	13	British Isles, Ireland, Co. Cork, S.of Kilbrittain	51·37 N	08·35 W	<50	RJA (03.10.91)
	14	British Isles, England, Norwich, waste ground at railway station	52·40 N	01·20 E	<50	PAA (08.07.90)
	15	British Isles, England, Bristol, wasteground, Coronation Rd	51·30 N	02·37 W	<50	JAI (06.09.90)
	16	British Isles, England, Weymouth, waste ground	50·37 N	02·29 W	<50	JAI (03.09.90)
	17	British Isles, Wales, Cardiff, waste ground, Colington Rd	51·30 N	03·10 W	<50	JAI (07.09.90)
	18	British Isles, Scotland, Kirriemuir, waste ground	56·43 N	03·00 W	<150	JAI (07.07.91)
S. vernalis	19	Germany, Schüsseläcker Weide, Eppelheim (nr. Heidelberg)	49·24 N	08·38 E	c.50	JWK (21.05.88)

*Populations included in morphometric analysis.
Collectors: RJA (R. J. Abbott), PAA (P. A. Ashton), JAI (J. A. Irwin), JWK (J. W. Kadereit).

TABLE 2. CHARACTER MEANS OF *SENECIO* POPULATIONS

	Populations						
	S. aet.	*S. chr.*	*S. a* × *S. c*		*S. squalidus*		*P*
	3	4	7	8	11	12	
Height mm	606^{ab}	703^{b}	585^{ab}	695^{b}	593^{ab}	483^{a}	**
Inflorescence length mm	28·8^{bc}	21·7^{ab}	21·6^{a}	27·8^{abc}	31·3^{c}	24·4^{abc}	*
Capitulum length mm	11·5^{b}	9·9^{a}	10·4^{ab}	10·5^{ab}	10·8^{ab}	10·7^{ab}	*
Pedicel length mm	17·3^{bc}	11·8^{ab}	11·1^{a}	17·3^{bc}	20·5^{c}	13·8^{abc}	*
No. of pedicel bracts	3·0^{b}	3·4^{b}	2·0^{a}	5·3^{b}	3·2^{b}	2·4^{ab}	***
Capitulum apical width mm	5·6^{b}	4·2^{a}	4·9^{b}	5·5^{b}	5·7^{b}	4·9^{b}	*
Capitulum basal width mm	5·8	4·7	5·8	5·3	5·9	5·7	ns
No. of phyllary bracts	21·8^{b}	21·2^{a}	21·0^{a}	21·0^{a}	22·8^{b}	21·6^{b}	**
No. of calyculus bracts	7·6^{bc}	5·0^{a}	9·0^{c}	6·7^{ab}	8·2^{bc}	9·0^{c}	**
Calyculus bract length mm	3·1^{ab}	2·3^{a}	2·9^{a}	3·5^{b}	3·0^{ab}	2·8^{a}	*
No. of florets per capitulum	116^{ab}	97^{a}	106^{ab}	119^{bc}	136^{c}	106^{ab}	**
Ray floret length mm	13·2^{c}	9·5^{a}	9·4^{a}	10·3^{ab}	13·0^{c}	12·5^{abc}	**
Ray floret width mm	3·7^{b}	2·6^{a}	3·1^{b}	3·6^{b}	3·7^{b}	3·6^{b}	***
No. of disc florets	105^{bc}	84^{a}	93^{ab}	106^{bc}	123^{c}	93^{ab}	***
Disc floret length mm	8·0^{b}	6·8^{a}	6·9^{a}	7·3^{ab}	7·2^{ab}	7·0^{a}	*
Disc floret corolla length mm	2·8^{b}	2·4^{a}	2·5^{a}	2·8^{b}	2·5^{ab}	2·6^{a}	***
Disc floret corolla width mm	0·99	0·9	0·89	0·94	0·92	0·87	ns
Longest leaf length mm	133^{a}	128^{a}	138^{a}	144^{ab}	177^{b}	152^{ab}	*
Midleaf length mm	107	108	116	124	125	126	ns
Midleaf lobe number	3·4^{a}	12·4^{b}	13·0^{b}	11·0^{b}	11·6^{b}	12·4^{b}	***
Midleaf maximum left width mm	18·7^{a}	42·1^{b}	42·7^{b}	37·5^{b}	38·7^{b}	41·7^{b}	***
Midleaf maximum right width mm	19·4^{a}	46·0^{b}	48·0^{b}	37·7^{b}	45·1^{b}	42·9^{b}	***
Midleaf basal length (left) mm	61·7	51·9	46·5	72·0	64·9	72·6	ns
Midleaf basal length (right) mm	65·9^{b}	44·8^{a}	82·0^{b}	64·0^{b}	71·9^{b}	71·6^{b}	*
Midleaf apical angle A† (deg)	109·0^{c}	62·7^{a}	69·0^{a}	98·3^{bc}	97·4^{bc}	83·6^{ab}	***
Midleaf apical angle B†† (deg)	41·4^{a}	58·6^{ab}	74·5^{abc}	96·0^{bc}	104·2^{c}	95·0^{bc}	**
Midleaf secondary vein angle‡ (deg)	20·4^{a}	66·6^{d}	61·0^{cd}	44·0^{bc}	40·2^{b}	53·8^{bcd}	***
Square root of midleaf area mm	52	44	49	54	56	51	ns
Midleaf perimeter mm	328^{a}	1062^{bc}	1240^{c}	797^{b}	1186^{c}	1240^{c}	***
Square root midleaf area/length mm	0·49	0·40	0·42	0·44	0·44	0·40	ns
Midleaf perimeter/length mm	3·1^{a}	9·8^{c}	10·7^{c}	6·4^{b}	9·4^{c}	9·9^{c}	***
Midleaf dissection‡‡	6·2^{a}	24·1^{c}	25·4^{c}	14·6^{b}	21·1^{c}	24·8^{c}	***

