Plant introductions, hybridization and gene flow

Richard J. Abbott[*], Juliet K. James, Richard I. Milne and Amanda C. M. Gillies

Division of Environmental and Evolutionary Biology, School of Biology, Mitchell Building, University of St Andrews, St Andrews, Fife KY16 9TH, UK

Many regional floras contain a high proportion of recently introduced plant species. Occasionally, hybridization between an introduced species and another species (introduced or native) can result in interspecific gene flow. This may occur even in instances where the F_1 hybrid shows very high sterility, but occasionally produces a few viable gametes. We provide examples of gene flow occurring between some rhododendrons recently introduced to the British flora, and between an introduced and native *Senecio* species. Neutral molecular markers have normally been employed to obtain evidence of interspecific gene flow, but the challenge now is to isolate and characterize functional introgressed genes and to determine how they affect the fitness of introgressants and whether they improve adaptation to novel habitats allowing introgressants to expand the range of a species. We outline a candidate gene approach for isolating and characterizing an allele of the *RAY* gene in *Senecio vulgaris,* which is believed to have introgressed from *S. squalidus*, and which causes the production of ray florets in flower heads. We discuss the effects of this introgressed allele on individual fitness, including those that originate directly from the production of ray florets plus those that may arise from pleiotropy and/or linkage.

Keywords: introduced plants; hybridization; gene flow; introgression; candidate genes; adaptation

1. INTRODUCTION

Plant species are often geographically isolated from their relatives due to past vicariance or long-distance dispersal events that were important precursors of divergence. In the past, such geographically disjunct species may have eventually gained contact with related species if, for example, changes in global climate or geology opened corridors for migration across what had previously been impenetrable barriers. Other plant species may occur in close geographical proximity to their relatives, but be ecologically isolated from them. In these cases, natural disturbance could either decrease the effectiveness of ecological barriers or curtail them. Under both sets of circumstances, opportunities for hybridization would increase between plants that were previously isolated from each other, provided that they had not diverged to an extent that cross-pollination never occurs and/or viable and fertile F_1 offspring are never produced.

The likelihood of contact occurring between species that were previously geographically or ecologically isolated from each other has risen dramatically in recent times due to human activities (Mooney & Cleland 2001). The development of world trade and international travel, in particular, has led to plants being introduced either intentionally or accidentally to parts of the world far from where they are native. In addition, landscape modification due to agriculture and urbanization has resulted in mass-

ive habitat disturbance and the breakdown of ecological barriers. It is not surprising, therefore, that a region such as the British Isles, which has been a centre for world trade for over 500 years, and which has a landscape largely altered by humans, possesses a flora containing many recently introduced species and numerous interspecific hybrids (Stace 1975, 1997; Abbott 1992; Preston *et al.* 2002; Williamson 2002).

The *New atlas of the British & Irish flora* (Preston *et al.* 2002) shows that approximately half of the species that compose this flora are native and the other half are introduced (table 1). If these values are adjusted to include only those species found in more than 50 of the 2837 10 km squares surveyed, then the proportion of introduced species is reduced to approximately one-third of the total, with *ca.* 25% of all species comprising those introduced in the last 500 years. Among the hybrids in the British Isles flora listed by Preston *et al.* (2002), 87 are the product of hybridization between a native and an introduced species while 26 originated from crosses between two alien species. These values are reduced to 22 and 2, respectively, when only hybrids present in more than fifty 10 km squares are considered.

Although interspecific hybridization is frequently recorded in the plant kingdom, it tends to be restricted to particular taxa. For example, hybrids are present in 34% of families and 16% of genera that comprise the British Isles flora, while in Hawaii these values fall to 16% and 6%, respectively (Ellstrand *et al.* 1996). At present, there is no information on how often a hybridization event is of considerable evolutionary significance, resulting, for example, in interspecific gene flow—introgression (Rieseberg & Wendel 1993)—the origin of stabilized

Phil. Trans. R. Soc. Lond. B (2003) **358**, 1123–1132
DOI 10.1098/rstb.2003.1289

1123

© 2003 The Royal Society

Table 1. Number and proportion of native and introduced species in the British and Irish flora (after Preston *et al.* 2002).

	all species[a]	species present in more than fifty 10 km squares
total number of species	2711	2010
native species	1363 (50.28%)	1324 (65.87%)
native/alien species	44 (1.62%)	42 (2.09%)
introduced species:	1304 (48.10%)	644 (32.04%)
archeophytes	149 (5.05%)	146 (7.26%)
neophytes	1155 (42.60%)	498 (24.78%)

[a] Based on a survey of 2837 10 km squares covering the whole of the British Isles. Archeophytes were introduced before AD 1500; neophytes were introduced after AD 1500. Native/alien species is a group in which species could not be accurately categorized as either native or introduced.

introgressants recognized as varieties or subspecies in the recipient species (Abbott 1992), or the origin of a stable hybrid species through allopolyploidy (Soltis & Soltis 1993, 1999) or homoploid hybrid speciation (Arnold 1997; Rieseberg 1997). Although there may often be no consequences beyond F_1 formation, detailed investigation of some plant groups has revealed that all or several of the above evolutionary consequences of interspecific hybridization have occurred (e.g. in *Helianthus* (Rieseberg *et al.* 1990; Rieseberg 1997), *Iris* (Arnold 1993, 2000) and *Senecio* (Abbott & Lowe 1996; Abbott *et al.* 2000, 2002)). Such hybrid products may exhibit high invasiveness as reflected by an ability to colonize rapidly areas closed to parents (Ellstrand & Schierenbeck 2000).

Identifying hybrid taxa and reconstructing their ancestry is most successfully done if they are of relatively recent origin. New hybrid derivatives are expected to combine the genes of their parents to varying degrees, and not to have diverged genetically such that they contain genes not found in either parent. If the history of hybridization and its products is recent and documented, it is easy to rule out alternative evolutionary scenarios for why a taxon might appear to combine the genes of its putative parents, but is not in fact hybrid. Such misidentification could result from convergent evolution or symplesiomorphy, i.e. the joint sharing of ancestral traits (Rieseberg *et al.* 1990). In the latter case, it is often not possible to distinguish between whether a putative ancient hybrid taxon is indeed hybrid or the ancestor of the putative parents without undertaking a phylogenetic analysis of related species and determining which taxa are ancestral.

We focus on two recent examples of hybridization and gene flow in the British flora that involve species that were intentionally introduced for cultivation. The first of these concerns hybridization between some introduced rhododendrons, while the second concerns the evolutionary consequences of hybridization between an introduced and a native species of *Senecio*. Both examples represent systems worthy of future detailed investigation that should lead to improvements in our present understanding of the process of interspecific gene flow in plants. Although there are many examples of introgressive gene flow recorded in flowering plants (Rieseberg & Wendel 1993), we remain ignorant of how often the process is adaptive, i.e. has increased the fitness of the introgressed species in its indigenous habitat and/or allowed it to increase its range by invading new habitats (Lewontin & Birch 1966). In addition, we currently have no detailed information on the

molecular genetics and development of introgressed traits that alter phenotype and affect fitness. We also consider, therefore, some approaches for isolating and characterizing genes that control introgressed traits, and the need to examine whether such introgressed genes are adaptive.

