MULTIPLE ORIGINS OF 'SENECIO CAMBRENSIS' ROSSER AND RELATED EVOLUTIONARY STUDIES OF BRITISH 'SENECIO'

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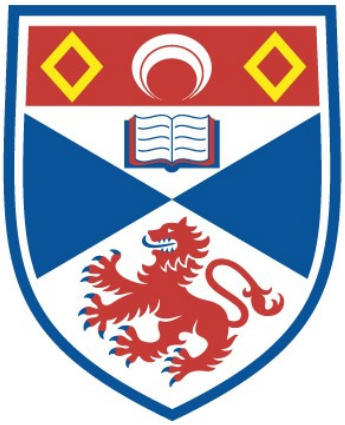
A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1991

Multiple Origins of *Senecio cambrensis* Rosser, and Related Evolutionary Studies In British *Senecio.*

by

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A thesis submitted to the

University of St. Andrews for

the degree of Doctor of Philosophy

Department of Biology and Pre-Clinical Medicine

University of St. Andrews

June 1990

The exercise of the will consumes us; the exercise of power destroys us; but the pursuit of knowledge leaves our infirm constitution in a state of perpetual calm.

Honoré de Balzac, in *La Peau de Chagrin* (*The Wild Ass's Skin*), 1831.

ABSTRACT

The most important finding to emerge from the studies reported in this thesis was the discovery that the newly arisen allopolyploid species, *S. cambrensis* Rosser, has originated on more than one occasion in Britain. A survey of isozyme variation for acid phosphatase (ACP) and α-esterase (α-EST) in *S. cambrensis* (2n = 60) and its putative parents, the Oxford Ragwort, *Senecio squalidus* L. (2n = 20) and the Common Groundsel, *S. vulgaris* L. (2n = 40), produced clear evidence that the Scottish and Welsh populations of *S. cambrensis* have separate origins. In addition, isozyme banding patterns for glutamate oxaloacetate transaminase (GOT) indicated that populations of *S. cambrensis* from Wrexham and Mochdre in N. Wales also represent independent origins of the species in Britain.

Extending the isozyme survey to include other populations of the two parental species led to the confirmation that the radiate allele in *S. vulgaris* has an introgressive origin from *S. squalidus*. Evidence for this came from an analysis of variation at the *Got-1* locus. It was established that the *Got-1a* allele which is present in British *S. squalidus* populations at high frequency also occurs in the radiate morph of *S. vulgaris*, but is virtually absent from the non-radiate morph. The greater allelic variation found at the *Got-1* locus in the radiate morph, compared to the non-radiate morph, was considered to be a direct result

of this introgression. In contrast, at other loci, the non-radiate morph exhibited greater allelic variation than the radiate morph, despite having a higher level of inbreeding. The reduced level of genetic variation in the radiate variant at these loci is presumed to be due to the recent origin of the radiate morph in Britain.

Of additional interest was the finding that *S. squalidus* contains a low level of genetic variation compared with most other outcrossing species that have been surveyed to date, probably due to a genetic bottleneck experienced by the species during its colonisation of Britain. Nevertheless, the level of variation within *S. squalidus* was still higher than that observed in the predominantly selfing *S. vulgaris*.

Finally, evidence from the electrophoretic survey has confirmed the close evolutionary relationship of several other members of *Senecio* section Annui (*S. viscosus, S. sylvaticus, S. vulgaris* ssp. *denticulatus, S. teneriffae* and *S. vernalis*) to *S. vulgaris* var. *vulgaris* and *S. squalidus,* but has failed to support the hypothesis that *S. vulgaris* originated from *S. vernalis* via autopolyploidy.

DECLARATION

I, Paul Allan Ashton, hereby certify that this thesis has been composed by myself, that it is a record of my own work, and that it has not been accepted in partial or complete fulfilment of any other degree of professional qualification.

Paul Ashton

June, 1990.

STATEMENT

I was admitted to the Faculty of Science of the University of St. Andrews under the Ordinance General No. 12 on October 1st, 1985, and as a candidate for the degree of Ph. D. on October 1st, 1986.

Paul Ashton

June, 1990.

CERTIFICATE

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate to the Degree of Ph. D.

R. J. Abbott

June, 1990.

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Dr. Richard J. Abbott and Dr. Ruth Ingram, for their constructive criticism, help and advice throughout the research project. I would also like to thank David Forbes for his technical assistance. The financial assistance of a Natural Environment Research Council research student grant is gratefully acknowledged. Finally I wish to thank Janis, my wife, for her eternal patience and encouragement.

Frontispiece. The allopolyploid *Senecio cambrensis* Rosser (centre) with its putative parents, *S. vulgaris* L., the Common Groundsel (left), and *S. squalidus* L., the Oxford Ragwort (right).

CONTENTS

CHAPTER 1: INTRODUCTION

1. 1. POLYPLOIDY AND PLANT EVOLUTION.

1. 1. 1. The Importance of Polyploidy

Polyploidy, the possession by an organism of more than two sets of homologous chromosomes, is widespread in the plant kingdom. Estimates of the degree of polyploidy in the flowering plants vary depending on the method of calculation. Stebbins (1950) listed 30-35% of angiosperms that were definitely polyploid because they have extant diploid relatives. Later Grant (1963), taking the haploid chromosome value of n>13 as indicative of polyploid origin, estimated that 47% of flowering plants were polyploid (43% dicots, 58% monocots). Since Grant's figure was published much more data on chromosome numbers have become available. Using this increased data base and taking n<11 to be diploid, Goldblatt (1980) has considered that 68% of monocots are polyploid, while Lewis (1980), using similar criteria, listed a figure of 70% for dicots, although he believed that the value may reach 80%, as the data for dicots tend to be less comprehensive than that for monocots. Depending on the figure which is chosen to delimit diploids, the percentage of flowering plants that are polyploid seems to lie between 50 and 70% (Stace, 1987). This generalisation, nevertheless, masks differences between disparate groups of plants, as polyploidy is common in some groups and rare or absent in others (Lewis, 1980).

Traditionally, a distinction has been made between auto- and allopolyploidy, based on whether the doubling of chromosome number is from one genome (autopolyploidy) or more than one (allopolyploidy). Both types of polyploidy are known to have given rise to new species; for instance *Galax urceolata* (Poir.) Brumitt has an autopolyploid origin (Davis and Heywood, 1963), while *Tragopogon miscellus* Ownbey and *T. mirus* Ownbey each have allopolyploid origins (Ownbey, 1950).

The criteria for distinguishing between a species origin via autopolyploidy or allopolyploidy have traditionally been based on chromosome behaviour, fertility, segregation ratios and morphology. However, the relationship between these characters and the type of polyploid origin may be confused. For instance, the high frequency of multivalent formation of autopolyploids when undergoing meiosis, is well known. However, such multivalent formation may not always occur. Soltis and Soltis (1988) found that during meiosis in the autopolyploid *Tolmiea menziesii* (Pursh.) T & G. all chromosomes formed bivalents at diakinesis and metaphase 1. There is also evidence that multivalent formation in an autopolyploid can be suppressed by selection: de Wet and Harlan (1972) reported that experimentally produced tetraploids, of *Zea mays* L. and *Sorghum bicolor* Moench, Meth. with an initial high frequency of multivalents, showed almost complete bivalent formation within a few generations of selection for this trait. This potential lack of accord between the pairing behaviour of

chromosomes and the origin of a species via polyploidy has led to the adoption of newer techniques, such as gel electrophoresis and DNA hybridisation, for the investigation of polyploid origins.

The relative importance of the two types of polyploid origin in plant species evolution is difficult to assess. Stebbins (1947) surveyed several plant groups containing polyploid members and concluded that allopolyploids were far more frequent than autopolyploids, a conclusion endorsed by Grant (1971). Despite the general acceptance of this conclusion, recent electrophoretic evidence has led Soltis and Soltis (1989) to suggest that autopolyploidy is not such an insignificant mode of evolution as previously proposed, at least in some groups, notably the Saxifragaceae.

Despite the accepted importance of polyploidy as a general evolutionary mechanism in plants, the actual number of detailed studies of the phenomenon is small compared to the work published on gradual modes of evolution. Reasons for this are considered below.

1. 1. 2. The Study of Polyploids

Darwin in "The Origin of Species" (1859) explained how selection might act on a species and gradually alter its characteristics until eventually it evolved into a new species. The rediscovery of Mendel's work revealed the mechanism whereby characters are inherited. During the 1930's, the writings of J. B. S. Haldane, Sewall

Wright and R. A. Fisher integrated the work of Mendel and Darwin into the modern synthesis of evolution (see Kimura, 1983; Lewontin, 1974). Subsequently biologists set out to show that evolution by gradual modification does indeed result from a change in gene frequency in response to changing environmental conditions.

The first evidence of such evolutionary change came from studies on animals [e.g. The Peppered moth *Biston betularia* (Kettlewell, 1955)] and this, coupled with the greater number of animal researchers, possibly had the effect of defining the field for plant evolutionists. Thus in studying how plants evolve, workers have tended to search for a unification of evolutionary mechanisms, rather than to seek "as many solutions as there are species...in the plant and animal kingdoms" (Montalenti, 1982). Although gradual evolution is of undoubted importance in the plant kingdom, the figures already quoted for the frequency of polyploids suggest that the sudden origin of new species through polyploidy must be of considerable significance and hence warrants detailed investigation.

There are, of course, considerable practical difficulties in studying the origins of polyploids. These problems stem from the difficulty of actually finding suitable species to work on and, until recently, detailed analysis of origins. Most polyploids are of ancient origin and consequently their evolution is extremely difficult, if not impossible, to reconstruct. The progenitors may be extinct or the derived

polyploid species may have evolved to such a degree since it arose that its origin is obscured. Clearly we need to study polyploids that have arisen in recent historical time, though such polyploids are *per force* extremely rare. The most frequently quoted examples of recently arisen allopolyploids are *Tragopogon miscellus* Ownbey and *T. mirus* Ownbey (Ownbey, 1950), *Spartina anglica* C. E. Hubbard (Marchant, 1967) and, one of the main subjects of this thesis, *Senecio cambrensis* Rosser (Rosser, 1955).

The difficulty of studying polyploids is borne out by the amount of work that has been conducted in attempting to resolve the origin of bread wheat (*Triticum aestivum* L.). The position of this hexaploid species as one of the world's major crops provides an incentive into conducting research into its origin that is matched by few other species. Its ancestry is of particular interest to plant breeders who may wish to introduce new characters into the species. However, despite its obvious importance, the pathway by which breadwheat has originated is still not completely resolved (e.g. Simmonds, 1976; Graur et al, 1989).

When analysing the origin of a polyploid species, a traditional method of studying plants, via morphological analysis, may be of limited use because the characters surveyed frequently exhibit considerable phenotypic plasticity. Other approaches are, therefore, necessary. During the last sixty years several alternative procedures have been developed and incorporated into the current methodology used for studying the

origin of polyploids. Occasionally a single method will yield great success; for instance Muntzing (1930) used a series of crosses to reveal the origin of the natural tetraploid *Galeopsis tetrahit* L. (2n = 32). The two putative parental species *G. pubescens* Beu. and *G. speciosa* Mill (both 2n = 16) were crossed to produce an F1 hybrid that was selfed to produce an F2. One individual of the F2 generation was found to be triploid and was backcrossed to *G. pubescens* to yield a single viable tetraploid F3 individual. This individual was very similar to the wild *G. tetrahit.* and it was concluded that it was via the process of hybridisation and backcrossing that *G. tetrahit* originated in the wild. Such clear cut indications of the origin of a polyploid based on a single methodology are, however, infrequent and the best approach is to use a variety of appropriate methods.

Until the 1960's, researchers had no procedure for assessing gene products which had not been modified by the environment. However, the introduction and rapid development of gel-electrophoresis helped clear this stumbling block. In the 1950's the procedure for detecting enzyme activity in plant homogenates separated by paper chromatography was developed. Refinements over the next few years, including the use of starch gels (Smithies, 1955), improved the technique and broadened the number of enzymes that could be investigated. Initially, the technique was developed by enzymologists to investigate the localisation of enzymes, improve purification and detect their

sensitivity to inhibitors etc. However, the identification of a number of different enzymes with multiple forms (Shaw, 1965) suggested that such variation, termed 'isozyme variation', could be useful in genetical investigations. The work of Hubby and Lewontin (1966), on *Drosophila*, and Harris (1966), on Man, which investigated levels of genetic polymorphism for enzyme coding loci, are generally considered to be the first evolutionary studies to utilise the new technique of gel electrophoresis.

Prior to the development of gel-electrophoresis, geneticists had relied on morphological characters under simple genetic control for the genetic analysis of plant populations and evolutionary events. Often these variants were rare in many species which thus precluded their use in investigations. With the advent of the technique of gel -electrophoresis, genotypes became accessible and large numbers of gene loci could be quickly surveyed. The value of the technique to investigations aimed at determining the origins of polyploids lay in the fact that the gene products of the putative parents could be easily compared to those of the polyploid offspring thus tracing ancestry. The general usefulness of gel-electrophoresis to all evolutionary biologists led to it becoming rapidly established as a major tool in the study of the genetics and evolution of species (Lewontin, 1974).

1. 2. THE GENUS *Senecio*

1. 2. 1. Background.

Senecio is one of the largest plant genera comprising approximately 1500 species (Mabberley, 1987). It is a cosmopolitan, diverse genus with individuals occupying a wide variety of ecological niches encompassing weedy annuals, perennial herbs and trees.

Until a recent review of the genus resulted in the removal of several satellite genera (e.g. *Sinosenecio, Ligularia*) 2000-3000 species were placed in the genus (Willis, 1973). Offsetting the deletion, new species of *Senecio* are continually being discovered in different parts of the world. For instance, Belcher (1983, 1986) has described three new species from Australia; Alva and Zardini (1982) have listed one new Peruvian species; and Cabrera (1984) has recently described seven new Bolivian species.

There is a high degree of variation in genome size in the genus (Lewis, 1980). Major evolutionary lines are typified by smallish chromosomes such as those found in the British members of the section Annui (Crisp, 1972); while both large and very small chromosomes tend to be associated with atypical sections of the genus, such as the African succulents and the African *Dendrosenecio* (Hedb.) Nordenstam (Crisp, 1972).

Senecio has centres of genetic endemism in the Andes, the West Indies and south and tropical Africa (Nordenstam, 1977). However, the

fact that Africa contains all but one of the 14 genera within the Senecioneae that have a base chromosome number of x=5 or 10, implies that this region is the place of origin of the tribe. Diversification of the genus has occurred not at the base chromosome number, but at a higher 'ploidy level. This phenomenon is described by Stebbins (1971) as the secondary cycle of polyploidy. In *Senecio* this has occurred mainly at the tetraploid level, 41% of investigated taxa having 2n=40 (Lawrence, 1980).

Polyploidy is obviously a major method of speciation in the group with at least three major ploidy levels being found on each major continent (Lawrence, 1980). Debate over the base chromosome number of the genus (x=5 or x=10) has focused on the interpretation of the absence or rarity of species with haploid chromosome numbers of n=15, 25 or 35. Ornduff *et al.* (1963, 1967) believed this to be evidence that x=10, with species possessing haploid numbers of less than n=10 being the products of aneuploid reduction. Species at the lower end of an aneuploid reduction series are often annuals (Stebbins, 1950, Grant, 1958) and this is true in those *Senecio* species in which n=5.

Turner and Lewis (1965) considered x=5 to be the base number, with strong selective pressures having prevented the evolution of odd numbered ploidy levels. They were not convinced that the existence of only one species (*S. arenarius* Thunb. n=9) with a haploid chromosome number between 5 and 10 constituted an aneuploid reduction series. Similarly, Lawrence (1980) has argued that the ancestral *Senecio* species

had a base number of x=5, and that this group gave rise to the specialised African annuals in which n=5, and to the species with n=10 by polyploidy. She considered that the absence of species with n=15, 25 etc. as due to the early extinctions of unspecialised plants with n=5.

Regardless of whether the issue has been completely resolved, for practical purposes most workers consider x=10 to be the base number. Consequently *S. vulgaris* with 2n=40 is invariably regarded as tetraploid while *S. squalidus* with 2n=20 is considered diploid.

1. 2. 2. British *Senecio* and the aims of the study

The most recent edition of "The Flora of the British Isles" (Clapham et al, 1987) lists seventeen species of *Senecio* found in Britain. Of these, two have recently become extinct or are almost extinct [*S. congestus* (R. Br.)DC. and *S. paludosus* L.]. Several are recent introductions, occasional aliens or escapees from cultivation and of limited distribution(*S. doria L., S. fluviatilis* Wallr., *S. cineraria* DC., *S. smithii DC., S. mikanoides* Otto and *S. inaequidens* DC.). In the present study, none of these species have been investigated. In addition, the native *S . integrifolius* (L.) Clairv., *S. jacobaea* L., *S. aquaticus* Hill. and *S. erucifolius* L. were also omitted from analysis.

The main body of work has focused on three species of *Senecio* : The native common Groundsel, *S. vulgaris* L., the introduced Oxford Ragwort, *S. squalidus* L. and the allohexaploid species, *S. cambrensis*

Rosser which is believed to have originated following hybridisation between *S. vulgaris* and *S. squalidus*. *S. cambrensis* occupies an almost unique status in the British flora, as along with *Spartina anglica,* it is a polyploid of recent origin whose parentage, appearance and establishment has been subject to some investigation. As such it forms excellent material for the study of polyploid evolution, investigated in this instance using electrophoretic techniques.

In the study reported in this thesis isozymes were used to investigate specific questions regarding the origin of *S. cambrensis* . A major aim was to confirm that *S. cambrensis* is an allopolyploid of *S. vulgaris* and *S. squalidus* and to determine whether there have been several independent origins of the allopolyploid species in Britain. Gel electrophoresis was employed in the analysis, as allopolyploids of recent origin typically exhibit the enzymes that are present in both parents - i. e. an 'additive' phenotype (Crawford, 1983).

In the course of conducting the analysis of origins of different populations of *S. cambrensis,* the survey of isozyme variation in the parental species was broadened to incorporate an analysis of the population genetic structure of both *S. vulgaris* and *S. squalidus.* Finally the survey was extended in a limited way to two related species in Britain, *S. sylvaticus* L. *and S. viscosus* L., and also to two European species, *S. vernalis* Waldst. & Kit., and *S. teneriffae* Schultz-Bip., to assess the degree of relatedness between each of these species and *S.*

vulgaris and *S. squalidus* .

CHAPTER 2 : ENZYME RESOLUTION IN *SENECIO VULGARIS* AND *S. SQUALIDUS* BY MEANS OF STARCH GEL ELECTROPHORESIS

2. 1. INTRODUCTION

Gel-electrophoresis is a technique that enables the separation of proteins by size or charge in a suitable medium whilst an electrical charge is applied to that medium (Lewontin, 1974; Ferguson, 1980). The experimental technique is very simple. An extract of plant proteins is prepared by crushing plant tissue in a buffer that preserves the activity of any enzymes present. This homogenate is then absorbed onto small pieces of filter paper which are inserted into a gel. The gel is usually made from starch or polyacrylamide and a suitable buffer. An electric current is applied to the gel, with contact between the gel and the electrode being mediated by an electrode buffer. After a sufficient amount of time has passed to allow movement of the proteins through the gel, the current is switched off and the gel is cut into several horizontal slices. Each slice is stained for a specific enzyme, after which the presence of an enzyme is revealed by the appearance of coloured bands on the gel.

Success in using the method depends on the suitability of four

the enzyme stain, each of which must be optimised in order to resolve an enzyme as a band on the gel. There is a confusing plethora of recipes in the literature for the extraction, separation and staining of enzymes (e. g. Scandalios, 1969; Brewer, 1970; Soltis *et al.* 1983) and frequently authors report different recipes for the same enzyme systems examined in different species. As Carr and Johnson (1980) have pointed out, there is no established protocol for researchers when starting an investigation on an electrophoretically 'naive' species. Success is largely a matter of perseverance.

At the outset of the present investigation it was decided to use an electrode and a gel buffer i. e. Lithium Borate and Tris citrate buffer respectively (Scandalios, 1969), that had been successfully used by numerous authors for the resolution of isozyme variation within a variety of plant species [e. g. Cardy *et al.* (1981); Gottlieb (1981b, 1984a); Layton and Ganders (1984)]. No attempt was made to apply the techniques developed by Koniuszek and Verkleij (1982) for *Senecio viscosus* and *S. sylvaticus* (close relatives of *S. vulgaris*) using acrylamide gels, as earlier attempts by a previous worker using their methods in our laboratory had proved unsuccessful. The main effort that ultimately led to the resolution of enzymes of *Senecio* on starch gels centred on the development of an appropriate extraction buffer and

stain solution.

In this chapter the procedures that were developed to resolve enzyme activity and variation in *Senecio vulgaris* and *S. squalidus* are described, followed by a description of the isozyme patterns resolved.

2. 2. MATERIALS AND METHODS

2. 2. 1. The development of appropriate extraction and staining procedures

Unless active enzymes are extracted from a plant the subsequent procedures of electrophoresis will fail to resolve enzyme variants on a gel, regardless of the appropriateness of conditions. Consequently an extraction buffer is required which maintains the activity and integrity of the plant enzymes. The main threat to the activity of the enzymes is the action of phenols (Carr and Johnson, 1980), and to prevent such interference an extraction buffer was developed for *S. vulgaris* which contained Polyvinylpolypyrolidone (PVPP) and Mercaptoethanol in a Tris citrate buffer. The PVPP and mercaptoethanol bind to the active site of the phenols, thus preventing contact with enzymes during crushing (Kelley and Adams, 1979). A third chemical performing a similar function was Triton X-100. This had been successfully used by Gottlieb on *Clarkia* (1984a) and was also employed in the present study.

In the development of a suitable extraction buffer these three compounds were included in all possible combinations. Alternatively, only ascorbic acid was included in the Tris citrate buffer as recommended by Soltis *et al.* (1983). Initially all extraction buffer systems were used in an attempt to resolve a limited number of enzymes; Peroxidase (PER), Phosphoglucose Isomerase (PGI), Acid Phosphatase (ACP), Isocitrate

Dehydrogenase (IDH) and Malate Dehydrogenase (MDH). The buffer that included all three phenol eliminating compounds was successful in resolving bands of PGI, ACP and MDH on gels, while the Ascorbic acid extraction buffer was only successful for PER. No success was obtained in the resolution of IDH irrespective of which extraction buffer was utilised.

Having developed appropriate buffers for the extraction of PGI, ACP, MDH and PER, further work involved the use of the same two extraction buffers to resolve additional enzymes of *S. vulgaris* on starch gels. It quickly became apparent that the ascorbic acid buffer was only successful for resolving PER, while it was possible to resolve other enzymes using the alternative extraction buffer system.

Several factors were found to influence the effectiveness of enzyme stain solutions; e. g. the composition of the stain, pH of the staining solution and the gel slice stained. Patient manipulation of these variables usually led to an improvement of staining intensity for a given enzyme. Solutions used to stain enzymes typically consist of three components; the staining buffer, the substrate and the specific dye. The buffer provides the required conditions for the enzyme on the gel to act upon the substrate and alter its chemical nature. This initiates a sequence of reactions that concludes with the dye binding to the site of the enzyme activity, thus revealing its location on the gel. Co-factors or catalysts may also be involved in this pathway.

It was discovered that varying the composition of the dye had little effect on the staining of most enzymes and a standard tetrazolium dye was employed. In contrast, the pH of the solution was found to be important. A compromise has to be found between the potentially different pH optima for the running conditions of the gel, enzyme activity and the stain reaction. Significantly different results could be obtained by varying the pH by just 0. 5. A series of repeat runs for the same enzyme, altering only the pH by this degree, was performed to identify the optimum pH. Increasing the amount of substrate in a recipe tended also to improve resolution, though excess substrate sometimes inhibited enzyme activity. Occasionally, resolution was improved almost by chance. For example, staining for Phosphoglucomutase (PGM) and Glutamate dehydrogenase (GDH) initially produced very faint, or no bands; however, the addition of ATP led to a significant improvement. This emphasises the empirical nature of the development of suitable procedures in electrophoretic work. In the course of optimising the staining procedures, thirty enzyme systems were examined. Of these, approximately half were successfully resolved.

Extracts were initially made from leaves of young healthy plants of *S. vulgaris* at the preflowering or early flowering stage. This was followed by examining a range of plant tissues that had been subject to various pre-treatments. Extracts from leaves, stems, roots, buds and

flowers were tested and it was established that for nearly all enzymes investigated, leaf tissue yielded the best results. Extracts from the other tissues usually produced either blank or faint banding patterns. The one exception concerned esterase enzymes which were best resolved from extracts of bud tissue. For certain enzymes, repotting plants into fresh compost a few days prior to screening, or subjecting them to cold conditions overnight before they were screened, improved resolution. Submerging pots in water to induce Alcohol dehydrogenase (ADH) activity was unsuccessful.

An attempt was made to extract isozymes from the pollen of *S. vulgaris* and *S. squalidus* . The results were disappointing with, at best, only a smudge appearing on the gel. It seems likely that successful extraction of enzymes from pollen requires conditions quite different from those that are successful with leaf material.

2. 2. 2. General Experimental Procedure

Seed from individual plants growing in the wild was sown out in pots containing Levingtons Universal Compost in a glasshouse. After germination, seedlings were thinned out to one per pot and regularly watered. The plants were treated with a range of insecticides and fungicides throughout development. During the course of the various studies, plants were raised under either natural daylight or daylight

supplemented with mercury vapour lighting. In no instance did the minimal photoperiod fall below ten hours.

Material was taken for enzyme extraction at the juvenile or early flowering stage, although material from older plants gave equivalent results. Normally plants were left outside overnight before samples were collected. In a few studies that were conducted towards the end of the research period, extracts were taken from material collected directly from the wild. This material was placed in water before use.

For enzyme extraction, small amounts of leaf tissue (or bud tissue in the case of EST), were placed in the well of an extraction tray to which 1-2 drops of extraction buffer were added. The tray, which had been previously chilled, was placed on ice and samples were crushed with a glass rod. After crushing, samples were placed in a refrigerator for up to half an hour until required.

Gels were prepared on the day they were to be used from an 11% starch solution (Hydrolysed potato starch) containing 33gms of starch and 300ml of gel buffer. The solution was simultaneously swirled and heated over a Bunsen flame until its consistency thickened appropriately. Air bubbles in the liquid gel were evacuated using a vacuum pump and the gel was poured into a glass mould to form a slab approximately 25 x 25 x 6 mm in size. A glass plate was then placed on top of the gel, which was left to cool, initially on a window sill, and later,

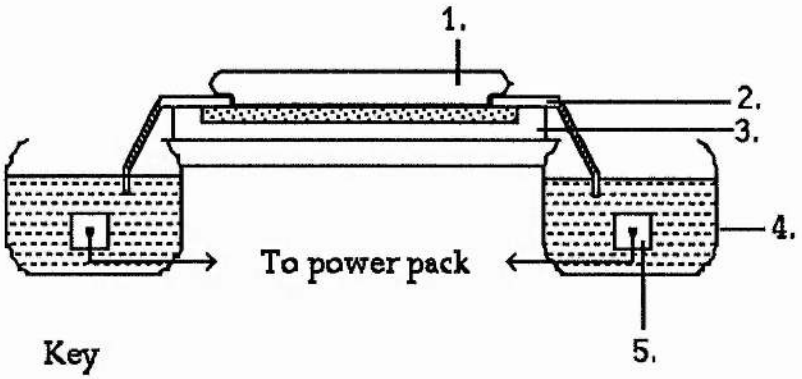
once it had set, in a refrigerator.

Once the gel had set and cooled, the glass plate was carefully removed allowing extracts to be loaded. Running parallel to, and positioned five cm from one end of the gel, a row of slits was cut in the gel into which were inserted squares of Whatman No 5 filter paper (5 x 4 mm) which had been dipped into extract. Gels typically held 25 inserts. A tracker dye of bromophenol blue was placed on one of the inserts, to indicate the "front" on the gel during electrophoresis. The gel was covered with cling film to prevent drying out and placed on the electrode tray (see Fig. 2. 1.). Cellulose sponges on each end of the gel provided contact between the gel and the electrode buffer which was contained in reservoirs at each end of the tray. An ice bag was placed on the gel, which together with the gel tray was placed in a refridgerator. Electrodes within each reservoir on the gel tray were connected to a power pack and a current of 70mA, 250v constant voltage, was supplied to each gel for approximately five hours. At the end of a run when the tracker dye had moved 8cm from the origin towards the anode, the power was switched off and the gel was removed for staining.

Before staining, wicks were removed and the slab containing the separated enzymes was cut into five thin horizontal slices with fishing line, yielding slices which were 1-2 mm thick. The top and bottom slices were discarded, leaving three slices which were placed in glass trays to

which an appropriate enzyme stain was added. Stains were made up immediately prior to use to prevent loss of activity. As noted above, some stains only produced optimal results on a specific slice. Gels were incubated at 37°C until bands were resolved. They were then removed from the stain, rinsed twice in tap water and preserved in a 1:1 solution of glycerol and water. For some systems the glycerol/water solution required two changes in order to clear the gel of background staining.

By the end of the period that was given over to the development of procedures for resolving enzymes of *S. vulgaris* and *S. squalidus* on starch gels (approximately twelve months), a total of 30 different enzyme systems had been examined (see Table 2. 1.). Of these, successful resolution was achieved for 13 systems and partial success with a further three. Full details of the components of the extraction, gel and electrode buffers and staining solutions used in the successful or partially successful resolution of *S. vulgaris* and *S. squalidus* enzymes are given in the Appendix.



Key

Starch gel containing plant extracts

Electrode buffer

1. Ice bag
2. Sponges connecting electrode buffer to the gel.
3. Gel mould
4. Electrode buffer reservoir
5. Platinum electrode

Fig 2. 1. Diagrammatic representation of the electrophoretic apparatus for separating enzymes in starch gels.

Table 2. 1. Enzymes stained and source of stain recipe

Enzyme	Abbrev.	E. C. No.	Stain reference
Successfully resolved enzymes			
Acid Phosphatase	ACP	3. 1. 3. 2.	Ad*. Scandalios (1969)
Glutamate Oxaloacetate Transaminase	GOT	2. 6. 1. 1.	Ad. Gottlieb (1973)
Peroxidase	PER	1. 11. 1. 7.	Graham *et al.* (1964)
α-Esterase	α-EST	3. 1. 1. 1.	Scandalios (1969)
β-Esterase	β-EST	3. 1. 1. 1.	Scandalios (1969)
Phosphoglucose Isomerase	PGI	5. 3. 1. 9.	Tanksley (1980)
Phosphoglucomutase	PGM	2. 7. 5. 1.	Tanksley (1979)
Glyceraldehyde-3-Phosphate Dehyd.	G-3-PD	1. 2. 1. 12.	Scandalios (1969)
6-Phosphogluconate Dehydrogenase	6-PGD	1. 1. 1. 44.	Sing & Brewer (1969)
Glucose-6-Phosphate Dehydrogenase	G-6-PDH	1. 1. 1. 49.	Sing & Brewer (1969)
Malic Enzyme	ME	1. 1. 1. 40.	Soltis *et al.* (1983)
Malic Dehydrogenase	MDH	1. 1. 1. 37.	Brown *et al.* (1978)
Glutamate Dehydrogenase	GDH	1. 4. 1. 2.	Hartmann *et al.* (1973)
Partially resolved enzymes			
Hexokinase	Hex	2. 7. 1. 1.	Ad. Eaton (1966)
Aldolase	ALD	4. 1. 2. 13.	Soltis *et al.* (1983)
Triose Phosphate Isomerase	TPI	5. 3. 1. 1.	Brewer (1970)
Unresolved enzymes			
Adenylate Kinase	ADKIN	2. 7. 4. 3.	Scandalios (1969)
Alcohol Dehydrogenase	ADH	1. 1. 1. 1.	Tanksley (1979)
Alkaline Phosphatase	ALKPH	3. 1. 3. 1.	Scandalios (1969)
Fumarase	FUM	4. 2. 1. 2.	Brewer (1970)
Galactose Dehydrogenase	GaDH	1. 1. 1. 48.	Cuatrecasas*et al.* (1966)
Glucose Oxidase	GO	1. 1. 3. 4.	Kilburn &Taylor (1969)
Isocitrate Dehydrogenase	IDH	1. 1. 1. 42.	Fine & Costello (1963)
Lactate Dehydrogenase	LDH	1. 1. 1. 27.	Shaw & Prasad (1970)
Leucine Amino Peptidase	LAP	3. 4. 11. 1.	Scandalios (1969)
Shikimic Dehydrogenase	Sh.DH	1. 1. 1. 25.	Tanksley & Rick (1980)
Succinate Dehydrogenase	Su.DH	1. 3. 49. 1.	Brewer (1970)
Superoxide Dismutase	SOD	1. 15. 1. 1.	Scandalios (1969)
Tyrosinase	TYR	1. 10. 3. 1.	Scandalios (1969)
Xanthine Dehydrogenase	XDH	1. 2. 1. 37.	Brewer (1970)

*Ad = Adapted from.

2. 3. RESULTS.

The results presented below describe the isozyme patterns that were clearly resolved on starch gels. These results summarise the findings obtained from a survey of almost 1000 individuals of *S. vulgaris* derived from 15 different populations, and over 650 specimens of *S. squalidus* from 20 populations. In addition to the description given, an interpretation is made of the genetic basis of each banding pattern and of any variation that occurred.

Isozymes, as defined by Markert and Moller (1959), are multiple molecular forms of particular enzymes. Isozymes coded by different alleles of the same gene are termed allozymes (Prakash et al., 1969). Where two or more isozymes are resolved on a gel and are considered to be the products of genes at different loci, they are denoted *isozyme-1*, *isozyme-2* etc. with *isozyme-1* being that which has run most slowly through the gel, remaining nearest to the origin. Allozymes coded by different alleles of the same gene are differentiated by lower case letters such that an allozyme denoted with an *a* is that positioned nearest to the origin. Occasionally blanks were present on gels where bands were expected. These were interpreted as due to the action of a null allele at a locus, i. e. an allele which fails to produce an active enzyme product. Such null alleles are denoted with an *n* . The relative mobilities of the various allozymes present in each enzyme system (R_f values) are listed in Table 2. 2., along with the sub-unit number of the enzyme, where this

has become apparent during the study. In addition to the plate accompanying the description of each enzyme system, the isozyme patterns observed for each species are summarised as zymograms in Fig. 2. 3.

Enzymes showing banding variation within either *S. vulgaris* or *S. squalidus* .

Acid Phosphatase

Staining for ACP revealed three zones of high enzyme activity (see Plate 2. 1.). The zone nearest to the origin, *Acp-1* , appeared to be composed of three bands and exhibited no variation in either *S. vulgaris* or *S. squalidus* . The most anodal band, *Acp-3* , showed only weak activity, and appeared to consist of a single band. The most interesting region of activity was in the zone denoted as *Acp*-2 . Within this region individuals of *S. squalidus* produced either a single slow allozyme-*Acp-2a* , a single fast allozyme-*Acp-2b* , both allozymes plus an intermediate band (i. e. were triple banded) or no bands at all. The variation present in the *Acp-2* region was interpreted as the product of allelic variation at a single locus (hereafter referred to as the *Acp*-2 locus). In *S. squalidus* three alleles were considered to occur at this locus; a slow allele *Acp-2a* , a fast allele *Acp-2b* and a null allele *Acp-2n*. Triple banded individuals, producing both fast and slow allozymes plus the additional intermediate isozyme were interpreted as heterozygous for the *Acp-2a* and *Acp-2b* alleles. This implies that in *Senecio* ACP is a

dimeric enzyme. In *S. vulgaris* all individuals tested produced only the fast allozyme at the *Acp-2* locus. *S. vulgaris* can therefore be regarded as monomorphic for the *Acp-2b* allele.

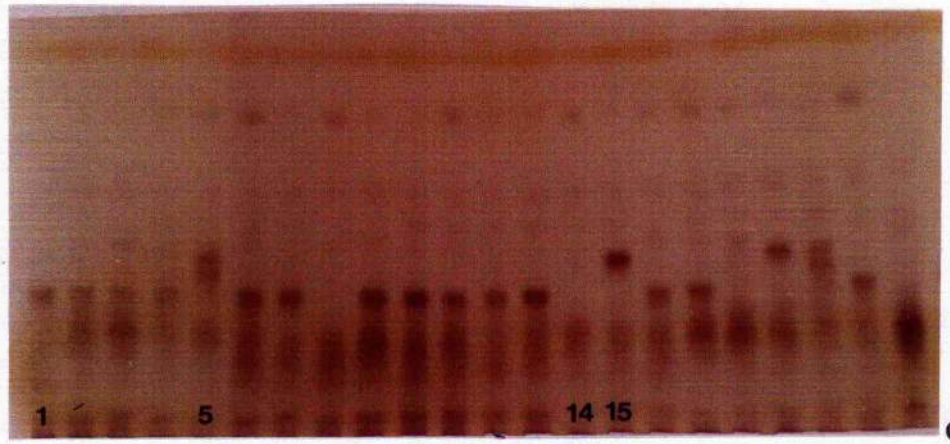


Plate 2.1 Banding patterns for ACP in *S. squalidus* . At the *Acp-2* locus three alleles are believed to occur - *Acp-2a* producing the slow allozyme (track 1), *Acp-2b* producing the fast allozyme (track 15) and *Acp-2n* yielding no allozyme (track 14). The triple banded *Acp-2* (track 5) phenotype represents the heterozygote *Acp-2a* / *Acp-2b* .

Glutamate Oxaloacetate Transaminase - GOT

Three zones of activity (see Plates 2. 2. a and b) were detected on gels stained for GOT. Two of these zones (*Got-2* and *Got-3*) were located close to each other, approximately equidistant between the origin and the anodal front of the gel (Plate 2. 2a). In both *S. vulgaris* and *S. squalidus* , each of these zones contained either a single band or no band at all. Individuals were frequently double banded, producing both the *Got-2* and *Got-3* isozymes, or were single banded, expressing only the band

closest to the origin (*Got-2*), or that closest to the anode (*Got-3*). However, no individuals were found that lacked both of these bands. This pattern of banding is not easily explained in terms of a simple genetic model as will be discussed more fully in the next chapter.

Variation in the zone of activity closest to the origin (*Got-1*) is more easily understood (Fig. 2. 2, Plate 2. 2b.). Within this zone *S. squalidus* individuals produced either a single slow allozyme, *Got-1a*, a single fast allozyme, *Got-1b* or were triple banded, exhibiting both allozymes and an intermediate band. This pattern of variation in *S. squalidus* is considered to be the product of two alleles at a single locus; a slow allele, *Got-1a* and a fast allele, *Got-1b*. The *Got-1a* / *Got-1b* genotype produces both fast and slow allozymes plus an additional intermediate allozyme to exhibit a triple banded phenotype. Triple banded phenotypes produced by heterozygotes would be evidence that GOT is a dimeric enzyme in *Senecio* .

S. vulgaris individuals at the *Got-1* locus were typically three banded, although six banded phenotypes were also found (Fig. 2. 2b). A total of three different three banded phenotypes were identified, each seemingly the product of a genotype in which heterozygosity was fixed. Such individuals would possess two copies of the *Got-1* locus being homozygous for different alleles at each of these two loci. Duplicated copies of a locus may be expected to occur in *S. vulgaris* which is tetraploid. Two of the allozymes (*Got-1a* and *Got-1b*) produced by *S. vulgaris* were also found in *S. squalidus* . A third allozyme, *Got-1c*, less

proximal to the origin than the other two, was unique to *S. vulgaris* . As shown in Fig. 2. 2. *S. vulgaris* individuals produced either the *Got-1a* and *Got-1b* allozymes, the *Got-1a* and *Got-1c* allozymes or the *Got-1b* and *Got-1c* allozymes. In addition to the two allozymes produced by each individual, a third intermediate band was also observed. The genotypes that produced these phenotypes are considered to be *Got-1a / Got-1b* ; *Got-1a / Got-1c* ; and *Got-1b / Got-1c* respectively. The absence of any single banded types in *S. vulgaris* at this locus suggests that the heterozygote is inherited as a single character i. e. individuals are 'fixed' heterozygotes.

Rarely, a six banded phenotype was observed in *S. vulgaris*. (Fig. 2. 2). This may be considered as the product of a cross between parents that differ in their fixed heterozygous genotype. The presence of all three alleles in such individuals (*Got-1a , Got-1b* and *Got-1c*) plus the three intermediate bands formed by the hybridisation of subunits produced by each allele, leads to the appearance of six bands on the gel.

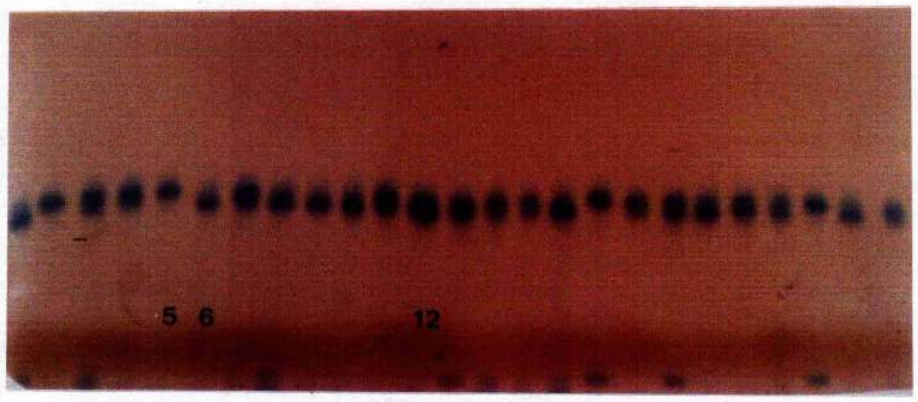


Plate 2. 2a. Banding patterns for *Got-2* and *Got-3* . Individuals may possess bands at both of these loci (e. g. track 12) or may be single banded, with only one of the two loci producing a band (e. g. track 6 showing the *Got-2* band and track 5, containing the *Got-3* product).

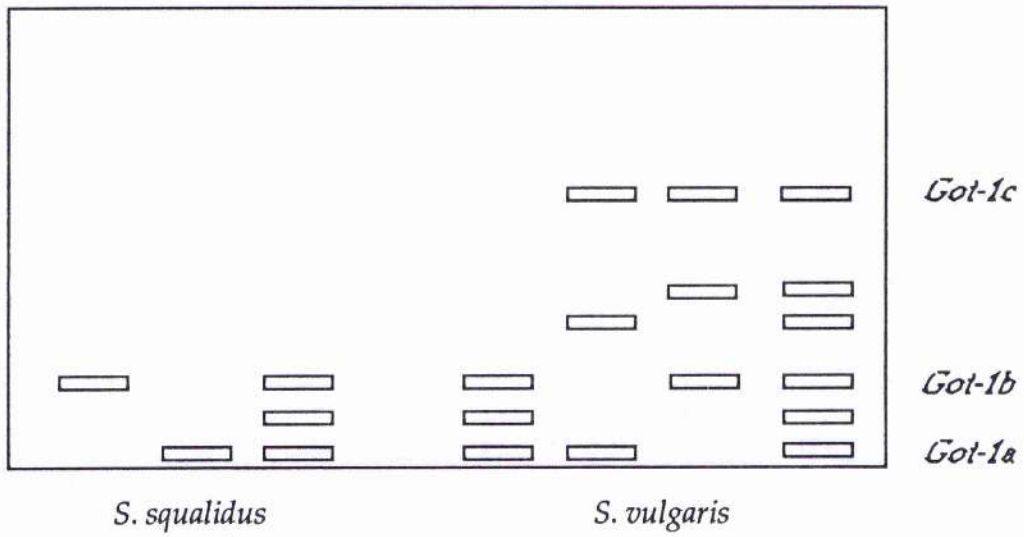
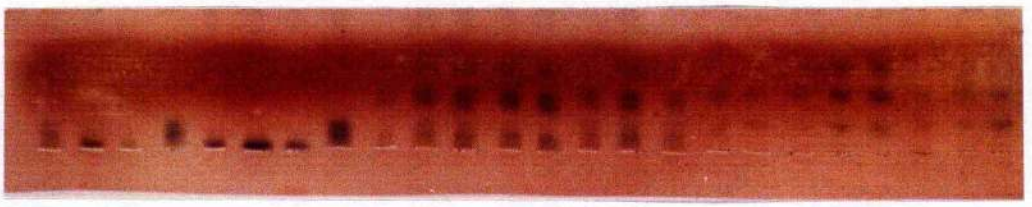


Fig. 2. 2. Zymogram of contrasting banding patterns found at the *Got-1* locus in *S. squalidus* and *S. vulgaris* . Note the three triple-banded patterns in *S. vulgaris* plus the 'hybrid' six banded pattern.