P = level of significant difference: * < 0·05; ** <0·01; *** <0·001.
Means followed by the same superscript letter are not significantly different at *P* = 0·05.
† angle between the apex of the primary vein and the apices of the adjacent marginal tooth sinuses.
†† angle between the apex of the primary vein and the apices of the adjacent secondary veins.
‡ angle between the midlobe secondary vein and the primary vein.
‡‡ perimeter of the midleaf divided by the square root of its area.

1997) to detect differences between population means. Variables that were not normally distributed were transformed into natural logarithms before analysis. Data were then standardized by subtracting the character mean of the total sample from each individual value and then dividing by the character standard deviation before a principal component analysis (PCA) was conducted using NTSYSpc software (version 2.0, Rohlf 1998) on the derived trait correlation matrix.

ALLOZYME ANALYSIS

Allozyme variation was assayed for six enzymes by means of starch gel electrophoresis procedures described previously (Ashton 1990; Abbott *et al.* 1992a; Glover & Abbott 1995). The enzymes assayed were acid phosphatase (*Acp*), aspartate aminotransferase (*Aat*), β–esterase (*β-Est*), glyceraldehyde-3-phosphate dehydrogenase (*G3pd*), phosphoglucoisomerase (*Pgi*), and phosphoglucomutase (*Pgm*). Buffer systems and staining procedures for these enzymes were as described by Comes & Abbott (1998).

The genetics of electrophoretic variants of *Aat, β–Est*, and *Pgi* were deduced from previous studies of their inheritance in *S. squalidus* and *S. vulgaris* L. (Abbott *et al.* 1992a,b; Ashton & Abbott 1992b). The genetic interpretation of the other enzymes (*Acp, G3pd*, and *Pgm*) were based on the generally conserved enzyme sub-structure, subcellular location, and isozyme number in higher plants (Weeden & Wendel 1989). Three to four standards were run within each gel. Isozymes and allozymes were designated numerically and alphabetically, respectively, according to decreasing mobility rate.

Allozyme variation was estimated by computing the mean number of alleles per locus (*A*) (including monomorphic loci), mean percentage of polymorphic loci (*P*), and mean unbiased expected heterozygosities (H_e) using the BIOSYS-1 software (vers. 1.7; Swofford and Selander 1989). Hardy-Weinberg equilibrium was tested across loci in all populations using the GENEPOP software (vers. 3; Raymond & Rousset 1995) probability test option (Fisher's method). Estimates of genetic distance between populations were calculated (after Nei 1972) and subjected to an unweighted pair group analysis (UPGMA) using BIOSYS-1.

F-statistics (Wright 1951) were estimated as outlined by Weir (1990) using the computer program FSTAT (vers 1.2, Goudet 1995). Mean values of *F*, θ and *f* [Weir & Cockerham's (1984) estimators of Wright's (1978) parameters of total inbreeding (*Fit*), subdivision among populations (*Fst*), and inbreeding within populations (*Fis*)] were computed separately for *S. squalidus*, and their standard deviations were calculated by jackknifing over loci. An association between genetic and geographical distance between populations of *S. squalidus* was examined in terms of the correlation between θ and distance (km). The significance of the product-moment correlation was evaluated by Mantel's test (Mantel 1967) with 10,000 random permutations using GENEPOP.

RESULTS

MORPHOMETRIC ANALYSIS

Univariate analysis of variance detected significant differences between population means for all but six of the 32 traits examined (Table 2). Duncan's multiple range tests indicated that *S. aethnensis* and *S. chrysanthemifolius* differed for 17 measured traits. *S. aethnensis* produced large capitula and entire leaves, whereas *S. chrysanthemifolius* bore relatively small capitula and highly dissected leaves. Comparisons between *S. aethnensis* and *S. squalidus* showed that the main differences were in leaf shape. *Senecio squalidus* plants produced more dissected leaves than *S. aethnensis*, but tended to have capitula of similar size. Differences between the two *S. squalidus* populations and *S. chrysanthemifolius* were less straightforward. Whereas *S. squalidus* from Oxford (population 11) produced larger capitula and leaves of a different shape to those of *S. chrysanthemifolius*, *S. squalidus* plants from Edinburgh (population 12) were more similar in morphology to *S. chrysanthemifolius*. Interestingly, the means of the two *S. squalidus* samples did not differ significantly from the means of one or other of the two hybrid samples examined (populations 7 and 8) for nearly all traits examined, indicating a close resembance between *S. squalidus* and hybrid material. However, the two hybrid samples did differ in mean for several traits and it was evident that whereas the sample from population 7 tended towards *S. chrysanthemifolius* in morphology, the other hybrid sample (population 8) was more similar in morphology to *S. aethnensis*.