2. HYBRIDIZATION AND GENE FLOW BETWEEN RHODODENDRONS INTRODUCED TO THE BRITISH ISLES

Rhododendron ponticum is a species widely cultivated in the British Isles. It is native to the area south of the Black Sea (i.e. the Caucasus, northern Turkey and the southeast corner of Bulgaria), and also to a small part of the Lebanon and three small areas in the Iberian Peninsula, i.e. southwest Spain, and southern and central Portugal (Jessen *et al.* 1959; Cross 1975). The species was first introduced into the British Isles for cultivation in 1763 from southwest Spain (Curtis 1803; Coats 1963), but subsequent introductions have certainly occurred, of which some were from the Black Sea area (Curtis 1803; Loudon 1838). Cultivated material of *R. ponticum* was soon recorded growing wild in the British Isles and is now extensively naturalized in these islands (Preston *et al.* 2002). It is currently recognized as a threat to native communities (e.g. in Killarney, southwest Ireland (Turner & Watt 1939; Cross 1981; Kelly 1981); Snowdonia, North Wales (Thomson *et al.* 1993); the Lake District, northwest England, and the west Highlands of Scotland (see also Williamson 2002)) and is a pest of forestry (Brown 1953, 1954; Simons 1988). A comparative survey of cpDNA RFLPs in naturalized material of *R. ponticum* sampled from wild populations throughout the British Isles, and also from native material in Turkey and the Iberian Peninsula, has revealed that 99% out of 260 British accessions examined were of Iberian origin, i.e. possessed cpDNA haplotypes found in Iberian but not Turkish material (Milne & Abbott 2000). Moreover, 89% had a cpDNA that characterized native material from southern Spain while 10% had a cpDNA unique to Portuguese material. The remaining 1% of accessions examined possessed the cpDNA of other introduced rhododendron species that are cultivated in the British Isles, but which have never escaped to the wild. This last finding indicates that *R. ponticum* is partly introgressed with the cpDNA of other rhododendron species introduced into the British Isles.

Further evidence that naturalized *R. ponticum* material is partly introgressed with genes from other introduced

rhododendrons emerged from a survey of nuclear rDNA RFLPs. Based on cpDNA and rDNA RFLPs combined, it was evident that 27 British accessions (*ca.* 10% of the total sample) were introgressed with genes from *Rhododendron catawbiense*, a species native to North America and known to be more cold tolerant than native *R. ponticum* (Davidian 1982; Nilsen 1991). Interestingly, these introgressed accessions were significantly more abundant in Britain's coldest region, eastern Scotland, than elsewhere, implying that the introgression may have been adaptive favouring an extension of the distribution of *R. ponticum* to parts of the British Isles to which material from the Iberian Peninsula was not preadapted. However, this association could simply reflect the chance release of introgressed material into this region rather than being indicative of adaptive introgression. Further research is required to distinguish between these two possibilities.

If variation throughout the nuclear genome were surveyed, rather than only that within the rDNA region, it is feasible that gene introgression from *R. catawbiense* and perhaps other introduced rhododendrons might be shown to be more widespread than has been recorded so far. Certainly, many British accessions of *R. ponticum* that contain neither the cpDNA nor rDNA of *R. catawbiense* express morphological traits characteristic of the species, therefore indicating that they are introgressed despite the current absence of molecular evidence. For example, ovary hair, which is absent from native material of *R. ponticum*, but present in *R. catawbiense*, was shown to occur to some degree in almost half of the accessions surveyed (41 out of 83) from Scottish wild populations of *R. ponticum* (Milne & Abbott 2000). Further analysis of *R. ponticum* would be worthwhile to determine how widespread introgression is in introduced material and whether it is adaptive.

3. THE EVOLUTIONARY CONSEQUENCES OF HYBRIDIZATION BETWEEN AN INTRODUCED AND A NATIVE *SENECIO* IN THE BRITISH ISLES

Hybridization between *Senecio squalidus* (Oxford ragwort, $2n = 20$) and *S. vulgaris* (groundsel, $2n = 40$) occurs very rarely in the wild in the British Isles (Marshall & Abbott 1980) and produces initially a highly sterile triploid F$_1$ hybrid ($2n = 30$), or very occasionally a fertile tetraploid F$_1$ (Lowe & Abbott 2000). *Senecio squalidus* was introduced to the British Isles from Sicily, in the early eighteenth century (Harris 2002). After a period of cultivation in gardens at Oxford and nearby, it was seen growing on walls and waste sites in Oxford by the end of the eighteenth century and has subsequently spread to many parts of the British Isles over the past 200 years (Abbott 1992; Preston *et al.* 2002). It now frequently co-occurs with the native *S. vulgaris* on disturbed ground, usually in urban areas and, very occasionally, hybrids are formed between the two species at such sites (Marshall & Abbott 1980). Although the triploid hybrid is highly sterile it produces some viable gametes, probably with balanced chromosome numbers, i.e. $x = 10$, 20 or 30. There is good evidence that three new stable hybrid taxa have evolved in the British Isles from the cross between *S. vulgaris* and *S. squalidus* during the past 170 years. These are as follows: (i) a variant form of *S. vulgaris*, named *S. vulgaris*

var. *hibernicus* Syme, which was first recorded in Britain in 1832 (Abbott 1992) and is now found at many locations in the British Isles, although it is more common in Wales, the Midlands and southern parts of England than elsewhere (figure 1*a*). This variant produces radiate capitula (flower heads) containing an outer whorl of female ray florets in addition to a complement of hermaphrodite disc florets. It contrasts with *S. vulgaris* var. *vulgaris*, which produces discoid capitula containing only hermaphrodite disc florets. (ii) A tetraploid hybrid species, *S. eboracensis* Abbott & Lowe, first recorded in the British Isles at York in 1979 (Irwin & Abbott 1992) and still restricted to that locality (figure 1*b*). This species is intermediate to the two parent species in a range of morphological traits, but possesses some novel characters (Irwin & Abbott 1992; Lowe & Abbott 2003). (iii) An allohexaploid hybrid species, *S. cambrensis* Rosser, first discovered in Britain in 1948 near Wrexham, Wales (Rosser 1955) and since recorded at other sites in Wales as well as in Edinburgh (figure 1*b*). This species is also morphologically intermediate, but again possesses some novel traits (Rosser 1955; Lowe & Abbott 1996). The Edinburgh population has recently been reported to have become extinct, but Welsh populations continue to thrive (Abbott & Forbes 2002).