S. squalidus *S. vulgaris*

Plate 2. 2b Banding patterns at the *Got-1* locus. Tracks 1 - 8 are from *S. squalidus*, tracks 9 - 24 are from *S. vulgaris*. The *S. squalidus* individuals on tracks 1, 4 and 8 are heterozygous exhibiting the *Got-1a* allozyme, the *Got-1b* allozyme plus a third intermediate band. The other *S. squalidus* individuals (e. g. track 6) are single banded exhibiting only the *Got-1a* allozyme.

Two banding patterns are evident among the *S. vulgaris* individuals. The plants on tracks 17 - 24 exhibit the *Got-1b/Got-1c* fixed heterozygote, with allozymes appearing at at the *Got-1b* and *Got-1c* positions plus a third band intermediate to these two. Individuals on tracks 9 - 16 have six bands, with *Got-1a*, *Got-1b* and *Got-1c* allozymes present plus three bands corresponding to the intermediate position of the three pairs of allozymes (i. e. *Got-1a/Got-1b*, *Got-1a/Got-1c* and *Got-1b/Got-1c*).

α-Esterase - α-EST

α-Esterase activity was detectable over much of the area of each gel stained for the enzyme (see Plate 2. 3.). However, most of the activity was typically faint and was not resolved into bands. Two populations of *S. vulgaris* possessed an apparently simple banding pattern in the zone close to the anodal end of the gel (*α-Est-2*). Individuals in these populations possessed either a slow band (*α-Est-2a)* , a fast band (*α-Est-2b)* or both of these bands. This pattern suggested single locus control with two alleles segregating at the locus.

Strongly stained bands of α-Esterase were obtained for *S. vulgaris* within a zone close to the origin. Within this zone *S. vulgaris* individuals produced either a single slow allozyme, *α-Est-1a,* a single fast allozyme, *α-Est-1b,* or both allozymes. This variation was considered to be due to the presence of two alleles, a slow allele (*α-Est-1a)* and a fast allele (*α–Est-1b*) segregating at a locus designated as *α-Est-1*. It is assumed that heterozygotes, *α-Est-1a / α-Est-1b,* produced both fast and slow allozymes. The two banded heterozygous phenotype at this locus, and at the *α–Est-2* locus, within some populations, leads to the conclusion that α-EST is a monomeric enzyme in *Senecio* .

No *S. squalidus* individuals surveyed produced isozymes at either of the two α-EST loci resolved in *S. vulgaris* .

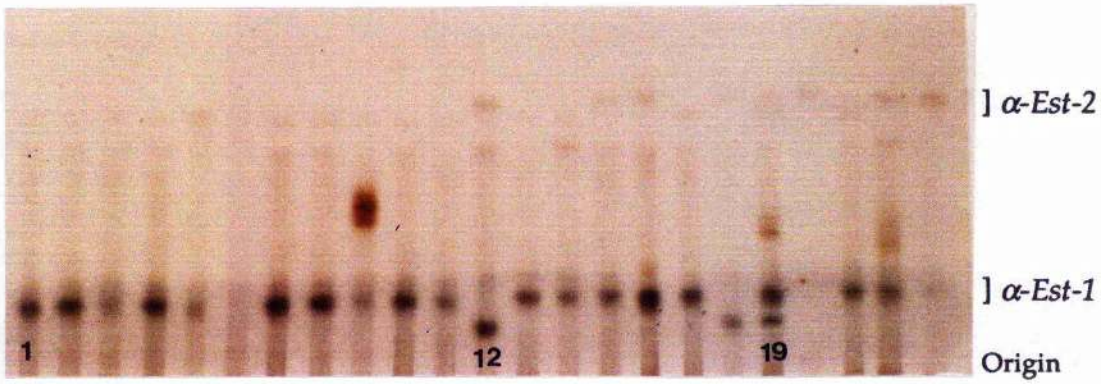


Plate 2. 3. α-EST banding patterns in *S. vulgaris* . At the *α-Est-1* locus two alleles occur; *α–Est-1a* producing the slow allozyme (track 12) and *α–Est-1b* producing the fast allozyme (track 1). The double banded phenotype (track 19) represents the heterozygous genotype *α-Est-1a* / *α-Est-1b*. Note also the variation at the *α-Est-2* locus; track 1 has a slow allozyme, track 12 the fast allozyme and track 19 the double banded fast and slow allozymes.

β- Esterase - β- EST

Faint enzyme activity was found over much of the area of each gel stained for β-Esterase. Distinct bands of the enzyme were clearly resolved within three zones (see Plate 2. 4.). The most anodal of these

three zones was designated as *β-Est-3*, and was present only in *S. squalidus* . Often two bands occurred together in the *β-Est-3* zone, although single bands were also observed. There was significant variation in the strength of staining within this zone and consequently the banding variation was not subjected to a detailed survey over populations.

Within the two other zones of activity - *β-Est-1* and *β-Est-2* - clear banding patterns were observed. The *β-Est-1* zone was located relatively close to the origin, while the *β-Est-2* zone was approximately equidistant between the *β-Est-1* and *β-Est-3* zones. Occasionally odd single bands appeared in the zone between *β-Est-1* and *β-Est-2*, however these stained inconsistently and were ignored.

Six *β-Est-1* phenotypes were recorded in *S. squalidus* (Plate 2. 4 and Fig 2. 3). Individuals produced either a single allozyme of which there were three types; a slow (*β-Est-1a*), a medium (*β-Est-1b*) and a fast (*β-Est-1c*), or were double-banded for any two of the three allozymes. All three possible double banded phenotypes were recorded (*β-Est-1a* / *β-Est-1b; β-Est-1a* / *β-Est-1c* ; and *β-Est-1b* / *β-Est-1c*). The pattern of variation within the *β-Est-2* zone in *S. squalidus* (Fig. 2. 3.) was

identical to that found at *β-Est-1* , with six phenotypes - three single banded and three double banded types - resulting from the various permutations of the slow (*β-Est-2a*), medium (*β-Est-2b*) and fast (*β-Est-2c)* allozymes. No triple banded loci were recorded at either the *β-Est-1* or the *β-Est-2* loci.

The patterns of variation within the *β-Est-1* and *β-Est-2* zones were interpreted as the product of allelic variation at two different loci. At each locus three alleles are believed to occur; a 'slow' allele, a 'medium' allele and a 'fast' allele. These have been designated as *β-Est-1a, β-Est-1b* and *β-Est-1c* respectively at the *β-Est-1* locus, and *β-Est-2a, β-Est-2b* and *β-Est-2c* respectively at the *β-Est-2* locus. Heterozygotes are double banded, indicating that, in *Senecio* β-EST is a monomeric enzyme.

The pattern of variation at the *β–Est–1* and the *β-Est-2* loci in *S. vulgaris* was similar to that found in *S. squalidus* . However only three phenotypes were recorded at each locus due to the absence of the *β-Est-1c* and *β-Est-2c* allozymes. Consequently, at the *β-Est-1* locus, *S. vulgaris* individuals exhibited either the *β-Est-1a* or the *β-Est-1b* single banded allozyme or were double banded producing both allozymes. An identical

situation existed at the *β-Est-2* locus.

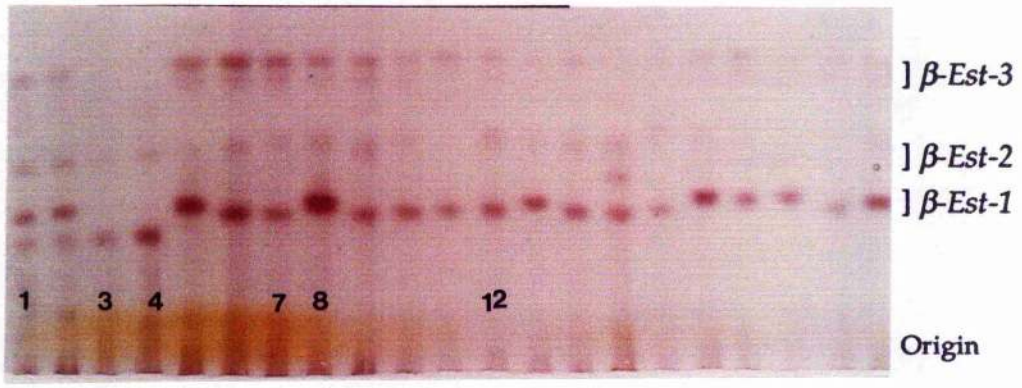


Plate 2. 4. Banding variation in gels stained for β-EST .

Clearly observed on this gel at the *β-Est-1* locus are the slow allozyme,*β-Est-1a*, (track 3), the medium allozyme, *β-Est-1b* , (track 7) and the fast allozyme, *β-Est-1c* , (track 8), together with the double banded slow/ medium heterozygote, *β-Est-1a / β-Est-1b* , (track 1). At the *β-Est-2* locus the slow allozyme, *β-Est-2a* , (track 1), the fast allozyme, *β-Est-2c*, (track 7) and the double banded medium / fast heterozygote, *β-Est-2b / β-Est-2c* , (track 12) are evident.

Note also that tracks 3 and 4, containing material from *S. vulgaris* , lack the *β-Est-3* bands, which were produced by *S. squalidus* individuals in the other tracks.

Phosphoglucose Isomerase - PGI

There were two distinct zones of enzyme activity on gels stained for PGI (see Plate 2. 5. and Fig 2. 3). An additional band of activity

occasionally occurred between these two zones but was inconsistent in its appearance (see Chapter 3). The most anodal zone of activity was monomorphic for a single band (*Pgi-2*) in both species. In *S. vulgaris* three different banding patterns were recorded within the zone of activity closest to the origin (*Pgi-1*). Within this zone, individuals of *S. vulgaris* produced either a single allozyme (*Pgi-1b*) or one of two triple banded phenotypes. Both triple banded phenotypes contained the *Pgi-1b* allozyme and in addition, either a slower allozyme (*Pgi-1a*) plus an intermediate band, or a faster allozyme (*Pgi-1c*) plus an intermediate band. It is proposed that there are two loci controlling the expression of PGI (*Pgi-1* and *Pgi-2*) of which *Pgi-1* is polymorphic and *Pgi-2* is monomorphic. The lack of individuals that are single banded for the *Pgi-1a* and the *Pgi-1c* allozymes suggests that individuals which are triple banded at the *Pgi-1* locus contain two copies of the *Pgi-1* gene, each of which produces a slightly different form of *Pgi-1* enzyme. Individuals which possess this gene duplication exhibit 'fixed heterozygosity' for *Pgi-1* and are therefore considered homozygous for different alleles at each locus. PGI has been shown to have a dimeric structure in all plant species in which it has been studied, hence the presence of the intermediate band and triple banded phenotype of the fixed heterozygotes in *S. vulgaris* . It is possible that individuals which are single banded for *Pgi-1b* also possess the gene duplication but the action of one of the genes is suppressed or both loci are homozygous for

Pgi-1b .

In *S. squalidus* *Pgi-1* was monomorphic, with all individuals producing only the *Pgi-1b* allozyme.

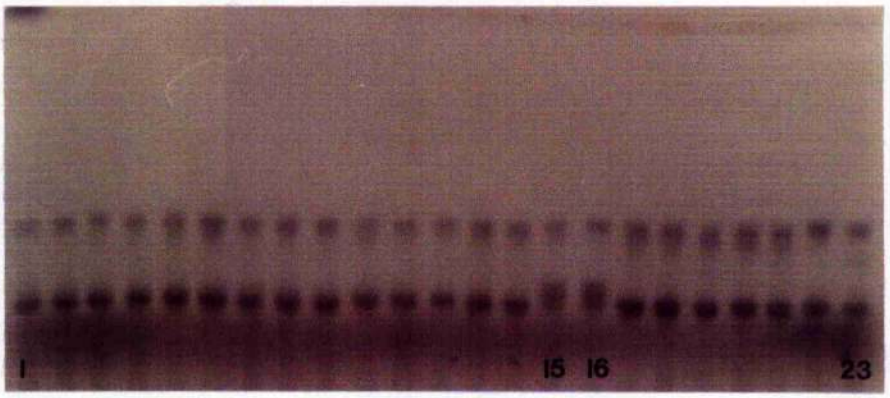
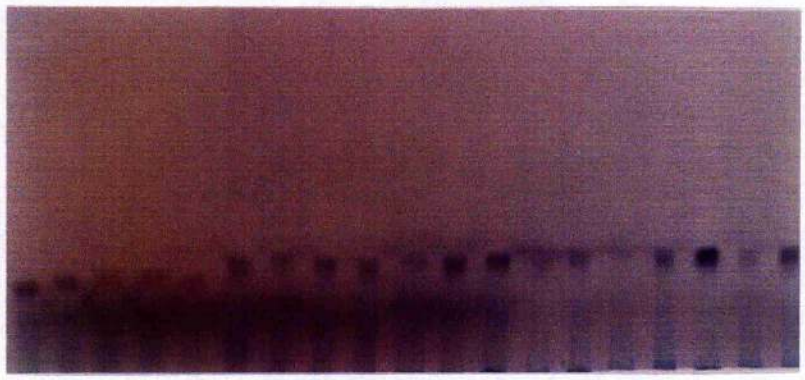


Plate 2. 5. Isozyme variation in PGI in *S. vulgaris* .
S. vulgaris is monomorphic for the *Pgi-2* isozyme. Within the *Pgi-1* zone, individuals may produce single bands (track 1), of the *Pgi-1b* isozyme, or be triple banded. Tracks 15 and 16 show the triple banded phenotype produced by *Pgi-1b / Pgi-1c* fixed heterozygotes. The phenotype produced by *Pgi-1a / Pgi-1b* heterozygotes is not shown on the plate. The additional band of PGI occasionally observed in extracts of some *S. vulgaris* individuals is present in track 23 as a faint band between the *Pgi-1* and *Pgi-2* zones.

Enzymes that are monomorphic within species but show polymorphism between *S. vulgaris* and *S. squalidus.*

Glutamate Dehydrogenase - GDH

On gels stained for GDH only one zone of activity was resolved (see Plate 2. 6. and Fig 2. 3.). In both *S. vulgaris* and *S. squalidus* this appeared as a single band; however, the band was located closer to the origin in *S. vulgaris* . This single band can be viewed as the product of a single locus (*Gdh-1*) which is fixed for different alleles in the two *Senecio* species.



] *Gdh-1*

Origin

[-*S. vulg.* -] [----------*S. squalidus* ----------]

Plate 2. 6. The GDH bands of *S. vulgaris* and *S. squalidus* . Tracks 1-5 show the band produced by *S. vulgaris* , which is closer to the origin than the band produced by *S. squalidus* (all other tracks).

Enzymes showing no variation within or between *S. vulgaris* and *S. squalidus* .

The enzyme systems described below were resolved clearly on starch gels but showed no variation either within or between *S. vulgaris* or *S. squalidus*. Because of the lack of variation at these loci it is not possible to speculate on the structure of these enzymes in *Senecio* .

Phosphoglucomutase - PGM

Two zones of enzyme activity occurred on gels stained for PGM (see Plate 2. 7. and Fig 2. 3). A third band also occasionally appeared, closer to the origin than the other two bands. It is considered that the two bands of PGM which stained consistently were coded for by two separate loci, designated as *Pgm-1* and *Pgm-2* . These loci were monomorphic in both *S. vulgaris* and *S. squalidus,* with only single bands being produced.

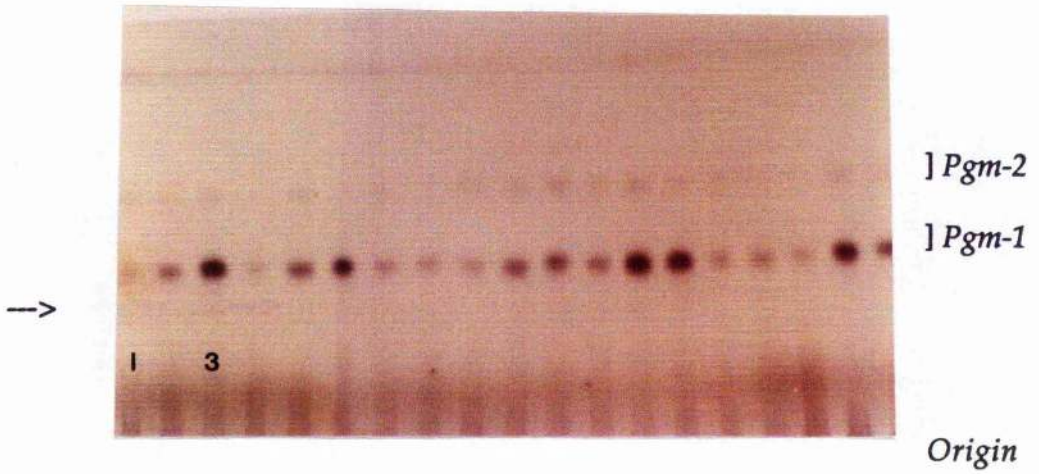


Plate 2. 7. PGM bands in *S. squalidus* . All tracks show single banded phenotypes for *Pgm-1* and *Pgm-2* . Tracks 1 and 3 contain an additional faint band of PGM (arrowed) found in some individuals.

Peroxidase - PER

PER was unique among the enzymes surveyed in that the isozymes detected moved towards the cathodal rather than the anodal end of the gel. Gels stained for PER exhibited an intensely stained band (see Plate 2. 8. and Fig 2. 3.), which was uniform, and located approximately midway between the origin and the cathodal end of the gel. Additional activity close to the origin also occurred, but was faint and poorly defined. A second band, produced closer to the cathodal end, appeared intermittently in some individuals but was ignored in the subsequent surveys of isozyme variation in the populations. The single band of *Per-1* was interpreted as the product of a monomorphic locus in

both *S. vulgaris* and *S. squalidus* .

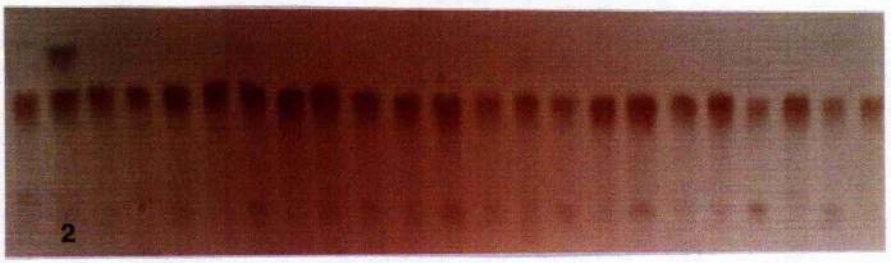


Plate 2. 8 . PER banding pattern in *S. vulgaris* and *S. squalidus* . Note the additional, more cathodal, band in track 2.

Malate dehydrogenase - MDH

There were two zones of activity on gels stained for MDH (see Plate 2. 9. and Fig. 2. 3.). These appeared as two bands situated close together, and located approximately halfway between the origin and the anodal end of the gel. The slower of the two bands was always slightly diffused and much thicker than the faster band. The two bands of MDH are taken as the products of two separate loci, *Mdh-1* (the lower, thicker band) and *Mdh-2*, both of which are monomorphic in *S. vulgaris* and *S. squalidus*.

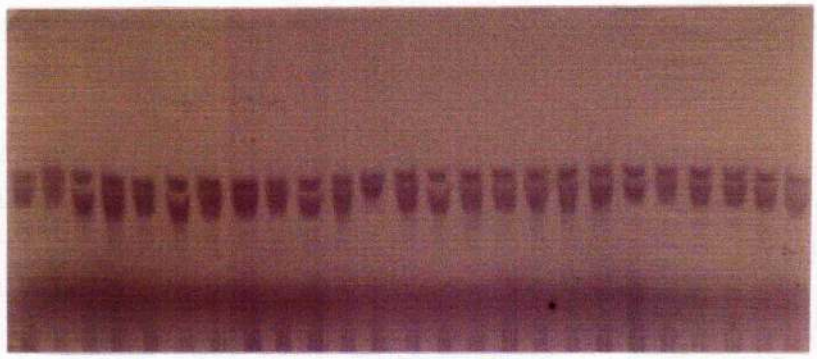


Plate 2. 9. Bands of MDH found in both *S. vulgaris* and *S. squalidus* . The bands are considered to be the products of two separate, monomorphic loci (*Mdh-1* and *Mdh-2*).

Malic enzyme - ME

Staining gels for the presence of ME activity revealed two zones of staining (Plate 2. 10. and Fig 2. 3.). The first zone was close to the origin and faintly stained, though clear single bands appeared sporadically. The second zone contained a single uniform band, which was considered to be the product of a monomorphic locus designated as*Me-1* .

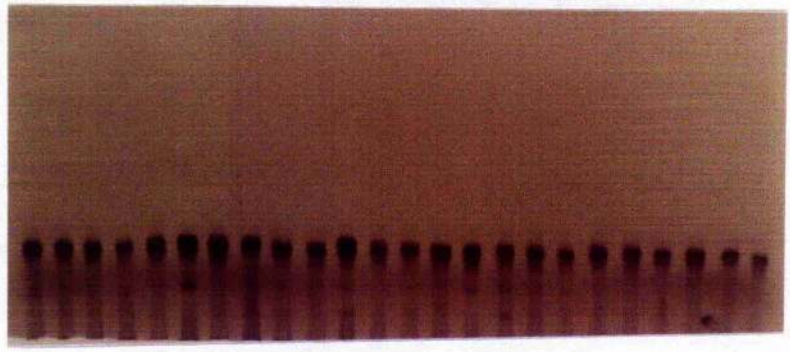


Plate 2. 10. The uniform single band produced on gels stained for ME. Note the additional faintly stained band midway between the origin and *Me-1* .

Glyceraldehyde-3-Phosphate dehydrogenase - G-3-PD

Staining gels for G-3-PD (Plate 2. 11. and Fig 2. 3.) revealed two zones of activity, each of which appeared as a single band. The band closer to the anodal end of the gel (*G-3-pd-2*) was only occasionally present on gels, and was never strongly stained (It is not illustrated in Plate 2. 11.). The second band was located nearer to the origin and was always quite distinct. The two bands were taken to be the product of two separate monomorphic loci (*G-3-pd-1* and *G-3-pd-2*). The sporadic appearance of the *G-3-pd-2* band was probably due to the isozyme requiring different electrophoretic conditions to the *G-3-pd-1* isozyme.

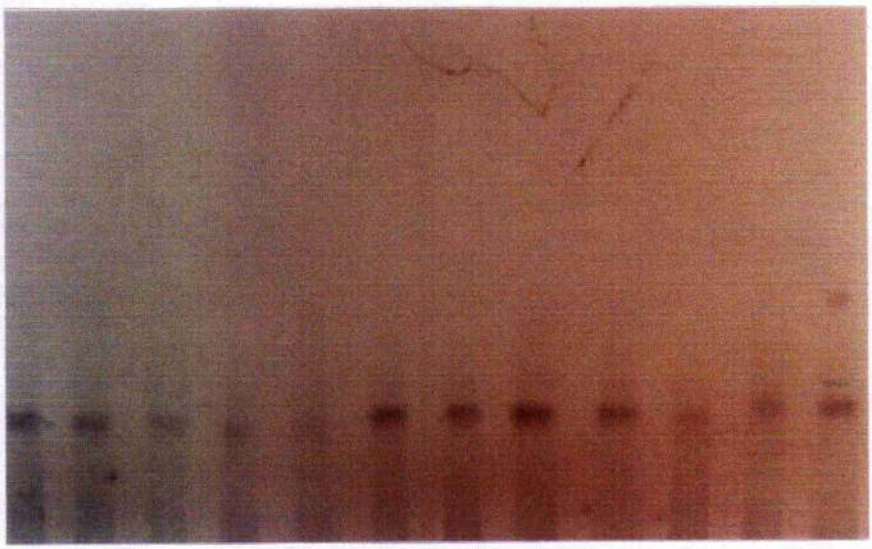


Plate 2. 11. The single band produced by the *G-3-pd-1* locus and the site of *G-3-pd-2* activity.

Glucose-6-phosphate dehydrogenase - G-6-PDH

Two zones of activity were apparent on gels stained for G-6-PDH activity (Plate 2. 12. and Fig. 2. 3.) One of these, close to the origin, either stained faintly or not at all, while the other contained a more strongly stained single band. The faintly stained zone appeared to consist of a double band (not shown on plate). Both zones were interpreted as the product of single loci (*G-6-pdh-1* and *G-6-pdh-2*) which were monomorphic in *S. vulgaris* and *S. squalidus*.

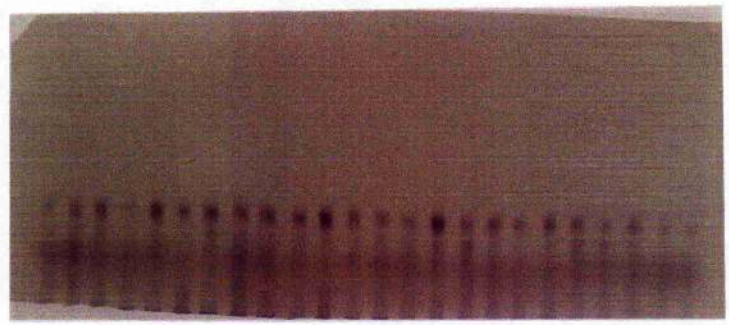


Plate 2. 12. A gel showing the single band of *G-6- pdh-2* . The site of *G-6-pdh-1* activity is also shown.

6- Phosphoglucodehydrogenase - 6-PGD

Gels stained for 6-PGD exhibited a single zone of activity, which appeared as a single band almost midway between the origin and the anodal end of the gel (Plate 2. 13 and Fig 2. 3.). The isozyme that formed this band was considered to be the product of a single monomorphic gene, designated *6-Pgd-1* .

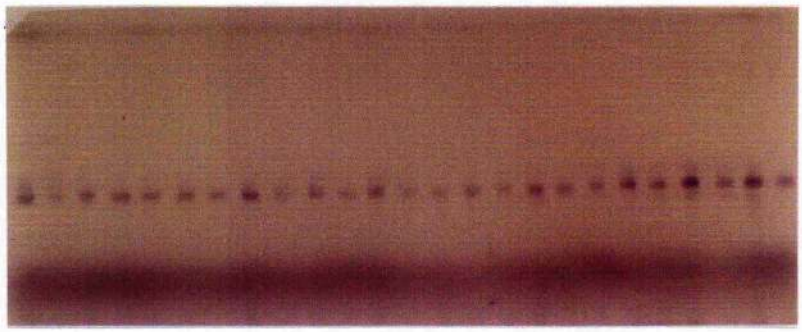
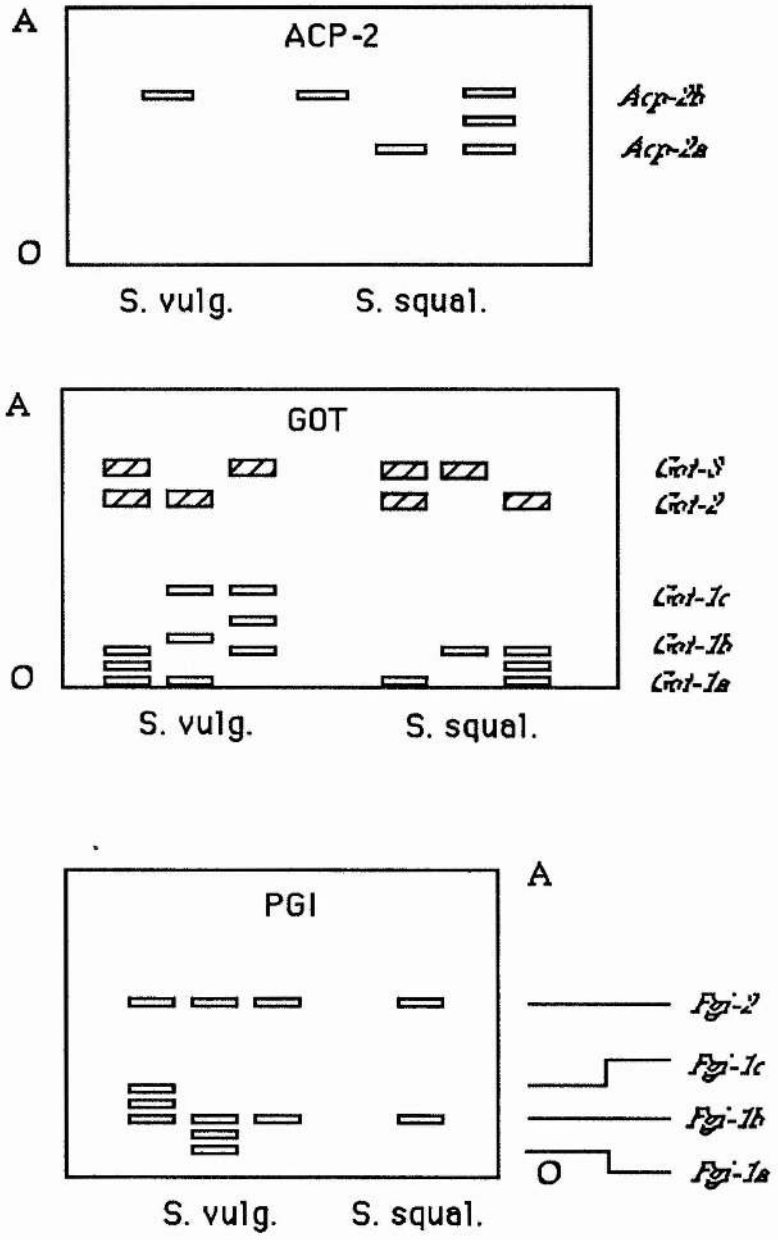


Plate 2. 13. illustrating the single band found on gels stained for 6-PGD.

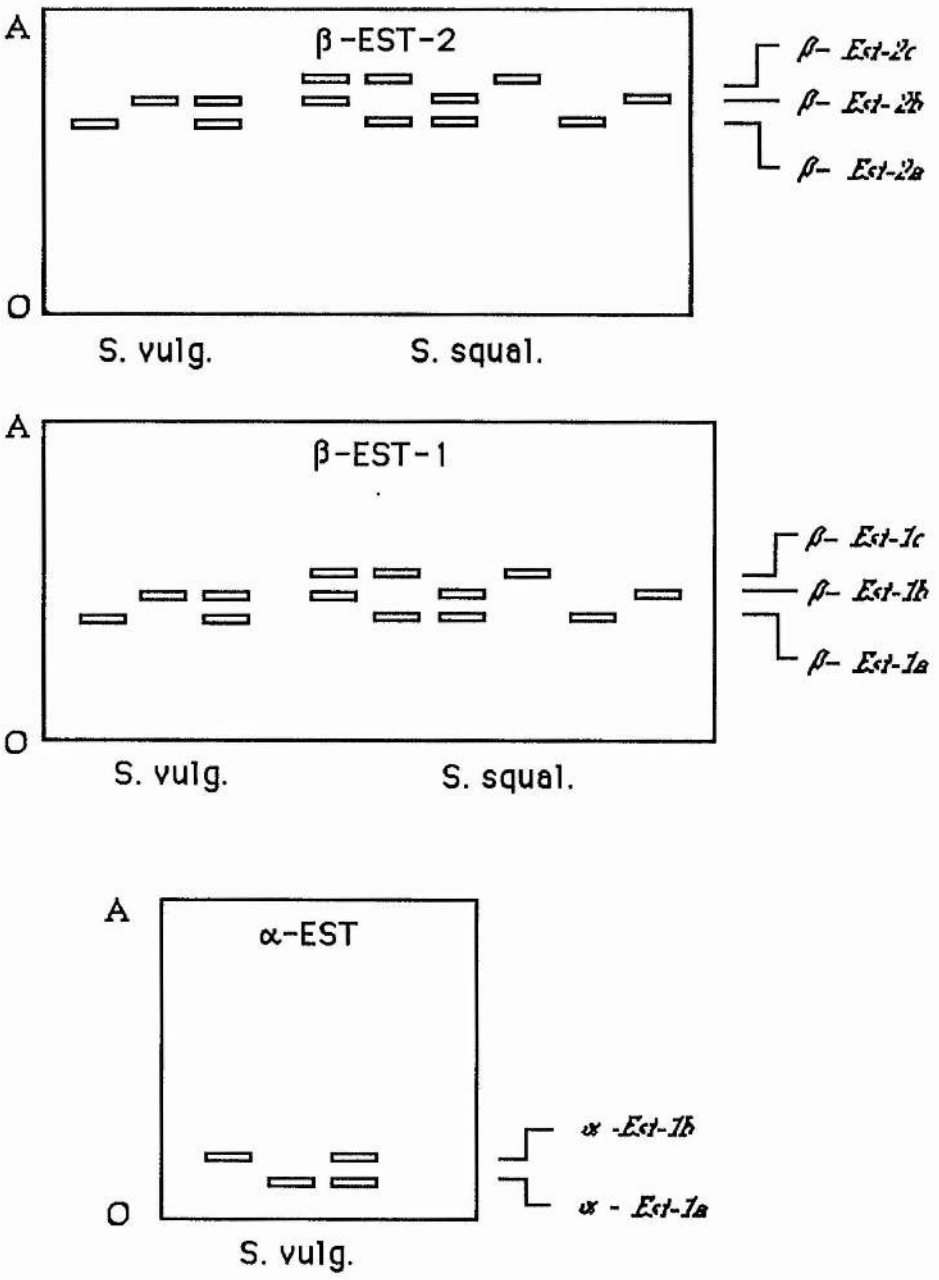
Fig. 2. 3. Zymograms of banding patterns obtained for thirteen enzyme systems in *S. vulgaris* and *S. squalidus*.



Enzymes move from the origin (O) to the anode (A).

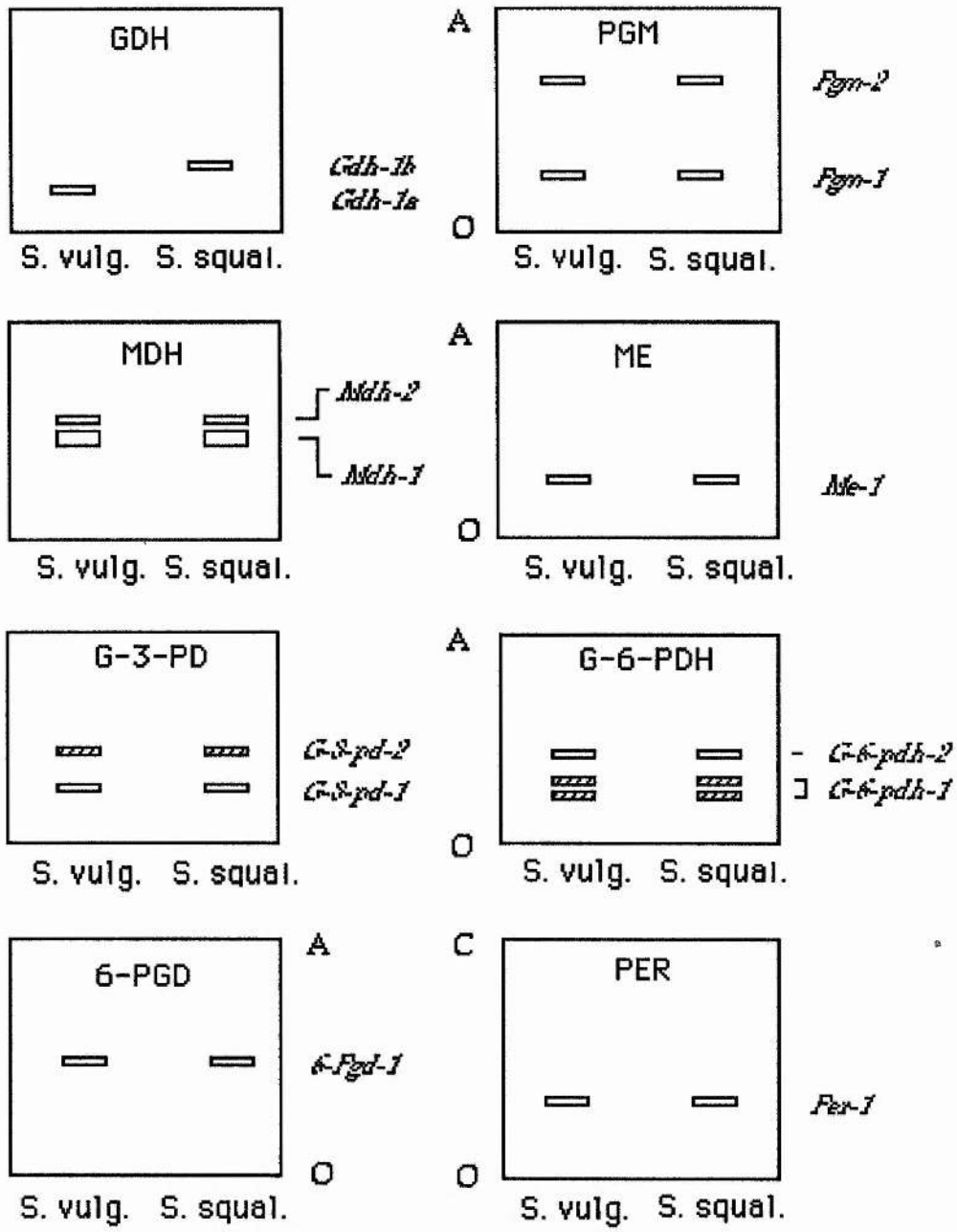
Zymograms are not to scale. R_f values are listed in Table 2. 2.

Fig. 2. 3. Continued.



Note that *S. squalidus* does not exhibit α– *Est* bands.

Fig. 2. 3. continued



The hatched bands (on G-3-PD and G-6-PDH) only appeared intermittently, and were stained very faintly.
The isozyme in PER moves from the origin to the cathode.

Table 2. 2. The relative positions on the gels of the allozymes found in each species.

Allozyme	R_f Value. (max=1. 0)	Species in which allozyme is detected		Structure (if apparent)
		S. vulg.	*S. squal.*	
Acp-2a	0.4	-	x	Dimeric
Acp-2b	0.5	x	x	
Acp-2n	Null	-	x	
Got-1a	0.01	x	x	Dimeric
Got-1b	0.06	x	x	
Got-1c	0.22	x	-	
Got-2a	0.48 - 0.51	x	x	
Got-3a	0.52 - 0.54	x	x	
α-Est-1a	0.10	x	-	Monomeric
α–Est-1b	0.12	x	-	
α–Est-1c	0.19	x	-	
β–Est-1a	0.31	x	x	Monomeric
β–Est-1b	0.37	x	x	
β–Est-1c	0.43	-	x	
β–Est-2a	0.57	x	x	Monomeric
β–Est-2b	0.61	x	x	
β–Est-2c	0.65	-	x	
Pgi-1a	0.19	x	-	Dimeric
Pgi-1b	0.26	x	x	
Pgi-1c	0.32	x	-	
Pgi-2	0.49	x	x	
Pgm-1	0.47	x	x	
Pgm-2	0.69	x	x	
Per-1	0.61	x	x	
Gdh-1a	0.26	x	-	
Gdh-1b	0.29	-	x	
Mdh-1	0.51 - 0.55	x	x	
Mdh-2	0.59	x	x	
Me-1	0.3	x	x	
G-3-pd -1	0.19	x	x	
G-6-pdh-2	0.36	x	x	
6-Pgd -1	0.51	x	x	

2. 4. DISCUSSION.

The most significant result to emerge from the survey of isozyme patterns in *S. squalidus* and *S. vulgaris* was the discovery of isozyme variation within and between the two species. Such variation is essential for an analysis to be conducted of the origins of different populations of *S. cambrensis* in Britain. Essentially, the additive phenotype that characterises an allopolyploid will only be recognised if variation exists between the two parental species. Furthermore, evidence of multiple origins of *S. cambrensis* in Britain will only be indicated if there is isozyme variation between populations of *S. cambrensis* , and this can only occur if such variation is present in at least one of the parental species. Therefore, the variation present in one or other of *S. squalidus* and *S. vulgaris* at the *Got-1* , *α-Est-1*, *β-Est-1* , *β-Est-2* and *Acp-2* loci, and between the two species at the *Gdh-1* locus, provides the basis for determining the respective origins of different populations of *S. cambrensis* in Britain.

The significance of the number of isozymes found for a given enzyme system in *S. squalidus* and *S. vulgaris* can be assessed in the light of the work of Gottlieb (1981a, 1982), who investigated the number and subcellular location of the most commonly surveyed plant isozymes. Enzymes common to the glycolytic and oxidative pentose phosphate pathways (e. g. PGI, PGM, G-3-PD, G-6-PDH and 6-PGD)

usually have two isozymes, each participating in the same metabolic processes but at different subcellular locations; one is active in the chloroplast, the other in the cytoplasm, although the expression of both isozymes is controlled by nuclear DNA. Of the two isozymes present for each system, the one active in the chloroplast is typically located on a gel in the more anodal position than the isozyme active in the cytoplasm. The isozyme located in the plastid is also often less likely to exhibit variation than the form active in the cytosol, i. e. is more "conserved".

Of the enzymes examined in the present study, PGI, PGM, G-3-PD, 6-PGD and G-6-PDH ought to have both cytosol and plastid located isozymes. Two isozymes were clearly evident for both PGI and PGM (see Fig 2. 3). There was also evidence of two isozymes occurring for G-3-PD and G-6-PDH, although only one of the isozymes stained regularly. The sporadic appearance of the other isozyme for each of these enzymes may be attributable to each isozyme requiring different electrophoretic conditions, with the conditions used in the experiment optimal for one of the isozymes but not the other. In contrast with the aforementioned systems, 6-PGD always produced just one isozyme on a gel. There was no indication of a second isozyme for this system.

The single isozyme for GDH found in both *S. vulgaris* and *S. squalidus* accords with the pattern observed in other species. Gottlieb (1982) lists 14 species, each showing only a single band for GDH on starch gels. *In vitro,* GDH may utilise both NAD and NADP, however the

enzyme stain for GDH incorporated NAD, rather than NADP, and there is normally only one form of NAD dependent GDH found in the mitochondria (Bonner, 1973).

