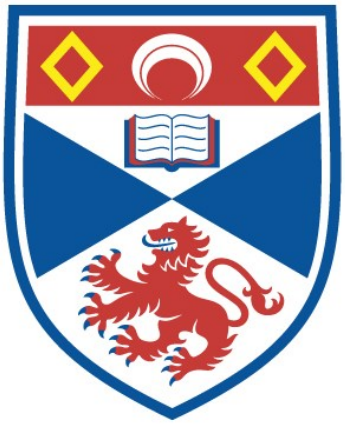
MALE COMPETITION AND OUTCROSSING RATE IN A HERMAPHRODITE PLANT

Judith A. Irwin

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



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MALE COMPETITION AND OUTCROSSING RATE IN A HERMAPHRODITE PLANT.

by Judith A. Irwin.

A thesis presented for the Degree of Doctor of Philosophy at the University of St. Andrews.

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ABSTRACT.

The principal aim of the research presented in this thesis was to investigate factors affecting the "male competition" component of sexual selection in the hermaphroditic species, *Senecio vulgaris*. As male reproductive function consists of attracting pollinators and the success of pollen in contributing genes to the next generation, sexual selection will act on both the sporophytic and gametophytic stages of the life cycle. The potential for, and consequences of male competition were analysed at both pre- and post-pollination stages.

A comparison of the relative attractiveness of the radiate and non-radiate morphs of *S.vulgaris* to pollinators revealed that in mixed stands, pollinators discriminated in favour of the radiate morph irrespective of the frequency of the two morphs in a population. Measurement of intramorph and intermorph maternal outcrossing rates showed the radiate morph always outcrossed at higher levels than the non-radiate morph. Both morphs exhibited levels of male outcrossing, the radiate morph exhibited higher levels of intramorph paternal outcrossing while non-radiate pollen was more successful than radiate pollen in intermorph crosses. The potential levels of intermorph and intramorph pollen

competitionexperienced by the radiate and non-radiate pollen types suggest radiate pollen was subjected to greater levels of competition for access to ovules than non-radiate pollen.

Examination of post-pollination events suggested that radiate pollen germinates faster than non-radiate pollen when applied to stigmas of either morph. However, no consistent evidence of radiate pollen tubes outcompeting non-radiate pollen tubes in the style and consequently fertilising a disproportionate share of available ovules was obtained. The problems associated with measuring pollen competitive ability are discussed.

In addition to the research on male competition, a study was also conducted to examine the origin of the radiate morph of *S.vulgaris*. Morphometric and electrophoretic analyses provided strong evidence that a radiate variant from York possessed more 'squalidus-like' characters than are generally found in radiate *S.vulgaris*. It is suggested that this radiate form may represent an early stage in the origin of radiate *S.vulgaris* via introgression of *S.squalidus* into *S.vulgaris*.

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DECLARATION.

I, Judith Ann Irwin, 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 or professional qualification.

Judith A. Irwin.

February 1990.

STATEMENT.

I was admitted to the Faculty of Science of the University of St. Andrews under Ordinance General No.12 in October 1986 and as a candidate for the degree of Ph.D. in October 1987.

Judith A. Irwin.

February 1990.

CERTIFICATE

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

R. J. Abbott.

February 1990.

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Plate 1 Radiate (right) and non-radiate capitula of *Senecio vulgaris* L.

CONTENTS.

CHAPTER 1.

GENERAL INTRODUCTION.

GENERAL INTRODUCTION.

The term sexual selection was first used by Darwin (1859,p87) in 'The Origin of Species' to describe a form of natural selection which depends "...not on a struggle for existence in relation to other organic beings or external conditions, but on a struggle between the individuals of one sex, generally the males, for possession of the other sex". Darwin pointed out that the result of this form of selection "is not death to the unsuccessful competitor, but few or no offspring". Subsequently, Darwin (1871) drew attention to sexual selection operating on the traits of an individual that determine its access to potential mates, and, in doing so, sought to explain the evolution of decorative characters such as a stag's antlers and a peacock's tail that (intuitively) would seem to decrease the chance of survival of males that possess them.

It is now recognised that two factors are important in sexual selection: (i) competition among members of one sex for reproductive access to the other sex i.e. intrasexual selection; and (ii) preference by members of one sex for certain members of the other sex i.e. intersexual selection (Huxley 1938; Willson 1979; Stephenson and Bertin 1983). Because female gametes are larger and more costly in terms

of resource allocation than male gametes, typically males compete with each other for females - "male competition" - while females exercise a choice of mate - "female choice" (Bateman 1948; Trivers 1972).

Numerous aspects of animal reproductive morphology and behaviour have since been ascribed to the effects of sexual selection especially in species with polygamous or promiscuous mating systems, in which the opportunities for male competition and female choice are considerable (Fisher 1958; Trivers 1972; Halliday 1978). For example competition among males for access to females has been considered responsible for large size in elephant seals (*Mirounga angustirostris*; Cox and Le Boeuf 1977) and large horns in mountain sheep (*Ovis canadensis*; Geist 1971). Similarly female preference is considered as the cause of long tailed males of the African widowbird (*Euplectes poryne*; Anderrson 1982). Until recently, however, the possibility of sexual selection being an important factor in plant evolution was largely ignored. This situation prevailed, despite the fact that Haldane (1932) noted that stigmas often contain more pollen than is needed to fertilise all the ovules of a gynoecium creating conditions for competition among pollen grains for the successful fertilisation of ovules by male gametes (see also Huxley 1942).

Bateman (1948) was among the first to suggest that sexual selection occurred in plants as a result of the size difference in male and female gametes. He suggested that male competition should lead to increased pollen production and that the effects of such competition should be more apparent in monoecious or dioecious plants than in hermaphrodites. Janzen (1977), while not explicitly referring to sexual selection, proposed that selection pressures on male and female functions differ greatly, that all pollen donors are not of equal fitness and that female sporophytes should be selective in their production of offspring with respect to pollen donors. Subsequently, Willson (1979) stressed the potential importance of sexual selection in explaining several reproductive phenomena in plants including the evolution of pollinia and breeding systems.

Because most plants are hermaphrodites, it might seem at first that sexual selection will play only a minor role in plant evolution. However Charnov (1979) and Willson (1979) have argued that sexual selection will in fact act separately on the male and female functions of outcrossing hermaphrodites given that seed/fruit production is not limited by pollination. Such selection would lead to a specialisation of the male and/or female role within a hermaphroditic species which, in turn, might provide the

basis for the evolution of dioecy from hermaphroditism (Willson 1979). With this in mind, there has been much recent debate about the potential for hermaphroditic plants to show variation in functional gender, although traditionally such plants were assumed to function equally as male and female parents (Lloyd 1979; Ross and Gregorius 1983).

Theory has tended to outstrip empirical evidence on the role of sexual selection in plant evolution. In a review of sexual selection in plants Stephenson and Bertin (1983) emphasised this point, stating that :- "Although there is a growing body of theory surrounding sexual selection as it applies to plants, and, although sexual selection is now often invoked as an explanation for the evolution of breeding systems and reproductive strategies, there have been few attempts to examine in detail the potential mechanisms underlying intrasexual competition and intersexual choice in plants".

More recently, Charlesworth, Schemske and Sork (1987) again drew attention to this deficiency in our knowledge, stating that :- "The important point is that our knowledge of these selection processes in plants is still very imperfect. We need much more evidence of variation in plant reproductive success measured as a contribution to progeny and the

relationship between flowering-time traits and the male component of fitness".

One problem with obtaining evidence of the mechanisms of sexual selection in hermaphrodites concerns the availability of appropriate material for investigation. Several studies which have addressed the problem have focussed on hermaphroditic species that produce far more flowers than actually set fruit. Having established that fruit set is not limited by pollination, the question posed by investigators is whether the excess production of flowers is a consequence of selection for increased flower number as a response to "male competition", or the result of a "selective abortion" of fertilised ovules based on paternity - "female choice". In the latter case only embryos that are genetically more vigorous, or compatible with the maternal genotype reach maturity.

Two studies which have attempted to differentiate between these two explanations have produced evidence that in *Asclepias exaltata* (Queller 1983) and *Thymus drucei* (Couvet, Henry and Gouyon 1985) excess flower number has evolved as a consequence of "male competition" rather than "female choice". However, two other studies indicate that selective abortion according to paternity may occur in *Campsis radicans* (Bertin 1982) and *Asclepias specosia*

(Bookman 1984). Clearly many more studies are needed before it can be determined whether both "male competition" and "female choice" are equally important in hermaphrodites or whether the former is the more important. That said, it is worth mentioning that Bell (1985) has recently advocated that the hermaphroditic flower is predominantly male in function. From the results of several experimental studies involving the manipulation of hermaphrodite flowers he concluded that :- "Female function (fertilisation of ovules) is almost completely satisfied by a single insect visit, or with very few visits, which will be made even to very small flowers, while successful male function (dispersal of pollen) requires repeated visits, which can be procured only by substantial investment in attractive structures. Any increment in secondary allocation beyond a certain minimal value increases the fraction of pollen that is dispersed but has little or no effect on the fraction of ovules that are fertilised".

In the research to be reported in this thesis, attention was focused on the "male competition" component of sexual selection. In contrast with the previous work of Queller (1983) and Couvet *et al* (1985) which provided circumstantial evidence of "male competition" occurring in hermaphrodites, a major objective was to obtain direct evidence of "male competition" occurring in a hermaphrodite

species. As pointed out by Stephenson and Bertin (1983) "...direct evidence for the existence of male competition and measurements of its intensity in natural populations of a hermaphrodite plant are lacking". The aim of the studies reported therefore has been to fill this gap.

The approach adopted has taken advantage of the fact that sexual selection is expected to occur in outcrossing hermaphrodites but not in selfing hermaphrodites (Charnov 1982; Willson 1983). In an obligate selfing hermaphrodite there would appear to be no opportunities for either "male competition" or "female choice" to take place. This being so, given suitable variation for outcrossing rate either within or between species, it is to be expected that mechanisms favouring "male competition" will have evolved in outcrossing taxa (morphs, populations or species) but not in selfing taxa. Appropriate comparisons between such taxa should, therefore, provide direct evidence of "male competition". As Stephenson and Bertin (1983 p116) have pointed out:- "The selfing/outcrossing comparison is useful because if the degrees of outcrossing of the plant species are known, one can evaluate the importance of adaptations hypothesised to be associated with intense male competition".

Charlesworth and Charlesworth (1981) have argued that

populations or species having greater outcrossing rates would be expected to have a more male-biased allocation of resources. This hypothesis has been tested by Schoen (1982) in *Gilia achilleifolia* and McKone (1986) in a range of *Bromus* species. From a comparison among populations of *Gilia achilleifolia*, Schoen (1982) found that the relative allocation of resources to pollen production was highly correlated with outcrossing rate. McKone (1987) reported similar findings based on a study of five species of *Bromus* that differed for outcrossing rate. He found that the obligately outcrossing *Bromus inermis* allocated nearly half of its reproductive effort to pollen production while the highly selfing species *B. tectorum* invested less than two percent of its reproductive effort in pollen production.

In the common Groundsel, *Senecio vulgaris* (Compositae), it has been demonstrated that many British populations are polymorphic for capitulum type and associated outcrossing rate. Such material would therefore seem ideally suited for an analysis involving detailed comparisons aimed at elucidating mechanisms of "male competition" which have evolved in an outcrossing taxon. *Senecio vulgaris* is an abundant, short-lived, self-compatible pioneer species of open ground in the British Isles. Although the majority of U.K. populations contain plants that produce capitula which bear only hermaphrodite disc florets, many also contain a

rayed form with capitula in which the outer whorl is composed of 8-13 ligulate ray florets which are male sterile, in addition to a complement of hermaphrodite disc florets. There are in fact two radiate forms of *S.vulgaris* in Britain; *Senecio vulgaris* subsp *denticulatus* which has an exclusively maritime distribution outside the Mediterranean where it occurs in montane habitats (Kadereit 1984a), and inland radiate groundsel *S.vulgaris subsp vulgaris* var *hibernicus* Syme, first recorded in Cork, Eire in 1866 (Syme 1875). The latter is the one investigated in this thesis and a fuller discussion of its origin is given in chapter 7.

Trow (1912) first showed that the presence/absence of ray florets in capitula of *S.vulgaris* is controlled by a pair of alternative alleles R and r at a single locus designated the 'ray floret' locus. The heterozygote Rr produces ray florets shorter than those of the radiate homozygote RR - expression of the character in the heterozygote being variable due to incomplete dominance. The three morphs radiate, non-radiate and intermediate are now designated Tr.Tr.; Tn.Tn. and Tr.Tn. respectively (Hull 1974). The capitulum polymorphism in *S.vulgaris* is of interest here because Marshall and Abbott (1982, 1984a) found a large and significant difference between radiate and non-radiate plants in female intermorph outcrossing frequency (using the ray floret locus as a marker). The radiate morph, with

capitula bearing an outer ring of conspicuous ray florets in addition to a complement of hermaphrodite disc florets, showed greater outcrossing (up to 35%) than the non-radiate morph (producing capitula bearing only hermaphrodite disc florets) which generally outcrossed at levels between 1-3%. The greater outcrossing rate of the radiate morph has been partly explained by the fact that the female ray florets outcross at significantly higher levels than the hermaphrodite disc florets of either radiate or non-radiate plants (Marshall and Abbott 1984b). (A more detailed description of the findings of Marshall and Abbott is given in chapters 2,3 and 4).

Although the outcrossing rates estimated by Marshall and Abbott (1982,1984a) were maternal outcrossing rates, it has since been shown by Abbott and Irwin (1988) that the radiate morph (R) of *S.vulgaris* is more attractive to pollinators than the non-radiate morph (N) and that the frequency of pollinator movements between radiate plants (R-R transitions) is significantly greater than any of the other possible transitions (i.e. R-N, N-R or N-N) that occur in stands composed of the two morphs. This would imply that the radiate morph, in addition to having the greater maternal outcrossing rate, would also exhibit a significantly greater paternal outcrossing rate than the non-radiate morph.

Based on these findings the radiate and non-radiate morphs of *Senecio vulgaris* would appear to provide a model system with which to investigate the effect of "male competition" within a hermaphrodite species. By studying material of the non-radiate and radiate morphs of *S.vulgaris* it should be possible to conduct an analysis of the expected association between outcrossing rate and the level of "male competition" in a hermaphrodite plant.

A detailed and comprehensive study of "male competition" requires analysis at both pre- and post-pollination phases. Male reproductive function consists of attracting pollinators and the success of pollen in contributing genes to seeds (i.e. getting pollen transferred to another individual, having that pollen outcompete other pollen for fertilisation of ovules and having those ovules incorporated into seeds and fruits - Charnov 1979). Thus it is feasible, indeed likely, that sexual selection will act on both sporophytic and gametophytic phases of the life cycle and that mechanisms at each of these phases have evolved to improve male function. Bearing this in mind, the aims of the study presented in this thesis were as follows :

(i) To analyse factors likely to affect "male competition" in the pre-pollination phase. In particular, to compare the relative attractiveness of each morph to pollinators and

the relative abilities of morphs to disperse pollen to plants of the same morph or the alternative morph.

(ii) In conjunction with (i) above, to obtain accurate estimates of male and female outcrossing rates of the radiate and non-radiate morphs of *S.vulgaris* using an electrophoretic marker in addition to the ray floret locus employed in previous studies. Estimates of male outcrossing are required both within and between morphs to provide information on whether "male competition" is likely to be greater in the radiate than the non-radiate morph.

(iii) To examine evidence of male competition in the post-pollination phase. Following deposition of pollen on the stigma, "male competition" will be expected to proceed whenever several pollen grains of different genotype have access to the same ovule. Under such conditions the success of a pollen grain in achieving fertilisation will depend on its speed of germination and rate of pollen tube growth.

As a preliminary to the studies involved in (ii) above, it was necessary to survey a large number of monomorphic and polymorphic populations of *S.vulgaris* for electrophoretic variation. The results of this survey and the analysis of the electrophoretic variants resolved, are presented in this thesis before the analysis of "male competition". In

addition, as an outcome of the electrophoretic survey, a chapter is also included at the end of the thesis which presents new evidence relating to the origin of the radiate morph of *S.vulgaris* in Britain.

CHAPTER 2.

DEVELOPMENT OF AN ISOZYME MARKER SYSTEM IN *SENECIO VULGARIS* L. FOR MEASURING OUTCROSSING RATE.

INTRODUCTION.

The mating system of many plants is often described in terms of the mixed mating model in which a certain proportion of zygotes is derived from self-fertilsation (selfing) and the remainder is derived from mating at random with other plants in the population (outcrossing), (Ritland 1983). To estimate the rate of outcrossing within a population, a reliable marker gene system is required to identify self from cross progeny. Historically, a number of alternative methods have been used to estimate natural outcrossing rates (see Jain 1979 for review). These are based on a diversity of data sets and include methods where (i) qualitative measures of outcrossing are based on tests of autofertility and inbreeding depression; (ii) natural populations are assumed to be at inbreeding equilibrium and outcrossing (t) is derived from Wrights Fixation Index F (i.e. the departure from heterozygosity level expected under panmixia) using the equation $t = (1-F)/(1+F)$ (Fyfe and Bailey, 1951); (iii) simultaneous estimation of allele frequencies and outcrossing rates from progeny data using a maximum likelihood procedure (Brown and Allard 1970).

Traditionally, direct estimates of outcrossing rates were limited by the necessity of there being present within a

population a morphological (visual) trait showing discontinuous variation due to the segregation of allelic variation controlling that trait (Imam and Allard 1965; Horovitz and Harding 1972; Bond and Pope 1974). Such variation, though providing a useful source of genetic markers for outcrossing estimation, often suffers from several disadvantages: (i) the frequent absence of such diversity in many populations of wild plants; (ii) an inability to differentiate between the heterozygotes and one of the homozygotes due to dominance; (iii) the direct effect of the chosen marker locus itself on floral traits and therefore the outcrossing rate of plants tested. The latter problem is particularly critical if the variation and dominance relationships are such that outcrossing is measured in terms of that exhibited by a rare homozygous recessive floral mutant in a population (Snape and Lawrence 1971; Humpreys and Gale 1974). Although such a procedure provides an accurate measure of the selfing/outcrossing rate of the recessive mutant, it is unlikely to reflect that of the wild type.

Previous studies of outcrossing rate in *Senecio vulgaris* have all employed marker genes affecting floral traits (Hull 1974; Campbell and Abbott 1976; Marshall and Abbott 1982,1984a,1984b; Warren 1988). The marker genes most commonly used have been those at the ray floret locus

controlling the capitulum polymorphism in *S.vulgaris* first described by Trow (1912). This locus has been used to estimate outcrossing in *S.vulgaris* by Trow (1912), Hull (1974), Campbell and Abbott (1976), and Marshall and Abbott (1982, 1984a). Recently, Warren (1988) employed an additional marker locus controlling the colour of the calyculus bract tips of the capitulum (green/black) to estimate levels of outcrossing both within and between the radiate and non-radiate morphs of *S.vulgaris* in artifical stands.

Both the markers at the ray floret and calyculus bract tip colour loci may affect estimates of outcrossing to some degree. This certainly would seem to be the case for the ray floret markers as it has been shown that insect pollinators of *S.vulgaris* exhibit a preference for the rayed morph which might result in a degree of assortative mating within polymorphic populations (Abbott and Irwin, 1988) such that most outcrossing occurs between radiate plants. In addition, the ray floret markers suffer from the disadvantage that when used alone it is not possible to obtain estimates of outcrossing within each capitulum morph. For such estimates to be obtained, a second locus which segregates independently from the ray floret locus (e.g. that controlling calyculus bract tip colour, Warren 1988) is required. Whether differences in calyculus bract tip colour

also affect pollinator behaviour/outcrossing rate remains to be determined.

In order to circumvent the problems involved with using floral trait and other morphological markers, many workers have employed isozyme marker loci to estimate outcrossing rates (eg Schoen and Clegg 1985; Epperson and Clegg 1987; Ritland 1983-review). Using these variants as markers has a number of advantages (Brown and Weir 1983) e.g. (i) enzyme specificity allows alleles to be attributed to loci; (ii) each allelic difference is detected as a mobility difference which is independent of the functional role or the overall level of variation of the enzyme in question; (iii) allelic expression is usually codominant and free of epistatic or environmental effects; (iv) an array of enzymatic loci can be assayed conveniently on one individual using small amounts of material.

The aim of the study reported in this chapter was to find a polymorphic isozyme marker locus that could be used to estimate outcrossing rates within and between the radiate and non-radiate morphs of *S.vulgaris*. An initial survey of isozyme variation within radiate and non-radiate plants from Newhaven Road, Edinburgh (grid ref NT261762) revealed no variation for any of several enzyme systems investigated. Subsequently a method of resolving esterase isozymes was

developed from that of Scandalios (1969) and variation within three separate zones on a gel was found. A genetic analysis of the three putative esterase loci controlling this variation was conducted. It transpired that there were three polymorphic esterase loci, two of which possessed two alternative alleles and another at which three possible alleles were present. There appeared to be a dominance/recessive relationship between the two alleles at one of the three loci, while allelic expression at the other two loci was codominant. A survey of natural populations of *S.vulgaris* indicated that in general the frequency of heterozygotes within natural populations was low. In addition, the results indicated that in populations polymorphic for capitulum type, more genetic variation was present within the non-radiate than the radiate morph.

MATERIALS AND METHODS.

Resolution of isozyme variation.

Plants subjected to electrophoretic analysis were raised from seed to the flowering stage in a glasshouse. Seed collected from individuals in the field was sown out on Arthur Bowers universal compost contained in 7cm pots . Seedlings were thinned to two per pot at a height of 1cm, and to prevent competition between plants at later stages, seedlings were subsequently thinned to one per pot at a height of 2-3cm. The plants were illuminated for 16 hours per day using 400 watt mercury vapour lamps and watered when necessary.

Plant material derived from a population polymorphic for capitulum type at Newhaven Road, Edinburgh (grid ref NT 261762) was screened for isozyme variation over a number of enzyme systems using starch gel electrophoresis. Full details of the protocols used in the analysis (previously developed by Ashton - unpublished) are presented in appendix A. Plants were initially screened for 16 different systems. For ten of these, Acid phosphatase (ACP), Phosphoglucose isomerase (PGI), Phosphoglucomutase (PGM), Malate dehydrogenase (MDH), Glutamate oxaloacetate transaminase

(GOT), Malic enzyme (ME), Glucose-6-phosophate dehydrogenase (G-6-PDH), Glyceraldehyde-3-phosphate dehygrogenase (G-3-PD), Malate dehydrogenase (MDH), and Glutamate dehydrogenase (GDH), the material surveyed proved to be monomorphic i.e. showed no variation for the banding patterns that were resolved. For the remaining six systems:- Hexokinase (HEX), Leucine amino peptidase (LAP), Triosephosphate isomerase (TPI), Alcohol dehydrogenase (ADH), Xanthine dehydrogenase (XDH), and Succinate dehydrogenase (SuDH), banding patterns showed weak and variable expression over gels and were not examined further. A system was developed eventually (after Scandalios, 1969) to resolve esterase isozymes from extracts of young developing flower buds that were ground up in an extraction buffer containing 37mg potassium chloride, 10mg magnesium chloride, 10mg EDTA, 50mg PVPP, 0.5ml triton-x-100, and 2ml mercaptoethanol in 50mls of gel buffer (pH 8.3)*. A lithium - borate buffer system was used to resolve the enzyme using 12% starch gels (Sigma starch hydrolysed for electrophoresis). Gels were run at 250V/70mA for 3-4 hours

* Components of electrode and gel buffers and procedures for extraction, loading of extracts, and staining for esterase isozymes are given in appendix A.

or until the front had migrated 8cm towards the anode. Gels were sliced horizotally into four slices and the second and third slices were stained using α- or β-napthyl acetate respectively as substrate (Scandalios 1969).

Crossing programme.

Electrophoretic analysis of plants from the Newhaven road population revealed three zones of staining on gels (a,b and c) within each of which band variation was observed. Full details of the banding patterns observed are described in the results section. In order to determine how many loci controlled the three zones of activity, a large crossing programme was undertaken. Seeds collected from the polymorphic population at Newhaven road, Edinburgh were raised to flowering as described above and the plants screened for esterase isozyme variation. Ten plants that showed variation in banding pattern in one or more of the three zones were selected for use in the crossing programme. These 10 parental lines were crossed in the following combinations :- (i) crosses between individuals showing different banding in zone a, zone b or zone c; (ii) between individuals showing different banding in two zones (a and b, a and c, or b and c); (iii) between individuals showing different banding in all three zones. In all, a total of

twelve different combinations were used. Crosses were performed by emasculating a capitulum on the selected female parent plant. This procedure was carried out by removing the top 1-2mm of the capitulum just prior to opening using a scalpel blade. This removed the fused anther tube present at the top of each floret but left the stigma undamaged as, at this point in development the stigma was situated well below the anthers in the corolla tube. Once emasculated the capitulum was bagged and left for 72 hours for the stigmas to grow through, open out and become receptive. After checking with a hand lens that no pollen had accidently come into contact with the stigmas, the stigmas were pollinated with pollen from the male parent by gently brushing a capitulum from the male parent (bearing freshly produced pollen) across the surface of the stigmas of the female. The capitulum was then rebagged and left to set seed. Each cross was replicated 10 times (five crosses using each plant as the maternal parent) and seed was collected from all crosses noting the amount of seed set per cross. F_1 progenies were raised from three replicate capitula of each reciprocal cross to provide two plants that were scored for esterase genotype. In all F_1's examined, the expected heterozygous banding patterns were obtained except, zone c. The bands in zone c proved difficult to resolve clearly and in many cases the presence/absence of a codominant heterozygous banding pattern was uncertain.

Once the heterozygous genotypes of the F_1 plants had been confirmed, F_1's from seven crosses were selected and selfed (by bagging capitula before anthesis to prevent any cross pollination) to produce an F_2 generation. The F_1's chosen had produced heterozygous banding patterns in at least two of the three zones of activity. One F_1 appeared to exhibit a heterozygous banding pattern in all three zones; (however there was some uncertainty about this due to poor resolution of bands in zone c). Following selfing, 100 seeds were sown out from each of the plants that had shown heterozygous banding patterns in two of the three zones and 200 from that suspected of being heterozygous in all three. These progenies were raised to flowering in trays of Arthur Bowers universal compost under the same conditions as described previously and screened for esterase isozyme variation using starch gel electrophoresis.

Survey of natural populations.

A survey of esterase variation was conducted in nineteen populations of *S.vulgaris* polymorphic for capitulum type and eight populations monomorphic for the non-radiate type to determine genotype frequencies at two esterase loci where alleles had been shown to exhibit codominance, (full details

of the genetic analysis of the banding patterns found in *S.vulgaris* are described in the results section). Table 2.1 (overleaf) lists the populations screened. For all populations except those from Newhailes and Salamander st. (where genotypes were scored directly from material collected in the field), seed was collected from up to 100 individuals per population, sown out and raised to flowering before electrophoresis. For populations monomorphic for the non-radiate morph, approximately 50 individuals were screened per population, while for populations polymorphic for capitulum type, up to 50 individuals of each of the radiate and non-radiate morphs were surveyed.

Table 2.1. Details of populations of *Senecio vulgaris* screened for esterase isozyme variation using starch gel electrophoresis.

Location	Grid Reference	Site description.
(i) Polymorphic populations.		
Edinburgh		
Newhaven road	NT261762	Site used by building contractors to dump soil and rubble together with other demolition waste. Population non-linear and extensive. Sampled June 1985.
Old Craighall	NT335708	Population growing along the banking on either side of a new bypass. Very extensive non-linear population, frequency of radiate plants low. Sampled April 1987.
Leith*	NT268765	Plants sampled from a wide area surrounding the Water of Leith. Plants growing both clumped and linearly along the side of pathways. Sampled April and May 1987.
Newhailes	NT337725	Sites sampled within a recently planted shrubbery. Population extensive and non-linear. Sampled April and May 1987.
Salamander Street	NT276763	Waste site situated in the dock area of Leith. Used for dumping soil and rubble. Non-linear population. Sampled June 1987.
Methil*	NT376995	Polymorphic population collected from the ballast of an infrequently used rail-link to the docks. Sampled July 1988.
Grangemouth		
Devon Street*	NS977814	Open, recently disturbed ground adjacent to the River Carron (Taylor 1984). Sampled May 1988.
Kinneil Tip*	NS913823	Municipal dump on the south side of the river Forth. Population sited in an area where tipping had ceased. Sampled May 1988.
Glasgow		
Kelvingrove*	NS578664	Population growing in clumps situated in neglected shrubberies. Sampled May 1988.
South Street*	NS534671	Population collected linearly for about half a mile. Grid reference represents the centre of the site. Sampled May 1988.

* Material sampled by Ashton.

Table 2.1. Continued.

Location	Grid Reference	Site description.
(i) Polymorphic populations (continued).		
Wrexham		
Ffrith	SJ286556	Linear population growing along the edge of a minor road. Sampled September 1987.
Rhostyllen	SJ312492	Adjacent to new building site. Ground recently disturbed. Population non-linear but quite scattered. Sampled September 1987.
Southsea	SJ306515	Roadside population growing in an area where recent alterations to the road layout had produced open areas on the verge. Non-linear. Sampled September 1987.
Brymbo	SJ296539	Population located on waste ground adjacent to the car park of a local public house and the road side leading up to it. Sampled September 1987.
Mochdre*	SH822781	Roadside linearly distributed population. Sampled October 1986.
Cardiff	ST173733	Disturbed ground beneath site of a new flyover towards the area of a marina and new housing development. Sparse, scattered population. Sampled September 1987.
Birmingham	SP045835	Material supplied By Dr.D.F. Marshall, from the University campus, Birmingham. Sampled July 1988.
St.Helens*	SJ524944	Sampled from waste ground in August 1988.
Netherlands		Supplied by Dr. Koniuscek in 1984 from Rotterdam docks.

* Material sampled by Ashton.

Table 2.1. Continued.

Location	Grid Reference	Site description.
(ii) Monomorphic populations.		
Dundee	NO373295	Waste land adjacent to the railway station and used for dumping rubble and other waste. Sampled April 1988.
Aberffraw	SH366656	Sand dune population. For details see Abbott 1976.
Puffin Island	SH653824	Nitrogen, phosphorus and potassium rich bird sanctuary. For details again see Abbott 1976a,b.
Switzerland		A small number of plants were collected from shrubbery or flowerbed locations in Grindelwald, Interlaken and Basel in August 1987.
Spain		Material collected from waste sites at Matalascanas adjacent to the dunes of the Parque Nacional de Donana, southern Spain in April 1987.
Methil*	NT376995	Car park on recently reclaimed land between the docks and the power station. Sampled July 1988.
Tower Hamlets (London)	TQ349807	Plants collected by Dr. R.J.Abbott from waste sites and roadsides over a wide area. The grid reference represents the centre of the sites sampled in April 1988.
Migvie	NJ437068	Population growing in a garden in Aberdeenshire. Sampled by Dr R.J. Abbott in October 1987.

* Material sampled by Ashton.

RESULTS.

Genetic basis of esterase variation in S.vulgaris.

Banding patterns for esterase were resolved in three zones of each gel (Fig 2.1, Plates 2.1 and 2.2). The bands in each zone were postulated to be the allelic products of a single locus and therefore three loci (one per zone) were assumed to code for esterase. The type and number of genotypes found in F_1 and F_2 families raised from crosses between parental lines of different esterase phenotype are presented in Table 2.2. All crosses between parents with different phenotypes at two of the proposed loci (α and β-1) produced heterozygous F_1 progeny. The third set of bands (at the β-2 locus) resolved poorly and it was difficult to see if heterozygotes were present in the F_1; however some were apparent in the F_1 progeny selected for selfing to produce the F_2. The two alleles (a and b) at the α-est locus segregated in a ratio not significantly different from 1:2:1 (X^2 = 1.208) while at the β-est-1 locus, where three alleles were present (a, b and c), progenies from heterozygotes bearing any two of the three alleles again segregated in ratios not significantly different from 1:2:1 (X^2 = 1.571, 1.778). At both of these loci the alleles were codominant with heterozygotes producing a double banded phenotype

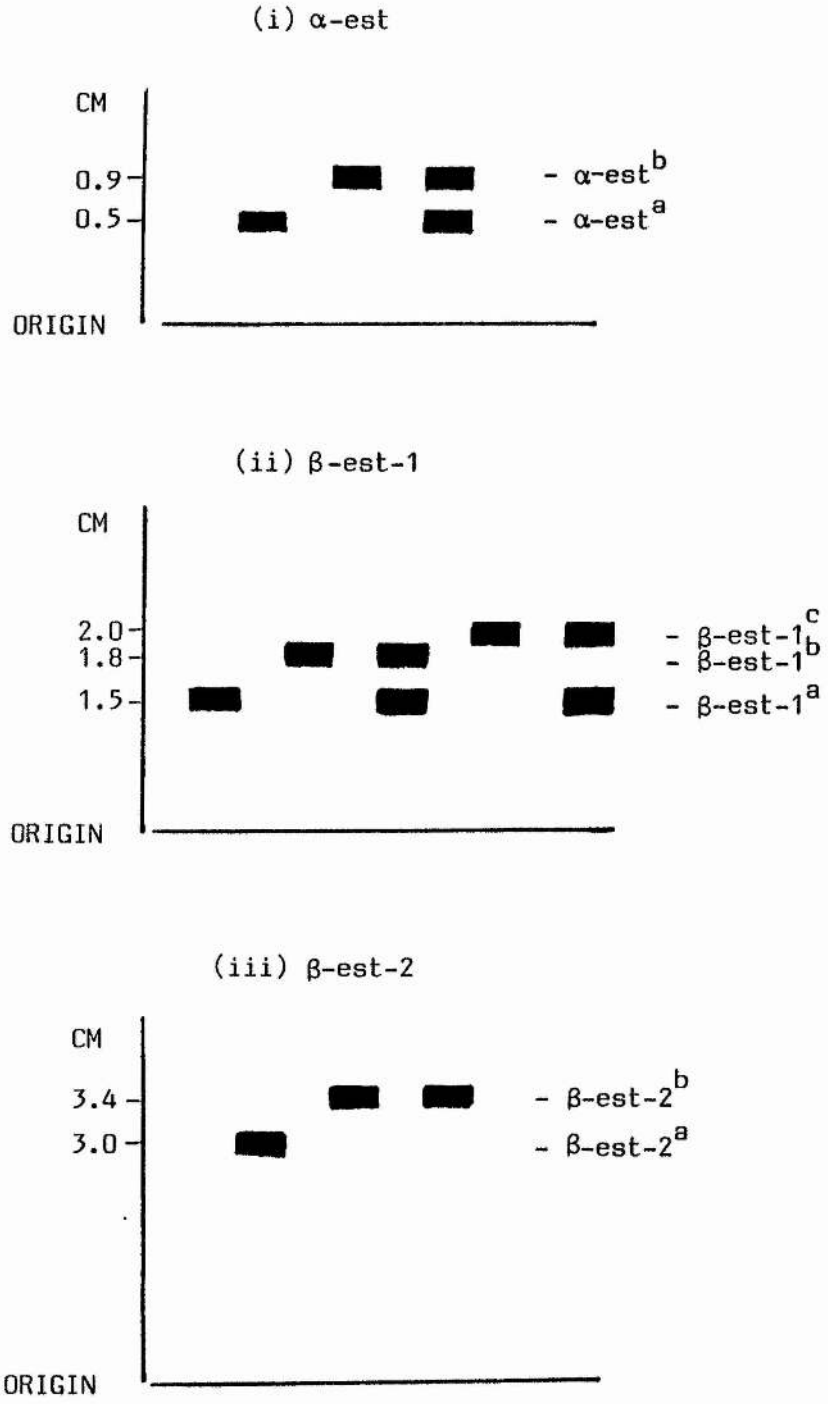


Fig. 2.1 Diagramatic representation of banding patterns obtained at the three putative esterase loci.

Plate 2.1 Banding patterns for esterase resolved in S. vulgaris following staining of a gel using α-napthyl acetate as substrate.

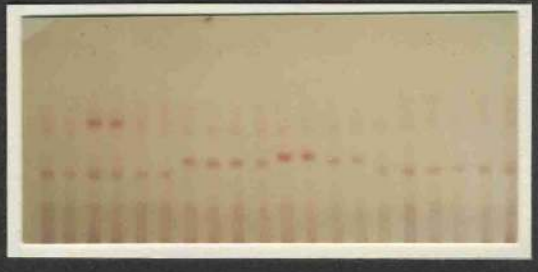


Plate 2.2 Banding patterns for esterase resolved in S. vulgaris following staining of a gel using β-napthyl acetate as substrate.

TABLE 2.2. Inheritance of alleles at the α-est, β-est-1 and β-est-2 loci in *Senecio vulgaris*. X^2 values are presented for tests of goodness of fit to a 1:2:1 ratio at the α-est and β-est-1 loci, and to a 3:1 ratio at the β-est-2 locus.

Parental genotypes					F_2 genotype				
Female		Male	F_1	locus	bb	ba	aa	X^2	
α β_1 β_2		α β_1 β_2							
bb aa bb	x	aa cc aa	Triple het.	α-est	42	61	42	3.65	0.1 < p < 0.2
				β-est-1*	39	77	29	1.94	0.4 < p < 0.5
				β-est-2	112	-	32	0.59	0.7 < p < 0.8
bb bb aa	x	aa aa bb	β-est-2/ β-est-1	β-est-2	72	-	28	0.48	0.4 < p < 0.5
				β-est-1	17	58	28	3.99	0.1 < p < 0.2
bb aa bb	x	bb cc aa	β-est-2/ β-est-1	β-est-2	79	-	21	0.85	0.3 < p < 0.4
				β-est-1*	16	60	24	5.28	.05 < p < 0.1
bb aa bb	x	aa aa aa	β-est-2/ α-est	β-est-2	66	-	25	0.30	0.5 < p < 0.6
				α-est	24	50	25	0.03	.98 < p < .99
aa aa aa	x	bb bb aa	β-est-1/ α-est	β-est-1	21	46	28	1.13	0.5 < p < 0.6
				α-est	21	43	31	2.96	0.2 < p < 0.3
aa cc aa	x	bb bb aa	β-est-1/ α-est	β-est-1	22	45	15	1.97	0.4 < p < 0.5
				α-est	25	43	14	3.14	0.1 < p < 0.2
bb aa aa	x	aa cc aa	β-est-1/ α-est	β-est-1*	27	44	24	0.70	0.7 < p < 0.8
				α-est	21	48	26	0.54	0.7 < p < 0.8

*the alleles at this locus were β-est-1^c and β-est-1^a.
X^2 for 2 degrees of freedom where there are 3 classes of F_2 progeny and 1 degree of freedom where 2 classes of progeny are present.

(i.e.a/b). At the β-est-2 locus, where resolution was weak, crosses between parent plants which produced either a slow a or fast b band yielded an F_1 which sometimes appeared to have a double banded phenotype a/b. These F_1 individuals, on selfing produced plants of either the fast or slow phenotype in a 3:1 ratio in the F_2. Double banded individuals occurred only at a very low frequency (approx 1.5%). It is concluded that in most heterozygotes the fast allele b is dominant to the slow allele a at the β-est-2 locus.

A linkage analysis was carried out between the three esterase loci and between the esterase and ray floret loci to determine if (i) the three esterase loci were linked; (ii) the esterase loci were linked to the ray floret locus. The analysis was conducted using the LINKAGE-1 program (Suiter *et al* 1983) and results are given in Table 2.3. The analysis shows that the α-est and β-est-1 loci are loosely linked; however the β-est-2 locus segregates independently from these two loci. Of particular importance was the finding that none of the esterase loci are linked to the ray floret locus of *S.vulgaris*.

Table 2.3. Results of a linkage analysis conducted between the three esterase loci and the ray floret locus.

Loci	X^2	df	p	r	SE
α-est/ β-est-2	1.731	2	0.421	0.491	0.065
α-est/ β-est-1	41.866	4	0.000	0.296	0.029
α-est/ β-est-1*	15.289	4	0.004	0.324	0.043
β-est-1/ β-est-2	1.702	2	0.427	0.415	0.059
β-est-1*/ β-est-2	1.644	2	0.439	0.413	0.060
α-est/ ray	5.263	4	0.261	0.410	0.054
β-est-2/ ray	0.265	2	0.876	0.460	0.072
β-est-1/ ray	4.11	4	0.390	0.499	0.057

r = recombination fraction.
SE = standard error on recombination fraction.

* the alleles at this locus were est^c and est^a.

Survey of natural populations of S. vulgaris.

(i) Single locus variation.

Table 2.4 shows that in most of the populations surveyed that were polymorphic for capitulum type, the α-est^b allele predominates at the α-est locus. In six populations, both the radiate and non-radiate morphs were fixed for this allele, while in another six populations, the allele was fixed in the radiate morph and occurred at high frequency in the non-radiate morph. Only among non-radiate plants in two Edinburgh populations (at Newhailes and Salamander street) was the α-est^b allele present at low frequency.

In all populations the frequency of heterozygotes at the α-est locus among radiate plants was low, heterozygous individuals occurring in only four of the nineteen populations surveyed. In contrast relatively high frequencies of heterozygotes at the α-est locus were found for the non-radiate morph at the Salamander street (0.279) and Old Craighall (0.294) sites. In addition, five out of the remaining seventeen populations surveyed exhibited low levels of heterozygotes at the α-est locus among non-radiate plants.

Table 2.5 shows the corresponding results for genotypic and

Table 2.4 Genotype and allele frequencies at the α-est locus among plants of the radiate and non-radiate morphs sampled from populations of *S.vulgaris* polymorphic for capitulum type.