Evidence to support an introgressive origin of the radiate variant of *S. vulgaris* (i.e. var. *hibernicus*) is based on an ability to artificially resynthesize plants with similar morphology after backcrossing the hybrid to *S. vulgaris* (Ingram *et al.* 1980; Lowe & Abbott 2000). In addition, an allozyme marker, which is present at high frequency in *S. squalidus* but is absent from native allopatric populations of *S. vulgaris*, occurs at an intermediate frequency in the radiate variant (Abbott *et al.* 1992). Artificial resynthesis and allozyme markers also provide evidence for *S. vulgaris* and *S. squalidus* being the parents of the tetraploid *S. eboracensis* (Irwin & Abbott 1992; Lowe & Abbott 2000), and the hexaploid *S. cambrensis* (Weir & Ingram 1980; Ashton & Abbott 1992). The parentage of *S. cambrensis* has also been confirmed by the results of a survey of chloroplast and nuclear rDNA RFLPs (Harris & Ingram 1992; Lowe & Abbott 1996).

It is expected that the allohexaploid *S. cambrensis* should contain the genomes of both parents and therefore exhibit a complete additive genetic profile. By contrast, we currently have no precise idea of the expected genomic contributions of the parents to the other two hybrid taxa, although morphology indicates that there should be a larger proportion of the *S. squalidus* genome in *S. eboracensis* than in *S. vulgaris* (var. *hibernicus*). To obtain information on the genomic contributions of parents to the three different *Senecio* hybrid taxa, we have undertaken a preliminary survey of the presence/absence in these taxa of a suite of RAPD and ISSR markers that are diagnostic of *S. squalidus* and *S. vulgaris* var. *vulgaris*. We present the results of this study in the next section.

4. GENOMIC COMPOSITION OF HYBRID DERIVATIVES OF *SENECIO SQUALIDUS* AND *SENECIO VULGARIS*

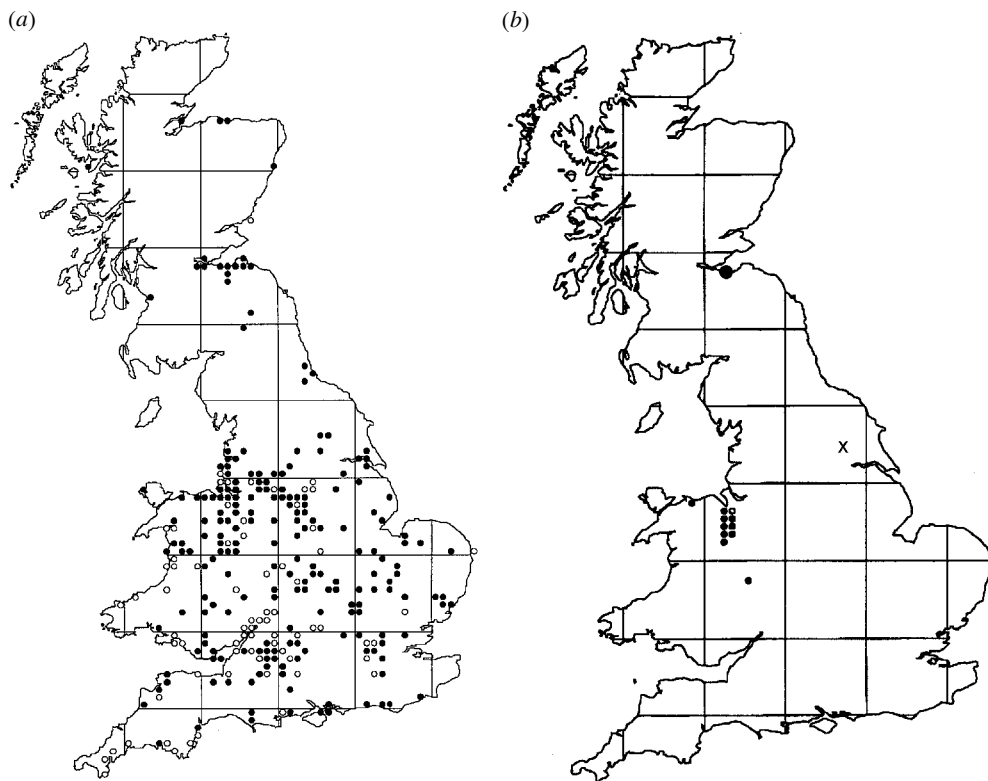RAPD and ISSR markers that distinguish *S. squalidus* from *S. vulgaris* var. *vulgaris* were sought from a sample

Figure 1. The distributions of three recently originated hybrid taxa of *Senecio vulgaris* × *S. squalidus* in the British Isles: (*a*) *Senecio vulgaris* var. *hibernicus* and (*b*) *S. eboracensis* (x) and *S. cambrensis*. For *S. vulgaris* var. *hibernicus* and *S. cambrensis*, each dot represents at least one record in a 10 km square of the National Grid. Pre-1950 (open circles); 1950 onwards (filled circles) mapped by the Biological Records Centre, Centre for Ecology and Hydrology.

of 10 individuals of *S. squalidus* and 18 individuals of *S. vulgaris* var. *vulgaris*. Individuals of *S. squalidus* and *S. vulgaris* were grown from seed collected from six and five different populations, respectively, with populations of *S. vulgaris* chosen because they were geographically isolated and therefore unlikely to be introgressed with germplasm of *S. squalidus* and other *Senecio* taxa investigated in the current study (table 1). A previous screen of a large number of RAPD and ISSR primers produced a set of 19 primers that appeared suitable for the analysis proposed based on reliable amplification and level of polymorphism in *S. squalidus* (James 1999).

DNA extracts from the individuals of *S. squalidus* and isolated *S. vulgaris* were surveyed for species diagnostic markers using this set of 19 primers. Protocols for DNA extraction and RAPD/ISSR amplification and resolution followed broadly those of Comes & Abbott (2001) and are described in detail by James (1999). Twelve *S. vulgaris* var. *vulgaris* marker bands and nine *S. squalidus* marker bands were identified for 10 of the 19 primers employed. Sequences (5′-3′) of these 10 primers were RAPDS: primer A9, GGGTAACGCC; primer C2, GTGAGG CGTC; primer C5, GATGACCGCC; primer C20, ACT TCGCCAC; primer F12, ACGGTACCAG; primer G5, CTGAGACGGA; primer G6, GTGCCTAACC; primer G12, CAGCTCACGA; primer H7, CTGCATCGTG; ISSR: primer 8, AGAGAGAGAGAGAGAGC. Presence or absence of parent diagnostic marker bands was examined in varying numbers of individuals of (i) *S. vulgaris* var. *hibernicus*; (ii) *S. vulgaris* var. *vulgaris* that co-occurred with var. *hibernicus* or *S. eboracensis* in mixed populations