The other enzymes that were resolved in the present study (MDH, ME, ACP, GOT, PER and EST) are generally less well understood than the systems previously discussed. They also have more diverse functions and, therefore, usually have a greater number of isozymes (Gottlieb, 1981a). Normally at least three isozymes of MDH are recorded in plant species (up to six have been found), each form being under independent genetic control. Two MDH isozymes were recorded in *Senecio* here. Arulsekar et al (1986) has noted that on starch gels overlapping isozymes, originating from different subcellular regions, may occur for MDH. It is possible, therefore, that the *Mdh-1* band in *Senecio*, which appeared as a much thicker, less distinct band than *Mdh-2*, could be comprised of two or more isozymes, each showing very similar mobility (see Plate 2. 9.). By comparison, ME, a chloroplast located NADP-dependent, form of MDH usually exists as one band (Gottlieb, 1981a), which complies with the result obtained here.

Plants usually possess at least three and possibly four different forms of the GOT enzyme. In the present study, two distinct zones of GOT activity were recorded on gels for *S. vulgaris* and *S. squalidus* (*Got-1* and *Got-2 / Got-3*) and it is possible that the *Got-2 / Got-3* zone (see Plate 2. 2a) is the product of two loci. GOT is usually reported to

possess a dimeric structure as found here in *Senecio,* although exceptions to this have been recorded (e. g. in *Taraxacum,* Hughes and Richards, 1985).

Gels stained for ACP generally exhibit several isozymes, with variation typically detected at 1-3 of the controlling loci. In *S. vulgaris* and *S. squalidus* , variation has been revealed within one of several zones on a gel that stained for ACP. This variation is interpreted as due to the action of three alleles at a single locus (see Plate 2. 1.) The triple banded phenotype of what is regarded as the heterozygote at this locus shows that the enzyme in *Senecio* is dimeric as is normally the case in most other plant species.

High numbers of isozymes yielding complex banding patterns on gels are typical of PER and EST. Such a pattern was observed for EST (see Plates 2. 3 and 2. 4.), but for PER only one band was observed (Plate 2. 8.). Abbott and Gomes (1989) also reported only a single banded PER phenotype in *Arabidopsis thaliana* while single banded PER systems have also been noted by Nash and Davies (1975) and Srivastava and van Huystee (1977) in other plant species. It is possible that more peroxidases are present in *Senecio,* as there was faint activity for both species on gels close to the origin. The success in resolving the esterases of *Senecio* into comprehensible patterns of variation is notable. Esterase is frequently ignored by many workers due to the difficulty in interpreting the high numbers of variable bands found in this system (e. g. Hughes and

Richards, 1985). Hull (1974b) has previously analysed the frequency of EST patterns found in populations of *S. vulgaris* and *S. squalidus* across central Scotland. However, he did not assign the bands recorded to specific loci and consequently his results are not comparable to the results reported for EST above.

2. 5. CONCLUSION.

Isozymes were successfully resolved in protein extracts from *S. vulgaris* and *S. squalidus* for thirteen different enzyme systems. It is considered that these isozymes are controlled by seventeen different loci. Of these, six (*Acp-2, Got-1, α-Est-1, β–Est-1, β–Est-2* and *Pgi-1*) showed intraspecific variation (i. e. produced different allozymes) in at least one of the species examined. In addition, there appeared to be interspecific variation for GDH controlled by the putative *Gdh-1* locus, while one locus,*α-Est-1,* was present in *S. vulgaris* but not *S. squalidus* . Other loci were monomorphic in both species.

The resolution of several enzyme systems showing variation either within or between *S. vulgaris* and *S. squalidus* provides a tool for investigating the origin of different populations of *S. cambrensis* in Britain.

CHAPTER 3 CROSSING PROGRAMME

3. 1. INTRODUCTION.

In Chapter 2 a description and putative genetical interpretation was presented for the banding pattern variation found for several different enzyme systems resolved on starch gels, following electrophoresis of extracts from *S. squalidus* and *S. vulgaris* over several different enzyme systems. To confirm the genetic control of this variation, a crossing programme was undertaken, the results of which are presented below. The programme was aimed specifically at gaining an understanding of the genetics of variants for the enzymes ACP, GOT, PGI, PGM and PER. A genetic analysis of α- and β-EST variation in *S. squalidus* and *S. vulgaris* was not required as this had been carried out by Irwin (1990) as part of another project taking place in the Department. The results of Irwin's analysis confirmed the interpretation for esterase variation presented in Chapter 2 with one additional point of interest. At the *β-Est-2* locus the *β-Est-2b* allele tended to be dominant to *β-Est-2a* , thus among F2 progeny double banded phenotypes were rare and, in general, heterozygotes could not be identified as distinct from the *β-Est-2b* homozygote.

3. 2. MATERIALS AND METHODS.

3. 2. 1. Selection and crossing of parent plants

Healthy plants of known electrophoretic phenotype and possessing a high number of capitula were selected as parents. In *S. squalidus,* individuals were chosen from different populations so as to improve the probability of cross-compatibility. Once selected, individuals were repotted into larger pots to facilitate continued growth.

Both before and after crossing, plants were maintained under conditions similar to those described in Chapter 2, with particular care taken to prevent capitula from becoming wet during watering.

Parent plants of *S. squalidus* were crossed reciprocally as follows. Initially floral buds were covered with bags made from lens tissue prior to capitula opening so as to prevent access to pollinators. When a capitulum was fully open (i. e. all anthers showing) and pollen was abundant, the bag was removed enabling the capitulum to be cross-pollinated by gently rubbing it with a capitulum from a different plant. After crossing in this way, the capitulum was rebagged, and the plant was left in the glasshouse. Approximately two weeks later, seed was collected for progeny testing. The genetics of enzyme variation common to both *S. squalidus* and *S. vulgaris* were investigated on *S. squalidus* alone. The high degree of self-incompatibility in *S. squalidus* allows a crossing programme to be undertaken without recourse to emasculation, such as is necessary with the self-fertile *S. vulgaris*. *S.*

vulgaris was only used in the analysis of PGI variation as such variation was not present in *S. squalidus* .

Acp-2 variation

Two types of crosses were carried out;

(i) between individuals that exhibited either the 'fast ' or 'slow' single banded phenotype in the *Acp-2* zone (see Chapter 2, Fig. 2. 3.) i. e. *Acp-2a x Acp-2b,*

(ii) between individuals that were triple banded for fast, intermediate and slow bands in the *Acp-2* zone, and which were presumed to be heterozygotes *(i. e. Acp-2a/ Acp-2b x Acp-2a/Acp-2b).*

Got-1 variation

Variation in the *Got-1* zone (Chapter 2, Fig. 2. 2.) was only resolved on gels late in the project and consequently a complete formal genetic analysis could not be conducted. However, by examining the *Got-1* phenotypes of plants utilised in the genetic analysis of *Acp-2* variation, it was discovered that crosses had been made which also provided some information on the genetics of *Got-1* variation in *S. squalidus* . These crosses involved;

(i) Crosses between individuals with the two different (fast or slow) single banded *Got-1* phenotypes *(i. e. Got-1a x Got-1b)* .

(ii) Crosses between individuals with the single banded, slow phenotype, *Got-1a* , and the triple banded heterozygous phenotype,

denoted as *Got-1a/ Got-1b* (see Chapter 2, Fig. 2. 2b).

Got-2 & 3 variation

The two bands that appeared in the middle of a gel stained for GOT (Chapter 2, Fig. 2. 2a.) were initially considered to be the products of two independent loci. Three different phenotypes were resolved with respect to these two bands: Both bands (*Got-2* and *Got-3*) present - p/p; *Got-2* present, *Got-3* absent - p/a; *Got-2* absent, *Got-3* present - a/p. Using individuals with these phenotypes as parents, progeny of the following crosses were examined:

(i) p/a x a/p,

(ii) p/a x p/p,

(iii) p/p x a/p.

PGI, PGM and PER extra band variation

The inheritance of the extra band resolved for PGI, PGM and PER in certain individuals (Chapter 2, Figs. 2. 5, 2. 7. and 2. 8. respectively) was investigated by producing an F1 generation from a cross between a parent which produced the extra band and another which lacked it, followed by crossing the F1's from different parents to produce an F2 generation.

Pgi-1 variation in *S. vulgaris*

Triple banded phenotypes on gels that stained for *Pgi-1* were

only found in *S. vulgaris* (Chapter 2, Plate 2. 5.). To determine whether these phenotypes represented heterozygous genotypes that were fixed or segregated freely, they were selfed to produce progeny that were subjected to electrophoresis. Selfed progeny of individuals with either the *Pgi-1a/ Pgi-1b* or the *Pgi-1c /Pgi-1b* triple banded phenotypes were examined.

Self-fertilisation of *S. vulgaris* individuals was ensured by bagging floral buds prior to opening. Seed was collected approximately three weeks after bagging and stored for two weeks at 4°C, before germinating and subjecting the offspring to electrophoresis.

3. 3. RESULTS.

Acp-2

The results obtained from two reciprocal crosses (i. e. Gm. 3 x Gl. 24 and Wr. 7 x Mo. 42; see Table 3. 1), clearly indicated that both parents were not homozygous for different alleles at the *Acp*-2 locus. The appearance of individuals which had the null allozyme implies that both parents must have been heterozygous for the null allele. The most likely explanation is that the cross was made between the following genotypes; *Acp-2a/Acp-2n x Acp-2b/Acp-2n* . This would account for the diverse allozyme patterns obtained in the offspring. Only the third reciprocal cross (i. e. Gm. 10 x Mo. 37) followed the pattern expected when each parent is homozygous for a different allele, with all offspring exhibiting the triple banded heterozygous F1 genotype.

The problem of an unknown genotype containing a null allele does not arise when both parents involved in a cross produce the triple banded phenotype. The results of crosses between such individuals are detailed in Table 3. 2.

A chi-squared test conducted on the totals show that the observed values do not differ significantly from the expected 1 : 2 : 1 ratio. Thus the two alleles, *Acp-2a* and *Acp-2b* , at the *Acp*-2 locus exhibit Mendelian inheritance.

Table 3. 1. Phenotypes of offspring produced from reciprocal crosses between putative fast and slow homozygotes (i. e. *Acp-2a x Acp-2b*).

Parental phenotype				Offspring Phenotypes		
Acp-2a x	*Acp-2b*	(no.)	a	b	a/b	n
Gm. 3 (female)	Gl. 24 (male)	2	-	-	-	2
Gm. 3 (male)	Gl. 24 (female)	9	2	4	3	-
Wr. 7 (female)	Mo. 42 (male)	4	4	-	-	-
Wr. 7 (male)	Mo. 42 (female)	3	-	-	-	3
Gm. 10 (female)	Mo. 37 (male)	8	-	-	8	-
Gm. 10 (male)	Mo. 37 (female)	12	-	-	12	-

N. B. n indicates a null phenotype i. e. neither band produced.
The origin of parents is as follows; Ch, Chesterfield; Gl, Glasgow; Gm, Grangemouth; Mo, Mochdre; Ox, Oxford; Wr, Wrexham.

Table 3. 2. Genotypes of offspring produced from reciprocal crosses between triple banded heterozygous parents (i. e. *Acp-2a / Acp-2b x Acp-2a / Acp-2b*).

Parental Genotype				Offspring Genotypes		
Acp-2a/Acp-2b x	*Acp-2a/Acp-2b*	(no.)	a/a	b/b	a/b	n
Ch. 7 (female)	Ox. 10 (male)	25	4	5	16	-
Ch. 7 (male)	Ox. 10 (female)	25	11	4	10	-
Wr. 7 (female)	Mo. 42 (male)	52	16	15	21	-
Wr. 7 (male)	Mo. 42 (female)	3	11	10	15	-
Total		138	42	34	62	-

$\chi^2 = 1.76$ N. S.

Got-1 variation

The phenotypes produced among the offspring of each reciprocal cross between *Got-1* variants of *S. squalidus* were as expected (Table 3. 3 and 3. 4.). Crosses between slow and fast banded parents (i. e. *Got-1a/1a x Got-1b/1b*) produced a triple banded phenotype indicative of the heteroozygote (Table 3. 3.). Similarly the cross between slow and triple banded phenotypes produced the same phenotypes among the offspring in the expected 1: 1 homozygote : heterozygote ratio (Table 3. 4.). It is concluded that the *Got-1a* and *1b* bands are coded for by different alleles at the *Got-1* locus and show Mendelian inheritance.

Table 3. 3. Genotypes of offspring produced from reciprocal crosses between putative *Got-1a* and *Got-1b* homozygous parents.

Parental Genotype			Offspring Genotype		
Got-1a/1a x	*Got-1b/1b*	(no.)	a/a	a/b	b/b
Gm. 3 (female)	Gl. 24 (male)	2	-	2	-
Gm. 3 (male)	Gl. 24 (female)	9	-	9	-
Gl. 20 (female)	Ca. 4 (male)	12	-	12	-
Gl. 20 (male)	Ca. 4 (female)	6	-	6	-
Total		29	-	29	-

Table 3. 4. Genotypes of offspring produced from reciprocal crosses between putative *Got-1a/Got-1b* heterozygous parents and *Got-1a/Got-1a* homozygous parents.

Parental Genotype			Offspring Genotype		
Got-1a/1a x	*Got-1a/1b*	(no.)	a/a	a/b	b/b
Ox. 10 (female)	Ch.7 (male)	25	13	12	-
Ox.10 (male)	Ch.7 (female)	25	13	12	-
Ch.13 (female)	Gm. 8 (male)	52	28	24	-
Ch. 13 (male)	Gm. 8 (female)	36	19	17	-
Total		138	73	65	-

The origin of parents is as follows; Ca, Cardiff; Ch, Chesterfield; Gl, Glasgow; Gm, Grangemouth; Ox, Oxford;

Got-2 and *Got-3* variation

The results of reciprocal crosses between parents with different combinations of the *Got-2* and *Got-3* bands (Tables 3. 5. and 3. 6.) did not confirm any simple pattern of Mendelian inheritance for the presence/absence of these two bands. For example, if the presence/absence of each band was controlled by loci at each of which one of two alleles was a null, then a cross between a plant which exhibited only the *Got-2* band and an individual with only the *Got-3* band should produce: only double

banded (p/p) if the two parents were homozygotes; four different phenotypes (p/p, p/a, a/p and a/a) among progeny, if both parents were heterozygous; and two phenotypes (p/a and p/p) if one parent was homozygous at its band coding locus while the other parent was heterozygous at the other band coding locus. In the event, no such cross yielded an individual among the offspring which lacked both bands (Table 3. 5.). Although such a result might be expected, the fact that no individual lacking both bands has been found in the wild despite extensive surveys of *S. squalidus* populations suggests that the presence/absence of the *Got-2* and *-3* bands is subject to more complex control than that proposed.

In support, a cross between a line which is single banded for *Got-2* (p/a), and a line that produces both the *Got-2* and *Got-3* bands (p/p) can only produce more than two phenotypes among its progeny if each of the loci which produces a band in the two parents is heterozygous. In this case four phenotypes should occur among the progeny including one lacking both bands. In the event, such crosses (Table 3. 6) always yielded three phenotypes in the progeny with the phenotype lacking both bands being absent. Only if the absence of both bands from an individual was lethal could the simple Mendelian model proposed have been accepted. Even then much more detailed genetic analysis would be required to confirm the model.

The possibility of simple maternal inheritance is rejected as if

this occurred each individual in the F1 generation would exhibit the same banding pattern as that of the female parent. This occasionally happens (i. e. in crosses where Gl. 20, Gm. 8 and Wr. 7 acted as the female parent, Table 3. 5.), however the small numbers of offspring produced from these plants suggest that this is most likely due to chance. In contrast, none of the crosses detailed in Table 3. 6. produced a total set of progeny that were identical to the female parent.

Table 3. 5. Phenotypes of offspring produced from reciprocal crosses between a parent with the *Got-2* band only and a parent with the *Got-3* band only.

Parental phenotype			Offspring phenotype		
Got-2 only x (p/a)	*Got-3 only* (a/p)	(no.)	p/a	a/p	p/p
Gl. 20 (female)	Gm. 8 (male)	2	2	-	-
Gl. 20 (male)	Gm. 8 (female)	4	-	4	-
Mo. 42 (female)	Wr. 7 (male)	3	-	2	1
Mo. 42 (male)	Wr. 7 (female)	4	-	4	-
Total		13	2	10	1

Table 3. 6. Phenotypes of offspring produced from reciprocal crosses between a single banded parent, with either the *Got-2* or the *Got-3* band only, and a parent with both bands.

Parental phenotype		Offspring phenotype			
Got-2 only (p/a) x	*Got-2 &3* (p/p)	(no.)	p/a	a/p	p/p
Ox. 10 (female)	Ch. 7 (male)	25	8	10	7
Gl. 20 (male)	Gm. 8 (female)	25	20	4	1
Got-3 only (a/p) x	*Got-2 &3* (p/p)	(no.)	p/a	a/p	p/p
Ch. 13 (female)	Gm. 8 (male)	52	13	20	19
Ch. 13 (male)	Gm. 8 (female)	36	10	11	17

The origin of parents is as follows; Ch, Chesterfield; Gl, Glasgow; Gm, Grangemouth; Mo, Mochdre; Ox, Oxford; Wr, Wrexham:

PGI, PGM and PER Extra bands

The possibility that the presence/absence of extra bands for PGI, PGM or PER is under simple genetic control is eliminated by the results of the crossing experiments. The additional bands only appeared at a very low frequency in the F2 generation on gels stained for PGI and PER gels (see Tables 3. 8. and 3. 12.), and were absent for these systems in the F1 generation (see Tables 3. 7. and 3. 11.). Moreover, in gels stained for PGM the extra band did not appear among progeny in either the F1 or F2 generation (Tables 3. 9. and 3.10.) despite being present in one of the original parents. Similarly, maternal inheritance of the extra band for each system, with each individual exhibiting the same banding pattern as its maternal parent, is discounted by the results.

Table 3. 7. Phenotypes of the offspring produced by reciprocal crosses carried out between parents that differed for the presence/absence of the extra PGI band.

Parental phenotype			Phenotypes produced		
Extra PGI band	x	No extra PGI band	(no.)	Extra PGI band	No extra PGI band
Gl. 24 (female)		Ox. 10 (male)	11	-	11
Gl. 24 (male)		Ox. 10(female)	8	-	8
Mo. 37 (female)		Wr. 70 (male)	5	-	5
Mo. 37 (male)		Wr. 70 (female)	7	-	7
Total			31	-	31

Table 3. 8. Phenotypes of the F2 generation monitored for the appearance of PGI extra bands. These were produced by crossing individuals from the F1 generations above.

F1 Parents			Phenotypes produced		
Female	x	Male	(no.)	Extra PGI band	No extra PGI band
Gl. 24 -1		Mo. 37-1	11	-	11
Mo. 37-1		Gl. 24 -1	4	-	4
Gl. 24 -5		Wr. 70-1	20	-	20
Wr. 70-1		Gl. 24 -5	43	3	40
Wr. 70-3		Ox. 10-8	11	-	11
Ox. 10-8		Wr. 70-3	27	1	26
Mo. 37-5		Ox. 10-1	13	-	13
Ox. 10-1		Mo. 37 -5	12	-	12
Total			141	4	137

The origin of parents is as follows; Gl, Glasgow; Mo, Mochdre; Ox, Oxford; Wr, Wrexham: Gm, Grangemouth; Sh, Sheffield; Ch, Chesterfield.

Table 3. 9. Phenotypes of the offspring produced by reciprocal crosses carried out between parents that differed for the presence or absence of the extra PGM band.

Parental phenotype			Phenotypes produced	
Extra PGM band	x No extra PGM band	(no.)	Extra PGM band	No extra PGM band
Gl. 24 (female)	Ox. 10 (male)	11	-	11
Gl. 24 (male)	Ox. 10(female)	8	-	8
Wr. 7 (female)	Mo. 42 (male)	4	-	4
Wr. 7 (male)	Mo. 42 (female)	3	-	3
Total		26	-	26

Table 3. 10. Phenotypes of the F2 generation surveyed for the appearance of the extra PGM extra bands. These were produced by crossing individuals from the F1 generations above.

F1 Parents			Phenotypes produced	
Female x	Male	(no.)	Extra PGM band	No extra PGM band
Gl. 24 -2	Mo. 42-1	9	-	9
Mo. 42-1	Gl. 24 -2	20	-	20
Gl. 24 -7	Wr. 7-2	66	-	66
Wr. 7-2	Gl. 24 -7	1	-	1
Wr. 7-1	Ox. 10-5	12	-	12
Ox. 10-5	Wr. 7-1	-	-	-
Mo. 42-2	Ox. 10-3	20	-	20
Ox. 10-3	Mo. 42 -2	15	-	15
Total		143	-	143

Table 3. 11. Phenotypes of the offspring produced by reciprocal crosses carried out between parents that differed for the presence or absence of the extra PER band.

Parental phenotype			Phenotypes produced	
Extra PER band	x No extra PER band	(no.)	Extra PER band	No extra PER band
Gl. 24 (female)	Ox. 10 (male)	11	-	11
Gl. 24 (male)	Ox. 10(female)	8	-	8
Wr. 7 (female)	Mo. 42 (male)	4	-	4
Wr. 7 (male)	Mo. 42 (female)	3	-	3
Gm. 8a (female)	Sh. 20 (male)	2	-	2
Gm. 8a(male)	Sh. 20(female)	3	-	3
Gm. 8b (female)	Ch. 13 (male)	36	-	36
Gm. 8b (male)	Ch. 13 (female)	52	-	52
Total		119	-	119

Table 3. 12. Phenotypes of the F2 generation surveyed for the appearance of the extra PER extra bands. These were produced by crossing individuals from the F1 generations above.

F1 Parents			Phenotypes produced	
Female	x Male	(no.)	Extra PER band	No extra PER band.
Gl. 24 -2	Mo. 42-1	9	-	9
Mo. 42-1	Gl. 24 -2	20	-	20
Gl. 24 -7	Wr. 7-2	66	-	66
Wr. 7-2	Gl. 24 -7	1	-	1
Gm. 8a-7	Wr. 7-4	20	-	20
Wr. 7-4	Gm. 8a-7	15	-	15
Total		112	2	110

Pgi-1 variation in *S. vulgaris*

All the offspring produced by selfing exhibited *Pgi-1* phenotypes identical to the parents (see Table 3. 13.). Thus it seems that the triple banded pattern at the *Pgi-1* locus present in certain individuals of *S. vulgaris* reflects fixed heterozygosity due to the locus being duplicated. The expected Mendelian ratio of 1: 2: 1. among the progeny of a freely segregating triple banded heterozygote was not observed.

Table 3. 13. Phenotypes of parents and F1 generation at the *Pgi-1* locus in *S. vulgaris*, after selfing the parents.

Parental phenotype	Origin	(n)	F1 phenotype
Pgi-1a/ Pgi-1b triple band	Spain	95	All *Pgi-1a/ Pgi-1b* triple band
Pgi-1b/ Pgi-1c triple band	Switzerland	45	All *Pgi-1b/ Pgi-1c* triple band
Pgi-1b/ Pgi-1b single band	Spain and Switzerland	45	All *Pgi-1b/ Pgi-1b* single band

3. 4. DISCUSSION.

Despite the fact that a comprehensive genetic analysis of variation at the *Acp-2* and *Got-1* loci was not completed, the results from the crosses that were made confirmed that allozymes encoded by these loci were inherited in a Mendelian manner. It was evident that a null allele segregated at the *Acp-2* locus which if present in a population, would create difficulties in determining the genotype of a single banded phenotype.

Genetic analysis of the variation for the *Got-2* and -3 bands failed to exhibit a Mendelian model for their inheritance. Heywood (1986) reported a similar banding pattern for malic enzyme in *Gaillardia pulchella* to that found here for *Got-2* and -3 in *Senecio*. He reported that crossing experiments did not yield an expected Mendelian banding pattern based on a two locus system, and suggested that allozymes of ME in *G. pulchella* were controlled by multiple loci, possibly with some directional dominance. When gels were stained over a long period for GOT in *Senecio* (to resolve clearly the allozymes encoded by the *Got-1* locus) some additional faint satellite bands were resolved above and below each of the usual *Got-2 &-3* bands. It is possible that the two satellite bands are allozymes of two different alleles present at each of two GOT loci - *Got-2* and *Got-3* while the middle satellite band is the heterodimer containing a sub-unit of each of these allozymes. A similar pattern of variation for GOT with faint satellite bands above and below

each of two closely situated intense bands has been described in *Spartina anglica* by Raybould (1988). Raybould considered that two loci were involved and that the two intensively stained bands were the heterodimers produced by two alleles at each locus.

Genetic analysis of the additional single bands for PGI, PGM and PER failed to establish a Mendelian pattern of inheritance. In this regard, it is of interest that PGI catalyses the reversible isomerization of glucose-6-phosphate and fructose-6-phosphate, while PGM catalyses the reversible isomerization of glucose-1-phosphate and glucose-6-phosphate. Glucose-6-phosphate is the product of the action of both enzymes in the staining recipe which, in turn, may be used as a substrate by G-6-PDH. As the G-6-PDH stain utilises the same conditions and tetrazolium dye as PGI and PGM, it is possible that G-6-PDH could appear on a gel stained for PGI and PGM. Thus the additional single bands which occasionally appear on gels stained for PGI and PGM may in fact be a G-6-PDH isozyme. Evidence in support of this suggestion comes from the finding that the extra bands of PGI and PGM are located on gels at a very similar position to the *G-6-pdh-1* band on gels stained for G-6-PDH. Furthermore, the extra bands only appear after the other bands have become evident, i. e. presumably after the substrate for G-6-PDH has been synthesised.

It is feasible that the presence of extra bands found on some gels stained for peroxidase (PER) is due to an 'environmental' effect

rather than a genetic factor. Srivastava and van Huystee (1977) showed that one PER isozyme of Peanut could form up to five bands after interaction with phenolic compounds. It is possible that different *Senecio* plants may vary in the level of phenolic compounds within their tissues and, consequently, the expression of PER is altered by these compounds. In this connection, it was of interest that the extra bands occurred more frequently in older plants which may be expected to contain greater concentrations of phenolic compounds in their tissues.

Finally the genetic analysis of the triple banded variant for *Pgi-1* in *S. vulgaris* showed that individuals of this phenotype were fixed for two alleles of *Pgi-1*. Fixed heterozygosity is not unexpected in tetraploid species such as *S. vulgaris* in which duplicated loci may frequently be homozygous for alternative 'alleles'.

CHAPTER 4. THE ORIGIN OF SENECIO CAMBRENSIS

4. 1. INTRODUCTION.

4. 1. 1. The discovery and distribution of *S. cambrensis*..

The Welsh Groundsel, *S. cambrensis*, was first described by Rosser (1955) from a specimen collected from Ffrith, 6 km north-west of Wrexham. According to Rosser, the species was initially detected by H. E. Green at Cefn-y-Bedd in 1948. Rosser considered the plant to be an allopolyploid (2n=60) of the hybrid between *S. vulgaris* (2n=40) and *S. squalidus* (2n=20). Typically an annual, the species may overwinter, and individuals which exhibit such longevity are characterised by having a woody stem.

An herbarium specimen that had been previously considered to be the triploid hybrid *S. vulgaris x S. squalidus* (2n = 30), collected at Denbigh by Jones, in 1925, was later re-identified by Rosser as *S. cambrensis* (Crisp, 1972). This raises the possibility that the species may have originated sometime prior to its discovery by Green in 1948. However, on re-examining Jones' specimen, Crisp (1972) could not distinguish the plant from a tetraploid *S. vulgaris x S. squalidus* hybrid which he had found on wasteland at Bromley, in East London. Even if the Denbigh specimen was *S. cambrensis*, the distance of 30km between

Denbigh and Wrexham raises doubts as to whether the Denbigh individual was ancestral to the population at Ffrith.

Due to the ambiguity over the identity of the specimen collected in 1925, there is confusion over the date of the origin of *S. cambrensis* in Britain. However, the species would seem to have arisen between 1910, when *S. squalidus* was first recorded in the Wrexham area (at Brymbo; Kent, 1963), and 1948, when *S. cambrensis* was first recorded at Ffrith.

According to Green (in Rosser, 1955), *S. cambrensis* showed little signs of spread between 1948 and 1955. Crisp (1972) also reported that *S. cambrensis* had "spread only slightly" from its original site following surveys he conducted at Ffrith and the surrounding area in 1967 and 1968. This situation contrasts with the current distribution of *S. cambrensis* around Wrexham. Presently, *S. cambrensis* is well established at several sites in the Wrexham area which run roughly in a semi-circle around the west of Wrexham, from Ffrith in the north to Rhostyllen in the south (see Fig 4. 1.). It is also found at Ruabon and Chirk, small towns respectively 8 and 16km south of Wrexham. It would seem that either Crisp did not survey the Wrexham area comprehensively, or the species has spread to these 'new' sites over the last twenty years.

In addition to being present at sites in and around the Wrexham

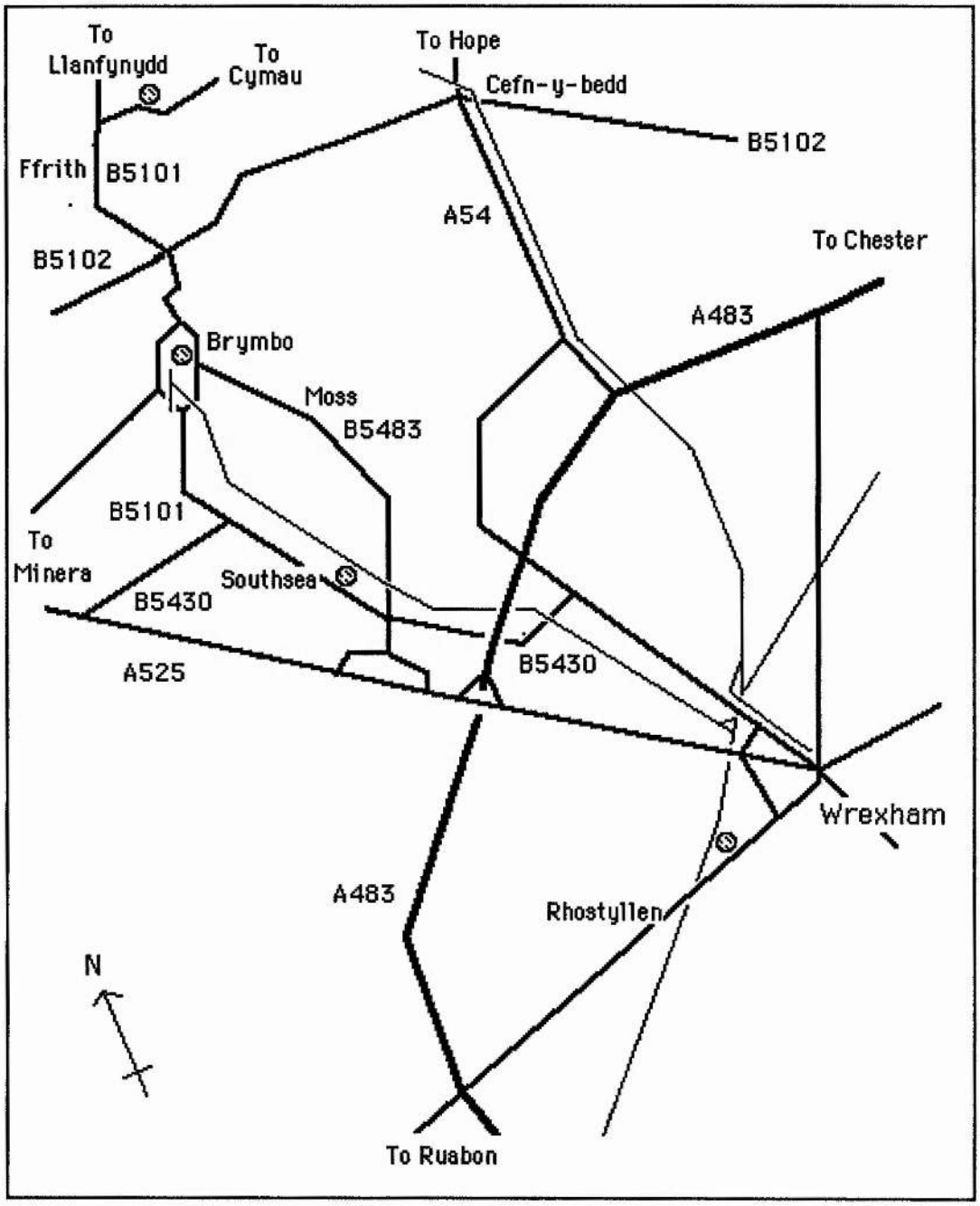


Fig. 4.1. Populations of *S. cambrensis* near to Wrexham (1985-1988)

area, *S. cambrensis* has been found growing in natural populations at three other locations in Britain; at Ness (SJ 307 764), on the Wirral; near Colwyn Bay, in North Wales (SH 822 781); and at Leith, Edinburgh (NT 268 765). The population at Ness was derived from material introduced to the area by H. E. Green. It is not known if this population is still extant. The population close to Colwyn Bay was discovered by Benoit in 1966 (Plant Records, 1971), who recorded it growing on a verge by the A55 (Map. Ref. 23/83 79). This road has now been renamed the A547 and the map reference given by Benoit is very close to the *S. cambrensis* population currently found in this area at Mochdre. At Leith the population was discovered by Abbott as recently as 1982 (Abbott et. al., 1983a). Noltie subsequently identified an herbarium specimen of *S. cambrensis* at the Royal Botanic Garden, Edinburgh collected from the Western Docks, Leith, in 1974 by Mrs. O. M. Stewart. This important find had previously escaped detection because it had been wrongly identified as the S. *squalidus x S. vulgaris* triploid hybrid, (Abbott et al., 1983a). Discovery of this specimen suggests that *S. cambrensis* has been present in the Leith area from at least 1974.

The fact that *S. cambrensis* currently occurs in Britain at three widely separated sites; at Wrexham, Mochdre and Leith (See Fig. 4. 2.), raises the question as to how the species has come to have such a

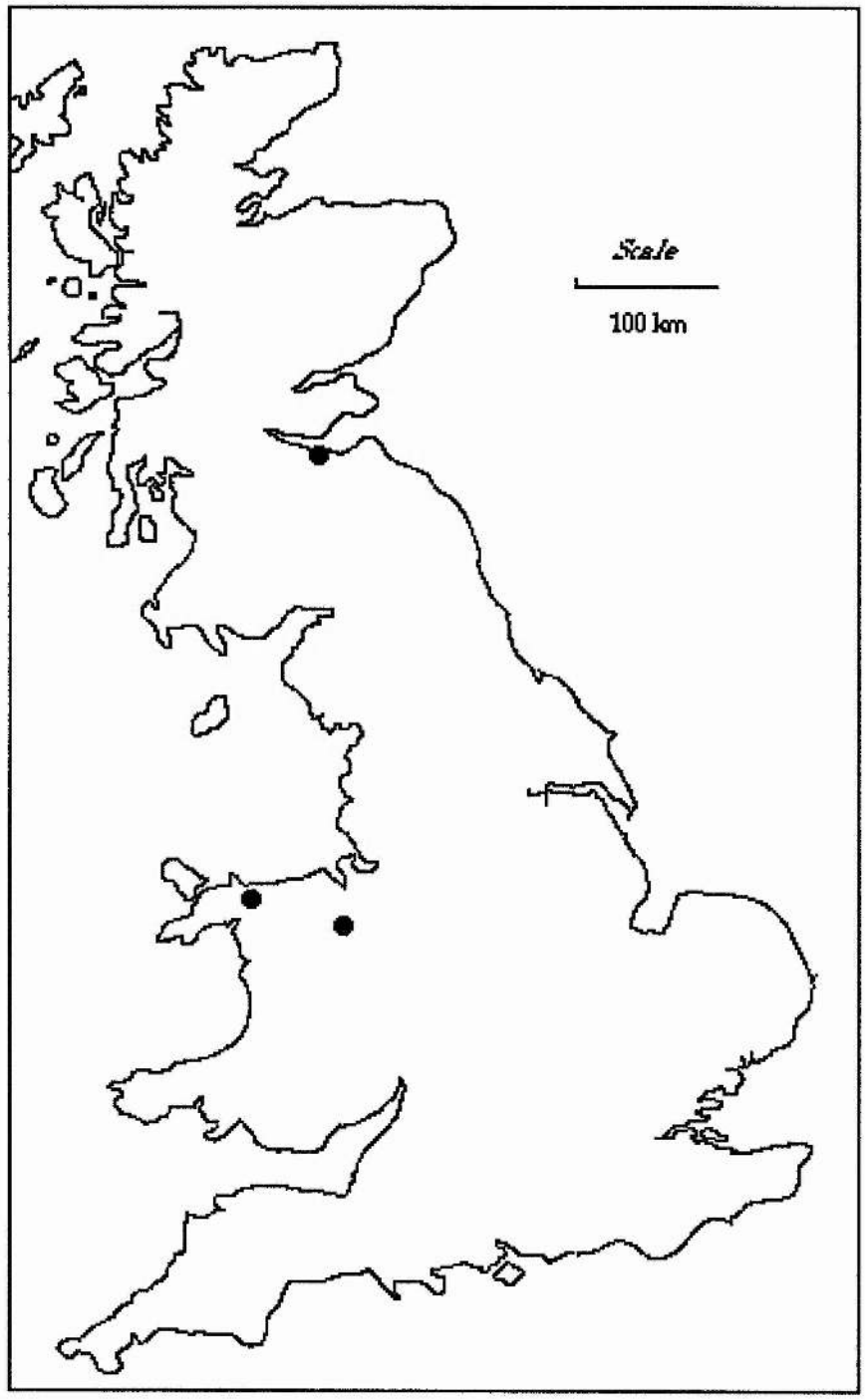


Fig. 4. 2. Current distribution of *S. cambrensis*.

disjunct distribution. There would seem to be two possible causes, either the plant has been dispersed by chance to these sites, possibly with the help of man, or alternatively the species has originated independently at each site following local hybridisation between *S. vulgaris* and *S. squalidus* .

The possibility that the Mochdre population of *S. cambrensis* originated independently at or near to its current location has not been advanced by any authors, and it is quite feasible that the population became established following long distance dispersal from Wrexham, approximately 60km away. There is also uncertainty concerning the origin of the population at Leith. Abbott et al. (1983b) considered that long distance dispersal of *S. cambrensis* to Leith, from Wales, was unlikely due to the absence of the species from intermediate sites. Certainly *S. cambrensis* has not been recorded between North Wales and Edinburgh; however, this does not necessarily eliminate long distance dispersal as the mode of spread of *S. cambrensis*. The ability of weedy species to travel long distances is highlighted by considering the spread of *S. squalidus* . Kent (1963) identified the arrival of *S. squalidus* in the Wrexham area as a result of frequent delivery of red iron ore, contaminated with seed of *S. squalidus*, to the foundry at Brymbo, probably from Warwick or Leamington. There is no reason why a

similar process could not occur for *S. cambrensis*, with seed being transported from Wrexham to Leith. Both Wrexham and Leith are industrial areas, and thus transport of seed via road or rail between the two sites is a distinct possibility.

Alternatively, *S. cambrensis* may have been deliberately introduced into the Leith area. The possibility that *S. cambrensis* was introduced into Edinburgh for horticultural reasons, seems unlikely, in that it is an aesthetically unremarkable species (a weed!). However, Crisp (1972) reported that *S. cambrensis* had been cultivated at the Royal Botanic Garden, Edinburgh, although this was checked by Abbott et al. (1983b) who concluded, following personal communications from J. Ratter at RBGE and P. M. Smith at Edinburgh University, that there was no evidence to support Crisp's statement.

The second option is that *S. cambrensis* originated *de novo* at Leith. Interestingly, Mabberley (1987), in discussing the *Senecio* genus, lists *S. cambrensis* as having a polytopic origin, but provides no evidence in support. If *S. cambrensis* originated independently at Leith, the speciation event must have occurred sometime between 1955 when *S. squalidus* was first reported in the Edinburgh area (Kent, 1966) and 1974, the year in which *S. cambrensis* was first collected from an Edinburgh site. Consequently the Leith population is potentially of a

more recent origin than the one at Wrexham, always assuming that the several populations at Wrexham have themselves a common ancestor. Should the population at Mochdre also be of separate origin, then this population too is likely to have originated in relatively recent times, i. e. sometime between the mid-1950's, when *S. squalidus* first became common in the Colwyn Bay area (Kent, 1963), and 1966, when the population was discovered.

After discussing the possible reasons for the disjunct distribution of *S. cambrensis* in Britain, Abbott et al. (1983b) suggested that an electrophoretic survey of isozyme variation in both parental species and the allopolyploid would be an appropriate method for investigating the likelihood of an independent origin of *S. cambrensis* at each different location where it is found. This suggestion has led to the present study being conducted.

4. 1. 2. Evidence for the polyploid origin of *S. cambrensis*

Experimental Evidence

S. cambrensis (2n=60) is considered to be an allopolyploid, produced from the chromosome doubling of an F1 triploid hybrid from a cross between *S. vulgaris* (2n=40) and *S. squalidus* (2n=20). Experimental evidence for such an origin has come from the artificial

synthesis of *S. cambrensis*.

Harland (1954) was first to resynthesise *S. cambrensis*. He made artificial crosses between the two putative parents (with non-radiate *S. vulgaris* acting as the female parent) and followed by treating the floral buds of the F1 hybrid with colchicine. In this manner he produced plants with a chromosome number of 2n=60 which were similar in morphology to the *S. cambrensis* found in the wild. The attempt to produce a hybrid between *S. squalidus* and *S. vulgaris* using *S. squalidus* as the female parent has never succeeded. Indeed, not all attempts to synthesise F1 hybrids from crosses between *S. vulgaris* and *S. squalidus* with *S. vulgaris* as the female parent have been successful (Crisp, 1972; Alexander, 1975; Kadereit, 1984a). However, Harland's procedure was repeated by Ingram (1977), who in turn produced artificial *S. cambrensis* from the F1 hybrid (Weir and Ingram, 1980), following colchicine treatment. The *S. cambrensis* synthesised by Weir and Ingram (1980) showed high levels of pollen fertility comparable to those found in natural populations. In addition, there was a strong similarity between the morphology of this synthesised *S. cambrensis* and those individuals from natural populations.

"Natural" Evidence

As the origin of *S. cambrensis* involves two separate steps, i.e.

the production of the triploid *S. vulgaris x S. squalidus* hybrid, followed by chromosome doubling, it is of interest to seek evidence for both of these stages occurring in the wild.

Evidence for the production of the F1 hybrid has been reported several times. However, if only cytotypically verified examples are accepted, as Stace (1977) recommends, then, to date, the triploid *S. vulgaris x S. squalidus* hybrid has been recorded in the wild on only five different occasions; these include records from Manchester (1971) and Leicester (1973) (in Stace, 1977); between Toft and Bourne, Cambridgeshire (Brettell and Leslie, 1977); Liverpool (Taylor, 1984) and Edinburgh (Marshall and Abbott, 1980). It is likely, however, that the *S. vulgaris x S. squalidus* hybrid is more common than is suggested by these rare records. It is probable that the hybrid goes undetected to all but the highly experienced observer of *Senecio* in the field. The best evidence for the frequency at which the hybrid is produced in the wild comes from a study by Marshall and Abbott (1980) who screened approximately 16, 000 offspring of 50 radiate and 50 non-radiate *S. vulgaris* plants which co-occurred with *S. squalidus* individuals in large populations at a site in Edinburgh. Of the total progeny scored, only two were confirmed as *S. vulgaris x S. squalidus* hybrids. Quite clearly the first stage of the origin of *S. cambrensis* in the wild does occur, albeit at a

low frequency.

Not surprisingly, direct evidence of the second stage, i. e. the doubling up of the hybrid chromosome number, is scarce. Weir and Ingram (1980) found a spontaneous hexaploid among artificial *S. vulgaris x S. squalidus* hybrids that they had produced, but were unable to ascertain if this was a product of chromosome doubling or due to the fusion of two sets of unreduced gametes.