The first three components derived from principal component analysis (PCA) extracted 33·61, 19·93 and 7·45% of the total variance, respectively. A plot of principal component 1 (PC1) against component 2 (PC2) separated plants in two-dimensions (Fig. 2). The two Sicilian taxa were widely separated along the first axis with *S. chrysanthemifolius* plants exhibiting high scores and *S. aethnensis* individuals having low scores. The majority of *S. squalidus* and hybrid plants (*S. aethnensis* × *S. chrysanthemifolius)* exhibited intermediate scores along this axis, although one *S. squalidus* plant (from Edinburgh) and a hybrid plant (from population 7) clustered with *S. chrysanthemifolius* individuals. Examination of character coefficients (loadings) showed that PC1 was largely influenced by capitulum size and leaf width and leaf shape characters (total-sample standardized coefficients >0·5; Table 3). Thus, *S. chrysanthemifolius* possessed small capitula and highly dissected leaves in contrast to *S. aethnensis* which produced large capitula and almost entire leaves (Fig. 2, Table 2). *Senecio squalidus* plants with capitula of intermediate size and less

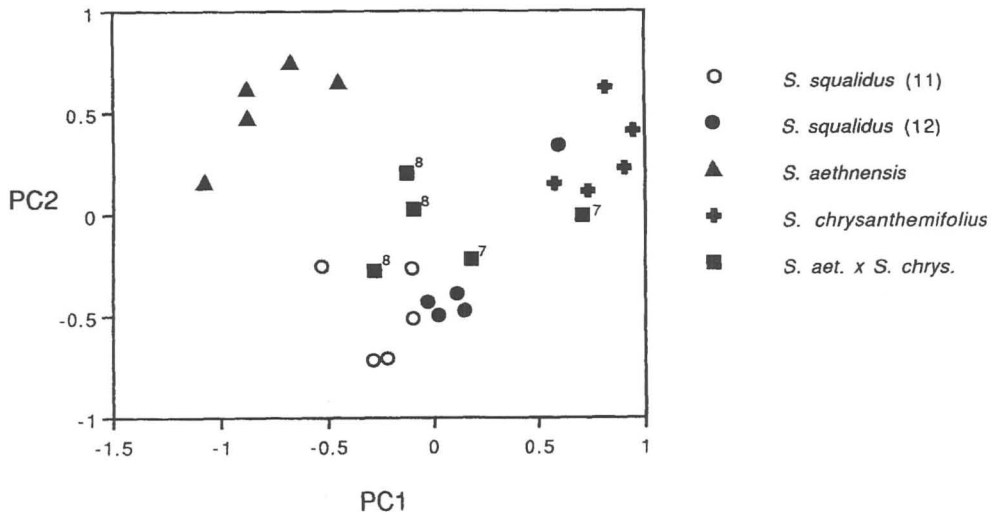

FIGURE 2. Plots for individuals of *S. aethnensis, S. chrysanthemifolius, S. aethnensis* × *S. chrysanthemifolius* (populations 7 and 8), and *S. squalidus* (populations 11 and 12) on the first two principal components axes.

dissected leaves than *S. chrysanthemifolius* were also clearly separated from both Sicilian taxa along PC2, but remained clustered with certain hybrid plants. PC2 was largely influenced by leaf size and shape characters (Table 3).

ALLOZYME ANALYSIS

Three of the eight loci investigated (*G3pd-1*, *Pgi-1* and *Pgm-1*) were monomorphic in all 19 populations surveyed. The total number of alleles detected over the five polymorphic loci was 14 (range 2–3 per locus) (Appendix 1). Of particular interest was the pattern of variation at the *Acp-2* locus. All three populations of *S. chrysanthemifolius* were monomorphic for the *Acp-2b* allele (Appendix 1). In contrast, all of the *S. aethnensis* and hybrid (*S. aethnensis* × *S. chrysanthemifolius*) populations investigated were polymorphic for the *Acp-2a* and *Acp-2b* alleles as were five of the nine populations of *S. squalidus*. The remaining four populations of *S. squalidus* were monomorphic for the *Acp-2b* allele (Appendix 1). When data were pooled over populations (Table 4) it was evident that the frequencies of the two *Acp-2* alleles in *S. squalidus* were very similar to those in the pooled hybrid material (*S. aethnensis* × *S. chrysanthemifolius*), with the *Acp-2b* allele occurring at a higher frequency (approximately 0·8) than in *S. aethnensis* material (approximatey 0·35).

At other polymorphic loci there were no major differences in allele frequencies between the two Sicilian taxa, nor between them and their hybrid populations (Table 4). However, there was a contrast between Sicilian material and *S. squalidus* in that the latter was monomorphic at the *β–Est-3* locus, and contained the *Aat-3c* allele at a much higher frequency.

UPGMA analysis of Nei's genetic distances between populations based on allele frequencies at all eight loci separated populations into two main clusters with *S. vernalis* positioned as a basal group (Fig. 3). One cluster comprised seven populations of *S. squalidus,* while the other contained the remaining populations. Within this second cluster, two subgroups were evident, one of which contained three populations of *S. aethnensis* and one of the hybrid populations (population 9), while the other consisted of the three populations of *S. chrysanthemifolius*, two hybrid populations, and two *S. squalidus* populations.