of the two variants; (iii) *S. eboracensis*; and (iv) *S. cambrensis*, using the same 10 primers and laboratory protocols as before (see table 2 for locations from which material was sampled). The results (table 3) showed that all 12 bands that were diagnostic of isolated *S. vulgaris* var. *vulgaris* were present in *S. vulgaris* var. *hibernicus* and co-occurring *S. vulgaris* var. *vulgaris*. These bands were also present in the majority of *S. cambrensis* individuals surveyed apart from one band, C5-500, which was present in Welsh material, but absent from all plants of Edinburgh *S. cambrensis* examined. By contrast, three *S. vulgaris* bands (C5-750, F12-1000, G12-400) were absent from all individuals of *S. eboracensis* examined. Out of the *S. squalidus* diagnostic marker bands surveyed, none was present in either *S. vulgaris* var. *hibernicus* or co-occurring var. *vulgaris*, seven occurred in *S. cambrensis* and six were found in *S. eboracensis*. One of the two markers not present in *S. cambrensis* was found in *S. eboracensis*.

The main conclusion drawn from these results is that the amount of *S. squalidus* genome present in *S. vulgaris* var. *hibernicus* must be considerably smaller than that present in either *S. eboracensis* or *S. cambrensis*. In fact, the RAPD and ISSR markers provided no evidence for var. *hibernicus* being introgressed. This is not wholly unexpected, as the two variants of *S. vulgaris* are very similar morphologically (Irwin & Abbott 1992). Consequently, very few genes of *S. squalidus* are likely to have been introgressed into *S. vulgaris* to produce var. *hibernicus*. By contrast, the tetraploid *S. eboracensis* may contain most of the genome of *S. squalidus* and consequently less of the genome of *S. vulgaris*.

Table 2. *Locations from where* Senecio *material was sampled for RAPD and ISSR analysis.*
(Numbers in brackets are the number of individuals surveyed per locality. Where two taxa were sampled from the same locality, the first number in brackets is the number of individuals surveyed for the first taxon listed for that locality.)

species	locality	latitude	longitude
S. vulgaris var. *vulgaris*	Tomintoul, Moray, Scotland (3)	57.14 N	03.29 W
	Letham, Angus, Scotland (3)	56.36 N	02.42 W
	Glenluce, Scotland (2)	54.45 N	04.44 W
	Puffin Island, Wales (5)	53.13 N	04.09 W
	Aberffraw, Wales (5)	53.09 N	04.34 W
S. vulgaris var. *hibernicus*	Edinburgh, Scotland (5), (5)	55.58 N	03.07 W
and *S. vulgaris* var. *vulgaris*	St Helens, England (2), (3)	53.23 N	02.45 W
	Brymbo, Wales (2), (3)	53.04 N	03.03 W
	Birmingham, England (3), (5)	52.25 N	01.54 W
	Cork, Ireland (5), (5)	51.55 N	08.27 W
	Cardiff, Wales (5), (4)	51.25 N	03.09 W
	Bristol, England (5), (5)	53.53 N	01.04 W
S. eboracensis and *S. vulgaris*	York, England (10) (5)	53.53 N	01.04 W
S. cambrensis, Edinburgh	Leith, Edinburgh, Scotland (21)	55.58 N	03.07 W
S. cambrensis, Welsh	Mochdre, Wales (5)	53.15 N	03.47 W
	Ffrith, Wales (1)	53.05 N	03.04 W
	Southsea, Wales (3)	53.03 N	03.02 W
	Wrexham, Wales (4)	53.02 N	03.00 W
S. squalidus	Kirkcaldy, Scotland (2)	56.08 N	03.10 W
	Hull, England (1)	53.40 N	00.18 W
	Norwich, England (2)	52.40 N	01.20 E
	Oxford, England (2)	51.45 N	01.09 W
	Cardiff, Wales (2)	51.24 N	03.08 W
	Portland Bill, England (1)	50.28 N	02.26 W

Rather surprising was the absence of a complete additive marker profile in *S. cambrensis*. Typically, this species lacked two of the nine *S. squalidus* marker bands, while Welsh material of the species also lacked one *S. vulgaris* band. The absence of some parental markers from *S. cambrensis* might be caused by certain markers not being entirely diagnostic of a parent species, and because the species has originated on at least two separate occasions. Welsh and Edinburgh material of *S. cambrensis* are known to have originated independently of each other (Ashton & Abbott 1992; Harris & Ingram 1992). Consequently, the presence of the C5-500 band in Welsh, but not Edinburgh, material of *S. cambrensis* might be due to different *S. vulgaris* parent genotypes being involved in the separate origins of the species at the two locations. Alternatively, diagnostic marker bands of parents may have been lost from *S. cambrensis* due to rapid genomic change, which is believed to occur in new allopolyploids more commonly than previously assumed (Soltis & Soltis 1999, 2000). A third possibility is that band absence is due to the effect of ploidy upon competition processes in RAPD-PCR reactions (Wolff & Peters-Van Rijn 1993; Weising *et al.* 1995). Because *S. cambrensis* contains a larger genome than *S. squalidus,* it is expected to possess more sites of 100% sequence similarity to a particular primer. Therefore, bands amplified with a degree of primer to priming-site mismatch in *S. squalidus* might not be amplified in *S. cambrensis*.

The study reported here is preliminary with regard to the sample sizes of the two parent taxa for which diagnostic markers were sought and the number of such markers detected. A more detailed comparative analysis of the genomic compositions of these hybrid taxa is now required to

obtain more accurate results and to detect what proportion of the *S. squalidus* genome has been introgressed into *S. vulgaris* var. *hibernicus*. This will require the construction and alignment of genomic maps for each hybrid taxon and its parents using a large number of dominant and co-dominant molecular markers.

5. FINDING INTROGRESSED GENES THAT MATTER

Molecular markers have proved to be extremely useful in determining whether a species is introgressed with genes from another species (e.g. Rieseberg *et al.* 1990; Martinsen *et al.* 2001). However, once introgression is proved, the challenge is to isolate and characterize the genes that control the development and expression of introgressed traits and to determine how these affect the fitness of introgressed individuals. Most molecular markers are assumed to be neutral to the effects of selection and can be used to map segments of chromosome that contain genes that directly affect fitness. Such segments of chromosome may affect the expression of a continuous trait, in which case they are referred to as QTLs. Alternatively, they may contain major genes that affect phenotype. Several laboratories are currently attempting to isolate and characterize introgressed genes that control what may be adaptive traits using mapping populations generated from inter- and intraspecific crosses (Rieseberg *et al.* 2000; Arnold 2000).