Such circumstantial evidence that chromosome doubling is feasible in the wild may be all that can be hoped for. To obtain direct evidence of the second stage, a screening programme of the chromosome numbers of the progeny of the F1 hybrid will be required. In view of the high infertility of the F1 hybrid, the likelihood of finding any offspring with 2n = 60 would be extremely low.

Despite the lack of evidence, chromosome doubling can be envisaged as occurring in the appropriate hybrid at a low but regular frequency. This has been found by others working with hybrids of other species. Grant (1965) reported that four of the artificial interspecific hybrids that he had produced within the *Gilia laciniata* group had spontaneously doubled in chromosome number, producing fertile or partially fertile allopolyploid progeny. More recently, Eckenwalder and Brown (1986) have resynthesised a tetraploid species of *Ipomoea, I.*

sloteri by crossing the two putative diploid parent species, followed by selfing F1 individuals. The artificial cross between the two diploid species, *I. coccinea* and *I. quamoclit,* produced 8 viable seeds amongst the 280, 000 ovules screened. The subsequent F2 individuals were tetraploid, although Eckenwalder and Brown considered that the tetraploids were most likely formed from the fusion of unreduced gametes, rather than the chromosome doubling of F1 individuals.

4. 1. 3. Possible Alternative Origins for *S. cambrensis.*

The strength of the experimental and circumstantial evidence for the origin of *S. cambrensis* via allopolyploidy has led to only a cursory examination of alternative means of origin.

Crisp (1972), after considering the additive nature of the ligule promoting genes, drew attention to the fact that the predominant ligule length of ray florets in *S. cambrensis* was between 4. 0 -7. 5 mm, which was smaller than that found in *S. squalidus.* As the artificial hexaploid of *S. viscosus x S. squalidus* was found to possess longer ligules than the corresponding F1 hybrid, Crisp argued that *S. cambrensis,* should have ligules (if it had arisen by polyploidy), at least 10-13mm long, i. e. similar in length to those of *S. squalidus.* Crisp suggested that *S. cambrensis*

may therefore not have arisen by doubling of the F1 genome.

The problem concerning ligule length of *S. cambrensis* has since been resolved by the work of Ingram and Noltie (1984) which showed that individuals of *S. cambrensis* with medium rayed ligules (the predominant form in natural populations) are produced when the allohexaploid is synthesised from the F1 between non-radiate *S. vulgaris* and *S. squalidus*.

Experimental evidence for the origin of *S. cambrensis* via non reduction of gametes is extremely scarce. *S. cambrensis* could theoretically arise in one step if an unreduced *S. squalidus* gamete successfully combined with an unreduced *S. vulgaris* gamete. There are records of tetraploid F1 *S. vulgaris x S. squalidus* hybrids. Taylor (1984) produced such a hybrid from a *S. vulgaris x S. squalidus* cross, and Crisp (1972) found an individual on waste ground adjacent to Bromley-by-Bow gasworks in London that was morphologically similar to *S. cambrensis*. Unfortunately before it could be karyotyped the individual died in cultivation. However, progeny of the plant had chromosome numbers around 2n=40, suggesting that the parent was probably a tetraploid F1 hybrid. The existence of such hybrids demonstrates that unreduced gametes can produce viable offspring, however, the probability of a cross between unreduced gametes from both parents must be extremely small.

4. 1. 4. Novel Features and Variation in *S. cambrensis* .

The morphology of *S. cambrensis* is clearly related to both of its parental species. This view was reinforced by morphometric studies undertaken by Taylor (1984), using both discriminant function and principal component analyses. Both these methods placed *S. cambrensis* equidistant between its parents based on an analysis of 64 capitulum and vegetative traits. Nevertheless, there are some features of *S. cambrensis* which cannot be ascribed to either parent. Rosser (1955) identified two such characteristics; four pored pollen grains [three pores are found in *S. vulgaris* and *S. squalidus*] and larger cypselae. Both of these studies were also found in artificially synthesised *S. cambrensis* (Weir and Ingram, 1980). Crisp (1972) has listed two other characteristics which he believed were unique to *S. cambrensis* : the lack of black tipped bracts and the possession of chromosomes with sub-terminal centromeres. However, subsequent investigations by Ingram (pers. comm.) have revealed that both of these characters are quite common in one or other of the parental species. Consequently, the two characters described by Rosser remain the only diagnostic differences between *S. cambrensis* and its parental species.

Variation between individuals of *S. cambrensis* occurs for several characters, e. g. height, leaf shape etc; however, such variation

will have a large environmental component and the most striking evidence of genetic variation in *S. cambrensis* is for ligule length. Four classes of ligule type are recognised: rayless, short-rayed (2. 0-3. 5 mm), medium-rayed (4. 0-7. 5 mm) and long-rayed (8. 0-11. 0 mm) with the medium rayed variant being the most common (Ingram and Noltie, 1984). Curiously, both Rosser (1955) and Crisp (1972) reported that the short-rayed form was the most common phenotype but this is explained by the fact that they classified rays that were 4. 8 mm in length as short. Rather surprisingly neither Rosser (1955) nor Harland (1954) mentioned the occurrence of a rayless form of *S. cambrensis* ; and the first specimen of this morph was not reported until M. Gillison collected it from a trading estate at Wrexham in 1968 (Crisp, 1972).

The genetic basis of ligule variation in *S. cambrensis* was investigated by Ingram and Noltie (1984). Earlier studies had revealed that ligule length was under simple genetic control in both *S. vulgaris* (Trow, 1912) and *S. squalidus* (Ingram and Taylor, 1982) with alleles showing co-dominance. The artificial synthesis of long and medium rayed *S. cambrensis* from crosses between *S. squalidus* and radiate and non-radiate *S. vulgaris* respectively (Weir and Ingram, 1980) revealed that the genes controlling ligule length in *S. cambrensis* acted in a similar additive manner to those in *S. vulgaris*. However, the greater

number of different classes of ligule length in *S. cambrensis,* compared with *S. vulgaris,* was caused by an increase in the number of alleles present at the higher ploidy level. By making controlled crosses, Ingram and Noltie (1984) found that the long rayed form of *S. cambrensis* possessed four or three ray producing alleles (R), the medium rayed form contained two ray producing alleles and two non-radiate alleles (r), while the rayless individuals of *S. cambrensis* possessed four "r" alleles (see Table 4. 1.).

This variation in *S. cambrensis* will have arisen since the origin of the species (most probably of the mid-rayed type) through the effects of homoeologous pairing. Although preferential pairing of homologous chromosomes usually occurs in *S. cambrensis* , both Crisp (1972) and Ingram and Noltie (1984) reported occasional quadrivalent formation, presumably due to the close genomic relationship of the parental species (Ingram, 1978). Crisp recorded that between 27-28 bivalents and 2-3 quadrivalents may form during meiosis.

Table 4. 1. Control of ray length in *S. cambrensis* (after Ingram and Noltie, 1984).

Genotype		Phenotype
r, r,	r, r .	Rayless
R, r,	r, r .	Short rayed
r, r, R, r,	R, R. R, r.	Medium rayed " "
R, r,	R, R.	Medium-Long rayed*
R, r, R, R,	R, R. R, R.	Long rayed " "

KEY.
R; Ray producing allele. r; allele producing no rays.
* The medium-long rayed class is not completely separable from the medium rayed class.

4. 2. AIMS.

An electrophoretic survey was carried out using material of *S. cambrensis* and the two putative parental species, *S. vulgaris* and *S. squalidus* , sampled from populations located at Leith, Mochdre and the four sites at Wrexham. The aims of this study were threefold:-

1. To confirm that *S. cambrensis* is an allopolyploid which originated following hybridisation between *S. squalidus* and *S. vulgaris.*

2. To investigate the possibility that *S. cambrensis* originated in Britain on more than one occasion. This was done by comparing the isozyme patterns of *S. cambrensis* plants from the three sites (Wrexham, Mochdre and Leith) and relating the between population variation found in *S. cambrensis* to the electrophoretic patterns exhibited in the parental species *S. vulgaris* and *S. squalidus* at each site.

3. To examine the level of electrophoretic variation within each *S. cambrensis* population, and the possible occurrence of novel isozymes in the species resulting from additive effects of the two parental genomes.

4. 3. MATERIALS AND METHODS

Seed was collected of *S. cambrensis* , *S. vulgaris* and *S. squalidus* from sites at Leith (Edinburgh), Mochdre (N. Wales), and four sites near to Wrexham (N. Wales); Ffrith, Brymbo, Southsea and Rhostyllen (see Fig. 4. 1.). The four sites in the Wrexham area were chosen because they had large, relatively stable, populations of *S. cambrensis* which had persisted over a number of years (Ingram and Noltie, in preparation). Seed from both non-radiate and radiate morphs of *S. vulgaris* were collected and kept separate. Map references of the collection sites for each species are listed in Table 4. 2, along with the approximate size of the population at the time of collection and the number of plants sampled. During the course of the study, each site was visited on several occasions to monitor any drastic fluctuation in population size.

Site descriptions

Ffrith. At Ffrith, *S. cambrensis* grows along both sides of a narrow stone-walled road for approximately 50m. The plant occurs at the foot of the wall, where it grows along with *S. vulgaris* in a thin layer of organic debris, and also on the wall where it is rooted between the stones. *S. squalidus* was not found at this site at any time during the study period and, therefore, could not be sampled for electrophoretic analysis.

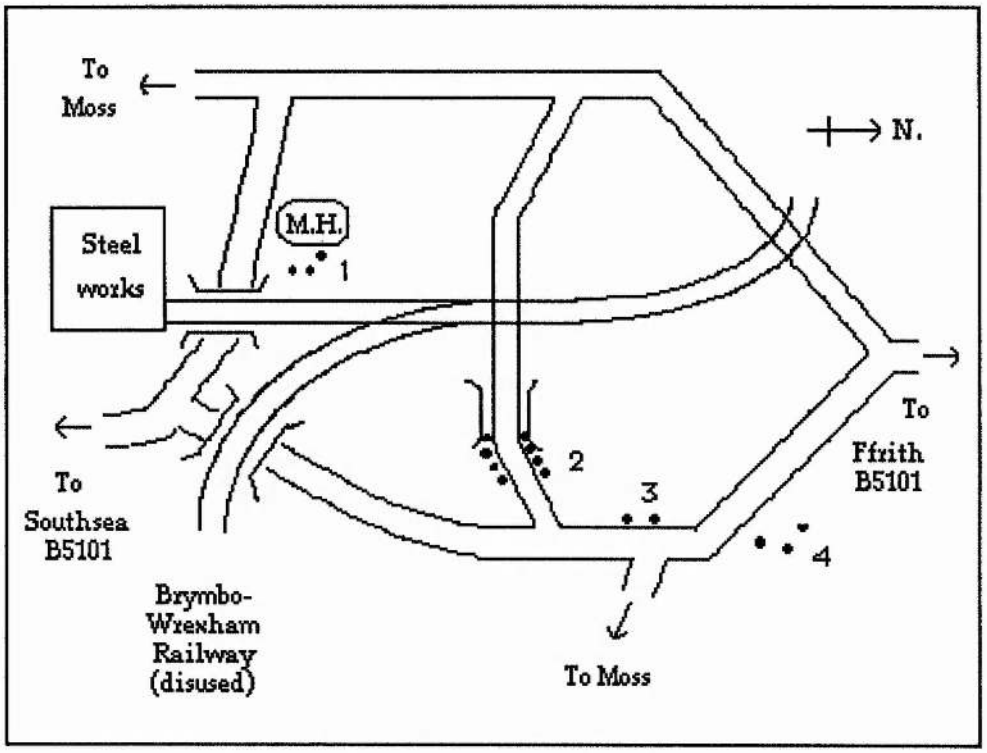
Table 4. 2. Location, collection date, population and sample size of species surveyed.

Location	Species	Grid ref.	Approx. Popn. size	Sample size	Collection date
Ffrith	S. camb.	SJ 286 556	30	25	03-10-86
(Fr)	S. vulg.	" " "	20/50	16/37	"
Brymbo	S. camb.	SJ 296 539	40	20	30-05-87
(Br)	S. vulg.	" " "	40/100+	21/38	"
	S. . squal.	" " "	100+	33	"
Southsea	S. camb.	SJ 308 515	80+	40	30-05-87
(So)	S. vulg.	SJ 306 515	50/100+	32/65	"
	S. squal.	SJ 309 514	50+	25	03-10-86
Rhostyllen	S. camb.	SJ 322 492	40	21	30-05-87
(Rh)	S. vulg.	SJ 312 492	15/50+	15/28	"
	S. squal.	SJ 314 485	50	27	"
Mochdre	S. camb.	SH 822 781	100+	57	03-10-86
(Mo)	S. vulg.	SH 822 781	100+/100+	40/45	"
	S. squal.	SH 818 778	40	26	"
Leith	S. camb.	NT 268 765	30	28	28-05-86
(Le)	S. vulg.	" " "	80+/100+	50/48	"
	S. squal.	" " "	100+	44	"

Note that there are two values for population and sample size of *S. vulgaris* at each site. The first figure refers to radiate *S. vulgaris* , the second to non-radiate *S. vulgaris.*

Brymbo. At Brymbo, the population of *S. cambrensis* is widely distributed over several different sites (see Fig. 4. 3.). The largest sub-population of the species was found growing in a thin layer of soil beneath a hedge, on both sides of a path that crossed the track bed of the former railway to Ffrith; *S. vulgaris* also grew at this site. *S. cambrensis* was also found at a similar site at the edge of the Mount Hotel car park, and again on the more open areas of the compacted ballast of the former railway line to Ffrith, where it grew along with *S. squalidus*. Close to this site, a few individuals were recorded growing in the stone wall adjacent to the road, similar to the site at Ffrith. Both *S. squalidus* and *S. vulgaris* were recorded in abundance at Brymbo; however, *S. cambrensis* was typically found growing in close association with *S. vulgaris* , rather than *S. squalidus*. Only at the Mount Hotel car park site were *S. squalidus* and *S. cambrensis* individuals found growing together.

Southsea. The largest population of *S. cambrensis* was found at Southsea. This population grew on a grassy bank adjacent to a footpath and in the thin layer of soil between the footpath and the bank, along with a few *S. squalidus* individuals. *S. squalidus* from this locality was collected 50m distant from the *S. cambrensis* population, where it was sufficiently abundant. Seed of *S. vulgaris* was collected approximately 100m away from the *S. cambrensis* population.



0 m 100

Approximate scale

KEY

- Location of *S. cambrensis* populations

1. Car park of Mount Hotel (M. H.)
2. Path over disused railway
3. On top of stone wall
4. On impacted ballast of disused railway to Ffrith

Fig. 4. 3. Sites of *S. cambrensis* at Brymbo (1986-1988).

Rhostyllen. At Rhostyllen, *S. cambrensis* occurred with non-radiate *S. vulgaris* along a 50m strip, growing adjacent to the footpath, beneath a hedge, on the west side of the A483. Seed of both non-radiate *S. vulgaris* and *S. cambrensis* were collected from this site. The few radiate *S. vulgaris* individuals sampled from Rhostyllen were found on a building site, 50m from the main road. *S. squalidus* seed was sampled from the area around the local colliery, approximately 800m from the *S. cambrensis* population; this was the only site in the Rhostyllen area where *S. squalidus* was found.

Mochdre. At Mochdre, *S. cambrensis* occurred growing with both morphs of *S. vulgaris* , along a 50-80m stretch on both sides of the A547 (see Plate 4. 1.). A few individuals of *S. cambrensis* also grew in a wall at the roadside as at Ffrith and Brymbo. The plants at Mochdre appeared different from those at Leith and in the Wrexham area They were larger, leafier and exhibited an upright growth form, unlike the trailing habit often shown by older plants at other sites. It is not known whether this variation is due to environmental or genetic effects.

No *S. squalidus* individuals were found in the immediate vicinity of the Mochdre site, consequently *S. squalidus* was collected from the nearest roadside population, about 1 km west.

Plate 4. 1. *S. cambrensis* growing at Mochdre on the south side of the A547

Leith Two distinct populations of *S. cambrensis* occurred at Leith: by the Water of Leith, and at the roadside of Salamander St (A149), close to the docks. The two sites were approximately 1500m apart. By the Water of Leith, *S. cambrensis* grew on a narrow ledge above the water, about 1m below a paved embankment (see Plate 4. 2.). Both *S. squalidus* and *S. viscosus* were also recorded growing alongside *S. cambrensis* at this site where plants flowered from spring until autumn and frequently

overwintered. *S. squalidus* also grew alongside *S. cambrensis* at the Salamander St. site, but both species tended to be excluded by grasses from the site during the summer.

Both radiate and non-radiate morphs of *S.vulgaris* were common in the Leith area but were not found growing in close association with *S. cambrensis*. Samples of seed of the two morphs were collected near to the localities of the two *S. cambrensis* populations.

Plate 4. 2. *S. cambrensis* and *S. squalidus* growing by the Water of Leith in Edinburgh (autumn, 1988).

Methods

Seed was collected from up to fifty individuals within each population. Following collection, seed of each species from each population was sown on potting compost in a heated glasshouse, each genotype being sown in a separate pot. Following germination the seedlings were thinned out to one plant per pot. Approximately 6 - 10 weeks after sowing when the plants were at the pre-flowering or early flowering stage, an electrophoretic survey of 14 enzymes was carried out on each individual plant using the techniques described previously in Chapter 2.

4. 4. RESULTS.

4. 4. 1. Comparison of *S. squalidus* and *S. vulgaris*

The electrophoretic survey revealed a close similarity between *S. vulgaris* and *S. squalidus* for all enzyme systems studied. In all populations, the isozyme banding patterns for eight enzyme systems [PGI, PGM, PER, MDH, ME, 6-PGD, G-3-PD and G-6-PDH] were the same in the two parental species (see Chapter 2 for description). However, for five systems and over a total of five different loci examined [*Acp-2*, *Got-1*, *α-Est-1*, *β-Est-1* and *Gdh-1*] differences between the two parental species were found. One locus (*α-Est-1*) was present only in *S. vulgaris*, showing no expression in *S. squalidus*, while at the *Acp-2* and *β-Est-1* loci, *S. squalidus* possessed more alleles than *S. vulgaris* with two alleles present at the *Acp-2* locus in *S. squalidus* rather than one in *S. vulgaris* (see Fig 4. 4.), and three alleles occurring at the *β-Est-1* locus rather than two in *S. vulgaris*. For *Got-1*, *S. vulgaris* individuals normally exhibited any one of three triple banded heterozygote patterns and occasionally a six banded pattern (presumably resulting from a cross between two different fixed heterozygotes). Individuals of *S. squalidus* on the other hand, were either homozygous or heterozygous for *Got-1a/Got-1b* bands (see Chap. 2., Fig. 2. 2.). Finally, for *Gdh-1* both species exhibited a single band but there was variation between species with the single band appearing on gels at a different position for *S.*

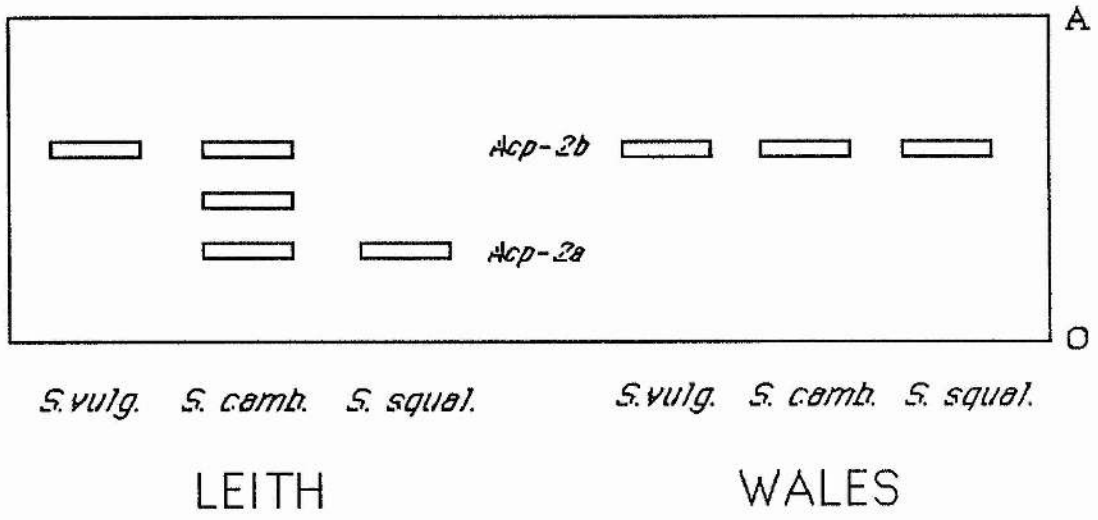


Fig. 4. 4. Zymograms showing the allozyme patterns resolved at the *Acp*-2 locus in plants of *S. cambrensis* from populations at Leith and in Wales. Also presented are the zymograms at the locus for individuals of the parental species (*S. vulgaris* and *S. squalidus*) which occur at these locations and which are most likely to have crossed to produce the different forms of *S. cambrensis* found at Leith and at Welsh sites.

vulgaris than for *S. squalidus*. The relative frequencies of the various isozyme phenotypes found for these five variable loci surveyed in populations of *S. vulgaris* and *S. squalidus* from each site are presented in Tables 4. 3. - 4. 7. A full description and discussion of this variation will be given in Chapters 5 and 6.

4. 4. 2. Isozyme variation and the evidence for a multiple origin of *S. cambrensis.*

The variation found between and within the two parental species over sites at Wrexham, Leith and Mochdre was sufficient to examine the possibility of different origins of *S. cambrensis* at each of the three locations. Examination of the banding patterns resolved at the *Acp-2*, *Got-1* and *α-Est-1* loci provided strong evidence that *S. cambrensis* has indeed originated on more than one occasion in Britain. A comparison of the banding patterns at all of these loci showed that marked differences occur between the Leith and Welsh populations of *S. cambrensis* in their respective isozyme profiles. In addition, it was also evident that the populations in N. Wales, at the Wrexham and the Mochdre sites, differ at the *Got-1* locus. As shown below, by comparing the isozyme patterns found for *S. cambrensis* at each location with the banding patterns found in the parental species at the same sites, it is possible to determine whether *S. cambrensis* at each location is the

product of a separate origin of the species.

The *Acp-2* locus

Individuals of *S. cambrensis* in the Wrexham and Mochdre populations exhibited only a single fast allozyme *(Acp-2b)* at the *Acp-2* locus (Table 4. 3. and Fig. 4. 4.). In contrast, all *S. cambrensis* plants from Leith produced three allozymes at this locus and due to non-segregation, can be considered as of fixed heterozygote genotype, *Acp-2a/ Acp-2b.* By considering the *Acp-2* banding patterns observed in the parental species at each site, differences between the Welsh and Scottish populations of *S. cambrensis* in regard to the *Acp-2* profile can be readily interpreted. At both Welsh locations, and at Leith, *S. vulgaris* was monomorphic at the *Acp-2* locus, for the allele producing the fast allozyme *(Acp-2b),* and can, therefore, be considered as a donor of this allele to an F1 hybrid that initially gave rise to *S. cambrensis.* In contrast, *S. squalidus* was variable at the *Acp-2* locus in all Welsh populations surveyed, but monomorphic for the slow allozyme producing allele *(Acp-2a)* in the Leith population (Table 4. 3). Due to the variability found at this locus in the Welsh population, it is feasible that *S. squalidus* could have contributed any one of three alleles, *Acp-2a, Acp-2b* or the null allele, to an F1 that gave rise to *S. cambrensis.* However, given that all *S. cambrensis* in Wales is monomorphic for the fast allozyme *(Acp-2b)* we may conclude that it originated following a cross between an *S. squalidus*

TABLE 4. 3. Phenotype frequencies at the *Acp*-2 and *Gdh* loci for *S. cambrensis*, *S. squalidus* and *S. vulgaris*.

			Phenotypes					
Locus			*Acp*-2			:	*Gdh*	
			2a/2a	2a/2b	2b/2b	:	1a/1a	1b/1b
Spp.	Lcn.	(n)	—	—	—	:	—	—
S. vulg	Le.	(48)	-	-	1.00	:	1.00	-
N. rad.	Mo.	(45)	-	-	1.00	:	1.00	-
	Fr.	(37)	-	-	1.00	:	1.00	-
	Br.	(38)	-	-	1.00	:	1.00	-
	So.	(65)	-	-	1.00	:	1.00	-
	Rh.	(28)	-	-	1.00	:	1.00	-
S. vulg	Le.	(50)	-	-	1.00	:	1.00	-
Rad.	Mo.	(40)	-	-	1.00	:	1.00	-
	Fr.	(16)	-	-	1.00	:	1.00	-
	Br.	(21)	-	-	1.00	:	1.00	-
	So.	(32)	-	-	1.00	:	1.00	-
	Rh.	(15)	-	-	1.00	:	1.00	-
S.	Le.	(28)	-	1.00	-	:	1.00	-
camb.	Mo.	(57)	-	-	1.00	:	1.00	-
	Fr.	(25)	-	-	1.00	:	1.00	-
	Br.	(20)	-	-	1.00	:	1.00	-
	So.	(40)	-	-	1.00	:	1.00	-
	Rh.	(21)	-	-	1.00	:	1.00	-
S.	Le.	(44)	1.00	-	-	:	-	1.00
squal.	Mo.	(26)	.31	.38	.31	:	-	1.00
	Fr.	(0)						
	Br.	(33)	.51	.42	.06	:	-	1.00
	So.	(25)	.20	.28	.52	:	-	1.00
	Rh.	(27)	.44	.30	.26	:	-	1.00

N. B. Individuals homozygous for the null allele were recorded at the *Acp*-2 locus in three populations (Mochdre, Brymbo and Rhostyllen) in very low numbers (2, 1 and 3 respectively). These are omitted from the Table (see also Chapter 6.).
N. Rad. refers to the non radiate form of *S. vulgaris*, Rad. to the radiate form.

individual which donated either the *Acp-2b* or null allele and an *S. vulgaris* plant which donated only the *Acp-2b* allele to the hybrid.

In contrast, the form of *S. cambrensis* found at Leith would appear to have originated following a cross between an *S. squalidus* which donated the *Acp-2a* allele, and a *S. vulgaris* individual which again donated the *Acp-2b* allele.

The fact that the two parental species at Leith are monomorphic for these alternative alleles is strong evidence that the local form of *S. cambrensis* originated in the Leith area.

The *α-Est-1* locus

All *S. cambrensis* individuals from Leith produced a single slow allozyme of α-esterase (*α-Est-1b*), whereas plants of *S. cambrensis* in Wales produced only the fast allozyme (*α–Est-1c*), (see Table 4. 4.). These differences at the *α-Est-1* locus in *S. cambrensis* must be attributable to the allelic variation at the locus in *S. vulgaris,* as the gene for *α-Est-1* is absent or not expressed in *S. squalidus*. It is concluded, therefore that the *α-Est-1b* allozyme present in *S. cambrensis* at Leith has been inherited following a cross involving an *S. vulgaris* individual which donated the *α-Est-1b* allele, while Welsh material of *S. cambrensis* originated following a cross which involved an *S. vulgaris*

TABLE 4.4. Phenotype frequencies at the *α-Est-1* locus in populations of *S. cambrensis*, *S. squalidus* and *S. vulgaris*.

			Phenotype		
			1b/1b	1c/1c	1b/1c
Spp.	Lcn.	(n)	—	—	—
S. vulg.	Le.	(48)	.33	.63	.04
N. rad.	Mo.	(45)	.38	.62	-
	Fr.	(37)	-	1.00	-
	Br.	(38)	.29	.68	.02
	So.	(65)	.48	.49	.03
	Rh.	(28)	.04	.96	-
S. vulg.	Le.	(50)	-	1.00	-
Rad.	Mo.	(40)	-	1.00	-
	Fr.	(16)	-	1.00	-
	Br.	(21)	.10	.85	.05
	So.	(32)	-	1.00	-
	Rh.	(15)	-	1.00	-
S.	Le.	(28)	1.00	-	-
camb.	Mo.	(57)	-	1.00	-
	Fr.	(25)	-	1.00	-
	Br.	(20)	-	1.00	-
	So.	(40)	-	1.00	-
	Rh.	(21)	-	1.00	-
S.	Le.	(44)	-	-	-
squal.	Mo.	(26)	-	-	-
	Fr.	(0)			
	Br.	(33)	-	-	-
	So.	(25)	-	-	-
	Rh.	(27)	-	-	-

plant which donated the *α-Est-1c* allele.

The *Got-1* locus.

Variation at the *Got-1* locus is unique among the loci surveyed in that it is the only locus for which different variant phenotypes of *S. cambrensis* were found at each of the Mochdre, Wrexham and Leith sites. In addition, at two Wrexham sub-sites (Rhostyllen and Brymbo), more than one isozyme pattern was observed in the same population of *S. cambrensis* (Table 4. 5.).

In *S. cambrensis,* phenotypic variation at the *Got-1* locus includes two different three banded variants and a six banded phenotype (Table 4. 5 and Fig. 4. 5.). The two different three banded phenotypes were also present in some populations of *S. vulgaris* (Table 4. 6.) and have been designated as fixed heterozygotes of genotype *Got-1a/Got-1c* and *Got-1b/Got-1c* respectively. The six banded phenotype, which was also found at very low frequency in *S. vulgaris* (Table 4. 5.), produces the *Got-1a, Got-1b* and *Got-1c* allozymes, plus three intermediate hybrid bands between each of the three possible allozyme pairs. In contrast, *S. squalidus* plants at each site are predominantly single banded for either the *Got-1a* or *Got-1b* allozymes. Triple banded heterozygotes for the alleles which encode these enzymes are also found, and such phenotypes also occur in some *S. vulgaris* populations where they are designated as

fixed heterozygotes.

At Leith, all plants of *S. cambrensis* exhibit only the six banded *Got-1* phenotype (Fig. 4. 5.) and are most likely to have originated from a cross between an *S. vulgaris* individual that exhibited fixed heterozygosity for *Got-1b/Got-1c,* and an *S. squalidus* plant that donated the *Got-1a* allele. The same six banded pattern could have arisen from hybridisation between an *S. vulgaris* individual donating the *Got-1a/Got-1c* alleles and an *S. squalidus* plant donating the *Got-1b* allele, however individuals of the parental species which possess the required phenotypes for such an origin are infrequent in the Leith population.

The six banded phenotype which characterised *S. cambrensis* at Leith was also recorded in Wrexham populations at Brymbo and Rhostyllen; however, it was the less common of two phenotypes found in *S. cambrensis* at these sites. By far the most common phenotype in populations at Wrexham sites was the *Got-1a/Got-1c* triple banded variant. Populations at Ffrith and Southsea were monomorphic for this variant, and at Brymbo and Rhostyllen the same variant was the more common. In contrast, at Mochdre, a third allozyme pattern at the *Got-1* locus was present in plants of *S. cambrensis,* with each individual exhibiting the triple banded *Got-1b/Got-1c* phenotype (see Table 4. 5.). The possible derivation of these distinctively different isozyme profiles for *Got-1* in plants of the Mochdre and Wrexham populations of *S.*

TABLE 4. 5. Phenotype frequencies for *Got-1* in populations of *S. cambrensis, S. squalidus* and *S. vulgaris* .

		Phenotypes						
					—	—	—	<--*Got-1c*
						—	—	
					—		—	
			—	—		—	—	<--*Got-1b*
				—			—	
		—		—	—		—	<--*Got-1a*
Spp.	Lcn. (n)	1a/1a	1b/1b	1a/1b	1a/1c	1b/1c	1a/1b/1c	
S. vulg.	Le. (27)	-	-	.04	.04	.92	-	
N. rad.	Mo. (20)	-	-	-	-	1.00	-	
	Fr. (10)	-	-	-	-	1.00	-	
	Br. (38)	-	-	.03	.03	.87	.07	
	So. (44)	-	-	.02	-	.96	.02	
	Rh. (23)	-	-	.04	-	.96	-	
S. vulg.	Le. (25)	-	-	.64	.08	.28	-	
Rad.	Mo. (24)	-	-	.04	.88	.08	-	
	Fr. (16)	-	-	.12	.88	-	-	
	Br. (21)	-	-	.29	.33	.38	-	
	So. (24)	-	-	.50	.17	.25	.08	
	Rh. (15)	-	-	.27	-	.73	-	
S.	Le. (28)	-	-	-	-	-	1.00	
camb.	Mo. (34)	-	-	-	-	1.00	-	
	Fr. (20)	-	-	-	1.00	-	-	
	Br. (20)	-	-	-	.80	-	.20	
	So. (28)	-	-	-	1.00	-	-	
	Rh. (21)	-	-	-	.65	-	.35	
S.	Le. (44)	.73	.02	.25	-	-	-	
squal.	Mo. (26)	.42	.27	.31	-	-	-	
	Fr. (0)							
	Br. (33)	.76	.06	.18	-	-	-	
	So. (25)	1.00	-	-	-	-	-	
	Rh. (27)	.56	.19	.26	-	-	-	

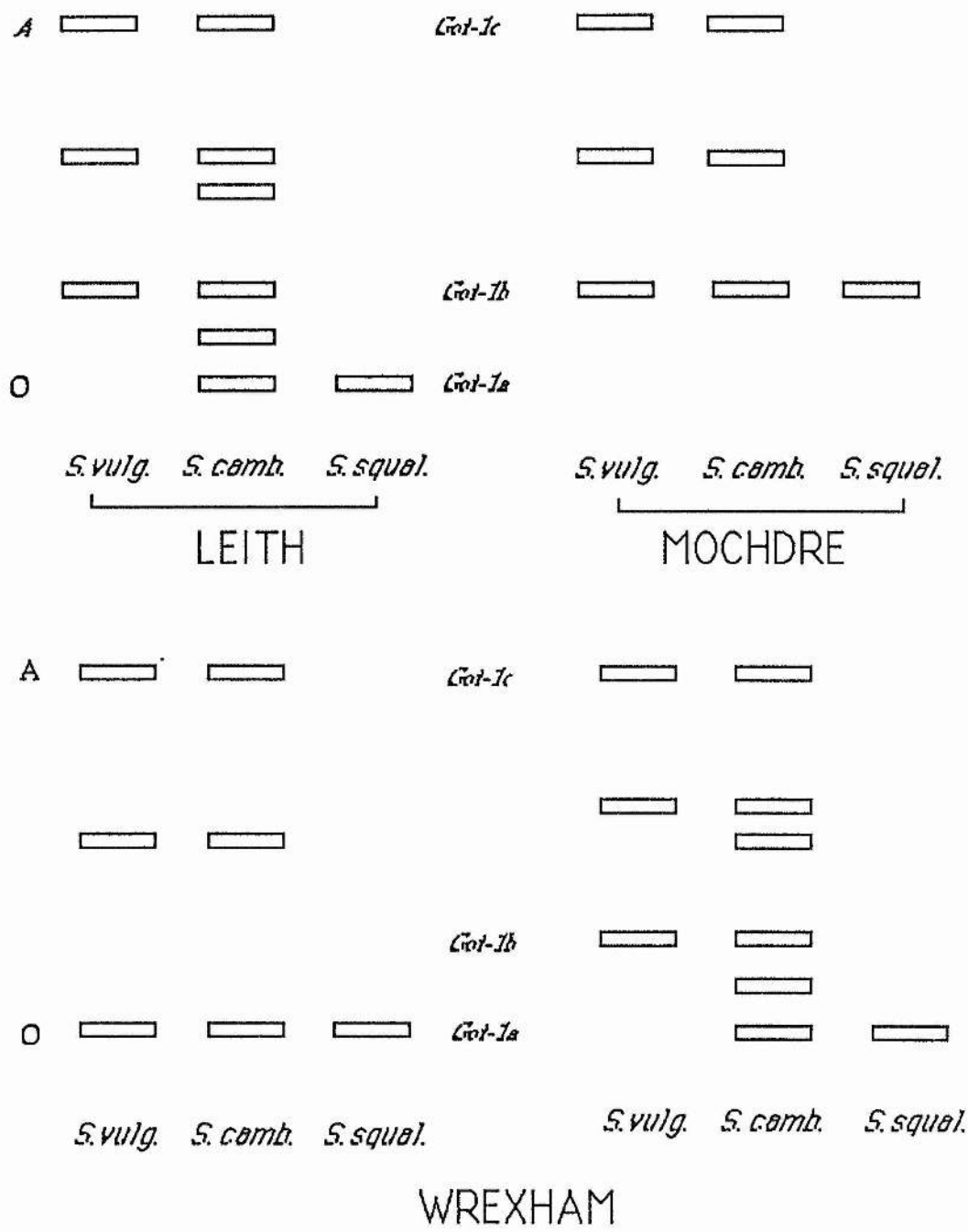


Fig. 4. 5. Zymograms of isozyme patterns observed at the *Got-1* locus in populations of *S. cambrensis* at Leith, Mochdre and various sites near to Wrexham. Also presented are some of the *Got-1* phenotypes of the two parental species, *S. vulgaris* and *S. squalidus*, found at these sites, and which are the likely parents of the respective *S. cambrensis* phenotypes.

cambrensis will be considered in the Discussion.

4. 4. 3. Isozyme variation at the *β-Est-1* locus.

S. cambrensis was monomorphic at Leith for the *β-Est-1a / β-Est-1b* phenotype (indicating fixed heterozygosity), while at Mochdre and at two sites in Wrexham (Ffrith and Southsea) was polymorphic containing both single banded phenotypes (*β–Est-1a / β-Est-1a* and *β–Est-1b / β-Est-1b)* as well as the double banded (heterozygous) phenotype (Table 4. 6.) Populations at Brymbo and Rhostyllen were monomorphic for the *β-Est-1a* allele. The fact that all plants of *S. cambrensis* at Leith exhibited a double banded phenotype indicates that they originated from a cross between parents which each contributed a different allele. Given the current genotype frequencies for *β–Est-1* in the Leith populations of *S. vulgaris* and *S. squalidus,* it is highly likely that *S. cambrensis* arose there from a cross between an *S. vulgaris* individual donating the *β-Est-1a* allele and an *S. squalidus* individual which donated the *β-Est-1b* allele.

The phenotypic variation found in Welsh populations of *S. cambrensis* at the *β-Est-1* locus prevents an accurate interpretation of which parental genotypes were most likely to have given rise to the

TABLE 4. 6. Genotype frequencies at the *β-Est-1* locus in populations of *S. cambrensis*, *S. squalidus* and *S. vulgaris* .

			Phenotype					
			1a/1a	1b/1b	1c/1c	1a/1b	1b/1c	1a/1c
Spp.	Lcn.	(n)	—	—	—	—	—	—
S. vulg.	Le.	(48)	.78	.22	-	-	-	-
N. rad.	Mo.	(45)	1.00	-	-	-	-	-
	Fr.	(37)	1.00	-	-	-	-	-
	Br.	(38)	1.00	-	-	-	-	-
	So.	(65)	.98	.02	-	-	-	-
	Rh.	(28)	1.00	-	-	-	-	-
S. vulg.	Le.	(50)	.98	-	-	.02	-	-
Rad.	Mo.	(40)	.35	.625	-	.025	-	-
	Fr.	(16)	1.00	-	-	-	-	-
	Br.	(21)	1.00	-	-	-	-	-
	So.	(32)	.97	.03	-	-	-	-
	Rh.	(15)	1.00	-	-	-	-	-
S.	Le.	(28)	-	-	-	1.00	-	-
camb.	Mo.	(57)	.37	.07	-	.56	-	-
	Fr.	(25)	.60	.04	-	.36	-	-
	Br.	(20)	1.00	-	-	-	-	-
	So.	(40)	.88	-	-	.12	-	-
	Rh.	(21)	1.00	-	-	-	-	-
S.	Le.	(44)	-	.82	-	.16	.02	-
squal.	Mo.	(26)	-	.81	-	.19	-	-
	Fr.	(0)						
	Br.	(33)	-	.85	-	.15	-	-
	So.	(25)	-	.96	-	.04	-	-
	Rh.	(27)	-	1.00	-	-	-	-

species at these sites. However, an absence in *S. cambrensis* of allozymes of the superfast allele (*β-Est-1c*), which was present in *S. squalidus*, suggests that *S. squalidus* individuals possessing this allele were not involved in the origin of *S. cambrensis*.

Perhaps the most reasonable explanation for the presence of the three phenotypes at the *β-Est-1* locus within both Mochdre and Wrexham populations of *S. cambrensis* is that all plants in these populations were originally double banded fixed heterozygotes, as is currently the case in the Leith population, and originated from a cross where each parent species contributed a different allele. Since the origin, however, variation has segregated out from the fixed heterozygote due to non-homologous pairing. On the evidence of the current allozyme frequencies within each parental species at each Welsh site, *S. vulgaris* is most likely to be the donor of the *β-Est-1a* allele while *S. squalidus* is most likely to have donated the *β-Est-1b* allele.

4. 4. 4. GDH Variation

All populations of *S. cambrensis* surveyed were monomorphic for the *Gdh-1a/ Gdh-1a* phenotype (Table 4. 3.). This was surprising given the monomorphism for alternative phenotypes at this locus in the two parental species; *S. vulgaris* showing the *Gdh-1a/Gdh-1a* phenotype and *S. squalidus* exhibiting only the *Gdh-1b /Gdh-1b* phenotype. Thus *S.*

cambrensis did not exhibit the additive phenotype that is expected in an allopolyploid.

4. 5. DISCUSSION

4. 5. 1. The allopolyploid origin of *S cambrensis*

The additive isozyme banding pattern that would confirm the allopolyploid origin of *S cambrensis* occurred at the *Acp-2*, *Got-1* and *β-Est-1* loci in all plants of *S. cambrensis* found at Leith (Tables 4. 3, 4. 5, and 4. 6.).

Populations of the parental species, *S. squalidus* and *S. vulgaris* at Leith were monomorphic for alternative alleles at the *Acp-2* locus while all plants of *S. cambrensis* possessed both alleles and exhibited the additive banding pattern of a fixed heterozygote. For *Got-1* and *β-Est-1*, the additive isozyme banding patterns found in *S. cambrensis*, reflected the combined isozyme patterns expressed by the most common genotypes of *S. squalidus* and *S. vulgaris* in the Leith population.

At Mochdre and the sites at Wrexham, it was not possible to state conclusively that the isozyme phenotype of *S. cambrensis* was the additive phenotype of banding patterns that occurred in the parental species for *Acp-2*, *Got-1* or *β–Est-1*. Indeed for each locus at these sites, the isozyme phenotype(s) present in *S. cambrensis* were also found in one or other of the two parental species (Tables 4. 3, 4. 5, and 4. 6.). That said, the variability resolved in the parental species at these sites was such that the isozyme phenotype of *S. cambrensis* could easily be interpreted as a product of banding patterns present in individuals of the

parental species that had crossed to give rise to an F1 hybrid from which the *S. cambrensis* was derived.

The one variable enzyme for which an expected additive pattern was not found in *S. cambrensis* was GDH. For this system, the parental species were monomorphic for alternative allozymes but *S. cambrensis* only produced the allozyme produced by *S. vulgaris* (Table 4. 3). It seems unlikely that *S. cambrensis* originally possessed both GDH bands, with that from *S. squalidus* being subsequently suppressed. This is because the same single banded isozyme phenotype was found in both Welsh and Scottish material of *S. cambrensis* , and the possibility that identical gene suppression has occurred in such disparate populations seems remote, assuming that the populations in Wales and Scotland are derived from separate origins of *S. cambrensis*. It is more probable that *S. cambrensis* has only ever expressed one form of GDH, the *Gdh-1a* allozyme.