Levels of diversity varied across populations (Tables 5). Lower levels of diversity were present in *S. squalidus* populations relative to populations of other taxa examined, based on measures of *A* (mean number of alleles per locus), *P* (percentage of polymorphic loci) and H_e (unbiased expected

TABLE 3. TOTAL - SAMPLE STANDARDIZED COEFFICIENTS (LOADINGS) AND EIGENVALUES FOR THE FIRST THREE PRINCIPAL COMPONENTS OF INDIVIDUAL VALUES OF QUANTITATIVE TRAITS

	Principal Components		
	1	2	3
Height	0·131	0·241	0·172
Inflorescence length	- 0·630	- 0·233	0·423
Capitulum length	- 0·793	- 0·008	0·226
Pedicel length	- 0·579	- 0·241	0·416
No. of pedicel bracts	- 0·784	- 0·357	- 0·064
Capitulum apical width	- 0·612	- 0·370	0·415
Capitulum basal width	- 0·286	- 0·277	0·432
No. of phyllary bracts	- 0·337	- 0·320	0·267
No. of calyculus bracts	- 0·590	- 0·163	- 0·070
Calyculus bract length	- 0·031	0·170	0·331
No. of florets per capitulum	- 0·606	- 0·468	0·349
Ray floret length	- 0·636	- 0·338	- 0·376
Ray floret width	- 0·675	- 0·403	- 0·375
No. of disc florets	- 0·629	- 0·448	0·344
Disc floret length	- 0·693	0·290	0·207
Disc floret corolla length	- 0·649	0·272	- 0·184
Disc floret corolla width	- 0·317	0·202	- 0·410
Longest leaf length	- 0·296	- 0·743	- 0·057
Midleaf length	- 0·143	- 0·788	- 0·141
Midleaf lobe number	0·709	- 0·607	0·020
Midleaf maximum left width	0·663	- 0·643	0·018
Midleaf maximum right width	0·683	- 0·610	0·115
Midleaf basal length (left side)	- 0·366	- 0·389	- 0·169
Midleaf basal length (right side)	- 0·457	- 0·414	- 0·124
Midleaf apical angle A	- 0·750	- 0·022	0·103
Midleaf apical angle B	0·020	- 0·778	0·104
Midleaf secondary vein angle	0·863	- 0·056	0·322
Square root of midleaf area	- 0·494	- 0·575	- 0·398
Midleaf perimeter	0·582	- 0·759	- 0·226
Square root midleaf area/length	- 0·557	0·075	- 0·397
Midleaf perimeter/length	0·727	- 0·573	- 0·177
Midleaf dissection	0·796	- 0·559	0·029
Eigenvalue	10·757	6·379	2·386
Variance (%)	33·616	19·935	7·455
Cumulative variance (%)	33·616	53·551	61·006

heterozygosity). More diversity was present in *S. aethnensis* than in *S. chrysanthemifolius* and their hybrid populations. Only in very few populations was the coefficient of inbreeding (*f*) high and deviations from Hardy-Weinberg equilibrium significant. Thus in most populations random mating was indicated.

The mean level of subdivision between *S. squalidus* populations ($\theta = F_{st}$) over all loci (Table 6) was significantly different from zero (as determined by bootstrap estimates of 95% CIs), indicating significant population differentiation within this species in the British Isles. However, examination of the UPGMA tree (Fig. 3) did not indicate any obvious geographical pattern to this structure, and Mantel's test of the correlation of θ and geographical distance between populations was not significant ($r = 0{\cdot}106$, $P = 0{\cdot}280$).

Although the mean coefficient of inbreeding within *S. squalidus* populations ($f = Fis$) was not significant, a high f value was calculated at the *Acp-2* locus (Table 6). This might stem from the