An alternative approach to the same problem is to use 'candidate' genes. A large number of genes that control the development and expression of diverse traits in model plants such as *Arabidopsis thaliana, Antirrhinum majus* and *Zea mays* are becoming available as 'candidates' for con-

Table 3. *Presence (+) or absence (−) of markers diagnostic of S. vulgaris and S. squalidus in hybrid derivatives of the two species.*
(*Abbreviations: W, Welsh material; Ed, Edinburgh material.*)

primer	band size (bp)	S. vulgaris vulgaris (n = 18)	S. vulgaris hibernicus (n = 27)	S. vulgaris vulgaris[a] (n = 35)	S. eboracensis (n = 10)	S. cambrensis W (n = 13, 21)	S. cambrensis Ed	S. squalidus (n = 10)
S. vulgaris var. *vulgaris* diagnostic markers								
C2	1000	+	+	+	+	+	+	−
C5	750	+	+	+	−	+	+	−
C5	500	+	+	+	+	+	−	−
C20	850	+	+	+	+	+	+	−
C20	450	+	+	+	+	+	+	−
F12	1000	+	+	+	−	+	+	−
G5	850	+	+	+	+	+	+	−
G6	800	+	+	+	+	+	+	−
G12	1400	+	+	+	+	+	+	−
G12	400	+	+	+	−	+	+	−
H7	750	+	+	+	+	+	+	−
18	1100	+	+	+	−	+	+	−
S. squalidus diagnostic markers								
A9	600	−	−	−	+	+	+	+
C5	1400	−	−	−	+	+	+	+
C5	800	−	−	−	+	+	+	+
C20	900	−	−	−	−	+	+	+
G5	550	−	−	−	+	−	−	+
G5	400	−	−	−	+	+	+	+
G6	1400	−	−	−	−	−	−	+
G12	800	−	−	−	+	+	+	+
H7	950	−	−	−	−	+	+	+

[a] *S. vulgaris* var. *vulgaris* co-occurring with var. *hibernicus* or *S. eboracensis*.

trolling similar traits in 'non-model' plant species. We are currently using a candidate gene approach to isolate and characterize the gene(s) controlling the production of radiate versus discoid flower heads in *S. vulgaris*. In the course of doing this we hope to prove that the gene(s) controlling presence of ray florets in the heads of *S. vulgaris* var. *hibernicus* has been introgressed from *S. squalidus*. The chances of such an approach succeeding increases when the trait of interest is under the control of one or very few genes of major effect.

Trow (1912) demonstrated that the presence/absence of ray florets in flower heads of *S. vulgaris* is under single gene control with the radiate condition dominant to the discoid condition. Heterozygotes are distinguished by the possession of ray florets with rays approximately half the length of those produced by the radiate homozygote. Consequently, the alleles at this locus (henceforth called *RAY*) exhibit co-dominance, and have an additive effect on ray length. Ingram & Taylor (1982) showed that the radiate versus discoid flower head type in *S. squalidus* is also under single gene control.

The radiate capitulum in *Senecio*, with a central disk of symmetrical (actinomorphic) disk florets surrounded by peripheral asymmetrical (zygomorphic) ray florets resembles, to some extent, the *cen* phenotype of *A. majus*. In this *Antirrhinum* mutant, the central terminal flower is radially symmetrical and the axillary flowers that surround it are zygomorphic. In both cases, the activation of genes controlling floral asymmetry seems to be restricted to a peripheral region around the inflorescence apex. In *cen*

this gives rise to all of the axillary flowers, while in radiate capitula it produces just the outermost florets (Coen *et al.* 1995).

This similarity between *cen* and the radiate capitulum type suggests that similar genes may be involved in controlling development of asymmetrical flowers in both of them (Coen *et al.* 1995). One of the genes known to control dorsoventral asymmetry in *A. majus* is *cycloidea* (*cyc*; Luo *et al.* 1996). *Cyc* is a member of the TCP gene family and is thought to function as a DNA-binding protein and a transcription factor (Cubas *et al.* 1999*a*; Cubas 2002). It has been proposed that production of zygomorphic wild-type *Antirrhinum* flowers is dependent on *cyc* activity establishing an axis of dorsoventral asymmetry. Activity is predicted to be greatest in the dorsal regions of the floral meristem and to decline towards the more ventral regions; this would account for the fact that flowers in *cyc* mutants are ventralized (Coen & Nugent 1994; Luo *et al.* 1996, 1999).

Asymmetrical flower structure is thought to have evolved independently many times (Stebbins 1974; Coen & Nugent 1994; Donoghue *et al.* 1998). Plants with actinomorphic instead of zygomorphic flowers, termed peloric mutants, have been described in several species in addition to *Antirrhinum* and it is likely that *cycloidea* homologues are controlling dorsoventral asymmetry in all of them (but see Donoghue *et al.* 1998). Genetic analysis of the naturally occurring peloric mutants in *Linaria vulgaris*, for example, has shown that the mutation is controlled by *cyc*-like genes (Cubas *et al.* 1999*b*). It is also speculated

that *cyc*-like genes may be performing a similar function in *Saintpaulia velutina* and *Sinningia speciosa* (Citerne *et al*. 2000). Therefore, it is reasonable to suggest that development of the ray floret in *Senecio* is controlled in a similar manner (Coen & Nugent 1994). We are currently using the polymorphism for radiate versus discoid capitula in *S. vulgaris* to test the theory that the *ray* gene in this species is a *cycloidea* homologue. If this theory proves to be correct, we shall determine whether the *cycloidea* homologue that produces ray florets in capitula of *S. vulgaris* var. *hibernicus* has been introgressed from *S. squalidus*. The homologue in both of these taxa would be expected to have an identical sequence and function.

The candidate gene approach could be widely adopted for isolating and characterizing introgressed genes that control important traits. However, as emphasized earlier, this approach is most likely to be successful where the trait of interest is under the control of one or a few genes that have a major effect on phenotype. Another method that can be used for isolating introgressed genes of importance involves the utilization of microarrays for monitoring simultaneously the expression of hundreds to thousands of genes in different tissues of an organism (Brown & Botstein 1999). This provides a powerful approach for identifying introgressed genes and determining how they might affect the differential expression of 'host' genes in the recipient plant. Microarraying the genomes of donor, recipient and introgressed taxa under diverse conditions that relate to the natural environments in which they grow will identify which genes are differentially expressed and may, therefore, indicate which introgressed genes are adaptive.

6. DETERMINING WHETHER INTROGRESSION IS ADAPTIVE

Isolating and characterizing the genes controlling introgressed traits is only a step in determining whether such traits are adaptive and therefore whether a particular example of introgression is adaptive (Lewontin & Birch 1966). Next, it has to be demonstrated whether such traits raise the fitness of the introgressed taxon in its native habitat and/or allows it to invade new habitats. In many cases this is not easy to do, owing to problems associated with tight linkage between the gene(s) of interest and other introgressed genes, and also pleiotropy, which independently or in combination may affect the expression of a wide range of different traits simultaneously.