The stain used in the present study to resolve GDH detects enzymes that are NAD dependent, and these forms of GDH are active in the mitochondria (Lees and Dennis, 1981). The enzyme, nonetheless, is probably coded for by nuclear genes as Pryor (1974) found that in maize, inheritance of GDH was Mendelian, rather than maternal. Bearing this in mind, the same banding pattern for *Gdh-1* in *S. cambrensis* and *S. vulgaris* could be due to a form of modification under the control of mitochondrial DNA. Although GDH is encoded by nuclear DNA, its

mobility could be affected after translation by conditions in the mitochondrion, which themselves are controlled by mitochondrial DNA. As the proposed origin of *S. cambrensis* postulates that *S. vulgaris* was the female parent, the mitochondrial genome of *S. cambrensis* will be closely related to that of *S. vulgaris* .

4. 5. 2. The Multiple Origin of *S. cambrensis*

The electrophoretic survey of isozyme variation at the *Acp-2* locus has revealed that the Welsh and Scottish populations of *S. cambrensis* are most likely to have originated independently from each other in that their isozyme profiles suggest they had different *S. squalidus* parents. Furthermore, the patterns for *α–Est-1* also indicate that the *S. vulgaris* parents, were not the same for Welsh and Scottish *S. cambrensis* (Table 4. 4). Taken together, therefore, the electrophoretic data for *Acp-2* and *α-Est-1* provides strong evidence for a separate origin of *S. cambrensis* in Wales and Scotland. In addition, the different banding patterns for *Got-1* in *S . cambrensis* and the two parental species at the Mochdre site suggests that the local population there may also be derived from a separate origin of the species in Britain (Table 4. 5.). Interpretation of the patterns observed at the *Got-1* locus as evidence for a third origin of *S. cambrensis* at Mochdre need to be treated with some caution, however, as alternative explanations of the patterns can

be invoked.

The contrasting isozyme phenotypes observed at the *Got-1* locus in *S. cambrensis* at the Wrexham and Mochdre sites can be explained either in terms of the populations at these two sites having a different origin, as stated above, or in other ways as explained below. Assuming an independent origin at each site, the *Got-1b/Got-1c* phenotype found in *S. cambrensis* at Mochdre would be derived from a cross between an *S. vulgaris* individual exhibiting the *Got-1b/Got-1c* triple banded phenotype (the only phenotype found amongst plants of non-radiate *S. vulgaris* surveyed at Mochdre), and an *S. squalidus* individual donating the *Got-1b* allele, the frequency of this allele was 0. 445 in Mochdre *S. squalidus* (see Table 4. 5. and Fig. 4. 5.). By comparison, the *Got-1a/Got-1c* triple banded phenotype, which is predominant among *S. cambrensis* at Wrexham sites, is likely to have resulted from hybridisation between an *S. vulgaris* individual with the *Got-1a/Got-1c* phenotype (common among the radiate but rare among the non-radiate plants at Wrexham) and an *S. squalidus* individual donating the *Got-1a* allele (the most common allele in this species at Wrexham).

Although a separate origin of *S. cambrensis* at Mochdre and in the Wrexham area is probably the cause of the different *Got-1* triple banded phenotypes found at each location there are alternative explanations for the disparity. Within some Wrexham populations a six banded *Got-1* phenotype was present and it is possible that all *S.*

cambrensis plants in Wales initially exhibited this phenotype, (which is identical to that currently exhibited by all plants at Leith). Over time, however, the frequency of this phenotype may have decreased in the Welsh population after homoeologous pairing produced the range of triple banded phenotypes which is expected to segregate out from a six banded fixed heterozygote. There are three possible triple banded phenotypes that would result from homoeologous pairing in a six banded hexaploid; these are the slow/fast *(Got-1a/Got-1c)*, slow/medium *(Got-1a/Got-1b)* and medium/fast *(Got-1b/Got-1c)* phenotypes. Despite the expected occurrence of these phenotypes, the slow/medium *(Got-1a/Got-1b)* phenotype has not been observed in any population of *S. cambrensis*, while Wrexham populations contain only one of the expected triple banded variants, i. e. the slow/fast *(Got-1a/Got-1c)* phenotype. Thus, if homoeologous pairing within a six banded individual did lead to the appearance of the triple banded phenotypes in Wales, the presence of only one such variant among *S. cambrensis* in Wrexham populations and another (a different phenotype) in the Mochdre population needs to be explained. It is possible that founder effects or selection have been important in fixing particular populations; however, should this be the case, it is surprising that the phenotype which occurs at Mochdre *(Got-1b/Got-1c)* disappeared from the Wrexham populations from within which it should have originated. The fact that only two electrophoretic *Got-1* phenotypes have been

found in Wrexham populations of *S. cambrensis* may indicate that the species has originated on at least two different occasions in the Wrexham area, each origin resulting in the production of a different phenotype. If this were the case, the six banded phenotype would have been produced in the same way as has been outlined for *S. cambrensis* at Leith, while the slow/fast *(Got-1a/Got-1c)* phenotype would have originated following a cross between an *S. vulgaris* individual exhibiting a slow/fast *(Got-1a/Got-1c)* phenotype and an *S. squalidus* individual containing the *Got-1a* allele.

One other way by which the six banded *S. cambrensis* phenotype may have arisen in Welsh populations is from a cross between an individual of *S. cambrensis* possessing the common slow/fast *(Got-1a/Got-1c)* triple banded phenotype and either an *S. vulgaris* plant exhibiting the medium/fast *(Got-1b/Got-1c)* phenotype (the most common phenotype among *S. vulgaris* at Wrexham) or one possessing the slow/medium *(Got-1a/Got-1b)* phenotype (common in radiate *S. vulgaris*). This cross, however, would produce a pentaploid individual which is likely to be meiotically unstable and consequently an unlikely progenitor of the stable six banded *S. cambrensis* individual, currently found in Wrexham populations.

The finding of a different *Got-1* phenotype in the Mochdre population of *S. cambrensis* from those which occur in populations near Wrexham may be due to long distance dispersal of the appropriate

variant from Wrexham, following homoeologous pairing in the six banded phenotype which occurs in some Wrexham populations. Alternatively, the particular phenotype found only at Mochdre, may be considered as the product of an independent origin of *S. cambrensis* in the Mochdre area. Although it is not yet possible to differentiate between these two alternative origins, the electrophoretic evidence indicates that the latter origin is the more likely.

4. 5. 3. Variation In *S. cambrensis*

Variation within populations of *S. cambrensis* was recorded, predominantly at the *β-Est-1* locus in material from Wrexham and Mochdre sites (see Table 4. 4.), and also as mentioned above, at the *Got-1* locus in two Wrexham populations.

The presence of two single banded phenotypes for *β-Est-1* at both Wrexham and Mochdre sites, in addition to the double banded phenotype, may have resulted from either of two processes, assuming that the original phenotype in these populations was the double banded type. It is possible that one of the bands has been silenced or that homoeologous pairing in individuals exhibiting the double banded phenotype has led to the segregation of single banded types within a population.

There is little evidence to suggest that alleles encoding allozymes

are silenced in a young polyploid. The loss of expression of a duplicated locus has been demonstrated in plants, but only in plants of ancient origin. Hart and Langston (1977) reported that while most loci of hexaploid wheat are present in triplicate, reflecting the origin of the species, this is not the case for loci encoding β-amylase, peroxidase and endopeptidase. In addition, it has been established that within ancient polyploids, most noticeably ferns [e. g. *Lycopodium lucidulum* Michx. (Levin and Crepet, 1973); *Pellaea andromedaefolia* (Kaulf.) Fee (Gastony and Gottlieb, 1982)] the expected expression of duplicated loci does not occur.

More relevant to the current study, however, is work carried out on polyploids of more recent origin. A study by Lack and Kay (1988) on *Polygala vulgaris* L., which is considered to be the allopolyploid of *P. serpyllifolia* Hose and *P. comosa* Schk. revealed no evidence of loci silencing. Similarly in *Talinum teretifolium* Pursh,. which is also believed to be an allopolyploid, Murdy and Carter, (1985) found the expected additive isozyme pattern of the two putative parental species to be present. Both of these allopolyploids are considered to be of relatively recent origin, for instance *T. teretifolium* is believed to have originated since the last ice age. *S. cambrensis,* however, is of much more recent origin. Of a more comparable age to *S. cambrensis* are the two allopolyploids of *Tragopogon, T. mirus* and *T. miscellus,* which are

known also to have originated within the last 100 years. These two species have been studied by Roose and Gottlieb (1976), and more recently by Rieseberg and Warner (1987); however, neither study produced evidence of loss of expression of duplicated loci within an allopolyploid.

A more plausible explanation for the presence within populations of single banded phenotypes at the *β-Est-1* locus is that they originated following homoeologous pairing in individuals possessing the double banded phenotype. It has already been proposed that homoeologous pairing in *S. cambrensis* has resulted in the variation found for ligule length in the species (Ingram and Noltie, 1984) ,and it is likely, therefore that this mechanism has also led to the variation observed at the *β-Est-1* locus.

4. 5. 4. Isozyme Studies On Other Allopolyploid Species

Isozymes have been used to study the proposed lineages of many crops that are considered to be allopolyploid. Such studies have been carried out on wheat (Mitra and Bhatia, 1971), tobacco (Smith et al, 1970; Reddy and Garber, 1971), cotton (Cherry et al, 1972) and safflower (Efron et al, 1973). However, similar studies on wild angiosperm allopolyploids of recent origin are less common.

Raybould (1989) recently completed an electrophoretic study of

the origin of *Spartina anglica* C. E. Hubbard. Like *S. cambrensis*, this allopolyploid species has a recent, well documented history (Marchant, 1967). The putative parents, *S. maritima* (Cart.) Fernald and *S. alterniflora* Loisel, were found to possess unique phenotypes for some enzyme systems while *S. anglica* expressed the expected additive phenotype, with all isozyme bands from both parents present. This confirmed the allopolyploid origin of the species. However, a complete lack of enzyme variation in both parental species, precluded any consideration that *S. anglica* may have originated more than once in Britain.

The allopolyploids, *Tragopogon mirus* and *T. miscellus*, were first discovered by Ownbey (1950) growing in southeastern Washington state and adjacent Idaho in the U. S. A. Ownbey proposed that *T. mirus* was the allopolyploid derivative of the diploid species *T. porrifolius* L. and *T. dubius* Scop., while *T. miscellus* was the allopolyploid product of *T. dubius* and *T. pratensis* L.(also diploid). This hypothesis was supported by morphological, cytological (Ownbey and McCollum, 1953) and biochemical studies (Belzer and Ownbey, 1971).

The hypothetical origins of the two allopolyploids of *Tragopogon* were investigated further by Roose and Gottlieb (1976), using electrophoretic techniques. Despite very low levels of variation within populations of the parental species, they were sufficiently differentiated from each to allow Roose and Gottlieb to confirm the

identity of the parents of both *T. mirus* and *T. miscellus* . Furthermore three geographically separate populations of *T. mirus* were genetically differentiated at three loci, with each population having inherited different alleles from one of the parents. This implied that *T. mirus* had originated on at least three separate occasions. Flavonoid evidence reported by Ownbey and McCollum (1953) also indicated that *T. miscellus* may have originated more than once; however, the uniform isozyme profile found in this species by Roose and Gottlieb (1976) has failed to confirm this possibility.

Comparable evidence for a multiple origin of a polyploid species has been reported for both pteridophyte and bryophyte species. An isozyme investigation into the origins of the allopolyploid members of the fern genus *Asplenium* from the Ozark - Appalachian region of the U. S. A. has revealed that two allotetraploid species, *A. bradleyi* D. C. Eaton and *A. pinnatifidum* Nutt. were genetically differentiated between populations at four loci (Werth et al, 1985). Each of these four loci were polymorphic in the putative diploid parental species, thus indicating that each allopolyploid species has originated more than once.

Similarly the moss *Plagiomnium medium* (Br. Eur.) Kop. has been shown by Wyatt et. al. (1988) to be the allopolyploid of *P. ellipticum* Brid. and *P. insigne* (Br. Eur.) Kop. Variation between different populations of *P. medium* has been resolved for several enzyme loci and is attributed to the species having originated on more than one

occasion.

Murdy and Carter (1985) have screened four diploid species of *Talinum* in order to identify the diploid progenitors of the tetraploid *T. teretifolium*. *T. teretifolium* was found to exhibit the characteristic additive phenotype of a recently evolved allopolyploid, with its banding patterns reflecting the combined isozyme phenotypes of *T. mengesii* Wolf. and *T. parviflorum* Nutt. The other two diploid species examined, possessed genes that were not found in *T. teretifolium* and were therefore excluded as possible parents. Interestingly, *T. teretifolium*, which has originated since the last ice age and is consequently much older than *S. cambrensis*, shows no intraspecific variation in isozyme phenotype.

A similar study to that of Murdy and Carter (1985) on *Talinum* has been undertaken by Bayer and Crawford (1986) to identify the progenitors of two polyploid species of *Antennaria; A. parlinii* Fern. and *A. neodioica* Greene. The ancestry of both of these species is confused, with three species considered to be involved in the evolution of the former species and four in the latter. Some of the diploid species are believed to be implicated in the ancestry of both polyploids. Unfortunately, minimal allelic divergence of the isozyme genes at the diploid level prevented a complete interpretation of how the polyploid species originated. Nevertheless, an electrophoretic survey revealed the identity of two of the contributory genomes to *A. parlinii* and one to *A.*

neodioica.

It is clear from the few studies completed so far, that a detailed understanding of how allopolyploids have originated and, in particular, whether multiple origins have occurred, requires the presence of isozyme variation between and within the species viewed to be the parents. When these conditions are satisfied, as in the case of *S. cambrensis*, *Tragopogon mirus*, *Asplenium bradleyi*, *A. pinnatifidum* and *Plagiomnium medium*, it is apparent that multiple origins of each species have occurred. It may be concluded, therefore, that based on the present evidence, allopolyploid species frequently evolve through a multiple rather than a single origin.

CHAPTER 5 ISOZYME VARIATION IN *SENECIO VULGARIS* AND THE ORIGIN OF THE RADIATE VARIANT, *S. VULGARIS* L. VAR. *HIBERNICUS* SYME.

5. 1. INTRODUCTION

Senecio vulgaris L., the Common Groundsel, is a tetraploid, monocarpic annual which reproduces via predominant self-fertilisation (Trow, 1912; Hull 1974a; Marshall and Abbott, 1982). A native of the British Isles, the species is ubiquitous on open and waste ground. It typically flowers from April through to October (Marshall and Abbott, 1982), though given suitable conditions this period can be extended throughout the year. Germination may occur throughout the growing season, however two main flushes of germination occur in spring and autumn (Popay and Roberts, 1970).

There is some dispute over the origin of *S. vulgaris* in the recent literature. Weir and Ingram (1980), on the basis of the regularity of chromosome pairing at meiosis, considered the plant to be of allopolyploid origin, with half of its genome homologous to that of *S. squalidus*. However, Kadereit (1984a) based on the results of experimental hybridisations and associated cytological studies, has argued that *S. vulgaris* (2n=40) is more likely to have originated as an autopolyploid from European *S. vernalis* L. (2n=40) via *S. vulgaris* ssp. *denticulatus* (O. F. Muell.) P. D. Sell. (see Chapter 7.).

The taxonomy of *S. vulgaris* at the subspecific level is confused. Trow (1912) described several microspecies based on morphological characters. In contrast, Clapham et al (1987), consider only two subspecific taxa based on inflorescence characters: the non-radiate, f. *vulgaris* (with tubular hermaphroditic flowers) and the radiate, f. *radiatus,* (with an outer whorl of female radiate flowers). Perhaps the most acceptable approach is that taken by Allen (1967) who recognised three varieties in the species;

1. var. *vulgaris* , the common non-radiate form;
2. var. *hibernicus,* an inland radiate type, morphologically similar to var. *vulgaris* , except for capitulum type.
3. var *denticulatus,* a radiate form, which in Britain is found only on maritime dunes and which is both morphologically and ecologically distinct from the inland variants.

Allen's approach essentially clarifies the differences within the species, though I consider that P. D. Sell's (1967) approach in assigning subspecific status to the maritime form more nearly reflects the biological situation. Hence in the subsequent text, 'the radiate type' refers to var. *hibernicus* Syme *sensu* Allen. *S. vulgaris* ssp. *denticulatus* will be considered more fully in Chapter 7.

The capitulum polymorphism, reflected in the taxonomy of *S. vulgaris* has been of interest to geneticists for many years. The genetic control of the polymorphism was first investigated by Trow (1912), who

found it to be the expression of a single gene, with two alleles showing incomplete dominance. Since Trow, the alleles at the "ray floret" locus have been denoted by different symbols by various authors; however, I have adopted the most commonly used nomenclature employed by Trow (1912), Richards (1975) and Stace (1977). Hence the radiate morph, which is homozygous for the radiate allele, is designated RR; the non-radiate morph which is homozygous for the non-radiate allele is designated rr; and the heterozygote, with an intermediate capitulum, possessing short stubby rays, due to incomplete dominance, is designated Rr.

The different capitula of the radiate and non-radiate morphs of *S. vulgaris* have an effect on the degree of outcrossing exhibited by a plant. Marshall and Abbott (1982, 1984a), found that the radiate morph had a rate of outcrossing that ranged between 3-35% and was much higher than that of the non-radiate morph (usually ≤1% outcrossing). This difference was chiefly accounted for by the pistillate ray florets outcrossing at significantly higher levels than the disc florets (Marshall and Abbott, 1984b).

The relationship between the relative fecundity of the two morphs has been investigated by a number of authors in an attempt to explain the maintenance of the capitulum polymorphism. Oxford and Andrews (1977), working on material from sites at Selby (Yorkshire) and The Mumbles and Barry in S. Wales, found that the radiate morph

produced more seed per plant than non-radiates from the same population. However, Kadereit and Briggs (1985) could find no such differences between the two morphs from four Cambridgeshire populations, when progenies were raised under controlled conditions, and Marshall and Abbott (1987) found that in several Edinburgh populations, the radiate morph exhibited either equal or, more often, lower relative fitness than the non-radiate morph based on measurements of seed output. More recently, Horrill (1989) has established that seed of the two morphs sampled from an Edinburgh population differed in germination behaviour such that seed of the non-radiate morph produced in autumn typically germinate soon after dispersal overwintering as seedlings. In contrast, a greater proportion of the seed of the radiate morph produced at the same time of year delay germination until the following spring. The relative success of each pattern of germination behaviour is dependent upon the severity of the winter. If the winter is marked by repeated heavy frosts, large numbers of seedlings are killed, leaving only the seed bank, which is comprised predominantly of radiate seed, to form the spring population. However, if the winter is mild, non-radiate seedlings that germinated in autumn develop into plants that are much larger than the radiate plants produced from spring germinated seed. As a consequence the large non-radiate plants produce greater numbers of seed than plants of the radiate morph.

It has been suggested that the radiate morph may have originated in *S. vulgaris* in two possible ways: via introgression of the radiate gene from *S. squalidus* into *S. vulgaris* or by a simple gene mutation in *S. vulgaris* .

Mutation of the non-radiate gene to produce a radiate form would essentially be a back mutation as the Mediterranean group from which *S. vulgaris* is thought to have evolved is typically radiate (Stace, 1977). However, mutations causing change from a radiate to non-radiate form (e. g. in *Aster tripolium* L.) and from non-radiate to the radiate forms (e. g. in *Bidens cernua* L.) are well known in the Asteraceae (Stace, 1977), and thus both types of mutation could occur within *Senecio*.

The initial evidence for an origin via introgression was largely circumstantial, with the appearance of the radiate form in an area generally coinciding with the spread of *S. squalidus*. Consequently, Stace (1977) in reassessing the evidence for the origin of the radiate morph, commented that the possibility of an introgressive origin had been overstated, at the expense of mutation. He pointed out that the coincidence of the radiate morph with the occurrence of *S. squalidus* was not perfect, and that the radiate morph frequently did not occur in areas were *S. squalidus* had been present for a long time, particularly in the south-east of England. Furthermore, he questioned the findings of Hull (1974b), who had carried out an electrophoretic study on central Scottish populations of *S. vulgaris* and concluded that there were

differences between the two capitulum morphs and that this reflected the influence of the genes that had been introduced into the radiate form of *S. vulgaris* from *S. squalidus* . Stace (1977) considered that the survey carried out by Hull revealed no significant differences between the two morphs of *S. vulgaris* . Following his review of the evidence available at the time, Stace (1977) concluded that neither of the two theories of the evolution of the radiate form of *S. vulgaris* had an overwhelming degree of support.

Much stronger evidence for the introgressive origin of radiate *S. vulgaris* has come from work carried out by Ingram et al (1980). They showed that a tetraploid resembling the radiate form found in the wild could be artificially generated by backcrossing the artificial triploid hybrid between *S. vulgaris* var. *vulgaris* and *S. squalidus* to the *S. vulgaris* var. *vulgaris* parent. The low degree of fertility of the artificially produced triploid, led Ingram et al (1980) to accept Stace's view that the radiate form had only a very restricted origin.

However, both Kadereit and Briggs (1985) and Abbott (1986), while commenting that the evidence of Ingram et al (1980) considerably strengthens the hypothesis of an introgressive origin of the radiate form, questioned the assertion that such an origin had occurred on only one or two occasions. They reported that differences between morphs in life history traits were not consistent over populations, and this may reflect a polytopic origin of radiate groundsel.

A major aim of the electrophoretic survey reported in this chapter was to examine the possibility of an introgressive origin of the radiate morph of *S. vulgaris* yet further. In the course of carrying out this analysis, the survey was extended to investigate the genetic structure of populations of *S. vulgaris* (monomorphic and polymorphic for capitulum type) within the British Isles. Furthermore, a comparison of the variation present in British and Continental populations was made to place the British results in a wider context.

5. 2. MATERIALS AND METHODS

Fifteen polymorphic and eleven monomorphic populations of *S. vulgaris* were subjected to an electrophoretic survey of isozyme variation using the methods described in Chapter 2. Details of the sites from which the populations were sampled are listed in Table 5. 1. Of the 26 populations surveyed, five were from mainland Europe, while the remainder were from Britain.

The sites of populations sampled from Wrexham, Mochdre and Leith were described in the Chapter 4. The remaining populations were from open or waste ground, or the thin layer of soil which occurs along the edge of paths and pavements.

Most populations were sampled from within a short radius of the given map reference (Table 5. 1). However, samples from Cardiff, Glasgow (South St.) and Tower Hamlets (London) were collected over a wider area. The Glasgow (South St.) population was sampled linearly over approximately 800m, while plants at Cardiff were collected within a 400m radius, and at Tower Hamlets (London) within a 1500m radius from the given map reference. Details of the sites and populations at Methil, Grangemouth, Migvie, Aberffraw, Puffin Island and mainland Europe are as follows.

Methil : Both a monomorphic and a polymorphic population were

Table 5. 1. Location, grid reference and collection date of *S. vulgaris* populations surveyed.

Location	Nat. Grid ref.	Collection date	Notes
Polymorphic populations			
Wrexham			
Rhostyllen	SJ 312 492	30-05-87	
Brymbo	SJ 296 539	30-05-87	
Ffrith	SJ 286 556	03-10-86	
Southsea	SJ 306 515	30-05-87	
Mochdre	SH 822 781	03-10-86	
Birmingham	SP 045 835	23-07-88	1.
Cardiff	ST 173 733	02-06-87	2.
St. Helens	SJ 524 944	07-08-88	1.
Methil (Fife)	NT 376 995	14-07-88	2.
Leith	NT 268 765	28-05-86	
Grangemouth			
Kinneil Tip	NS 977 814	12-05-88	1.
Devon St.	NS 913 823	12-05-88	1.
Glasgow			
Kelvingrove	NS 578 664	09-05-88	1.
South St.	NS 534 671	09-05-88	1.
Rotterdam docks		1984	
Monomorphic populations			
Tower Hamlets (London)	TQ 349 807	12-07-88	
Migvie (Aberdeenshire)	NJ 437 068	28-10-87	1, 2.
Dundee			
Railway yard	NO 373 295	04-10-87	1.
Tip	NO 394 295	04-10-87	1.
Puffin Island (Anglesey)	SH 653 824	1968	
Aberffraw	SH 366 656	1968	
Methil	NT 377 998	05-09-87	
Matalascañas (S. Spain)		04-87	
Basel (Switzerland)		09-87	
Interlaken (Switzerland)		09-87	
Grindelwald (Switzerland)		09-87	

Notes to table. 1. Samples were taken directly from the wild rather than grown up from seed. 2. Samples were surveyed jointly with Irwin

sampled from Methil in Fife. The polymorphic population was located on the ballast of an infrequently used rail-link to the docks. Also present were *S. squalidus* and *S. viscosus*. The monomorphic population occurred in a car park on recently reclaimed land between the docks and a power station and was located approximately 400m from the polymorphic population.

Grangemouth : The Kinneil tip at Grangemouth, West Lothian, is a municipal dump on the south side of the River Forth. The second population sampled from Grangemouth occurred on open, recently disturbed ground, adjacent to the River Carron. Material from both Grangemouth sites had been previously subjected to morphometric analysis by Taylor (1984).

Migvie : The population sampled from a garden at Migvie, Aberdeenshire, represents a "pure" non-radiate population. i. e. neither *S. squalidus* nor radiate *S. vulgaris* have been reported from this part of Scotland, and thus the genome of *S. vulgaris* at this site is unlikely to have been affected by introgressed genes from *S. squalidus*.

Aberffraw and Puffin Island : The seed of plants from these two Welsh populations came from stock collected originally by Abbott (1976). Seed was collected from inbred lines derived from this stock. The population

at Puffin Island occurs on soils rich in nitrogen, phosphorus and potassium, while the population on the sand dunes at Aberffraw occurs on soils deficient in these nutrients. Further details of the populations at both sites is given in Abbott (1976).

Mainland Europe : The populations sampled at Basel, Grindelwald and Interlaken in Switzerland were small, occurring on open and disturbed sites. Material from Matalascañas (southern Spain) was collected from waste ground adjacent to sand dunes. Seed of this population has been shown to exhibit innate seed dormancy which can be broken by a short period of stratification in wet sand at 4°C (Abbott, pers. comm.).

Polymorphic material from the Netherlands came from a site adjacent to the Rotterdam docks and was supplied by Dr. Koniuszek in 1984. Although the seed was collected from several parents, samples were bulked following collection to provide a sample of radiate and non-radiate seed. Since 1984 a number of inbred lines have been derived from each of these bulked samples.

Statistical analysis

Following estimation of electrophoretic phenotype/genotype and allele frequencies within morphs in each population, the degree of genetic variation within *S. vulgaris* was quantified using Nei's gene diversity statistics (1973). These statistics compartmentalise the total gene

diversity (H_T) of the species' population into gene diversity between and within sub-populations (D_{ST} and H_S respectively), such that

$$H_T = D_{ST} + H_S.$$

H_T is measured in terms of the total expected heterozygosity and is calculated from the mean allele frequencies, across populations,

$$\text{i. e. } H_T = 1 - \Sigma^K_{i=1} \bar{x}_i^2,$$

where $\bar{x}_i$ is the mean frequency of the ith of K alleles for the populations surveyed. H_S is the mean of the expected heterozygosities within each sub-population, (expected heterozygosity $H_S = 1 - \Sigma^K_{i=1} x_i^2$, where x_i is the ith of K alleles in a given sub-population) a "sub-population" being the sample of *S. vulgaris* at a particular site and the "population" referring to *S. vulgaris* throughout the country.

These diversity indices are used to calculate further measures of genetic variation; i. e. G_{ST}, the gene diversity between sub-populations relative to the combined populations ($G_{ST} = D_{ST}/H_T$), and R_{ST}, the between population gene diversity relative to the within population gene diversity ($R_{ST} = D_m/H_S$), where D_m is a measure of between sub-populational diversity calculated from D_{ST} by excluding the comparisons of sub-populations with themselves. D_m is estimated using

the formula $D_m = sD_{ST}/(s-1)$ where s is the number of sub-populations surveyed.

In addition to estimating the above indices of gene diversity, the genetic structure of sub-populations was analysed using F-statistics (Wright, 1951). This approach distinguishes two levels of population structure : F_{IS}, the inbreeding co-efficient, describes the divergence of observed heterozygosity from the expected level of heterozygosity within the sub-populations assuming panmixia, and F_{ST}, the fixation index, describes the reduction in heterozygosity within sub-populations relative to the total population due to selection or drift. (F_{ST} is equivalent to the gene diversity between sub-populations, i. e. G_{ST}; Nei, 1975). Finally, F_{IT}, the overall inbreeding coefficient, describes the reduction of heterozygosity within individuals relative to the total population due to non-random mating within sub-populations (F_{IS}) and population sub-division (F_{ST}).

The three F-statistics may be calculated directly from gene diversity indices (after Hartl, 1981) as follows:

$$F_{IS} = (H_S - H_I) / H_S ;$$

$$F_{ST} = (H_T - H_S) / H_T ;$$

$$F_{IT} = (H_T - H_I) / H_T ;$$

where H_I is the mean observed heterozygosity per individual, and H_S and H_T are as previously defined. The three F-statistics are related as follows :

$$1-F_{IT} = (1-F_{IS})\ (1-F_{ST})$$

Other methods have been utilised to calculate F values, particularly F_{ST}. Nei (1987), following Kirby (1975), incorporated weighted means into the method of calculation. Otherwise his method was the same as that used by Hartl (1981). Such increased sophistication is of dubious value given that population size is liable to fluctuate from one generation to the next (Nei, 1987). Soltis and Soltis (1987) have estimated F_{ST} from the variance of the allele frequencies among the sub-populations divided by the product of the mean allele frequencies. Guries and Ledig (1982) also used this approach, but incorporated a correction factor to account for the effect of variation in population size. A different approach has been taken by Weir and Cockerham (1984), using a method of estimation based on an analysis of variance of gene frequencies.

To determine the most appropriate method of calculating Wright's F-statistics in the present study, two data sets were analysed using the methods of Hartl (1981), Nei (1987), Soltis and Soltis (1987) and Guries and Ledig (1982) in turn. The two sets of data differed in regard to the distribution of allele frequencies. In the first data set, one of two

alleles occurred at high frequency, while in the second both alleles occurred at intermediate frequencies. Each method of estimation produced very similar values for the three F-statistics and it was decided, therefore, to use the simplest method, that of Hartl (1981), on the total data set.

The statistical significance of F_{ST} values was tested for each locus by a χ^2-test where $\chi^2 = 2NF_{ST}(k-1)$, with (k-1) (s-1) degrees of freedom, where N is the total sample size, k is the number of alleles at the locus and s is the number of sub-populations (Workman and Niswander, 1970).

Four measures of "allelic richness" (Glover and Barrett, 1987) were also calculated:- P - the percentage of polymorphic loci, (a polymorphic locus being defined as one where the frequency of the most common allele is < 0.95); L - the mean number of alleles per polymorphic locus; K - the mean no. of alleles per locus, where the frequency of the least common allele is > 0. 05, calculated per population [K (Popn.)] or per species [K (Spec.)]; and H_O - the mean observed heterozygosity.

5. 3. RESULTS.

Phenotype/Genotype frequencies in populations

The electrophoretic analysis of isozyme variation revealed that five of the seventeen enzyme coding loci surveyed were polymorphic over the 26 populations of *S. vulgaris* examined (29. 4%). The polymorphic loci were *α–Est-1*, *β–Est-1*, *β–Est-2*, *Got-1* and *Pgi-1*. Genotype and allele frequencies at each polymorphic locus in each population are presented in Tables 5. 2 - 5. 6., except for the *β–Est-2* locus where due to dominance only phenotype frequencies are presented.

The *α-Est-1* locus

In all populations, bar that from Migvie (Aberdeenshire), the individuals of *S. vulgaris* produced one or other or both allozymes, denoted as slow(*α-Est-1b*) and fast (*α-Est-1c*), with the heterozygote expressing the two banded phenotype. In the populations monomorphic for non-radiate capitulum type (Table 5. 2b.), allelic variation at the *α-Est-1* locus was limited and most individuals in a population were homozygous for the same allele. Individuals expressing the double banded 'heterozygous' genotype were rare. In contrast, the Migvie population was composed of double banded individuals (*α-Est-1b* / *α-Est-1c*) plus individuals homozygous for the *α-Est-1c* allele. A test

Table 5.2a. *α-Est -1* genotype and allele frequencies within morphs in polymorphic populations of *S. vulgaris*

			Genotype frequency.			;	Allele frequency.	
Location	morph	(n)	b/b	b/c	c/c	;	b	c
Wrexham								
Rhostyllen	RR	14	-	-	1.00	;	-	1.00
	rr	28	.04	-	.96	;	.04	.96
Brymbo	RR	21	.10	.05	.85	;	.12	.88
	rr	38	.29	.02	.68	;	.31	.69
Ffrith	RR	16	-	-	1.00	;	-	1.00
	rr	37	-	-	1.00	;	-	1.00
Southsea	RR	32	-	-	1.00	;	-	1.00
	rr	65	.48	.03	.49	;	.49	.51
Mochdre	RR	40	-	-	1.00	;	-	1.00
	rr	45	.38	-	.62	;	.38	.62
Birmingham	RR	50	-	-	1.00	;	-	1.00
	rr	50	-	-	1.00	;	-	1.00
Cardiff	RR	27	-	-	1.00	;	-	1.00
	rr	33	.06	-	.94	;	.06	.94
St. Helens	RR	40	-	-	1.00	;	-	1.00
	rr	40	-	-	1.00	;	-	1.00
Methil	RR	36	-	-	1.00	;	-	1.00
	rr	32	.19	.09	.72	;	.23	.77
Leith	RR	50	.50	.06	.44	;	.53	.47
	rr	48	.33	.04	.63	;	.35	.65
Grangemouth								
Devon St.	RR	25	-	.04	.96	;	.02	.98
	rr	25	.16	-	.84	;	.16	.84
Kinneil Tip	RR	25	.04	-	.96	;	.04	.96
	rr	25	.40	.04	.56	;	.42	.58
Glasgow								
Kelvingrove	RR	25	-	-	1.00	;	-	1.00
	rr	25	-	-	1.00	;	-	1.00
South St.	RR	25	-	-	1.00	;	-	1.00
	rr	25	-	-	1.00	;	-	1.00
Netherlands	RR	20	-	-	1.00	;	-	1.00
	rr	20	-	-	1.00	;	-	1.00

Table 5. 2b. *α-Est -1* genotype and allele frequencies in populations of *S. vulgaris* monomorphic for the non-radiate morph.

Population	(n)	Genotype frequency a/a	b/b	b/c	c/c	;	Allele frequency a	b	c
Tower Hamlets	(45)	-	.98	.02	-	;	-	.99	.01
Migvie	(55)	.11	.09	.53	.27	;	.11	.35	.54
Dundee									
Railway yard	(27)	-	-	-	1.00	;	-	-	1.00
Tip	(18)	-	-	.17	.83	;	-	.08	.92
Puffin Island	(30)	-	-	.03	.97	;	-	.02	.98
Aberffraw	(24)	-	-	.04	.96	;	-	.02	.98
Methil	(25)	-	1.00	-	-	;	-	1.00	-
Matalascañas	(37)	-	.64	-	.36	;	-	.64	.36
Basel	(11)	-	-	-	1.00	;	-	-	1.00
Interlaken	(4)	-	-	-	1.00	;	-	-	1.00
Grindelwald	(4)	-	-	-	1.00	;	-	-	1.00

by Irwin (1990) has shown that the double banded individuals in this population failed to segregate and may be considered as fixed heterozygotes. The Migvie population was also characterised by the presence of an allele (*α-Est-1a*), albeit at a low frequency, not found in any other population.

In eight of the eleven non-radiate populations surveyed (Table 5. 2b), the most common allele was *α-Est-1c* . Only in the populations sampled from Tower Hamlets, Methil and Matalascañas was the *α-Est-1b* allele more common. In five of the populations polymorphic for capitulum type (at Ffrith, Birmingham, St. Helens and both Glasgow sites.) the *α-Est-1c* allele was fixed in both the radiate and non-radiate morphs (Table 5. 2a.). In nearly all other populations surveyed, the radiate morph was monomorphic or nearly so for the *α-Est-1c* allele whereas the non-radiate morph was polymorphic for both *α-Est-1c* and *α-Est-1b* alleles. Only in the population at Leith (Edinburgh) was the *α-Est-1b* allele present along with the *α-Est-1c* allele at a high intermediate frequency in the radiate morph. The general trend, therefore, was for the non-radiate morph to be far more polymorphic than the radiate morph at the *α-Est-1* locus in populations containing both morphs.

It is of interest to compare the allele frequencies at the *α-Est-1* locus of morphs in different populations within the same area i. e. at Glasgow, Dundee, Grangemouth, Wrexham and Methil. In both populations in Glasgow, each morph was fixed for the *α-Est-1c* allele, while at Dundee the non-radiate morph exhibited a similar frequency for the same allele in both populations. In contrast, marked differences in allele frequencies within the non-radiate morph occurred between local populations within the Wrexham area and also at Grangemouth and Methil, possibly due to founder effects.

The *β-Est-1* locus

Three alleles were detected at the *β–Est-1* locus (Table 5. 3a & b). One of these, denoted as *β-Est-1c*, was only found in the St. Helens population where it occurred in the radiate morph at a very low frequency (.02). The *β-Est-1c* allele produced an allozyme of comparable mobility to an equivalent isozyme present in *S. squalidus*.

The *β-Est-1a* allele occurred at high frequency (> .84) in all populations monomorphic for the non-radiate morph, except that from Basel which was fixed for the *β–Est-1b* allele. Only in one population, at the Dundee tip site, were plants heterozygous for the *β–Est-1a* and

Table 5. 3a. *β-Est-1* genotype and allele frequencies within morphs in polymorphic populations of *S. vulgaris*.

Location	morph	(n)	Genotype frequency a/a	a/b	b/b	;	Allele frequency a.	b.
Wrexham								
Rhostyllen	RR	15	1.00	-	-	;	1.00	-
	rr	28	1.00	-	-	;	1.00	-
Brymbo	RR	21	1.00	-	-	;	1.00	-
	rr	38	1.00	-	-	;	1.00	-
Ffrith	RR	16	1.00	-	-	;	1.00	-
	rr	37	1.00	-	-	;	1.00	-
Sou thsea	RR	32	.97	-	.03	;	.97	.03
	rr	65	.98	-	.02	;	.98	.02
Mochdre	RR	40	.35	.025	.625	;	.36	.64
	rr	45	1.00	-	-	;	1.00	-
Birmingham	RR	50	.88	.10	.02	;	.93	.07
	rr	50	.98	-	.02	;	.98	.02
Cardiff	RR	27	.89	.04	.07	;	.91	.09
	rr	33	.27	-	.73	;	.27	.73
St. Helens	RR	40	1.00	-	-	;	1.00	-
	rr	40	.325	.275	.35*	;	.46	.52*
Methil	RR	36	1.00	-	-	;	1.00	-
	rr	32	.97	-	.03	;	.97	.03
Leith	RR	50	.98	.02	-	;	.99	.01
	rr	48	.78	-	.22	;	.78	.22
Grangemouth								
Devon St.	RR	25	1.00	-	-	;	1.00	-
	rr	25	1.00	-	-	;	1.00	-
Kinneil Tip	RR	25	1.00	-	-	;	1.00	-
	rr	25	1.00	-	-	;	1.00	-
Glasgow								
Kelvingrove	RR	25	1.00	-	-	;	1.00	-
	rr	25	.72	-	.28	;	.72	.28
South St.	RR	25	1.00	-	-	;	1.00	-
	rr	25	.96	-	.04	;	.96	.04
Netherlands	RR	20	1.00	-	-	;	1.00	-
	rr	20	1.00	-	-	;	1.00	-

Notes to table 5.3a; * The St Helens sample contained two individuals with a fast/superfast banding pattern (A frequency of .05). The superfast allele was present at a frequency of .02.

Table 5. 3b. *β–Est-1* Genotype and allele frequencies in populations of *S. vulgaris* monomrphic for the non-radiate morph.

		Genotype frequency			;	Allele frequency	
Population	(n)	a/a	a/b	b/b	;	a	b
Tower Hamlets	(45)	1.00	-	-	;	1.00	-
Migvie	(55)	.92	-	.08	;	.92	.08
Dundee							
Railway yard	(27)	.96	-	.04	;	.96	.04
Tip	(18)	.83	.17	-	;	.92	.08
Puffin Island	(30)	.84	-	.16	;	.84	.16
Aberffraw	(24)	.04	-	.96	;	.04	.96
Methil	(25)	1.00	-	-	;	1.00	-
Matalascañas	(37)	.97	-	.03	;	.97	.03
Basel	(11)	-	-	1.00	;	-	1.00
Interlaken	(4)	1.00	-	-	;	1.00	-
Grindelwald	(4)	1.00	-	-	;	1.00	-

β–Est-1b alleles.

The *β-Est-1a* allele was also the most common allele in polymorphic populations (Table 5. 3a.). Only in three instances was this not the case i. e. among radiate plants at Mochdre and within the non-radiate morph at Cardiff and St. Helens.

The trend, previously recorded at the *α-Est-1* locus, of the non-radiate morph being more polymorphic than the radiate morph was also found at the *β-Est-1* locus in the populations at Cardiff, St. Helens, Leith and Glasgow Kelvingrove. However, in two samples, i. e. from Mochdre and Birmingham this pattern was reversed. Radiate plants that were heterozygous at the *β-Est-1* locus were recorded in samples from Mochdre, Birmingham, Cardiff and Leith. In contrast, heterozygous non-radiate plants were only recorded in the St. Helens sample where they occurred at a surprisingly high frequency.

In general, local populations from the same areas exhibited very similar allele frequencies at the *β-Est-1* locus. The exception was in Glasgow where the non-radiate plants in the Kelvingrove sample exhibited the *β-Est-1b* allele at a higher frequency than in the samples from South St.

β-Est-2

The dominance recorded at the *β-Est-2* locus (Irwin, 1990) makes identification of heterozygotes at this locus uncertain. Consequently, this prevents accurate calculation of genotype frequencies and, therefore, only phenotype frequencies may be considered (Table 5. 4a & b). Three phenotypes were detected at the *β-Est-2* locus (Table 5. 4a & b). One of these, which was considered to be homozygous for *β–Est-2c*, was only found in the Migvie population, where it occurred in the non-radiate morph at a very low frequency (.02). This phenotype, which was previously undetected in *S. vulgaris* (see Chapter 2), produced an allozyme which had a comparable mobility to the *β–Est-2c* isozyme found in *S. squalidus*.

In monomorphic populations the predominant phenotype was that which produced the *β-Est-2a* allozyme (see Table 5. 4b.). Only in samples from Puffin Island and Aberffraw was this not the case. Again in the majority of populations polymorphic for capitulum type, the *β–Est-2a* phenotype was the most common type both in the radiate and non-radiate morph (Table 5. 4a.). In seven populations i. e. from Rhostyllen, Ffrith, St. Helens, Methil, Leith and both Glasgow populations, the radiate morph was fixed for the *β–Est-2a* phenotype

Table 5. 4a. *β-Est-2* phenotype frequencies within morphs in polymorphic populations of *S. vulgaris*.