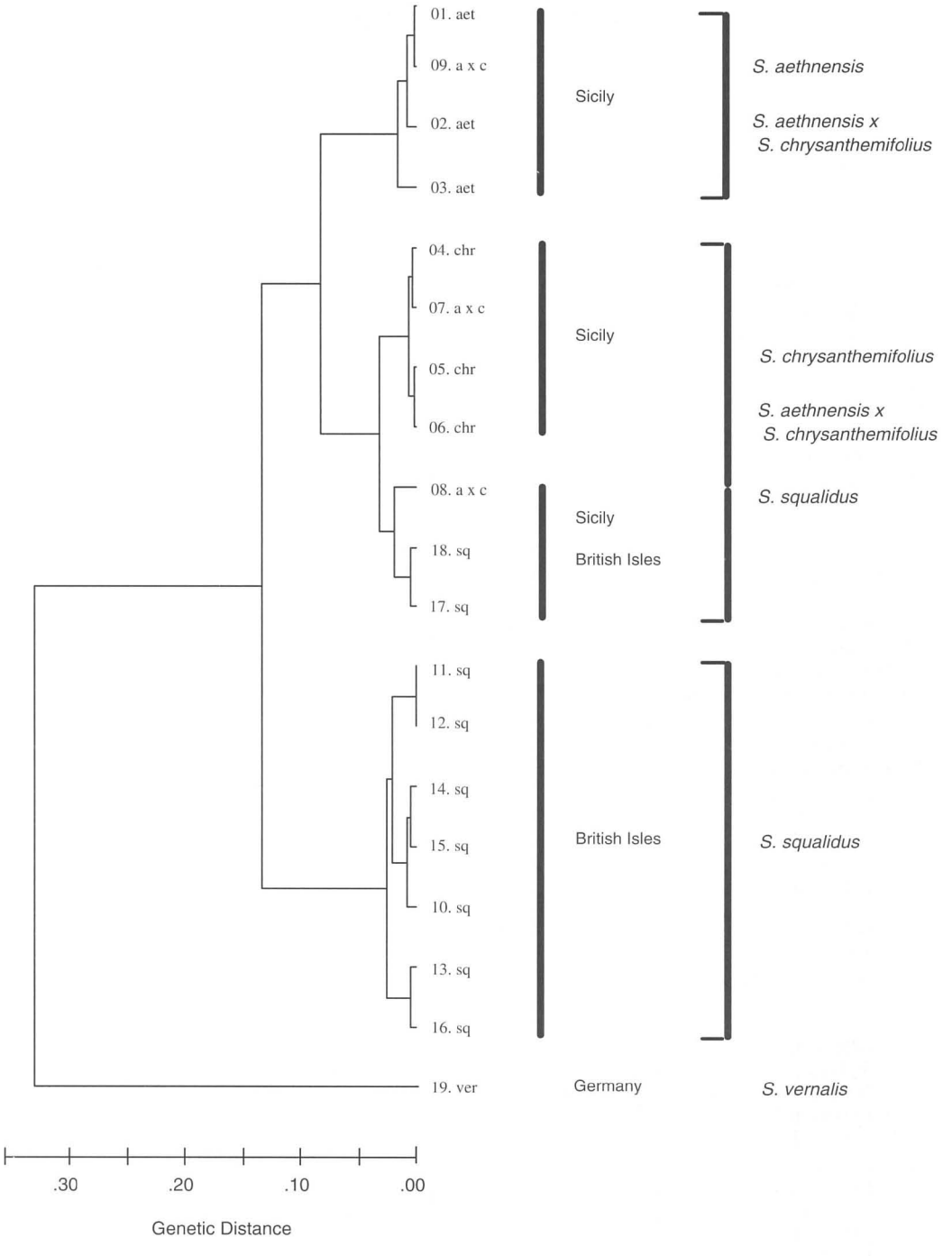

FIGURE 3. UPGMA tree constructed from genetic distances (after Nei, 1972) between *Senecio* populations. Key to population numbers is given in Table 1.

TABLE 4. ALLELE FREQUENCIES AT POLYMORPHIC LOCI IN *SENECIO* TAXA AFTER POOLING DATA OVER POPULATIONS. (N = SAMPLE SIZE)

Locus/allele		Taxon *S. aethnensis*	*S. chrysanth.*	*S. aet.* × *S. chrys.*	*S. squalidus*
Acp-2	N	(62)	(95)	(76)	(209)
a		0·645	----	0·204	0·170
b		0·355	1·000	0·796	0·830
Aat-3	N	(62)	(94)	(76)	(214)
b		0·911	0·925	0·960	0·327
c		0·089	0·075	0·040	0·673
β–Est-3	N	(52)	(87)	(82)	(215)
a		0·673	0·632	0·591	-----
b		0·327	0·368	0·409	1·000
Pgi-2	N	(61)	(88)	(84)	(211)
a		0·107	-----	-----	-----
b		0·893	1·000	1·000	1·000
Pgm-2	N	(59)	(85)	(81)	(237)
a		-----	0·023	-----	-----
b		0·881	0·906	0·920	0·996
c		0·119	0·071	0·080	0·004

occurrence of substructure within populations. However, previous studies (Abbott, unpublished) have detected a null allele segregating at low frequency at the *Acp-2* locus in some *S. squalidus* populations. In the present study, null homozygotes were not recorded; however, it can not be ruled out that a null allele was present at low frequency in some of these populations and affected the correct identification of genotypes. The presence of a null allele for this dimeric enzyme would lead to inflation of the number of homozygotes recorded due to masking of the null allele in heterozygotes and, in turn, would result in inflation of the coefficient of inbreeding (*f*).

DISCUSSION

HYBRID ORIGIN OF *SENECIO SQUALIDUS*

The hypothesis that *Senecio squalidus* originated from hybrid material between *S. aethnensis* and *S. chrysanthemifolius* introduced from Mt Etna, Sicily, to the British Isles in the 17th century (Crisp 1972) is supported by the results of the surveys of morphometric and allozyme variation reported here. Morphometric analysis showed that nine of ten *S. squalidus* plants raised under glass bore a close morphological resemblance to hybrid plants derived from material sampled from the hybrid zone on Mt Etna. Both types of plants produced phenotypes intermediate between *S. aethnensis* and *S. chrysanthemifolius*. Thus, whereas *S. aethnensis* produced large capitula and entire leaves, while *S. chrysanthemofolius* had smaller capitula and highly dissected leaves, *S. squalidus* and the hybrid plants tended to produce capitula of intermediate size and leaves of an intermediate degree of dissection. The only exceptions to this concerned one *S. squalidus* individual and one hybrid plant which exhibited a phenotype similar to that of *S. chrysanthemifolius*.