Population.	(N)		Genotype bb	ba	aa	Allele b	a
EDINBURGH							
Newhaven rd.	(44)	NR	0.820	0.040	0.140	0.840	0.160
	(40)	RR	0.950	-	0.050	0.950	0.050
Salamander st.	(43)	NR	0.186	0.279	0.535	0.325	0.675
	(45)	RR	0.800	0.090	0.110	0.845	0.155
Leith	(50)	NR	0.620	0.040	0.340	0.640	0.360
	(70)	RR	0.440	0.060	0.500	0.475	0.525
Old Craighall	(17)	NR	0.648	0.294	0.058	0.795	0.205
	(13)	RR	0.923	-	0.077	0.923	0.077
Newhailes	(50)	NR	0.020	-	0.980	0.020	0.980
	(50)	RR	1.000	-	-	1.000	-
METHIL	(32)	NR	0.720	0.090	0.190	0.765	0.235
	(36)	RR	1.000	-	-	1.000	-
GRANGEMOUTH							
Devon st.	(25)	NR	0.840	-	0.160	0.840	0.160
	(25)	RR	0.960	0.040	-	0.980	0.020
Kinneil tip	(25)	NR	0.560	0.040	0.400	0.580	0.420
	(25)	RR	0.960	-	0.040	0.960	0.040
GLASGOW							
Kelvingrove	(25)	NR	1.000	-	-	1.000	-
	(25)	RR	1.000	-	-	1.000	-
South st.	(25)	NR	1.000	-	-	1.000	-
	(25)	RR	1.000	-	-	1.000	-
WREXHAM							
Ffrith	(37)	NR	1.000	-	-	1.000	-
	(16)	RR	1.000	-	-	1.000	-
Rhostyllen	(28)	NR	0.960	-	0.040	0.980	-
	(14)	RR	1.000	-	-	1.000	-
Southsea	(65)	NR	0.490	0.030	0.480	0.505	0.495
	(32)	RR	1.000	-	-	1.000	-
Brymbo	(38)	NR	0.685	0.020	0.295	0.695	0.305
	(21)	RR	0.850	0.050	0.100	0.875	0.125
MOCHDRE	(45)	NR	0.620	-	0.380	0.620	0.380
	(40)	RR	1.000	-	-	1.000	-
CARDIFF	(33)	NR	0.940	-	0.060	0.940	0.060
	(27)	RR	1.000	-	-	1.000	-
BIRMINGHAM	(50)	NR	1.000	-	-	1.000	-
	(50)	RR	1.000	-	-	1.000	-
ST. HELENS	(40)	NR	1.000	-	-	1.000	-
	(40)	RR	1.000	-	-	1.000	-
NETHERLANDS	(20)	NR	1.000	-	-	1.000	-
	(20)	RR	1.000	-	-	1.000	-

Table 2.5 Genotype and allele frequencies at the β-est-1 locus among plants of the radiate and non-radiate morphs sampled from populations of *S.vulgaris* polymorphic for capitulum type.

Population.	(N)		Genotype bb	ba	aa	Allele b	a
EDINBURGH							
Newhaven rd.	(44)	NR	0.120	0.020	0.860	0.130	0.870
	(40)	RR	0.020	–	0.980	0.020	0.980
Salamander st.	(43)	NR	0.442	0.023	0.535	0.453	0.547
	(45)	RR	0.800	–	0.200	0.800	0.200
Leith	(50)	NR	0.620	0.040	0.340	0.640	0.360
	(70)	RR	0.440	0.070	0.490	0.475	0.525
Old Craighall	(17)	NR	0.294	–	0.706	0.294	0.706
	(13)	RR	–	–	1.000	–	1.000
Newhailes	(50)	NR	0.420	0.120	0.460	0.480	0.520
	(50)	RR	0.060	0.020	0.920	0.070	0.930
METHIL	(32)	NR	0.030	–	0.970	0.030	0.970
	(36)	RR	–	–	1.000	–	1.000
GRANGEMOUTH							
Devon st.	(25)	NR	–	–	1.000	–	1.000
	(25)	RR	–	–	1.000	–	1.000
Kinneil tip	(25)	NR	–	–	1.000	–	1.000
	(25)	RR	–	–	1.000	–	1.000
GLASGOW							
Kelvingrove	(25)	NR	0.280	–	0.720	0.280	0.720
	(25)	RR	–	–	1.000	–	1.000
South st.	(25)	NR	0.040	–	0.960	0.040	0.960
	(25)	RR	–	–	1.000	–	1.000
WREXHAM							
Ffrith	(37)	NR	–	–	1.000	–	1.000
	(16)	RR	–	–	1.000	–	1.000
Rhostyllen	(28)	NR	–	–	1.000	–	1.000
	(14)	RR	–	–	1.000	–	1.000
Southsea	(65)	NR	0.020	–	0.980	0.020	0.980
	(32)	RR	0.030	–	0.970	0.030	0.970
Brymbo	(38)	NR	–	–	1.000	–	1.000
	(21)	RR	–	–	1.000	–	1.000
MOCHDRE	(45)	NR	–	–	1.000	–	1.000
	(40)	RR	0.625	0.025	0.350	0.637	0.363
CARDIFF	(33)	NR	0.730	–	0.270	0.730	0.270
	(27)	RR	0.070	0.040	0.890	0.090	0.910
BIRMINGHAM	(50)	NR	0.020	–	0.980	0.020	0.980
	(50)	RR	0.020	0.100	0.880	0.070	0.930
ST. HELENS	(40)	NR	0.350	0.275	0.325*	0.520	0.460
	(40)	RR	–	–	1.000	–	1.000
NETHERLANDS	(20)	NR	–	–	1.000	–	1.000
	(20)	RR	–	–	1.000	–	1.000

*The St. Helens population contain two individuals with an est bb/estcc banding pattern (frequency = 0.05). Therefore the estcc allele has a frequency of 0.02 in this population (Ashton per.comm).

allelic variation at the β-est-1 locus in polymorphic populations. At this locus, the β-est-1^a allele tended to predominate in both morphs. Again in six populations, both morphs were fixed for the β-est-1^a allele. In five other populations the radiate morph was fixed for the same allele while in another population it was the non-radiate morph that was fixed. Only among radiate plants at the Salamander street and Mochdre sites, and non-radiate plants at Cardiff and Leith was the β-est-1^b allele the more common of the two alleles.

At St. Helens where the radiate morph was fixed for β-est-1^b, the β-est-1^b and β-est-1^a alleles occurred at approximately equal frequency in the non-radiate morph. An interesting feature of this population was that a third allele, the β-est-1^c allele, was also present in the non-radiate morph albeit at a frequency of 0.02. Only in the population from St.Helens was the β-est-1^c allele found.

The frequency of heterozygotes at the β-est-1 locus was generally low in both morphs (Table 2.5). Only four of the nineteen populations surveyed contained some radiate individuals producing heterozygous banding patterns; and only five populations contained some non-radiate plants that were heterozygous at this locus. In the St.Helens population however, heterozygotes were present at a frequency of 0.275

among plants of the non-radiate morph.

Allele frequencies at the α- and β-est-1 loci in populations monomorphic for the non-radiate morph are presented in Table 2.6. Again one allele at each locus was found to predominate over the populations surveyed. Given that a population is defined as polymorphic when the rare allele is present at a frequency of > 1% then six of the eight populations surveyed were polymorphic at the α-est locus and four were polymorphic at the β-est-1 locus. In five populations, the α-est^{b} allele predominated, being fixed in two populations and present at a level greater than 90% in another three. Only in two of the eight populations examined was the α-est^{a} allele the more common allele. At the β-est-1 locus, two populations were fixed for the β-est-1^{a} allele, two were fixed for the β-est-1^{b} allele, and in the four remaining populations surveyed, the β-est-1^{a} allele predominated in three while the β-est-1^{b} allele was the more common in one.

In general, the frequency of heterozygotes at both the α- and β-est-1 loci was low in the populations surveyed. However, in the population from Migvie, Aberdeenshire, a surprisingly high frequency of heterozygotes (0.58) was recorded at the α-est locus (Table 2.6). To determine whether these heterozygotes were of the 'fixed' type or

Table 2.6. Genotype and allele frequencies at the α-est and β-est-loci among plants sampled from populations of *S.vulgaris* monomorphic for the non-radiate morph.

Population.	(N)		Genotype bb	ba	aa	Allele b	a
(i) α-est							
Dundee	(30)	NR	0.935	0.033	0.032	0.952	0.048
Aberfraw	(25)	NR	0.960	0.040	-	0.980	0.020
Puffin Is.	(25)	NR	0.970	0.030	-	0.985	0.015
Switzerland	(15)	NR	1.000	-	-	1.000	-
Spain	(20)	NR	0.200	-	0.800	0.200	0.800
Methil	(25)	NR	1.000	-	-	1.000	-
Tower Hamlets	(45)	NR	-	0.020	0.980	0.010	0.990
Migvie	(50)	NR	0.300	0.580	0.010	0.590	0.300
(ii) β-est-1							
Dundee	(30)	NR	-	0.035	0.965	0.018	0.982
Aberfraw	(25)	NR	1.000	-	-	1.000	-
Puffin Is.	(25)	NR	1.000	-	-	1.000	-
Switzerland	(15)	NR	0.930	-	0.070	0.930	0.070
Spain	(20)	NR	0.200	-	0.800	0.200	0.800
Methil	(25)	NR	1.000	-	-	-	1.000
Tower Hamlets	(45)	NR	-	-	1.000	-	1.000
Migvie	(50)	NR	0.060	-	0.940	0.060	0.940

segregated freely on selfing, selfed progenies of plants showing the double banded heterozygous phenotype were tested for genotype at the α-est locus. All the progeny bred true to parental type and it was concluded that these individuals were indeed fixed heterozygotes for the α-est^{a} and α-est^{b} alleles. The population at Migvie also contained a few individuals homozygous for a very slow allele at the α-est locus (α-est^{1a}) This allele was not found in any other population surveyed.

(ii) Two locus variation.

The frequencies, over all monomorphic and polymorphic populations surveyed, of the various two-locus genotypes (based on α-est and β-est-1 variation) are given in Table 2.7. Table 2.7 highlights the fact that one allele predominates at each of these two loci. In both polymorphic and monomorphic populations, the α-est^{b} α-est^{b} / β-est-1^{a} β-est1^{a} two-locus genotype is, by far the most common, with 83% of radiates and 61% of non-radiates in polymorphic populations being of this type, and 50% of non-radiate plants in monomorphic populations exhibiting this same genotype. The next most common two-locus genotype is that homozygous for the 'a' allele at each locus.

Table 2.7. Multilocus genotype frequencies. Frequencies of two locus genotypes in populations monomorphic for the non-radiate morph and in radiate and non-radiate morphs in populations polymorphic for capitulum type.

α-est/ β-est-1	monomorphic	polymorphic	
		NR	RR
bb/bb	0.146	0.113	0.054
bb/ba	0.005	0.028	0.029
bb/aa	0.498	0.614	0.826
ba/bb	0.011	0.020	-
ba/ba	-	-	0.002
ba/aa	0.005	0.016	0.016
aa/bb	-	0.026	0.002
aa/ba	0.005	0.006	0.002
aa/aa	0.330	0.177	0.069

DISCUSSION.

Esterase isozyme variation.

Isozymes offer a powerful tool for providing codominant alleles at single marker loci that can be used in many ways for the genetic analysis of plant populations (Brown 1979; Brown and Weir 1983; Clegg 1980; Gottleib 1981; Rick, Forbes and Tanksley 1979; Rick and Tanksley 1981). Thus, the most important outcome of the research reported in this chapter was the resolution of genetic variability within and between the radiate and non-radiate morphs of *Senecio vulgaris* at two esterase loci. Without resolving this variation, it would not have been possible to conduct some of the research reported in chapter 4 of this thesis concerning estimation of intramorph outcrossing rates (outcrossing occuring between individuals of the same capitulum morph).

The allelic variation at the α- and β-est loci was shown to be inherited in a mendelian manner. The alleles present at the α- and β-est-1 loci were codominant and therefore, individuals producing a double banded heterozygous phenotype at each of these loci could readily be identified from homozygotes (Fig.2.1). Neither the α-est nor the β-est-1 loci were linked to the ray floret locus in *S.vulgaris*.

Therefore both of these isozyme loci can be regarded as suitable for use as markers to estimate outcrossing within morphs and in conjunction with the ray floret locus, can be used also to measure levels of outcrossing between the radiate and non-radiate morphs of *S.vulgaris*.

The inheritance of variants at the β-est-2 locus (Table 2.2) was of interest in that there appeared to be a dominance/recessive relationship between the alleles at this locus, with the β-est-2^b allele being dominant to the β-est-2^a allele. This dominance was not always complete however, and a small number of two banded heterozygotes (@ 1.5%) occurred among the selfed progeny of F_1 individuals produced from crosses between individuals homozygous for the β-est-2^b allele and others homozygous for the β-est-2^a allele. The rare occurrence of the two banded heterozygote phenotype may be explained in two ways: (a) expression of the alternative alleles at the β-est-2 locus is variable due to incomplete dominance and as a result, two-banded heterozygotes are occasionally detected; (b) the techniques of starch gel electrophoresis are not sensitive enough to detect reliably the two banded heterozygous phenotype at the β-est-2 locus. The latter explanation is supported by the fact that the product of the est^a allele at the β-est-2 locus tends to stain very lightly in homozygous individuals and therefore is itself difficult to resolve. The problem of

staining would be increased in the heterozygote where only half the amount of β-est-2^a product is expected.

In a study of the putative progenitors of the D-genome in hexaploid wheat, Nakai (1979) reported a dominance/ recessive relationship between two alleles at the Est-5 locus. However, later Lagudah and Halloran (1989) using isoelectric focussing clearly resolved the two banded heterozygote from a cross between parental lines homozygous for each of the two alleles in question. On selfing, these heterozygotes segregated in a ratio not significantly different from the expected 1:2:1. Based on this finding in wheat, in would be worth applying isoelectric focussing to the variants at the β-est-2 locus of *Senecio vulgaris* to determine whether the dominance exhibited is indeed an artifact of the technique of starch gel electrophoresis that has been used.

To summarise, the analysis of esterase isozyme variation in *S.vulgaris* has resolved three polymorphic esterase loci. The alleles present at the α-est and β-est-1 loci are codominant, segregating in a ratio not significantly different from 1:2:1 in the F_2 generation. These two loci are loosely linked but segregate independently from the ray floret locus in *S.vulgaris*. There appears to be a dominance/recessive relationship between the alleles at the

β-est-2 locus. This third locus is not linked to either the α-est, β-est-1 or the ray floret locus of *S.vulgaris*.

Esterase variation in natural populations.

The effects of the breeding system on the amount and organisation of genetic variation within and between populations has been treated extensively within mathematical and conceptual contexts by Wright (1931,1932,1946,1964); Mather (1943); Stebbins (1950,1957,1958); Allard *et al.* (1968) and others. These workers have argued that predominantly self-fertilising species (such as *Senecio vulgaris*) will contain much less genetic variation than cross-fertilising species all other factors being equal. However, Allard (1970,1975) and Jain (1976) have pointed out that this is somewhat of an oversimplification and that a fixed pattern of variation is not confined to one group of species or the other. Evidence in support of the latter, comes from several empirical studies. For example Arroyo (1975) reported that allogamous and autogamous species of *Limnanthes* contained similar levels of allozymic variation within and between populations while Hillel *et al* (1973) found that inbreeding species of *Triticum* possessed greater total genetic variability as well as within family variability compared to outbreeding species of the same genus. On the other hand, a comparison by Layton and

Ganders (1984), of genetic variation based on isozyme variation at 12 different loci of *Plectritis brachystemon* (autogamous) and *P. congesta* (predominantly allogamous) showed that the outcrosser *P. congesta* maintained much more genetic variation within populations. This situation in *Plectritis* now appears to be representative of a general one and recent comparisons made by Gottlieb (1981) and Loveless and Hamrick (1984) over a number of plant species, have indeed confirmed that overall, outcrossers contain more total genetic variation than selfers.

The results of the survey of variation at the α-est and β-est-1 loci in natural populations of *S.vulgaris* has indicated that little genetic variability is present within most populations. At each locus one allele tends to predominate. The expected consequence of self-fertilisation on the genetic structure of a plant population is to reduce heterozygosity within that population (Jain 1976). Thus, the lack of genetic variability at the esterase loci found within natural populations of *S.vulgaris* is expected given that repeated generations of selfing will produce homozygous lines of plants which may, in turn, become fixed in a population either through chance (e.g. founder effects) or selection.

In a colonising species such as *S.vulgaris*, genetic

bottlenecks following colonisation are likely to have important effects on the level of gene diversity in natural populations. As colonisation often involves just a few founding individuals, only a limited sample of genetic variability from the source population is likely to be present in the founding group (see Barrett 1982 - review). Table 2.7 shows that in the populations of *S.vulgaris* surveyed, out of nine possible two-locus genotypes for α- and β-est-1 only three commonly occurred. This was true both for populations monomorphic for the non-radiate morph and for populations polymorphic for capitulum type. In *S.vulgaris* the $\alpha\text{-est}^{b}$ allele predominates at the α-est locus and the $\beta\text{-est-1}^{a}$ allele at the β-est-1 locus. A similar situation to that found in *S.vulgaris* for the two polymorphic esterase loci was found in Californian populations of *Avena barbata* where particular alleles were favoured at four esterase loci (Allard *et al* 1972).

Despite the reduced levels of genetic variation in nearly all populations of *S.vulgaris* surveyed it was apparent that in populations polymorphic for capitulum type the non-radiate morph contained more variabilty than the radiate morph at both esterase loci (Tables 2.4 and 2.5). This result is somewhat surprising as the radiate morph is known to exhibit greater rates of intermorph outcrossing than the non-radiate morph in natural populations polymorphic for

capitulum type (Marshall and Abbott 1982, 1984a) and would be expected to contain more genetic variation (Gouyon and Vernet 1982). Reasons why the opposite has been found are not obvious; however, it is feasible that despite the outcrossing which has been recorded between the radiate and non-radiate morphs in natural populations, the two morphs remain effectively isolated from each other due to low fitness of the heterozygous intermediate phenotype. There is some evidence to suggest that this is the case in populations polymorphic for capitulum type.

Marshall (1982), found that in all populations of *Senecio vulgaris* he studied which were polymorphic at the ray floret locus, there was a deficiency of heterozygotes relative to expected values based on the measured

Table 2.8 Observed and expected values of Wrights fixation index (F) together with values for delta F for five polymorphic populations of *Senecio vulgaris*.

Population	Year	F(obs)	F(exp)	delta F
Leith	1978	0.950	0.872	0.078
Newhaven road	1978	0.980	0.911	0.069
Newhaven road	1979	0.964	0.815	0.149
Newhaven road	1980	0.972	0.949	0.028
Leeds	1979	0.951	0.931	0.020
Cardiff	1979	0.985	0.922	0.063
Rhos.	1979	0.829	0.684	0.245

From Marshall (1982) Studies on the breeding system of *Senecio vulgaris*. PhD thesis University of St.Andrews.

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 in these populations, (Table 2.8). In all populations the observed value was larger than expected and therefore delta F was positive in all cases. Brown (1979) has described several factors which may affect delta F values, some of which may be important in causing positive delta F values in populations of *S.vulgaris*. Of these, negative heterosis may be of principal importance. If the radiate allele originated through introgression from *Senecio squalidus* into *S.vulgaris* (Ingram, Weir and Abbott,1980; Marshall and Abbott,1980) it is possible that heterozygotes are genetically imbalanced and therefore of reduced fitness. Richards (1975) demonstrated that heterozygotes at the ray floret locus had slower growth rates than either homozygote. In addition, Ross and Abbott (1987) reported the intermediate morph to exhibit a lower fitness than either homozygote in one Edinburgh population based on measures of fertility components. If, as this evidence suggests, the radiate and non-radiate morphs are genetically isolated from each other this might explain the reduced level of genetic variation in the radiate morph which is of recent origin and therefore, likely to be in possession of only a small sample of the variation present in the non-radiate morph.

To conclude, the major objective in surveying isozyme

variation in populations of *S.vulgaris* that were either monomorphic or polymorphic for capitulum type, was to find a suitable isozyme marker for use in estimating outcrossing within and between the radiate and non-radiate morphs. This objective was achieved. Using starch gel electrophoresis, genetic variation was detected at three esterase loci. The alleles at two of these loci (the α-est and β-est-1 loci) were shown to be codominant, with progeny of selfed heterozygotes segregating in a ratio not significantly different from 1:2:1 in the F_2 generation. Both loci segregated independently from the ray floret locus and are therefore suitable for estimating outcrossing both within and between the radiate and non-radiate morphs of *S.vulgaris*.

CHAPTER 3.

POLLINATOR BEHAVIOUR IN MONOMORPHIC AND POLYMORPHIC STANDS Of *SENECIO VULGARIS*.

INTRODUCTION.

The behavioural characteristics of insect pollinators are known to have a profound influence on the mating system of insect pollinated plants (Biezychudek 1981; Lee and Hartgerink 1986; Waser and Price 1983; Wyatt 1983; Stanton, Snow and Handel 1986). The disperal of male gametes (pollen) between such plants is largely controlled by the vectors utilised in transferring pollen from anther (male) to stigma (female), which in turn affects the paternity of the progeny produced.

Pollinators often exhibit a preference when presented with a choice of flower colour or morphology within a species. For example, in *Ipomoea purpurea* a white-flowered morph was found to be undervisited by pollinators relative to a blue-flowered morph (Ennos, 1981; Ennos and Clegg, 1983; Brown and Clegg, 1984; Schoen and Clegg, 1985;). Such a preference may be constant or frequency dependent. In *Ipomoea purpurea* Epperson and Clegg (1987) showed that while the white morph was undervisited when at low frequency this was not the case when it occurred at high frequency in a population. If constancy prevails at all frequencies, then it can result in 'stabilising' selection for the preferred morph. Waser and Price (1981, 1983) have investigated the effect of pollinator

choice on stabilising selection for the blue colour flower morph of *Delphinium nelsonii* in populations which also contained a rare albino flower morph. They found that pollinators visited the blue morph, from which it was easier to extract nectar, more frequently than the albino which had inferior honey guides with the result that seed set in the white-flowered morph was reduced by 20% compared to that of the blue-flowered morph.

In populations of *Senecio vulgaris* that are polymorphic for capitulum type, radiate plants exhibit significantly greater female outcrossing rates than non-radiate plants (Marshall and Abbott, 1982, 1984a). This conclusion has been based on estimates of outcrossing between morphs. Marshall and Abbott used the two alleles at the ray floret locus as markers in estimating outcrossing rates of the two capitulum morphs and therefore were able to identify crosses between but not within morphs. In one of two populations subjected to further study by Marshall and Abbott (1984b) it was established that the greater female outcrossing of the radiate compared to the non-radiate morph was entirely accounted for by the greater outcrossing of the pistillate (female) ray florets relative to hermaphrodite disc florets. Within capitula of radiate plants in this population, disc florets outcrossed at a rate equivalent to that recorded for disc florets of non-radiate plants. The greater female outcrossing rate of the ray

florets can be attributed to the occurrence of functional protogyny within radiate capitula (Burtt, 1977). The whorls of florets within a capitulum of *S.vulgaris* open serially in a centripetal fashion. Thus in radiate capitula, the outer whorl is composed entirely of male sterile ray florets which are the first to open. Each radiate capitulum is therefore functionally protogynous being female until the inner whorls of hermaphrodite disc florets open.

In a second population studied by Marshall and Abbott (1984b), the difference between the two morphs in outcrossing rate was only partially explained by the greater outcrossing of the ray florets. In this population, though disc florets of radiate plants outcrossed at lower rates than ray florets, their outcrossing rate was nonetheless significantly greater than that of equivalent florets in non-radiate capitula. Marshall and Abbott (1984b) suggested two possible causes for the higher outcrossing rate of disc florets of radiate plants relative to non-radiate plants in this population:- (a) Disc florets of radiate plants may exhibit some form of cryptic self-incompatibility mechanism that inhibits the germination and/or pollen tube growth of self-pollen. Evidence against this has since been presented by Warren *et al* (1988) who found that cross pollen (radiate or non-radiate) germinated in greater quantities than self pollen on stigmas of both radiate and non-radiate individuals, and there appeared to be

no difference between morphs in the expression of cryptic self-incompatibility in disc florets. (b) Radiate plants are more attractive to pollinators in polymorphic populations.

In regard to the latter, Marshall and Abbott postulated that one effect of the radiate morph being preferentially visited relative to the non-radiate morph might be to cause pollinators to move more frequently from non-radiate to radiate plants than in the opposite direction. However, simple preference for the radiate morph held at a constant level throughout the period that a pollinator forages a polymorphic population would not in itself lead to a higher frequency of pollinator flights between morphs in one direction rather than the other. Polarised movement from the non-radiate to the radiate morph would occur only if preference for the radiate type increases as a pollinator moves from the initial to subsequent plants during a flight sequence.

Abbott and Irwin (1988) recently showed that in polymorphic stands of *Senecio vulgaris* in which the two morphs were present at equal frequency, radiate plants were indeed more attractive to pollinators and moreover, pollinators were biased in their movements between morphs with flights from non-radiate to radiate plants occurring more frequently than those in the opposite direction. In polymorphic stands where

the two morphs were present at equal frequency, pollinators made 72% of their visits to radiate plants. There was also a distinct preference for transition flights between radiate plants, followed by non-radiate to radiate and then radiate to non-radiate plants. Transitions between non-radiate plants were very infrequent. Based on these findings it would be expected that most outcrossing in polymorphic populations of *S.vulgaris* would occur between plants of the radiate morph (intramorph outcrossing), while intramorph crossing between non-radiate plants should be negligible. In terms of intramorph crossing, therefore, the radiate morph is expected to donate and receive more cross pollen than the non-radiate morph and therefore show significantly greater levels of male as well as female intramorph outcrossing. In regard to intermorph outcrossing however, a different situation might be anticipated. Here the greater frequency of flights between morphs in the non-radiate to radiate direction relative to flights from radiate to non-radiate is likely to favour the radiate morph as a recipient of cross pollen and therefore increase its female intermorph outcrossing rate relative to the non-radiate morph. At the same time however, the non-radiate morph would be favoured as a pollen donor and thus be expected to show a higher rate of male intermorph outcrossing than the radiate morph.

The results obtained by Abbott and Irwin (1988) are of

particular interest because of the way pollinator movement may affect outcrossing rate (as indicated above). However, their study was conducted exclusively in artifical stands in which the radiate and non-radiate morphs of *S.vulgaris* were present at equal frequency. In view of the possibility that morph preference may be frequency dependent (see Epperson and Clegg 1987) it is necessary to broaden the study to investigate pollinator movement and the relative attractiveness of the two morphs in stands in which the frequency of radiate and non-radiate plants is varied. To this end several experiments have been conducted aimed at : (i) observing the pattern of pollinator visitation and movements in stands of *S.vulgaris* that varied in the relative frequency of the radiate and non-radiate morphs; and (ii) measurement of the outcrossing rates of the two morphs in these stands and relating these estimates to the observed foraging pattern of pollinators. In this chapter the pattern of pollinator behaviour observed in the stands is described and discussed while estimates of morph outcrossing rates are considered in detail in chapter 4.

MATERIALS AND METHODS.

Pollinator identification.

Pollinators were caught for subsequent identification while foraging three different natural populations of *Senecio vulgaris*. One population, from a disused airfield at Crail, N.E. Fife (NO632089) was monomorphic for the non-radiate morph and consisted of a linear population of plants growing along with the related *Senecio jacobea* amongst clover and grass species. This was the site at which previous pollinator studies on artifically constructed stands had been conducted by Abbott and Irwin (1988). Two other populations from which pollinators were collected were located respectively at Newhailes (NT337725) and Leith (NT268765) Edinburgh (populations previously surveyed for esterase isozyme variation in chapter 2). Each of these populations was polymorphic for capitulum type. The population at Newhailes occurred on a neglected shrubbery while plants of the Leith population were dispersed over a large area in Leith (the grid reference representing the centre of the site). At Leith most pollinators were collected from a building site adjacent to the Water of Leith where vegetation was sparse and *S.vulgaris* and *S.squalidus* were the predominant species.

In addition to collecting pollinators from wild populations of *S.vulgaris*, pollinators which visited the artifical stands (monomorphic and polymorphic) constructed on the experimental plot at the Botanic Gardens, St. Andrews during August and September 1988 were also collected (see below).

Pollinators were captured using a butterfly net and transferred to small specimen bottles each containing a square of filter paper soaked in ethyl acetate. Exposure to ethyl acetate quickly killed the insects which were then removed and mounted in specimen boxes ready for identification.

Pollinator behaviour.

Seed from plant material derived from the population of *S.vulgaris* at Newhailes, Edinburgh (NT377725), was sown out on Arthur Bowers universal compost in 7.0cm pots in a glasshouse at the Botanic gardens, St.Andrews, in June 1988. Radiate and non-radiate lines of plants homozygous for either the β-est-1^b allele, or the β-est-1^a allele, were raised to flowering. Seedlings were thinned to two per pot at a height of 1cm and potted on into 11.5cm pots at a height of approximately 3cm. Plants were illuminated for 16 hours per day using 400 watt mercury vapour lamps and

watered when necessary.

Using these plants, five different plot designs were constructed (see Table 3.1).

Table 3.1 Artifical plot designs constructed at the Botanic Gardens St.Andrews.

	Plot Design.
(i)	Monomorphic non-radiate.
(ii)	Monomorphic radiate.
(iii)	0.5 : 0.5 radiate : non-radiate.
(iv)	0.8 : 0.2 radiate : non-radiate.
(v)	0.2 : 0.8 radiate : non-radiate.

Within each plot 20 plants were set out in a 5 x 4 rectangular grid with 1m spacing between nearest neighbours (Fig.3.1). All plants used were doubly homozygous for alleles at the codominant β-est-1 locus and the ray floret locus.

In plots monomorphic for capitulum type (designs (i) and (ii) in Table 3.1 above) 10 plants were homozygous for the β-est-1^a allele and 10 homozygous for the β-est-1^b allele. Genotypes were randomly distributed among the 20 positions within the plot.

In all polymorphic plots the plants were chosen so that there were 10 β-est-$1^{a\,a}$ and 10 β-est-$1^{b\,b}$ homozygotes present

Fig.3.1. 0.5:0.5 radiate:non-radiate polymorphic stand. Plants randomly arranged in a 5 x 4 grid at intervals of 1m.

R	N	R	N	N
R	R	N	R	R
N	R	R	R	N
N	R	N	N	N

Fig.3.2. 0.2:0.8 radiate:non-radiate polymorphic stand. Plants placed 1M apart with the 4 radiate plants in positions 7,9,12 and 14 in the 5 x 4 grid.

N	N	N	N	N
N	R	N	R	N
N	R	N	R	N
N	N	N	N	N

in each plot. In plots where the radiate and non-radiate morphs were present at equal frequency (design (iii) - Table 3.1) 5 plants of each capitulum morph were homozygous for the β-est-1^a allele while 5 were homozygous for the β-est-1^b allele. Plants were randomly distributed throughout the plot with respect to genotype at both the ray floret and the β-est-1 loci (see Fig.3.1). In designs (iv) and (v) where one capitulum morph was present in a majority (80%), the four 'minority' plants were placed in positions 7,9,12 and 14 in the plot (See Fig 3.2). With respect to esterase genotype, 8 plants of the 'majority' capitulum morph were homozygous for one of the two alternative alleles at the β-est-1 locus while the remaining 8 plants were homozygous for the other allele. Similarly in the 'minority' group, half the plants were homozygous for β-est-1^a and the other half were homozygous for β-est-1^b. In the plots, plants of the 'majority' morph were randomly distributed with respect to genotype at the β-est-1 locus over the 16 positions indicated in Fig 3.2. 'Minority' plants were arranged in two ways so that the nearest neighbour plants in positions 7 and 12, and 9 and 14 were homozygous for the same or alternative alleles at the β-est-1 locus (referred to in chapter 4 as designs A and B).

Within each plot the number of open capitula on each plant was trimmed to 5 or 6 per plant so that for each experiment

there were between 100 and 120 capitula available for visitation within the plot. The five different experimental designs listed in Table 3.1 were monitored in August and September 1988 such that pure stands of the radiate and non-radiate morphs were each observed on two separate days. Pollinator behaviour in design (iii) (0.5:0.5 radiate:non-radiate) was recorded on four different days, and in designs (iv) and (v) (0.8:0.2 radiate:non-radiate and 0.2:0.8 radiate:non-radiate - Table 3.1) on each of two days respectively.

Plots were monitored between 11.30am and 3.00pm on each given day. The primary pollinators were syrphid flies (*Diptera*) though the occasional honeybee from nearby hives was also recorded (a number of small black flies were also observed crawling over capitula but these were ignored). Pollinators were deemed to visit a plant if they settled on a capitulum and commenced feeding; pollinators that merely rested on capitula and did not feed were ignored. For each pollinator that entered a stand a record was taken of the sequence of radiate and non-radiate plants visited during a flight sequence i.e. until the pollinator left the stand or ceased feeding. Movement both between plants within the stand and between capitula on each plant was recorded. From these records the number of plants visited and the number of capitula foraged by each pollinator were noted.

A record of the weather conditions was taken on each day that plots were monitored. In addition, offical weather records for each of these days was obtained from the nearest weather station at RAF Leuchars.

RESULTS.

Pollinators of Senecio vulgaris L.

Based on insects caught visiting *S.vulgaris* plants in wild populations and artifical stands (Table 3.2) the main pollinators of the species in Scotland are hoverflies (Diptera). Three of the nine species observed foraging on *S.vulgaris* were found visiting both natural and artifical stands: these were *Episyrphus balteatus, Metasyrphus corollae* and *Platycheirus albimanus*, (Table 3.3). Most of the pollinators that visited the natural population monomorphic for the non-radiate morph at Crail (NO632089), were also observed foraging both morphs in polymorphic populations of *S.vulgaris* in Edinburgh. This shows that monomorphic and polymorphic populations tend to share the same pollinators.

In general pollinator activity was affected by weather conditions with most flights recorded on days when it was sunny.

Pollinator behaviour.

In the mixed stands of *S.vulgaris* raised at St.Andrews in

Table 3.2 Details of pollinators that foraged material in populations of *S. vulgaris* at either Crail, Newhailes and Leith or the artifical stands at the Botanic Gardens, St.Andrews.

Species	Notes
Episyrphus balteatus (Degeer 1776)	Very distinctive species. Common all year round.
Metasyrphus corollae (Fabricus 1794)	One of the most common species of hoverfly in Britain. Frequents open habitats, patches of flowers in arable fields, meadows, roadside verges, hedgerows, gardens and waste ground. April to October.
Platycheirus albimanus (Fabricus 1781)	Familiar throughout the British Isles in wood margins, hedgerows and gardens. April to November.
Syrphus ribesii (Linnaeus 1758)	Abundant in gardens, hedgerows and waste ground. April to November.
Syrphus vitripennis (Meigen 1822)	Wide range of habitats. March to November.
Syritta pipiens (Linnaeus 1758)	Frequently found in urban areas, rough meadows, hedgerows and marshy areas. May to October (often abundant in July and August).
Melanostoma mellinum (Linnaeus 1758)	One of the most common hoverflies. Often abundant in grassland areas. May to November.
Eristalis arbustorum (Linnaeus 1758)	Common in gardens, urban waste ground and other open habitats. April to Octobr.
Platycheirus manicatus	Very widespread. Common in dry open grassland especially on calcareous or neutral soils. May to October.

Table 3.3. Pollinators that visited *S.vulgaris* in three natural populations and the artifical stands at St.Andrews.

Location of stand/population	Grid reference	Pollinator
*+St.Andrews.	NO503162	*Episyrphus balteatus* *Metasyrphus corollae* *Platycheirus albimanus* *Syritta pipiens* *Syrphus ribesii* *Syrphus vitripennis* *Melanostoma mellium* *Eristalis arbustorum*
*Leith	NT268765	*Episyrphus balteatus* *Metasyrphus corollae* *Platycheirus albimanus* *Melanostoma mellium*
*Newhailes	NT337725	*Episyrphus balteatus* *Metasyrphus corollae* *Platycheirus albimanus* *Melanostoma mellium*
+Crail	NO632089	*Episyrphus balteatus* *Metasyrphus corollae* *Platycheirus albimanus* *Syritta pipens* *Platycheirus manicatus*

* stand/population polymorphic for capitulum type.
+ stand/population monomorphic for capitulum type.

which both morphs were present, it was evident that pollinators discriminated in favour of the radiate morph (Tables 3.4 and 3.5). Over all days that records were taken, the frequency of pollinator visits to radiate plants was respectively 66%, 84% and 40% in stands in which the frequency of the radiate morph equalled 50%, 80% and 20% (Table 3.5). Only in the stand in which the frequency of the radiate morph was high (80%) was there no significant difference from the expected frequency of visits to the radiate morph (i.e. assuming equal attractiveness of the two morphs). Pollinator discrimination in favour of the radiate morph was even more marked based on a comparison of pollinator visits to radiate and non-radiate capitula (Tables 3.4 and 3.5). Pollinators made 69%, 90% and 55% of their visits to radiate capitula in stands where the frequency of radiate capitula was equal to 50%, 80% and 20% respectively (Table 3.5). Thus, it was apparent that pollinators tended to forage more radiate than non-radiate capitula during visits to individual plants (Table 3.6). This was so even for pure stands where more capitula per plant were visited in stands monomorphic for the radiate type than in stands monomorphic for the non-radiate morph. In stands monomorphic for the non-radiate morph, 71 pollinators were observed visiting 223 capitula at an average of 1.92 +/- 0.130 capitula per plant, while in stands monomorphic for the radiate morph 88 pollinators made

Table 3.4. Observed frequencies of pollinator visits to (i) plants and (ii) capitula of the radiate and non-radiate morphs of *Senecio vulgaris* in mixed stands.

(i) PLANTS.

DESIGN:	0.5R:0.5N				0.8R:0.2N		0.2R:0.8N	
DAY:	1	2	3	4	1	2	1	2
R	0.694	0.513	0.770	0.681	0.848	0.833	0.429	0.384
N	0.306	0.487	0.230	0.319	0.152	0.167	0.571	0.616
$_{(1)}X^2$	10.89**	0.053	17.85**	15.54**	1.49	0.126	22.86**	15.37**
N_1	72	76	61	119	105	90	70	73

(ii) CAPITULA

DESIGN:	0.5R:0.5N				0.8R:0.2N		0.2R:0.8N	
DAY:	1	2	3	4	1	2	1	2
R	0.745	0.566	0.789	0.696	0.892	0.912	0.561	0.540
N	0.255	0.435	0.211	0.304	0.108	0.088	0.439	0.460
$_{(2)}X^2$	37.76**	2.77	40.98**	36.82**	12.01**	13.47**	100.17**	99.07**
N_2	157	159	123	240	204	171	123	137

$_{(1)}X^2$ (1 df) is for the null hypothesis of equal attractiveness of the radiate and non-radiate morphs.
$_{(2)}X^2$ (1 df) is for the null hypothesis of equal attractiveness of radiate and non-radiate capitula (the number of capitula per plant of each morph being equal in each plot).
N_1 and N_2 are the number of visits to plants and capitula respectively.

Table 3.5 Pooled data for each experimental design in Table 3.4.

(i) PLANTS.

DESIGN:	0.5R:0.5N	0.8R:0.2N	0.2R:0.8N
R	0.662	0.841	0.406
N	0.338	0.159	0.594
$_{(1)}X^2$	34.26**	2.05	39.38**
N_1	328	195	143

(ii) CAPITULA.

DESIGN:	0.5R:0.5N	0.8R:0.2N	0.2R:0.8N
R	0.694	0.901	0.550
N	0.306	0.099	0.450
$_{(2)}X^2$	101.88**	24.06**	199.06**
N_2	679	375	260

$_{(1)}X^2$ (1 df) is for the null hypothesis of equal attractiveness of the radiate and non-radiate morphs.
$_{(2)}X^2$ (1 df) is for the null hypothesis of equal attractiveness of radiate and non-radiate capitula (the number of capitula per plant of each morph being equal in each plot).
N_1 and N_2 are the number of visits to plants and capitula respectively.

Table 3.6 Mean number of capitula visited per plant in pure and mixed stands.

Design	Day	R	N	t	N_1
Pure non-radiate(N)	1	-	1.66(.135)		34
	2	-	2.14(.206)		37
	$\bar{x}$	-	1.92(0.130)		71
Pure radiate(R)	1	2.25(.142)	-		46
	2	2.22(.117)	-		42
	$\bar{x}$	2.25(0.095)	-		88
0.5R:0.5N	1	2.29(.215)	1.96(.249)	1.000 N.S.	52
	2	2.22(.313)	1.97(.206)	0.667 N.S.	46
	3	2.06(.211)	1.86(.376)	0.494 N.S.	46
	4	2.01(.112)	2.03(.242)	0.117 N.S.	69
	$\bar{x}$	2.12(.098)	1.97(.251)	0.928 N.S.	213
0.8R:0.2N	1	2.04(.112)	1.37(.294)	3.576 ***	54
	2	2.04(.099)	1.13(.162)	6.734 ***	53
	$\bar{x}$	2.04(.076)	1.26(.178)	6.590 ***	107
0.2R:0.8N	1	2.30(.245)	1.35(.203)	3.566 ***	41
	2	2.73(.240)	1.40(.183)	5.950 ***	45
	$\bar{x}$	2.50(.175)	1.38(.136)	5.159 ***	86

N_1 = Number of pollinators observed foraging the stand.
Standard errors given in parentheses.

308 visits to different capitula at an average of 2.20 +/- 0.095 capitula per plant.

Analysis of pollinator flights between plants i.e. transitions (Tables 3.7 and 3.8), showed that in mixtures containing an equal proportion of the two morphs (0.5R:0.5N) there was a preference for radiate to radiate (R-R), followed by non-radiate to radiate transitions (N-R) on three out of the four days that records were taken. In contrast, transitions from radiate to non-radiate (R-N) plants and between plants of the non-radiate morph (N-N) occurred at lower than expected frequencies in these stands. In stands in which the frequency of the radiate morph was increased to 80%, the frequencies of R-R and N-N transitions were respectively greater and lower than expected on the first day that records were taken (Table 3.7). However on day 2, the relative frequencies of each of these transitions were very close to the expected values. A particular point of interest was that on each of these days N-R transitions occurred at a much higher frequency than R-N transitions. (On day 1 the N-R transition was not much more frequent than expected; however, on both days, R-N transitions occurred at very low frequencies i.e. 0.077 and 0.054 compared to the expected value of 0.16 (Table 3.7)). In stands in which the frequency of the radiate morph was reduced to 20%, the frequency of transitions between radiate plants (R-R) was

Table 3.7 Transition frequencies for pollinator flights between plants in mixed stands.