Location	morph	(n)	Phenotype fr. a/a	a/b	b/b
Wrexham					
Rhostyllen	RR	15	1.00	-	-
	rr	28	.89	-	.11
Brymbo	RR	21	.95	.05	-
	rr	38	1.00	-	-
Ffrith	RR	16	1.00	-	-
	rr	37	.59	-	.41
Southsea	RR	32	-	.06	.94
	rr	65	.88	-	.12
Mochdre	RR	40	.20	.05	.75
	rr	45	.89	-	.11
Birmingham	RR	50	.30	.04	.66
	rr	50	.04	.02	.94
Cardiff	RR	27	.11	-	.89
	rr	33	.52	-	.48
St. Helens	RR	40	1.00	-	-
	rr	40	.925	-	.075
Methil	RR	36	1.00	-	-
	rr	32	.97	-	.03
Leith	RR	50	1.00	-	-
	rr	48	.88	.02	.10
Grangemouth					
Devon St.	RR	25	.76	-	.24
	rr	25	.48	.08	.44
Kinneil Tip	RR	25	.04	-	.96
	rr	25	.76	.04	.20
Glasgow					
Kelvingrove	RR	25	1.00	-	-
	rr	25	.92	.04	.04
South St.	RR	25	1.00	-	-
	rr	25	.96	-	.04
Netherlands	RR	20	1.00	-	-
	rr	20	1.00	-	-

Table 5. 4b *β-Est-2* phenotype frequencies in populations of *S. vulgaris* monomorphic for the non-radiate morph.

Population	(n)	Phenotype fr a/a	a/b	b/b
Tower Hamlets	(45)	1.00	-	-
Migvie	(55)	.85	-	.13*
Dundee				
Railway yard	(27)	.74	-	.26
Tip	(18)	.61	-	.39
Puffin Island	(30)	.07	-	.93
Aberffraw	(24)	-	-	1.00
Methil	(25)	1.00	-	-
Matalascañas	(37)	.82	-	.18
Basel	(11)	1.00	-	-
Interlaken	(4)	1.00	-	-
Grindelwald	(4)	1.00	-	-

*The Migvie population contained an individual exhibiting a fast/superfast phenotype at a frequency equalling 0 .02.

while both phenotypes, *β–Est-2a* and *β–Est-2b*, were recorded in the non-radiates morph. Only in the sample from Brymbo was the reverse situation recorded.

A point of particular note to emerge in several of the populations surveyed was the finding that the most common phenotype in one morph for *β-Est-2* was the least common in the other morph. This was the case in samples from Southsea, Mochdre, Cardiff and Kinneil Tip.

Got-1

As previously described in Chapter 2, three 3-banded *Got-1* phenotypes were found in *S. vulgaris*. Individuals exhibited either a slow /medium *(Got-1a /Got-1b)* ; slow /fast *(Got-1a /Got-1c)* or medium /fast *(Got-1b /Got-1c)* phenotype. A rare six banded phenotype also occurred *(Got-1a /Got-1b /Got-1c)*, which is believed to be the product of crosses between individuals of different three-banded phenotype.

In populations monomorphic for the non-radiate morph (Table 5. 5b), only the *Got-1b /Got-1c* phenotype was recorded. Further analysis of over 100 individuals sampled from monomorphic populations in north Fife has confirmed this uniformity of the non-radiate morph. Phenotypic variation at the *Got-1* locus was present in both morphs within polymorphic populations, but was much more common in the

Table 5. 5a. *Got-1* phenotype and gamete type frequencies within morphs in polymorphic populations of *S. vulgaris.*

Location	morph	(n)	Phenotype frequency ; a/b	a/c	b/c	a/b/c	;	Gamete frequency a/b	a/c	b/c
Wrexham										
Rhostyllen	RR	15	.27	-	.73	-	;	.27	-	.73
	rr	23	.04	-	.96	-	;	.04	-	.96
Brymbo	RR	21	.29	.33	.38	-	;	.29	.33	.38
	rr	38	.03	.03	.87	.07	;	.05	.05	.90
Ffrith	RR	16	.12	.88	-	-	;	.12	.88	-
	rr	10	-	-	1.00	-	;	-	-	1.00
Southsea	RR	24	.50	.17	.25	.08	;	.53	.19	.28
	rr	44	.02	-	.96	.02	;	.03	-	.97
Mochdre	RR	24	.04	.88	.08	-	;	.04	.88	.08
	rr	20	-	-	1.00	-	;	-	-	1.00
Birmingham	RR	50	-	-	1.00	-	;	-	-	1.00
	rr	50	-	-	1.00	-	;	-	-	1.00
St. Helens	RR	40	.025	.125	.85	-	;	.025	.125	.85
	rr	40	-	-	1.00	-	;	-	-	1.00
Methil	RR	36	-	1.00	-	-	;	-	1.00	-
	rr	32	-	-	1.00	-	;	-	-	1.00
Leith	RR	25	.64	.08	.28	-	;	.64	.08	.28
	rr	27	.04	.04	.92	-	;	.04	.04	.92
Grangemouth										
Devon St.	RR	25	-	.68	.32	-	;	-	.68	.32
	rr	25	.04	-	.96	-	;	.04	-	.96
Kinneil Tip	RR	25	-	-	1.00	-	;	-	-	1.00
	rr	25	-	-	1.00	-	;	-	-	1.00
Glasgow										
Kelvingrove	RR	25	-	-	1.00	-	;	-	-	1.00
	rr	25	-	-	1.00	-	;	-	-	1.00
South St.	RR	25	.08	.48	.44	-	;	.08	.48	.44
	rr	25	-	-	1.00	-	;	-	-	1.00
Netherlands	RR	10	-	-	1.00	-	;	-	-	1.00
	rr	6	-	-	1.00	-	;	-	-	1.00

N. B. As the parentage of the six banded heterozygote is unknown, calculation of gametic type frequencies in a population where the six banded heterozygote occurs is performed by dividing the frequency of the heterozygote equally between the three alleles.

Table 5. 5b. *Got-1* phenotype frequencies in non-radiate populations of *S. vulgaris.*

Population	(n)	a/b	a/c	b/c	a/b/c
Tower Hamlets	(45)	-	-	1.00	-
Dundee					
Railway yard	(27)	-	-	1.00	-
Tip	(18)	-	-	1.00	-
Puffin Island	(10)	-	-	1.00	-
Aberffraw	(10)	-	-	1.00	-
Matalascañas	(37)	-	-	1.00	-
Basel	(11)	-	-	1.00	-
Interlaken	(4)	-	-	1.00	-
Grindelwald	(4)	-	-	1.00	-

Table 5. 6. Frequency of *Pgi-1* Phenotypes in *S. vulgaris*

Population	(n)	a/b	b/b	b/c
Matalascañas	(37)	.65	.35	-
Basel	(11)	-	.45	.55
Interlaken	(4)	-	.75	.25
Grindelwald	(4)	-	1.00	-

N. B. All other populations surveyed were monomorphic for the *Pgi-1b* allele.

radiate morph (Table 5. 5a.). Within polymorphic populations, the *Got-1b /Got-1c* phenotype was the most common phenotype among non-radiate plants being fixed in samples of the non-radiate morph from Ffrith, Mochdre, Birmingham, St. Helens, Methil, Kinneil tip the Netherlands and both Glasgow sites. At Birmingham, Kinneil tip, the Netherlands site and Kelvingrove the radiate morph was also fixed for this phenotype. In contrast, at Methil the radiate morph was fixed for the *Got-1a/Got-1b* phenotype, while at other sites the radiate morph was polymorphic for two or more phenotypes. Thus in contrast to what was established at the esterase coding loci, the radiate morph was more polymorphic than the non-radiate morph for *Got-1* .

Comparison of phenotype frequencies in the radiate morph over the four Wrexham populations revealed marked differences between sites. Similarly there were marked discrepancies in phenotype frequencies within the radiate morph between sites in Glasgow and Grangemouth.

Pgi-1

The *Pgi-1* locus was monomorphic for the *Pgi-1b* allele in all British populations surveyed. However, two fixed heterozygotes were detected in continental populations (see Chapters 2 and 3), along with individuals exhibiting the *Pgi-1b / Pgi-1b* single banded phenotype (Table 5. 6). The triple banded *Pgi-1a/ Pgi-1b* fixed heterozygote was

recorded in the sample collected from Matalascañas (S. Spain), while the *Pgi-1b* / *Pgi-1c* heterozygote was found in two of the three Swiss populations screened.

Allelic Richness

Levels of allelic richness are presented in Table 5. 7a & b. Data from the survey of variation at the *β-Est-2* locus (Table 5. 4a, b) are excluded from these estimates in view of the known dominance at the locus and the inability, therefore, to score accurately genotype and allele frequencies. Also, fixed heterozygotes for *Got-1* were treated as homozygotes in the calculation of H_O. The percentage of polymorphic loci (P) was greater in polymorphic than monomorphic populations, with radiate plants showing the highest value. Values for L (mean number of alleles per polymorphic locus), K (mean number of alleles per locus), and H_O (mean observed heterozygosity) were very similar in all three classes of plants investigated (i. e. among radiate and non-radiate plants from polymorphic populations and non-radiate plants in monomorphic populations). Values of allelic richness tended to fall within the range of values recorded for other selfing species (Gottlieb, 1981a) and, therefore, were much lower than is normal for outcrossing species.

Gene Diversity

Measures of gene diversity, i. e. H_T, H_S, D_{ST}, G_{ST} and R_{ST}, in populations of *S. vulgaris* are presented in Table 5. 8. [The zero values for the *Got-1* locus in monomorphic populations are due to the invariant nature of this locus in these populations.]. Total gene diversity

Table 5. 7a. Percentage of loci polymorphic (P), mean number of alleles per polymorphic locus (L), mean number of alleles per locus (K) and mean observed heterozygosity (Ho) in *S. vulgaris* populations polymorphic for capitulum type..

Location	morph	no. loci	P	L	K	H_o
Wrexham						
Rhostyllen	RR	16	6.25	2.	1.06	0.
	rr	16	0.	-	1.	0.
Brymbo	RR	16	12.5	2.5	1.19	.003
	rr	16	12.5	2.5	1.19	.006
Ffrith	RR	16	6.25	2.	1.06	0.
	rr	16	0.	-	1.	0.
Southsea	RR	16	6.25	3.	1.125	.005
	rr	16	6.25	2.	1.06	.003
Mochdre	RR	16	12.5	2.	1.125	.002
	rr	16	6.25	2.	1.06	0.
Birmingham	RR	16	6.25	2.	1.06	.006
	rr	16	0.	-	1.	0.
Cardiff	RR	15	6.67	2.	1.07	.0025
	rr	15	13.33	2.	1.13	0.
St. Helens	RR	16	6.25	2.	1.06	0.
	rr	16	6.25	2.	1.06	.017
Methil	RR	16	0.	-	1	0.
	rr	16	6.25	2.	1.06	.006
Leith	RR	16	12.5	2.5	1.19	.005
	rr	16	12.5	2.	1.125	.0025
Grangemouth						
Devon St.	RR	16	6.25	2.	1.06	.0025
	rr	16	6.25	2.	1.06	0.
Kinneil Tip	RR	16	0.	-	1	0.
	rr	16	6.25	2.	1.06	.0025
Glasgow						
Kelvingrove	RR	16	0.	-	1	0.
	rr	16	6.25	2.	1.06	0.
South St.	RR	16	6.25	3.	1.125	0.
	rr	16	0.	-	1	0.
MEAN VALUES	RR	15.93	6.28	2.27	1.08	.0019
	rr	15.93	5.86	2.05	1.06	.0027

Table 5. 7b. Percentage of loci polymorphic (P), mean number of alleles per polymorphic locus (L), mean number of alleles per locus (K) and mean observed heterozygosity (Ho) in *S.vulgaris* populations monomorphic for capitulum type.

Location	no. of loci.	P	L	K	H_o
Tower Hamlets	16	0.	-	1.	.001
Migvie	16	12.5	2.5	1.19	0.*
Dundee					
Railway yard	16	0.	-	1.	0.
Tip	16	12.5	2	1.125	.021
Puffin Island	16	6.25	2	1.06	.002
Aberffraw	16	0.	-	1.	.0025
Methil	16	0.	-	1.	0.
MEAN	16	4.46	2.17	1.05	.0038

N.B . In the calculation of these indices the *Got-1* locus was taken as monomorphic in populations monomorphic for capitulum type.

* The observed heterozygosity (H_o) for the Migvie population excludes the fixed heterozygote at the *α-Est 1* locus. If included the H_o value for Migvie would be .03 and the overall mean value would equal. 008.

Table 5.8. Levels of gene diversity in the radiate (RR) and non-radiate (rr_p) morphs sampled from polymorphic populations, and the non-radiate morph (rr_m) from monomorphic populations of *S. vulgaris* as measured by Nei's gene diversity statistics.

Locus	Morph	H_T	H_S	D_{ST}	G_{ST}	R_{ST}
α-Est-1	RR	.0963	.0590	.0373	.3873	.6808
	rr_p	.2878	.2227	.0651	.2262	.3148
	rr_m	.4560	.0409	.4151	.9103	12.1790
β-Est-1	RR	.1128	.0595	.0533	.4725	.9647
	rr_p	.2329	.1337	.0992	.4259	.7990
	rr_m	.3061	.1024	.2037	.6655	2.3208
Got-1	RR	.6095	.2971	.3124	.5126	1.1391
	rr_p	.0438	.0421	.0017	.0388	.0471
	rr_m	0.	0.	0.	0.	0.
Locus	Morph	H_T	H_S	D_{ST}	G_{ST}	R_{ST}
Mean	RR	.2727	.1385	.1342	.4921	1.0454
values	rr_p	.1882	.1328	.0554	.2944	.4501
	rr_m	.2540	.0478	.2062	.8118	5.0750

N. B. The mean values for G_{ST}, D_m and R_{ST} were calculated from the mean values of H_T, H_S and D_{ST}.

The European populations were omitted from the statistical analysis due to small sample sizes (rr_p) or uncertainty over the origin of the sample (RR). The allele frequencies at the *α-Est-1* locus for the Migvie populations were omitted from the calculations due to the fixed heterozygotes found at this locus in this population. *Got-1* variation was included in the estimates by equating alleles to gamete type as in Tables 5. 5 a, b.

(H_T) in each class of plants surveyed (radiates and non-radiates from polymorphic populations, and non-radiates from monomorphic populations) was generally low, although a high value was recorded for the radiate morph at the *Got-1* locus and for the non-radiate morph (monomorphic) at the *α-Est-1* locus. Averaged over all loci the low values for H_T are typical of an annual, selfing species (Loveless and Hamrick, 1984).

Examination of R_{ST} values for the radiate morph, and the non-radiate morph occurring in monomorphic populations, shows that a greater proportion of total diversity is due to between population than to within population diversity (H_S). However, the reverse seems to be the case in the non-radiate morph present in polymorphic populations.

Genetic Structure

Estimates of the total inbreeding co-efficient (F_{IT}) were high in all cases (Table 5. 9), as expected for a predominantly selfing species such as *S. vulgaris*. At most loci, F_{IS} was greater than F_{ST}, indicating that high values of F_{IT} were due to a lack of heterozygosity within individuals. In general, the values of F_{IS} were high and positive, but at the *α–Est-1* locus an F_{IS} value close to zero was calculated for individuals in non-radiate monomorphic populations, indicating a

Table 5. 9. The genetic structure of *S. vulgaris* investigated by F-statistics (Hartl, 1981, after Wright, 1953).

Locus	Morph	F_{IS}	F_{ST}	F_{IT}
α-Est-1	RR	.8186	.3873**	.8889
	rr_p	.9295	.2262**	.9454
	rr_m	-0.0587	.9103**	.9050
β-Est-1	RR	.7781	.4725 **	.8830
	rr_p	.8534	.4259 **	.9158
	rr_m	.7627	.6655 **	.9206
Got-1	RR	.9791	.5126 **	.9898
	rr_p	.8425	.0388 **	.8425
	rr_m	0.	0.	0.
Locus	Morph	F_{IS}	F_{ST}	F_{IT}
Mean	RR	.2780	.4921	.9633
	rr_p	.8938	.2944	.9251
	rr_m	.5293	.8118	.9114

N. B. The significance of the F_{ST} values for each locus (i. e. whether significantly different from zero) were calculated using a χ^2-test.

** indicates significant at the 99% level.

The mean values for F_{IS}, F_{ST} and F_{IT} were calculated from the mean values of H_I, H_S and H_T.

The European populations were omitted from the statistical analysis due to small sample sizes (rr_p) or uncertainty over the origin of the sample (RR).

slight excess of heterozygotes compared to the expected value (assuming panmixia). This anomalous result was probably due to a sampling effect caused by a low probability of finding rare alleles in homozygous form, rather than selection favouring heterozygous individuals (Cuguen et. al. 1988). The F_{ST} value was also high at this locus for the same material, indicating a considerable degree of genetic heterogeneity among populations.

5. 4. DISCUSSION

An interesting finding to emerge from the survey of isozyme variation in *S. vulgaris* was the frequent difference between capitulum morphs within the same population, in level of isozyme polymorphism. At the three esterase loci surveyed (*α-Est-1*, and *β-Est-1* & -2), the radiate morph was typically monomorphic while the non-radiate morph was often polymorphic (Tables 5. 2a, 5. 3a and 5. 4a). In contrast, at the *Got-1* locus the radiate morph was often polymorphic while the non-radiate morph was usually monomorphic (Table 5. 5a). Occasionally this difference between morphs in degree of polymorphism resulted in a common isozyme phenotype in one morph being rare in the other morph. This was the case in 5 out of 15 polymorphic populations screened for *β-Est-2* (Table 5. 4a), and 2 of the 15 populations screened for *Got-1* (Table 5. 5a).

These observations raise two questions. First, what causes such differences between morphs to occur, and, second, how might such differences be maintained?

Differences between morphs within a population may be maintained in the absence of effective outcrossing. However outcrossing between morphs in polymorphic populations does occur (Marshall & Abbott, 1982; 1984a) and it is expected, therefore, that in time genetic differences between morphs would tend to disappear except for genes

that are closely linked to the radiate gene or which form a co-adapted gene complex with either allele at the ray floret locus. Despite the occurrence of intermorph outcrossing, there is evidence from Marshall (1982) that the two capitulum morphs remain effectively isolated from each other due, possibly, to the low fitness of the heterozygous intermediate phenotype. Marshall found that in all populations of *S. vulgaris* studied which were polymorphic at the ray floret locus, there was a deficiency of heterozygotes relative to expected values based on the measured outcrossing rates of the three morphs in these populations. This deficiency of heterozygotes was evident from a comparison of the observed and expected values of Wrights fixation index (F) in these populations (Table 5. 9). In all populations, the observed value was larger than expected and, therefore, ΔF was positive in all cases. If the radiate morph originated through introgression from *Senecio squalidus* into *S. vulgaris* (Ingram *et al.* 1980), it is feasible that heterozygotes are genetically unbalanced and, therefore, of reduced fitness.

Although reproductive isolation is the most likely reason for the maintenance of the genetic differences between the two morphs, it does not explain the cause of such differences. In order to understand the origin of these differences, it is necessary to consider the GOT and EST results separately.

Table 5.9. Observed and expected values of Wright's fixation index (F) at the ray floret locus together with values for ΔF for five polymorphic populations of *Senecio vulgaris*, (From Marshall , 1982).

Population	Year	$F_{(obs)}$	$F_{(expt)}$	ΔF
Leith	1978	.950	.872	+ .078
Newhaven Road (Edinburgh)	1978	.980	.911	+ .069
Newhaven Road	1979	.964	.815	+ .149
Newhaven Road	1980	.977	.949	+ .028
Leeds	1979	.951	.931	+ .020
Cardiff	1979	.985	.922	+ .063
Rhos (Wrexham)	1979	.829	.684	+ .245

In populations of *S. vulgaris* polymorphic for capitulum type, three triple banded *Got-1* phenotypes occurred (Table 5. 5a) plus a rare six banded phenotype which is most likely produced following a cross between individuals exhibiting any two of the three triple banded phenotypes (see Chapters 2, 3 and 4 for details of *Got-1* phenotypes). In contrast, in populations monomorphic for the non-radiate variant, only one triple banded phenotype (*Got-1b/Got-1c*) was found (Table 5. 5b). Essentially, the difference is due to the absence of the *Got-1a* allele from non-radiate monomorphic populations and, therefore, an absence of the *Got-1a* band produced by this allele. Comparison of *Got-1* phenotypes within the two capitulum morphs that form polymorphic populations, also showed that *Got-1* phenotypes containing the *Got-1a* band (i. e. *Got-1a/Got-1b, Got-1a/Got-1c* and *Got-1a/Got-1b/Got-1c)* though common in the radiate morph, are extremely rare in the non-radiate

morph.

The survey of *Got-1* phenotypes in *S. vulgaris* clearly shows that the *Got-1a* allele is common in the radiate morph but very rare among non-radiate plants that co-occur with the radiate variant in polymorphic populations, and absent from non-radiate plants growing in monomorphic populations. These facts are made particularly evident when the frequency data for each morph is pooled over populations (Table 5. 10).

Table 5. 10. Frequency of *Got-1* phenotypes among (i) non-radiate plants sampled from populations of *S. vulgaris* monomorphic for capitulum type; and (ii) non-radiate and radiate plants sampled from populations polymorphic for capitulum type.

		Got-1 Phenotypes			
Capitulum morphs	(n)	Got-1a/ Got-1b	Got-1a/ Got-1c	Got-1b/ Got-1c	Got-1a/ Got-1b/ Got-1c
			–	–	–
				–	–
			–		–
		–		–	–
		–			–
		–	–		–
(i) Monomorphic populations*					
(a) non-radiate	266			1.00	
(ii)Polymorphic populations†					
(a) non-radiate	390	.01	.01	.97	.01
(b) radiate	360	.12	.33	.55	(.003)

* 10 populations surveyed (This includes 100 individuals sampled from a N. E. Fife population).
† 14 populations surveyed .

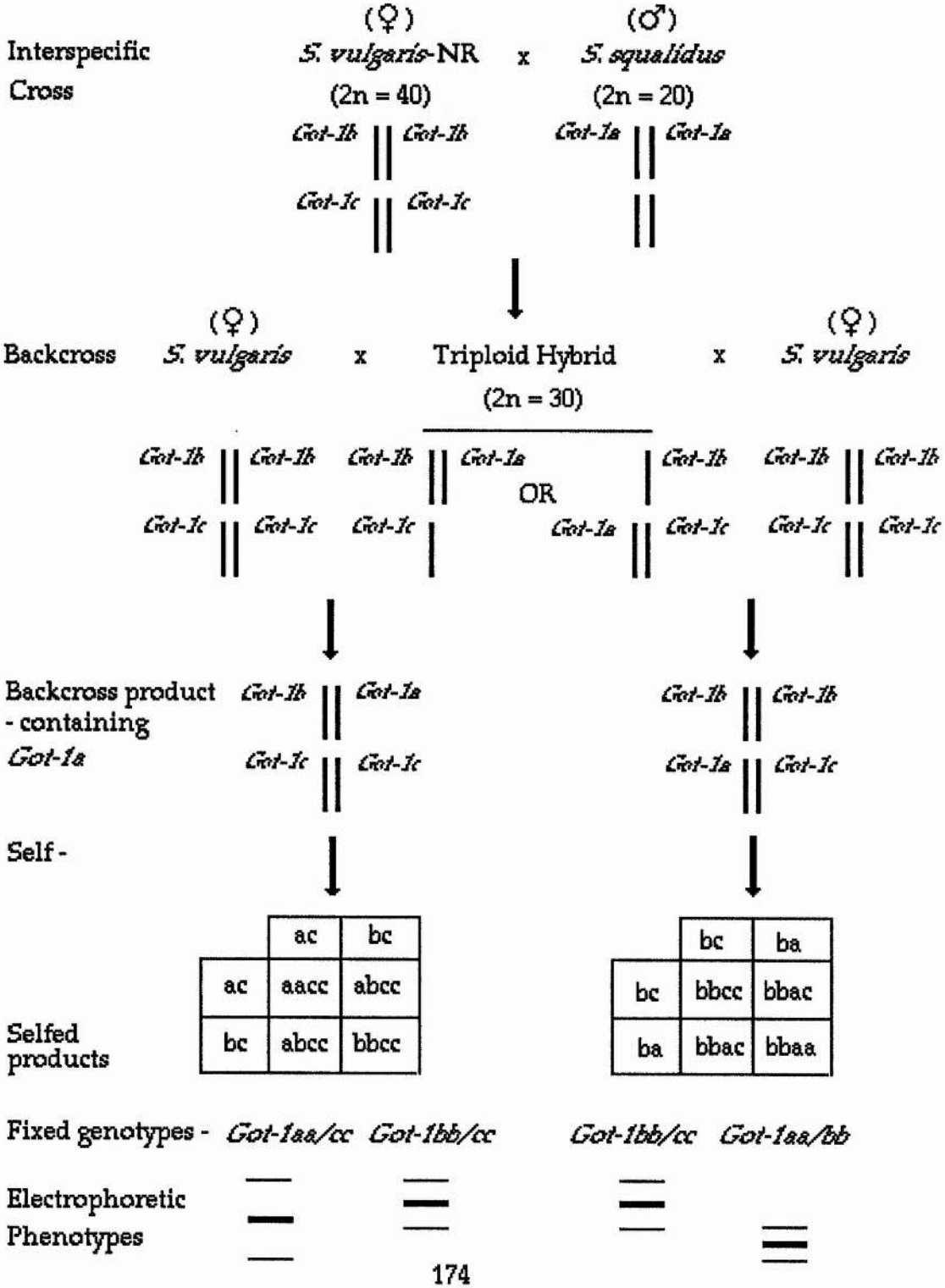
The differences between morphs at the *Got-1* locus, raises the question as to why the *Got-1a* allele is common in radiate *S. vulgaris*, but rare or absent in non-radiate *S. vulgaris*. A possible explanation is that within the radiate morph either the *Got-1b* or the *Got-1c* allele mutated to produce the *Got-1a* allele which subsequently increased in frequency within the British population of the radiate morph. However, if the mutation occurred at only one of the two duplicated loci (either *Got-1b* or *Got-1c*), and assuming strict homologous pairing during meiosis, then only two triple banded *Got-1* phenotypes would be recorded in the radiate morph;- the original *Got-1b/Got-1c* fixed heterozygote phenotype, and a second triple banded phenotype produced by the combined expression of the *Got-1a* allele and the remaining unaltered *Got-1* allele at the other locus. To produce the third triple banded phenotype that is present in the radiate morph, homoeologous pairing would be necessary in a product of a cross between the two triple banded phenotypes produced after the mutational event. Such pairing, although perhaps possible, would also lead to the production of single banded *Got-1* phenotypes not found in *S. vulgaris*..

If mutation is the reason for the presence of the *Got-1a* allele in radiate *S. vulgaris*, it is likely that the mutation occurred on a very limited number of occasions, and the resulting allele has spread during the last fifty years along with the radiate morph with which it is associated.

Although an origin of the *Got-1a* allele in radiate *S. vulgaris* via mutation cannot be ruled out, it is nevertheless surprising that such a mutation might occur and spread in the radiate morph yet not in the non-radiate morph. An alternative and simpler explanation of the frequent occurrence of the *Got-1a* allele in the radiate morph would seem to be required. A much more likely cause of the presence of the *Got-1a* allele in the radiate morph is that the allele was introgressed from *S. squalidus* into *S. vulgaris* along with the radiate allele. A survey of variation at the *Got-1* locus in *S. squalidus* involving a total of 687 plants sampled from 20 populations throughout Britain (see Chapter 6) showed that the *Got-1a* allele was the most common allele at the locus in *S. squalidus* with an estimated frequency equal to 0. 74. A scheme by which this allele may have been introgressed into *S. vulgaris* to produce all three triple banded phenotypes currently found in the radiate morph is illustrated in Fig. 5. 1. The model assumes that the triploid hybrid produced from a cross between non-radiate *S. vulgaris* and *S. squalidus* forms balanced gametes (i. e. containing 20 chromosomes) which are of either *Got-1a/Got-1c* or *Got-1a/Got-1b* genotype. In a backcross to non-radiate *S. vulgaris,* these gametes would fuse with *Got-1b/Got-1c* gametes to produce zygotes that segregate to yield fixed heterozygotes which produce the three different triple banded phenotypes found in radiate *S. vulgaris.*

Two important assumptions of the model are (i) two backcross

Fig. 5. 1. A model for the introgression of the *Got-1a* allele into *S. vulgaris* from *S. squalidus* by means of hybridisation and backcrossing.



products (one of *Got-1b, Got-1a/Got-1c, Got-1c* genotype, the other of *Got-1b, Got-1b/Got-1c, Got-1a* genotype) are required to yield the three different fixed heterozygotes found in the radiate morph; and (ii) in the triploid hybrid, the *S. squalidus* chromosome carrying the *Got-1a* allele may pair with either *S. vulgaris* chromosomes containing the *Got-1* locus, but in the backcross product will pair only with the *S. vulgaris* chromosome for which there is no homologue. If homoeologous pairing occurs within a backcross product, then all three fixed heterozygotes could be produced following just one backcross event.

However, such homoeologous pairing would also yield genotypes homozygous for the same allele at each duplicated locus which, in turn, would produce a single banded phenotype not found in the radiate morph.

The model proposed here follows closely the one advanced previously by Ingram (1978) to explain the introgression of the radiate gene from *S. squalidus* into *S. vulgaris*. It differs, however, in the assumption that an *S. squalidus* chromosome in the triploid hybrid may pair with either of the two *S. vulgaris* chromosomes with which it shares genes rather than with just one of them with which it is presumed to be genetically homologous. The close association found between the two *S. squalidus* characters - the *Got-1a* allozyme and the radiate capitulum - transferred to *S. vulgaris*, suggests that the controlling genes are most likely linked on the same chromosome.

Recombination of these genes would seem to occur occasionally to produce non-radiate variants containing the *Got-1a* allele.

In theory, interspecific gene flow via hybridisation followed by introgression is viewed as an important mechanism in plant evolution (e. g. Stebbins, 1969; Grant, 1981). However, clear evidence of the permanent transfer of genetic material from one species to another is scarce. Most data for introgression is based on morphological analyses which sometimes might be unreliable - witness the failure of Reiseberg et al (1988) to confirm the introgression of genes from *Helianthus annuus* L. into *H. bolanderi* A. Gray from a comparison of chloroplast and ribosomal DNA. Evidence of interspecific transfer of alleles encoding isozymes by means of introgression has been reported in *Phlox* (Levin, 1975), *Sabatania* (Bell and Lester, 1978) *Zea* (Doebley et al 1984), *Pinus* (Plessas and Strauss, 1986) and *Arctostaphylos* (Ellstrand et al, 1987). The current finding in *Senecio* of transfer from *S. squalidus* to *S. vulgaris* of the *Got-1a* allele along with the radiate gene, provides additional evidence of interspecific introgression that is of particular significance.

Having discussed in some detail the cause of the difference between radiate and non-radiate *S. vulgaris* in regard to *Got-1* variation, morph differences for esterase variation will now be considered. In contrast to the results at the *Got-1* locus the non-radiate morph was more polymorphic than the radiate morph at both esterase loci. This

greater polymorphism in the non-radiate morph is unexpected given the higher outcrossing rate of the radiate morph (Marshall and Abbott, 1982, 1984a) yet accords with a morph difference in variation for life history traits previously reported by Abbott (1986). Gouyon and Vernet (1982) have shown that within a polymorphic population, an outcrossing morph (e. g. the radiate morph) is expected to contain greater levels of genetic variation than a selfing morph (e. g. the non-radiate morph). Why then is this not so for the esterases surveyed in this study and the life history traits investigated by Abbott ?

Perhaps the most likely explanation rests on the fact that the radiate morph of *S. vulgaris* is of recent origin (Stace, 1977), and therefore contains a much narrower gene pool than the non-radiate morph. Consequently for most characters the radiate morph exhibits less genetic variation. The radiate morph was first recorded in Britain in 1866 (Syme, 1875) but has only become common since the late 1950's (Stace, 1977). Given the presence of restricted gene flow between the two morphs as argued above, it is not surprising that the radiate morph is less polymorphic than the non-radiate morph for esterases.

Finally it is worth commenting on the differences found between British and Continental populations of *S. vulgaris* for *Pgi-1* . In contrast to British populations, all of which were monomorphic for the *Pgi-1b* allele, three of the four populations of *S. vulgaris* sampled from the Continent were polymorphic containing some triple banded

individuals presumably of fixed heterozygous genotype (either *Pgi-1a/Pgi-1b* at Matalascañas or *Pgi-1b/Pgi-1c* at Basel and Interlaken) together with some single banded individuals homozygous for the *Pgi-1b* allele (Table 5. 6). Given the fixed nature of the heterozygotes, it is evident that the *Pgi-1* locus is duplicated in *S. vulgaris* .

PGI duplications have been recorded in several other polyploid species; e. g. *Festuca microstachys* Nutt. (Adams and Allard, 1977), wheat (Hart, 1979), *Clarkia* (Gottlieb and Weeden, 1979), strawberries (Arulsekar et al, 1981) *Polygala vulgaris* (Lack and Kay, 1986), and *Dactylis glomerata* L. (Lumaret, 1986). Such duplications may arise from the combination of genomes when a new allopolyploid originates (Ohno, 1970; Roose and Gottlieb, 1976; Gottlieb and Higgins, 1984). As *S. vulgaris* is polyploid (Ingram and Weir, 1980) such a process could be the cause of the duplication for PGI in this species.

The only instance where a similar pattern of variation to that for *Pgi-1* was found at another locus i. e. presence of fixed heterozygotes and homozygotes in the same population, was at the *α-Est-1* locus in material from Migvie, Aberdeenshire (Table 5. 2b). In all other material surveyed for *α-Est-1*, variation results from the expression of allelic diversity at a single locus (Irwin, 1990). The fact that the *α-Est-1* locus is clearly duplicated in Migvie plants, indicates that in other populations one of the two duplicated loci present in the genome has been silenced.

Similarly at the *Pgi-1* locus in British populations, one of the duplicated loci may have been silenced or, alternatively, individuals are homozygous for the same allele at both loci.

CHAPTER 6 ; ISOZYME VARIATION AND GENETIC STRUCTURE IN *SENECIO SQUALIDUS.*

6. 1. INTRODUCTION.

The Oxford Ragwort *Senecio squalidus* L. is a diploid (2n=20) self-incompatible species that is widespread and common as an annual or short-lived perennial of ruderal habitats in Britain. It is an introduced species, spreading originally from Oxford in the 19th century via the railway system, after being introduced to the Botanic gardens at Oxford from Mount Etna, Sicily, prior to 1690 (Druce, 1927).

The species was cultivated at the Oxford Botanic Gardens from at least 1690 (Kent, 1956), becoming established as an escape in the city over one hundred years later, where it was widespread by the start of the 19th century (Smith, 1828). It was subsequently introduced to various parts of the country by collectors, and Kent (1956) believes that its initial small scale spread in Britain was due to such introductions. Its spread from Oxford occurred rapidly after it reached the railway there in 1880 (Druce, 1927), its means of spread being eloquently described by Druce (1927) as follows;

> "The vortex of air following the express trains carries the fruits in its wake. I have seen them enter a railway-carriage window near Oxford and remain suspended in the air in the compartment until they found an exit near Tilehurst".

The spread of the species in Britain has been chronicled by Kent

(1955, 1956, 1957, 1960, 1963, 1964a, b, c, 1966). This shows that rather than an even spread from Oxford, the plant frequently became established at locations at a distance from other populations. It is presumed that this form of dispersal may have occurred by the transport of ballast, via the railway, from one site to another which then served as a base for the spread of the plant in the surrounding area, as at Cardiff and Brymbo (Kent, 1963).

By the outbreak of the Second World War, the plant was well established over large areas of southern and central England and south Wales, and spreading to the Midlands, north of England, though always in the vicinity of railways or large towns (Kent, 1960). The increased opportunity for the spread of the species away from railway sites and onto waste ground, roadsides, canal paths and walls was provided by the Second World War. Bomb sites provided an ideal open habitat for the species to invade (Kent, 1964a) and such sites were particularly common at sea ports and industrial sites throughout Britain (Kent, 1964a, 1964c). Subsequent clearing of these sites and dumping into gravel pits will have caused further dispersal of the species.

After becoming common in the north of England in the 1940's and 1950's, *S. squalidus* reached Scotland in the late 1950's following its spread along the north-east coast of England. Today the species is common in Scotland at suitable habitats below the Forth-Clyde line (see Plate 6. 1), but its presence north of this line is slight. Small, apparently

Plate 6. 1. *S. squalidus* growing on a disused railway at Grangemouth docks, summer 1988.

stable populations occur on the east coast, at Kirkcaldy and Methil in Fife and the current northern limit of the species range in Britain is defined by a well established road population on a verge at Kirriemuir, Angus. Small populations at Dundee and Aberdeen have been recorded (personal observation), but appear to be ephemeral.

During the period of its establishment in Scotland, *S. squalidus* has spread to many suitable sites throughout England and Wales, but remains absent or very local in rural areas such as Central Wales and the Lake District where suitable habitats are lacking.

There are two particularly important features about the history

of *S. squalidus* in Britain. First the delay of over a century between its introduction to the Oxford Botanic Gardens and its spread to the city; second the success of the species in rapidly colonising vast areas of the country following its arrival at the railway.

Crisp (1972) postulated that the spread of the species into Oxford from its initial site of cultivation must have occurred following catastrophic selection which resulted in the plant becoming adapted to British conditions. He suggested that mutation or the introgression of characters from related species may have aided in the development of a genome adapted to Britain, and concluded that mutation was the more likely reason, given the lack of recognisable characters from neighbouring taxa within *S. squalidus* .

It is not known whether one or several introductions of *Senecio* material from Sicily were made to the Oxford Botanic Gardens during the 17th century as herbarium records for the 1700's have unfortunately been lost. Rather surprisingly for a colonising species, *S. squalidus* is self-incompatible and reproduces by outcrossing. Crisp (1972) considered that the species within Britain may exhibit a weaker level of self-incompatability compared with its Sicilian progenitor, and this may have facilitated its spread. On this point Gibbs *et al.* (1975) reported a low level of seed set (approx. 4-5%) on selfing indicating that, though not complete, self-incompatibility is strong within the species. It is worth noting that for a sporophytic self-incompatibility system to function, as

in *S. squalidus,* at least four self-incompatibility alleles must be segregating in a population (assuming dominance is absent). This means that the offspring of at least two different introduced plants must have been cultivated initially at the Oxford Botanic Garden.

In attempting to explain the successful spread of *S. squalidus* from Oxford via the railways, it is important to recognise that the species was most likely pre-adapted to the railway habitat. Its lack of success outside Oxford prior to 1880 may be accounted for by the absence of such suitable habitats. The area around Oxford during the 19th century was, without exception, rural and *S. squalidus* is still absent from such areas. However, the arrival of the railway in Oxford in 1844 provided the open habitat and edaphic conditions to which the plant was familiar. Druce (1927) considered that the clinker-ash comprising the permanent way was very similar to the laval soils of Sicily on which the species normally grew. Thus the creation of a suitable habitat, coupled with the high levels of traffic on the railways, combined to provide *S. squalidus* with ideal conditions for dispersal. It is of interest that this crucial relationship between the railway habitat and the spread of a plant is not restricted to *S. squalidus* : indeed railways have long been known to be important in the introduction of alien species, and the spread of both alien and native species (Muehlenbach, 1979).

Despite the importance of the railway system to the spread of the species in Britain, Sargent (1984) in a vegetation survey of the permanent

way and railway verges, recorded *S. squalidus* as present at less than 5% of sites surveyed. It is possible that the species has been over-recorded as a railway species in the literature due to its alien origin and conspicuous capitula, alternatively there may be a genuine decline in its abundance on the railways in recent years due to a change in weed control practice or an alteration in the composition of ballast since the end of steam in 1968 (Sargent, 1984). The species, nevertheless, remains a common denizen of railway yards and their environs and such sites normally support large populations for survey.

Identifying the Sicilian antecedents of the British *S. squalidus* is rendered difficult by the loss of herbarium records from the opening of Oxford Botanic Garden in 1621 until 1690, and the ambiguity surrounding the close European relatives of *S. squalidus* . Walters (1964) has remarked, that

> "The taxonomy of plants closely related to the familiar Oxford Ragwort is extremely confused".

Despite two revisions of the taxonomy of European *Senecio* (Tutin *et al.*, 1976; Alexander, 1979)) this confusion remains.

Crisp (1972) has described three species from Mount Etna, Sicily which he believed were the possible antecedents of British *S. squalidus* . These are *S. aethnensis* Jan. a montane type from open ground at altitudes above 1500m with glaucous, entire or widely toothed leaves, and capitula bearing large ligules (~12mm); *S. chrysanthemifolius* Poir. a

lowland type (sea level to 1000m) distinguished from *S. aethnensis* by non-glaucous, highly dissected leaves and capitula with smaller ligules (~8mm); and *S. incisus* Presl. which is morphologically intermediate between *S. aethnensis* and *S. chrysanthemifolius* and occurs at an intermediate altitude of 1000 - 1400m. Due to similarities in morphology, Crisp (1972) considered that British *S. squalidus* is most likely descended from *S. incisus* , which is a hybrid of *S. aethnensis* and *S. chrysanthemifolius*. However, he emphasised that there was significant morphological variation within both *S. aethnensis* and *S. chrysanthemifolius* and so either of these two species could be the source of *S. squalidus* in Britain. From my own observations, specimens of *S. chrysanthemifolius* raised in the greenhouse are very similar to *S. squalidus* .

Alexander (1979) while acknowledging Crisp's theory that *S. squalidus* may have arisen from a hybrid of *S. aethnensis* and *S. chrysanthemifolius* (both of which he recognised), has described three sub-species of *S. squalidus,* two of which occur on Sicily; ssp *squalidus* and ssp. *aurasiacus* . The third subspecies, ssp. *araneosus,* is restricted to Morocco. Alexander lists seven synonyms for *S. squalidus* ssp. *squalidus,* including *S. rupestris* Waldst & Kit. a native of Central Europe which Walters (1964) considered to be identical to British *S. squalidus*. British *S. squalidus* is identified as ssp. *squalidus,* but considered to be untypical of the subspecies, most probably because of the

limited number of individuals that founded the British population. Tutin *et al.* (1976) have recognised *S. aethnensis* but not *S. chrysanthemifolius* . Furthermore they describe *S. incisus* as probably a hybrid between *S. aethnensis* and *S. siculus* All., a lowland species of open sandy ground and they remark that some *S. incisus* individuals resemble *S. squalidus* .

Clearly, historical and morphological evidence point to a Sicilian origin of British *S. squalidus* , although the exact identity of the antecedent is unclear. However, the possibility exists that further introductions into Britain of *S. squalidus*, or morphologically similar inter-fertile relatives, have occurred, and the close similarities of the British plant to its close European relatives, most notably *S. rupestris* , increases the prospect that such introductions would go undetected.

6. 2. AIMS

The principal aim of the studies reported in this chapter was to investigate the level and pattern of genetic variation within and between British populations of *S. squalidus* by means of an electrophoretic analysis of isozyme variation. From the data obtained it has been possible to compare the variation present in *S. squalidus* with that recorded in *S. vulgaris* (Chapter 5) and further to determine whether along the Forth-Clyde valley of Scotland there is clinal variation for isozyme frequency in *S. vulgaris* due to different degrees of introgression from *S. squalidus* as previously reported for esterase by Hull(1974b).

6. 3. MATERIALS AND METHODS

Plant material was subjected to an electrophoretic analysis of isozyme variation using the procedures described in Chapter 2. Plants from twenty three populations were surveyed with an average of 35. 7 individuals per population. Populations were sampled from a wide range of locations in Britain and included material from Oxford and also from one of the most northern populations of the species, located at Methil in Fife. The populations occurred on open ground of roadsides, railway yards or docks, and plants were collected within a short radius (≤300m) of the given map reference (see Table 6. 1.) for each population.