The survey of allozyme variation across populations confirmed a close similarity of certain populations of *S. squalidus* (populations 17 and 18) to *S. aethnensis* × *S. chrysanthemifolius* hybrid material (populations 7 and 8), and also to *S. chrysanthemifolius*. These materials were placed in a separate sub-cluster from *S. aethnensis* and one of the hybrid populations (9), in a UPGMA tree constructed using Nei's genetic distances between populations (Fig. 3). Other populations of *S. squalidus* (populations 11–16) were positioned in a different cluster to Sicilian material, although based on the genetic distances recorded they were closely related to this

TABLE 5. ALLOZYME VARIABILITY ESTIMATES AND WEIR & COCKERHAM'S (1984) COEFFICIENT OF INBREEDING (*F* = *FIS*) IN SAMPLED POPULATIONS OF *SENECIO*. TESTS OF HARDY-WEINBERG EQUILIBRIUM ACROSS LOCI WITHIN SAMPLES (FISHER'S METHOD) ARE INDICATED.

Species		Popn.	Mean sample size†	*A*†	*P*	H_e†	*f*††
S. aethnensis		1	12·4 (1·2)	1·3 (0·2)	25·0	0·129 (0·084)	-0·071
		2	23·9 (0·1)	1·6 (0·2)	62·5	0·184 (0·068)	0·058
		3	23·8 (0·6)	1·6 (0·2)	62·5	0·208 (0·067)	0·209
	Mean			1·5	50·0	0·174	
S. chrysanthemifolius		4	41·0 (1·1)	1·5 (0·3)	37·5	0·107 (0·061)	-0·071
		5	23·5 (1·1)	1·5 (0·3)	37·5	0·094 (0·065)	0·054
		6	24·8 (0·2)	1·5 (0·3)	37·5	0·094 (0·062)	0·030
	Mean			1·5	37·5	0·098	
S. aet. x S. chrys.		7	37·8 (1·3)	1·5 (0·2)	50·0	0·076 (0·060)	-0·319
		8	27·6 (0·2)	1·5 (0·2)	50·0	0·138 (0·059)	-0·159
		9	15·6 (0·2)	1·5 (0·2)	50·0	0·138 (0·077)	0·365*
	Mean			1·5	50·0	0·117	
S. squalidus		10	28·4 (0•6)	1·3 (0·2)	25·0	0·022 (0·015)	-0·031
		11	24·8 (1·3)	1·3 (0·2)	25·0	0·091 (0·059)	0·045
		12	26·4 (1·7)	1·1 (0·1)	12·5	0·005 (0·005)	0·000
		13	22·4 (2·1)	1·1 (0·1)	12·5	0·049 (0·049)	-0·139
		14	25·4 (0·5)	1·3 (0·2)	25·0	0·113 (0·075)	0·467**
		15	25·5 (1·5)	1·3 (0·2)	25·0	0·081 (0·060)	0·208
		16	27·4 (0·3)	1·3 (0·2)	25·0	0·085 (0·063)	0·364*
		17	18·8 (0·6)	1•1 (0·1)	12·5	0·019 (0·019)	-0·059
		18	25·0 (0·7)	1·3 (0·2)	25·0	0·102 (0·069)	0·217
	Mean			1·2	20·8	0·063	
S. vernalis		37	27·0 (0·0)	1·3 (0·2)	25·0	0·103 (0·068)	0·241

† Standard errors in parentheses
†† Hardy-Weinberg equilibrium rejected at * $P < 0·05$ and ** $P < 0·01$
A: mean no. of alleles per locus
P: percentage of polymorphic loci
H_e: mean expected heterozygosity

material. Strong evidence that *S. squalidus* is of hybrid origin, rather than a derivative of *S. chrysanthemifolius*, was the presence of both *Acp-2a* and *Acp-2b* alleles in *S. squalidus*. Our results showed that *S. chrysanthemifolius* is monomorphic for the *Acp-2b* allele and consequently another taxon must have donated the *Acp-2a* allele to *S. squalidus*, which we propose is *S. aethnensis*. It could be argued, of course, that *S. squalidus* might be a derivative of *S. aethnensis* rather than of hybrid origin, as all the allozyme alleles present in *S. squalidus*, including both alleles at the *Acp-2* locus, were also found in populations of *S. aethnensis* (Table 4). However, there is good reason to believe that material of *S. aethnensis* we sampled from Mt Etna was introgressed. One of us (James 1999) has since found that populations of *S. aethnensis* that grow at much higher altitudes on the mountain (>2,500 m) are monomorphic for the *Acp-2a* allele. We propose, therefore, that *S. squalidus* is derived from hybrids between *S. aethnensis* and *S. chrysanthemifolius* rather than being derived directly from either parent.

A hybrid origin of *S. squalidus* assumes that the hybrid zone on Mt Etna formed following secondary contact between *S. aethnensis* and *S. chrysanthemifolius*, and that material regarded as hybrid on Mt Etna is not ancestral to *S. aethnensis* and *S. chrysanthemifolius*. If the latter were the case, then *S. squalidus* would also be considered as directly derived from this ancestral taxon.