It is of interest that the introgressant taxon, *S. vulgaris* var. *hibernicus*, has spread rapidly in the British Isles since it was first recorded in 1832 (figure 1*a*), indicating that the radiate condition increases the fitness of an individual in its native habitat. However, the manner in which this trait affects the fitness of an individual is not fully resolved, and appears complex. Marshall & Abbott (1982, 1984*a*) showed that in wild polymorphic populations, the radiate morph has a higher maternal outcrossing rate (6–36%) than the discoid morph (1–15%). This is because pistillate ray florets exhibit greater outcrossing than hermaphroditic disc florets (Marshall & Abbott 1984*b*), and because radiate plants are more attractive to pollinators (Abbott & Irwin 1988). The higher outcrossing rate of the radiate morph places it at an immediate disadvantage to the

highly selfing discoid morph in polymorphic populations, due to the 'cost of outcrossing' that results from the inherent transmission advantage of a selfing gene relative to an outcrossing gene (Fisher 1941; Maynard Smith 1978). Thus, one direct effect of the introgressed trait is to lower the fitness of radiate relative to non-radiate plants!

Studies of factors that may counter this disadvantage and be responsible for the spread of the radiate morph have shown that it frequently produces more seeds per plant relative to the discoid morph both in the wild and under controlled conditions (Oxford & Andrews 1977; Abbott 1985). This difference is not due to inbreeding depression in the discoid morph (Abbott 1985) and is normally of sufficient magnitude to cause the radiate morph to spread to fixation in a population, at least in theory (Marshall & Abbott 1987). However, in certain populations, there is no difference in seed production between the two morphs or the difference is reversed (Abbott 1985; Marshall & Abbott 1987; Abbott & Horrill 1991). Oxford *et al*. (1996) have shown that associations in natural populations between flower head morphs and a suite of developmental, morphological and reproductive characters are largely due to genetic linkage in some populations, and linkage disequilibrium between the *ray* locus and unlinked loci in other populations. They have argued that linkage disequilibrium would be established at the time of the introgressive origin of the radiate morph and will be maintained over a number of generations due to low levels of outcrossing between the two morphs in the wild. However, ultimately, genetic linkages will be broken, linkage equilibrium generated and only associations due to pleiotropy maintained.

Abbott *et al*. (1998) reported that the radiate and discoid morphs of *S. vulgaris* consistently differ in their germination behaviour, with the radiate morph producing a greater proportion of seed showing delayed germination. It is not known whether this consistent association results from tight genetic linkage or pleiotropy. However, it is not due to any difference between seed produced by ray and disc florets of radiate plants as both types exhibit similar germination behaviour. The difference in germination behaviour between the radiate and discoid morphs greatly affects their relative fitness under different conditions in the wild. When mortality of early-germinated seedlings is high, the radiate morph holds advantage, whereas the discoid morph has a much higher relative fitness under conditions of low mortality of early-germinated plants (Abbott *et al*. 1998).

Detailed investigation of the establishment of the radiate variant of *S. vulgaris* in the British Isles suggests that complex genetic and ecological factors are involved. It is feasible that the establishment and maintenance of this morph are largely dependent on its recurrent formation by introgressive hybridization, the type of associations between flower head type and fitness characters generated by this process and maintained over numerous generations, and the opposing force of the 'cost of outcrossing'.

7. CONCLUSIONS

Over the past 500 years, opportunities for hybridization to occur between plant species that were previously geographically and/or ecologically isolated from each other

have risen dramatically. The introduction of plant species by humans, either intentionally or accidentally, to sites where they are non-native has been an important factor in this. Hybridization between introduced species or between introduced and a native species can lead to important evolutionary consequences including interspecific gene flow (introgression). Molecular markers have frequently been used to demonstrate introgression, but now the challenge is to isolate and characterize the genes that control introgressed traits, and to demonstrate when introgression is adaptive. QTL analysis, use of candidate genes and microarrays are three approaches that can be used for isolating and characterizing introgressed genes. Determining whether such genes improve the adaptation of the recipient species to its own or a novel habitat remains an important challenge, and will require detailed examination of the conditions that favour the spread of such genes in the wild. Although we have focused on the situation where introgression of genes controlling a specific trait could improve the adaptation of the recipient species, it should be emphasized that introgression may also improve adaptation in subtly different ways. For example, hybridization and introgression can create variation due to transgressive segregation that may allow adaptation to novel or unique habitats (Rieseberg *et al.* 1999), or raise the fitness of the recipient species by purging genetic load. Future detailed examination of these aspects together with those discussed at length in the present paper, will lead to a much improved understanding of the importance of introgressive hybridization in plant evolution.

REFERENCES

Abbott, R. J. 1985 Maintenance of a polymorphism for outcrossing frequency in a predominantly selfing plant. In *Structure and functioning of plant populations, 2* (ed. J. Haeck & J. Woldendorp), pp. 227–286. Amsterdam: North-Holland Publishing.

Abbott, R. J. 1992 Plant invasions, interspecific hybridisation and the evolution of plant taxa. *Trends Ecol. Evol.* 7, 401–405.

Abbott, R. J. & Forbes, D. G. 2002 Extinction of the Edinburgh lineage of the allopolyploid neospecies, *Senecio cambrensis* Rosser (Asteraceae). *Heredity* 88, 267–269.

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

Abbott, R. J. & Irwin, J. A. 1988 Pollinator movements and the polymorphism for outcrossing rate at the ray floret locus in groundsel, *Senecio vulgaris*. *Heredity* 60, 295–299.

Abbott, R. J. & Lowe, A. J. 1996 A review of hybridisation and evolution in British *Senecio*. In *Compositae: systematics* (ed. D. J. N. Hind), pp. 679–689. London: Royal Botanic Garden.

Abbott, R. J., Ashton, P. A. & Forbes, D. G. 1992 Introgressive origin of the radiate groundsel, *Senecio vulgaris* L. var. *hibernicus* Syme: *Aat-3* evidence. *Heredity* 68, 425–435.

Abbott, R. J., Bretagnolle, F. C. & Thébaud, C. 1998 Evolution of a polymorphism for outcrossing rate in *Senecio vulgaris*: influence of germination behavior. *Evolution* 52, 1593–1601.

Abbott, R. J., James, J. K., Irwin, J. A. & Comes, H. P. 2000 Hybrid origin of the Oxford ragwort, *Senecio squalidus* L. *Watsonia* 23, 123–138.

Abbott, R. J., James, J. K., Forbes, D. G. & Comes, H. P. 2002 Hybrid origin of the Oxford ragwort, *Senecio squalidus* L: morphological and allozyme differences between *S. squalidus* and *S. rupestris* Waldst. & Kit. *Watsonia* 24, 17–29.