For each isozyme system resolved, the allele frequencies at each locus were calculated and used to estimate measures of allelic richness, measures of genetic variation (Nei's gene diversity statistics), and measures of population structure (Wright's F-statistics respectively) in the species. For details of calculations see Chapter 5. A problem arose at the *Acp*-2 locus, due to the presence of a null allele in several populations. In theory the frequency of a null allele (r) can be calculated from the frequency of individuals exhibiting the null phenotype (r^2) assuming that the population is at Hardy - Weinberg equilibrium. Clearly, if populations are not at Hardy-Weinberg equilibrium this procedure is inaccurate. Consequently it was decided to ignore the null allele at the *Acp*-2 locus and calculate allele frequencies based on a two allele system in all populations.

Table 6. 1. Locations of *S. squalidus* populations surveyed.

Location	National grid reference	Collection date	Date of first † record	Reference.
Oxford	SP 505 065	18-09-87	1800	Kent(1956)
Kingston-upon-Thames	TQ 191 691	08-06-87	1913	Kent (1960)
Dartford	TQ 555 743	16-07-86	1923	Kent (1960)
Banbury	SP 463 404	18-09-87	1900	Kent (1960)
Warwick	SP 286 655	18-09-87	1917	Kent (1960)
Birmingham	SP 092 876	18-09-87	1939	Kent (1964b)
Wrexham				
Brymbo	SJ 296 539	03-07-87	1910	Kent (1960)
Rhostyllen	SJ 312 492	12-10-87	1915	Kent (1963)
Southsea	SJ 306 515	03-07-87	1915	Kent (1963)
Cardiff	ST 173 733	02-06-87	1900	Kent(1963)
Mochdre	SH 813 774	29-06-86	1950	Kent (1963)
Stoke-on-Trent	SJ 886 407	07-07-87	1940	Kent (1964b)
Derby	SK 362 356	07-07-87	1945	Kent (1964b)
Sheffield	SK 395 845	07-07-87	1945	Kent (1964b)
Chesterfield	SK 383 713	07-07-87	1941	Clapham (1969)
*St. Helens	SJ 524 926	19-09-87	1940	Kent (1964c)
Ince (Wigan)	SD 594 034	08-07-87	1950	Kent (1964c)
*York	SE 612 518	20-05-87	1950	Kent (1964c)
Darlington	NZ 296 146	23-06-86	1952	Kent (1964c)
*Glasgow (South St.)	NS 534 671	10-10-87	1965	Kent (1966)
*Grangemouth (Docks)	NS 945 825	10-10-87	1969	Plant records (1971)
Leith	NT 268 765	12-09-86	1960	Kent (1966)
*Methil	NT 376 995	08-10-87	1978	Taylor (1984)

†. Date of first record of population at site is taken from the first report of *S. squalidus* in locality as stated in reference.

*. Electrophoresis conducted on material sampled directly from the field. For populations not marked with an asterisk electrophoresis was conducted on plants raised from seed collected from wild populations.

6. 4. RESULTS

It was established that *S. squalidus* is genetically polymorphic at four of the seventeen loci studied (*Acp-2*, *Got-1*, *β–Est-1* and *β-Est-2*). Details of the genotype and allele frequencies at the *Acp-2*, *Got-1* and *β–Est-1* loci in each population are included in Tables 6. 2. to 6. 4. Because dominance may occur at the *β-Est-2* locus (as established for *S. vulgaris*) only phenotype frequencies are presented for this locus in Table 6. 5.

Genotype and Allele frequencies

Of the two alleles present at the *Acp-2* locus, the *Acp-2a* allele (slow) was the more common (Table 6. 2), although only two populations (at Leith and Cardiff) were fixed for the allele. Among populations polymorphic for the *Acp-2* locus, individuals with an heterozygous genotype were recorded at all sites except Sheffield.

At the *Got-1* locus (Table 6. 3.), the *Got-1a* allele was more common than the *Got-1b* allele in all populations and was fixed in the population at Southsea. In most populations the frequency of the *Got-1b* allele did not fall below .15. Heterozygous individuals were recorded in all but two populations polymorphic for the *Got-1* locus, with the frequency of the heterozygotes ranging from .18 to .5.

At the *β-Est-1* locus, the *β-Est-1b* allele was by far the most

Table 6. 2. Genotype and Allele frequencies at the *Acp-2* locus in *S.squalidus*

Location	(number surveyed + no. null phenotypes)	Genotype frequency			;	Allele frequency	
		a/a	a/b	b/b	;	a	b
Oxford	(46 + 1)	.61	.20	.20	;	.71	.29
Kingston-upon-Thames	(48 + 1)	.90	.02	.08	;	.91	.09
Dartford	(25 + 0)	.72	.20	.08	;	.82	.18
Banbury	(50 + 1)	.96	.02	.02	;	.97	.03
Warwick	(44 + 0)	.66	.34	-	;	.83	.17
Birmingham	(21 + 1)	.62	.38	-	;	.81	.19
Wrexham							
Brymbo	(33 + 1)	.51	.42	.06	;	.72	.28
Rhostyllen	(27 + 3)	.44	.30	.26	;	.59	.41
Southsea	(25 + 0)	.20	.28	.52	;	.34	.66
Cardiff	(22 + 2)	1.00	-	-	;	1.00	-
Mochdre	(26 + 2)	.31	.38	.31	;	.50	.50
Stoke-on-Trent	(49 + 1)	.82	.10	.08	;	.87	.13
Derby	(44 + 1)	.75	.16	.09	;	.83	.17
Sheffield	(50 + 0)	.80	-	.20	;	.80	.20
Chesterfield	(50 + 0)	.48	.22	.30	;	.59	.41
St. Helens	(46 + 2)	.57	.24	.19	;	.69	.31
Ince (Wigan)	(36 + 1)	.75	.11	.14	;	.81	.19
York	(26 + 0)	.38	.31	.31	;	.54	.46
Darlington	(15 + 1)	.53	.27	.20	;	.67	.33
Glasgow							
(South St.)	(34 + 1)	.65	.13	.24	;	.71	.29
Grangemouth							
(Docks)	(35 + 0)	.86	.14	-	;	.93	.07
Leith	(44 + 0)	1.00	-	-	;	1.00	-
Methil	(26 + 0)	.61	.35	.04	;	.79	.21

Table 6. 3. Genotype and Allele frequencies at the *Got-1* locus in *S. squalidus.*

Location	(n)	Genotype frequency			;	Allele frequency	
		a/a	a/b	b/b	;	a	b
Oxford	(46)	.46	.43	.11	;	.67	.33
Kingston-upon-Thames	(48)	.69	.31	-	;	.84	.16
Dartford	(25)	.48	.40	.12	;	.68	.32
Banbury	(50)	.48	.36	.16	;	.66	.34
Warwick	(44)	.27	.5	.23	;	.52	.48
Birmingham	(21)	.52	.33	.14	;	.69	.31
Wrexham							
Brymbo	(33)	.76	.18	.06	;	.85	.15
Rhostyllen	(27)	.56	.26	.19	;	.69	.31
Southsea	(25)	1.00	-	-	;	1.00	-
Cardiff	(22)	.68	-	.32	;	.68	.32
Mochdre	(26)	.42	.31	.27	;	.58	.42
Stoke-on-Trent	(49)	.82	.18	-	;	.91	.09
Derby	(44)	.48	.45	.07	;	.70	.30
Sheffield	(50)	Not recorded					
Chesterfield	(50)	Not recorded					
St. Helens	(46)	.63	.30	.07	;	.78	.22
Ince (Wigan)	(36)	.69	.25	.06	;	.82	.18
York	(26)	.35	.5	.15	;	.6	.4
Darlington	(15)	.67	-	.33	;	.67	.33
Glasgow							
(South St.)	(34)	.71	.20	.09	;	.81	.19
Grangemouth							
(Docks)	(35)	Not recorded					
Leith	(44)	.73	.25	.02	;	.85	.15
Methil	(26)	.42	.46	.12	;	.65	.35

Table 6. 4. Genotype and Allele frequencies at the *β-Est-1* locus in *S.squalidus*

Location	(n)	Genotype frequency						;	Allele frequency		
		a/a	b/b	c/c	a/b	b/c	a/c	;	a	b	c
Oxford	(46)	.04	.83	-	.11	-	.02	;	.10	.88	.01
Kingston-upon-Thames	(48)	-	1.00	-	-	-	-	;	-	1.00	-
Dartford	(25)	-	.96	.04	-	-	-	;	-	.96	.04
Banbury	(50)	.02	.92	-	.04	.02	-	;	.04	.95	.01
Warwick	(44)	-	.95	-	.05	-	-	;	.02	.98	-
Birmingham	(21)	-	.90	-	.05	-	.05	;	.05	.93	.02
Wrexham											
Brymbo	(33)	-	.85	-	.15	-	-	;	.08	.92	-
Rhostyllen	(27)	-	1.00	-	-	-	-	;	-	1.00	-
Southsea	(25)	-	.96	-	.04	-	-	;	.02	.98	-
Cardiff	(22)	-	.95	-	.05	-	-	;	.02	.98	
Mochdre	(26)	-	.81	-	.19	-	-	;	.10	.90	
Stoke-on-Trent	(49)	.06	.57	-	.37	-	-	;	.24	.76	
Derby	(44)	-	1.00	-	-	-	-	;	-	1.00	-
Sheffield	(50)	.10	.68	-	.22	-	-	;	.21	.79	-
Chesterfield	(50)	.02	.62	.02	.26	.04	.04	;	.17	.77	.06
St. Helens	(46)	-	.87	-	.13	-	-	;	.07	.93	-
Ince (Wigan)	(36)	-	1.00	-	-	-	-	;	-	1.00	-
York	(26)	-	1.00	-	-	-	-	;	-	1.00	-
Darlington	(15)	-	.8	.13	.07	-	-	;	.03	.83	.13
Glasgow											
(South St.)	(34)	-	.85	-	-	.15	-	;	-	.93	.07
Grangemouth											
(Docks)	(35)	.11	.89	-	-	-	-	;	.11	.89	-
Leith	(44)	-	.82	-	.16	.02	-	;	.08	.91	.01
Methil	(26)	-	.92	-	.08	-	-	;	.04	.96	-

Table 6. 5. Phenotype frequencies at the *β-Est-2* locus in *S.squalidus*

Location	(n)	Phenotye frequency					
		a/a	b/b	c/c	a/b	b/c	a/c
Oxford	(46)	.22	.41	.22	.09	.07	-
Kingston-upon-Thames	(48)	.375	.19	.19	.125	.125	-
Dartford	(25)	.12	.72	.12	-	.04	-
Banbury	(50)	.16	.30	.12	.24	.08	.10
Warwick	(44)	.36	.11	.18	.11	.18	.05
Birmingham	(21)	.29	.24	.14	.10	.19	.05
Wrexham							
Brymbo	(33)	.45	.21	.09	.12	.09	.03
Rhostyllen	(27)	.41	.22	.22	.07	.07	-
Southsea	(25)	.32	.20	.12	.08	.12	.16
Cardiff	(22)	.36	.32	-	.05	.14	.14
Mochdre	(26)	.08	.42	.19	.08	.23	-
Stoke-on-Trent	(49)	.08	.25	.16	.16	.22	.12
Derby	(44)	.36	.25	.16	.09	.09	.05
Sheffield	(50)	-	.62	.22	-	.14	.02
Chesterfield	(50)	.08	.40	.26	.04	.22	-
St. Helens	(46)	.28	.15	.11	.15	.26	.04
Ince (Wigan)	(36)	.25	.28	.22	.06	.08	.11
York	(26)	.12	.42	.19	.15	.08	.04
Darlington	(15)	.33	.33	.07	.27	-	-
Glasgow							
(South St.)	(34)	.24	.24	.03	.18	.32	-
Grangemouth							
(Docks)	(35)	.09	.83	.03	.06	-	-
Leith	(44)	.16	.45	.14	.05	.16	.05
Methil	(26)	.19	.35	.08	.27	.11	-

common in all populations surveyed (Table 6. 4). Five populations were fixed for this allele (Kingston-upon-Thames, Rhostyllen, Derby, Ince and York) and in the remaining populations the frequency of the allele never fell below .76. The *β-Est-1c* allele was present in only a third of the populations surveyed, and can be considered as rare in the species. Heterozygotes were found in 16 of the 18 populations polymorphic for this locus. In these populations the *β-Est-1a/ β-Est-1b* heterozygote was the most common, being present in 15 populations. The other two heterozygous genotypes (*β-Est-1b* /β-*Est-1c* and *β-Est-1a* /*β-Est-1c*) were uncommon being found only in 4 and 3 populations respectively.

The six phenotypes observed at the *β-Est-2* locus were present in 11 of the 23 populations surveyed (Table 6. 5). Of the remaining 12 populations, 8 possessed 5 of the 6 possible phenotypes. Single banded phenotypes were recorded at a higher frequency than double banded phenotypes in most populations, with the *β-Est-1b* band the most common single banded phenotype in 13 populations.

Allelic Richness, Gene diversity and Genetic structure

Measures of allelic richness are presented in Table 6. 6. The mean number of alleles per polymorphic locus (L) was virtually uniform over populations with only one population (Chesterfield) having a

Table 6. 6. Percentages of loci polymorphic (P) , mean number of alleles per polymorphic locus (L), mean number of alleles per locus (K) and mean observed heterozygosity (H_o) in populations of *S. squalidus*.

Location	no. of loci.	P	L	K	H_o
Oxford	15	20	2.	1.2	.051
Kingston-upon-Thames	15	13.33	2.	1.13	.022
Dartford	15	13.33	2.	1.2	.028
Banbury	15	6.66	2.	1.13	.029
Warwick	15	13.33	2.	1.13	.029
Birmingham	15	13.33	2.	1.2	.054
Wrexham					
Brymbo	15	20	2.	1.2	.05
Rhostyllen	15	13.33	2.	1.13	.037
Southsea	15	6.66	2.	1.07	.021
Cardiff	15	13.33	2.	1.07	.003
Mochdre	15	20	2.	1.2	.059
Stoke-on-Trent	15	20	2.	1.2	.043
Derby	15	13.33	2.	1.13	.041
Sheffield	14	14.29	2.	1.14	.016
Chesterfield	14	14.29	2.5	1.21	.04
St. Helens	15	13.33	2.	1.2	.045
Ince (Wigan)	15	13.33	2.	1.13	.024
York	15	20	2.	1.13	.024
Darlington	15	20	2.	1.2	.023
Glasgow (South St.)	15	20	2.	1.2	.032
Grangemouth (Docks)	14	14.29	2.	1.14	.01
Leith	15	13.33	2.	1.13	.029
Methil	15	13.33	2.	1.13	.059
MEAN	14.87	14.91	2.02	1.16	.033

N. B. The lower number of loci screened at Chesterfield, Sheffield and Grangemouth is due to the *Got-1* locus being omitted from these populations

value above 2. Similarly there was little variation in mean number of alleles per locus (K), ranging between 1. 07 and 1. 21. All populations exhibited at least one polymorphic locus (P), with the majority of populations (21 out of 23) containing two or three polymorphic loci (P > 6. 66%). Observed heterozygosity ranged between .003 and .059, with a mean of .033, which is low for an outcrossing species (Gottlieb, 1981a).

Measures of gene diversity (Table 6. 7) reveal that the within population diversity (H_S) accounts for most of the total gene diversity (H_T) present within the species. Between population diversity (D_{ST}) is low. This is also reflected in the low values of R_{ST} and G_{ST}. Nevertheless the genetic differentiation between populations, as measured in terms of F_{ST} (to which G_{ST} is equivalent) was significant (Table 6. 7). Finally, the positive F_{IS} values recorded (Table 6. 8) clearly reveal the deficiency of observed heterozygotes from expected values at all three polymorphic loci surveyed.

Table 6. 7. Gene diversity statistics for populations of *S. squalidus* .

Locus	H_T	H_S	D_{ST}	G_{ST}	R_{ST}
Acp-2	.3671	.3137	.0534	.1455	.1780
Got-1	.3919	.3645	.0274	.0699	.0790
β-Est-1	.1409	.1267	.0142	.1008	.1168
Mean	.3000	.2683	.0317	.1057	.1235

The arithmetic mean values of H_T, H_S and D_{ST} were used to calculate the mean values of G_{ST}, and R_{ST}.

Table 6. 8. Genetic structure statistics for populations of *S. squalidus* .

Locus	F_{IS}	F_{ST}	F_{IT}
Acp-2	.3715	.1455**	.3776
Got-1	.2222	.0699**	.2766
β-Est-1	.2076	.1008**	.2874
Mean	.2393	.1057	.3197

N. B. Mean values of F_{IS}, F_{ST} and F_{IT} are calculated from the mean values of H_I, H_S and H_T (See Chapter 5 for details).

6. 5. DISCUSSION

6. 5. 1. Level of Genetic Variation in *Senecio squalidus.*

It is of interest to compare the level of genetic variation within *S. squalidus* with that found in other outcrossing species (Table 6. 9.). Mean values for other outcrossing species are taken from Gottlieb (1981a).

Table 6. 9. Measures of allelic richness in *S. squalidus* compared with means for other outcrossing species (data from Gottlieb, 1981).

	Mean no. loci	P (Popn.)	L	K	P(Spec.)	H_o
S. squalidus	14. 87	14. 91	2. 02	1. 16	20	.033
Mean outcrossing species.	17. 57	37	2. 90	1. 97	51	.086

P = average percentage polymorphic loci, L = Mean number of alleles per polymorphic locus,

K = mean number of alleles per locus, H_o = mean observed heterozygosity.

N. B. Gottlieb does not list a value for K, however this was calculated from his data.

It is evident that over all measures of allelic richness, *S. squalidus* exhibits less genetic variation than is normal for an outcrossing species. This indicates that British *S. squalidus* is genetically depauperate, possibly due to loss of allelic variability that occurred during the colonisation of Britain. To establish whether the latter is so, it

would be necessary to survey levels of genetic variation within ancestral populations. Losses of allelic variation in introduced species have been recorded in *Sarracenia purpurea* L. (Schwaegerle and Schaal, 1979) and *Emex spinosa* (L.) Campd. (Marshall and Weiss, 1982), following colonisation of Cranberry Island bog, Ohio and Australia respectively. However, such reductions in genetic variation are not always evident: in both *Trifolium hirtum* All. (Martins and Jain, 1980) and *Apera spica-venti* L. (Warwick *et al.*, 1987) no reduction of genetic variation was found among introduced relative to ancestral populations. In these two cases, however, there may have been repeated introductions of the species during the process of colonisation.

Repeated introductions of *S. squalidus* may also have occurred in Britain during the colonisation phase. Kent (1960) has suggested that an early population at Bristol Docks was possibly due to a separate introduction from that at Oxford, and, more recently, Hanson and Mason (1985) recorded viable *S. squalidus* seed in imported bird seed mixtures. Thus *S. squalidus* may simply have a lower than average level of genetic variation for an outcrossing species, with the level of variation found in Britain reflecting that which is present in the species as a whole. Certainly the levels of allelic variation present in *S. squalidus* are not unknown in other outcrossing species. For example, similar levels have been recorded in the outbreeders *Phlox roemariana* Scheele (Levin, 1975) and *Oenothera argillicola* (Levy and Levin, 1975).

Despite the apparent paucity of genetic variation in *S. squalidus*, the species, nonetheless, contains more gene diversity than is present in *S. vulgaris* (Tables 6. 10 - 6. 11). This was evident for all measures of allelic richness recorded in the two species (Table 6. 10), except, that is, for mean number of alleles per polymorphic locus (L), which was slightly higher in *S. vulgaris*. As expected for an outcrossing species, most of the total gene diversity (H_T) recorded within *S. squalidus* occurred within populations (H_S), whereas in *S. vulgaris*, most diversity was distributed between populations (D_{ST}). Similarly, the inbreeding co-efficient (F_{IS}) and overall inbreeding co-efficient (F_{IT} - see Table 6. 12.) was greater in *S. vulgaris* than *S. squalidus*.

Several other studies in which levels of gene diversity have been compared between related taxa exhibiting contrasting breeding systems have produced results similar to those obtained from the comparison between *S. squalidus* and *S. vulgaris* [e. g. Levin (1978), for *Phlox drummondii* Hook. and *P . roemariana* Scheele (both outcrossers) with *P . cuspidata* (a selfer); Brown and Jain (1979), *Limnanthes alba*, (outcrosser), v *L. floccosa*, (selfer); Ellstrand and Levin (1980), for *Oenothera grandis*, (outcrosser), *O . mexicana*, (selfer), and *O. laciniata* (asexual); and Layton and Ganders (1984), for *Plectritis congesta* , (outcrosser) v *P. brachystemon*, (selfer)]. In addition, Hughes and Richards (1988) have reported similar differences in regard to level of

Table 6. 10. Comparison of allelic richness of *S. vulgaris* and *S. squalidus.*

Species		No. loci	P (Popn.)	L	K	P (Spec.)	H_o
S. vulgaris.	RR	15. 93	6.28	2.27	1.08	18.75	.0019
	rr_p	15.93	5.86	2.05	1.06	12.5	.0027
	rr_m	16	4.46	2.17	1.05	12.5	.0038
S. squalidus		14.87	14.91	2.02	1.16	20	.033

P ; Mean percentage of polymorphic loci, per population [P (Popn.)] or per species [P(Spec.)]. L ; Mean number of alleles per polymorphic locus.
K ; Mean number of alleles per locus. H_o ; Mean observed heterozygosity per population per locus.

Table 6. 11. Comparison of Nei's gene diversity statistics betwen the three clases of *S. vulgaris* and *S. squalidus.*

Species	Morph	H_T	H_S	D_{ST}	G_{ST}	R_{ST}
S. vulgaris	RR	.2727	.1385	.1342	.4921	1.0454
	rr_p	.1882	.1328	.0554	.2944	.4501
	rr_m	.2540	.0478	.2062	.8118	5.0750
S. squalidus		.3000	.2683	.0317	.1057	.1235

Table 6. 12. Comparison of the genetic structure of *S. vulgaris* and *S. squalidus.*

Species	Morph	F_{IS}	F_{ST}	F_{IT}
S. vulgaris	RR	.2780	.4921	.9633
	rr_p	.8938	.2944	.9251
	rr_m	.5293	.8118	.9114
S. squalidus		.2393	.1057	.3197

gene diversity between outcrossing and selfing species of *Taraxacum*, however, inbreeding species contained greater number of alleles per locus, possibly as a result of the greater geographic and taxonomic diversity of the inbreeding relative to outbreeding samples.

Given the history of *S. squalidus* in Britain, and particularly, the known dates at which the species first colonised particular sites in Britain (Kent, 1956, 1957, 1960, 1963, 1964a, b, c, 1966), it is of interest to examine if an association exists between the amount of gene diversity within a population and the date of colonisation of an area containing a population. One expectation might be that populations in recently colonised areas contain less genetic variation than populations from areas colonised early during the spread of *S. squalidus* in Britain. Dates of first colonisation, distance from Oxford and measures of within population gene diversity (H_S), are presented in Table 6. 13.

It is evident that the level of gene diversity (H_S) is correlated neither with date of first colonisation (r = -0. 0378) nor linear distance from Oxford (r = 0.093). Because of the temporary nature of *S. squalidus* populations in the wild, and the repeated genetic bottlenecks that populations are likely to pass through during re-establishment in an area, it is to be expected that amounts of genetic variation in populations will be greatly affected by sampling effects. It is not surprising therefore, that level of gene diversity in British populations of *S. squalidus* is

Table 6. 13. Mean gene diversity per population (H_S), distance from Oxford of sample sites and date of first record of *S. squalidus* in an area containing a population.

Location	Date of first record	Linear distance from Oxford (km)	Mean H_S
Oxford	1800	0	.3565
Kingston-upon-Thames	1913	80	.109
Dartford	1923	115	.2111
Banbury	1900	30	.2004
Warwick	1917	65	.2735
Birmingham	1939	80	.2893
Wrexham			
Brymbo	1910	185	.2685
Rhostyllen	1915	180	.3039
Southsea	1915	185	.1627
Cardiff	1900	140	.1581
Mochdre	1950	215	.2813
Stoke-on-Trent	1940	160	.2516
Derby	1945	130	.2341
St. Helens	1940	215	.3004
Ince (Wigan)	1950	215	.201
York	1950	250	.3256
Darlington	1952	325	.4059
Glasgow			
(South St.)	1965	500	.2833
Leith	1960	490	.1417
Methil	1978	510	.2878

N. B. The populations at Sheffield, Chesterfield and Grangemouth were omitted from the analysis due to the absence of data for variation at the *Got-1* locus at these sites.

randomly distributed rather than being associated with the two variables examined here.

6. 5. 2. Deficiency of Heterozygotes in Populations

An important finding to emerge from the survey of isozyme variation in *S. squalidus* was the deficiency of heterozygotes within populations relative to frequencies expected assuming panmixia. This was evident from the positive F_{IS} values estimated for each polymorphic locus investigated. Positive F_{IS} values often occur in outbreeding plants and may result from presence of dominant or null alleles at the loci surveyed, partial self-pollination, selection against heterozygotes or population sub-division (Brown, 1979).

A null allele was identified at the *Acp-2* locus and could account for the deficiency of heterozygotes observed at this locus. For instance if two alleles, neither of which are null alleles, are present in a population at equal frequency, then the expected number of heterozygotes at Hardy-Weinberg equilibrium is 0.5. If a null allele is introduced into the population at low frequency, say 0. 1, with the other two alleles remaining at equal frequency, then the expected number of observed heterozygotes will decrease to .405, a reduction of almost 0.1. Individuals heterozygous for the null allele will appear as homozygotes. The effect will be more pronounced when the frequencies of the two "visible" alleles within a population are unequal, as was found at the

Acp-2 locus in the vast majority of *S. squalidus* populations surveyed. At the other two loci included in the analysis of F-statistics, *Got-1* and *β-Est-1*, there was no evidence that either dominance or the occurrence of null alleles was a cause of the positive F_{IS} values recorded.

Zero F_{IS} values will only occur in large populations where reproduction occurs through obligate outcrossing. *S. squalidus* has been reported to exhibit some self-compatability (Gibbs et al, 1975), and further investigation is now required of the expression of self-compatability across a range of populations and environments. It is feasible that some selfing occurs in most populations in the wild, and is wholly or partly responsible for the deficit of heterozygotes observed in the present study.

The possibility that selection might be responsible for positive F_{IS} values, raises the whole issue of the adaptive significance of allozyme variation, (see Kimura, 1983). Currently only the work of Koehn and co-workers on the LAP locus in the common mussel *Mytilus edulis*, have provided a clear demonstration of the effect of selection on electrophoretic variation at an enzyme coding locus (summarised in Koehn and Hilbisch, 1987). If selection is responsible for a deficit of heterozygotes, it must be acting against the heterozygotes. In this regard, there is a considerable literature relating heterozygosity to fitness in species (reviewed by Zouros and Foltz, 1987), from which no consistent trend has emerged. For instance, associations have been found between

the success of clones of the grass *Spartina patens* and a lack of heterozygosity (Silander, 1984), while Mitton and Grant (1980) have found that the fastest growing individuals of quaking aspen, *Populus tremuloides,* exhibited the highest levels of isozyme heterozygosity. In contrast, Strauss (1986) reported no relationship between heterozygosity and growth rate in *Pinus attenuata.* If an association occurs in *S. squalidus,* it would be expected to be positive, as heterozygote disadvantage is virtually unknown in outcrossing species.

The most likely reason often given for the lack of heterozygotes in a population of outcrossing individuals is population sub-division. This may result from two interlinked phenomena, small neighbourhood size and the Wahlund effect. Bradshaw (1972) has stressed that natural plant populations are liable to fracture into sub-populations which are genetically differentiated from each other. Such differentiation may occur over very short distances. If such genetic sub-division has occurred within a sample of *S. squalidus* from a given site, then treating the sample as one population would lead to a deficiency of heterozygotes from that expected, assuming panmixia. This is known as the Wahlund effect (Brown, 1979).

The degree of sub-division within a given area and hence the severity of the Wahlund effect, is partially dependent upon the neighbourhood size of the species. No estimates of the neighbourhood size have been calculated for *S. squalidus* . However, it is significant that

the greatest deficiency of heterozygotes is commonly found in short lived, animal pollinated species (Brown, 1979), implying that population sub-division in a species such as *S. squalidus* may be quite marked.

6. 5. 3. Allozyme variation in *S. squalidus* and *S. vulgaris* across central Scotland.

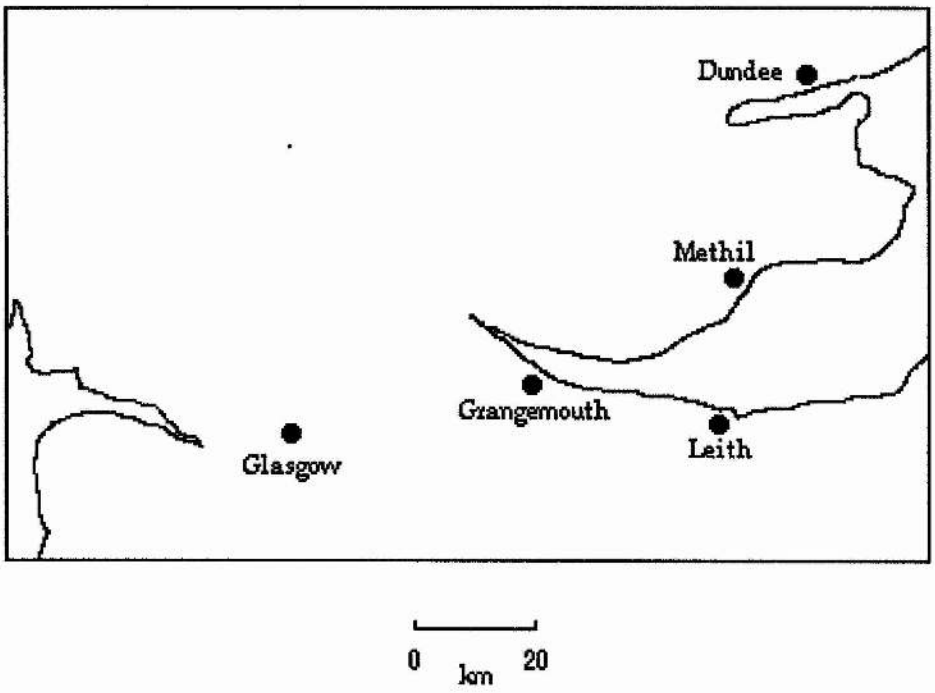
The only previous study of electrophoretic variation in *S. squalidus* and *S. vulgaris* was conducted by Hull (1974b) and focussed on the frequency distribution of esterase bands in *S. squalidus* and populations of *S. vulgaris* (monomorphic and polymorphic for capitulum type) across the central belt of Scotland. No genetic analysis was made of the esterase bands resolved, but instead, a description was given of the frequency at which a band was present in a sample. From this form of analysis, Hull found a difference in esterase distribution between *S. squalidus* and *S. vulgaris* in all populations surveyed, which he attributed to differences between *S. squalidus* and non-radiate *S. vulgaris* , there being no significant difference between *S. squalidus* and radiate *S. vulgaris*. Hull considered that the similarity in frequency distribution of esterase bands in *S. squalidus* and radiate *S. vulgaris* would be expected based on an introgressive origin of radiate Groundsel. Moreover, the similarity should be greater in the east of central Scotland than the west, as was found, due to the fact that *S. squalidus* had colonised the east first (Kent, 1966) and, therefore there had been longer

for introgression to have occurred.

From the results of the present survey, it is possible to re-examine esterase variation, together with variation for *Got-1*, in populations of *S. squalidus* and *S. vulgaris* across central Scotland, but in terms of allelic variation rather than band frequencies. Relevant data are available from polymorphic *S. vulgaris*, and *S. squalidus* populations located at Glasgow, Grangemouth, Edinburgh(Leith) and Methil, and for monomorphic *S. vulgaris* populations at Dundee and Methil (see Fig. 6. 1.).

Within polymorphic *S. vulgaris* populations, the frequency of the *α-Est-1c* allele in both morphs was substantially lower in Leith than in Glasgow (Table 6. 14). Moreover at Grangemouth, which is located mid-way between Leith and Glasgow, the *α-Est-1c* allele occurred at an intermediate frequency in the non-radiate morph. As the *α-Est-1* locus is absent from *S. squalidus*, variation in degree of introgression from *S. squalidus* into *S. vulgaris* over sites, is ruled out as a factor responsible for any difference in allele frequency in *S. vulgaris*. Instead differences must result from the effects of chance or selection. Given the higher frequency of the *α-Est-1c* allele within the polymorphic and monomorphic populations at Methil and in both monomorphic populations from Dundee, there would seem no good evidence that the

Fig. 6. 1. Location of *S. vulgaris* and *S. squalidus* populations surveyed in central Scotland.



N. B. The Dundee site contained only non-radiate *S. vulgaris*.

Table 6. 14. Frequencies of *α-Est-1c*, *β-Est-1a* alleles and *Got-1* allozymes across central Scotland polymorphic populations (Glasgow - Methil Docks) and among monomorphic populations (Methil Power station and Dundee).

	G'gow Docks	G'gow K'side	G'mth D. St.	G'mth Tip	Leith	Mthl Docks	Mthl P. Stn.	D'dee Rlwy	D'Dee Tip
α-Est-1c allele frequency									
S. vulgaris - rr	1.00	1.00	.84	.58	.65	.77	1.00	1.00	.92
S. vulgaris - RR	1.00	1.00	.98	.96	.47	1.00	-	-	-
β-Est-1a allele frequency									
S. vulgaris - rr	.96	.72	1.00	1.00	.78	.97	1.00	.96	.92
S. vulgaris - RR	1.00	1.00	1.00	1.00	.99	1.00	-	-	-
S. squalidus	0.00	-	.11	-	.08	.04	-	-	-
Got-1a allozyme frequency									
S. vulgaris - rr	0.00	0.00	0.00	0.00	.08	0.00	0.00	0.00	0.00
S. vulgaris - RR	.58	0.00	.68	0.00	.72	1.00	-	-	-
S. squalidus	.81	-	N. R.	-	.85	.65	-	-	-

N. B.

- indicates that this species or morph was absent from this site.

N. R. - allozyme frequency was not recorded.

Full name of abbreviated sites is as follows:- G'gow Docks, Glasgow docks (South St.); G'gow K'side, Glasgow Kelvinside; G'mth D. St., Grangemouth Devon St (adjacent to docks); G'mth Tip, Grangemouth Kinneil tip; Mthl P. Stn, Methil Power Station; D'dee Rlwy, Dundee railway yard.

frequency of the *α-Est-1c* allele is affected by climatic change across central Scotland.

For alleles at the *β-Est-1* locus, there was no evidence of an association between the frequency of the *β-Est-1a* allele in *S. squalidus* and polymorphic *S. vulgaris*. In all *S. vulgaris* material the *β-Est-1a* allele was common, whereas in all *S. squalidus* populations it was absent or rare.

Evidence for clinal variation in *S. vulgaris* across the central belt of Scotland due to greater levels of introgression from *S. squalidus* having occurred in the east (Hull, 1974b) would most likely be found for the *Got-1a* allele (see Chapter 5). In the radiate morph the *Got-1a* allele occurred at a higher frequency in both eastern populations (Leith and Methil) and at decreasing frequencies moving westwards (through Grangemouth to Glasgow). Thus there is some evidence of a cline in *Got-1a* frequency. However, at both Grangemouth and Glasgow there were marked differences between local populations in allele frequency, and it is clear that more intensive sampling is required at all sites before firm conclusions can be drawn.

CHAPTER 7. ISOZYME STUDIES ON ADDITIONAL SPECIES OF *SENECIO* SECTION ANNUI

7. 1. INTRODUCTION.

This chapter reports electrophoretic studies conducted on the relationship between the inland types of *S. vulgaris* and the maritime form *S. vulgaris* ssp. *denticulatus*, and between *S. vulgaris* and four other *Senecio* species from section Annui viz; *S. viscosus* L., *S. sylvaticus* L., *S. vernalis* Waldst. & Kit. and *S. teneriffae* Schultz-Bip.

S. sylvaticus and *S. viscosus*

S. viscosus and *S. sylvaticus* are closely related, self-compatible annual species with a diploid chromosome number of 2n = 40 (Gibbs *et al.*, 1975). Both species are native to the British Isles and occur at early stages of succession. *S. sylvaticus* is locally common and occurs on light, non-calcareous sandy soils. It has a narrow ecological niche (West and Chilcote, 1968), apparently due to restricted mineral tolerance (De Neeling and Ernst, 1986). *S. viscosus* is more widespread, and is found on open sites, rubbish dumps and railway yards, often with *S. squalidus* , with which it frequently hybridises to produce highly infertile offspring (Crisp and Jones, 1978). There is evidence (e. g. Edees, 1972) that *S. viscosus* has become more widespread in Britain in the last hundred or so years, as a result of increasing habitat availability, and possibly due to the introgression of beneficial genes from *S. squalidus* (Crisp and Jones,

1978).

An electrophoretic study of populations of these two species in the Netherlands has been conducted by Koniuszek and Verkleij (1982). They found both species to be monomorphic at all loci surveyed, with some interspecific differences evident for certain enzymes. One of the aims of the present study on the two species was to determine whether British populations are also monomorphic at enzyme coding loci.

S. teneriffae Schultz Bip.

S. teneriffae is a self-fertile, short-lived, annual which is endemic to the Canary Isles. It produces radiate capitula, and in many respects is morphologically similar to *S. vulgaris*. Its status as a distinct species has been confirmed by Kadereit (1984c), who established that it was hexaploid (2n = 60), and could be distinguished from *S. vulgaris* by leaf shape, an unbranched habit, shorter ligules and larger achenes. Within section Annui, only two other hexaploids have been recorded; *S. cambrensis* and the annual African succulent, *S. hoggariensis* Batt. and Trab. (Crisp, 1972).

S. teneriffae most closely resembles *S. vulgaris* ssp. *denticulatus* and Kadereit (1984c) has proposed that it most likely evolved from an ancestor similar to *S. vulgaris* ssp. *denticulatus* which reached the Canary Isles by long distance dispersal from the western Mediterranean. An alternative hypothesis is that *S. teneriffae* and *S. vulgaris* ssp. *denticulatus* owe their morphological similarity to convergent

evolution. An electrophoretic study was conducted to examine the resemblance of allozyme patterns between *S. vulgaris* and *S. teneriffae* to assess their degree of relatedness.

S. vernalis and *S. vulgaris* ssp. *denticulatus*

S. vernalis is an annual, outcrossing species with a diploid chromosome number of 2n = 20 (Alexander, 1979). It commonly occurs on dry, open ground and is native to the eastern Mediterranean, although it is also found in the western Mediterranean region (Polunin, 1965) and occasionally in Britain (Clapham *et al*,. 1987). The taxonomic position of the species is not completely resolved, with most authors (e. g. Tutin *et al.*, 1976) assigning it specific status, however Alexander (1979) has considered it to be a variety of *S. leucanthemifolius* Poiret.

S. vulgaris ssp. *denticulatus* is a winter annual that is morphologically and ecologically distinct from other forms of *S. vulgaris*. Morphologically, ssp. *denticulatus* differs from var. *hibernicus* possessing shorter ray florets (2.3 - 3.0 mm vs. 3.5 - 5.5 mm in var. *hibernicus*) that are angled upwards. In addition, ssp. *denticulatus* is usually covered by a dense arachnoid indumentum and its leaves are generally darker and more ligulate. Kadereit (1984b) considers it to be morphologically intermediate between *S. vulgaris* var. *vulgaris* and *S. vernalis* .

Developmental differences in the various forms of *S. vulgaris* have been investigated by Kadereit (1984b), who found ssp. *denticulatus*

took longer to flower and had a lower reproductive output than either var. *vulgaris* or var. *hibernicus*. Furthermore, the achenes of ssp *denticulatus* were larger than those of the other types of *S. vulgaris* and exhibited innate dormancy. Such dormancy is not typical of seed of other forms of *S. vulgaris* and has only been recorded in one population to date (a non-radiate population of *S. vulgaris* from Matalascañas, S. Spain - Abbott, pers. comm.). The dormancy in ssp. *denticulatus* is broken by a prolonged cold spell and it is this mechanism which limits the plant to one generation per year, with the life cycle completed by the onset of summer. Kadereit (1984b) has produced evidence, based on crossing experiments, to suggest that seed dormancy is controlled by a major gene exhibiting dominance.

Kadereit (1984b) also recorded differences between populations of ssp. *denticulatus*, for generation time and capitulum number per plant. Personal observations following cultivation, have shown that material collected from Ainsdale (S. Lancs.) differs in appearance from material sampled from Jersey.

The distribution of ssp. *denticulatus* in the Britain Isles is highly localised. Allen (1967) and Perring and Sell (1968) have recorded it as present in Devon, Cornwall, Cheshire, S. Lancs., the Isle of Man and the Channel Islands. However, the majority of these records are old and it is possible that the plant is now extinct at many of these locations. Nevertheless, wild populations of the subspecies still occur on dunes at

Ainsdale (S. Lancs.) and Jersey (Preston, NCC records section, pers, comm.). In Europe, ssp. *denticulatus* has been recorded on several islands in the Baltic and North Sea (Bornholm, Lolland and the Freisian Islands), and also on the Baltic coasts of mainland Sweden and Denmark. It also occurs on the coast of Normandy and is present as a montane element of the Mediterranean flora (Kadereit (1984b). The present distribution of the species is most probably a remnant of a once widespread range (Allen, 1967). Kadereit (1984b) has proposed that *S. vulgaris* var. *vulgaris* (non-radiate Groundsel) may have evolved from ssp. *denticulatus* on several occasions in keeping with the view that denizens of maritime habitats are frequently the progenitors of ruderal species (e. g. Godwin, 1975; Ellenberg, 1978).

The aim of the studies conducted on *S. vernalis* and *S. vulgaris* ssp. *denticulatus,* was to investigate the allozyme patterns in the two species and to relate these to the patterns observed in *S. vulgaris* var. *vulgaris.* In so doing it was hoped to test the hypothesis of Kadereit (1984a, b) that *S. vernalis* gave rise to *S. vulgaris* ssp. *denticulatus,* via autopolyploidy, and that ssp. *denticulatus* is the ancestor of *S. vulgaris* var. *vulgaris* .

7. 2. MATERIALS AND METHODS

Brief details of the samples of *S. viscosus, S. sylvaticus, S. teneriffae, S. vernalis* and *S. vulgaris* ssp. *denticulatus* investigated are presented in Table 7. 1. Plants were subjected to electrophoretic survey using the methods described previously (Chapter 2). All material was raised from seed in a glasshouse except for samples of *S. sylvaticus* which were collected directly from the wild and maintained in water in the laboratory before electrophoresis. Samples of *S. vulgaris* and *S. squalidus* were included on all gels to aid comparison of isozyme patterns.

S. viscosus was sampled from Ince Moss Tip, Wigan, an open area within a triangle of railway lines. The "soil" was composed mainly of clinker-ash, and supported a population of *S. squalidus* in addition to the population of *S. viscosus*. Material of *S. sylvaticus* was sampled from two widely separated sites. A population from Tentsmuir, Fife grew in shade on sandy soil at the periphery of the dune system, while the population at Thirlmere grew on a shale substrate, under trees.

Seed of *S. teneriffae* was collected from individuals which grew under pine trees by a roadside at an altitude of 1620m. Material for analysis was raised from bulked seed and it is thus not possible to guarantee that each individual analysed was produced by a separate parent.