DESIGN:	0.5R:0.5N				0.8R:0.2N		0.2R:0.8N	
DAY:	1	2	3	4	1	2	1	2
R-R	0.450	0.133	0.600	0.460	0.731	0.649	0.135	0.179
N-R	0.400	0.467	0.200	0.240	0.173	0.243	0.207	0.250
R-N	0.050	0.233	0.067	0.100	0.077	0.054	0.276	0.107
N-N	0.100	0.167	0.133	0.200	0.019	0.054	0.379	0.464
X^2	10.0*	8.13*	10.34*	13.84**	3.53	4.39	12.86**	16.70**
N_1	20	30	15	50	52	37	29	28

X^2 (3 df) for null hypothesis of equal attractiveness of the two morphs.
N_1 equals the number of pollinator flights.

Table 3.8. Pooled data from Table 3.7

DESIGN:	0.5R:0.5N	0.8R:0.2N	0.2R:0.8N
R-R	0.391 (0.25)	0.697 (0.64)	0.158 (0.04)
N-R	0.322 (0.25)	0.202 (0.16)	0.228 (0.16)
R-N	0.130 (0.25)	0.067 (0.16)	0.193 (0.16)
N-N	0.157 (0.25)	0.034 (0.04)	0.421 (0.64)
X^2	22.48**	6.30	26.11**
N_1	115	89	57

Figures in parentheses are the expected transition frequencies assuming that pollinators do not discriminate between morphs.
X^2 (3 df) for null hypothesis of equal attractiveness of the two morphs.
N_1 equals the number of pollinator flights.
For Tables 3.7 and 3.8 : Transitions were recorded between
radiate to radiate (R-R)
non-radiate to radiate (N-R)
radiate to non-radiate (R-N)
non-radiate to non-radiate (N-N).

again significantly greater than expected on both days that records were taken (0.135 and 0.179 compared to an expected frequency of 0.04 - Table 3.7). In contrast N-N transitions occurred at a significantly lower frequency than expected (0.39 and 0.464 compared to 0.64 - Table 3.7). On day 1 R-N transitions occurred at a frequency greater than expected; however on day 2 the relative frequency of this transition dropped to a value lower than expected. In contrast, on each of these days the frequency of N-R transitions was greater than expected.

An understanding of why the frequency of N-R transitions is consistently greater than that of R-N transitions in all polymorphic stands (only in one instance was this not found i.e. on day 1 in the 0.2R:0.8N stand) comes from an examination of the change in level of attractivness of radiate plants to pollinators as pollinators move from plant to plant during a flight sequence. Table 3.9 presents the observed frequencies of pollinator visits to the radiate morph for the initial and subsequent plants visited in a flight sequence in each polymorphic stand. In the 0.5R:0.5N stands, the preference shown by pollinators for the radiate morph increased as pollinators moved from the first to the second plant during a flight sequence. Thereafter preference for this morph tends to decrease. With respect to the latter it should be noted that only a small number of

Table 3.9. Observed frequencies of pollinator visits to the radiate morph for the initial and subsequent plants visited over a flight sequence.

Design.	Day	Order of plants visited during a flight sequence 1	n	2	n	3	n
0.5R:0.5N	1	0.61(.07)	52	0.94(.06)	16	0.50(.25)	4
	2	0.48(.07)	46	0.50(.13)	16	0.78(.14)	9
	3	0.76(.06)	46	0.80(.13)	10	0.67(.27)	3
	4	0.67(.03)	69	0.80(.07)	30	0.63(.17)	8
0.8R:0.2N	1	0.80(.06)	54	0.92(.05)	25	0.92(.08)	12
	2	0.79(.06)	53	0.88(.07)	25	1.00	9
0.2R:0.8N	1	0.49(.08)	41	0.40(.13)	15	0.33(.19)	6
	2	0.36(.07)	45	0.53(.12)	17	0.29(.17)	7

n = number of pollinators observed visiting position in flight sequence
standard errors given in parentheses.

flights by pollinators extend beyond two plants (Table 3.9), and therefore, the preference values for plant 3 in a flight sequence will be subject to a large sampling error. In the 0.8R:0.2N stand preference for the radiate morph again increased as pollinators moved from the first to the second plant in a flight sequence; however the same trend was only evident in the 0.2R:0.8N stand on one of the two days that this stand was studied. In this stand on the first day that records were taken, there was a decrease in the frequency of visits to radiate plants (49% to 40%) as pollinators moved from the first to the second plant. In contrast, on day 2 the reverse was found with visits to radiate plants increasing from 35% to 53% over the first two plants visited.

A deeper understanding of the change in relative attractiveness of the two morphs during a pollinator flight sequence emerges from a comparison of observed and expected transition frequencies over the first two plants visited (Tables 3.10 and 3.11). In this analysis the expected frequency of visits to the second plant are based on the relative attractiveness of morphs (R or N) to pollinators of the first plant visited in a flight sequence. In the 0.5R:0.5N stand there is evidence that the increase in attractiveness of the radiate morph to pollinators was due largely to transition flights between the first and second

Table 3.10 Frequency of visits to radiate (R) or non-radiate (N) morph for the second plant visited, after the pollinator had first visited the radiate morph during a flight sequence.

Design	Day	Frequency of visits First plant R	Second plant R	Second plant N	(n)	X^2	X^2_{HET}
0.5R:0.5N	1	0.615	0.615	0.000	(7)	2.91	
	2	0.478	0.120	0.358	(4)	0.14	3.77
	3	0.761	0.761	0.000	(5)	0.53	
	4	0.667	0.556	0.111	(18)	1.55	
	Pooled	0.634	0.522	0.112	(34)	4.48*	
0.8R:0.2N	1	0.796	0.708	0.088	(18)	0.46	
	2	0.792	0.748	0.044	(18)	1.71	0.16
	Pooled	0.794	0.728	0.066	(36)	2.63	
0.2R:0.8N	1	0.488	0.209	0.279	(7)	0.01	
	2	0.355	0.355	0.000	(7)	4.72*	5.25*
	Pooled	0.419	0.266	0.153	(11)	1.32	

X^2 = Goodness of fit of observed to expected values for visits to second plant.

X^2_{het} = test of heterogeneity.

Note : Expected frequencies of visits to either morph type (R or N) of second plant visited are computed from the relative attractiveness of the two morphs to pollinators for the first plant visited in a flight sequence.

Table 3.11 Frequency of visits to radiate (R) or non-radiate (N) morph for the second plant visited, after the pollinator had first visited the non-radiate morph during a flight sequence.

Design	Day	Frequency of visits First plant N	Second plant R	Second plant N	(n)	X^2	X^2_{HET}
0.5R:0.5N	1	0.385	0.346	0.039	(8)	1.31	
	2	0.522	0.304	0.218	(12)	0.19	3.14
	3	0.239	0.143	0.096	(5)	0.11	
	4	0.333	0.250	0.083	(12)	0.09	
	Pooled	0.366	0.257	0.109	(37)	0.49	
0.8R:0.2N	1	0.204	0.178	0.026	(8)	0.16	
	2	0.208	0.149	0.059	(7)	0.01	0.58
	Pooled	0.206	0.165	0.040	(15)	0.05	
0.2R:0.8N	1	0.512	0.192	0.320	(8)	0.08	
	2	0.645	0.248	0.397	(13)	0.01	0.34
	Pooled	0.581	0.221	0.360	(21)	0.02	

X^2 = Goodness of fit of observed to expected values for visits to second plant.

X^2_{het} = test of heterogeneity.

Note : Expected frequencies of visits to either morph type (R or N) of second plant visited are computed from the relative attractiveness of the two morphs to pollinators for the first plant visited in a flight sequence.

plant in the flight sequence being of the R-R type more often than expected (Table 3.10). There was a tendency for N-R transitions also to occur more often than expected over the first two plants visited but this was not significant (Table 3.11). In the 0.8R:0.2N and 0.2R:0.8N stands increased attractiveness of the radiate morph was entirely due to pollinators that first visited radiate plants showing an increased preference for this morph when moving to the next plant in the flight sequence (Table 3.10). In contrast, pollinators in these stands that first visited non-radiate plants chose the second plant in the flight sequence in accordance with preferences shown for the first plant i.e. as expected with no increased preference for the radiate morph (Table 3.11).

DISCUSSION.

The study has shown that the most frequent pollinators of *Senecio vulgaris* in Scotland are syrphid flies (hoverflies). Pollination by syrphids and bee flies is known as myophily and occurs most commonly in actinomorphic flowers that lack nectar guides (Baker and Hurd 1969; Faegri and van der Pijl 1979 - cited in Wyatt 1983). In *Senecio vulgaris* there is no evidence that ray florets of the radiate morph possess nectar guides. Warren (1987) has examined radiate capitula under ultraviolet light and found that the ray florets do not reflect at these wavelengths.

Hoverflies visit flowers to seek either pollen, nectar or both of these floral rewards (Gilbert 1981 cited in Stubbs and Faulk 1983; Meeuse 1978; Valentine 1978). It has been established that the pollen proteins and amino acids obtained are required for maturation of the reproductive system in these Diptera (Gilbert 1986). Only traces of the required substances are contained in nectar which is therefore not adequate in itself to promote reproductive maturity. Some genera of syrphids feed exclusively on pollen e.g. *Melanostoma, Episyrphus*; whereas others (including *Metasyrphus*) utilise both pollen and nectar. Most hoverflies tend to visit composite, umbelliferous or rosaceous flowers

selected mainly by colour i.e. yellow or white flowers (Gilbert 1986); for example *Eristalis tenax* is known to show a preference for yellow flowers (Ilse 1949; Kay 1976). Some hoverflies specialise in visiting certain groups of plants: two *Melanostoma* species and *Platycheirus clypetris* visit and remove pollen from wind pollinated grasses and plantains e.g. *Plantago lanceolata* (Stelleman 1978). While very little in known about the pollinating abilities of hoverflies, it has been established by following individuals and noting their visits, that hoverflies do tend to visit flowers of one type in succession (i.e. show constancy) particularly with composites and umbellifers (Gilbert 1986). Gilbert has also reported that hoverflies are thought to be important economically as pollinators of such species as onions, carrots and fruit trees.

The results of the investigation of pollinator behaviour within stands of *S.vulgaris* polymorphic for capitulum type produced clear evidence that pollinators discriminate in favour of the radiate morph (Tables 3.4 and 3.5). Moreover, analysis of pollinator flights between plants revealed that in all stands, transitions between radiate individuals (R-R) occurred at frequencies greater than expected (i.e. assuming equal attractiveness to pollinators - Tables 3.7 and 3.8). This was so even in stands where the radiate morph was present at low frequency (20%). The preponderance of

pollinator movement between radiate plants is likely to favour the radiate morph as both a male and female parent in intramorph outcrossing compared to the non-radiate morph as transitions between non-radiate plants occur at much lower frequencies than expected even when the non-radiate morph is present at high frequency (80%) in the population.

In all polymorphic stands transitions from non-radiate to radiate plants (N-R) occurred at greater frequency than expected assuming equal attractivness of the two morphs while R-N transitions were nearly always less frequent. If it is assumed that pollen production of the two morphs is equivalent (as recorded for some natural populations for plants that produce equal numbers of capitula - see Ross and Abbott 1987), and pollen pick-up, retention and carry over by pollinators is the same for each morph, this observed pattern of intermorph transitions could lead to a greater female outcrossing rate in the radiate morph relative to the non-radiate morph based on intermorph outcrossing. This in turn would explain (at least in part) the higher outcrossing rate of radiate relative to non-radiate disc florets recorded in some polymorphic populations (Marshall and Abbott 1984b).

The higher than expected frequency of transitions from non-radiate to radiate plants is partly explained by the

increased attractiveness of the radiate morph to pollinators as the latter move from the first to the second plant in a flight sequence. One possible explanation for this increase is that hoverflies, which visit *S.vulgaris* to collect pollen, find the non-radiate morph offers poor floral rewards for energy expended in extracting pollen from capitula when compared to the radiate morph. If this is the case, a pollinator initially visiting a non-radiate plant may tend to switch quickly to a more attractive radiate plant as it moves to the second plant in a flight sequence. In contrast, if the floral rewards from radiate plants are sufficient to satisfy the demands of the pollinator because, for example, radiate capitula are more easily manipulated by the pollinator when extracting pollen, then the preference exhibited for the radiate morph will be maintained or increased when the pollinator moves from the first to the second plant in a flight sequence. Analysis of the change in relative attractiveness of the two morphs during a pollinator flight sequence (Tables 3.10 and 3.11) has indicated that pollinators do indeed show an increase in preference for the radiate morph following an initial visit to a radiate plant when pollinators move from the first to the second plant in a flight sequence. In contrast, pollinators first visiting a non-radiate plant showed no significant increase in preference for the radiate morph but chose the second plant in the flight sequence in accordance

with preferences shown for the first. The one exception to this was in the 0.5R:0.5N stand where there was a tendency for more N-R transitions than expected to occur as pollinators moved from the first to the second plant foraged.

In a previous study of pollinator behaviour in artifical stands where the two morphs were present at equal frequency (Abbott and Irwin 1988) the pattern of pollinator behaviour was as shown in Table 3.12.

Table 3.12 Transition frequencies for pollinator flights between the first and second plants visited in a flight sequence in mixed stands in which the radiate and non-radiate morphs were present at equal frequency.

Transition	Day 1	2	3
R-R	0.606 (0.465)	0.373 (0.425)	0.413 (0.399)
N-R	0.299 (0.217)	0.348 (0.227)	0.343 (0.233)
R-N	0.076 (0.217)	0.219 (0.227)	0.219 (0.233)
N-N	0.019 (0.101)	0.000 (0.121)	0.025 (0.135)
$X_{(3)}^2$	10.498*	3.997 N.S.	5.816 N.S.
N	44	19	42

Expected frequencies given in parentheses.
Taken from data presented in Abbott and Irwin (1988).

It is clear that on two of the three days that records were taken, the frequency of R-R transitions was greater than expected, while on all three days, N-R transitions occurred more frequently than expected (measured over the first two plants visited in a flight sequence).

When the results of Abbott and Irwin (1988) are taken together with those from the present study it would seem that following a visit to a radiate plant, pollinators appear to 'learn' from the experience and show an increased preference for the radiate morph. In contrast, following a visit to a non-radiate plant, pollinators sometimes show an increase in preference for the radiate morph (Abbott and Irwin 1988) but more often, no change in behaviour occurs and the relative attractiveness of the two morphs remains the same.

Waser (1983) suggested that floral traits could influence pollinators in any of three sequential stages: (i) as the pollinator approaches the plant; (ii) as the pollinator forages on the plant; and (iii) as the pollinator leaves the plant to travel to others. In some plants, flowers no longer receptive to pollination are retained as they improve the overall attractiveness of the individual to pollinators (eg *Lupinus argenteus*, Gori 1989). However, this effect is qualified by small changes within the flower that direct the pollinator to more rewarding/receptive flowers. The radiate morph of *S.vulgaris* possesses an outer whorl of showy ray florets which are likely to make radiate capitula initially more conspicuous to pollinators. It is feasible also that the ray florets provide a stable platform for hoverflies to

land on particularly in windy conditions and thus make extraction of pollen easier. If the ray florets do facilitate a more efficient mode of pollen collection for pollinators, it follows that subsequent pollinator visits are more likely to be to radiate than non-radiate plants because the cost/benefit ratio is more benefical to the hoverfly and thus the level of attractiveness of the radiate morph increases as the pollinator moves from the first to the second plant in a flight sequence.

It has been realised for many years that pollinators discriminate between different flowers in the same population and that features leading to discrimination are often heritable. In natural and artifical populations of the wild radish *Raphanus raphanistrum*, Kay (1978) and Stanton, Snow and Handel (1986) showed that pollinators discriminate between the white and yellow flowered morphs. Levin (1969) has provided evidence of butterfly pollinators discriminating between cultivars of *Phlox drummondii* with different corolla shapes in addition to differences in flower colour, and has also shown (Levin 1972) that interspecific differences in corolla outline between two sympatric species of *Phlox, P.bifida* and *P. divaricata*, were probably responsible for discrimination between these species by Lepidopteran pollinators. Intraspecific discrimination according to the height of flowers above

ground has also been demonstrated for dwarf and tall forms of *Lythrum salicaria* (Levin and Kerster 1973) and by Eisikowitch (1978) for artifically mixed populations of the tall inland *Nigella arvensis* ssp *tuberculata* and the dwarf maritime *N.arvensis* ssp *divaricata*.

A series of studies on the white- and blue-flower colour morphs of the common morning glory (*Ipomoea purpurea*) have shown that natural populations are polymorphic for flower colour genes which bias pollinator service and hence the rate of outcrossing exhibited by each morph (Brown and Clegg 1984; Schoen and Clegg 1985). Epperson and Clegg (1987) showed that the white-flowered morph was undervisited by the primary pollinators of *Ipomoea purpurea* (bumblebees) and had a lower outcrossing rate than the blue flowered morph when the white morph was present at low frequency in a population. However, when the white morph was present at high frequency in the population, pollinators did not discriminate against it. Such discrimination against a rare morph has been described in other species e.g. *Raphanus raphanistrum* (Kay 1976,1978) and *Delphinium nelsonii* (Waser and Price 1981,1983). In contrast, pollinators discriminate against the non-radiate morph of *S.vulgaris* irrespective of morph frequency. Even when the non-radiate morph was present at high frequency (80%) pollinators made significantly fewer visits to non-radiate plants compared to minority radiate

individuals (Tables 3.4 - 3.8).

Pollinator discrimination can therefore affect the mating system of a plant population as typified by the increase in selfing exhibited by the white-flowered morph of *Ipomoea purpurea* (Brown and Clegg 1984; Schoen and Clegg 1985; Epperson and Clegg 1987). Stanton et al (1986) have attempted to ascertain the impact of such pollinator discrimination on male fitness in *Raphanus raphanistrum* pollinated by a variety of generalised pollinators including *Pieris* butterflies and syrphids. Stanton et al (1986) tested the effect of such discrimination on the performance of the yellow and white morphs as male parents. They found that the male performance of yellows was highly correlated with positive pollinator discrimination with 72% of progeny from yellow and a predominance of progeny from white-flowered plants being sired by yellow-flowered plants. Thus in *Raphanus raphanistrum* pollinator discrimination appears to have a profound effect on the relative paternal success of yellow and white flower-colour morphs.

If a similar relationship between pollinator discrimination and paternal success exists in populations of *S.vulgaris* polymorphic for capitulum type, it is to be expected that the radiate morph will have a greater rate of male outcrossing than the non-radiate morph. The following

chapter (chapter 4) describes the results of experiments conducted to estimate male and female intermorph and intramorph outcrossing rates using the same monomorphic and polymorphic stands of *S.vulgaris* in which pollinator behaviour was observed. It is proposed from the pattern of pollinator behaviour described in the present chapter, that the radiate morph will exhibit greater rates of female intermorph outcrossing (due to the higher than expected frequency of N-R transitions) and also greater rates of both male and female intramorph outcrossing (given that R-R transitions were favoured by pollinators) compared to the non-radiate morph.

CHAPTER 4.

OUTCROSSING IN *SENECIO VULGARIS* L.:

ESTIMATION OF MALE AND FEMALE OUTCROSSING RATES.

INTRODUCTION.

In the preceeding chapter, the pattern of pollinator visitation and movements in monomorphic and polymorphic stands of radiate and non-radiate *Senecio vulgaris* was described and discussed. The results obtained were of particular interest in terms of how pollinator movements may affect the male and female outcrossing rates of the two morphs in polymorphic populations over a range of morph frequencies. Most previous estimates of outcrossing rates in *Senecio vulgaris* have employed the alleles controlling the capitulum polymorphism first described by Trow (1912). Employment of these markers provides a measure of intermorph female outcrossing rate by identifying crosses between but not within capitulum morphs. Using these markers, Trow (1912) first estimated outcrossing in *S.vulgaris* to be around 1% between morphs, though he considered that 'the absence of selfing may reach 10% or more'. Much later, Haskell (1953) reviewed the information available on the breeding system of *S.vulgaris* and concluded that levels of outcrossing were low as a result of sporadic visits by insect pollinators. This was confirmed by Hull (1974), who from population rather than progeny data, calculated that average outcrossing in populations of *S.vulgaris* from central Scotland was 1%, although at some sites, levels as

high as 15% could occur. Subsequently Campbell and Abbott (1976) found that in experimental plots, the average outcrossing rate of the non-radiate morph could rise to 22%.

Detailed analysis of the outcrossing rates of the radiate and non-radiate morphs in natural populations polymorphic for capitulum type were first conducted by Marshall and Abbott (1982, 1984a) using the progeny test procedure of Allard and Workman (1963). They found that radiate plants exhibited a greater intermorph female outcrossing rate than non-radiate plants and that this difference was consistent over several populations studied. In one of two populations which were subjected to more detailed investigation, they established that the morph difference in outcrossing rate was entirely due to the greater female outcrossing of pistillate ray florets relative to hermaphrodite disc florets in the same capitulum. Marshall and Abbott attributed this increased outcrossing in the ray florets to functional protogyny within radiate capitula (Burtt 1977). In the second population studied by Marshall and Abbott (1984b), the difference in outcrossing between the two morphs was only partly explained on the basis of greater outcrossing in the ray florets. In this population, the disc florets of radiate plants also outcrossed at a greater level than the disc florets of non-radiate plants (9.8% compared to 0.7%, $X^2_{(1)} = 126$ ***). Consequently, Marshall

and Abbott (1984b) proposed two additional properties of the radiate morph that may contribute to the greater female intermorph outcrossing rate of radiate compared to non-radiate plants : (i) increased relative attractiveness of the radiate morph to pollinators such that insects move more frequently from non-radiate to radiate plants than expected (see chapter 3 for full discussion); and (ii) greater expression of cryptic self-incompatibility in the radiate morph.

Estimates of intermorph maternal outcrossing rate have been derived in the past from the proportion of total seed resulting from cross pollination by pollen from a plant of the opposite capitulum morph (i.e. radiate by non-radiate or non-radiate by radiate). Such estimates of maternal outcrossing between capitulum morphs assume that both capitulum morphs contribute equally to the available outcross pollen pool and that pollen dispersal among maternal parent plants is random. However, in view of the pollinator behaviour described in chapter 3, it is unlikely that pollen transfer between the two capitulum morphs of *Senecio vulgaris* is random. Pollinators exhibit a preference for the radiate morph, causing them to make more visits to radiate plants than would be expected (if the two morphs were equally attractive to pollinators) in all polymorphic stands (chapter 3). One consequence of this

preference is that more transitions (flights) occur from non-radiate to radiate plants than in the opposite direction; thus, it is likely that most radiate pollen transfer will occur between radiate plants and very little will be transferred from radiate to non-radiate plants. In contrast, for the non-radiate morph, while some pollen is transferred between non-radiate plants, non-radiate pollen is likely to be directed towards radiate plants as a result of polarised pollinator movement.

All previous estimates of maternal intermorph outcrossing between capitulum morphs which have used the ray floret locus as a marker suffer from the assumption that radiate and non-radiate maternal plants receive pollen from an equivalent pollen pool. In the light of the pollinator behaviour described in chapter 3, these estimates of maternal intermorph outcrossing can, at best, reflect a measure of gene flow between the two capitulum morphs and should not be considered as accurate measures of maternal outcrossing rate. Despite this shortcoming, and in view of the considerable body of information which exists in the literature concerning estimates of maternal intermorph outcrossing in *S.vulgaris*, the term intermorph outcrossing rate will be retained here. It is emphasised, however, that this measure is one which reflects gene flow between radiate and non-radiate plants and is not an accurate estimate of

maternal intermorph outcrossing rate.

When considering outcrossing within capitulum morphs, it is reasonable to assume that maternal plants sample pollen from an equivalent outcross pollen pool. In this case radiate and non-radiate plants sample pollen from separate outcross pollen pools. Only one study has so far attempted to estimate the level of outcrossing which occurs within capitulum morphs (Warren 1987) and it would seem of value therefore, to obtain further estimates of such outcrossing.

The frequency of intramorph maternal outcrossing is calculated from the proportion of total seed produced as a result of cross pollination with pollen from another plant of the same capitulum morph. In addition to maternal outcrossing, a measure of paternal intramorph outcrossing can be estimated, where, male oucrossing of a given sporophyte is the binomial probability of its male gametes uniting with female gametes of a different genotype (Horovitz and Harding 1972). In practice, paternal intramorph outcrossing is determined by adding the cases in which an individual acts as a male parent in crosses, and dividing these by the sum of all cases in which the individual acts as a pollen parent (for both selfs and crosses). Identification of male and female outcrossing within the capitulum morphs of *S.vulgaris* does, of course,

require the presence of a second marker locus at which alleles segregate independently from the alleles at the ray floret locus.

This chapter reports an investigation aimed at estimating the male and female outcrossing rates of the radiate and non-radiate morphs of *Senecio vulgaris* raised in monomorphic and polymorphic stands on the experimental plot at St.Andrews in 1988. The types and behaviour of pollinators that visited these stands was discussed previously in chapter 3.

MATERIALS AND METHODS.

Experimental details of the monomorphic and polymorphic stands of *Senecio vulgaris* set out on the experimental plot at the Botanic Gardens, St.Andrews were described in chapter 3. In the 0.8R:0.2N and 0.2R:0.8N stands, 'minority' plants were placed in positions 7,9,12 and 14 in two different ways according to genotype at the β-est-1 marker locus. In design A, plants were postioned with nearest neighbours bearing the same esterase allele, while in design B, nearest neighbours bore alternative alleles at the β-est-1 locus i.e.

Design A	bb	aa	Design B	bb	aa
	bb	aa		aa	bb

From each of the 20 plants included in each experimental design, one test capitulum (just receptive to pollination) was tagged at the base of the pedicel, using a fine indelible marker, prior to the plants being set out on the plot. Plants in each stand were left on the plot for 72 hours to allow free pollinator activity. After 72 hours, the marked capitula were bagged before returning plants to the glasshouse to allow test capitula to set seed. From September 1988 onwards, 25 seeds from each test capitulum

were sown out on trays of Arthur Bowers universal potting compost. Progeny were raised to flowering under a 16 hour photoperiod (supplied by 400 watt mercury vapour lamps) and watered when necessary. Once in flower, progeny were scored for capitulum type and assayed electrophoretically to determine esterase genotype at the β-est-1 locus. Details of the electrophoretic procedure are given in Appendix A.

Estimation of intramorph outcrossing rates and gene flow between radiate and non-radiate plants.

(i) Intramorph maternal outcrossing.

In each monomorphic and polymorphic stand, an estimate of female intramorph outcrossing rate, t, for radiate and non-radiate plants was obtained by dividing the frequency of progeny produced by each electromorph (β-est-$1^{a\,a}$ or β-est-$1^{b\,b}$) that were heterozygous at the β-est-1 locus, by the expected proportion 'q' of identifiable cross pollen in the outcross pollen pool. 'q' was taken to be equal to the frequency of plants in the stand that have genotypes different from the maternal parent at the β-est-1 locus. For example, consider the radiate morph in an 0.5R : 0.5N mixture, where maternal radiate plants can be subdivided into two electromorphs. In this design five radiate plants are homozygous for the β-est-1^{a} allele and five are

homozygous for the β-est-1^b allele. Therefore, for the β-est-1^{aa} electromorph, the frequency of heterozygotes at the β-est-1 locus is equal to the number of progeny produced by β-est-1^{aa} maternal parents heterozygous at the β-est-1 locus (β-est-1^{ba}) divided by the total number of β-est-1^{aa} progeny screened :-

$$h_{intra} = \frac{\beta\text{-est-}1^{ba}}{\text{Total number of radiate } \beta\text{-est-}1^{aa} \text{ progeny screened}}$$

This value, h_{intra}, is weighted by the frequency of identifiable intramorph outcross pollen parents, 'q', to obtain an estimate of maternal intramorph outcrossing. For any individual (radiate or non-radiate) within the 0.5R:0.5N stand, there are nine possible intramorph cross pollen parents (i.e. nine other plants of the same capitulum morph), five of which carry the alternative allele at the β-est-1 locus. Therefore, in an 0.5R:0.5N stand, 'q' is equal to 5/9. Thus the radiate intramorph maternal outcrossing rate of the β-est-1^{aa} electromorph is equal to:-

$$t_{intra} = \frac{h_{intra}}{5/9}$$

In the 0.2R:0.8N and 0.8R:0.2N stands, the method of estimating radiate and non-radiate intramorph maternal outcrossing rates follows the same procedure as that given above except that the weighting factor 'q' varies according to the number of identifiable outcross pollen parents in the stand. Table 4.1 lists the values of 'q' for the radiate

morph in pure stand and each of three polymorphic stands investigated.

Table 4.1 Proportion of cross pollen parents (q) that are identifiable in estimating the intramorph maternal outcrossing rate of radiate plants using the β-est-1 marker locus.

Experimental Design	q
Pure Radiate.	10/19
0.8R:0.2N	8/15
0.5R:0.5N	5/9
0.2R:0.8N	2/3

Standard errors are attached to each estimate of outcrossing as follows :

Given that $t = h/q$

the standard error of $t = \sqrt{\frac{h(1-h)/n}{q^2}}$

Significant differences may be tested using the following statistic:

$$t_1 - t_2 \quad +/- \ 1.96 \quad \sqrt{(\text{var}t_1 + \text{var}t_2)}$$

When testing for one specific value to be greater than another, this becomes a one sided test of significance and is modified as follows:-

$t_1 - t_2 > 1.645 \sqrt{\text{var}t_1 + \text{var}t_2}$ *

$t_1 - t_2 > 2.326 \sqrt{\text{var}t_1 + \text{var}t_2}$ **

$t_1 - t_2 > 3.09 \sqrt{\text{var}t_1 + \text{var}t_2}$ ***

(ii) Paternal intramorph outcrossing.

The male outcrossing rate of a given sporophyte is defined as the binomial probability of its male gametes uniting with female gametes produced on a different sporophyte (Horovitz and Harding 1972). Thus the male outcrossing frequency in a genotype can be determined by adding together the cases of outcrossing in which it acts as a male parent and dividing these by the sum of all cases in which it acts as a pollen parent (cross and selfs). Therefore for the radiate β-est-1^{aa} electromorph, the male intramorph outcrossing rate is given by:

$$\frac{(\text{RRba from RRbb}) + (\text{RRaa from RRaa} \times t_{\text{intra RRaa}})}{(\text{RRba from RRbb}) + (\text{RRaa from RRaa})}$$

where

RRba from RRbb = number of progeny produced by RRbb maternal parent plants following cross fertilisation by pollen from RRaa plants

RRaa from RRaa = number of progeny produced by RRaa maternal plants following fertilisation by pollen from RRaa plants (selfs and crosses).

RRaa from RRaa x t_{intra} RRaa = number of progeny produced by RRaa maternal parent plants following fertilisation by pollen from other RRaa plants.

t_{intra} RRaa = female intramorph outcrossing rate of the RRaa electromorph.

This calculation can be performed for each electromorph of both radiate and non-radiate plants in monomorphic and polymorphic designs.

(iii) Gene flow between radiate and non-radiate plants

A similar method to that used to estimate intramorph outcrossing can be used to estimate the amount of gene flow between the two capitulum morphs. Using the radiate morph in the 0.5R:0.5N stand as an example, progeny of the radiate morph that are heterozygous at the ray floret locus are first expressed as a proportion of the total number of radiate progeny screened - h_{ray}. This value is then divided by 'q', the proportion of possible outcross pollen parents which are identifiable using the ray floret locus as a marker. For any one radiate plant there are 19 possible outcross pollen parents, ten of which are identifiable using the ray floret locus as a marker, therefore in this case 'q' is equal to 10/19. Hence:

$$t_{inter} = \frac{h_{ray}}{\text{Number of radiate progeny screened}} \Big/ \frac{10}{19}$$

Note. As explained in the introduction, t_{inter} is actually a measure of gene flow as described and not of intermorph outcrossing. The term t_{inter} is retained to facilitate comparisons with earlier estimates of intermorph maternal outcrossing reported in the literature.

In the 0.2R:0.8N and 0.8R:0.2N stands the method of estimation is the same except that the weighting factor for 'q' varies according to the number of identifiable outcross pollen parents in the stand. Table 4.2 lists the values of 'q' for the radiate morph in the three designs used.

Table 4.2. Proportion of outcross pollen parents (q) identifiable in estimating t_{inter} for radiate maternal parent plants using the ray floret marker locus.

Experimental design.	q
0.8R:0.2N	4/19
0.5R:0.5N	10/19
0.2R:0.8N	16/19

(iv) Gene flow between radiate and non-radiate plants measured in terms of restriction of pollen transfer between capitulum morphs.

It is not possible to estimate a measure of male outcrossing for either morph based entirely on intermorph crossing. Measures of male outcrossing which include intermorph crossing also contain a component of intramorph maternal outcrossing (Horovitz and Harding 1972). Thus an estimate of paternal outcrossing between morphs alone cannot be made. However, it is possible to measure the restriction in pollen flow from radiate to non-radiate plants and vice versa as follows. If both morphs contributed equally to the outcross pollen pool, and if pollen was randomly dispersed over maternal plants, then:

$$\frac{\text{frequency of non-radiate progeny fertilised by radiate pollen}}{q} = \alpha \times \text{maternal } t_{intra} \text{ for the non-radiate morph}$$

and similarly:

$$\frac{\text{frequency of radiate progeny sired by non-radiate pollen}}{q} = \beta \times \text{maternal } t_{intra} \text{ for the radiate morph}$$

where α and β equal 1.

It follows that where there is unequal contribution by morphs to the outcross pollen pool, and/or non-random

dispersal by pollen over maternal plants, the values of α and β provide a measure of the restriction in gene flow (pollen movement) from R - N and N - R respectively. In these equations, 'q' is equal to the number of identifiable pollen parents and varies according to experimental design.. Table 4.3 Lists the values of 'q' for the three designs used.

Table 4.3. Proportion of identifiable outcross pollen parents using the ray floret marker locus in polymorphic stands in which the radiate morph is present at a frequency of 0.8, 0.5 or 0.2.

Experimental Design.	q α	q β
0.8R:0.2N	16/19	4/19
0.5R:0.5N	10/19	10/19
0.2R:0.8N	4/19	16/19

Using the values of α and β obtained, an estimate of the relative fitness of outcross radiate pollen relative to outcross non-radiate pollen in crosses occurring between the two capitulum morphs can be obtained by dividing the rstriction in gene flow R-N (α) by that from N-R (β). Thus if the male fitness of non-radiate pollen is taken to be 1.0 and that of radiate pollen is m then:

$$m = \frac{\alpha}{\beta}$$

RESULTS.

Intramorph outcrossing.

In both replicates of the monomorphic non-radiate stand and in one of two replicates of the monomorphic radiate stand the β-est-$1^{a\,a}$ electromorph exhibited a higher level of maternal and paternal outcrossing than the β-est-$1^{b\,b}$ genotype(Tables 4.4 and 4.5). However, when data were pooled over both electromorphs, it was clear that the radiate morph showed higher levels of both male and female outcrossing compared to the non-radiate morph (Tables 4.6 and 4.7).

In stands where the radiate morph was present at frequencies of 0.8, 0.5, or 0.2, a significant difference between the two electromorphs within each capitulum morph was again found for female outcrossing, with the β-est-$1^{a\,a}$ electromorph usually exhibiting the higher level (table 4.8).

In most polymorphic stands, the radiate morph tended to exhibit a greater rate of both male and female outcrossing than the non-radiate morph (Tables 4.8, 4.9), although the difference within 0.5R:0.5N was not significant. In stands in which morph frequency was either 0.8 or 0.2, there was

Table 4.4 Maternal outcrossing rates of β-est-1^{aa} and β-est-1^{bb} electromorphs of radiate and non-radiate *Senecio vulgaris* in monomorphic stands.

Morph	outcrossing rate β-est-1aa	β-est-1bb
Radiate		
rep 1	15.92(3.94)	5.51(2.22)
rep2	2.09(1.47)	5.59(2.25)
pooled	8.95(2.12)	5.55(1.58)
Non-radiate		
rep1	5.91(2.20)	0.00
rep2	3.80(1.88)	0.00
pooled	4.92(1.46)	0.00

Standard errors given in parentheses.

Table 4.5 Paternal outcrossing rates of β-est-1^{aa} and β-est-1^{bb} electromorphs of radiate and non-radiate *Senecio vulgaris* in monomorphic stands.

Morph	outcrossing rate β-est-1aa	β-est-1bb
Radiate		
rep1	18.89	12.07
rep2	5.25	6.53
pooled	12.02	9.41
Non-radiate		
rep1	5.91	2.88
rep2	3.80	1.69
pooled	4.92	2.29

Table 4.6 Maternal outcrossing rates of the radiate and non-radiate morphs of *Senecio vulgaris* in monomorphic stands.

Morph	Replicate	t	n
Radiate			
R	1	10.34 (2.19)	386
R	2	3.94 (1.38)	386
Pooled		7.14 (1.30)	772
Non-radiate			
N	1	2.88 (1.08)	461
N	2	1.76 (0.87)	463
Pooled		2.34 (0.70)	894

$t_R - t_N > 3.09 \quad var t_1 + var t_2$ ***
t = outcrossing rate
n = number of progeny screened
Standard errors given in parentheses.

Table 4.7. Paternal outcrossing rates of the radiate and non-radiate morphs of *Senecio vulgaris* in monomorphic stands.

Morph	Replicate	t
Radiate		
R	1	15.29
R	2	5.89
Total		10.71
Non-radiate		
N	1	4.36
N	2	2.29
Total		3.54

t = outcrossing rate.

TABLE 4.8 Female outcrossing rate of the Est^{bb} and Est^{aa} electromorphs for radiate and non-radiate Senecio vulgaris in polymorphic stands in which the frequency of the radiate morph was 0.8, 0.5 or 0.2.

		RADIATE			NON-RADIATE		
		$t_{(Est^{aa})}$	$t_{(Est^{bb})}$	p	$t_{(Est^{aa})}$	$t_{(Est^{bb})}$	p
0.8R : 0.2N	(A)	12.499 (2.622)	0.580 (0.579)	***	1.613 (1.604)	0.00 (-)	N.S.
	(B)	12.784 (2.519)	1.062 (0.749)	***	0.00 (-)	0.00 (-)	-
0.5R : 0.5N		6.923 (2.400)	1.967 (1.383)	**	4.327 (1.912)	1.846 (1.299)	N.S.
0.2R : 0.8N	(A)	8.219 (3.995)	1.786 (1.775)	*	1.797 (0.843)	0.00 (-)	**
	(B)	0.00 (-)	0.00 (-)	-	2.633 (1.167)	0.00 (-)	***

$t_{(Est^{aa})}$, $t_{(Est^{bb})}$ = outcrossing rate

Standard Errors given in parentheses.

an interesting effect of experimental design (i.e. A or B) on outcrossing rate. In stands in which radiate plants were at high frequency (80%), both male and female intramorph outcrossing rates of the radiate morph were greater than those of the non-radiate morph (Tables 4.9 and 4.10). with the non-radiate morph exhibiting no intramorph outcrossing in stands employing design B. In contrast, in stands containing the radiate morph at low frequency (20%), it was the radiate plants which showed no outcrossing in plots using design B, while in plots of design A the radiate morph again exhibiting a greater rate of male and female outcrossing.

Gene flow between capitulum morphs.

In each of the polymorphic stands studied, t_{inter} for the radiate morph was significantly greater than that estimated for the non-radiate morph (Table 4.11). Furthermore, in all cases, t_{inter} of the radiate morph was greater than the intramorph maternal outcrossing rates recorded for radiate plants (Tables 4.9 and 4.11). In contrast, estimates of intramorph maternal outcrossing for the non-radiate morph were always greater than the estimated level of t_{inter}.

A comparison of the relative fitness of radiate and non-radiate pollen involved in outcrossing between the two

Table 4.9. Maternal intramorph outcrossing rates of radiate and non-radiate *Senecio vulgaris* in polymorphic stands in which the frequency of the radiate morph was 0.8, 0.5 or 0.2.

Experimental Design		t_R	t_N	*p*
0.8R:0.2N	(A)	6.47 (1.35)	0.85 (0.85)	***
	(B)	6.92 (1.33)	0.00 -	***
0.5R:0.5N		4.60 (1.44)	3.13 (1.17)	N.S.
0.2R:0.8N	(A)	4.78 (2.10)	0.84 (0.48)	*
	(B)	0.00 -	1.32 (0.59)	**

t_R = outcrossing rate of radiate morph
t_N = outcrossing rate of non-radiate morph
Standard errors given in parentheses.

Table 4.10. Paternal intramorph outcrossing rates for radiate and non-radiate *Senecio vulgaris* in polymorphic stands in which the frequency of the radiate morph was 0.8, 0.5 or 0.2.

Experimental Design		t_R	t_N
0.8R:0.2N	(A)	9.72	1.41
	(B)	10.47	0.00
0.5R:0.5N		6.91	4.82
0.2R:0.8N	(A)	7.95	1.28
	(B)	0.00	2.02

t_R = outcrossing rate of radiate morph.
t_N = outcrossing rate of non-radiate morph.

Table 4.11. Estimated gene flow between radiate and non-radiate plants in polymorphic stands of *Senecio vulgaris* in which the radiate morph was present at a frequency of 0.8, 0.5 or 0.2.

Experimental design		t_R	t_N	p
0.8R:0.2N	(A)	5.20 (1.96)	1.34 (0.94)	***
	(B)	14.82 (3.11)	0.66 (0.66)	***
0.5R:0.5N		9.23 (2.07)	1.41 (0.82)	***
0.2R:0.8N	(A)	5.29 (1.96)	0.70 (0.70)	**
	(B)	6.17 (2.12)	0.00 -	***

t_R = maternal outcrossing rate of radiate morph
t_N = maternal outcrossing rate of non-radiate morph.
Standard errors given in parentheses.

capitulum morphs (Table 4.12), showed that radiate pollen was more effective when radiate plants were at high frequency (80%) in a stand. However, when the radiate morph was present at an intermediate (50%) or low (20%) frequency, the opposite was the case and non-radiate pollen was the more effective in fertilising ovules of the other morph.

Table 4.12. Estimated relative fitness of radiate and non-radiate pollen in crosses between capitulum morphs in polymorphic stands of *Senecio vulgaris* in which the radiate morph was present at a frequency of 0.8, 0.5 or 0.2.