TABLE 6. WEIR & COCKERHAM'S (1984) ESTIMATES OF WRIGHT'S *F*-STATISTICS CALCULATED SEPARATELY FOR EACH LOCUS FOR ALL BRITISH POPULATIONS OF *SENECIO SQUALIDUS*

Locus	*F*	θ	*f*
Acp-2	0·659 (0·087)	0·239 (0·091)	0·546 (0·073)
Aat-3	0·443 (0·160)	0·426 (0·154)	0·028 (0·083)
Pgm-2	0·000 (0·000)	0·030 (0·014)	-0·031 (0·015)
Mean (Std. Dev.)	0·478 (0·140)	0·385 (0·122)	0·219 (0·332)
95% confidence interval	0·000 - 0·655	0·016 - 0·420	-0·016 - 0·546

Means and standard deviations were obtained by jackknifing over loci. Confidence intervals were obtained by bootstrapping over loci. Locus specific standard deviations were obtained by jackknifing over populations. F = *Fit* (within total), θ = *Fst* (among populations), f = *Fis* (within populations)

Although this possibility can not be excluded, it is considered to be highly unlikely. In addition to the fixation of different alleles at the *Acp-2* locus in *S. aethnensis* and *S. chrysanthemifolius*, James (1999) has found that populations of *S. aethnensis* and *S. chrysanthemifolius* from the extremes of the altitudinal range on Mt Etna are monomorphic or almost monomorphic for many different RAPD bands and also for cpDNA haplotype. Such nuclear and cytoplasmic differentiation between these two species indicates that they are likely to have originated in isolation and recently come into contact. Despite these molecular differences, *S. aethnensis* and *S. chrysanthemifolius* produce fertile F1 and F2 hybrid populations, and are interfertile with *S. squalidus* (Abbott & Forbes unpublished).

In another study (Abbott & Milne 1995) the *Acp-2b* allele, which occurs at high frequency in *S. squalidus* and in hybrid material on Mt Etna, was shown to be absent from 16 populations of *S. rupestris* sampled widely from the geographical distribution of this species. This shows that *S. squalidus* could not be derived entirely from *S. rupestris* material, although does not rule out the possibility that it might be derived in part from this source. However, we propose that the allozyme evidence combined with the absence of any known documentary evidence of an introduction of *S. rupestris* to the British Isles, provide a strong argument against the possibility of *S. rupestris* having contributed to the British taxon.

GENETIC STRUCTURE OF *SENECIO SQUALIDUS*

The level of allozyme diversity within *S. squalidus*, measured in terms of expected heterozygosity, was approximately one third of that recorded in *S. aethnensis* and approximately half that in the hybrid material from which we propose *S. squalidus* is derived (Table 5). This reduced level of genetic diversity in *S. squalidus* is probably due to a founder effect caused by the introduction to the British Isles of a small sample of hybrid material (and allelic diversity) from the hybrid zone on Mt Etna. Rieseberg (1997) has reviewed amounts of allozyme variation found in other homoploid hybrid species relative to their parents, and shown them to differ between species. In three *Helianthus* diploid hybrid species, levels of diversity were lower than expected, indicating a small number of parental individuals involved in their origin. In contrast, in other homoploid species, levels of diversity were approximately the same or slightly greater than those of parents. Despite the low diversity in *S. squalidus*, significant population differentiation was found for allozyme variation based on F statistics analysis (θ was significantly different from zero). However, there was no obvious geographical pattern to this variation according to the UPGMA tree constructed from genetic distances between populations (Fig. 3), and the non-significant correlation of θ and geographical distance between populations. It is likely that population differences in allozyme variation are the product of founder effects and genetic drift occurring during the colonization of new sites, and that the recent massive range expansion of *S. squalidus* in the British Isles has prevented a possible association of genetic and geographical distance from arising between populations due to isolation by distance.

The mean coefficient of inbreeding (f) in *S. squalidus* was not significantly different from zero, indicating that random mating is normal in the species - as noted by Abbott & Forbes (1993).

However, a high *f* value was evident at the *Acp-2* locus (across populations) and also in two populations (estimated across loci). The high *f* value at the *Acp-2* locus might stem from the presence of a null allele at this locus which, if true, most likely occurs at low frequency, given that null homozygotes were not recovered (see Results section). The high *f* values in two populations could be due to population sub-structure (the Wahlund effect) which would promote biparental inbreeding. Alternatively, some selfing might be occurring. Abbott & Forbes (1993) showed that *S. squalidus* is strongly self-incompatible; however, the self-incompatibility reaction might be weakened under certain conditions (Hodgkin *et al.* 1988) allowing selfing to take place.

Although *S. squalidus* was less variable than either of its parents on the basis of allozyme variation, this finding does not tally with its morphological variation. In the field, it exhibits considerable variation in leaf shape, with individuals at one extreme producing highly dissected leaves similar to *S. chrysanthemifolius*, and plants at the other extreme that bear almost entire leaves (var. *subinteger*, Druce 1927). However, most plants produce leaves intermediate between these two extremes though tending towards *S. chrysanthemifolius*. Other characters known to vary are capitulum size and seed type (Druce 1927). It would seem that morphologically the species might be converging towards an intermediate phenotype, but is not yet completely stabilized.