Arnold, M. L. 1993 *Iris nelsonii* (Iridaceae): origin and genetic composition of a homoploid hybrid species. *Am. J. Bot.* 80, 577–583.

Arnold, M. L. 1997 *Natural hybridization and evolution*. Oxford University Press.

Arnold, M. L. 2000 Anderson's paradigm: Louisianan irises and the study of evolutionary phenomena. *Mol. Ecol.* 9, 1687–1698.

Ashton, P. A. & Abbott, R. J. 1992 Multiple origins and genetic diversity in the newly arisen allopolyploid species, *Senecio cambrensis* Rosser (Compositae). *Heredity* 68, 25–32.

Brown, J. M. B. 1953 The *Rhododendron* problem in the woodlands of southern England. *Q. J. Forestry* 47, 239–253.

Brown, J. M. B. 1954 Rhododendron ponticum in British woodlands. In *Report forestry reserves 1953*. pp. 42–43. London: HMSO.

Brown, P. O. & Botstein, D. 1999 Exploring the new world of the genome with DNA microarrays. *Nature Genet.* 21(Suppl.), 33–37.

Citerne, H. L., Möller, M. & Cronk, Q. C. B. 2000 Diversity of *cycloidea*-like genes in Gesneriaceae in relation to floral symmetry. *Ann. Bot.* 86, 167–176.

Coats, A. M. 1963 *Garden shrubs and their histories*. London: Vista Books.

Coen, E. S. & Nugent, J. M. 1994 Evolution of flowers and inflorescences. *Development* 1994(Suppl.), 107–116.

Coen, E. S., Nugent, J. M., Luo, D., Bradley, D., Cubas, P., Chadwick, M., Copsey, L. & Carpenter, R. 1995 Evolution of floral symmetry. *Phil. Trans. R. Soc. Lond.* B 350, 35–38.

Comes, H. P. & Abbott, R. J. 2001 Molecular phylogeography, reticulation, and lineage sorting in Mediterranean *Senecio* Sect. *Senecio* (Asteraceae). *Evolution* 55, 1943–1962.

Cross, J. R. 1975 Biological flora of the British Isles: *Rhododendron ponticum*. *J. Ecol.* 63, 345–364.

Cross, J. R. 1981 The establishment of *Rhododendron ponticum* in the Killarney oakwoods, S. W. Ireland. *J. Ecol.* 69, 807–824.

Cubas, P. 2002 The role of TCP genes in the evolution of key morphological characters in Angiosperms. In *Developmental genetics and plant evolution* (ed. Q. C. B. Cronk, J. Hawkins & R. M. Bateman), pp. 247–266. London: Taylor & Francis.

Cubas, P., Lauter, N. & Coen, E. 1999a The TCP domain: a motif found in proteins regulating plant growth and development. *Plant J.* 18, 215–222.

Cubas, P., Vincent, C. & Coen, E. 1999b An epigenetic mutation responsible for variation in floral symmetry. *Nature* 401, 157–161.

Curtis, W. 1803 Rhododendron ponticum. *Bot. Mag.* 16, 650.

Davidian, H. H. 1982 Rhododendron *species I. Lepidote rhododendrons*. New York: Timber Press.

Donoghue, M. J., Ree, R. H. & Baum, D. A. 1998 Phylogeny and the evolution of flower symmetry in the Asteridae. *Trends Plant Sci.* 3, 311–317.

Ellstrand, N. C. & Schierenbeck, K. 2000 Hybridization as a stimulus for the evolution of invasiveness in plants? *Proc. Natl Acad. Sci. USA* 99, 7043–7050.

Ellstrand, N. C., Whitkus, R. & Rieseberg, L. H. 1996 Distribution of spontaneous plant hybrids. *Proc. Natl Acad. Sci. USA* **93**, 5090–5093.

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

Harris, S. A. 2002 Introduction of Oxford ragwort, *Senecio squalidus* L. (Asteraceae), to the United Kingdom. *Watsonia* **24**, 31–43.

Harris, S. A. & Ingram, R. 1992 Molecular systematics of the genus *Senecio* L. 1. Hybridisation in a British polyploid complex. *Heredity* **69**, 1–10.

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

Ingram, R., Weir, J. & Abbott, R. J. 1980 New evidence concerning the origin of inland radiate groundsel, *S. vulgaris* L. var. *hibernicus* Syme. *New Phytol.* **84**, 543–546.

Irwin, J. A. & Abbott, R. J. 1992 Morphometric and isozyme evidence for the hybrid origin of a new tetraploid radiate groundsel in York, England. *Heredity* **69**, 431–439.

James, J. K. 1999 Molecular analysis of the hybrid origin of Oxford ragwort, *Senecio squalidus* L., and related studies. PhD thesis, University of St Andrews, UK.

Jessen, K., Anderson, S. T. & Farrington, A. 1959 The interglacial flora of Killarney, S. W. Ireland; an ecological account. *Proc. R. Irish Acad.* B **60**, 1–77.

Kelly, D. L. 1981 The native forest vegetation of Killarney, S. W. Ireland; an ecological account. *J. Ecol.* **69**, 437–472.

Lewontin, R. C. & Birch, L. C. 1966 Hybridization as a source of variation for adaptation to new environments. *Evolution* **20**, 315–336.

Loudon, J. 1838 *Arboricetum et Fruticetum*, vol. II, part III, pp. 1130–1133. London.

Lowe, A. J. & Abbott, R. J. 1996 Origins of the new allopolyploid species *Senecio cambrensis* (Asteraceae) and its relationship to the Canary Islands endemic *Senecio teneriffae*. *Am. J. Bot.* **83**, 1365–1372.

Lowe, A. J. & Abbott, R. J. 2000 Routes of origin of two recently evolved hybrid taxa: *Senecio vulgaris* var. *hibernicus* and York radiate groundsel (Asteraceae). *Am. J. Bot.* **87**, 1159–1167.

Lowe, A. J. & Abbott, R. J. 2003 A new British species of *Senecio* (Asteraceae), another hybrid derivative of *S. vulgaris* L. and *S. squalidus* L. *Watsonia* **24**, 375–388.

Luo, D., Carpenter, R., Vincent, C., Copsey, L. & Coen, E. 1996 Origin of floral asymmetry in *Antirrhinum*. *Nature* **383**, 794–799.

Luo, D., Carpenter, R., Copsey, L., Vincent, C., Clark, J. & Coen, E. 1999 Control of organ asymmetry in flowers of *Antirrhinum*. *Cell* **99**, 367–376.

Marshall, D. F. & Abbott, R. J. 1980 On the frequency of introgression of the radiate (*Tr*) allele from *Senecio squalidus* L. into *Senecio vulgaris* L. *Heredity* **45**, 133–135.