Experimental design		R	N	R/N = m
0.8R:0.2N	(A)	1.58(3.08)	0.81(0.05)	1.97
	(B)	-	2.14(0.09)	-
pooled	(A+B)	2.38(4.37)	1.56(0.06)	1.52
0.5R:0.5N		0.45(0.28)	2.32(0.78)	0.20
0.2R:0.8N	(A)	0.84(0.15)	1.11(1.02)	0.76
	(B)	-	-	-
pooled	(A+B)	0.32(0.05)	2.67(1.93)	0.12

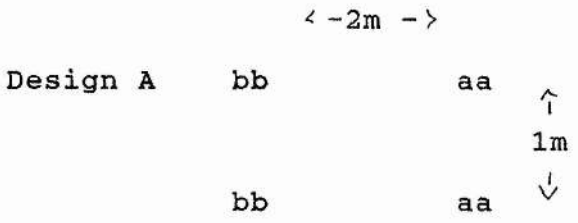
R/N = m gives the relative fitness of radiate outcross pollen compared to non-radiate outcross pollen in crosses between radiate and non-radiate plants.
Standard errors were calculated in the usual manner for a binomial distribution.

DISCUSSION.

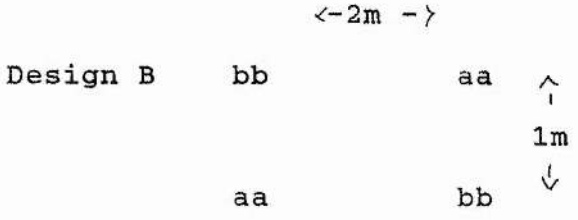
A number of important points have emerged from the results reported in this chapter. Of particular note was the finding that the intramorph female outcrossing rate of the radiate morph of *Senecio vulgaris* was nearly always greater than that of the non-radiate morph (Tables 4.6 and 4.9). This was so both in monomorphic and polymorphic stands. The one exception to this rule occurred in the 0.2R:0.8N stand which employed design B (Table 4.9). Here, no intramorph outcrossing was recorded in the radiate morph.

With regard to this latter result, it is of interest that in all of the four replicates where design B was employed, no intramorph outcrossing was detected in the rare morph be it of the radiate or non-radiate type. In contrast, using design A, three out of four replicates showed significant levels of intramorph outcrossing in the rare morph. This contrast suggests that design B may be less sensitive to intramorph outcrossing in the rare morph than design A. Why this should be so is difficult to explain although the cause may be related to an effect of wind on the direction of pollinator flights within a stand. The two designs (A and B) differed in the arrangement of the four individuals of the rare morph with respect to the β-est-1 genotype they

possessed. With design A, the β-est-1 genotypes of the rare morph were positioned as follows :-



Whereas in design B the arrangement was :-



It has previously been reported by Stellman (1978) that hoverflies visiting *Plantago lanceolata* tend to fly directly into the wind. The wind direction on the 12 days in question was most commonly diagonally across the plots from bottom right to top left, and averaged approximately 21 km per hour (weather records supplied by the Met. Office, Edinburgh for the nearest recording station at R.A.F. Leuchars). It is possible, therefore, that due to the wind, more identifiable intramorph crosses occurred in stands arranged according to design A than in stands in which design B was employed.

As explained in the introduction, it was expected that one result of pollinator preference for the radiate morph described in chapter 3, would be to cause more pollen to be transferred between capitulum morphs in the non-radiate to radiate direction than from radiate to non-radiate plants. The levels of gene flow between capitulum morphs estimated using the ray floret locus as marker (Table 4.11) suggest that this is indeed the case. Comparisons of intramorph outcrossing rate and intermorph gene flow (Tables 4.9 and 4.11) showed that in general t_{inter} for the radiate morph exceeded the intramorph estimate of radiate maternal outcrossing. In contrast, t_{inter} recorded for the non-radiate morph was less than the estimated rate of intramorph maternal outcrossing (at least when the non-radiate morph was present at intermediate or high frequency- Tables 4.9 and 4.11). These results suggest that radiate pollen has a tendency to be transferred to other radiate plants rather than to non-radiate individuals, while non-radiate pollen is more likely to be transferred to radiate individuals than to another non-radiate plant.

Comparisons of the relative fitness of radiate and non-radiate pollen in outcrossing between the two capitulum morphs (Table 4.12) support this view. When the radiate morph was present at intermediate or low frequency in a

stand, the relative fitness (m) of radiate pollen was lower than that of non-radiate pollen. Only when the radiate morph was present at high frequency (80%) in a stand did the relative fitness of outcross radiate pollen exceed that of outcross pollen from non-radiate individuals.

The differences recorded between morphs for intramorph and intermorph (t_{inter}) maternal outcrossing rate were in broad agreement with the differences for intermorph maternal outcrossing rate reported previously by Marshall and Abbott (1982, 1984a,1984b) From a study of two polymorphic populations of *S.vulgaris* from Edinburgh, Marshall and Abbott (1982) showed that during periods of peak flowering, radiate plants exhibited a mean maternal intermorph outcrossing rate that ranged between 13-20% while that of the non-radiate morph never exceeded 1%. Such differences between morphs in maternal outcrossing rate were confirmed when the survey was extended to include populations from Leeds, Cardiff and Rhosllanerchrugog (Clwyd)(Marshall and Abbott 1984a). In this context it needs to be emphasised once again that because pollinator movements are non-random in polymorphic stands, the measures of t_{inter} calculated in the present study, and by Marshall and Abbott, are not true measures of maternal outcrossing but rather reflect the level of gene flow between morphs as well as differences in maternal outcrossing.

Whereas the estimates of t_{inter} recorded in the present studies accord with those reported by Marshall and Abbott (1984a), both sets of results contrast markedly with those from a study reported by Warren (1988), see Fig 4.1. Warren used a marker locus controlling calyculus bract tip colour in addition to the ray floret locus to obtain estimates of inter- and intra-morph maternal outcrossing rates for the two capitulum morphs of *S.vulgaris*. Within artifical plots containing both morphs he recorded the intermorph maternal outcrossing rate

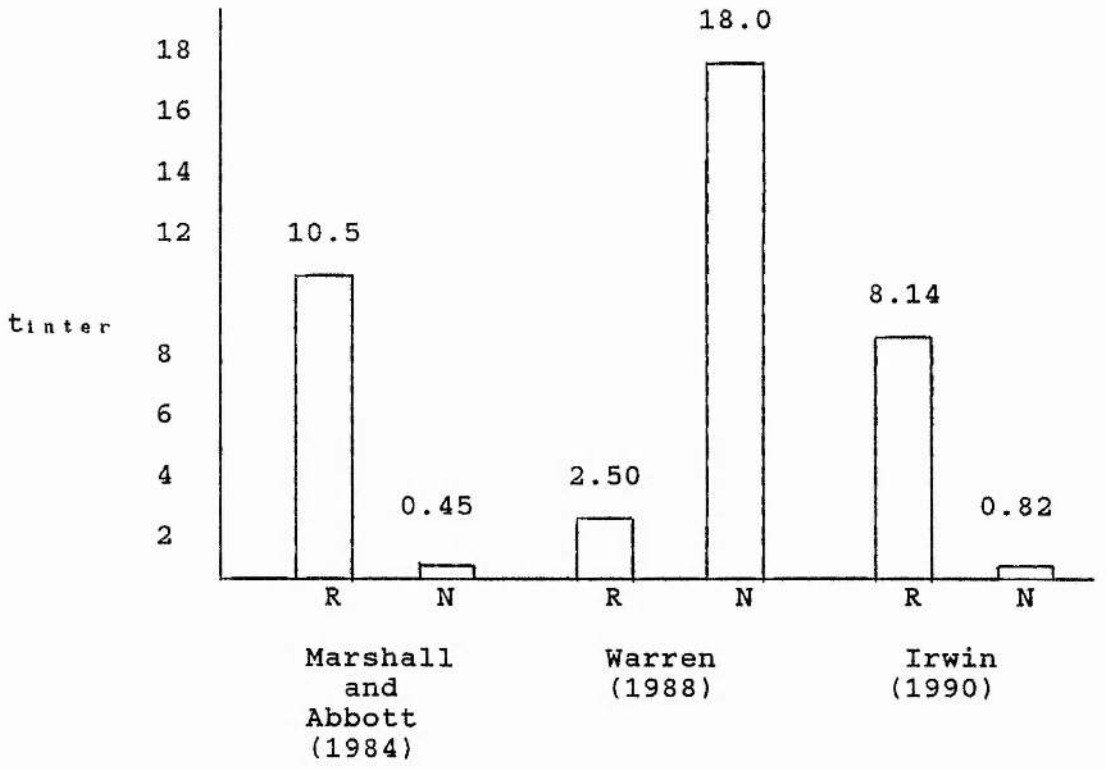


Fig.4.1. Average female outcrossing rates (t_{inter}) recorded for radiate and non-radiate *Senecio vulgaris* in natural populations and artifical polymorphic stands.

of the non-radiate morph to be 18% compared to 2.5% for the radiate morph. Thus the difference he found between the two morphs for intermorph maternal outcrossing rate was in the opposite direction to that recorded in the present studies and those previously reported by Marshall and Abbott (1982,1984a), Fig.4.1.

Again, in regard to intramorph outcrossing in monomorphic stands, the results of the present studies also conflict with those reported by Warren (1988). He found that in monomorphic plots, the radiate and non-radiate morphs exhibited similar levels of female outcrossing: 17.8% for the non-radiate and 15% for the radiate morph. In contrast, in the present study, there was a significant difference in intramorph outcrossing rate between the two morphs raised in monomorphic stands, with the radiate morph averaging 7.4% and the non-radiate showing 2.3% outcrossing. Only in regard to the results for intramorph maternal outcrossing rates of morphs in polymorphic stands was a similar pattern recorded by Warren to that found in the studies reported here. Warren recorded that the non-radiate morph outcrossed at 2.6% while the radiate showed a much higher level of 27%. These results agree in pattern if not in magnitude with those reported here for polymorphic stands of the two morphs where the radiate morph was present at a frequency of 0.2,

0.5, or 0.8 (Table 4.9).

Why the results obtained by Warren (1988) should contrast so sharply with other measured rates of outcrossing in polymorphic stands of *S.vulgaris* is unclear. Warren proposed two rules to explain his findings: (i) insects are equally attracted to all plots irrespective of the morphs present; and (ii) within plots, radiate plants are preferentially visited. With respect to the first of these rules, the results reported in chapter 3 of this thesis (Tables 3.4 - 3.11) suggest that all plots are not equally attractive to pollinators and results obtained for outcrossing rates in the monomorphic stands tend to confirm this. When raised in monomorphic plots it is evident that the radiate morph is much more attractive to pollinators than the non-radiate morph.

The second rule identified by Warren has been confirmed by the findings presented in chapter 3. As predicted by Marshall and Abbott (1984b), radiate plants are indeed preferentially visited in polymorphic plots. However, whereas Warren's findings indicate that such preference by pollinators for the radiate morph will raise the level of outcrossing within the radiate morph at the expense of its maternal outcrossing between morphs, the results here show that this was not the case in the present studies. Instead

the pollinator preference for the radiate morph and the pattern of movements (transitions) within and between morphs would appear to have had the effect of raising both the intra- and intermorph maternal outcrossing rate of the radiate morph relative to the non-radiate morph.

In addition to a greater outcrossing of the ray florets and pollinator discrimination in favour of the radiate morph, Marshall and Abbott (1984b) also suggested that an increased level of cryptic self-incompatibility associated with the radiate condition might be a factor in explaining increased female intermorph outcrossing rate of radiate compared to non-radiate plants. Although Warren *et al* (1988) reported no difference in cryptic self-incompatibility between morphs, they found in three populations surveyed (two in Yorkshire and one in Edinburgh) radiate plants exhibited a polymorphism for self-incompatibility in the ray florets. However, Abbott, Irwin and Forbes (in press) have failed to confirm the occurrence of radiate individuals bearing self-incompatible ray florets in Edinburgh populations, finding no such individuals among a sample of over 400 radiate plants tested.

Turning to the results for male intramorph outcrossing, it was evident that in all polymorphic stands, the radiate morph exhibited levels of intramorph paternal outcrossing

greater than those of the non-radiate morph in all but the 0.2R:0.8N stand which employed design B. In contrast, estimates of the relative fitness of radiate and non-radiate pollen from crossing occurring between the two capitulum morphs (Table 4.12) suggested that when the non-radiate morph was present at intermediate or high frequency, non-radiate pollen was more effective than radiate pollen in fertilising ovules of the opposite morph.

Estimates of paternal intramorph outcrossing rates and the relative fitness of radiate and non-radiate pollen in intermorph crossing provide a basis for comparing the relative abilities of radiate and non-radiate plants to disperse effective pollen within and between capitulum morphs, and thus give a measure of male fitness with respect to access to ovules. The results of the present study reveal that pollen from the non-radiate morph exhibited a higher fitness than pollen from the radiate morph in crosses between morphs when the non-radiate morph was present at intermediate or high frequency in the population. Hence, the non-radiate morph contributeed more pollen to the intermorph outcross pollen pool and consequently fertilised a higher proportion of radiate ovules than radiate pollen fertilised non-radiate ovules. Therefore, based on access to ovules of the opposite capitulum morph, the non-radiate morph has a higher male fitness than the radiate morph in these stands.

In contrast, the radiate morph exhibited higher levels of intramorph paternal outcrossing than the non-radiate morph in all polymorphic stands. Therefore, with respect to access to ovules of the same capitulum morph, the radiate morph has a higher male fitness than the non-radiate morph.

These estimates of relative pollen fitness in crossing within and between capitulum morphs are in agreement with what would be expected from the pattern of pollinator behaviour described in chapter 3. Pollinator preference for the radiate morph resulted in movements between radiate plants occurring most frequently and suggested that in consequence, the radiate morph would be favoured as both a donor and recipient of cross pollen within the radiate morph and as a recipient of non-radiate pollen in crossing between morphs. In a similar study in which pollinator data suggested one pollen donor to be favoured, subsequent genetic analysis of progeny data showed the opposite to be the case. In populations of *I.purpurea* polymorphic for flower colour, pollinators discriminated against a white-flowered morph suggesting it would not be favoured as a pollen donor (Schoen and Clegg 1985). In contrast, genetic data following progeny testing showed the white-flowered morph to be the favoured contributor to the male gametic pool. In other studies, observation of pollinator behaviour

has not always provided accurate data on gene flow e.g. Schaal (1980) on *Lupinus texensis* and Ennos and Clegg (1982) on *I. purpurea*. Therefore, while studies of pollinator behaviour provide useful indicators of how pollen flow proceeds between individuals in a population, such imformation is not always a reliable way of accurately describing the breeding system in plant populations.

The paternal outcrossing rates recorded in the present study for the two morphs of *S.vulgaris* have important implications with regard to the expected effects on male gametophytic competition within the two morphs. Due to pollen transfer between morphs, radiate and non-radiate pollen types will frequently compete with each other on radiate and non-radiate stigmas. The effects of such competition will be experienced reciprocally by both pollen types, although competition on the former type of stigmas will be more common. In contrast, because intramorph paternal outcrossing is greater in the radiate morph, radiate pollen will be subjected to a greater degree of intramorph pollen competition than is experienced by non-radiate pollen. Thus, while one must exercise a note of caution, it can be assumed that, relative to non-radiate pollen, radiate pollen of a particular genotype is likely to be subject to greater levels of competition from pollen of other genotypes in the course of gaining access to ovules.

In introducing the topic of male competition in *S.vulgaris*, it was assumed (without the benefit of estimates of male outcrossing rate for the radiate and non-radiate morphs) that the radiate morph would be subject to male competition but that the non-radiate morph would not. In light of the results of the present study of paternal outcrossing in the two morphs, it now appears that both radiate and non-radiate pollen are subject to competition for access to ovules; however, the DEGREE of pollen competition that each pollen type is subjected to differs between the morphs, it being greater in the radiate than the non-radiate morph.

CHAPTER 5

POLLEN COMPETITION IN *SENECIO VULGARIS* : A COMPARISON OF THE EFFECTIVENESS OF RADIATE AND NON-RADIATE PLANTS AS SELF AND CROSS POLLEN DONORS.

INTRODUCTION.

In flowering plants, the pollen grain acts as a vehicle for the transport and delivery of male gametes to the embryo sac for fertilisation (Knox, Williams and Dumas 1986). Many plants rely on external agents to mediate pollen transfer and consequently have little direct control over the number or genotype of pollen grains deposited on their stigmas. Although pollen deposition is somewhat haphazard, plants have evolved mechanisms to promote non-random fertilisation and thus possess some degree of control over the paternity and genetic quality of offspring; for example, self-incompatibility mechanisms, (de Nettancourt 1977); microgametophytic selection (e.g. in Alfalfa, Mulinix and Lezzoni 1988).

Gametophytic selection due to pollen competition is thought to play an important role in angiosperm evolution (Mulcahy 1979; Willson and Burley 1983; Crepet 1983). When many microgametophytes vie for access to a limited number of ovules, genetically based differences in pollen performance could lead to, (i) non-random fertilisation of ovules with respect to pollen genotype (Jones 1928; Bateman 1956; Levin 1975; Ottaviano *et al* 1975; van Breuklen 1982; Marshall and Ellstrand 1986), which in turn might affect, (ii) progeny

phenotype - intense pollen competition may favour genes which also increase seed germination and growth rates in the progeny and thus lead to a decrease in the amount of variation of phenotype in sporophytic offspring (Ter-Avanesian 1978a,1978b; Davis *et al* 1987; Schlichting *et al* 1987; Windsor *et al* 1987).

Opportunities for pollen competition occur when the number of pollen grains on the stigmatic surface exceeds the number of available ovules (Mulcahy 1975; Stephenson and Bertin 1983; Willson and Burley 1983). Competition among pollen grains arriving on the stigma is important in determining the relative outcrossing success of different genotypes in a population (Bateman 1956; Harding and Tucker 1969). It is expected that such competition should result in strong natural selection for faster rates of pollen tube growth correlated with higher rates of fertilisation and enhanced reproductive success (Mulcahy 1983).

Pollen competitive ability has been inferred from studies of pollen germinability and pollen tube growth rates (Pfahler 1970; Schemske and Fenster 1983) and from measurements of fruit set, seed set and seedling vigour (Bookman 1984; van der Kloet 1984). Several studies have shown that when many pollen grains are deposited on a stigma, only the fastest growing pollen tubes achieve fertilisation of ovules; in

contrast, when only a few pollen grains are deposited on the stigma, pollen grains producing either fast or slow-growing pollen tubes fertilise the available ovules (Correns 1914,1928; Mulcahy and Mulcahy 1975; Mulcahy *et al* 1975, 1978; Ter-Avanesian 1978). Recent electrophoretic (Tanksley *et al* 1981) and DNA-mRNA hybridisation studies (Willing and Mascarenhas 1984; Willing *et al* 1984) have shown that large portions of the microgametophyic genome are transcribed and translated in the pollen grain and most genes transcribed in the gametophytic phase of the life cycle are also expressed in the sporophyte. The observed correlation between pre- and postzygotic qualities (vigorous pollen tubes produce vigorous progeny, Mulcahy 1971; Mulcahy and Mulcahy 1975) is due, presumably, to the large number of genes expressed during both the gametophytic and sporophytic stages of the life cycle.

When a plant is self-pollinated, the number of pollen grains applied to the stigma may greatly exceed the number of ovules to be fertilised, creating a situation where selection for pollen tubes which penetrate the style very rapidly may occur. It is therefore reasonable to assume that if selfing continues for a number of generations, the result will be a pollen genotype highly selected for rapid growth in that stylar environment. If a mixture of self and cross pollen is subsequently applied to the stigma one would

expect that self pollen (the "specialist") would 'win out'. This is precisely what Jones (1928) found from studies of selective fertilisation in *Zea mays*. Johnson and Mulcahy (1978) also tested this hypothesis in *Zea mays* and found that percentage self-fertilisation did increase with increasing number of generations of selfing. Similarly, Currah (1983) reported that in some cases (though not all) self pollen of *Allium cepa* outcompeted non-self pollen. In contrast, Pfahler (1967) using F_1 hybrid *Zea mays* found no difference between self and cross pollen in relative competitive ability. However, Mulcahy (1984) has suggested that this result most probably occurred due to the population of gametes produced in an F_1 hybrid being "new and unselected" and, therefore, lacking the advantage of previously selected self pollen such that some pollen would be superior and some would not.

The concept of gametophytic selection favouring a highly specialised self-pollen type contrasts with the hypothesis of Charnov (1982) and Willson (1983) that male gametophytic selection will only occur in an outcrossing but not a selfing hermaphrodite. Charnov (1982) and Willson (1983) reason that because all pollen which fertilises an obligate selfing hermaphrodite will be of the same genotype, no opportunity exists in selfing hermaphrodites for male competition or female choice. Clearly in formulating their

ideas, Charnov and Wilson have discounted the importance of genetic variation produced via mutation within the pool of pollen that fertilises a selfing individual, whereas Mulcahy (1984) considers such variation to be of great significance. Currently, therefore, there are two alternative theories as to how sexual selection may act within hermaphrodites which reproduce by both selfing and outcrossing : (i) mechanisms favouring male competition will evolve in the outcrosser but not in the selfer (Charnov 1982; Willson 1983); or (ii) the selfing morph will produce pollen highly adapted to its own particular stylar environment (Mulcahy 1984).

In the preceeding two chapters the polymorphism for outcrossing rate in radiate and non-radiate *Senecio vulgaris* has been discussed. Estimation of male outcrossing rates, which provides a measure of male reproductive success, showed that intramorph male outcrossing was higher in the radiate morph than the non-radiate morph in all stands (Tables 4.5 and 4.7). As both radiate and non-radiate morphs exhibit intermorph outcrossing, radiate and non-radiate pollen types will both be subjected to competition with pollen types of the 'opposite' morph on both radiate and non-radiate stigmas. Over and above this, however, radiate pollen will be subjected to more competition with other radiate pollen types (intramorph competition) due to the higher intramorph male outcrossing rates exhibited by the

radiate morph. Therefore, it is expected that overall, the degree of pollen competition that radiate and non-radiate pollen types are subjected to will be greater for radiate relative to non-radiate pollen.

The aims of the studies presented in this chapter were (i) to examine the relative effectiveness of radiate and non-radiate pollen to fertilise ovules when a mixture of the two pollen types is applied to stigmas of both morph types; and (ii) to interpret the results obtained in the light of the contrasting views held by Mulcahy (1984), and Charnov (1982) and Willson (1983) in regard to how selection acts on pollen effectiveness in morphs that differ in male outcrossing rate. A pilot study of the effectiveness of radiate and non-radiate pollen in achieving fertilisation of a disproportionate share of available ovules (Irwin 1986) suggested that the radiate morph was more successful as both a self and cross (between morphs) pollen donor. If pollen of the radiate morph is subjected to more competition than pollen produced by the non-radiate morph (as would be expected according to the ideas of Charnov (1982) and Willson (1983)), then pollen from the radiate morph is likely to outcompete non-radiate pollen in the post-pollination phase. This possibility is subjected to more detailed analysis in the studies reported below.

MATERIALS AND METHODS.

Crossing programme 1.

Seed collected from inbred lines of radiate and non-radiate *Senecio vulgaris* derived from a Cardiff population (Irwin 1986) was sown out on 7cm pots containing Arthur Bowers Universal potting compost in a glass house at the Botanic Gardens, St.Andrews. Seedlings were thinned to two per pot at a height of about 1cm and to one per pot at about 3cm. Plants were subsequently repotted into 11.5cm pots at a height of about 5cm. Five pairs of plants were raised to flowering under a 16 hour daylength (supplied by 400 watt mercury vapour lamps) and watered when necessary. Each pair consisted of one radiate and one non-radiate plant. Once plants had commenced flowering and were producing several new capitula each day, a single capitulum on each plant was selected and emasculated by removing the top 1-2mm of the capitulum, prior to opening, using a scalpel blade. This removed the fused anther tube inside the top of each floret. Each emasculated capitulum was washed with water to remove any loose pollen (using a pasteur pipette) and covered by a small bag made of lens tissue which was secured with a pin to prevent contamination by foreign pollen. Capitula were then left to allow styles to elongate. Once the stigmas had

opened out and become receptive (after about 72 hours), self pollen followed immediately by cross pollen from the other plant of a selected pair, was applied to the stigmas of the emasculated capitulum. All pollen applied came from newly opened capitula and was of a similar age. For each of the five pairs of plants used, both plants were treated (reciprocally) in the same way. Each test capitulum was then rebagged and left in the glasshouse to set seed (about two weeks). Wherever possible five capitula per plant were treated in the same way. In addition, some capitula were emasculated and left untreated to act as controls. Seed produced by each test capitulum was collected, counted and sown out in seed trays on Arthur Bowers universal potting compost. The progenies produced were raised to flowering and scored for capitulum type.

Crossing programme 2.

The procedure used here was the same as for crossing programme 1 but expanded to allow the effect of temperature on pollen germination to be tested. Four pairs of radiate and non-radiate plants from the Cardiff population were selected which had shown in previous investigations (Irwin 1986, and crossing programme 1) highly significant deviations from an expected 1:1 ratio of progeny sired by radiate or non-radiate pollen following pollination with a

mixture of pollen from the two parent plants. Representatives of each of these lines were raised to flowering at the Botanic gardens as before. Once flowering had commenced, replicate pairs of plants were placed in growth rooms with the temperature in one room set at 15°C and in the other at 20°C. In all other respects the environmental conditions in the two rooms were the same. Five capitula on each plant were emasculated and pollinated with self and cross pollen, as described for crossing programme 1, producing a total of 80 test capitula. Seed was collected from each capitulum, counted and sown out on Arthur Bowers universal compost. Progenies were raised to flowering and subsequently scored for capitulum type.

Examination of segregation ratios in the F_2 of crosses between radiate and non-radiate morphs.

F_1 heterozygotes at the ray floret locus were selected from among the progeny raised from crosses produced in crossing programme 1 and used to test (i) the effect of emasculation on the effectiveness of pollen to fertilise ovules; and (ii) any deviation from the expected F_2 ratio which might point to a difference in the relative effectiveness of pollen carrying either the radiate or the non-radiate allele in fertilising ovules of the F_1.

Two heterozygotes produced among the progeny of each of five replicate crosses between lines FR9 and FN42 and between FR7 and FN32 were selected at random. This provided a total of 20 F_1 heterozygotes for use in the study. Two capitula per plant were bagged and left to set self seed, while another two capitula of the same plant were emasculated, pollinated with self pollen (once stigmas were receptive to pollen), rebagged and left to set seed. Seed from each F_1 was collected, counted and sown out on trays of Arthur Bowers universal compost at the Botanic gardens. Progeny were raised to flowering and scored for capitulum type.

RESULTS.

Crossing programme 1.

For two out of five pairs of inbred lines tested, FR9/FN42 and FR20/FN28 (Table 5.1), more progeny resulted from fertilisation of ovules by non-radiate pollen than by radiate pollen whether non-radiate pollen was applied as self or cross pollen. In another pair of lines, FR7/FN31, cross pollen, whether radiate or non-radiate, was more effective in achieving fertilisation than self pollen, while for the pair of lines, FR13/FN27, non-radiate pollen was more effective when used as cross pollen but showed no advantage over radiate pollen when applied as self pollen. Finally, comparisons involving the pair of lines FR15/FN32 showed no advantage to either pollen type used as self or cross pollen. In no instance, therefore, was there any evidence of radiate pollen being more effective than non-radiate pollen when used as both self and cross pollen within a particular pair of lines treated.

Crossing programme 2.

With the pair of lines FR18/FN42, more progeny resulted from fertilisation of ovules by non-radiate pollen than by

Table 5.1. Percentage progeny produced as a result of self or cross pollination where self and cross pollen were applied at the same time to emasculated capitula of radiate and non-radiate *Senecio vulgaris*.

Cross			(n)	Self progeny	Cross progeny	X^2	
Female		Male					
FR9	x	FN42	124	10.48	89.52	77.45	**
FN42	x	FR9	321	63.86	36.14	24.68	**
FR20	x	FN28	205	33.66	66.34	22.00	**
FN28	x	FR20	183	57.92	42.08	4.59	*
FR7	x	FN31	277	45.13	54.87	2.63	*
FN31	x	FR7	121	35.54	64.46	10.12	**
FR15	x	FN32	144	54.86	45.14	1.36	N.S.
FN32	x	FR15	241	53.53	46.47	1.20	N.S.
FR13	x	FN27	75	33.33	66.67	8.33	**
FN27	x	FR13	173	50.87	49.13	0.05	N.S.

FR = Radiate *S.vulgaris* from Cardiff.
FN = Non-radiate *S.vulgaris* from Cardiff.
X^2 (1 df) for the hypothesis of equal fertilisation by both pollen types.
$X^2 = 3.841$, $p = 0.05$ * $X^2 = 6.635$, $p = 0.01$ **

Table 5.2. Percentage progeny produced as the result of self or cross pollination at 15°C where self and cross pollen were applied at the same time to emasculated capitula of radiate and non-radiate *Senecio vulgaris*.

Cross			(n)	Self progeny	Cross progeny	X^2
Female		Male				
FR18	x	FN42	66	25.76	74.24	15.51 **
FN42	x	FR18	177	73.45	26.55	38.92 **
FR13	x	FN31	41	56.10	43.90	0.609 N.S.
FN31	x	FR13	112	34.82	65.18	10.32 **
FR20	x	FN28	89	70.79	29.21	15.38 **
FN28	x	FR20	97	46.39	53.61	0.505 N.S.
FR9	x	FN2	48	47.92	52.08	0.080 N.S.
FN2	x	FR9	180	47.22	52.88	0.555 N.S.

FR = Radiate *S.vulgaris* from Cardiff.
FN = Non-radiate *S.vulgaris* from Cardiff.
X^2 (1 df) for the hypothesis of equal fertilisation by both pollen types.
$X^2 = 3.841$, $p = 0.05$ * $X^2 = 6.635$, $p = 0.01$ **

Table 5.3. Percentage progeny produced as a result of self or cross pollination at 20°C where self and cross pollen were applied at the same to emasculated capitula of radiate and non-radiate *Senecio vulgaris*.

Cross			(n)	Self progeny	Cross progeny	X^2
Female		Male				
FR18	x	FN42	206	18.93	81.07	79.53 **
FN42	x	FR18	222	59.46	40.54	7.95 *
FR13	x	FN31	186	42.47	57.53	4.21 *
FN31	x	FR13	134	79.10	20.90	45.40 **
FR20	x	FN28	157	63.06	36.94	10.71 **
FN28	x	FR20	214	49.53	50.47	0.02 N.S.
FR9	x	FN2	136	55.15	44.85	1.44 N.S.
FN2	x	FR9	91	59.34	40.66	3.17 N.S.

FR = Radiate *S.vulgaris* from Cardiff.
FN = Non-radiate *S.vulgaris* from Cardiff.
X^2 (1 df) for the hypothesis of equal fertilisation by both pollen types.
$X^2 = 3.841$, $p = 0.05$ * $X^2 = 6.635$, $p = 0.01$ **

Table 5.4. Segregation of progeny following self-pollination of emasculated and unemasculated capitula from heterozygous individuals produced in crossing programme 1.

Heterozygote	RR	RN	NN	X^2
FR9/42	271	526	279	0.655 N.S.
FN42/9	257	491	266	1.169 N.S.
FR7/31	193	428	205	1.440 N.S.
FN31/7	218	375	170	6.262 *
FR9/42 (e)	257	496	267	0.965 N.S.
FN42/9 (e)	217	395	228	3.264 N.S.
FR7/31 (e)	176	352	162	0.852 N.S.
FN31/7 (e)	150	341	169	1.828 N.S.

FR = Radiate *S.vulgaris* from Cardiff.
FN = Non-radiate *S.Vulgaris* from Cardiff.
X^2 (2 df) = 5.991, p = 0.05 *
X^2 (2 df) = 9.210, p = 0.01 **

radiate pollen at both 15° C and 20° C (Tables 5.2 and 5.3). In contrast, comparisons involving lines FR13/FN31 showed that at 15° C radiate pollen was more effective as cross pollen but held no advantage when applied as self pollen (Table 5.2), while, rather surprisingly, when the same material was tested at 20° C, more ovules were fertilised by non-radiate than radiate pollen whether pollen was applied as self or cross pollen (Table 5.3). Comparisons involving the FR20/FN28 combination, at both 15° C and 20° C revealed that radiate pollen was more effective when applied as self pollen but showed no advantage as cross pollen. These results stand in marked contrast to the results for the same pair of lines obtained in crossing programme 1 (Table 5.1) where non-radiate pollen was seen to hold a clear advantage whether applied as self or cross pollen. Finally, comparisons involving the combination FR9/FN2, showed no advantage to either pollen type used as self or cross pollen at either 15° C or 20° C.

Segregation ratios in F_2's.

In general, F_2 progenies produced by selfing heterozygotes of FR9/FN42 and FR7/FN31 crosses exhibited the expected 1:2:1 segregation ratio (Table 5.4) whether (i) the F_1 was left to set seed by natural self-pollination; or (ii) capitula of the F_1 were emasculated and self pollen was

applied to stigmas. This result confirmed therefore, that the emasculation technique does not affect the ability of pollen bearing the Tr or the Tn alleles to germinate on stigmas and fertilise ovules. Only one class of heterozygote yielded a significant deviation from the expected 1:2:1 ratio in the F_2 following natural self pollination; this was the heterozygote produced from crossing FN31 and FR7 where FN31 was the maternal parent. In the F_2 of this cross more radiate progeny were produced than expected ($p<0.05$ - Table 5.4).

DISCUSSION.

The aim of the crossing programmes reported in this chapter was to examine the relative effectiveness of radiate and non-radiate pollen types to fertilise ovules when a mixture of the two was applied to stigmas of both morph types. No consistent pattern of results showing an advantage to radiate or non-radiate pollen types emerged from the two crossing programmes (Tables 5.1 - 5.3). In crossing programme 1, five of the seven significant deviations from the hypothesis of equal fertilisation by radiate and non-radiate pollen, showed an advantage to the non-radiate pollen type, (Table 5.1), while the two remaining deviations resulted from advantage shown by the radiate pollen type. For two pairs of lines tested (FR9/FN42 and FR20/FN28) non-radiate pollen held an advantage whether applied as self or cross pollen; however, within no pair of lines treated was a similar advantage found for radiate pollen.

In crossing programme 2, four significant deviations from the expected F_1 ratio of self and cross progeny were detected at 15° C (Table 5.2). For one pair of lines (FR18/FN42) non-radiate pollen was more effective whether used as self or cross pollen. Both other deviations were due to an advantage shown by radiate pollen. Similar results

were obtained for material tested at 20°C with the exception that in one pair of lines, FR13/FN31, the advantage of radiate pollen when used as cross pollen was lost, and, instead, non-radiate pollen held an advantage whether used as self or cross pollen. Rather surprisingly, radiate pollen held its advantage over non-radiate pollen at both temperatures when applied as self pollen in the FR20/FN28 combination. This was in marked contrast to what had been found in crossing programme 1 where non-radiate pollen held a clear advantage over radiate pollen whether applied as self or cross pollen (Table 5.1).

The pairs of lines used in crossing programme 2 were selected on the basis of previous results obtained by Irwin (1986) or from crossing programme 1, where large deviations from the expected 1:1 ratio of self:cross progeny had been obtained. When the results obtained in crossing programme 2 were compared with previous results (Table 5.5), the pattern of results showed no consistent trends. Results obtained by Irwin (1986) from studies of lines FR18/FN42, FR13/FN31 and FR9/FN2 all showed a very clear advantage to radiate pollen, (applied as either self or cross pollen in the first two pairs of lines tested and as self pollen in the third). In contrast, in crossing programme 2 the comparison involving lines FR18 and FN42 showed a significant deviation in favour of the non-radiate morph at both temperatures, while

Table 5.5. Comparison of results obtained following pollination of emasculated capitula of radiate (FR) and non-radiate (FN) S. vulgaris with a mixture of self and cross pollen. Previous comparisons are taken from +Irwin (1986) and crossing programme 1.

CROSS ♀ ♂	PREVIOUS STUDIES				CROSSING PROGRAMME 2							
					15°C				20°C			
	SELF	CROSS	(n)	X^2	SELF	CROSS	(n)	X^2	SELF	CROSS	(n)	X^2
+FR18 x FN42	95.50	4.50	(44)	36.36***	25.76	74.24	(66)	15.51***	18.93	81.07	(206)	79.53***
+FN42 x FR18	2.10	97.90	(48)	44.08***	73.45	26.55	(177)	38.92***	59.46	40.54	(222)	7.95*
+FR13 x FN31	84.40	16.60	(35)	15.11***	56.10	43.90	(41)	0.61 N.S.	42.47	57.53	(186)	4.21*
+FN31 x FR13	15.00	85.00	(40)	19.60***	34.82	65.18	(112)	10.32**	79.10	20.90	(134)	45.40***
FR20 x FN28	33.66	66.34	(205)	22.00***	70.79	29.21	(89)	15.38***	63.06	36.94	(157)	10.71**
FN28 x FR20	57.92	42.08	(183)	4.59*	46.39	53.61	(97)	0.51 N.S.	49.53	50.47	(214)	0.02 N.S.
+FR9 x FN2	87.50	12.50	(24)	13.50***	47.92	52.08	(48)	0.08 N.S.	55.15	44.85	(136)	1.44 N.S.
+FN2 x FR9	-	-	-		47.22	52.88	(180)	0.56 N.S.	59.34	40.66	(91)	3.17 N.S.

FR = radiate S. vulgaris; FN = non-radiate S. vulgaris

(n) = number of progeny screened

X^2 for the hypothesis of equal fertilisation by both pollen types

$X^2 = 3.841$ $p = 0.05^*$

$X^2 = 6.635$ $p = 0.01^{**}$

$X^2 = 10.827$ $p = 0.001^{***}$

for the FR13/FN31 combination non-radiate pollen was more effective at 20° C. No difference in the effectiveness of either pollen type to fertilise ovules was recorded for the FR9/FN2 combination at either temperature.

Turning to the results for segregtion ratios among F_2 families (Table 5.4), only one significant deviation was found from the expected 1:2:1 ratio. This occurred in the F_2 produced from the cross FN31 (female) x FR7 (male). The fact that only one significant deviation from a 1:2:1 ratio was detected suggests that neither pollen type (carrying either the Tr or the Tn allele) holds advantage over the other in ability to fertilise the ovules of the F_1 intermediate morph.

The results quoted from Irwin (1986) in Table 5.5 formed part of a pilot study into the effectiveness of radiate and non-radiate pollen in achieving fertilisation of a disproportionate share of available ovules. The study suggested that the radiate morph was more successful as both self and cross (between morphs) pollen donor. The results of the pilot study supported the idea that the radiate morph, being subject to more intramorph pollen competition as a consequence of a higher intramorph male outcrossing rate, would outcompete the non-radiate morph in the post-pollination phase according to the hypotheses of

Charnov (1982) and Willson (1983). However, the results of the present studies have failed to confirm this finding. As a result of this inconsistency in the results obtained, it is not possible to determine whether the views held by Mulcahy (1984) or those of Charnov (1982) and Willson (1983) most closely explain how selection acts on pollen effectiveness in morphs differing in male outcrossing rate.

The possible reasons why inconsistent results have been obtained from the studies conducted so far are : (a) there is no advantage to either pollen type- both being equally successful at achieving fertilisation. In this case the results are artifacts of the experimental technique employed; (b) pollen bearing the Tr allele does hold an advantage in some combinations of lines, as found in the pilot study by Irwin (1986), while pollen bearing the Tn allele holds advantage in other combinations (Table 5.1); however, again the experimental technique employed is too crude to detect these differences on every occasion.

When the experimental technique is examined critically, there is good reason to believe that the method of pollen application may have been too crude. One pollen type applied to the stigma immediatly following another, may have resulted in the first pollen type being "rubbed off" during application of the second type. Alternatively, the first

pollen type may have formed a layer over the stigmatic surface such that it was difficult for the second pollen type to come into contact with the stigmatic surface and hence to germinate.

With regard to the effects of the method of pollen application, Thomson (1988) has used a naturally occurring visual marker in pollen of *Erythronium grandiflorum* (Liliaceae) to investigate the effect of the spatial arrangement of pollen grains on germination time and the relationship between pollen density and mean germination rate. He found that when pollen was applied to stigmas of *Erythronium* by hand or by pollinators (bees), pollen load sizes and distributions frequently included clumps of 5-15 pollen grains. To see if clumping of pollen grains affected the rate of pollen germination, Thomson spread the clumped grains evenly over the surface of one of the stigmatic lobes leaving grains clumped together on the other lobe as a control. The results showed that the removal of clumps of pollen grains yielded faster germination rates i.e. clumping strongly delayed germination of pollen . A similar though less intense effect was observed when pollen was applied to stigmas at high density, i.e. a reduction in pollen germination.

Taking note of the findings of Thomson (1988) it is possible

that the inconsistent results obtained in the studies reported for *S.vulgaris* may be due to random variation in the degree of clumping of pollen grains applied to stigmas. This could have affected the germination rate of one or other pollen type and, in turn affected the relative ability of pollen types to fertilise ovules.

The order in which pollen types were applied to stigmas might also have affected which pollen type fertilised most ovules. Epperson and Clegg (1987) found that the first pollen applied to the stigma tended to hold advantage in *Ipomoea purpurea*. Second pollinations following immediatly after the first suffered a significant disadvantage being only half as effective as first pollinations. A delay of as little as 20 minutes rendered subsequent pollinations largely ineffective. However, results of other studies involving sequential pollinations are varied. In a similar study to that of Epperson and Clegg, Eenink (1982) found that in Witloof chicory (*Cichorum intybus*) second pollinations conducted 60 minutes or more after first pollinations were ineffective. Likewise, Marshall and Ellstrand (1985) found a 30 minute delay rendered second pollinations ineffective in *Raphanus sativus*. In contrast, in apple (Visser and Verhaegh 1980) and in pear (Visser and Marcucci 1983) second pollinations delayed 1 or 2 days after first pollination were more effective! In these two fruit

species, it seems that the first pollination "paves the way" for the second.

It is clearly difficult to draw any firm conclusions from the results presented in this chapter, although the findings are worthy of attention. The fact that there was one example of a significant deviation from the 1:2:1 ratio expected following the selfing of a heterozygote at the ray floret locus (Table 5.4) suggests that perhaps larger sample sizes might reveal further differences in the effectiveness of pollen carrying the Tr or Tn allele at least in some combinations of lines. Mulcahy and Kaplan (1979) stated that deviations from Mendelian ratios are rarely reported in the literature, not because they actually are rare, but because they are extremely difficult to detect. To be 90% certain of detecting a 1% deviation from an expected frequency of 50% (in say a backcross generation), they estimated it would be necessary to use a sample of at least 26,546 progeny. Very few studies, including the one presented here include sample sizes of that order. Taking this into account, together with the fact that some of the deviations from expected 1:1 ratio of self to cross progeny in crossing programme 2 occurred at both temperatures, the inconclusiveness of the results leaves room for more detailed investigations to be conducted.