Seed of *S. vernalis* was sampled from two sites; from a population that grew on a gravel pit near Heidelberg, Germany, and

from a single individual which was found growing on dry, sandy ground adjacent to the dunes at Matalascañas, Coto Doñana, southern Spain. Finally, seed of *S. vulgaris* spp. *denticulatus* from Jersey (Channel Islands) was collected originally from several individuals on dunes near Quennevais in 1977, that were subsequently maintained as selfed lines. Seed from the population at Ainsdale (S. Lancs.) was sampled directly from plants which were restricted to a small area (approx 1000m^2) of an extensive dune system.

Table 7. 1 Details of species and collection sites of taxa subjected to electrophoretic analysis.

Species	Location	(n)	Date of Collection	Collector/ Supplier
S. viscosus	Ince, Wigan	(24)	12-09-87	P. Ashton
S. sylvaticus	Tentsmuir, Fife.	(11)	12-06-88	P. Ashton
	Thirlmere	(24)	17-07-88	J. Irwin.
S. teneriffae	Mirador deortuno	(8)	05-77	J. H. Dickson/ J. W. Kadereit.
S. vernalis	Matalascañas	(1)	03-04-87	R. J. Abbott
	Schlusselacker Weide, Eppelheim nr. Heidelberg	(27)	21-05-88	J. W. Kadereit.
S. vulgaris	Ainsdale	(33)	30-05-87	P. Ashton
ssp. *denticulatus*	Quennevais.	(8)	05-86	C. D. Preston/
	N. of Pulente, Jersey,			J. W. Kadereit.

Map references are as follows; Wigan, SD 594 034; Tentsmuir, NO 499 241; Ainsdale, SD 295 124; Thirlmere, NY 315 155.

7. 3. RESULTS

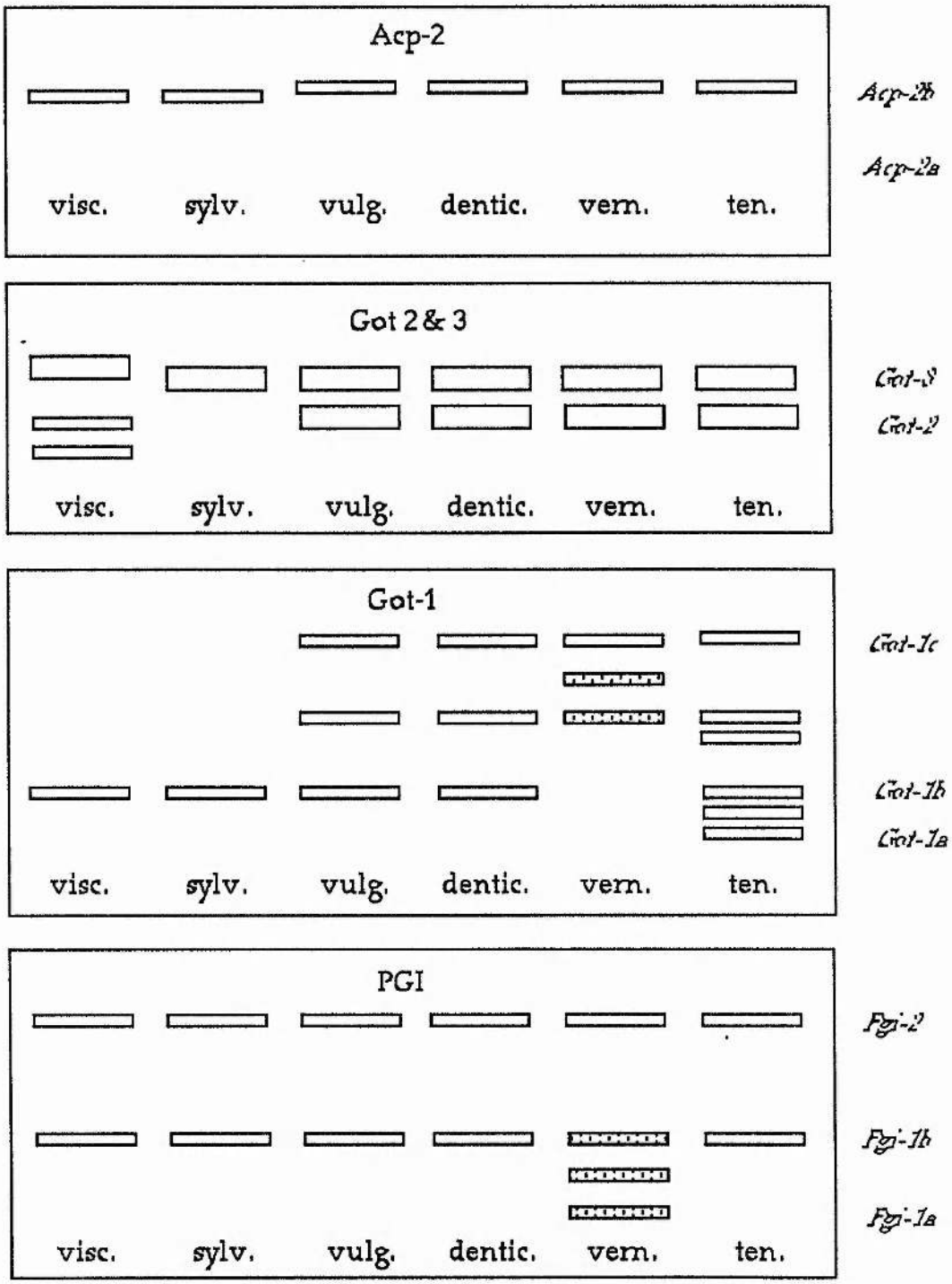
Patterns of isozyme variation found in the five species are presented in Figs. 7. 1 - 4). Also included for comparison are the patterns observed for *S. vulgaris* var. *vulgaris*. Both *S sylvaticus* and *S. teneriffae* were monomorphic for isozyme phenotype across all enzyme systems surveyed, while *S viscosus* and *S. vulgaris* ssp. *denticulatus* showed variation only for β-EST. In contrast, *S. vernalis* exhibited variation for β-EST, PGI and GOT.

S sylvaticus and *S viscosus*.

S. sylvaticus and *S. viscosus* differed from *S. vulgaris* (var. *vulgaris*) in electrophoretic phenotype for ten enzyme systems (ACP, GOT, PGM, MDH, 6-PGD, G-3-PD, G-6-PDH, GDH, β-EST and PER), but showed similar phenotypes for PGI, ME and α-EST (Figs. 7. 1. - 4.).

At the *β-Est-1* locus *S. viscosus* exhibited two different electrophoretic phenotypes. Most individuals (71%) in the Wigan population exhibited a double banded phenotype, while the remainder produced only a single band. All individuals of *S. sylvaticus* exhibited just the single banded phenotype for this isozyme. Differences between the two species were recorded only for four enzyme systems (GOT, PGM, MDH and G-6-PDH). The species, therefore, can be considered to be more closely related to each other than to the other species surveyed.

Fig. 7. 1. Comparative zymograms of five *Senecio* species for ACP, GOT and PGI.



Key. visc, S. viscosus; sylv. S. sylvaticus; vulg, S. vulgaris var. vulgaris; dentic, S. vulgaris ssp. denticulatus; vern, S. vernalis; ten, S teneriffae. denotes variable pattern (see text for further details).

Fig. 7. 2. Comparative zymograms of five *Senecio* species for PGM, MDH, ME and 6-PGD.

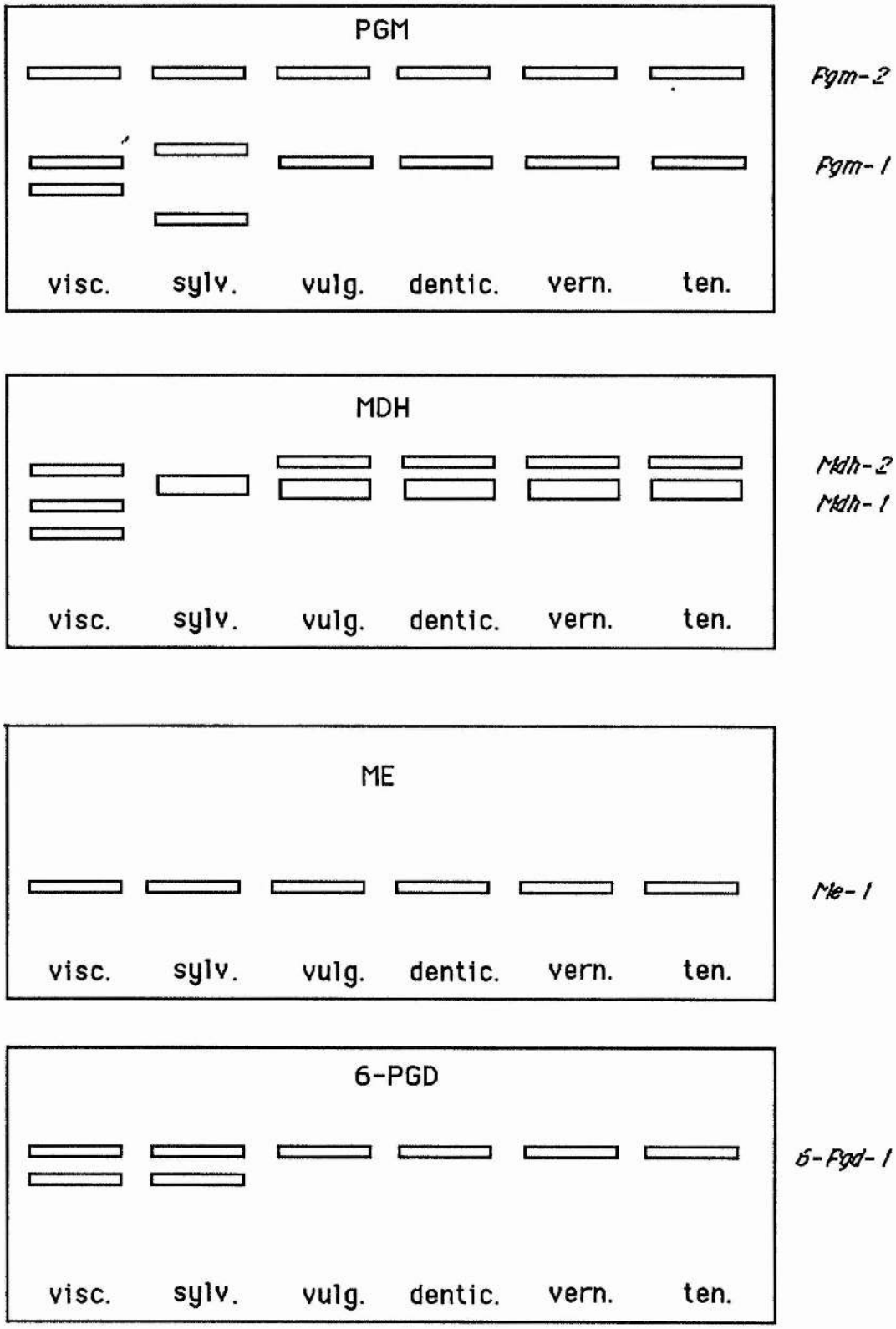
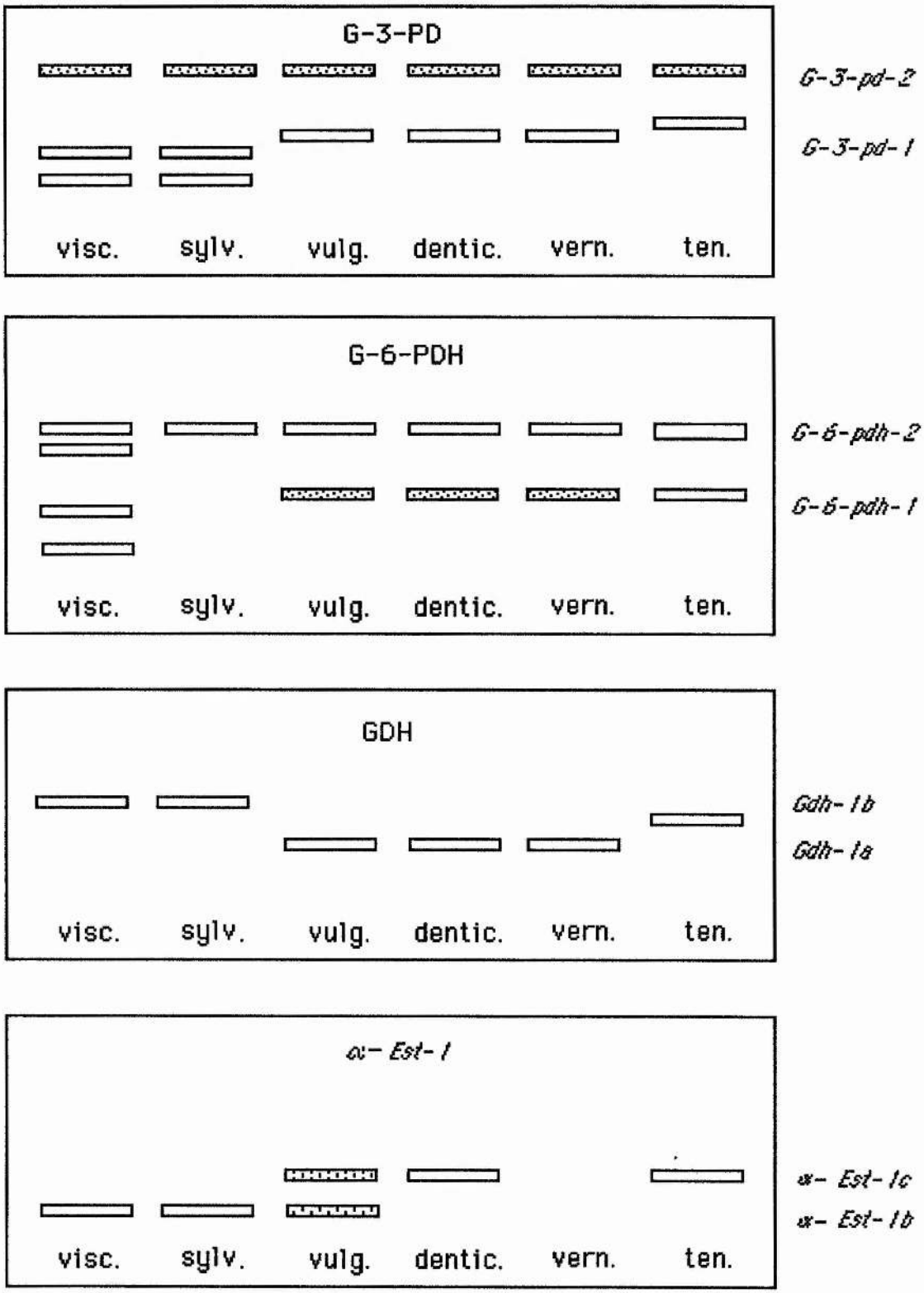
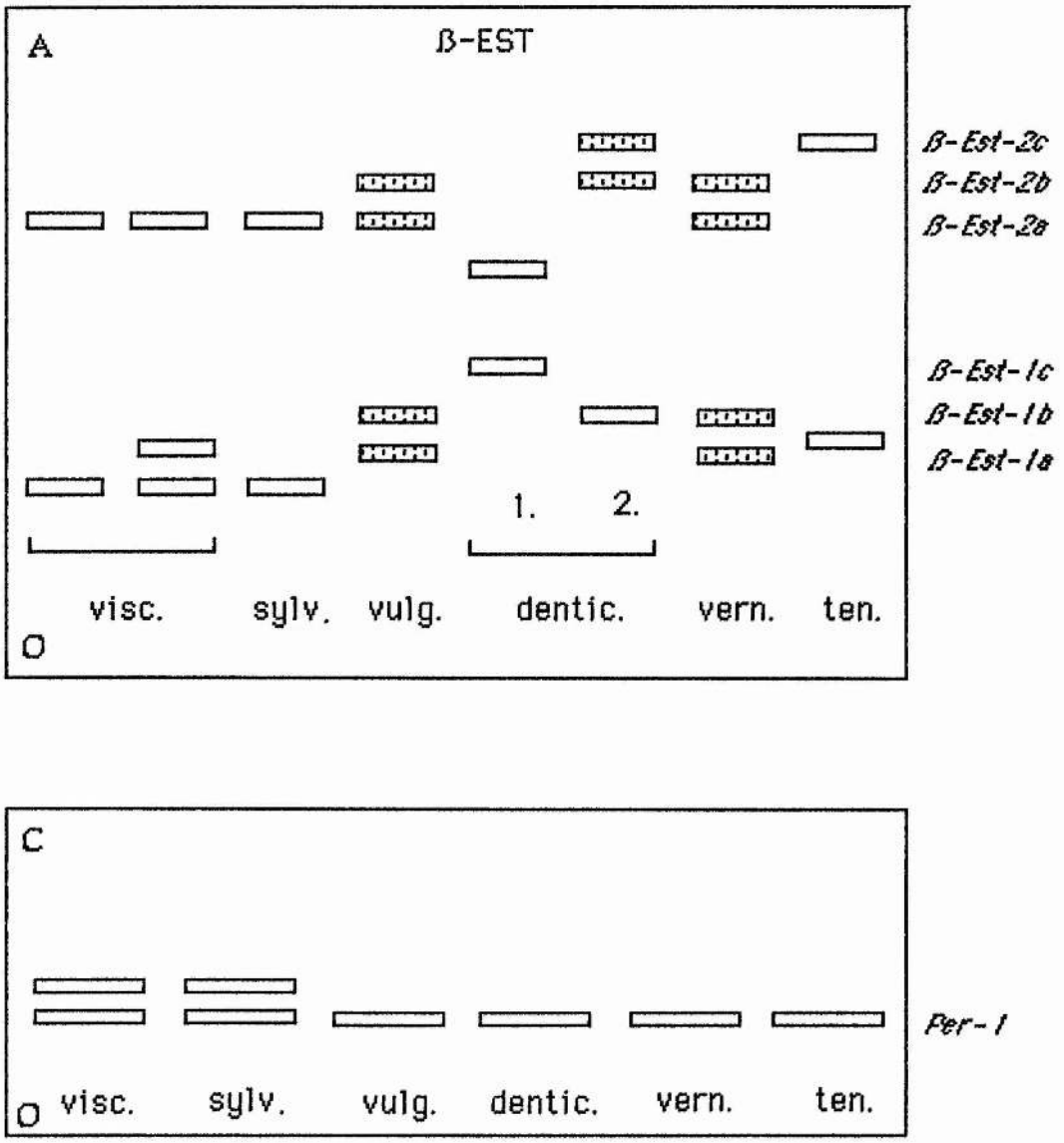


Fig. 7. 3. Comparison of zymograms of five *Senecio* species for G-3-PD, G-6-PDH, GDH and α-EST.



Key ▭ is a very faint band. ▭ is a variable pattern

Fig. 7. 4. Comparisons of zymograms obtained for five *Senecio* species for β-EST and PER.



Key. ▭ variable patterns (see text for further details).
O, Origin; C, Cathode; A, Anode.
Dentic 1 and 2 refer to the samples of *S. vulgaris* spp. *denticulatus* from Ainsdale and Jersey respectively.

S. teneriffae.

S. teneriffae showed a different electrophoretic phenotype from *S. vulgaris* at the *Got-1, G-3-pd-1, Gdh-1, G-6-pdh-2, β-Est-1* and *β-Est-2* loci. At five of these loci the difference was simply due to slight differences in the mobility of an allozyme.

Only at the *Got-1* locus was there a marked difference in the banding pattern in *S. teneriffae* compared with that which commonly occurs in *S. vulgaris* . Instead of the three banded *Got-1b/Got-1c* allozyme pattern found in *S. vulgaris* var. *vulgaris* , a six banded pattern was observed comprising allozymes at the *Got-1a, Got-1b* and *Got-1c* positions plus the appropriate intermediate bands between the these allozymes. This pattern was very similar to that found for *Got-1* in *S. cambrensis* from Leith (Chapter 4) and also in a few *S. vulgaris* var. *hibernicus* individuals from Wrexham (Chapter 5).

S. vulgaris ssp. *denticulatus* and *S. vernalis*

S. vulgaris var. *denticulatus* yielded isozyme patterns identical to those found in *S. vulgaris* var. *vulgaris,* except at the *β-Est -1* and the *β-Est -2* loci. Variation between samples from Jersey and Ainsdale was present at both of these loci, and within the Jersey sample there was additional variation at the *β-Est -2* locus. For *β-Est -1,* individuals from the Ainsdale population produced a single band which occurred at the same position as the extremely rare *β-Est-1c* band, found occasionally in

S. vulgaris var.*vulgaris* (Chapter 5). In contrast, plants from Jersey were monomorphic for the *β-Est-1b* band. At the *β-Est-2* locus Ainsdale individuals produced a single allozyme located on gels nearer to the origin than the *β-Est-2a* allozyme. This particular band was not found in any other species surveyed. A different set of phenotypes were produced by Jersey individuals with plants being either double banded (*β-Est-2b* / *β-Est-2c*) or single banded (*β-Est-2c*) at the *β-Est-2* locus.

In general, *S. vernalis* exhibited a very similar electrophoretic phenotype to *S. vulgaris* for most enzymes surveyed. There were differences, however, for *Got-1* and *Pgi-1*, and most notably at the *α-Est-1* locus, which was not expressed in *S. vernalis*.

At the *Pgi-1* locus, two allozymes were produced, *Pgi-1a* and *Pgi-1b*. In the population from Germany individuals producing one or other of these isozymes were present along with individuals producing a triple banded phenotype. Relative frequencies of the three phenotypes are presented in Table 7. 2. Assuming that phenotypes equate to genotypes (with the triple banded type representing the heterozygote) then phenotypic frequencies are at Hardy-Weinberg equilibrium ($\chi^2_{(1)}$ = 0. 149. n. s.).

For *Got-1* the German population was monomorphic, with individuals producing only the *Got-1c* allozyme. In contrast, the one individual from Spain produced a triple banded heterozygous phenotype

which included the *Got-1c* allozyme and a second allozyme mid-way between the positions of the *Got-1b* and the *Got-1c* allozymes found in *S. vulgaris* .

Variation was present at both *β-Est* loci in *S. vernalis* and may be interpreted in terms of two alleles segregating at each locus producing slow, fast and double banded phenotypes. Phenotype and putative allele frequencies at the *β-Est-1* locus within the Heidelberg population are presented in Table 7. 2. A χ^2 test shows that the deviation of expected from observed frequencies was close to being significantly different from Hardy-Weinberg expectations. It follows that the F-value (F_{IS}) for this locus is considerably greater than that for the *Pgi-1* locus. In view of the possibility of dominance occurring at the *β-Est-2* locus, as found in *S. vulgaris,* no attempt was made to equate phenotype to genotype frequencies.

Table 7. 2. Phenotype and Allele frequencies at the variable loci in the Heidelberg (W. Germany) population of *S. vernalis* .

Locus	Phenotype frequency			;	Allele frequency		;	F_{IS}
	a/a	a/b	b/b	;	a	b	;	
Pgi-1	.07	.34	.59	;	.24	.76	;	.096
β-Est-1	.52	.29	.19	;	.67	.33	;	.344*
β-Est-2	.04	.07	.89	;	Not calculated		;	-

* χ^2 value for this locus is 3. 033 (0. 10 > p > 0. 05)

7. 4. DISCUSSION

The similarity of electrophoretic phenotypes found in *S. vulgaris* and *S. teneriffae* is strong evidence in support of the theory of Kadereit (1984c) that *S. vulgaris* is the progenitor of *S. teneriffae*. The main difference between the two species, occurred at the *Got-1* locus where all individuals of *S. teneriffae* produced a six banded phenotype. Such a phenotype could have evolved following the duplication of one of the genes which code for the two *Got-1* allozymes found in *S. vulgaris*, i. e. during an increase in chromosome number from 2n = 40 (in *S. vulgaris*) to 2n = 60 (*S. teneriffae*). With the duplication of the gene the subsequent hybridisation of enzymes produced by the duplicated locus and the two loci already present, would produce a six banded phenotype for *Got-1*.

Alternatively, it is possible that *S. teneriffae* originated via allopolyploidy. Allopolyploidy could also lead to the production of a six banded phenotype for *Got-1* as found in the Leith population of *S. cambrensis* (Chapter 4). However, the lack of other allozyme patterns which may be interpreted as having an additive pattern in *S. teneriffae*, such as are found in *S. cambrensis* at the *Acp-2* locus in Leith and at the *β-Est-1* locus, tends to suggest that *S. teneriffae* may have evolved directly from *S. vulgaris* rather than through allopolyploidy.

The close similarity of the isozyme patterns observed in *S.*

viscosus and *S. sylvaticus* supports the accepted taxonomic view that these two species are closely related (Koniuszek and Verkleij, 1982). Differences found were once again primarily due to minor variation in allozyme mobility. Of particular interest was the result at the *Pgm-1* locus, where two allozymes were produced by all individuals in both species, presumably due to a duplication at the *Pgm-1* locus. Duplications of PGM loci have been recorded in *Camellia japonica* Chap. (Wendel and Parks, 1982),*Layia* (Gottlieb, 1987) and *Clarkia* (Soltis et al, 1987), and appear to be common for loci which produce enzymes that are active in the cytosol (Gottlieb, 1982). The presence of a duplication at the same locus in both *S. viscosus* and *S. sylvaticus* may be purely coincidental. However, the possibility exists that the duplication occurred in a common ancestor, and current differences in the mobility of the enzymes produced at this locus results from subsequent evolutionary divergence in *S. viscosus* and *S. sylvaticus* . In addition to the two *Pgm-1* isozymes, individuals of *S. viscosus* and *S. sylvaticus* also produced two isozymes for *6-Pgd-1*, *Per-1* and *G-3-pd-1*, whereas the other species investigated only produced single enzymes at these loci. It would seem, therefore, that within *S. viscosus* and *S. sylvaticus* many loci which encode enzymes are duplicated, functional and divergent.

A difference between *S. viscosus* and *S. sylvaticus* was evident on gels stained for G-6-PDH. Both loci encoding this enzyme appeared to be duplicated in *S. viscosus*, while in *S. sylvaticus* only a single band is

produced at the *G-6-pdh-2* locus, and *G-6-pdh-1* is not expressed. The two species also differed for MDH phenotype and again this may be due to a duplicated locus which is present in *S. viscosus* being absent or silent in *S. sylvaticus* .

Finally, for *β−Est-1* in *S. viscosus,,* the presence of some individuals with a double banded phenotype and others exhibiting a single slow band would indicate that all individuals carry duplicated copies of the *β-Est-1* locus and that both genes are switched on in double banded types while only one locus is functional in individuals showing the single banded phenotype.

It is of interest to compare the results obtained from the present electrophoretic analysis of *S. viscosus* and *S. sylvaticus* using starch gels, with those obtained previously by Koniuszek and Verkleij (1982) using polyacrylamide. The most noticeable differences to emerge were for ME and 6-PGD for which more bands were resolved on acrylamide, and for PGI which yielded only a single enzyme on acrylamide. For *G-6-pdh-1* both species produced two bands on acrylamide suggesting that the single band resolved in *S. sylvaticus* on starch (see above) may be an artifact of experimental conditions. In accordance with the findings of Koniuszek and Verkleij(1982), the present study has shown little electrophoretic variation to exist within either species. However, it is clear that within *S. viscosus* there is variation for *β-Est-1* which is an enzyme not previously

investigated by Koniuszek amd Verkleij.

The allozyme patterns found in *S. vulgaris* ssp. *denticulatus* and *S. vernalis* closely resembled those observed in *S. vulgaris* var. *vulgaris* . That said, the hypothesis of Kadereit (1984a) that *S. vulgaris* is an autopolyploid of *S. vernalis* is disputed by the fact that the *α-Est-1* locus expressed in *S. vulgaris* appears to be absent from *S. vernalis* . In theory, all loci that are expressed in an autotetraploid species should occur in the progenitor species (Crawford, 1983; Gottlieb, 1984b); this has been confirmed in the comparative studies of diploid and autopolyploid races of *Tolmiea menziesii* (Soltis and Rieseberg, 1986) and *Heuchera micrantha* Dougl. (Ness et al, 1989). Although the similarity of allozyme patterns in *S. vernalis* and *S. vulgaris* would indicate that the former may be a parental species of the latter, the absence of the *α-Est-1* locus in *S. vernalis* implies that this locus in *S. vulgaris* is derived from another species. Consequently, *S. vernalis* is unlikely to be the sole parent of *S. vulgaris*. Instead, *S. vulgaris* is more likely to be of allopolyploid origin. A survey of other diploid European taxa would be worthwhile, to establish which of these species possess the *α-Est-1* locus and thus may be parental to *S. vulgaris*.

One final point of interest worth mentioning, concerns the electrophoretic phenotypes of individuals in the two populations of *S. vulgaris* ssp. *denticulatus*. These were identical for all enzyme systems

except β–EST. Whereas individuals derived from the Jersey population produced β–EST phenotypes similar to those found in *S. vulgaris* var. *vulgaris* and *S. vernalis* , Ainsdale plants exhibited a unique phenotype that was monomorphic within the sample studied. In view of this difference, and also the differences which are evident in morphology between the two populations of ssp. *denticulatus* (personal observation), it would seem of value to conduct further investigations on the degree of relatedness of these populations, to each other and to *S. vulgaris* var. *vulgaris* and *S. vernalis*, using a range of biochemical and molecular techniques.

CHAPTER 8 FINAL COMMENTS

In the research reported in this thesis isozymes have proved to be a powerful tool in answering questions concerning the evolution of certain taxa within the genus *Senecio* section Annui. The most significant findings to emerge relate to the multiple origin of a new allopolyploid species, *S. cambrensis*, from *S. vulgaris* and *S. squalidus*, and the introgression of genes into *S. vulgaris* from *S. squalidus*. In addition, the electrophoretic analysis has provided an understanding of the population structure of *S. vulgaris* and *S. squalidus*, and at a higher taxonomic level, has underlined the close relationship between *S. viscosus* and *S. sylvaticus* plus their proximity to *S. vulgaris* and *S. squalidus*. Finally, electrophoretic evidence has shown that a close genetic relationship exists between *S. vulgaris* and *S.vernalis* , although the hypothesis that *S. vulgaris* arose via autopolyploidy from *S. vernalis* (Kadereit, 1984a) is disputed.

The results of the survey of variation at the *α-Est-1* and the *Acp-2* loci within *S. vulgaris, S. squalidus* and *S. cambrensis* have shown that the Scottish and Welsh populations of *S. cambrensis* are the products of independent origins. Furthermore, an analysis of the patterns of variation obtained at the *Got-1* locus, indicates that in Wales the populations at Wrexham and Mochdre may also have arisen

independently. Confirmation of the latter awaits further investigation, which may involve extending the survey of isozyme variation to other enzyme systems and an analysis of nuclear and plasmid DNA variation.

If multiple origins frequently occur among polyploid species, then this has important implications for plant evolution. In particular, if a new species has more than one opportunity to survive the rigours of selection then the chances of it eventually persisting may be increased. Furthermore, if a species can evolve in different places, the subsequent colonisation of other areas is not dependent upon the success of a single population. The relative frequency of multiple speciation via polyploidy is currently unknown due to the limited number of cases which have received detailed investigation. Nevertheless, as within the last fifteen years, six polyploid species from widely disparate plant groups have been shown to have polytopic origins (the autopolyploid *Heuchera micrantha;* Soltis et al., 1989; and the allopolyploids*Tragopogon mirus;* Roose and Gottlieb, 1976; *Asplenium bradleyii, A. pinnatifidum* ; Werth et al, 1985; *Plagiomnium medium* ; Wyatt et al, 1988 and now *S. cambrensis*), it is reasonable to suggest that multiple origins of polyploids may not be uncommon. While the origin of a species is a significant event, subsequent differentiation, and establishment, is also of paramount interest. Recognition of the recent origins of *S. cambrensis* will provide an almost unique opportunity to study these events.

Hybrids may exhibit characteristics that are not found in either

parental species, due to the interaction of the parental genomes in the hybrid. These new features may be morphological, e. g. leaf shape in *Ranunculus* subgenus *Batrachium* (Cook, 1970), or, more typically, biochemical novelties, for instance phenolic compounds in *Baptisia* (Alston and Turner, 1963) and enzyme proteins in *Triticosecale* (Barber, 1970). At the isozyme level, novel products are produced in the formation of multimeric enzymes following interaction between enzyme sub-units produced by different alleles inherited from the parents (Roose and Gottlieb, 1976). Barber (1970) and Manwell and Baker (1970) have proposed that allopolyploids owe their success, relative to their progenitors, due to this production of novel enzymes and also the combined expression of each parental set of enzymes. The selective pressure on a single enzyme to maintain the integrity of a biochemical pathway is likely to prevent maintenance of an altered structure caused by mutation. However, when several isozymes of the enzyme occur in an individual there may be opportunities for one or more to alter their function in some way (following mutation), which, in turn may increase the range of suitable habitats for the species (Stebbins, 1985). Lack and Kay (1986) suggest that this may have enabled the allopolyploid *Polygala vulgaris* to occupy a wider ecological range than either of its putative parents, *P. serpyllifolia* and *P. comosa* .

In *S. cambrensis,* however, no unique allozymes were detected in the present study. All the allozymes resolved in *S. cambrensis* were

recorded in at least one of the parental species.

The evidence for introgression in *S. vulgaris* provided by the survey of variation at the *Got-1* locus is important for two reasons. Firstly, coupled with the work carried out by Ingram et al (1980), it seems to confirm that the origin of the radiate morph of *S. vulgaris* is due to introgression, rather than mutation. Secondly, it forms an important addition to the literature on introgression, showing that two distinct genes, one for the radiate character, the other coding for a GOT allozyme, have been introduced into *S. vulgaris* from *S. squalidus*. The presence of an allele (*Got-1a*) in the radiate morph of *S. vulgaris*, that is absent from the vast majority of non-radiate morphs could be used in future studies of gene flow from the radiate morph to the non-radiate morph within a natural population. Similarly, it would be of interest to monitor changes in allele frequencies at the *Got-1* locus in *S. vulgaris* at locations that are colonised by *S. squalidus* in the future.

Finally, it is worth considering other important effects that the spread of *S. squalidus* may have had on members of the genus *Senecio* in Britain. In addition to *S. vulgaris*, two other species, *S. viscosus* and *S. sylvaticus*, are closely related to *S. squalidus*. It is feasible that the recent spread of *S. viscosus* is due to the influence of *S. squalidus* on the *S. viscosus* genome. The introgression of *S. squalidus* genes into *S. viscosus*, was investigated by Crisp and Jones (1978). Although they were unable to produce conclusive results, they believed that *S. squalidus* had

affected *S. viscosus* and other tetraploid British species, such as *S. vulgaris,* specifically by reducing the isolating barriers between the different members of British species in section Annui. They argued that the fertile hybrids formed between *S. sylvaticus* and *S. viscosus* , found in Britain, but absent from Europe (Crisp, 1972), could be attributed to the presence of *S. squalidus* in Britain. This led them to speculate that:-

> "*Senecio* section Annui in Britain can be postulated as being in the first phase of a burst of evolutionary development as a consequence of the escape of *S. squalidus* from Oxford Botanic Gardens"

In the light of the results reported in this thesis, this comment has proved to be particularly apposite. Since *S. squalidus* started to spread, just over a hundred years ago - a very short time in the evolutionary time scale - the species has had a profound influence on *Senecio* section Annui in Britain. Its spread has resulted in the introduction of new genes into *S. vulgaris* and possibly also into *S. viscosus,* and following hybridisation with *S. vulgaris* has given rise to a new species, *S. cambrensis,* on more than one occasion. In view of these findings, it would be of interest to establish whether other successful plant invaders of Britain (or elsewhere) have had equally profound effects on the evolution of indigenous plants. Perhaps all that is needed to uncover further examples are appropriate resources and patient investigation by evolutionary biologists.

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APPENDIX 1

Buffer Recipes

Electrode buffer:-

1.2g LiOH (.03M); 11.9g Boric Acid (.192M)

Add to 1l water. Fix at pH8.3 with dry ingredients.

Scandalios (1969).

Gel buffer:-

1.28g Citric Acid (.007M); 5.45g Tris base (.05M)

Add to 900ml water. Combine with 100ml electrode buffer. Fix at pH8.3 with conc. NaOH solution or dry Citric Acid. Each gel uses 300ml gel buffer.

Scandalios (1969).

Extraction buffers:- 50ml Gel buffer; 37mg KCl; 14mg $MgCl_2$; 18mg EDTA (Tetrasodium)

(1) For all enzymes except PER add; 2ml Mercaptoethanol; 0. 5ml Triton X-100; 25mg PVPP (40T).

Gottlieb (1984a).

(2) For PER add only 880mg Ascorbic Acid.

Adopted from Soltis et al (1983)

All chemicals used were supplied by Sigma. All water was distilled and de-ionised.

APPENDIX 2

Staining recipes

succesful stains.

The following dyes were used in the following stains where specified. These were made up 2-3 hours before use.

Dye 1; 10mg NADP	Dye 2 ; 10mg NAD
10mg MTT	10mg MTT
3mg PMS	3mg PMS
Dissolved in 1ml water	Dissolved in 1ml 10% $MgCl_2$ soln.

Acid Phosphatase (ACP) E. C. No. 3. 1. 3. 2.

Slice; Top or Middle

Stain; Presoak in 50ml 0.4M Acetate pH 5.0 soln. for 20 mins at 4^{o} C .

Pour off, then add 50ml 0.2M Acetate soln. pH 5.0, 50mg Na α-Napthyl acid phosphate, 0.5ml 10% $MgCl_2$ and 40mg Fast Garnet GBC.

Staining time; approx. 2 hours.

Reference; Scandalios (1969)

Glutamate Oxaloacetate Transaminase (GOT) E. C. No. 2. 6. 1. 1.

Slice; Top or Middle

Stain; 50ml 0.1M Tris HCl soln. pH 8.5,18mg α-ketoglutaric acid, 65mg Aspartic acid, 250mg PVP (40T), 25mg EDTA (Tetrasodium), 710mg Na_2HPO_4, 1mg Pyridoxal-5-Phosphate, 200mg Fast Blue BB.

Staining time; approx. 1/2 hour.

Reference; Gottlieb (1973).

Peroxidase (PER) E. C. No. 1. 11. 1. 7.

Slice; Top or Middle

Stain; 50ml 0.2M Acetate soln. pH 5.0, 1ml H_2O_2, 1ml 10% $CaCl_2$, 40mg 3-Amino-9-Ethylcarbazole dissolved in 5ml Dimethyl formamide

Staining time; approx. 1/2 hour.

Reference; Graham *et al.* (1964)

Note; Stain appears on the cathodal slice.

α-Esterase (α-EST) E. C. No.3. 1. 1. 1.

Slice; Top or Middle

Stain; 40ml Distilled water, 2ml 1% α-napthyl acetate, 50ml 0.2M NaH_2PO_4, 10ml 0.2M Na_2HPO_4, 125mg Fast Blue RR salt.

Staining time; approx. 1/2 hour.

Reference; Scandalios (1969)

ß-Esterase (ß-EST) E. C. No. 3. 1. 1. 1.

Slice; Top or Middle

Stain; 40ml Distilled water, 2ml 1% ß-napthyl acetate, 50ml 0.2M NaH_2PO_4, 10ml 0.2M Na_2HPO_4, 125mg Fast Blue RR salt.

Staining time; approx. 1/2 hour.

Reference; Scandalios (1969).

Phosphoglucose Isomerase (PGI) E. C. No. 5. 3. 1. 9.

Slice; Any

Stain; 50ml 0.1M Tris HCl soln. pH 8.5, 40mg Fructose-6-Phosphate (Disodium), 20 units Glucose-6-Phosphate dehydrogenase (G-6-Pdh), Dye 1.

Staining time; approx. 1/2 hour.

Reference; Adopted from Tanksley (1980).

Phosphoglucomutase (PGM) E. C. No. 2. 7. 5. 1.

Slice; Any

Stain; 50ml 0.1M Tris HCl soln. pH 7.5, 100mg Glucose-1-Phosphate, 30 units G-6-Pdh, 15mg ATP, Dye 1.

Staining time; approx. 2-3 hours.

Reference; Tanksley (1979).

Glyceraldehyde-3-Phosphate dehydrogenase (G-3-PD) E. C. No. 1. 2. 1. 12.

Slice; Any

Stain; 50ml 0.1M Tris HCl soln. pH 8.0, 100mg Fructose-1, 6-Diphosphate, 10 units Aldolase, 312mg Sodium Arsenate, Dye 1.

Staining time; approx. 2-3 hours.

Reference; Scandalios (1969).

6-Phosphogluconate Dehydrogenase (6-PGD) E. C. No. 1. 1. 1. 44.

Slice; Any

Stain; 50ml 0.1M Tris HCl soln. pH 8.0, 50mg 6-Phosphogluconic acid (Trisodium), Dye 1.

Staining time; approx. 1 hour.

Reference; Adopted from Sing and Brewer (1969).

Glucose-6-Phosphate dehydrogenase (G-6-PDH) E. C. No. 1. 1. 1. 49.

Slice; Any

Stain; 50ml 0.1M Tris HCl soln. pH 7.5, 50mg Glucose-6-Phosphate (Monosodium), Dye 1.

Staining time; approx. 2-3 hours.

Reference; Sing and Brewer (1969).

Malic Enzyme (ME) E. C. No. 1. 1. 1. 40.

Slice; Top or Middle

Stain; 50ml 0.1M Tris HCl soln. pH 7.5, 1g Malic acid, Dye 1.

Staining time; approx. 1/2 hour.

Reference; Soltis *et al.* (1983).

Malic Dehydrogenase (MDH) E. C. No. 1. 1. 1. 37.

Slice; Top or Middle

Stain; 50ml 0.1M Tris HCl soln. pH 8.5, 1g Malic acid, Dye 2.

Staining time; approx. 1/2 hour.

Reference; Brown *et al.* (1978).

Glutamate dehydrogenase (GDH) E. C. No. 1. 4. 1. 2.

Slice; Middle or Bottom

Stain; 50ml 0.1M Tris HCl soln. pH 7.5, 210mg Glutamic acid, 25mg ATP, Dye 2.

Staining time; approx. 2 hours.

Reference; Adopted from Hartmann *et al.* (1973).

<u>Partially succesful stains.</u>

The following three stains all gave good results on occasions. However the inconsistency of their resolution led to their omission from the study.

Hexokinase (Hex) E. C. No. 2. 7. 1. 1.

Slice; Middle

Stain; 50ml 0.1M Tris HCl soln. pH 8.0, 90mg Glucose, 20mg EDTA (Tetrasodium), 65mg ATP, 40 units G-6-Pdh, Dye 1.

Staining time; approx. 2-3 hours.

Reference; Adopted from Eaton *et al.* (1966).

Aldolase (ALD) E. C. No. 4. 1. 2. 13.

Slice; Middle

Stain; 50ml 0.1M Tris HCl soln. pH 8.0, 200mg Fructose-1, 6-Diphosphate (trisodium), 100 units G-3-pd, 312mg Sodium Arsenate, Dye 2.

Staining time; approx. 2-3 hours.

Reference; Soltis et al (1983).

Triose Phosphate Isomerase (TPI) E. C. No. 5. 3. 1. 1.

Slice; Any

Stain; 50ml 0.1M Tris HCl soln. pH 8.0, 10mg DHAP , 30 units G-3-pd, 100mg Sodium Arsenate, Dye 2.

Staining time; approx. 2-3 hours.

Reference; Brewer (1968).