ALLOPATRIC ORIGIN OF HOMOPLOID HYBRID SPECIES

The morphometric and allozyme evidence reported here, combined with previous documentary evidence (Druce 1927; Kent 1956; Crisp 1972), provide support for the hypothesis that *S. squalidus* is a new diploid hybrid species which originated allopatrically through stabilization of hybrid material of *S. aethnensis* and *S. chrysanthemifolius* introduced to the British Isles from the hybrid zone that occurs between the two species on Mt Etna, Sicily. Previous to this study, only ten plant species had been indicated to be of homoploid hybrid origin based on rigorous molecular analysis (Rieseberg 1997; Allan *et al.* 1997; Wolfe *et al.* 1998). Mechanisms of reproductive isolation between homoploid hybrid species and their parents have been reviewed by Rieseberg (1997) and Rieseberg & Carney (1998) and shown to involve postmating and/or premating barriers. Two homoploid hybrid species, *Pinus densata* Masters and *Encelia virginensis* A. Nels, are believed to be derived from parents that produce interspecific F_1 hybrids that are vigorous and fertile (Wang & Szmidt 1994; Allan *et al.* 1997) and, in the case of *E. virginensis*, are known to be interfertile with both parents. In these respects, therefore, they resemble *S. squalidus*, which is also derived from parents which when intercrossed form vigorous fertile hybrid offspring, and whose parents are interfertile with *S. squalidus* (Abbott & Forbes unpublished). Natural populations of *P. densata* and *E. virginensis* are mainly allopatric with their parents and also ecologically isolated from them. However, it has been argued that they are likely to have originated in sympatry with gene flow between hybrid and parent species prevented by ecological isolation, and only later became distributed allopatrically with their parents. Diploid hybrid species of *Paeonia* which are also allopatric with their parents may have originated in the same way (Sang *et al.* 1995). In contrast, *S. squalidus* is an example of a diploid hybrid species which originated through stabilization of hybrid material dispersed to locations geographically isolated from where hybridization initially took place. Although the first step in this speciation process involved hybridization between sympatric parents, the crucial process of stabilization resulting in the origin of a new hybrid species occurred at an allopatric site where there was no possibility of hybrid material backcrossing to either parent. During this process *S. squalidus* is likely to have also become ecologically isolated from both parents, as neither parent species appears able to form viable populations in the wild in the British Isles (Abbott, pers. obs.). Thus, the evolution of premating barriers due, for example, to pollinator behaviour and/or ecological isolation (e.g. in *Penstemon*, Wolfe *et al.* 1998; *Pinus*, Wang & Szmidt 1994; and *Encelia,* Allan *et al.* 1997), and postmating barriers resulting from chromosomal or genic sterility factors (e.g. in *Helianthus*, *Iris* and *Stephanomeria,* see Rieseberg (1997)), were not essential in the origin of *S. squalidus*, as isolation was maintained by geographical separation.

Because of the possible ease with which homoploid hybrid speciation might occur at allopatric sites, we may need to revise current views on the importance of this mode of plant speciation and the frequency at which it occurs (Rieseberg 1997). That said, in the case of *S. squalidus,* considerable assistance was required from man to aid the origin of this new species. Material from Mt Etna was cultivated in the Oxford Botanic Garden for approximately 100 years before it escaped to the surrounding area and started to spread beyond Oxford. It will be of interest in the

future to determine what genetic changes might have occurred in this material, due to recombination, segregation and mutation during this period, that could have led to the creation of a plant type that is now well adapted to conditions in the British Isles.

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Appendix 1. Allele frequencies at polymorphic loci in populations (1–19) of *Senecio* surveyed. Key to populations is given in Table 1.

Locus/ allele	Population 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Acp-2																			
a	·615	·542	·760	·000	·000	·000	·016	·232	·531	·000	·224	·000	·000	·522	·094	·407	·000	·180	1·000
b	·385	·458	·240	1·000	1·000	1·000	·984	·768	·469	1·000	·776	1·000	1·000	·478	·906	·593	1·000	·820	·000
Aat-3																			
a	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	1·000
b	1·000	·896	·880	·848	·979	·980	·969	·946	·969	·056	·239	·019	·741	·259	·365	·103	·921	·525	·000
c	·000	·104	·120	·152	·021	·020	·031	·054	·031	·944	·761	·981	·259	·741	·635	·897	·079	·475	·000
β–Est-3																			
a	·625	·761	·600	·363	·500	·620	·412	·185	·667	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000
b	·375	·239	·400	·637	·500	·380	·587	·815	·333	1·000	1·000	1·000	1·000	1·000	1·000	1·000	1·000	1·000	·333
c	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·667
Pgi-2																			
a	000	·063	·208	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000
b	1·000	·938	·792	1·000	1·000	1·000	1·000	1·000	1·000	1·000	1·000	1·000	1·000	1·000	1·000	1·000	1·000	1·000	·759
c	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·241
Pgm-2																			
a	·000	·000	·000	·013	·022	·042	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000	·000
b	1·000	·833	·857	·934	·891	·875	·987	·796	·967	·966	1·000	1·000	1·000	1·000	1·000	1·000	1·000	1·000	1·000
c	·000	·167	·143	·053	·087	·083	·013	·204	·033	·034	·000	·000	·000	·000	·000	·000	·000	·000	·000