The following chapter discusses a number of further experiments in which a different approach was taken to determine if either pollen type (radiate or non-radiate) has an advantage over the other over all maternal parents tested, or, on stigmas of only certain maternal plants.

CHAPTER 6.

POLLEN COMPETITION IN *SENECIO VULGARIS* : EFFECT OF POLLEN GERMINATION ON STYLE LENGTH.

INTRODUCTION.

The studies reported in chapter 5 failed to confirm a previous finding of Irwin (1986) that radiate pollen was more effective than non-radiate pollen in fertilising the available ovules after a mixture of radiate and non-radiate pollen was applied to stigmas of either the radiate or non-radiate morphs of *S.vulgaris*. The results obtained in chapter 5 showed no consistent pattern in regard to distorted segregation ratios and, bearing in mind the inadequacies of the technique employed, an alternative approach was required to further examine the possibility that pollen types of the two morphs might differ in their relative effectiveness to fertilise ovules.

The alternative approach employed in the studies reported in this chapter is based on a relationship between style length and pollen germination recently discovered in *S.vulgaris*. Studies by Ashton (1984), Irwin (1986) and Graham (1989) have revealed that in florets in which pollen has germinated on stigmas, styles are short, while in florets where pollen has failed to germinate on stigmas, styles are long. The first indication of this relationship came from studies by Ashton (1984), and Irwin (1986) which showed that the radiate morph from two Welsh populations of *S.vulgaris* (at

Cardiff and Rhosllanerchrugog) produced capitula which, when left to self, contained a higher frequency of florets in which the stigma was exserted above the level of the corolla rim (24 hours after stigmas became receptive to pollen) than were present in similar capitula of the non-radiate morph. An analysis of the cause of exserted stigmas in florets (Irwin 1986) indicated that pollination of virgin stigmas following emasculation resulted in shorter styles compared to styles of pistils within the same capitulum whose stigmas were not pollinated. Further examination of pollinated stigmas under the fluorescence microscope revealed that 99% of stigmas which had been treated with pollen bore germinating pollen grains indicating a link between pollen germination and failure of stigmas to be exserted from florets.

Graham (1989), in a subsequent study of the relationship between stigma exsertion and self pollen germination in *S.vulgaris*, found a correlation between the frequency of florets containing exserted stigmas in a capitulum and the percentage of stigmas that bore no germinating pollen grains. In capitula of both radiate and non-radiate plants left to self-pollinate in a glasshouse, more than 70% of exserted stigmas had no germinating pollen grains present on the stigmatic surface (78% in the radiate morph and 71% in the non-radiate morph) while the same was true for only

10-13% of inserted stigmas (i.e. those positioned below the level of the corolla rim 24 hours after becoming receptive to pollen). In his study, Graham (1989) also reported the number of germinating self-pollen grains present on the stigmatic surface in the radiate and non-radiate morphs of *S.vulgaris*. He established that radiate plants possessed less germinating pollen grains on both exserted and inserted stigmas than were present on the corresponding stigmas of non-radiate plants.

Based on the findings of Irwin (1986) and Graham (1989), it would seem that capitula of the radiate morph which are left to self pollinate, contain more florets with exserted stigmas than capitula of the non-radiate morph (as found by Ashton 1984) due to radiate self-pollen being slower to germinate on radiate stigmas than non-radiate self-pollen on non-radiate stigmas. This situation in capitula left to self pollinate, though of great interest, tends to obscure a difference between radiate and non-radiate pollen in relative effectiveness to germinate on stigmas after pollen is physically applied to self or cross stigmas.

As mentioned previously, Irwin (1986) established within both radiate and non-radiate capitula, that when florets were emasculated and pollination was prevented, styles grew long. In contrast, if stigmas were pollinated by physical

application of pollen following emasculation, short styles resulted. Of particular significance, however, was the finding that if capitula of both morphs were emasculated, divided in two, and treated such that one half was self-pollinated and the other half cross-pollinated (with cross pollen always coming from the 'other' morph) then styles of pistils self or cross pollinated with radiate pollen, were always shorter than styles self or cross pollinated with non-radiate pollen ($p < 0.01$ in both cases - see Irwin 1986). It was concluded from this result that following physical application of pollen to stigmas, radiate pollen is more rapid to germinate than non-radiate pollen and hence produces shorter styles in pistils pollinated with radiate pollen.

Given that short style length is a symptom of effective pollination/fertiliastion, this relationship provides a basis for a bioassay aimed at comparing the relative effectiveness of pollen from the two morphs of *S.vulgaris* in achieving pollination/fertilisation.

The aims of the experiments conducted in this chapter were: (i) to establish that short style length following the physical application of pollen to stigmas in emasculated capitula (reported by Irwin, 1986) is due to pollination rather than simply the effect of brushing stigmas; (ii) to

confirm the finding that the styles of pistils pollinated with self pollen are shorter than those of pistils to which no pollen is applied; (iii) to establish if the physical application of pollen to stigmas in emasculated capitula results in styles ceasing to elongate, or causes styles of pollinated stigmas to shrink; (iv) to compare the relative effectiveness of self and cross pollen from radiate and non-radiate pollen donors, over a range of radiate and non-radiate maternal parents, by means of a bioassay based on the relationship between style length and the physical application of pollen; and (v) to compare the speed of fertilisation of ovules by pollen from radiate and non-radiate pollen donors by removing the stigmas and styles from emasculated capitula at varying time intervals after pollen application.

MATERIALS AND METHODS.

Plant cultivation.

All plants used in these studies were inbred lines derived from a Cardiff population of *S.vulgaris* (see Chapter 5). Seed from inbred lines of the radiate and non-radiate morphs was sown out on 7cm pots containing Arthur Bowers universal compost in a glass house at the Botanic Gardens St. Andrews. Seedlings were thinned to 2 per pot at a height of approximately 1cm and to 1 per pot at about 3cm. Plants were subsequently repotted into 11.5cm pots at a height of about 5cm. All plants were raised to flowering under a 16 hour photoperiod (supplied by 400watt mercury vapour lamps) and watered when necessary. Once flowering commenced, plants were transferred to a growth room kept at 15° C.

Experiment 1 : Effect of brushing stigmas on style length.

One capitulum on each of two radiate and two non-radiate plants was emasculated as described in chapter 5, bagged, and left for the stigmas to become receptive to pollen (after about 72 hours). Once stigmas were receptive to pollen, each emasculated capitulum was divided into two by inserting a small square of acetate sheet vertically into

the capitulum. Stigmas on one side of the capitulum were left untreated, while stigmas on the other side were brushed gently using a fine paint brush to simulate the action of one capitulum being rubbed by another to effect pollination. Each of the 4 test capitula was rebagged and left for 24 hours. A sample of ten florets was removed at random from each half of the capitulum and mounted on a slide using sellotape. The length of each style from the base of the ovule to the level of the receptive stigma was measured to estimate style length using a vernier microscope.

Experiment 2 : Effect of pollination on style length.

One capitulum on each of two radiate and non-radiate plants was emasculated. Once stigmas were receptive to pollen, each emasculated capitulum was divided into two halves as described for experiment 1. Stigmas on one side of the capitulum were left untreated, while stigmas on the other side of each test capitulum were treated with self pollen from another capitulum of the same plant. Before each of the 4 test capitula was rebagged, ten florets were removed at random from each half capitulum and mounted on slides using sellotape. Capitula were rebagged and left for 4 hours. A further ten florets were then removed from each half capitulum, mounted on slides and the length of each style from the base of the ovule to the level of the receptive

stigma measured using a vernier microscope.

Experiment 3 : Change in style length over time following treatment of stigmas with self pollen.

Six capitula on each of two radiate and non-radiate plants were emasculated. Once stigmas were receptive to pollen, all capitula were divided into two halves using a piece of acetate film. Stigmas on one side of each capitulum were left untreated, while stigmas on the other side were treated with self pollen from another capitulum on the same plant. All 24 test capitula were rebagged, and at intervals of 1,3,6,12,18 and 24 hours after pollen application, ten florets were removed from each half of a single capitulum, mounted on slides, and measured as before using a vernier microscope.

Experiment 4 : Effect of self and between morph cross pollination on style length of radiate and non-radiate plants.

One capitulum on each of nine radiate and nine non-radiate plants was emasculated, bagged, and left for stigmas to become receptive to pollen (after about 72 hours). The plants were grouped into nine pairs, each pair consisting of one radiate and one non-radiate plant. Once stigmas were

receptive to pollen, each capitulum was divided into two halves using a small square of acetate sheet. Self pollen from another capitulum on the same plant was applied to stigmas on one side of each test capitulum, while the stigmas on the other side were treated with cross pollen using a capitulum from the plant of the opposite morph in each of the nine pairs. All 18 test capitula were rebagged and left for 24 hours. Ten florets were collected from each half capitulum, mounted on slides using sellotape before measuring the length of the style from the base of the ovule to the level of the receptive stigma by means of a vernier microscope.

Experiment 5 : Effect of self and cross pollen on style length : cross pollen coming from the same or a different morph.

Five capitula on three radiate and three non-radiate plants were emasculated. Once stigmas were receptive to pollen, each capitulum was divided into two halves using a small square of acetate sheet. Stigmas on one side of each capitulum were treated with self pollen from another capitulum on the same plant, while stigmas on the other side were treated with cross pollen from one of the five other plants. Thus, as Table 6.1 shows, pollen from each maternal plant was compared with five cross pollen types for effect

on style length.

Table 6.1 Cross pollination carried out to test the effectiveness of radiate and non-radiate pollen donors on six maternal parents.

	R_1	R_2	R_3	N_1	N_2	N_3
R_1	-	*	*	*	*	*
R_2	*	-	*	*	*	*
R_3	*	*	-	*	*	*
N_1	*	*	*	-	*	*
N_2	*	*	*	*	-	*
N_3	*	*	*	*	*	-

* Comparison made (one half of the capiulum subjected to self pollination and the other half to the cross pollination indicated).
\- Comparison not made

Following pollination, each test capitulum was rebagged and left for 24 hours. All test capitula were then removed from the plants. Ten florets were taken at random from each half capitulum, mounted on a slide with sellotape, and the length of the style from the base of the ovule to the level of the receptive stigma measured using a vernier microscope.

Experiment 6 : Speed of fertilisation of ovules by radiate and non-radiate pollen.

Eight capitula on representative plants of the same radiate and non-radiate lines used in experiment 5 were emasculated. The six plants were arranged in three pairs each consisting of one radiate and one non-radiate plant. Each test

capitulum was emasculated and, once stigmas were receptive to pollen, was divided into two using a small square of acetate sheet. Four of the test capitula on each plant were treated with self pollen and four with cross pollen from the other plant in the pair. All capitula were rebagged. One hour after pollination, the styles of individual florets in one selfed and one crossed capitulum on each plant were cut away just above the top of the ovules using a scaplel blade. This procedure was repeated on the other three selfed and crossed capitula on each plant at 3, 6 and 24 hours after pollen was applied. All capitula were then left for 10 to 14 days at which time a record was made of ovules which had set seed.

Analysis of results.

A nested analysis of variance was carried out on each set of results recorded in experiments 2 - 6 using the statisical computer package GENSTAT. Comparisons between treatments applied to each half of individual capitula in experiments 2 - 5 were made using least significant differences (L.S.D.'s) determined from the residual mean square in each analysis. An analysis of paired comparisons (Sokal and Rohlf p357) was carried out on treatment means within each inbred line in experiments 3 and 6 to ascertain whether differences were significant.

RESULTS.

Effect of brushing stigmas on style length.

The mean style length of pistils whose stigmas had been brushed to simulate pollen application was not significantly different from the mean style length of pistils whose stigmas were left untreated in emasculated capitula (Table 6.2). It is concluded that the effect of brushing stigmas in the course of physically applying pollen to stigmas does not in itself result in short style length.

Effect of self-pollination on style length.

The application of self pollen to virgin stigmas in emasculated capitula caused the styles of pollinated stigmas to shrink (Table 6.3). A small significant difference in style length between pistils which were either pollinated or left unpollinated was present at the time of pollen application with styles of pistils left unpollinated being slightly shorter than styles of pollinated pistils. In contrast, four hours after pollination, styles of pollinated pistils were much shorter than styles of pistils left unpollinated ($p < 0.001$ - Table 6.3). Styles of unpollinated pistils showed no increase in length over the same time

Table 6.2. Mean style length (mm) of pistils whose stigmas were brushed or left untreated in emasculated capitula.

Line	Brushed	Untreated	*p*
FR18	6.98 (0.17)	6.72 (0.13)	$0.2 < p < 0.3$
FR20	7.05 (0.18)	6.89 (0.15)	$0.5 < p < 0.6$
FN42	7.18 (0.18)	7.35 (0.09)	$0.2 < p < 0.3$
FN28	6.89 (0.14)	6.74 (0.08)	$0.3 < p < 0.4$

All readings are mean style lengths of 10 florets taken from each half capitulum.
FR= radiate *S.vulgaris*; FN= non-radiate *S.vulgaris*.
95% confidence limits given in parentheses.

Table 6.3. Mean style length (mm) of pistils which were self-pollinated or left unpollinated at the time of pollen application and four hours after pollination.

Treatment	Time	Line FR18	FR20	FN42	FN28
self pollen	0 hours	7.14 (0.17)	6.84 (0.13)	6.82 (0.11)	6.76 (0.22)
	4 hours	6.50 (0.08)	6.32 (0.14)	6.32 (0.10)	6.32 (0.14)
	t	3.449**	2.701*	3.363**	1.672 N.S.
no pollen	0 hours	7.03 (0.11)	6.75 (0.21)	6.93 (0.14)	6.67 (0.22)
	4 hours	7.00 (0.06)	6.74 (0.11)	6.76 (0.11)	6.68 (0.09)
	t	0.247 N.S.	0.038 N.S.	0.965 N.S.	0.034 N.S.

All readings are mean style lengths of 10 florets sampled from each half capitulum.
95% confidence limits given in parentheses.
Analysis of variance at 0 hours showed significant differences between treatments (self pollinated v unpollinated) within lines within morphs, $p < 0.05$.
L.S.D. (treatments within lines, 0 hours) = 0.117
Analysis of variance at 4 hours showed significant differences between treatments within lines within morphs, $p < 0.001$.
L.S.D. (treatments within lines, 4 hours) = 0.083

interval.

The clear demonstration that pollination of pistils causes styles to shrink 24 hours after treatment came as a surprise as previously it had been assumed that pollination caused styles to cease elongating rather than to shrink. It is possible that if pistils were pollinated immediatly after stigmas had unfurled and before styles had elongated, then pollination would prevent elongation. However, in the present study, styles had elongated to their maximum length before pollination, and the effect of this treatment on styles at this stage of development is clearly to cause shrinkage.

Change in style length over time following treatment of stigmas with self pollen.

As found in the previous study, styles from the side of emasculated capitula to which self pollen was applied were significantly shorter than styles on the side of capitula left unpollinated (Table 6.4). This difference was apparent just one hour following treatment and increased at each time interval up to 24 hours after treatment (Table 6.5).

Table 6.4. Mean style lengths (mm) of pollinated and unpollinated pistils at six different time intervals after treatment.

Time (hours)	Treatment	Line FR18	FR20	FN42	FN28
1	pollen	5.82 (0.16)	5.67 (0.14)	5.35 (0.10)	5.63 (0.11)
	no pollen	6.83 (0.09)	6.39 (0.21)	5.83 (0.10)	6.36 (0.20)
3	pollen	5.58 (0.10)	6.10 (0.21)	6.00 (0.22)	5.60 (0.13)
	no pollen	6.16 (0.07)	6.84 (0.17)	6.60 (0.10)	6.37 (0.15)
6	pollen	5.68 (0.24)	5.78 (0.11)	5.65 (0.13)	5.60 (0.11)
	no pollen	6.42 (0.13)	6.30 (0.19)	6.30 (0.14)	6.13 (0.12)
12	pollen	5.86 (0.20)	6.07 (0.27)	6.28 (0.28)	5.79 (0.18)
	no pollen	6.96 (0.24)	7.02 (0.15)	7.34 (0.16)	6.85 (0.10)
18	pollen	5.44 (0.20)	5.26 (0.18)	5.31 (0.24)	4.74 (0.14)
	no pollen	6.60 (0.12)	6.85 (0.15)	6.31 (0.18)	6.06 (0.19)
24	pollen	5.46 (0.20)	5.56 (0.09)	5.37 (0.04)	5.99 (0.16)
	no pollen	6.96 (0.24)	7.28 (0.20)	6.98 (0.11)	7.56 (0.12)

All readings are mean style lengths of 10 florets sampled from each half capitulum.
95% confidence limits given in parentheses.
Analysis of variance showed significant differences between lines within morphs x times, $p < 0.001$; and between treatments within lines within morphs x times, $p < 0.001$.
L.S.D. (treatments within times...) = 0.209
Analysis of variance of paired comparisons between means within individual lines over times showed significant differences between treatments for all lines, $p < 0.01$.

Table 6.5 Change in style length (mm) over time (hours).

Time (hours)	FR18	FR20	FN42	FN42
1	0.45	0.72	0.48	0.71
3	0.58	0.74	0.60	0.77
6	0.74	0.52	0.65	0.53
12	1.10	0.95	1.05	1.06
18	1.16	1.59	1.00	1.31
24	1.50	1.72	1.61	1.57

Least significant difference for treatment within lines within morphs x times (Table 6.4) showed all differences in style length between pollinated and unpollinated stigmas to be significant, $p < 0.001$.

Effect of self and between morph cross pollination on style length.

(i) Radiate maternal plants.

Five of the nine comparisons made between radiate and non-radiate pollen types on radiate maternal plants showed significant differences in style length between pistils pollinated with self (radiate) and cross (non-radiate) pollen (Table 6.6). In each case the styles of pistils pollinated with radiate self pollen were significantly shorter than styles of pistils pollinated with non-radiate cross pollen. In the four remaining comparisons styles of pistils pollinated with radiate self pollen were also shorter than styles of pistils pollinated with radiate cross pollen but differences were not judged to be significant.

Table 6.6. Mean style lengths (mm) of pistils of radiate *Senecio vulgaris* following treatment with self (radiate) pollen or cross (non-radiate) pollen.

Comparison	self-pollinated (radiate pollen)	cross-pollinated (non-radiate pollen)	L.S.D.
FR7 X FN32	6.53 (0.16)	6.57 (0.06)	N.S.
FR37 X FN29	5.40 (0.17)	5.85 (0.18)	***
FR26 X FN26	5.80 (0.16)	5.85 (0.13)	N.S.
FR14 X FN17	6.15 (0.06)	6.42 (0.20)	*
FR6 X FN31	6.41 (0.13)	6.58 (0.12)	N.S.
FR8 X FN27	5.70 (0.13)	6.55 (0.13)	***
FR18 X FN42	5.45 (0.07)	5.65 (0.07)	N.S.
FR20 X FN28	5.87 (0.11)	6.14 (0.09)	***
FR46 X FN26	6.22 (0.13)	6.91 (0.27)	***

All readings are mean style lengths from 10 florets sampled from each half capitulum.
95% confidence intervals are given in parentheses.
Analysis of variance showed significant differences between self and cross pollen treatments within pairs of lines, $p < 0.001$.
L.S.D. (treatment within pairs) = 0.204*, 0.268**,0.343***

Table 6.7. Mean style lengths (mm) of pistils of non-radiate *Senecio vulgaris* following treatment with self (non-radiate) pollen or cross (radiate) pollen

Comparison	self-pollinated (non-radiate pollen)	cross-pollinated (radiate pollen)	L.S.D
FN32 X FR7	7.08 (0.17)	6.93 (0.14)	N.S.
FN29 X FR37	5.89 (0.17)	5.58 (0.11)	**
FN26 X FR26	6.41 (0.11)	6.50 (0.11)	N.S.
FN17 X FR14	6.48 (0.20)	6.50 (0.14)	N.S.
FN31 X FR6	6.51 (0.10)	6.22 (0.10)	**
FN27 X FR8	6.49 (0.17)	6.13 (0.14)	***
FN42 X FR18	5.84 (0.08)	5.51 (0.17)	***
FN28 X FR20	6.58 (0.16)	6.29 (0.08)	**
FN26 X FR46	6.04 (0.10)	5.77 (0.18)	**

All readings are mean style lengths of 10 florets sampled from each half capitulum.
95% confidence intervals are given in parentheses.
Analysis of variance showed significant differences between self and cross pollen treatments within pairs of lines, $p < 0.001$.
L.S.D. (treatments within pairs) = 0.199*,0.261**,0.327***

(ii) Non-radiate maternal parents.

Six of the nine reciprocal comparisons showed that pistils of non-radiate plants pollinated with radiate cross pollen were significantly shorter than those pollinated with non-radiate self pollen (Table 6.7). For the remaining three comparisons, style length differences were not significant. It was of interest that for two of the comparisons where differences in style length were not significant, differences were also insignificant in the reciprocal comparisons (see Table 6.6).

Effect of self and cross pollen on style length : cross pollen coming from the same or a different morph.

(i) Between morph comparisons.

Styles of pistils of radiate maternal plants pollinated with cross pollen from three non-radiate cross pollen donors were significantly longer than styles of pistils pollinated with self (radiate) pollen (Table 6.8). This was established from an analysis of variance of the total data set with the difference between treatments (within pairs within maternal lines) proving to be significant. The least significant differences computed from the residual mean square of this ANOVA and also separate ANOVAs within each maternal line, indicated that treatment differences were not significant

Table 6.8. Mean style length (mm) of pistils of radiate *Senecio vulgaris* following treatment with self (radiate) pollen or cross (non-radiate) pollen.

Comparison	self-pollinated (radiate pollen)	cross-pollinated (non-radiate pollen)	L.S.D.$_1$	L.S.D.$_2$
FR18 X FN42	5.45 (0.07)	5.65 (0.07)	*	*
X FN28	5.91 (0.14)	5.97 (0.18)	N.S.	N.S.
X FN26	6.11 (0.12)	6.12 (0.14)	N.S.	N.S.
FR20 X FN42	5.52 (0.18)	6.56 (0.20)	***	***
X FN28	5.87 (0.11)	6.14 (0.09)	**	**
X FN26	5.99 (0.11)	6.14 (0.15)	N.S.	N.S.
FR46 X FN42	5.93 (0.04)	6.14 (0.10)	N.S.	*
X FN28	5.73 (0.07)	5.91 (0.10)	N.S.	N.S.
X FN26	6.22 (0.13)	6.91 (0.27)	***	***

All readings are mean style lengths of 10 florets sampled from each half capitulum.
95% confidence intervals are given in parentheses.
Analysis of variance showed significant differences between self and cross pollen treatments within pairs within maternal lines, $p < 0.001$.
Analysis of variance conducted within each maternal line in turn showed significant differences between pairs for FR18, $p < 0.001$, significant differences between treatments within pairs for FR20, $p < 0.001$, and significant differences between both pairs and treatments within pairs for FR46, $p < 0.001$.
L.S.D.$_1$ = least significant difference between treatments within pairs for analyses conducted within maternal lines, = 0.178 (FR18); 0.205 (FR20); 0.207 (FR46).
L.S.D.$_2$ = least significant difference between treatments within pairs within lines from analysis conducted over all maternal lines, = 0.192*, 0.248**, 0.323***.

for every pair of lines examined. Indeed, in tests using FR18 as the maternal parent plant, styles of self-pollinated pistils were significantly shorter than those of pistils cross pollinated with non-radiate pollen in only one of the three pairs of lines examined.

Reciprocal comparisons using non-radiate plants as maternal parents (Table 6.9) showed that, in all but one pair of lines (FN42 x FR20), the style lengths of pistils pollinated with radiate cross pollen were shorter than those of pistils pollinated with non-radiate self pollen. In six of the eight comparisons this difference was significant. Only for the FN42 x FR20 comparison were styles of pistils pollinated with self (FN42) pollen significantly shorter than those of pistils pollinated with radiate (FR20) cross pollen ($p < 0.05$).

(ii) Within morph comparisons.

Tables 6.10 and 6.11 show that very few significant differences were found between self and cross pollen treatments when both pollen sources were of the same capitulum morph. For the radiate morph, application of pollen from FR46 produced shorter styles than pollen from FR18 when FR46 was used as either the self or cross pollen parent (Table 6.10). In addition, FR20 pollen was more effective in reducing style length when compared to FR18

Table 6.9. Mean style length (mm) of pistils of non-radiate *Senecio vulgaris* following treatment with self (non-radiate) pollen or cross (radiate) pollen.

Comparison	self-pollinated (non-radiate pollen)	cross-pollinated (radiate pollen)	L.S.D.$_1$	L.S.D.$_2$
FN42 X FR18	5.84 (0.08)	5.51 (0.17)	***	***
X FR20	5.70 (0.11)	5.92 (0.14)	*	*
X FR46	6.46 (0.14)	5.96 (0.09)	***	***
FN28 X FR18	6.48 (0.08)	5.98 (0.21)	***	***
X FR20	6.58 (0.16)	6.29 (0.08)	**	**
X FR46	6.10 (0.07)	6.06 (0.12)	N.S.	N.S.
FN26 X FR18	6.02 (0.10)	6.00 (0.10)	N.S.	N.S.
X FR20	5.68 (0.11)	5.48 (0.10)	*	*
X FR46	6.04 (0.10)	5.77 (0.18)	**	**

All readings are mean style lengths of 10 florets sampled from each half capitulum.
95% confidence limits are given in parentheses.
Analysis of variance showed significant differences between self and cross pollen treatments within pairs within lines, $p < 0.001$.
Analysis of variance conducted within each maternal line in turn showed significant differences between pairs $p < 0.001$ for FN42, FN28 and FN26, and significant differences between treatments within pairs, $p < 0.001$, for FN42 and FN28, and $p < 0.05$ for FN26.
L.S.D.$_1$ = least significant difference between treatments within pairs for analyses conducted within maternal lines, = 0.185 (FN42); 0.193 (FN28); 0.176 (FN26).
L.S.D.$_2$ = least significant difference between treatments within pairs within lines from analysis conducted over all maternal lines, = 0.181*, 0.237**, 0.303***.

Table 6.10. Mean style length (mm) of pistils of radiate *Senecio vulgaris* following treatment with self or cross radiate pollen.

Comparison	self-pollinated	cross pollinated	L.S.D.$_1$	L.S.D.$_2$
FR18 X FR20	5.95 (0.20)	5.48 (0.08)	***	***
X FR46	6.47 (0.07)	6.30 (0.06)	N.S.	*
FR20 X FR18	6.11 (0.06)	6.08 (0.07)	N.S.	N.S.
X FR46	6.49 (0.11)	6.64 (0.16)	N.S.	N.S.
FR46 X FR18	6.14 (0.09)	6.27 (0.08)	*	N.S.
X FR20	5.40 (0.07)	5.41 (0.07)	N.S.	N.S.

All readings are mean style lengths of 10 florets sampled from each half capitulum.
95% confidence limits are given in parentheses.
Analysis of variance showed significant differences between self and cross pollen treatments within pairs within maternal lines, $p < 0.001$.
Analysis of variance conducted within each maternal line in turn showed significant differences between pairs for FR18, FR20 and FR46, $p < 0.001$; and a significant difference between treatments within pairs for FR18, $p < 0.001$, but not FR20 or FR46.
L.S.D.$_1$ = least significant difference between treatments within pairs for analyses conducted within maternal lines, = 0.204 (FR18); 0.160 (FR20) and 0.116 (FR46).
L.S.D.$_2$ = least significant difference between treatments within pairs within lines from analysis conducted over all maternal lines, = 0.160*, 0.212**, 0.274***.

Table 6.11. Mean style length (mm) within pistils of non-radiate *Senecio vulgaris* following treatment with self or cross non-radiate pollen.

Comparison	self-pollinated	cross pollinated	L.S.D.$_1$	L.S.D.$_2$
FN42 X FN28	5.34 (0.12)	5.57 (0.10)	*	N.S.
X FN26	5.25 (0.09)	5.29 (0.17)	N.S.	N.S.
FN28 X FN42	6.38 (0.10)	6.09 (0.12)	*	*
X FN26	5.87 (0.13)	5.81 (0.07)	N.S.	N.S.
FN26 X FN42	5.51 (0.15)	5.71 (0.10)	N.S.	N.S.
X FN28	6.27 (0.11)	6.13 (0.10)	N.S.	N.S.

All readings are mean style lengths of 10 florets sampled from each half capitulum.
95% confidence limits are given in parentheses.
Analysis of variance showed significant differences between self and cross pollen treatments within pairs within maternal lines, $p < 0.05$.
Analysis of variance conducted within each maternal line in turn showed significant differences between pairs for FN42,FN28,and FN26, $p < 0.001$; but no significant difference between treatments within pairs for FN42, FN28 or FN26.
L.S.D.$_1$ = least significant difference between treatments within pairs for analyses conducted within maternal lines, = 0.182 (FN42); 0.285 (FN28) and 0.251 (FN26).
L.S.D.$_2$ = least significant difference between treatments within pairs within lines from analysis conducted over all maternal lines, = 0.238*, 0.315**, 0.406***.

pollen as a cross but not a self pollen donor. In comparisons involving the non-radiate morph (Table 6.11), application of pollen from FN42 produced shorter styles compared to pollen from FN28 when used as either the self or cross pollen parent. However, there were no significant differences between self and cross pollen treatments in the other comparisons. Taken overall, the results revealed no consistent trend that either self or cross pollen was more effective in reducing style length when comparisons were made within morphs.

Speed of fertilisation of ovules.

Comparison of percentage seed set after removal of styles immediately above the level of the ovule following self or cross pollination (Tables 6.12 and 6.13) showed that, in general, seed set increased the longer the styles were left intact following pollination. Significant differences between times at which the styles were cut away were found for pollination treatments involving lines FR18 and FN42. A significant difference in seed set was also found between self and cross pollen treatments, when FN42 was the maternal parent, with more seed being set following self pollination (Table 6.13). While the results obtained for the FR46 and FN26 comparison (Table 6.12) indicated a marked change in seed set over the 4 time intervals, results were so variable

Table 6.12. Percentage seed set in emasculated capitula of radiate *Senecio vulgaris* following style removal at four different time intervals after pollination.

Comparison	Pollen	Time (Hours) 1	3	6	24
FR18 X FN42	Self	31.10	76.50	72.20	93.75
	Cross	29.51	50.91	80.00	84.20
FR20 X FN28	Self	89.04	87.10	75.00	86.67
	Cross	78.80	82.00	98.70	90.57
FR46 X FN26	Self	1.52	34.50	72.31	80.30
	Cross	36.00	94.60	66.70	62.50

Analysis carried out following arcsin transformation of data.
Analysis of variance showed significant differences between plants $p < 0.05$, but no significant differences between treatments within plants.
Analysis of variance of paired comparisons between treatment means over the 4 time intervals showed a significant difference between times for FR18 x FN42, $p < 0.05$, but no other significant differences.

Table 6.13. Percentage seed set in emasculated capitula of non-radiate *Senecio vulgaris* following style removal at four different time intervals after pollination.

Comparison	Pollen	Time (Hours) 1	3	6	24
FN42 X FR18	Self	56.25	82.67	93.94	85.33
	Cross	52.46	64.30	82.60	61.76
FN28 X FR20	Self	64.28	87.88	62.07	68.75
	Cross	50.72	88.90	86.07	83.33
FN26 X FR46	Self	83.33	89.40	94.10	92.50
	Cross	96.60	76.20	88.57	84.75

Analysis carried out following arcsin transformation of data.
Analysis of variance showed significant differences between plants, $p < 0.05$, but no significant differences between treatments within plants.
Analysis of variance of paired comparisons between treatment means over the four time intervals showed a significant difference between pollen treatments and between times within treatments for the FN42 x FR18 comparison but no other significant differences.

between pollination treatments that the analysis revealed no significant difference between either pollination treatments or times.

DISCUSSION.

The results of a preliminary study presented in this chapter showed that 24 hours after pollen was applied to virgin stigmas, the length of styles of pistils treated with self pollen was significantly shorter than those of pistils left unpollinated (Table 6.3). This effect was apparent one hour after pollination and the difference in style length between pollinated and unpollinated stigmas increased with time (Tables 6.4 and 6.5). The effect of pollination was to make the styles of pollinated stigmas shrink when compared to the styles of unpollinated stigmas. Tests to see if the method of emasculation affected the ability of pollen grains to germinate (see chapter 5), or whether the method of pollen application itself (i.e. brushing stigmas) had an effect on style length (Table 6.2), both proved negative. Therefore, any differences recorded in style length were due to pollination treatments and not an artifact of the experimental technique.

Comparisons between morphs in which self pollen was applied to stigmas on one side of the capitulum and cross pollen from a plant of the 'opposite' morph was applied to stigmas on the other side of the capitulum showed that, with one exception, whenever a difference in style length was found,

styles of pistils pollinated with radiate pollen were shorter (Tables 6.6 - 6.9). This suggested that radiate pollen either germinated faster than non-radiate pollen on the stigmatic surface, or that the pollen tube growth rate of radiate pollen was superior to that of non-radiate pollen in these comparisons. The exact cause of style shrinkage, i.e. germination of pollen on stigmas or fertilisation, is not yet known. It was reported in chapter 5 that radiate pollen is expected to be subjected to more intramorph pollen competition than non-radiate pollen due to the pattern of pollinator movements and male outcrossing rates occurring in polymorphic populations. This being so, the results obtained here, indicating a more 'effective' radiate pollen type are expected based on the hypothesis of Charnov (1982) and Willson (1983) - that in a morph which exhibits a high degree of outcrossing, selection will favour competitively superior pollen.

When self pollen was compared with cross pollen from another plant of the same morph (intramorph comparison) very few significant differences and no consistent trend that either self or cross pollen was more effective in reducing style length was found. The results of the studies that examined the speed of fertilisation of ovules of radiate and non-radiate pollen types showed that, in general, seed set increased the longer styles were left intact following

pollination. While some changes in seed set were found between self and cross pollen treatments, more of these studies are required to establish whether style length effects are correlated with the effectiveness of pollen from different pollen donors to fertilise ovules.

The finding that radiate pollen is more effective than non-radiate pollen in reducing style length is of particular interest in view of the fact that, when left to self pollinate in a glasshouse (i.e. when pollen is not applied to the stigma), unemasculated capitula of radiate plants contain more florets with exserted stigmas (long styles) than non-radiate capitula (Ashton 1984; Irwin 1986). Given that stigma exsertion is related to the presence of germinating pollen on the stigmatic surface (Graham 1989), radiate self pollen appears to be slower to germinate than non-radiate self pollen in untreated capitula, and is only superior to non-radiate pollen, therefore, when physically APPLIED to the stigmatic surface.

Previously it was assumed (Irwin 1986) that pollen germination stopped style elongation, while styles of unpollinated stigmas continued to elongate. The results in Table 6.3, however, show that pollination causes styles to shrink. In the study that showed this effect, the styles were fully elongated at the time pollen was applied to

stigmas and it is possible, therefore, that if pistils were pollinated immediately after stigmas had unfurled and before styles had fully elongated then pollination would prevent further elongation. Either way, cessation of style elongation or style shrinkage act as indicators of rapid pollen germination. The method of classification of exserted and inserted stigmas in unemasculated capitula (employed by Ashton 1984, Irwin 1986 and Graham 1989) was arbitrary, the corolla rim being used as a convenient point of reference to discriminate between stigma types. In practice, the style length of exserted stigmas (those positioned above the rim of the corolla tube - see Ashton 1984, Irwin 1986) varies, with some stigmas exserted well above the corolla rim while others are positioned only just above it. The variation in style length in unemasculated capitula observed by Ashton (1984), Irwin (1986) and Graham (1989) could, therefore, result from either or both of the following : (i) differences in rate of pollen germination causes stylar shrinkage to vary in relation to speed of pollen germination; (ii) rapid pollen germination causes styles to cease elongating while late pollen germination enables styles to elongate to maximum length. Why radiate pollen shows delayed germination in untreated capitula is unknown. However, one possibility is that self pollen lands on the stigma in clumps and hence finds rehydration and/or germination difficult because initially it forms a poor

contact with the stigmatic surface.

Although radiate pollen is slow to germinate in unemasculated capitula the reverse is true in emasculated capitula. However, comparisons show that differences in speed of germination (as reflected by style length differences) between the radiate and non-radiate pollen types following application of pollen to stigmas depends on the maternal parent plant to which pollen is applied (Tables 6.6 - 6.9). Similar findings have emerged from a study by Snow and Mazer (1988) of relative pollen effectiveness in *Raphanus raphanistrum*. In this species, significant differences in pollen competitive ability were found among 4 out of 5 pairs of pollen donors compared. For 3 of these pairs, competitive differences were apparent only on certain maternal parent plants.

Competition among pollen grains for access to ovules should result in selection for faster pollen tube growth rates provided there is genetic variation for this character in a population. While the results of the present study suggest differences in ability to germinate between radiate and non-radiate pollen types, the question remains as to whether a significant amount of genetic variation in pollen competitive ability is present in natural populations of *S.vulgaris*. The evidence presented here suggests that the

dominant factor in deciding which pollen type fertilises what proportion of ovules is genetic complimentarity between pollen grain and pistil. Thus in *S.vulgaris*, selection for improved pollen competitive ability may only be possible with respect to a given stylar environment or inbred line (see also Johnson and Mulcahy 1978; Ottaviano *et al* 1983; Yamada and Murikami 1983).

In their work on *Raphanus raphanistrum* Snow and Mazer (1988) also assessed the competitive ability of pollen derived from different lines relative to a standard unrelated pollen donor, using pollen mixtures applied to 6 wild maternal plants. They found that pollen produced by progeny of plants pollinated for two successive generations with 'heavy' pollen loads from a mixture of three pollen donors (i.e. subjected to intense pollen competition) was not more effective in fertilising ovules when compared to pollen produced by progeny of plants pollinated with 'light' pollen loads from a single pollen donor (i.e. subjected to low levels of pollen competition). On two maternal parent plants, pollen from progeny produced following the application of heavy pollen loads was actually inferior to pollen from progeny of plants subjected to low levels of pollen competition, contrary to expectation. Thus, Snow and Mazer found no evidence from *Raphanus* to support the theory of a genetic basis for the effects of pollen competition.

Documented examples of heritable variation in pollen performance therefore, are rare and often result from unusual characteristics of particular species. For example diploid pollen is competitively superior to haploid pollen in *Solanum tuberosum* (Van Breuklen 1982) and in dioecious *Rumex* species, pollen grains carrying the gene for 'femaleness' appear to be better competitors than their male counterparts (Rychlewski and Zarzycki 1975; Conn and Blum 1981).

Based on our current state of knowledge it seems that male-female interactions are more important than the male genotype alone in determining the outcome of pollen competition. Certainly in the study presented in this chapter they were an important factor, while in *Raphanus sativus*, both pollen donor identity and donor-recipient interactions influence the number of seeds sired by competing pollen types (Marshall and Ellstrand 1986, 1988), and in maize, the fertilising ability of a given genotype is known to be affected by male-female interactions as well as male-male interactions (Pfahler 1965,1967; Ottaviano *et al* 1975,1983; Sari-Gorla *et al* 1975). Male-Female interactions between specific plants would lead to the maintenance of variation for pollen tube growth if specific pollen types are favoured in differnt stylar environments. This complimentarity between pollen grain and pistil will lead to

a number of different pollen types being present in the population.

In conclusion, the comparison of the relative effectiveness of radiate and non-radiate pollen types in *S.vulgaris* described in this chapter has produced evidence that real differences in rate of pollen germination may occur. However, pollen germination is controlled not only by the genotype of the pollen grain but also by that of the maternal plant to which the pollen is applied. Taken in conjunction with other studies that have compared the competitive ability of different pollen genotypes, it is not yet clear whether true male-male competition occurs during pollen germination or the process of pollen tube growth in the style. It is possible, even likely, that such competition does occur, but the question of whether it leads to fixation of the gene(s) determining the most competitive pollen type or whether variation for pollen competitive ability is retained in natural populations due to variation in male-female interactions and/or an inverse association between gametophyte and sporophyte fitness remains unanswered.

CHAPTER 7

THE ORIGIN OF RADIATE *SENECIO VULGARIS*: MULTIVARIATE AND ELECTROPHORETIC ANALYSIS.

INTRODUCTION.

The hypothesis that inland radiate *Senecio vulgaris* (*Senecio vulgaris* L var *hibernicus* Syme) has arisen following introgression of genetic material from the introduced Mediterranean diploid species *Senecio squalidus* into the native non-radiate *S.vulgaris* (*S.vulgaris* var *vulgaris*) has been based on three lines of evidence :

(i) Parallel spread of *S.squalidus* and *S.vulgaris* var *hibernicus* in Britain over the past 150 years. *S.squalidus* was first recorded as an escape from the Oxford Botanic Gardens in 1794 and its spread since then has been well documented (Kent 1956, 1957, 1963, 1964a, 1964b, 1964c, 1964d, 1966). *S.vulgaris* var *hibernicus* was first recorded in Cork, Eire in 1866 (Syme 1875) but did not become common in Britain until after 1900. Crisp (1972) has presented evidence of a good correlation in the spread of *S.squalidus* and radiate *S.vulgaris* in England and Wales prior to 1930, with the radiate form appearing only after *S.squalidus* had become established in an area. In many places, however, the expected appearence of radiate groundsel has not always occurred following colonization by *Senecio squalidus* (Stace 1977). In particular, in London and S.E. England *S.squalidus* has been established since 1900, but radiate *S.vulgaris*

remains uncommon.

(ii) Studies which have suggested *S.vulgaris* var *hibernicus* is intermediate between *S.squalidus* and *S.vulgaris* var *vulgaris* for a range of characters, e.g. growth rate (Richards 1975), leaf characters (Monaghan and Hull 1976), relative fitness in terms of seed output (Oxford and Andrews 1977).