Marshall, D. F. & Abbott, R. J. 1982 Polymorphism for outcrossing frequency at the ray floret locus in *Senecio vulgaris*. I. Evidence. *Heredity* **48**, 227–235.

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

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

Marshall, D. F. & Abbott, R. J. 1987 Morph differences in seed output and the maintenance of the polymorphism for capitulum type and outcrossing rate in *Senecio vulgaris* L. *Trans. Bot. Soc. Edin.* **45**, 107–119.

Martinsen, G. D., Whitham, T. G., Turek, R. J. & Keim, P. 2001 Hybrid populations selectively filter gene introgression between species. *Evolution* **55**, 1325–1335.

Maynard Smith, J. 1978 The ecology of sex. In *Behavioural ecology: an evolutionary approach* (ed. J. R. Krebs & N. B. Davies), pp. 159–179. Oxford: Blackwell Scientific.

Milne, R. I. & Abbott, R. J. 2000 Origin and evolution of invasive naturalized material of *Rhododendron ponticum* L. in the British Isles. *Mol. Ecol.* **9**, 541–556.

Mooney, H. A. & Cleland, E. E. 2001 The evolutionary impact of invasive species. *Proc. Natl Acad. Sci. USA* **98**, 5446–5451.

Nilsen, E. T. 1991 The relationship between freezing tolerance and thermotropic leaf movement in five *Rhododendron* species. *Oecologia* **87**, 63–71.

Oxford, G. S. & Andrews, T. 1977 Variation in characters affecting fitness between radiate and non-radiate morphs in natural populations of groundsel (*Senecio vulgaris* L.). *Heredity* **38**, 367–371.

Oxford, G. S., Crawford, T. J. & Pernyes, K. 1996 Why are capitulum morphs associated with other characters in natural populations of *Senecio vulgaris* (groundsel)? *Heredity* **76**, 192–197.

Preston, C. D., Pearman, D. A. & Dines, T. D. 2002 *New atlas of the British flora*. Oxford University Press.

Rieseberg, L. H. 1997 Hybrid origins of plant species. *A. Rev. Ecol. Syst.* **28**, 359–389.

Rieseberg, L. H. & Wendel, J. F. 1993 Introgression and its consequences in plants. In *Hybrid zones and the evolutionary process* (ed. R. G. Harrison), pp. 70–90. Oxford University Press.

Rieseberg, L. H., Beckstrom-Sternberg, S. & Doan, K. 1990 *Helianthus annuus* ssp. *texanus* has chloroplast DNA and nuclear ribosomal RNA genes of *Helianthus debilis* ssp. *cucumerifolius*. *Proc. Natl Acad. Sci. USA* **87**, 593–597.

Rieseberg, L. H., Archer, M. A. & Wayne, R. K. 1999 Transgressive segregation, adaptation, and speciation. *Heredity* **83**, 363–372.

Rieseberg, L. H., Baird, S. J. E. & Gardner, K. A. 2000 Hybridization, introgression, and linkage evolution. *Plant Mol. Biol.* **42**, 205–224.

Rosser, E. M. 1955 A new British species of *Senecio*. *Watsonia* **3**, 228–232.

Simons, P. 1988 The day of the *Rhododendron*. *New Scientist* **119**, 50–54.

Soltis, D. E. & Soltis, P. S. 1993 Molecular data and the dynamic nature of polyploidy. *Crit. Rev. Plant Sci.* **12**, 243–273.

Soltis, D. E. & Soltis, P. S. 1999 Polyploidy: recurrent formation and genome evolution. *Trends Ecol. Evol.* **14**, 348–352.

Soltis, P. S. & Soltis, D. E. 2000 The role of genetic and genomic attributes in the success of polyploids. *Proc. Natl Acad. Sci. USA* **97**, 7051–7057.

Stace, C. A. (ed.) 1975 *Hybridisation and the British flora*. London: Academic Press.

Stace, C. A. 1997 *New flora of the British Isles*, 2nd edn. Cambridge University Press.

Stebbins, G. L. 1974 *Flowering plants: evolution above the species level*. Cambridge, MA: Harvard University Press.

Thomson, A. G., Radford, G. L., Norris, D. A. & Good, J. E. G. 1993 Factors affecting the distribution and spread of rhododendron in North Wales. *J. Environ. Mngmnt* **39**, 199–212.

Trow, A. H. 1912 On the inheritance of certain characters in the common groundsel—*Senecio vulgaris*—and its segregates. *J. Genet.* **2**, 239–276.

Turner, J. S. & Watt, A. S. 1939 The oakwoods (*Quercetum sessiflorae*) of Killarney, Co. Kerry, Ireland. *Proc. R. Irish Acad.* **66**, 9–26.

Weir, J. & Ingram, R. 1980 Ray morphology and cytological investigations of *Senecio cambrensis* Rosser. *New Phytol.* **86**, 237–241.

Weising, K., Nybom, H., Wolff, K. & Meyer, W. 1995 *DNA fingerprinting in plants and fungi.* Boca Raton, FL: CRC Press.

Williamson, M. 2002 Alien plants in the British Isles. In *Biological invasions* (ed. D. Pimentel), pp. 91–112. Boca Raton, FL: CRC Press.

Wolff, K. & Peters-Van Rijn, J. 1993 Rapid detection of genetic variability in chrysanthemum (*Dendranthema grandiflora* Tzvelev) using random primers. *Heredity* **71**, 335–341.

Discussion

R. Vinkenoog (*Department of Biology and Biochemistry, University of Bath, Bath, UK*). *Senecio jacobaea* in The Netherlands has two varieties—with and without ray florets. The distribution of these in chalk-rich and chalk-poor dunes suggests a link with fitness.

R. J. Abbott. I am aware of the presence of these two varieties of *S. jacobaea* and their European distribution. I was not aware of them occurring on different types of dune. It would be of interest to determine the underlying basis of this association.

R. Vinkenoog. *Senecio jacobaea* may be another good model for the *CYC*-like gene control of ray floret production.

R. J. Abbott. I have thought of this. It is a logical step to examine the influence of *CYC*-like genes on ray floret production in other *Senecio* once such control is fully established in *Senecio vulgaris*.

Z. J. Chen (*Department of Soil and Crop Science, Texas A&M University, TX, USA*). Is it possible that *CYC*-like genes are epigenetically modified, which contributes to flower variation?

R. J. Abbott. This has been demonstrated in *Linaria vulgaris* (see Cubas *et al.* 1999*b*). It will be of interest to examine this possibility in *Senecio*.

GLOSSARY

cpDNA: chloroplast DNA
ISSR: inter simple sequence repeat
QTL: quantitative trait locus
RAPD: randomly amplified polymorphic DNA
RFLP: restriction fragment length polymorphism