(iii) Studies of synthesised hybrids and backcrosses of *S.vulgaris* var *vulgaris* x *S.squalidus*. While some attempts to synthesise the F_1 hybrid *S.vulgaris* x *S.squalidus* have been unsuccessful (Crisp 1972, Alexander 1975, Kadereit 1984), others have been able to produce both non-radiate *S.vulgaris* x *S.squalidus* and radiate *S.vulgaris* x *S. squalidus* triploid hybrids (2n=3X=30) (Harland 1954, Gibbs 1971, Ingram 1977, Taylor 1984). Moreover, it has been shown that fertile progeny can be obtained from backcrossing a radiate *S.vulgaris* x *S.squalidus* hybrid to radiate *S.vulgaris* and a non-radiate *S.vulgaris* x *S.squalidus* hybrid to both radiate and non-radiate *S.vulgaris* (Ingram, Weir and Abbott 1980). By selfing the backcross of non-radiate *S.vulgaris* x *S.squalidus* to non-radiate *S.vulgaris*, Ingram *et al* (1980) produced tetraploid *S.vulgaris* which bore radiate capitula. More recently, Taylor (1984) obtained a tetraploid F_1 hybrid produced as the result of fusion

between an unreduced *S.squalidus* gamete and a haploid gamete from non-radiate *S.vulgaris*. Unlike the triplod hybrids produced by others, this tetraploid hybrid was self-fertile and fully interfertile with *S.vulgaris*. Triploid *S.vulgaris* x *S.squalidus* hybrids have been recorded from natural populations (Stace 1977; Bretel and Leslie 1978; Valentine 1979; Ingram, Weir and Abbott 1980; Marshall and Abbott 1980; Taylor 1984), while F_2 products are rare, only one being recorded by Crisp (1972) and three progeny from two of the tetraploid F_1 hybrids found by Taylor (1984).

An alternative hypothesis to an introgressive origin of the radiate form of *S.vulgaris* is that it arose by mutation. The ray floret character in *S.vulgaris* var *hibernicus* is controlled by a single gene (Trow 1912; Hull 1974). Stace (1977) lists a number of other members of the Compositae in which mutations from the radiate to non-radiate condition have occurred, e.g. the Sea Aster (*Aster tripolium*) and the Ox-eye daisy (*Leucantheum vulgare*), and from a non-radiate to radiate condition e.g. in the Nodding Bur-Marigold (*Bidens cernua*). Non-radiate variants have also been recorded in radiate *Senecio jacobaea* and *Senecio squalidus*. In *S. squalidus*, the non-radiate condition is controlled by a single gene with incomplete dominance like that in *S.vulgaris* (Ingram and Taylor 1982).

It is also possible that radiate *S.vulgaris* could have been introduced into Britain from abroad. Haskell (1953) reported that the variant was associated with western seaports although it is unclear whether he was referring to inland radiate *S.vulgaris* or to the maritime form *S.vulgaris* ssp *denticulatus*. Allen (1967) considered that early records of inland radiate *S.vulgaris* in Britain could have referred to stray alien immigrants, and indeed Richards (1975) found that the inland radiate *S.vulgaris* variant was similar to a Mediterranean race of non-radiate *S.vulgaris* in being slow growing. Richards believed, however, that this similarity was the product of introgression of genes from *S.squalidus* which is itself a Mediterranean species. Furthermore, Richards (1975) states that there is no evidence that radiate *S.vulgaris* is similar to radiate German plants that were described in 1843 (cited in Richards 1975).

While the present evidence favours the hybrid-introgression hypothesis for the origin of radiate *S.vulgaris*, it is not known whether the variant has originated more than once in Britain. Monaghan and Hull (1976) have argued that repeated hybridisation with *S.squalidus* was responsible for the leaf shape of radiate *S.vulgaris* showing greater similarity to that of *S.squalidus* in areas of central Scotland where the latter is abundant. Hull (1976) used the same argument to explain the increase in frequency of the Tr. allele in

S.vulgaris in areas where *S.squalidus* was common but not in areas where it was absent. This argument is supported by the fact that the spread of radiate *S.vulgaris* is generally similar to that of *S.squalidus*. Ingram (1977) reported that radiate *S.vulgaris* hybridised more readily with *S.squalidus* than non-radiate *S.vulgaris* suggesting that once introgression has occurred subsequent hybridisation events would be more likely.

Despite the suggestions of repeated origins of the radiate morph of *S.vulgaris*, and continuing introgression of *S.squalidus* genes into the *S.vulgaris* genome, there remain doubts on how frequent such events may be. In view of the low fertility of F_1 hybrids (wild and artifically synthesised), Ingram et al (1980) concluded that the spread of radiate *S.vulgaris* was probably due to dispersal following a single origin rather than to repeated origins. Stace (1977) also considered it unlikely that the spread of *S.vulgaris* was due to hybridisation events at each site, but rather was the result of a rapid colonisation of available sites. Finally, Taylor (1984) reported that while radiate *S.vulgaris* may have evolved by introgression from *S.squalidus* into *S.vulgaris*, there was no evidence of current introgression occurring.

The studies described in this chapter use electrophoretic

and morpholometric techniques to show that a radiate variant of *S.vulgaris* (plate 7.1) found in a polymorphic population of *S.vulgaris* at York by R.J. Abbott and D.F. Marshall in June 1979 possesses more 'squalidus like' characters than are generally found in radiate *S.vulgaris* and may represent an early stage in the origin of radiate *S.vulgaris* via introgression of *S.squalidus* into *S.vulgaris*.

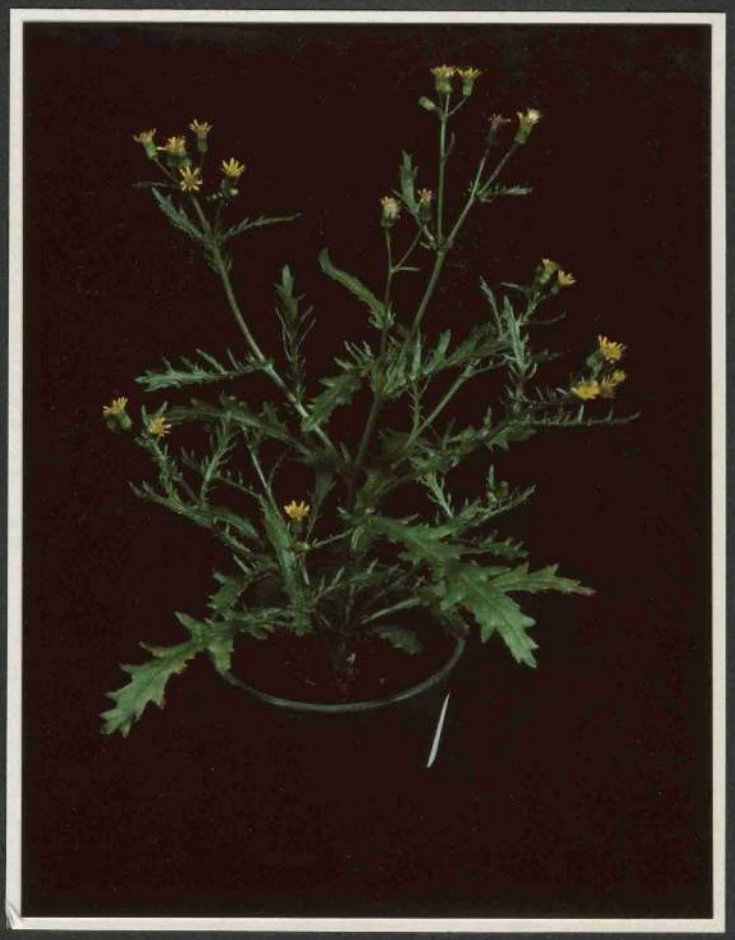


Plate 7.1 The York radiate variant of *S. vulgaris*.

MATERIALS AND METHODS.

(i) Plant material and cultivation.

Seed from radiate and non-radiate *Senecio vulgaris* and *Senecio squalidus* was sampled from populations in Edinburgh and York. The Edinburgh material was collected in June 1987 from the Salamander Street site (NT276763) previously mentioned in chapter 2. Radiate and non-radiate *S.vulgaris* from York was collected from a site adjacent to York railway station by R.J. Abbott and D.F. Marshall in 1979. The *S.squalidus* from York was sampled from locations in the same area in 1988.

Seed collected from ten representative plants of each of the six taxa was sown out on 7cm pots containing Arthur Bowers universal potting compost. Pots were arranged in a randomised 6 x 10 block on a bench in a glass house at the Botanic Gardens, St. Andrews. Seedlings were thinned to two per pot at a height of 1cm and, to prevent competition between developing plants, to one per pot at 2-3 cm. Seedlings were potted on into 11.5cm pots after reaching a height of 5cm. Plants were raised under a 16 hour photoperiod supplied by 400watt mercury vapour lamps and watered when necessary. Plants were harvested for

measurement on the first full day of anthesis of the apical capitulum.

(ii) Morphometrical Analysis

The character set examined was a reduced form of that investigated by Taylor (1984) in a study of introgression in the British *Senecio*. Capitulum morphology in *Senecio* is generally uniform and formal taxonomic treatments of the European members of the group (Chater and Walters, 1976) are largely based on quantitative differences in capitulum size and shape and ray floret development; and on vegetative characters such as growth habit and leaf shape. Thirty seven of the thirty eight quantitative characters analysed in the present study (see below and Table 7.1) were selected on the basis of a possible difference in the character or group of characters between *S.vulgaris* and *S.squalidus* as identified by Taylor (1984). Of the thirty eight characters studied, half were vegetative, sixteen of which described the shape of the mid-leaf. The remaining nineteen characters were descriptors of the capitulum, fifteen of which concerned dimensions of the capitulum and its component parts, the involucre (ring of bracts at the base of the capitulum) and the disc florets; the remaining four capitulum characters described the ray florets. The presence of ray florets is, of course, the diagnostic character for

distinguishing *S.vulgaris* var *vulgaris* (rayless) from *S.vulgaris* var *hibernicus*. There is evidence to suggest that the Tn allele controls the presence of disc florets rather than the absence of ray florets. Taylor showed that when the total number of florets in radiate and non-radiate capitula is compared, there is no significant difference between the two types. (e.g here mean number of florets per capitulum in the York population : radiate = 54.0 +/- 1.97, non-radiate = 56.8 +/- 2.50).

Measurement of characters C1,C2 and C3 (Plant height, inflorescence length and longest leaf length) were made on fresh material. Measurements were to the nearest millimetre. Following this, the leaf nearest to the midpoint of the plant height (the midleaf) was removed, placed in a polythene bag and frozen. Subsequently, the apical capitulum was dissected and characters C3-C10, C15 and C19 were measured or counted. The calyculus bracts, outer florets and a sample of 10 disc florets from the centre of the capitulum were placed on sellotape and mounted on microscope slides allowing measurement of characters C12-C14, C16-C18 and C20-C22 using a vernier microscope. Characters that were single measurements e.g. C4 (capitulum total length) were made to the nearest 0.02mm, and means or ranges of multiple measurements e.g. C12 (mean calyculus bract length) were calculated to the nearest 0.01mm After harvesting was

complete, characters C23-C38 were measured on frozen leaf material. Leaf measurements were made to the nearest 0.1mm and angles to the nearest 30 minutes. Leaf dissection was measured using a Delta T area meter produced by Delta T Devices, Cambridge. The perimeter of the leaf and its area were computed and dissection was then measured as the perimeter divided by the square root of the area, a high ratio indicating a highly divided leaf.

The Character Set.

C1 Plant height.
Length from the base of the stem, defined as the cotyledon node, to the level of the apical capitulum.

C2 Inflorescence length.
The length from the apical stem node, defined as the node subtending the apical capitulum, to the level of the apical capitulum. Length AG in Fig. 7.1(i).

C3 Longest leaf length.
Length of the longest leaf, measured parallel to the primary vein.

C4 Capitulum total length.
Defined as the length from the point at which the pedicel widens into the receptacle to the stigmatic surface of the apical capitulum. Length AB in Fig. 7.1(i).

C5 Capitulum apex width.
Diameter of the capitulum, measured at the level of the outermost ring of disc florets. Length CD in Fig. 7.1(i).

C6 Capitulum base width.
Diameter of the capitulum measured at the level of the base of the phyllaries. Length EF in Fig. 7.1(i).

C7 Pedicel length.
Maximum length of the pedicel from the apical stem node to the point at which the pedicel widens into the receptacle. Length BG in Fig.1(i).

C8 Number of Phyllaries.

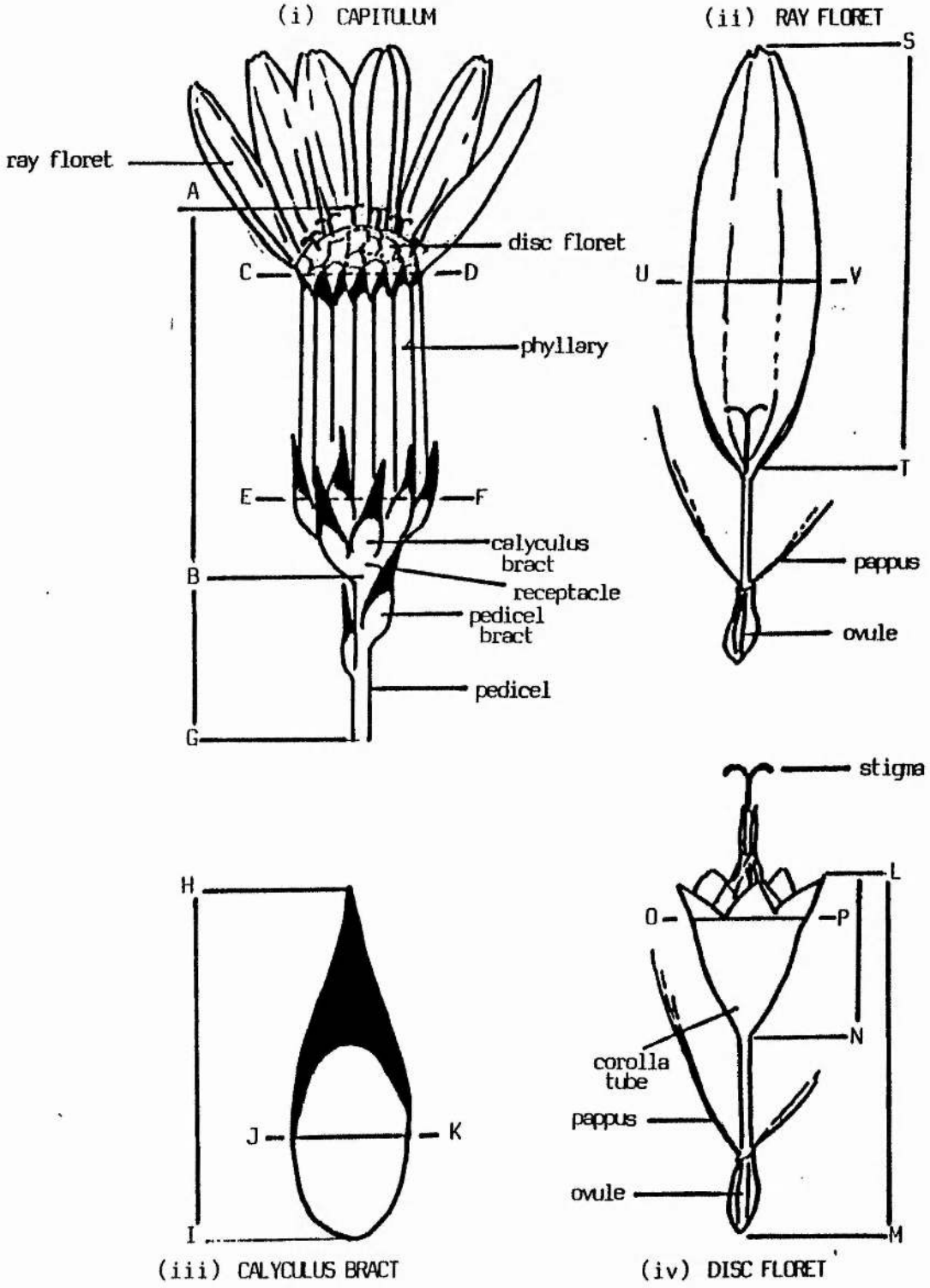


Fig. 7.1 Drawing of apical capitulum of S. vulgaris showing:

(i) Capitulum size characters C4 to C7
(ii) Ray floret characters C20 and C22
(iii) Calyculus bract characters C12 and C14
(iv) Disc floret characters C16 to C18.

(After Taylor 1984)

C9 Proportion of phyllaries with black tips.
Defined as the number of phyllaries with black and/or brown tips divided by the total number of phyllaries.

C10 Number of calyculus bracts.
Total number of bracts which are attached to the receptacle. i.e. bracts occurring above point B in Fig. 7.1(i).

C11 Number of pedicel bracts.
Total number of bracts which are attached to the pedicel. i.e. bracts occurring between points B and G in Fig. 7.1(i).

C12 Mean calyculus bract length.
Defined as the sum of the lengths of the calyculus bracts, length HI in Fig. 7.1(iii), divided by the number of calyculus bracts.

C13 Range of calyculus bract length.
Defined as the difference between the maximum and minimum calyculus bract lengths.

C14 Mean calyculus bract width.
Defined as the sum of the maximum calyculus bract widths (JK in Fig. 7.1(iii)) divided by the number of calyculus bracts.

C15 Number of disc florets.

C16 Mean disc floret total length.
The length from the base of the ovule to the apex of the corolla tube lobes. Length ML in Fig. 7.1(iv). Mean of a sample of 10 disc florets from the centre of the capitulum.

C17 Mean disc floret corolla length.
Length from the base of the corolla, defined as the point of attachment of the stamens, to the apex of the corolla lobes. Length NL in Fig. 7.1(iv). Mean of a sample of 10 disc florets used in C16.

C18 Mean disc floret corolla width.
Defined as half the circumference of the corolla tube, measured at the base of the corolla lobes. Length OP in Fig. 7.1(iv). Mean of a sample of 10 disc florets used in C16 and C17.

C19 Number of ray florets.

C20 Mean outer floret length.
Sum of the lengths of the outer florets, defined as the length from the base to the apex of the ligule, divided by the number of outer florets. Length TS in Fig. 7.1.(ii).

C21 Range of outer floret length.
Defined as being the difference between the maximum and minimum outer floret lengths.

C22 Mean outer floret width.
Sum of the maximum widths of the outer florets divided by the number of outer florets. Width UV in Fig.7.1(ii).

C23 Midleaf length.
Maximum length of the midleaf defined as the leaf attached to the stem nearest to the midpoint of the plant height (C1). Measured parallel to the apex of the primary vein. Length AB in Fig. 7.2(i).

C24 Midleaf maximum width L.
The maximum width of the midleaf, measured perpendicular to the primary vein on the left-hand side of the primary vein. Length EF in Fig. 7.2(i).

C25 Midleaf maximum width R.
Defined as C24 except measured on the rigth-hand side of the primary vein. Length CD in Fig. 7.2(i).

C26 Midleaf base to maximum width L.
Defined as the length from the base of the midleaf to the point at which C24 intersects with the primary vein. Length BF in Fig. 7.2(i).

C27 Midleaf base to maximum width R.
Defined as the length from the base of the midleaf to the point at which C25 intersects the primary vein. Length BD in Fig. 7.2(i).

C28 Midleaf number of lobes.
Defined as the number of secondary veins plus the apical lobe. The apical lobe is defined as originating at the point at which the secondary veins are of equal thickness to the primary vein. Point M in Fig. 7.2(ii).

C29 Midleaf apical lobe length.
Length of the apical lobe measured parallel to the primary vein. Length JK in Fig. 7.2(i).

C30 Midleaf apical lobe width.
The sum of the maximum widths of the apical lobe on both sides of the primary vein. Measured perpendicular to the primary vein. Length LM in Fig. 7.2(i).

C31 Midleaf midlobe maximum width A.
Defined as the maximum width of the midlobe on the apical side of the secondary vein measured perpendicular to the secondary vein. Length CD in Fig. 7.2(iii).

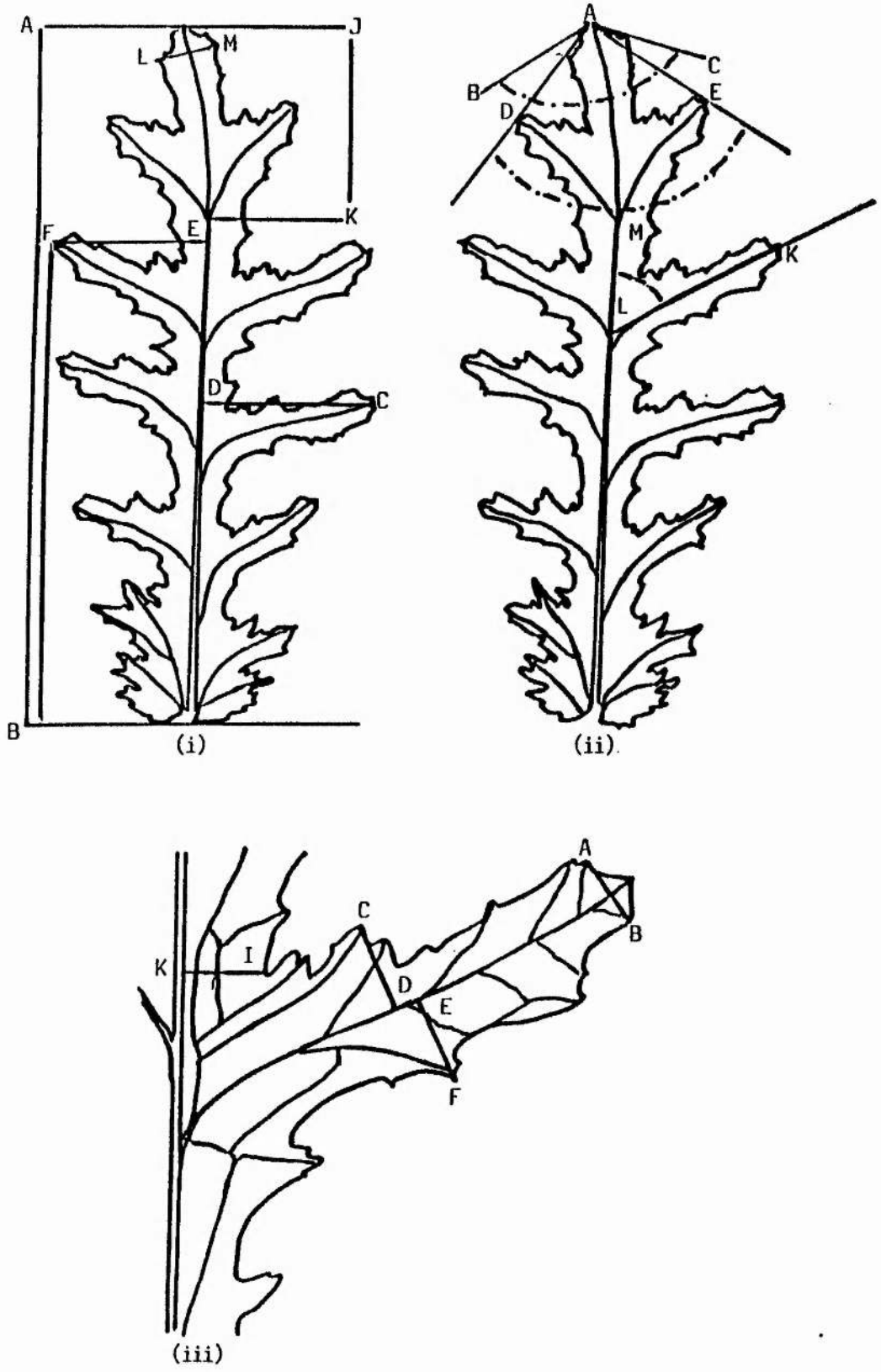


Fig. 7.2 Drawing of midleaf of S. vulgaris showing:

(i) Length characters C23 to C27, C29 and C30
(ii) Angular characters C35 to C37
(iii) Midlobe characters C31 to C34.

(After Taylor 1984)

C32 Midleaf midlobe maximum width B.
The maximum width of the midlobe on the basal side of the secondary vein measured perpendicular to the secondary vein. Length EF in Fig. 7.2(iii).

C33 Midleaf midlobe apical width.
Defined as the sum of the lengths from the marginal ends of the tertiary veins adjacent to the apex of the midlobe secondary vein to the ponts of intersetion with the secondary vein. Length AB in Fig. 7.2(iii).

C34 Midleaf midlobe lamina width.
The width of the primary lamina, from the centre of the primary vein to the point of intersection with the leaf margin. Measured perpendicular to the primary vein. Length IK in Fig. 7.2(iii).

C35 Midleaf apical angle A.
Defined as the angle between the apex of the primary vein and the apices of the adjacent marginal tooth sinuses. Angle BAE in Fig. 7.2(ii).

C36 Midleaf apical angle B.
Defined as the angle between the apex of the primary vein and the apices of the adjacent secondary veins. Angle DAE in Fig. 7.2(ii).

C37 Midleaf secondary vein angle.
Defined as the angle between the midlobe secondary vein and the primary vein.Angle KLMin Fig. 7.2(ii).

C38 Leaf dissection.
Defined as the ratio of the perimeter of the midleaf divided by the square root of the area.

Analysis.

Initially the data were analysed by subjecting each character, in turn to a two way analysis of variance to detect differences between sites and the significance of the site*taxa interaction, using the statistical package GENSTAT. In the course of the analysis the data set for each character was assessed to see if it satisfied the

assumptions of normality and homogeneity of variance. When these assumptions were not met, the data was subjected to a $\log_e$ transformation. A number of significant site*taxa interactions were revealed and subsequently the data for each site were separately analysed using a one way analysis of variance. Orthogonal contrasts were extracted for each character to test for significant differences between taxa (i.e. radiate (R) v non-radiate (N) *S.vulgaris*, and R+N *S.vulgaris* V *S.squalidus*).

Following analysis of variance, the whole data set was subjected to a multivariate principal components analysis (PCA) using the statistical package CLUSTAN. This analysis takes p variables X_1, X_2, ...X_p and finds combinations of these to produces indices Z_1, Z_2, ...Z_p that are uncorrelated (the Z indices are the principal components). Because the indices are uncorrelated, they are measuring different 'dimensions' of the data. The indices are ordered so that Z_1 displays the largest amount of variation, Z_2 the second etc.

i.e. $\text{var}(Z_1) > \text{var}(Z_2) > \text{var}(Z_p)$.

Eigenvalues were extracted to indicate the proportion of the original variance accounted for by each principal component while, factor loadings (Eigenvectors) were calculated to represent the contribution of each original variable to the new uncorrelated vector.

(ii) Electrophoresis.

The sixty individuals used in the morphometric analysis were also screened for isozyme variation over 12 enzyme systems: α-esterase, β-esterase, acid phosphatase (ACP), phosphoglucose isomerase (PGI), phosphoglutomutase (PGM), malate dehydrogenase (MDH), glutamate oxaloacetate transaminase (GOT), malic enzyme (ME), glucose-6-phosphate dehydrogenase (G-6-PDH), glyceraldehyde-3-phosphate dehydrogenase (G-3-PD), malate dehydrogenase (MDH), and glutamate dehydrogenase (GDH). Details of electrophoretic procedures are given in appendix A.

RESULTS.

(i) Morphometrical analysis.

Table 7.1 shows that there were significant differences between the two sites (Edinburgh and York) for 10 of the characters measured. All but 6 of the 38 characters showed significant differences between taxa and 24 of these were significant at the $P < 0.001$ level. Significant site*taxa interactions were found for 14 of the characters. When the characters were subjected in turn to a one way analysis of variance at each site (Tables 7.2 and 7.3) 25 of the characters showed significant differences between taxa from the Edinburgh population (Table 7.3) while 30 showed significant differences between taxa from York (Table 7.2).

Tables 7.2 and 7.3 also present levels of significance for differences between (i) the two morphs of *S.vulgaris* (R v N), and (ii) the mean of the two morphs of S.vulgaris and S.squalidus (R+N v S). These were obtained after partitioning the taxa mean square into two orthogonal contrasts (Sokal and Rohlf 1981).

In the Edinburgh population (Table 7.3) a significant difference between the radiate and non-radiate morphs of

TABLE 7.1. Results of twoway analysis of variance for the 38 consecutive characters measured.

	CHARACTER	SITE	TAXA	SITE*TAXA	
C1	Plant height	N.S.	***	N.S.	+
C2	Inflorescence length	*	***	N.S.	+
C3	Longest leaf length	***	***	***	
C4	Capitulum total length	N.S.	*	N.S.	+
C5	Capitulum apex width	N.S.	***	N.S.	
C6	Capitulum base width	N.S.	***	N.S.	
C7	Pedicel length	*	***	*	+
C8	Number of phyllaries	N.S.	**	*	
C9	Proportion of phyllaries with black tips	***	***	***	+
C10	Number of calyculus bracts	**	***	***	+
C11	Number of pedicel bracts	N.S.	***	N.S.	+
C12	Mean calyaulus bract length	N.S.	***	***	
C13	Range of calyculus bract length	*	*	N.S.	+
C14	Mean calyculus bract width	*	***	N.S.	
C15	Number of disc florets	N.S.	***	N.S.	
C16	Mean disc floret total length	N.S.	***	*	
C17	Mean disc floret corolla length	N.S.	***	**	
C18	Mean disc floret corolla width	N.S.	***	**	+
C19	Number of ray florets	*	***	*	
C20	Mean outer floret length	N.S.	***	N.S.	+
C21	Range of outer floret length	N.S.	***	N.S.	+
C22	Mean outer floret width	N.S.	***	N.S.	+
C23	Midleaf length	N.S.	***	***	
C24	Midleaf maximum width L	N.S.	N.S.	N.S.	
C25	Midleaf maximum width R	N.S.	N.S.	N.S.	
C26	Midleaf base to maximum width L	N.S.	N.S.	N.S.	
C27	Midleaf base to maximum width R	N.S.	*	N.S.	+
C28	Midleaf number of lobes	N.S.	***	***	
C29	Midleaf apical lobe length	N.S.	*	N.S.	
C30	Midleaf apical lobe width	N.S.	***	N.S.	
C31	Midleaf midlobe maximum width A	N.S.	**	*	
C32	Midleaf midlobe maximum width B	N.S.	N.S.	N.S.	
C33	Midleaf midlobe apical width	N.S.	N.S.	N.S.	
C34	Midleaf midlobe lamina width	N.S.	**	N.S.	+
C35	Midleaf apical angle A	N.S.	N.S.	N.S.	
C36	Midleaf apical angle B	***	***	N.S.	
C37	Midleaf secondary vein angle	N.S.	***	N.S.	
C38	Midleaf leaf dissection	*	**	**	

+ Analysis carried out on transformed data.

Table 7.2. Results of one way analysis of variance for 38 consecutive characters: S.squalidus, radiate S.vulgaris (R) and non-radiate S.vulgaris (N) from York.

CHARACTER	RADIATE	NON-RADIATE	SQUALIDUS	Taxa (RvNvS)	R v N	R+N v S	
C1	11.50	9.66	22.74	***	*	***	+
C2	15.40	11.10	25.10	***	**	***	+
C3	13.14	8.27	10.53	***	***	N.S.	
C4	9.11	7.91	9.09	N.S.	N.S.	N.S.	
C5	4.11	3.85	6.01	***	N.S.	***	
C6	3.66	3.35	5.08	***	*	***	
C7	6.49	3.21	16.05	***	***	***	+
C8	16.80	19.80	21.87	***	**	***	
C9	0.30	0.87	0.93	***	***	***	
C10	9.00	16.00	9.50	***	***	***	+
C11	0.50	1.60	7.87	***	*	***	+
C12	3.40	2.22	2.69	***	***	N.S.	
C13	0.95	1.08	0.51	*	N.S.	**	+
C14	0.71	0.59	0.70	**	**	N.S.	+
C15	45.20	56.80	91.90	***	**	***	
C16	6.83	5.79	6.91	***	***	*	
C17	2.08	1.67	2.86	***	***	***	
C18	0.77	0.59	1.36	***	***	***	+
C19	8.80	0.00	12.37	***	***	***	
C20	5.10	1.61	9.73	***	***	***	+
C21	0.91	0.20	2.33	***	***	***	
C22	1.49	0.57	2.93	***	***	***	+
C23	9.48	6.74	5.39	***	***	***	
C24	1.81	1.45	1.41	N.S.	N.S.	N.S.	
C25	1.99	1.57	1.54	N.S.	N.S.	N.S.	
C26	4.30	4.53	3.28	N.S.	N.S.	*	
C27	4.77	4.25	3.05	*	N.S.	**	
C28	11.40	7.60	7.25	***	***	***	
C29	0.91	0.77	0.67	N.S.	N.S.	N.S.	
C30	2.22	1.46	1.96	*	**	N.S.	
C31	0.25	0.33	0.22	N.S.	N.S.	N.S.	
C32	0.38	0.36	0.31	N.S.	N.S.	N.S.	
C33	0.38	0.32	0.34	N.S.	N.S.	N.S.	
C34	0.40	0.30	0.19	**	N.S.	**	+
C35	117.30	113.90	114.90	***	**	*	
C36	97.70	109.80	94.60	**	*	*	
C37	54.40	58.80	34.20	***	N.S.	***	
C38	6.53	4.81	5.84	**	**	N.S.	

+ Analysis carried out on transformed data.

Table 7.3. Results of one way analysis of variance for 38 consecutive characters: S.squalidus, radiate S.vulgaris (R) and non-radiate (N) S.vulgaris from Edinburgh.

CHARACTER	RADIATE	NON-RADIATE	SQUALIDUS	Taxa (RvNvS)	R v N	R+N v S	
C1	10.52	11.19	20.34	***	N.S.	***	
C2	11.80	11.70	21.36	***	N.S.	***	+
C3	8.60	8.82	10.64	***	N.S.	***	
C4	8.07	7.97	8.50	N.S.	N.S.	N.S.	
C5	3.69	3.79	5.99	***	N.S.	***	
C6	3.27	3.38	5.22	***	N.S.	***	+
C7	3.73	3.67	12.85	***	N.S.	***	+
C8	19.90	19.50	21.44	N.S.	N.S.	N.S.	
C9	0.96	0.91	0.91	N.S.	N.S.	N.S.	+
C10	16.10	16.00	10.11	***	N.S.	***	+
C11	1.00	1.20	10.30	***	N.S.	***	+
C12	2.22	2.33	3.03	***	N.S.	***	
C13	1.02	1.29	1.11	N.S.	N.S.	N.S.	+
C14	0.63	0.59	0.67	*	N.S.	**	
C15	42.90	48.80	90.30	***	N.S.	***	
C16	6.16	6.19	6.71	*	N.S.	*	
C17	1.87	1.83	2.76	***	N.S.	***	
C18	0.71	0.71	1.38	***	N.S.	***	
C19	10.60	0.00	12.33	***	***	***	
C20	4.68	1.77	9.69	***	***	***	
C21	0.51	0.22	1.88	***	***	***	+
C22	1.36	0.67	3.12	***	***	***	+
C23	6.84	6.22	6.73	N.S.	N.S.	N.S.	
C24	1.75	1.61	1.72	N.S.	N.S.	N.S.	
C25	1.76	1.50	1.79	N.S.	N.S.	N.S.	
C26	2.93	3.89	3.91	N.S.	N.S.	N.S.	
C27	4.46	3.65	3.97	N.S.	N.S.	N.S.	
C28	7.70	8.30	8.67	N.S.	N.S.	N.S.	
C29	0.94	0.61	0.74	*	**	N.S.	+
C30	1.82	1.33	2.34	***	*	***	
C31	0.38	0.31	0.24	**	N.S.	**	
C32	0.46	0.44	0.35	N.S.	N.S.	*	
C33	0.37	0.36	0.39	N.S.	N.S.	N.S.	
C34	0.29	0.29	0.23	N.S.	N.S.	N.S.	
C35	130.80	142.30	110.10	**	N.S.	**	
C36	112.10	119.40	105.40	*	N.S.	*	
C37	61.40	60.20	38.90	***	N.S.	***	
C38	4.51	4.47	6.01	**	N.S.	***	+

+ Analysis carried out on transformed data.

S.vulgaris, was present for only 6 of the characters recorded, C19-C22 (i.e. characters concerned with describing the ray florets of the radiate form, the diagnostic feature of radiate *S.vulgaris*) and C29-C30 (i.e.characters describing the dimensions of the apical lobe of the midleaf). Significant differences between *S.squalidus* and the pooled data for *S.vulgaris* were present for 13 of the 38 characters for material from the same site examined. In contrast, for material from York (Table 7.2) 25 of the 38 characters examined showed significant differences between radiate and non-radiate *S.vulgaris* while 12 showed differences between *S.squalidus* and *S.vulgaris*. Therefore in the York population, more of the variation between taxa is due to differences between radiate and non-radiate *S.vulgaris* than in the Edinburgh material where most of it is taken up by differences between *S.vulgaris* and *S.squalidus* and very little from differences between radiate and non-radiate *S.vulgaris*.

Principal components analysis (PCA) was carried out initially on the covariance matrix based on absolute values. However the results showed that one character (C15 - Number of disc florets) had an over-riding effect on the analysis and masked differences. Therefore, as is usual with such data sets (Manly 1988), the data was standardised to have zero mean and unit standard deviation to ensure that all

measurements had equal weight in the analysis. Thus the covariance matrix of the standardised variables provided the correlation matrix. Figures 7.3 - 7.5 present the number of significant correlations when *p* was set at $p < 0.05$; $p < 0.01$ and $p < 0.001$. It is apparent the when $p < 0.001$ (Fig 7.5) and < 0.01 (Fig 7.4) capitulum characters were correlated with each othere as were vegetative characters. However, when $p < 0.05$, several correlations between vegetative and capitulum characters also emerged.

The first and second principal components of the analysis accounted for 53.5% of the variance and the third explained a further 9% (Table 7.4). Based on their component specifications the 60 plants included in the analysis were plotted against principal components 1 (x axis) and 2 (Y axis) in fig. 7.6 and components 1 and 3 in fig. 7.7. Both figures show clearly that while the *S.squalidus* individuals from the York and Edinburgh populations group together on the right side of the plot, radiate and non-radiate Edinburgh *S.vulgaris* together with York non-radiate plants cluster on the left side with York radiate *S.vulgaris* located between these two groups.

(ii) Electrophoresis.

No difference in banding patterns was found between the

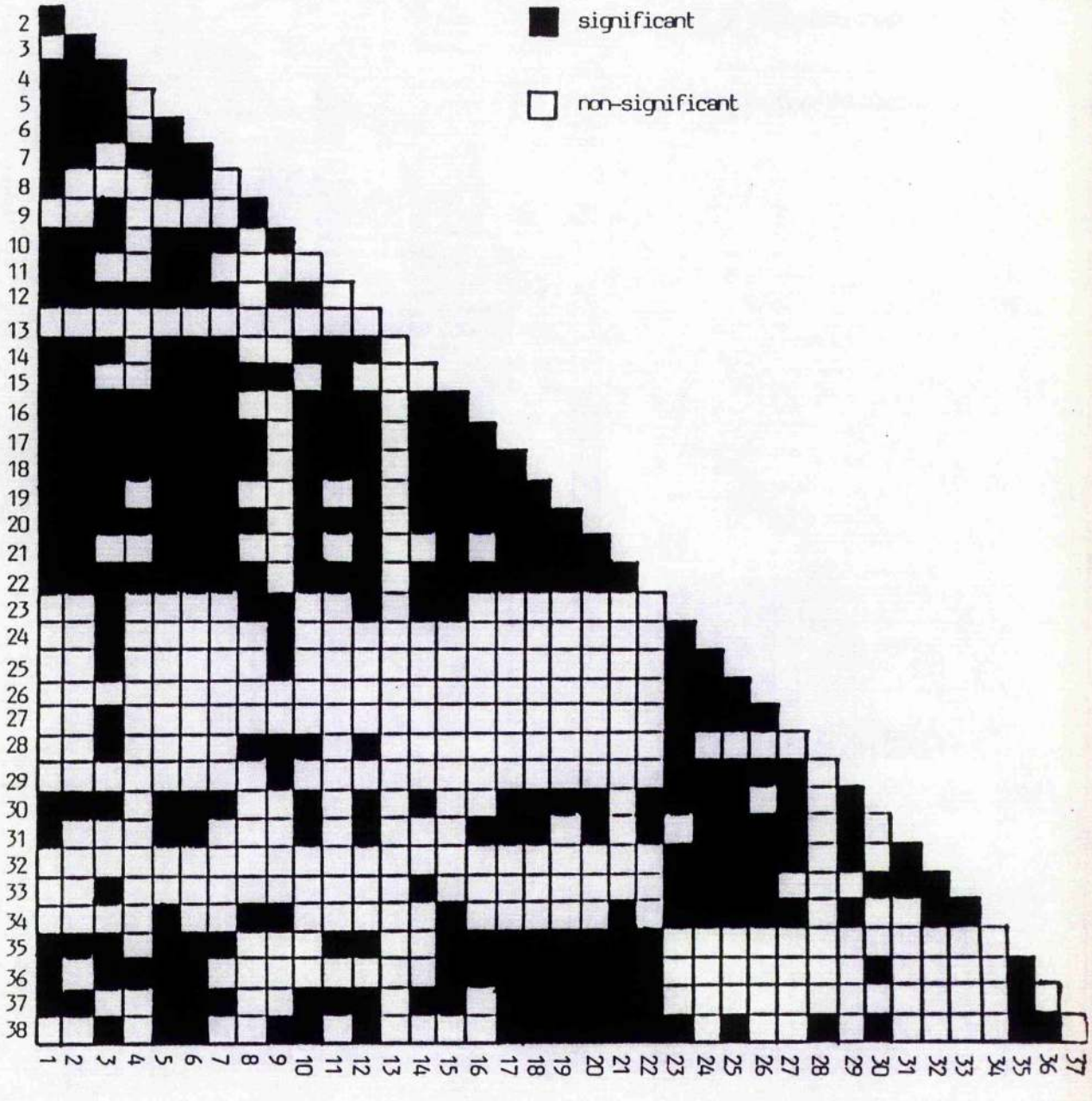


Fig. 7.3 Correlation matrix for principal components analysis where $p \leq 0.05$.

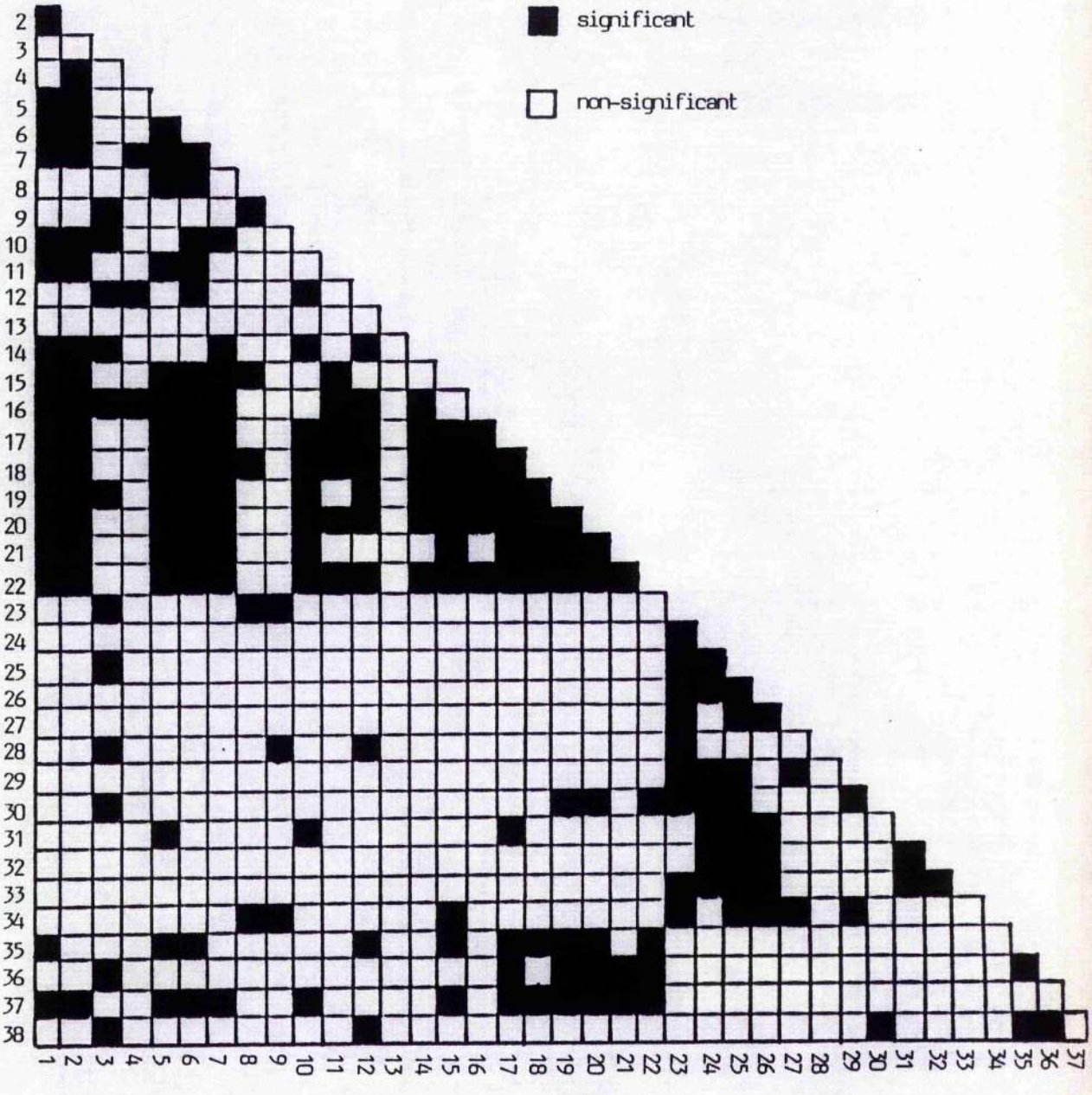


Fig. 7.4 Correlation matrix for principal components analysis where $p \leqslant 0.01$.

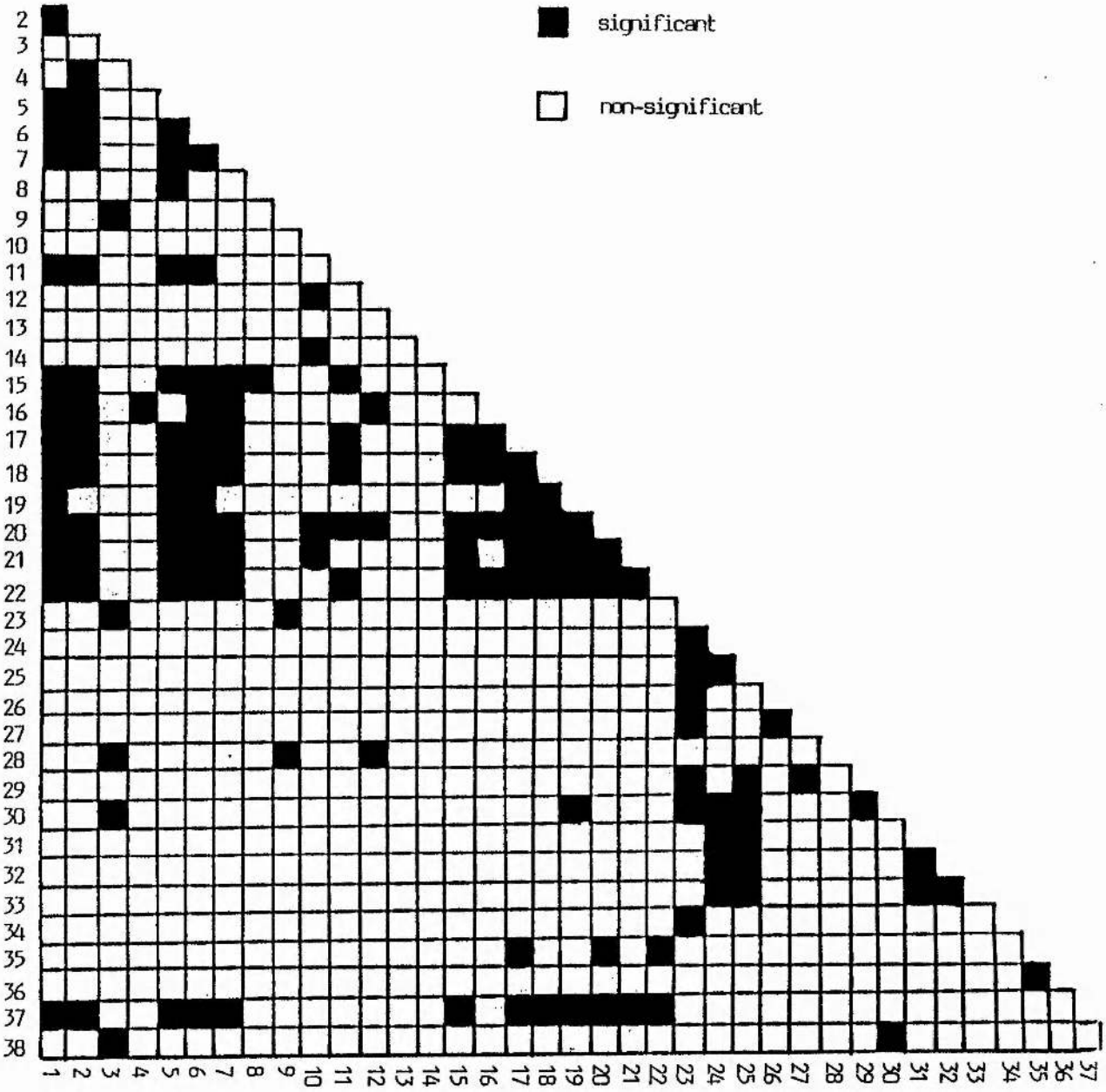


Fig. 7.5 Correlation matrix for principal components analysis where $p \leqslant 0.001$.

Table 7.4 The first ten eigenvalues (the variance of each principal component), percentage variaqnce and cumulative variance for the first 10 principal components of the analysis of 60 taxa examined.

Component	Eigenvalue	Percentage variance	Cumulative variance
1	13.36	35.15	35.15
2	6.98	18.36	53.51
3	3.42	9.00	62.51
4	1.93	5.08	67.59
5	1.57	4.13	71.72
6	1.45	3.83	75.55
7	1.25	3.30	78.85
8	1.06	2.78	81.62
9	0.94	2.48	84.10
10	0.76	2.00	86.10

Fig.7.6. A plot of the 60 individuals from the York and Edinburgh sites against the first two components extracted from a PCA of the total data set.

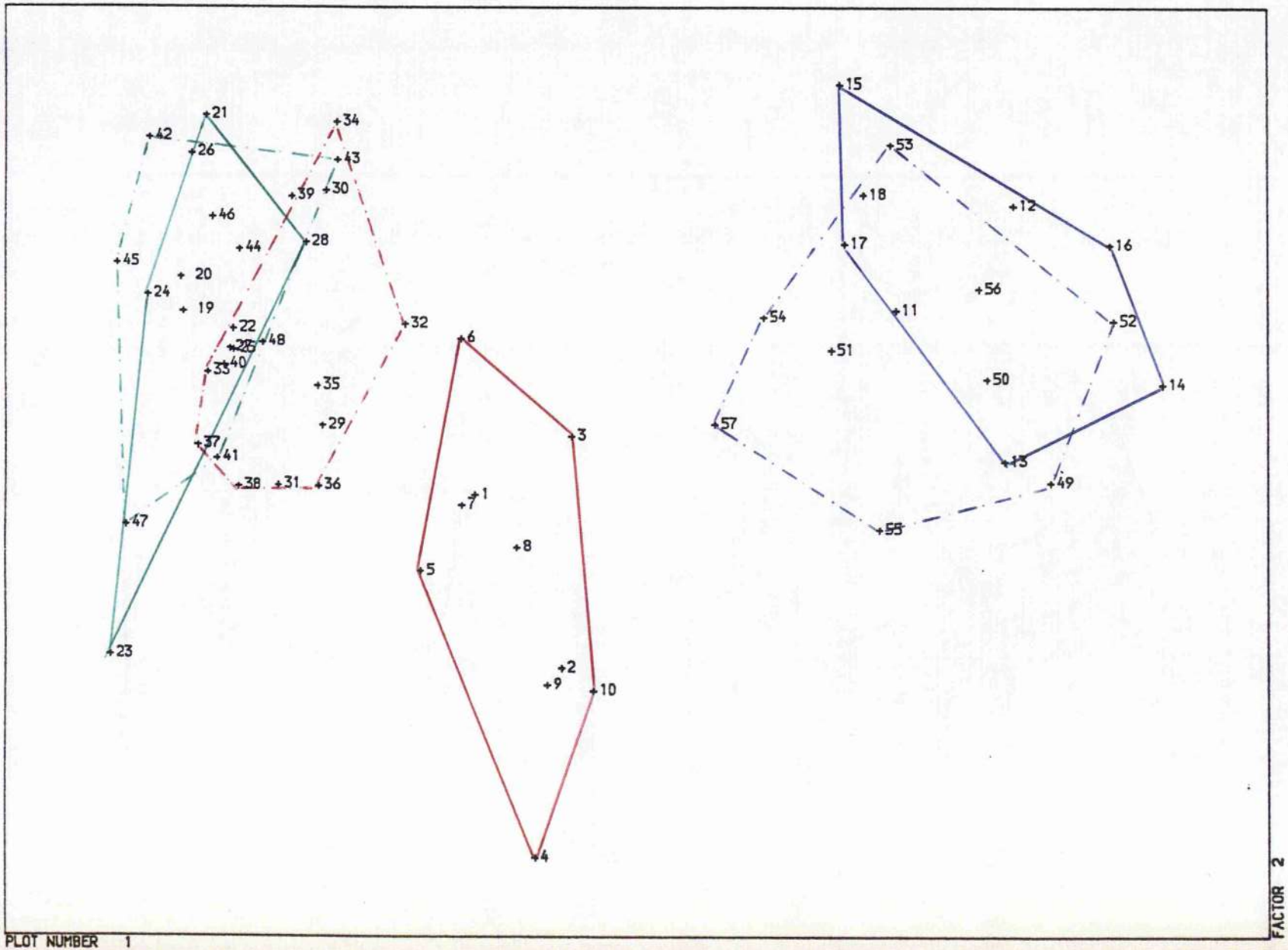

FACTOR 1
FACTOR 2
PLOT NUMBER 1
21
42
26
34
43
39
30
46
28
44
45
20
24
19
22
48
32
6
3
1
7
8
5
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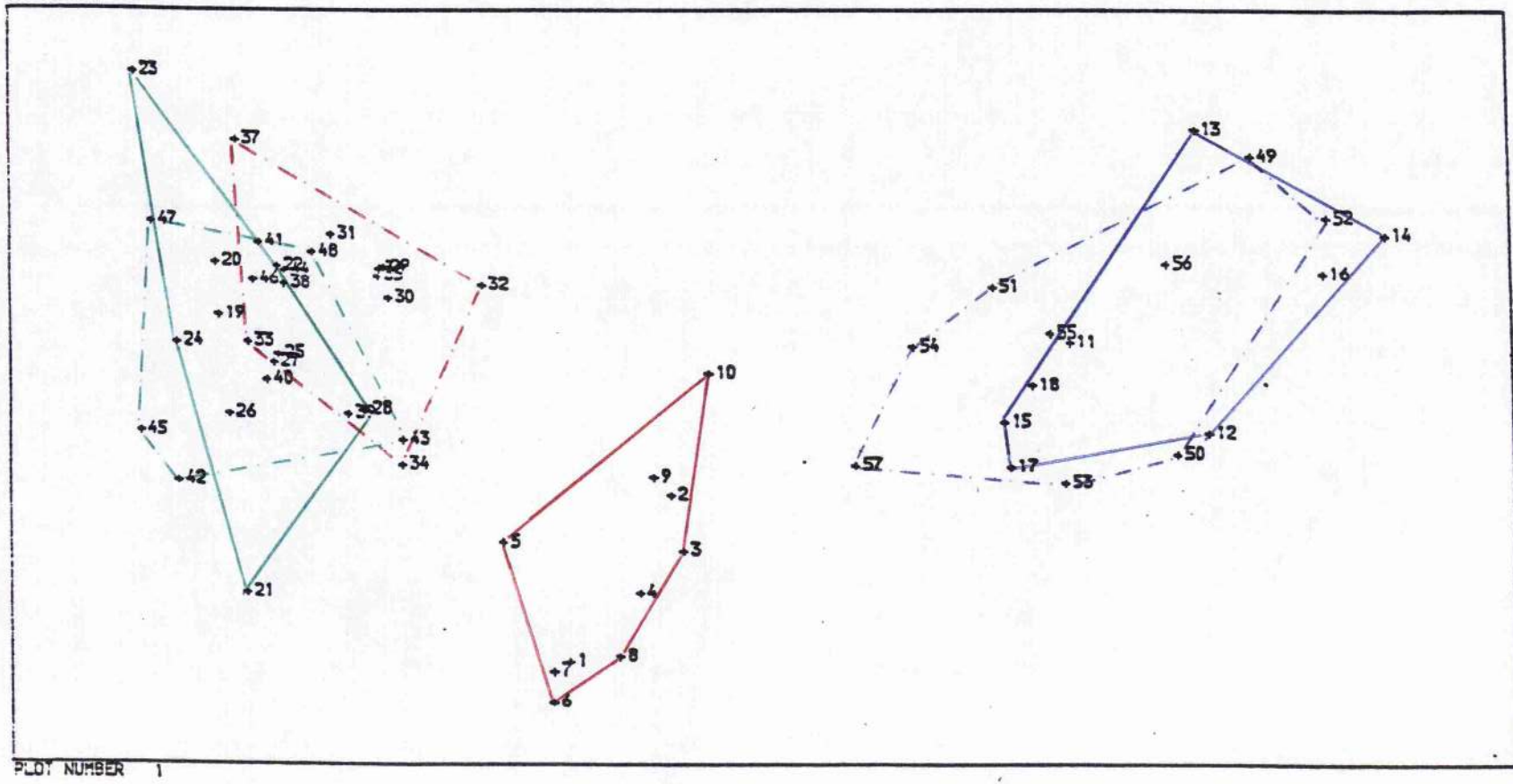
Fig.7.7. A plot of the 60 individuals from the York and Edinburgh sites against the first and third components extracted from a PCA of the total data set.

——— York *S.squalidus*.
–·–·– Edinburgh *S.squalidus*.
——— York radiate *S.vulgaris*.
–·–·– Edinburgh radiate *S.vulgaris*.
..... York non-radiate *S.vulgaris*.
..... Edinburgh non-radiate *S.vulgaris*.


FACTOR 1
FACTOR 3
PLOT NUMBER 1
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47
41
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33
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Edinburgh and York *S.vulgaris* for all enzyme systems except β-esterase. When the starch gel was stained using β-nathyl acetate as substrate the β-esterase banding pattern illustrated in figure 7.8 (plate 7.2) was obtained. It is evident that the radiate *S.vulgaris* from York possessed all three β-esterase loci corresponding to those possesssed by both Edinburgh and York *S.squalidus*. In contrast, the non-radiate *S.vulgaris* from York and both morphs from Edinburgh possessed only the β-est-1 and β-est-2 loci that are usually found in *S.vulgaris* (see chapter 2). This result suggests that the York radiate *S.vulgaris* contains an extra locus encoding β-esterase which is present in *S.squalidus* but not normally present in *S.vulgaris*.

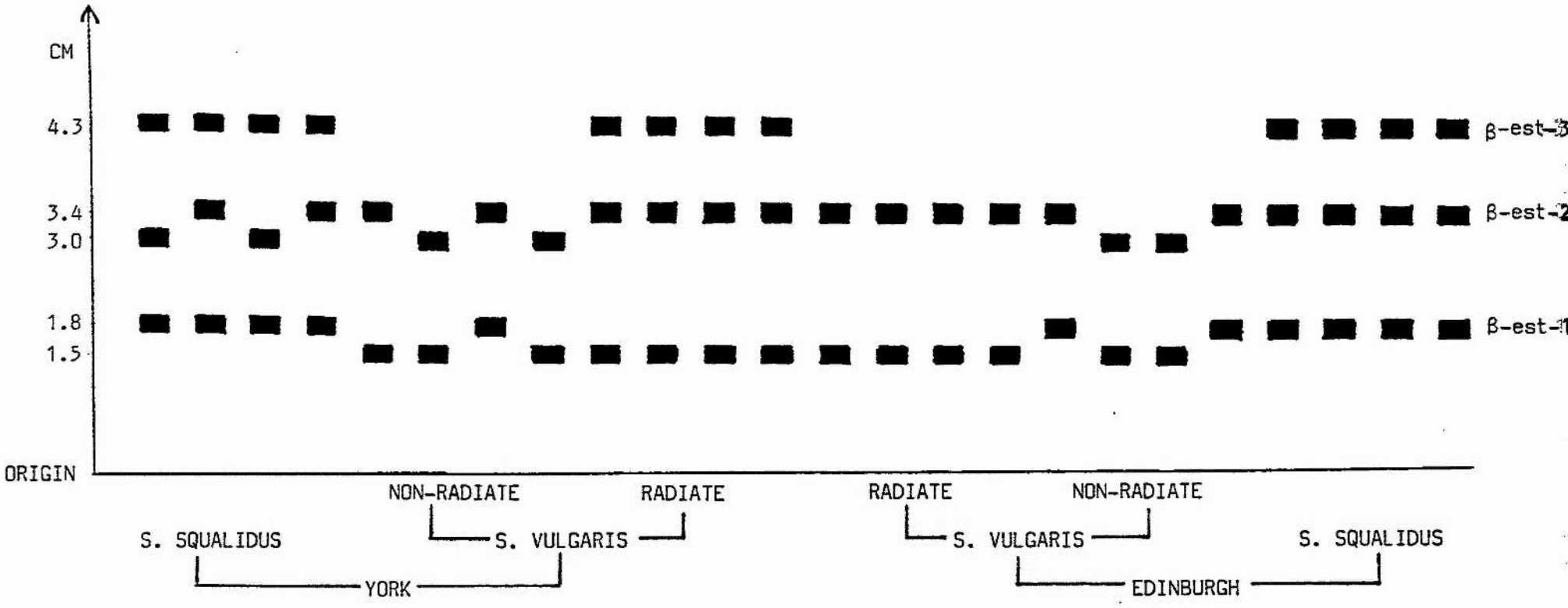

Fig. 7.8 Zymogram of β-esterase isozymes for radiate and non-radiate S. vulgaris and S. squalidus from York and Edinburgh.

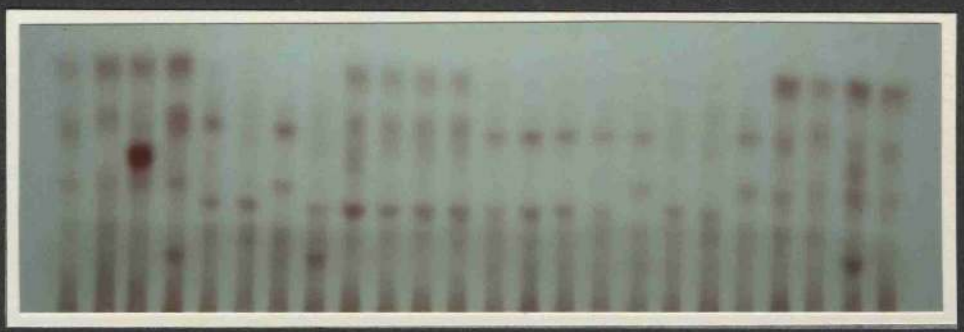

Plate 7.2 β-esterase banding patterns obtained for radiate and non-radiate S. vulgaris and S. squalidus from York (tracks 1-12) and Edinburgh (tracks 13-24).

Key given in Fig. 7.8.

DISCUSSION.

The results of both the morphometric and electrophoretic analyses provide strong evidence that radiate *S.vulgaris* from York is more "squalidus like" than other 'normal' radiate groundsel - typified by the radiate *S.vulgaris* from Edinburgh examined here. Visual comparision of the two radiate types (Plate 7.3) shows that the York variant is morphologically very different from the Edinburgh type, exhibiting a phenotype that is between Edinburgh *S.vulgaris* and *S.squalidus*.

Analyses of variance demonstrated that York radiate *S.vulgaris* was significantly different from York non-radiate *S.vulgaris* for 17 of the 19 capitulum characters and for 8 of the vegetative characters measured. By contrast Edinburgh radiate *S.vulgaris* differed from the non-radiate variant for only 4 capitulum characters all of which described the diagnostic ray florets of radiate capitula plus 2 vegetative characters.

The intermediate phenotype of the York radiate groundsel relative to non-radiate *S.vulgaris* and *S.squalidus* was made particularly clear by the principal components analysis (Fig. 7.6 and 7.7). Whereas the two Edinburgh forms of

Plate 7.3 York radiate *S. vulgaris* (left), Edinburgh radiate *S. vulgaris* (middle) and *S. squalidus* (right).

S.vulgaris together with the York non-radiate type clustered to one side of the plots, while *S.squalidus* from both populations formed another distinct group, radiate *S.vulgaris* from York was positioned as an independent group between these two extremes.

Electrophoretic evidence revealed that the York radiate *S.vulgaris* also possessed an additional β-esterase locus which is always present in *S.squalidus* but absent from other *S.vulgaris* samples (radiate or non-radiate) surveyed here or in chapter 2. This finding further supports the view that the York radiate variant has more "squalidus like" features than the other radiate *S.vulgaris* screened.

It remains to be determined if the third β-esterase locus in York radiate *S.vulgaris* is linked to the ray floret locus. If the β-est-3 locus is not linked to the ray floret locus, then it is easy to envisage how it might have been lost from radiate *S.vulgaris* during the introgression event involving repeated backcrossing to non-radiate *S.vulgaris*. In any introgression event, it is likely that only a limited amount of genetic material will be passed from *S.squalidus* to *S.vulgaris* and that this may well be a random transfer. Except for loci tightly linked to the ray floret locus, therefore, the presence or absence of genetic material affecting any character may very well be a random process.

However, if the β-est-3 locus is linked to the ray floret locus this raises the question as to why the third locus is not present in other radiate material.

The origin of the York radiate variant of *S.vulgaris* can, therefore, be explained in a number of ways.
(i) It is representative of an early stage in the introgressive process following a cross between non-radiate *S.vulgaris* and *S.squalidus* and the β-est-3 locus has not been lost in the subsequent backcrosses to the non-radiate morph.
(ii) York radiate *S.vulgaris* is the product of a hybrid-introgression event during which the random sample of genetic material transferred from *S.squalidus* into *S.vulgaris* encoded the β-est-3 locus. In other hybrid-introgression events, this part of the *S.squalidus* genome has not been transferred into *S.vulgaris* along with the ray floret locus.
(iii) The York variant is the product of a tetraploid hybrid produced following fusion of an unreduced *S.squalidus* gamete with a haploid gamete from *S.vulgaris*. Taylor (1984) synthesised just such a tetraploid hybrid.

If the third pathway gave rise to the York radiate variant of *S.vulgaris*, the whole *S.squalidus* genome would be present within these plants and thus they would be expected to

express many "squalidus like" features against a *S.vulgaris* background. Therefore if the York radiate *S.vulgaris* was indeed produced following the fusion of an unreduced *S.squalidus* gamete with a haploid gamete from non-radiate *S.vulgaris*, it is not surprising that it should contain the β-est-3 locus while other radiate forms produced following the formation of a triploid hybrid do not.

A further indication of a hybrid origin for the York radiate variant comes from the four-pored nature of some pollen grains produced by York radiate plants (pers. obs.). Both *S.vulgaris* and *S.squalidus* produce three-pored pollen grains while the allopolyploid *S.cambrensis* (2n=6x=60) produces four-pored grains (one of the diagnostic characters of the species). Non-radiate S.vulgaris from York that were examined produced 'normal' three-pored pollen, however, some York radiate individuals produced a mixture of three-pored and four-pored pollen grains.

In conclusion, both the morphometric and electrophoretic analyses have confirmed the intermediate nature of the York radiate *S.vulgaris* between 'normal' *S.vulgaris* and *S.squalidus*. However, the electrophoretic data does not exclude the possibility that 'normal' radiate *S.vulgaris* originated via mutation rather than introgression. Ingram et al (1980) reproduced a radiate form of *S.vulgaris* by

crossing *S.squalidus* and non-radiate *S.vulgaris* but only took the synthesis to the first selfed generation of the first backcross generation at which stage the material produced was still unstable. In future it will be necessary to resynthesise a stable radiate form of *S.vulgaris* via both triploid and tetraploid F_1's and subject these products to the form of morphometric and electrophoretic analysis performed here. In this way it might be possible to determine whether the York variant arose via the triploid or tetraploid hybrid and also establish the evolutionary pathway of the 'normal' radiate type.

CHAPTER 8.

GENERAL DISCUSSION.

GENERAL DISCUSSION

The research reported in this thesis focused on the occurrence of male gametophytic competition in the hermaphroditic species, *Senecio vulgaris*. Based on the assumption of Charnov (1982) and Willson (1983), that sexual selection is expected to act in outcrossing but not in selfing hermaphrodites, the polymorphism for outcrossing rate associated with the capitulum morphs of *Senecio vulgaris* (Marshall and Abbott 1982, 1984a, 1984b), provided a model system with which to investigate the occurrence and mechanisms of male competition within a hemaphrodite species. A major aim of this work, therefore, was to conduct an analysis of the expected association between outcrossing rate and the level and effects of male competition in the radiate and non-radiate morphs of *Senecio vulgaris*.

To examine male competition, it was necessary first to determine the amount of competition that pollen of each morph is subject to during the fertilisation of available ovules. Using an electrophoretic marker in conjunction with the alleles at the ray floret locus, levels of maternal and paternal outcrossing exhibited by each morph were estimated. Further, by apportioning the observed outcrossing rates into outcrossing that occurred due to pollen transfer between

plants of opposite capitulum morphs (intermorph outcrossing) and that following crosses between plants of the same capitulum morph (intramorph outcrossing), the level of competition between radiate and non-radiate pollen types and between different pollen types within the radiate and non-radiate morphs was assessed. Analysing pollinator behaviour within stands polymorphic for capitulum type (also used to estimate outcrossing), allowed the potential for pollinator-mediated pollen transfer within and between the two morphs to be related to the observed levels of outcrossing.

Records of pollinator activity revealed that radiate plants were always more attractive to pollinators, both in stands monomorphic for capitulum type, and in mixed stands in which the frequency of the radiate morph (relative to the non-radiate morph) was 80%, 50% or 20% respectively (chapter 3, tables 3.4 - 3.6). Further, the relative attractiveness of the radiate morph increased as pollinators moved from the first to the second plant during a flight sequence. Pollinators appeared to 'learn' from visiting a radiate capitulum such that the frequency with which the second plant visited in a flight sequence was of the radiate type was greater than would be expected based on the initial preference shown for the radiate morph (chapter 3 table 3.10). In contrast, when the first plant visited in a

flight sequence was of the non-radiate morph, preference for the two morphs when the pollinator moved to the second plant was the same as that exhibited when the pollinator initially began foraging the plot (chapter 3 table 3.11).

Pollinator preference for the radiate morph resulted in more transitions between radiate plants (R-R) than would be expected if the two morphs had been equally attractive to pollinators, while transitions between non-radiate plants (N-N) occurred at a lower than expected frequency (Chapter 3, tables 3.7 and 3.8). This was reflected in the maternal and paternal intramorph outcrossing rates exhibited by the two morphs (chapter 4, tables 4.4 - 4.8). In all plots, the radiate morph showed higher levels of intramorph outcrossing than the non-radiate morph. Thus, the radiate morph was more successful than the non-radiate morph as both a donor and recipient of intramorph cross pollen. In terms of the potential for intramorph pollen competition therefore, it is expected that radiate pollen will be subjected to more competition between different radiate pollen types, than non-radiate pollen will be to competition between non-radiate pollen types.

Transitions from non-radiate to radiate plants (N-R) occurred at higher than expected frequencies in all mixed stands while pollinators generally moved from radiate to

non-radiate plants (R-N) less often then expected; an exception to this occurred in stands containing the radiate morph at low frequency (20%) where R-N transitions occurred just above the expected frequency (Chapter 3, table 3.8). As a result of the pattern of pollinator movements and outcrossing between morphs, both radiate and non-radiate pollen types would be subject to intermorph pollen competition on the stigmas of radiate and non-radiate plants.

The outcome of the observed pattern of pollen movement within and between the radiate and non-radiate morphs is that instead of pollen of one morph (the 'outcrossing' morph of Charnov and Willson's hypothesis) being subject to pollen competition while pollen of the other morph (the 'selfing' morph) is subject to no competition at all, pollen of both morphs of *S.vulgaris* is subjected to some male gametophytic competition, but it is the level of competition which varies. Combining the potential for both intermorph and intramorph pollen competition, radiate pollen is expected to be exposed to more competition for access to available ovules than non-radiate pollen. Consequently, sexual selection for a highly effective pollen type is likely to act more strongly on radiate pollen than on non-radiate pollen in the race to fertilise available ovules.

The results in chapter 6 suggest that due to the greater sexual selection experienced by radiate pollen, a more effective radiate pollen type has indeed evolved. Radiate pollen was more effective in causing a reduction in style length than non-radiate pollen when both pollen types were applied to stigmas of emasculated capitula (chapter 6, tables 6.6 - 6.9). As stylar shrinkage is indicative of pollen germination, it is evident that radiate pollen is quicker to germinate than non-radiate pollen on the stigmas of either radiate or non-radiate plants. It should be borne in mind, however, that while the results consistently indicate a faster rate of germination for radiate pollen, they do not prove that radiate pollen tubes outcompete non-radiate pollen tubes in the style and consequently fertilise a disproportionate share of the ovules.

The experiments reported in chapter 5 were designed to test the relative effectiveness of radiate and non-radiate pollen to fertilise ovules by applying both pollen types to the stigmas of emasculated capitula and scoring the number of ovules fertilised by each pollen type. Unfortunately, the results obtained were inconsistent both between experiments (chapter 5, Table 5.5), and with the results of previous studies (Irwin 1986). A number of criticisms can be levelled at the experimental protocol employed which would suggest that the method of sequential pollen application

used in these studies may have been too crude. Pollen clumping on the stigmatic surface (Thomson 1988) or the order in which the pollen was applied (Epperson and Clegg 1987) may have affected the results (see chapter 5 for fuller discussion). An alternative approach in future would be to apply radiate pollen to one of the two lobes of each individual stigma and non-radiate pollen to the other. In this way the amount of pollen of each type applied could be controlled and subsequent progeny testing would reveal which pollen type more often achieves fertilisation of the ovule when in direct competition with the other pollen type.

Although the results presented in chapter 6 suggest that radiate pollen is faster to germinate than non-radiate pollen when physically applied to stigmas, the converse is true in untreated capitula. When radiate and non-radiate plants are left to set self seed in a glasshouse, radiate capitula contain more exserted stigmas than non-radiate capitula (Ashton 1984; Irwin 1986). Given the relationship between style length (and hence stigma exsertion) and rate of pollen germination (chapter 6), this indicates that in capitula allowed to self naturally, radiate self pollen is slower to germinate than non-radiate self pollen. To understand the cause of this contrast, it will be necessary to elucidate what happens once pollen arrives on the stigmatic surface. Self pollen lands on the stigma surface

once the pollen plug shatters (Marshall 1982). It may be that radiate pollen which is applied to the stigma is able to rehydrate more easily because it is in closer contact with the stigmatic surface than radiate self pollen in untreated capitula. For example, if radiate self pollen lands on the stigmatic surface in clumps and non-radiate pollen does not (or does so to a lesser degree), radiate self pollen might consequently be slower to germinate (see Thomson, 1988, for discussion of the effects of clumping on pollen germination). Whatever the reason, further investigation of the processes controlling pollen germination in *S.vulgaris* are needed.

In a wider context, it has to be emphasised that the interpretation of results obtained from various crossing programmes designed to test the relative effectiveness of different pollen types in achieving fertilisation of available ovules (see chapter 5), is anything but easy. How much variation in reproductive success among individuals is due to the influence of the male parent, the female parent or a combination of the two, and the extent to which pollen tube growth rate is a trait of pollen, competition between different types of pollen, of the stylar environment or of a combination of any or all of these factors will remain generally unknown. Recently, Lyons et al (1989) looking at the sources of variation in reproductive success, suggest

that comparing paternal success of different pollen donors on 'disabled' females (i.e. female rendered physiologically more passive by e.g. heat shock or treatment with carbon dioxide as used to break some types of self-incompatibility) might be one way to measure the effect of the female parent on the rate of pollen tube growth. If the 'normal' female parent discriminates between pollen types, but this difference diminishes when pollen is applied to the 'disabled' female, this would indicate that the female does influence relative male performance.

Despite the tendency in recent years to invoke sexual selection as a process by which variation in reproductive success occurs (e.g Stephenson and Bertin 1983; Willson 1979; Willson and Burley 1983), the extent to which such selection occurs in natural plant populations is unclear (see Charlesworth et al 1987 for critical review). As yet it has not been clearly demonstrated that competition between pollen tubes in the style occurs as a phenomenon separate from self-incompatibility and genetic complimentarity between pollen and pistil (e.g. Snow and Mazer 1988).

In conclusion, the results presented in this thesis have gone some way towards unravelling the level of pollen competition occurring in the two morphs of *Senecio vulgaris* which exhibit different levels of outcrossing. It has been

established that there is more potential for pollen competition in the radiate morph of *S. vulgaris* than in the non-radiate morph as a consequence of pollinator preference for the radiate morph leading to higher rates of pollen transfer between radiate than between non-radiate plants. Future investigations must now focus on the mechanisms controlling pollen germination on the stigmatic surface and pollen tube growth rate in the style. We may find that one pollen type is competitively superior to all others within a population either leading to it becoming fixed, or to variation for pollen competitive ability remaining in the population because the character is associated with lower fitness in another stage of the life cycle. Alternatively, if the major factor determining reproductive success is the interaction between specific male and female parents, selection will maintain variation in pollen tube growth rates within a population due to specific pollen types being specialists in particular stylar environments.

REFERENCES

ABBOTT R.J. 1976(a). Variation within common groundsel, *Senecio vulgaris* L. I. Genetic response to spatial variation of the environment. *New Phytol.* 76: 153-164.

ABBOTT R.J. 1976(b). Variation within common groundsel, *Senecio vulgaris* L. II. Local differences within cliff populations on Puffin Island. *New Phytol.* 76: 165-172.

ABBOTT R.J. and IRWIN J.A. 1988,. Pollinator movement and the polymorphism for outcrossing rate at the ray floret locus in Groundsel, *Senecio vulgaris* L. *Heredity* 60: 295-298.

ABBOTT R.J., IRWIN J.A. and FORBES D.G. (in press). Presence of self-incompatible ray florets in radiate *Senecio vulgaris* L. unconfirmed. *Heredity.*

ALLARD R.W. 1970. Population structure and sampling methods. pp 97-108 In : O.H. Frankel and E.Bennett (eds) *Genetic resources in Plants.* Blackwell, Oxford.

ALLARD R.W. 1975. The mating system and microevolution. *Genetics* 79: 115-126.

ALLARD R.W.; JAIN S.K. and WORKMAN P.L. 1968. The genetics of inbreeding populations. *Adv. Genetics* 14: 55-131.

ALLARD R.W.; BABBEL G.R.; CLEGG M.T.and KAHLER A.L. 1972. Evidence for coadaptation in *Avena barbata.* *Proc.Nat.Acad.Sci USA* 69: 3043-3048.

ALLARD R.W. and WORKMAN P.L. 1963. Population studies in predominantly self-pollinated species. IV Seasonal fluctuations in estimated values of genetic parameters in lima bean populations. *Evolution* 18 : 470-480.

ALEXANDER J.C.M. 1975. Experimental taxonomy of some annual species of *Senecio* from the Mediterranean area. Ph.D. thesis, University of Edinburgh.

ALLEN D.E. 1967. The taxonomy and nomenclature of the radiate variants of *Senecio vulgaris* L. *Watsonia* 6 : 280-282.

ANDERSSON M. 1982. Female choice selects for extreme tail length in a widowbird. *Nature* 199 : 818-820.

ARROYO M.T.K. 1975. Electrophoretic studies of genetic variation in natural populations of allogamous *Limnanthes alba* and autogamous *L.floccosa* (Limnanthaceae). *Heredity* 35: 153-164.

ASHTON P.A. 1984. An investigation of the reason for different outcrossing rates in the two morphs of *Senecio vulgaris*. BSc. Honours project, University of St. Andrews.

BAKER H.G. and HURD P.D. 1968. Intrafloral ecology. *Ann. Rev. Entomol.* 13 : 385-414.

BARRETT S.C.H. 1982. Genetic variation in weeds. pp 73-98. In : R Charudattan and H.L.Walker (eds) *Biological control of weeds with plant pathogens*. Wiley New York.

BATEMAN A.J. 1948. Intrasexual selection in Drosophila. *Heredity* 2 : 349-368.

BATEMAN A.J. 1956. Cryptic self-incompatibility in the wallflower *Cheiranthus cheri* L. *Heredity* : 257-261.

BELL G. 1985. On the function of flowers. *Proc.R.Soc.London B.* 224 : 223-265.

BERTIN R.I. 1982. Paternity and fruit production in trumpet creeper (*Campsis radicans*). *Amer. Nat.* 119 : 694-709.

BIERZYCHUDEK P. 1981. Pollinator limitation of plant reproductive effort. *Amer. Nat.* 117 : 838-844.

BOND D.A. and POPE M. 1974. Factors affecting the proportions of cross-bred and self-bred seed obtained from field bean (*Vicia faba* L.) crops. *J. Agric. Sci.* 83 : 343-351.

BOOKMAN S.S. 1984. Evidence for selective fruit abortion in *Asclepias*. *Evolution* 38 : 72-80.

BRETTEL R.I.S. and LESLIE A.C. 1978. *Senecio squalidus* L. x *Senecio vulgaris* L. in Cambridgeshire. *Watsonia* 12 : 155-156.

BROWN A.H.D. 1979. Enzyme polymorphisms in plant populations. *Theor.Pop.Biol.* 15: 1-42.

BROWN A.H.D. and ALLARD R.W. 1970. Estimation of the mating system in open-pollinated maize populations using isozyme polymorphisms. *Genetics* 66 : 133-145.

BROWN A.H.D. and WEIR B. 1983. Measuring genetic variability in plant populations. pp 219-239 In S.D. Tanksley and J.J. Orton (eds): *Isozymes in plant genetics and breeding*. Elsevier publications.

BROWN B.A. and CLEGG M.T. 1984. Influence of flower colour polymorphism on genetic transmission in a natural population of the common morning glory, *Ipomoea purpurea*. *Evolution* 38 : 796-803, 1984.

BURTT B.L. 1977. Aspects of diversification in the capitulum. pp41-59 In V.H. Heywood, J.B. Harborne and B.L.Turner *The Biology and Chemistry of the Compositae Vol 1*. Academic Press London.

CAMPBELL J.M. and ABBOTT R.J. 1976. Variability of outcrossing frequency in *Senecio vulgaris*. *Heredity* 36 : 267-274.

CHARLESWORTH D. and CHARLESWORTH B. 1981. Allocation of resources to male and female function in hermaphrodites. *Biol. J. Linn. Soc.* 15 : 57-74.

CHARLESWORTH D., SCHEMSKE D.W. and SORK V.L. 1987. The evolution of plant reproductive characters; sexual versus natural selection. pp317-335 In S.C. Stearns (ed) *The evolution of sex and its consequences*. Birkhauser-verlag, Basel-Boston.

CHARNOV E.L. 1979. Simultaneous hermaphroditism and sexual selection. *Proc. Nat. Acad. Sci.* USA. 76 : 2480-2484.

CHARNOV E.L. 1982. *The theory of sex allocation*. Princeton University Press.

CHATER A.O. and WALTERS S.M. 1976. *Senecio*. In Tutin, T.G. et al (eds) *Flora Europea* Vol 4. Cambridge Universtiy Press, Cambridge.

CLEGG M.T. 1980. Measuring plant mating systems. *Bioscience* 30 : 814-818.

CLUSTAN - 1987. Cluster analysis software. Copyright 1987 D.Wishart. University of St.Andrews (Pubs).

CONN J.S. and BLUM U. 1981. Sex ratio of *Rumex hastatus*: The effect of environmental factors and certation. *Evolution* 35 : 1108-1116.

CORRENS C. 1914. *Die Bestimmung und Vererbung des Geschlechtes nach neven Versuchen mit hoheren Pflanzen*. Borntraeger, Berlin.

CORRENS C. 1928. Bestimmung, Vererbung und Verteilung des Geschlechtes bei den hoheren Pflanzen. *Handbuch der Vererbungswissenschaft* 2 : 1-138.

COUVET D., HENRY J. and GOUYON P.H. 1985. Sexual selection in hermaphrodites. *Amer. Nat.* 126 : 294-299.

COX C.R. and LE BOEUF B.J. 1977. Female incitation of male competition : a mechanism for sexual selection. *Amer. Nat.* 111 : 317-335.

CREPET W.L. 1983. The role of insect pollination in the evolution of the angiosperms. pp1-50 In L.Real (ed) *Pollination Biology*. Academic Press New York.

CRISP P. 1972. Cytotaxonomic studies in the Section Annui of *Senecio*. PhD thesis, University of London.

CURRAH L. 1983. Pollen competition and the breeding system in onion (*Allium cepa* L.). pp 375-379 In D.L. Mulcahy, E. Ottaviano (eds) : *Pollen : Biology and Implications for Plant Breeding*. Elsevier, New York.

DARWIN C.R. 1859. *The Origin of Species*. Murray, London.

DARWIN C. 1871. *The Descent of Man and Selection in relation to Sex*. John Murray, London.

DAVIS L., STEPHENSON A.G. and WINDSOR J.A. 1987. Pollen competition improves performance and reproductive output of the common Zucchini Squash under field conditions. *J.Amer.Soc.Hort.Sci.* 112 : 712-716.

EENINK A.H. 1982. Compatibility and incompatibility in Witloof-chicory (*Cichonium intybus* L.) 3. Gametic competition after mixed pollinations and double pollination. *Euphytica* 31 : 773-786.

EISIKOWITCH D. 1978. Insect visiting of two species of *Nigella arvensis* under adverse seaside conditions. pp125-132 In A.J. Richards (ed) : *The Pollination of Flowers by Insects*. Linnean Society Symposium series 6, Academic Press, London.

ENNOS R.A. 1981. Quantitative studies of the mating system in two sympatric species of *Ipomoea* (Convolvulaceae). *Genetica* 57 : 93-98, 1981.

ENNOS R.A. and CLEGG M.T. 1982. Effect of population substructuring on estimates of outcrossing rate in plant populations. *Heredity* 48 : 283-292.

ENNOS R.A. and CLEGG M.T. 1983. Flower colour variation in the morning glory *Ipomoea purpurea*. *J.Hered.* 74 : 247-250, 1983.

EPPERSON B.K. and CLEGG M.T. 1987a. Frequency dependent variation for outcrossing rate among flower-colour morphs of *Ipomoea purpurea*. *Evolution* 41 : 1302-11.

EPPERSON B.K. and CLEGG M.T. 1987b. First-pollination primacy and pollen selection in the morning glorg *Ipomoea purpurea*. *Heredity* 58 : 5-14.

FAEGRI K. and VAN DER PIJL L. 1966, 1971, 1979. *The principles of pollination ecology*. Pergamon press, Toronto.

FISHER R.A. 1958. *The Genetical Theory of Natural Selection*. 2nd Edition. Dover, New York.

FYFE J.L. and BAILEY N.T. 1951. Plant breeding studies in leguminous forage crops. I. Natural and crossbreeding in winter beans. *J. Agric. Sci.* 41 : 371-378.

GEIST V. 1971. *Mountain Sheep*. University of Chicago Press, Chicago, USA.

GENSTAT - 1984. statistical computer package. Copyright 1984 Lawes Agricultural Trust (Rothamstead Experimental Station).

GIBBS P.E. 1971. Studies on synthetic hybrids of British species of *Senecio*, 1. *Senecio viscosus* x *S. vulgaris* L. *Trans. Bot. Soc. Edinb.* 41 : 213-218.

GILBERT F.S. 1981. Foraging ecology of Hoverflies: morphology of mouthparts in relation to feeding on nectar and pollen in some common urban species. *Ecol. Ent.* 6 : 245-262, 1981.

GILBERT F.S. 1986. *Hoverflies*. Cambridge University Press.

GORI D.F. 1989. Floral colour change in *Lupinus argenteus* (Fabaceae) : Why should plants advertise the location of unrewarding flowers to pollinators ? *Evolution* 43 : 870-881.

GOTTLIEB L.D. 1981. Electrophoretic evidence and plant populations. *Progress in Phytochemistry* 7 : 1-46.

GOUYON P.H. and VERNET P. 1982. The consequences of gynodioecy in natural populations of *Thymus vulgaris*. *T.A.G.* 61 : 315-320.

GRAHAM S.W. 1989. *In vivo* and *in vitro* investigations into pollen germination in radiate and non-radiate *Senecio vulgaris* L. BSc Honours project, University of St. Andrews.

HALDANE J.B.S. 1930, 1932. *The Causes of Evolution.* Longmans and Green, London 1930. Harper New York, 1932.

HALLIDAY T.R. 1978. Sexual selection and mate choice. pp180-213 In J.R. Kress, N.B. Davies (eds) : *Behavioral Ecology: An Evolutionary Approach.* Blackwell, Oxford.

HARDING J. and TUCKER C.L. 1969. Quantitative studies on mating system II Method for estimation of male gametophytic selective values and differential outcrossing rates. *Evolution* 23 : 85-95, 1969.

HARLAND S.C. 1954. The genus *Senecio* as a subject for cytological investigation. *Proc. Bot. Soc. Br. Isl.* 1 : 256-257.

HASKELL G. 1953. Adaptation and the breeding system in groundsel. *Genetica* 26 : 468-484.

HILLEL J.M., FELDMAN M.W. and SIMCHEN G. 1973. Mating systems and population structure in two closely related species of the wheat group. I. Variation between and within populations. *Heredity* 30 : 141-167.

HOROVITZ A. and HARDING J. 1972. The concept of male outcrossing in hermaphrodite higher plants. *Heredity* 29 : 223-236.

HULL P. 1974. Self-fertilisation and the distribution of the radiate form of *Senecio vulgaris L.* in central Scotland. *Watsonia* 10 : 69-75.

HULL P. 1976. The influence of different degrees of interspecific hybridisation with *Senecio squalidus* on the frequency of the two morphs of *Senecio vulgaris. Heredity* 36 : 67-72.

HUMPHREYS M.O. and GALE J.S. 1974. Variation in wild populations of *Papaver dubium.* VIII The mating system. *Heredity* 33 : 33-41.

HUXLEY J.S. 1938. The present standing of the theory of sexual selection. pp 11-42 In G.R. de Beer (ed) *Evolution: Essays on Aspects of Evolutionary Biology.* Oxford University Press, London.

HUXLEY J.S. 1942. *Evolution, the modern synthesis.* Allen and Unwin, London.

ILSE D. 1949. Colour discrimination in the Drone fly *Eristalis tenax. Nature* 163 : 255-256, 1949.

IMAM A.G. and ALLARD R.W. 1965. Population studies in predominantly self-pollinated species VI Genetic variability between and within natural populations of wild oats, *Avena fatua* L., from differing habitats in California. *Genetics* 51 : 49-62.

INGRAM R. 1977. Synthesis of the hybrid *S.squalidus* L. x *S.vulgaris* L. f. *radiatus* Hegi. *Heredity* 39 : 67-72.

INGRAM R. and TAYLOR L. 1982. The genetic control of a non-radiate condition in *Senecio squalidus* L. and some observations on the role of ray florets in the Compositae. *New Phytol.* 91 : 749-756.

INGRAM R., WEIR J. and ABBOTT R.J. 1980. New evidence concerning the origin of inland radiate groundsel *Senecio vulgaris* hybernicus syme. *New Phytologist* 84 : 543-546.

IRWIN J.A. 1986. An investigation into the causes of differential outcrossing frequency in the radiate and non-radiate morphs of *Senecio vulgaris* L. BSc Honours project, University of St.Andrews.

JAIN S.K. 1976. Evolution of inbreeding in plants. *Ann. Rev. Ecol. Syst.* 7 : 468-495.

JAIN S.K. 1979. Estimation of outcrossing rates : some alternative procedures. *Crop Science* 19 : 23-26.

JANZEN D.H. 1977. A note on optimal mate selection by plants. *Amer.Nat.* 111 : 365-375.

JOHNSON C.M. and MULCAHY D.L. 1978. Male gametophyte in maize II. Pollen vigour in inbred plants. *T.A.G.* 51 : 211-215.

JONES D.F. 1928. *Selective fertilisation.* University of Chicago Press.

KADEREIT J.W. 1984. The origin of *Senecio vulgaris* (Asteraceae). *Pl. Syst. Evol.* 145 : 135-153.

KAY Q.O.N. 1976. Preferential pollination of yellow flowered morphs of *Raphanus raphanustrum* by *Pieris* and *Eristalis species. Nature* 261 : 230-232.

KAY Q.O.N. 1978. The role of preferential and assortative pollination in the maintenance of flower colour polymorphisms. pp 175-190 In A.J. Richards (ed) *The Pollination of Flowers by Insects.*

KENT D.H. 1956. *Senecio squalidus* L. in the British Isles. 1. Early records (to 1877). *Proc. Bot. Soc. Br. Isl.* 2 : 115-118.

KENT D.H. 1957. *Senecio squalidus* L. in the British Isles. 3. East Anglia. *Trans. Norfolk and Norwich Nat. Soc.* 18(5) : 30 - 31.

KENT D.H. 1963. *Senecio squalidus* L. in the British Isles. 7. Wales. *Nature Wales* 8 : 175-178.

KENT D.H. 1964a. *Senecio squalidus* L. in the British Isles. 4. Southern England (1940-). *Proc. Bot. Soc. Br. Isl.* 5 : 210-213.

KENT D.H. 1964b. *Senecio squalidus* L. in the British Isles. 5. The Midlands (1940-). *Proc. Bot. Soc. Br. Isl.* 5 : 214-216.

KENT D.H. 1964c. *Senecio squalidus* L. in the British Isles. 6. Northern England (1940-). *Proc. Bot. Soc. Br. Isl.* 5 : 217-219.

KENT D.H. 1964d. *Senecio squalidus* L. in the British Isles. 9. Ireland. *Irish Nat. J.* 14 : 203-204.

KENT D.H. 1966. *Senecio squalidus* L. in the British Isles. 8. The recent spread in Scotland. *Glasgow Nat.* 18 : 407-408.

KNOX R.B, WILLIAMS E.G. and DUMAS C. 1986. Pollen, pistil and reproductive function in crop plants. *Plant Breeding reviews* 4 : 9-79.

LAGUDAH E.S.and HALLORAN G.M. 1989. Phylogenetic relationships of *Triticum tauschii*, the D genome donor to hexaploid wheat. *T.A.G.* 77 : 851-56, 1989.

LAYTON C.R. and GANDERS F.R. 1984. The genetic consequences of contrasting breeding systems in *Plectritis. Evolution* 38 : 1308-1325.

LEE T.D. and HARTGERINCK A.P. 1986. Pollination intensity, fruit maturation pattern and offspring quality. pp417-422 In D.L. Mulcahy. G.B. Mulcahy and E. Ottaviano (eds) *Biotechnology and Ecology of Pollen*. Springer-verlag, Berlin.

LEVIN D.A. 1969. The effect of corolla colour and outline on interspecific pollen flow in *Phlox*. *Evolution* 23 : 444-445.

LEVIN D.A. 1972. Low frequency disadvantage in the exploitation of pollinators by corolla variants in *Phlox*. *Amer. Nat.* 106 : 453-460.

LEVIN D.A. 1975. Gametophytic selection in Phlox. pp207-217 In D.L. Mulcahy (ed) *Gametic competition in plants and animals*. North Holland Amsterdam, Netherlands.

LEVIN D.A. and KERSTER H.W. 1973. Assortative pollination for stature in *Lythrum salicaria*. *Evolution* 27 : 144-152.

LOVELESS M.D. and HAMRICK J.L. 1984. Ecological determinants of genetic structure in plant populations. *Ann. Rev. Ecol. Syst.* 15 : 65-96.

LYONS E.A., WASER N.M., PRICE M.V., ANTONOVICS J. and MOTTEN A.F. 1989. Sources of variation in plant reproductive success and implications for concepts of sexual selection. *Amer. Nat.* 134 : 409-433.

MANLY B.J. 1988. *Multivariate Statistical Methods, A Primer*. Chapman and Hall.

MARSHALL D.F. 1982. Studies on the breeding system of *Senecio vulgaris* L. PhD thesis, University of St.Andrews.

MARSHALL D.F. and ABBOTT R.J. 1980. On the frequency of introgression of the radiate Tr allele from *Senecio squalidus* into *S.vulgaris*. *Heredity* 45 : 133-135.

MARSHALL D.F. and ABBOTT R.J. 1982. Polymorphism for outcrossing frequency at the ray floret locus in *Senecio vulgaris* L. I Evidence. *Heredity* 48 : 227-235.

MARSHALL D.F. and ABBOTT R.J. 1984a. Polymorphism for outcrossing frequency at the ray floret locus in *Senecio vulgaris L.* II. Confirmation. *Heredity* 52 : 331-336.

MARSHALL D.F and ABBOTT R.J. 1984b. Polymorphism for outcrossing frequency at the ray floret locus in *Senecio vulgaris* L. III Causes. *Heredity* 53 : 145-149.

MARSHALL D.R. and ELLSTRAND N.C. 1985. Proximal causes of multiple paternity in wild radish *Raphanus raphanistrum*. *Amer.Nat.* 126 : 596-605.

MARSHALL D.R. and ELLSTRAND N.C. 1986. Sexual selection in *Raphanus sativus*: Experimental data on non-random fertilisation, maternal choice and consequences of multiple paternity. *Amer.Nat.* 127 : 446-461.

MARSHALL D.R and ELLSTRAND N.C. 1988. Effective mate choice in wild radish: Evidence for selective seed abortion and its mechanism. *Amer.Nat.* 131 : 739-756.

MATHER K. 1943. Polygenic inheritance and Natural Selection. *Biological review* 18 : 32-64.

McKONE M.J. 1987. Sex allocation and outcrossing rate : A test of theoretical predictions using Bromograsses (*Bromus*). *Evolution* 41 : 591-598.

MEEUSE A.D.J. 1978. Entomophily in *Salix*. Theoretical considerations. pp47-50 In A.J. Richards : *The Pollination of Flowers by Insects*. Linnean Society Symposium series 6, Academic Press, London.

MONAGHAN J.L. and HULL P. 1976. Differences in vegetative characteristics among four populations of *Senecio vulgaris* L. possibly due to interspecific hybridisation. *Ann. Bot.* 40 : 125-128, 1976.

MULCAHY D.L. 1971. A correlation between gametophytic and sporophytic characteristics in *Zea mays* L. *Science* 171 : 1155-1156.

MULCAHY D.L. 1975. The biological significance of gamete competition. pp 1-4 In D.L.Mulcahy (ed) *Gamete competition in plants and animals*. North Holland Amsterdam, Netherlands.

MULCAHY D.L. 1979. The rise of angiosperms a genecological factor. *Science* 206 : 20-23.

MULCAHY D.L. 1983. Models of pollen-tube competition in *Geranium masculatum*. pp 151-161 In L.Real (ed) *Pollination Biology*. Academic Press, New York.

MULCAHY D.L. 1984. The relationships between self-incomptibility, pseudo-compatibility and self-compatibility. pp229-236 In W.F. Grant (ed) *Plant Biosystematics*. Academic Press, Toronto.

MULCAHY D.L. and KAPLAN S.M. 1979. Mendelian ratios despite non-random fertilisation? *Amer.Nat.* 113 : 419-425.

MULCAHY D.L. and MULCAHY G.B. 1975. The influence of gametophytic competition on sporophytic quality. *T.A.G.* 46 : 277-280.

MULCAHY D.L., MULCAHY G.B. and OTTAVIANO E. 1975. Sporophytic expression of gametophytic competition in *Petunia hybrida*. pp227-232 In D.L. Mulcahy (ed) *Gamete competition in plants and animals*. North Holland Amsterdam Netherlands.

MULINIX C.A. and LEZZONI A.F. 1988. Microgametophytic selection in two alfalfa (*Medicago sativa* L.) clones. *T.A.G.* 75 : 917-922.

NAKAI Y. 1979. Isozyme variation in *Aegilops* and *Triticum* 4. The origin of the common wheats revealed from the study on esterase isozymes in synthesised hexaploid wheats. *Jpn. J. Genet.* 54 : 175-189.

NETTANCOURT D. DE 1977. *Incompatibility in angiosperms.* Springer-verlag, Berlin.

OTTAVIANO E., SARI-GORLA M. and MULCAHY D.L. 1975. Genetic and intergametophytic influences on pollen-tube growth. pp125-134 In D.L.Mulcahy (ed) *Gamete competition in plants and animals.* North Holland, Amsterdam, Netherlands.

OTTAVIANO E., SARI-GORLA M. and ARENARI I. 1983. Male gametophyte competitive ability in maize. Selection and implications with regard to the breeding system. pp 367-373 In D.L. Mulcahy E.Ottaviano *Pollen : Biology and Implications for Plant Breeding.* Elsevier, New York.

OXFORD G.S. and ANDREWS T. 1977. Variation in characters affecting fitness between radiate and non-radiate morphs in natural populations of groundsel (*Senecio vulgaris L.*) *Heredity* 39 : 383-388.

PFAHLER P.L. 1965. Fertilisation ability of maize pollen grains. I. Pollen sources. *Genetics* 52 : 513-520.

PFAHLER P.L. 1967. Fertilisation ability of maize pollen grains II. Pollen genotype, female sporophyte and pollen storage interactions. *Genetics* 57 : 513-521.

PFAHLER P.L. 1970. *In vitro* germination and pollen-tube growth of maize (*Zea mays*) pollen. III The effect of pollen genotype and pollen source vigour. *Can J.Bot.* 48 : 111-115.

QUELLER D.C. 1983. Sexual selection in a hermaphrodite plant. *Nature* 305 : 706-707.

QUELLER D.C. 1985. Proximate and ultimate causes of low fruit production in *Asclepias exaltata*. *Oikos* 44 : 373-381.

RICHARDS A.J. 1975. The inheritance and behaviour of the rayed gene complex in *Senecio vulgaris*. *Heredity* 34 : 95-104.

RICK C.M. and TANKSLEY S.D. 1981. Genetic variation in *Solanum pennellii*. Comparisons with two other sympatric tomato species. *Plant Syst. Evol.* 139 : 11-45.

RICK C.M., FORBES J.F.and TANKSLEY S.D. 1979. Evolution of mating systems in *Lycopersicon hirsutum* as deduced from genetic variation in electrophoretic and morphological characters. *Plant Syst. Evol.* 132 : 279-298.

RITLAND K. 1983. Estimation of mating systems. pp 289-302 In S.D.Tanksley and T.J.Orton (eds) *Isozymes in Plant Genetics and Breeding A*. Elsevier Publications.

ROSS M.D. and ABBOTT R.J. 1987. Fitness, sexual asymmetry, functional gender and selfing in *Senecio vulgaris* L. *Evolutionary trends in Plants* 1 : 21-28.

ROSS M.D. and GREGORIUS H.R. 1983. Outcrossing and sex function in hermaphrodites : A resource allocation model. *Amer. Nat.* 121 : 204-222.

RYCHLEWSKI J. and ZARZYCKI K. 1975. Sex ratio in seeds of *Rumex acetosa* L. as a result of sparse or abundant pollination. *Acta. Biol. Crakov. Bot.* 18 : 101-114.

SARI-GORLA M.S., OTTAVIANO E. and FAINI D. 1975. Genetic variability of gametophyte growth rate in maize. *T.A.G.* 46 : 289-294.

SCANDALIOS J.G. 1969. Genetic control of multiple forms of enzymes : a review. *Biochemical Genetics* 3 : 37-79.

SCHAAL B. 1980. Measurement of gene flow in *Lupinus texensis*. *Nature* 284 : 450-451.

SCHEMSKE D.W. and FENSTER C. 1983. Pollen grain interactions in a neotropical *Costus*: Effects of clump size and competitiors. pp405-410 In D.L. Mulcahy E.Ottaviano (eds) *Pollen: Biology and implications for plant breeding.* Elsevier, New York.

SCHLICHTING C.D., STEPHENSON A.G., DAVIS L.E. and WINDSOR J.A. 1987. Pollen competition and offspring variance. *Evol.Trend.Plant.* 1 : 35-39.

SCHOEN D.J. 1982. The breeding system of *Gilia achilleifolia* : variation in floral characteristics and outcrossing rate. *Evolution* 36 : 352-360.

SCHOEN D.J. and CLEGG M.T. 1985. The influence of flower colour on outcrossing rate and male reproductive success in *Ipomoea purpurea*. *Evolution* 39 : 1242-1249.

SNAPE J.W. and LAWRENCE M.J. 1971. The breeding system of *Arabidopsis thaliana*. *Heredity* 27 : 299-302.

SNOW A.A. and MAZER S.J. 1988. Gametophytic selection in *Raphanus raphanistrum*: A test for heritable variation in pollen competitive ability. *Evolution* 42 : 1065-1075.

SOKAL R.R. and ROHLF F.J. 1981. *Biometry*. The principles and practice of statistics in Biological research. Freeman, New York.

STACE C.A. 1977. The origin of radiate *Senecio vulgaris* L. *Heredity* 39 : 383-388.

STANTON M.L., SNOW A.A. and HANDEL S.N. 1986. Floral evolution : attractiveness to pollinators increases male fitness. *Science* 232 : 1625-1627.

STEBBINS G.L. 1950. *Variation and Evolution in Plants*. Columbia University Press, New York.

STEBBINS G.L. 1957. Self-fertilisation and population variability in higher plants. *Amer.Nat.* 91 : 337-354.

STEBBINS G.L. 1958. Longevity, habitat and release of genetic variability in higher plants. *Cold Spring Harbour Symposium Quant. Biol.* 23 : 386-378.

STELLEMAN P. 1978. The possible role of insect visits in pollination of reputedly anemophilous plants exemplified by *Plantago lanceolata* and Syrphid flies. pp 41-46 In A.J.Richards: *The Pollination of Flowers by Insects*. Linnean Society Symposium series, Academic press, London.

STEPHENSON A.G. and BERTIN R.I. 1983. Male competition, female choice and sexual selection in plants. pp109-149 In L.Real (ed) *Pollination Biology*. Academic Press, New York.

STUBBS A.E and FAULK S.J. 1983. *British Hoverflies - An illustrated identification guide*. British Ent. and Nat. History Soc. London.

SUITER K.A., WENDEL J.F. and CASE J.S. 1983. *Linkage-1* version 3.50. Computer program for the detection and analysis of genetic linkage.

SYME J.T.B. 1875. *Senecio vulgaris* L. var *hibernica* mihi. *Botl. Exch. Club Rep. Curators 1872-1874* : 27-28.

TANKSLEY S.D., ZAMIR D. and RICK C.M. 1981. Evidence for extensive overlap of sporophytic and gametophytic gene expression in *Lycopersicion esculentum. Science* 213 : 453-455.

TAYLOR L. 1984. The potential for introgression in a British polyploid complex. PhD thesis, University of St. Andrews.

TER-AVANESIAN D.V. 1978a. The effect of varying the number of pollen grains used in fertilisation. *T.A.G.* 52 : 77-79.

TER-AVANESIAN D.V. 1978b. Significance of pollen amount for fertilisation. *Bull.Torr.Bot.Club.* 195 : 2-8.

THOMSON J.D. 1988. Germination schedules of pollen grains : implications for pollen selection. *Evolution* 43 : 220-223.

TRIVERS R.L. 1972. Parental investment and sexual selection. pp136-179 In B.Campbell (ed): *Sexual selection and the Descent of Man.* Aldine, Chicago.

TROW A.H. 1912. On the inheritance of certain characters in the common groundsel *Senecio vulgaris* L. and its segregates. *J.Genet.* 2 : 239-276.

VALENTINE D.H. 1978. The pollination of introduced species, with special reference to the British Isles and the genus *Impatiens.* pp117-124 In A.J. Richards : *The Pollination of Flowers by Insects.* Linnean Societuy Symposium series 6, Academic Press, London.

VALENTINE D.H. 1979. Experimental work on the British Flora. *Watsonia* 12 : 201.

VAN BREUKELEN E.W.M. 1982. Competition between 2X and X pollen in the styles of *Solanum tuberosum* determined by a quick *in vivo* method. *Euphytica* 31 : 585-590.

VAN DER KLOET S.P. 1982. Effects of pollen donors on seed production, seed weight, germination and seedling vigour in *Vaccinium corymbosum* L. *Amer.Midl.Nat.* 112 : 392-396.

VISSER T. and MARCUCCI M.C. 1983. Pollen and pollination experiments IX. The pioneer pollen effect in apple and pear related to the interval between pollinations and the temperature. *Euphytica* 32 : 703-709.

VISSER T. and VERHAEGH J.J. 1980. Pollen and pollination experiments II. The influence of the first pollination on the effectiveness of the second one in apple. *Euphytica* 29 : 385-390.

WARREN J.M. 1987. The origin and maintenance of the capitulum polymorphism in *Senecio vulgaris* L. (Groundsel). PhD thesis. University of York.

WARREN J.M. 1988. Outcrossing frequencies within and between capitulum morphs in Groundsel *Senecio vulgaris* L. *Heredity* 61 : 161-166.

WARREN J.M., CRAWFORD T.J. and OXFORD G.S. 1988. Inhibition of self-pollen germination in *Senecio vulgaris* L. *Heredity* 60 : 33-38.

WASER N.M. 1983. The adaptive nature of floral traits : ideas and evidence. pp241-285. In L. Real (ed) *Pollination Biology.* Academic Press New York.

WASER N.M. and PRICE M.V. 1981. Pollinator choice and stabilising selection for flower colour in *Delphinium nelsonii. Evolution* 35 : 376-390.

WASER N.M. and PRICE M.V. 1983. Optimal and actual outcrossing in plants and the nature of plant-pollinator interactions. pp 341-359 In C.E. Jones and R.J. Little (eds) *Handbook of Experimental Pollination Biology.* Van Nostrand Reinhold New York.

WILLING R.P. and MASCARENHAS J.P. 1984. Analysis of complexity and diversity of mRNA's from pollen and shoots of *Tradescantia. Plant Physiol.* 75 : 865-868.

WILLING R.P., EISBERG A. and MASCARENHAS J.P. 1984. Genes active during pollen development and the construction of cloned cDNA libraries to mRNA's from pollen. *Plant cell incompat. newsl.* 16 : 11-12.

WILLSON M.F. 1979. Sexual selection in plants. *Amer. Nat.* 113 : 777-790.

WILLSON M.F. 1983. *Plant Reproductive Ecology.* Wiley, New York.

WILLSON M.F. and BURLEY N. 1983. *Mate choice in plants : Tactics, mechanisms and consequences.* Princeton University Press Princeton New Jersey.

WINDSOR J.A., DAVIS L.E. and STEPHENSON A.G. 1987. The relationship between pollen load and fruit maturation and the effect of pollen load on offspring vigour in *Cucurbita pepo. Amer.Nat.* 129 : 643-656.

WRIGHT S. 1931. Evolution in Mendelian populations. *Genetics* 16 : 97-156.

WRIGHT S. 1932. The roles of mutation, inbreeding, cross breeding and selection in evolution. *Proc.VI Int.Cong.Genet.* 1 : 356-366.

WRIGHT S. , 1946. Isolation by distance under diverse systems of mating. *Genetics* 31 : 39-59.

WRIGHT S. 1964. Stochastic processes in evolution. pp199-244 In J.Gurland (ed) *Stochastic models in Medicine and Biology.* University of Wisconsin Press, Madison.

WYATT R. 1983. Pollinator-plant interactions and the evolution of breeding systems. pp 51-96 In L. Real (ed) *Pollination Biology.* Academic Press, New York.

YAMADA M. amd MURIKAMI K. 1983. Superiority in gamete competition of pollen derived from F_1 plants in maize. pp 389-395 In D.L. Mulcahy, E. Ottaviano *Pollen: Biology and Implications for Plant Breeding.* Elsevier, New York.

APPENDICES

Appendix (A).

Starch gel electrophoresis.

1. Preparing a gel.
33g of hydrolysed potato starch (Sigma S4501) are mixed with 300ml of gel buffer (constituents given below) in a 1 litre Buchner flask. The flask is sealed using aluminium foil. The flask is swirled vigorously above a hot bunsen flame until the solution thickens. (the consistency changes, the solution clears and becomes more viscous). The flask is then removed from the heat. A vacuum pump is used to evacuate the flask and remove air bubbles from the gel. The fluid is then poured rapidly into a gel mould. The mould is sealed by lowering a glass plate over the top of the hot starch. The gel is left to set.

2. Preparing and loading samples.
A young developing flowerbud is removed from each plant under test and placed in one of the cavities of a microtitre plate. One drop of extraction buffer (see below) is added to each well. The sample is crushed using a glass rod until a green solution remains. Once all samples have been crushed (24 per gel) the gel can be loaded. The glass plate is removed from the top of the cold gel and the excess starch wiped from the edges of the gel mould. A gel-comb is used to make 24 wells across the gel, 5cm from the base. the gel is then covered in cling film. Using a pair of fine forceps, a small chromatography paper wick is dipped into each well to soak up some of the extract. Wicks are then placed into the wells of the gel. Once all the samples have been loaded, a small drop of tracker dye is put onto the first track and the cling film cover replaced.

3. Running the gel.
The gel is loaded onto a gel-rig and run at 4°C (in a fridge) for approximately 3 to 4 hours at 250 volts. When the gel front, indicated by the tracker dye has moved 8cm from the origin, the current is switched off.

4. Slicing the gel.
The wicks are removed using fine forceps and the gel is cut round the edges so as to loosen it. A cut is made along the line of the wicks and across the top of the gel just above the front marked by the tracker dye. The gel is removed from the mould and placed on a glass plate with spacers on either side. The spacers are of two different thicknesses and guide a fishing line used to slice the gel. The gel is cut into four slices using the spacers in different combinations. The uppermost slice is discarded and the next two slices are placed in glass staining trays to which the

staining solution is added.

5. Staining the gel.
The recipies for the staining solutions are given below. The solution is poured onto a gel slice which is incubated at 37°C. Bands appear on the gel after 15-60 minutes (or longer) depending on the enzyme being assayed. Once the bands have appeared, the staining solution is poured off and the gel washed twice in distilled water. 50% glycerol is then poured over the gel which is left to clear of background stain.

6. Recipies for buffers and stains.

Electrode buffer.

11.9g Boric acid. (Sigma B0252)
1.2g Lithium hydroxide (Sigma L4256)
Dissolved in 1 litre distilled / deionised water. pH 8.3 adjusted using dry components.

Gel buffer.

5.45g Trizma base. (Sigma T1503)
1.28g Anhydrous citric acid. (Sigma C0759)
Dissolved in 900ml of distilled / deionised water and 100ml of electrode buffer. pH adjusted using 1M NaOH or 1M HCl.

Stains.
(i) Alpha-esterase
50ml water
40ml 0.2M sodium phosphate solution. (Sigma S0876)
10ml 0.2M sodium hydrogen phosphate sln. (Sigma S0751)
2ml 1% alpha-napthyl acetate (Sigma N8505) in acetone.
125mg Fast blue RR. (Sigma F0500)

(ii) Beta-esterase.
50ml water
40ml 0.2M sodium phosphate solution. (Sigma S0876)
10ml 0.2M sodium hydrogen phosphate sln.(Sigma S0751)
1ml 1% bate-napthyl acetate (Sigma N6875) in acetone.
125mg Fast blue RR. (Sigma F0500)

(iii) Phosphoglucose isomerase (PGI).
50ml 0.1M Tris-HCl buffer, pH8.5
40mg Fructose-6-phosphate (Disodium) (Sigma F3627)
50ug Glucose-6-phosphate dehydrogenase (Sigma G4134)
7mg NADP (Sigma N3886)
12mg MTT (Sigma M2128)
3mg PMS (Sigma P9625)
0.5ml 10% $MgCl_2$ (BDH)

(iv) Malate dehydrogenase (MDH).
50ml 0.1M Tris-HCl buffer pH 8.5.
1g Malic acid (Sigma M9138)
12mg NAD (Sigma N8881)
10mg MTT (Sigma M2128)
3mg PMS (Sigma P9625)

(v) Glutamate oxaloacetate transaminase (GOT).
50ml 0.1M Tris-HCl buffer pH 8.5
250mg PVP (40T)
710mg Na_2HPO_4 (Sigma S0876)
25mg EDTA (Tetrasodium) (Sigma ED4SS)
18mg alpha-keto glutaric acid (Sigma K3752)
65mg Aspartic acid (Sigma A9256)
300mg Fast blue BB (Sigma F0250)
1mg Pyridoxal-5-phosphate (Sigma P9255)

(vi) Glyceraldehyde-3-phosphate dehydrogenase (G-3-PD).
50ml 0.1M Tris-HCl buffer pH 8.5
70mg Fructose-1-6-diphosphate (trisodium) (Sigma 725-1)
10ug Aldolase (Sigma G1259)
10mg NADP (Sigma N3886)
15mg MTT (Sigma M2128)
1mg PMS (Sigma P9625)

(vii) Phosphoglucomutase (PGM).
50ml 0.1M Tris-HCl buffer pH 7.5
100mg Glucose-1-phosphate (Sigma G1259)
20 units Glucose-6-phosphate dehydrogenase(Sigma G4134)
15mg ATP (Sigma A2383)
15mg MTT (Sigma M2128)
10mg NADP (Sigma N3886)
1mg PMS (Sigma P9625)

(viii) Malic enzyme (ME)
50ml 0.1M Tris-HCl buffer pH 7.5
1g Malic acid (Sigma M9138)
10mg NADP (Sigma N3886)
15mg MTT (Sigma M2128)
3mg PMS (Sigma P9625)
0.5ml 10% $MgCl_2$ (BDH)

(ix) Acid phosphatase (ACP).
Presoak : 50ml 0.4M Acetate buffer.
50 ml 0.2M acetate buffer
50mg alpha-napthyl acid phosphate (Sigma N7000)
40mg Fast garnet GBC (Sigma F0875)
1ml 10% $MgCl_2$ (BDH)

(x) Glucose-6-phosphate dehydrogenase (G-6-PDH).
50ml 0.1M Tris-HCl buffer pH 7.5
50mg Glucose-6 phosphate (monosodium) (Sigma G7879)
10mg NADP (Sigma N3886)
15mg MTT (Sigma M2128)
3mg PMS (Sigma P9625)
1ml 10% $MgCl_2$ (BDH)

(xi) Glutamate dehydrogenase (GDH).
50ml 0.1M Tris-HCl buffer pH 7.5
210mg L-glutamic acid
10mg NAD (Sigma N8881)
10mg MTT (Sigma M2128)
25mg ATP (Sigma A2383)
3mg PMS (Sigma P9625)

(xii) Peroxidase (PER).
50ml 0.05M acetate buffer pH 5.0
40mg 3-amino-9-ethylcarbazole (Sigma A5754)
5ml Dimethyl formamide (Sigma D4254)
1ml $CaCl_2$ (BDH)
1ml H_2O_2 (BDH)

(xiii) Leucine amino peptidase (LAP).
Presoak : 100ml H_2O
3.09g Boric acid (Sigma B0252)
1ml 10% $MgCl_2$ (BDH)

50ml 0.1M Sodium phosphate buffer pH 5.8
75mg Fast black K (Sigma F7253)
15mg Leucyl-beta-napthylamide (Sigma L6377)
5ml Dimethyl formamide. (Sigma D4254)

(xiv) Hexokinase (HEX).
50ml 0.1M Tris-HCl buffer pH 8.0
90mg glucose (Sigma G5000)
20mg EDTA (tetrasodium) (Sigma ED4SS)
65mg ATP (Sigma A2383)
10mg NADP (Sigma N3886)
15mg MTT (Sigma M2128)
1mg PMS (Sigma P9625)
0.5ml 10% $MgCl_2$ (BDH)

(xv) Triosephosphate isomerase (TPI).
50ml 0.1M Tris-Hcl pH 8.0
100mg Arsenic (sodium salt) (Sigma A6756)
10mg Dihydroxyacetone phosphate (Sigma D7137)
0.15ml Glyceraldehyde-3-phosphate dehydrogenase (Sigma G9263)
10mg NAD (Sigma N8881)
10mg MTT (Sigma M2128)
3mg PMS (Sigma P9625)

(xvi) Succinate dehydrogenase (SuDH).
50ml 0.02M sodium phosphate buffer pH 7.0
210mg succinic acid (Sigma S2378)
185mg EDTA (disodium) (Sigma ED2SS)
25mg NAD (Sigma N8881)
20mg MTT (Sigma M2128)
25mg ATP (Sigma A2382)
3mg PMS (Sigma P9625)

(xvii) Alcohol dehydrogenase (ADH)
50ml 0.1M Tris-Hcl buffer pH 8.0
5ml Ethanol (BDH)
10mg NAD (Sigma N8881)
10mg MTT (Sigma M2128)
3mg PMS (Sigma P9625)

(xviii) Xanthine dehydrogenase (XDH).
50ml 0.05M Tris-HCl buffer pH 7.5
350mg hypoxanthine (Sigma H9377)
30mg NAD (Sigma N8881)
20mg MTT (Sigma M2128)
3mg PMS (Sigma P9625)

Appendix B.

Table B1. Intramorph maternal outcrossing rates of β-est-1^{aa} and β-est-1^{bb} electromorphs of radiate and non-radiate *S.vulgaris* in polymorphic stands where the radiate morph is present at high frequency (80%). Design A.

Morph	outcrossing rate β-est-1^{aa}	β-est-1^{bb}
Radiate		
rep. 1	16.42 (4.53)	1.23 (1.22)
rep. 2	9.48 (3.08)	0.00 -
Non-radiate		
rep. 1	3.19 (3.16)	0.00 -
rep. 2	0.00 -	0.00 -

Standard errors given in parentheses.

Table B2. Intramorph maternal outcrossing rates of β-est-1^{aa} and β-est-1^{bb} electromorphs of radiate and non-radiate *S.vulgaris* in polymorphic stands where the radiate morph is present at high frequency (80%). Design B.

Morph	outcrossing rate β-est-1^{aa}	β-est-1^{bb}
Radiate		
rep. 1	13.64 (3.79)	1.07 (1.07)
rep. 2	12.03 (3.36)	1.05 (1.05)
Non-radiate		
rep. 1	0.00 -	0.00 -
rep. 2	0.00 -	0.00 -

Standard errors given in parentheses.

Table B3. Intramorph maternal outcrossing rates of β-est-1^{aa} and β-est-1^{bb} electromorphs of radiate and non-radiate *S.vulgaris* in polymorphic stands where the the two morphs are present at equal frequency.

Morph	outcrossing rate β-est-1^{aa}	β-est-1^{bb}
Radiate		
rep. 1	8.49 (3.71)	1.87 (1.87)
rep. 2	5.29 (3.01)	2.07 (2.06)
Non-radiate		
rep. 1	5.35 (3.04)	3.30 (2.31)
rep. 2	3.36 (2.36)	0.00 -

Standard errors given in parentheses.

Table B4. Intramorph maternal outcrossing rates of β-est-1^{aa} and β-est-1^{bb} electromorphs of radiate and non-radiate *S.vulgaris* in polymorphic stands where the radiate morph is present at low frequency (20%). Design A.

Morph	outcrossing rate β-est-1^{aa}	β-est-1^{bb}
Radiate		
rep. 1	12.16 (6.73)	0.00 -
rep. 2	4.17 (4.11)	3.49 (3.45)
Non-radiate		
rep. 1	1.17 (1.16)	0.00 -
rep. 2	2.47 (1.73)	0.00 -

Standard errors given in parentheses.

Table B5. Intramorph maternal outcrossing rates of β-est-1^{aa} and β-est-1^{bb} electromorphs of radiate and non-radiate *S.vulgaris* in polymorphic stands where the radiate morph is present at high frequency (80%). Design B.

Morph	outcrossing rate β-est-1^{aa}	β-est-1^{bb}
Radiate		
rep. 1	0.00 -	0.00 -
rep. 2	0.00 -	0.00 -
Non-radiate		
rep. 1	2.94 (1.69)	0.00 -
rep. 2	2.27 (1.59)	0.00 -

Standard errors given in parentheses.

Table B6. Intramorph paternal outcrossing rates of β-est-1^{aa} and β-est-1^{bb} electromorphs of radiate and non-radiate *S.vulgaris* in polymorphic stands where the radiate morph is present at high frequency (80%). Design A.

Morph	outcrossing rate β-est-1^{aa}	β-est-1^{bb}
Radiate		
rep. 1	17.10	8.41
rep. 2	9.49	5.03
Non-radiate		
rep. 1	3.19	2.33
rep. 2	0.00	0.00

Table B7. Intramorph paternal outcrossing rates of β-est-1^{aa} and β-est-1^{bb} electromorphs of radiate and non-radiate *S.vulgaris* in polymorphic stands where the radiate morph is present at high frequency (80%). Design B.

Morph	outcrossing rate β-est-1^{aa}	β-est-1^{bb}
Radiate		
rep. 1	14.22	7.45
rep. 2	12.59	7.34
Non-radiate		
rep. 1	0.00	0.00
rep. 2	0.00	0.00

Table B8. Intramorph paternal outcrossing rates of β-est-1^{aa} and β-est-1^{bb} electromorphs of radiate and non-radiate *S.vulgaris* in polymorphic stands where the two morphs are present at equal frequency.

Morph	outcrossing rate β-est-1^{aa}	β-est-1^{bb}
Radiate		
rep. 1	8.49	6.78
rep. 2	6.26	5.45
Non-radiate		
rep. 1	7.26	5.96
rep. 2	3.36	2.30

Table B9. Intramorph paternal outcrossing rates of β-est-1^{aa} and β-est-1^{bb} electromorphs of radiate and non-radiate *S.vulgaris* in polymorphic stands where the radiate morph is present at low frequency (80%). Design A.

Morph	outcrossing rate β-est-1^{aa}	β-est-1^{bb}
Radiate		
rep. 1	12.16	6.82
rep. 2	7.36	5.73
Non-radiate		
rep. 1	1.17	0.53
rep. 2	2.47	1.14

Table B10. Intramorph paternal outcrossing rates of β-est-1^{aa} and β-est-1^{bb} electromorphs of radiate and non-radiate *S.vulgaris* in polymorphic stands where the radiate morph is present at low frequency (80%). Design B.

Morph	outcrossing rate β-est-1^{aa}	β-est-1^{bb}
Radiate		
rep. 1	0.00	0.00
rep. 2	0.00	0.00
Non-radiate		
rep. 1	2.95	1.56
rep. 2	2.27	1.20