DEMOGRAPHIC GENETICS OF THE POLYMORPHISM FOR CAPITULUM TYPE AND ASSOCIATED OUTCROSSING RATE IN 'SENECIO VULGARIS L.'

James Christopher Horrill

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

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DEMOGRAPHIC GENETICS OF THE POLYMORPHISM FOR CAPITULUM TYPE AND ASSOCIATED OUTCROSSING RATE IN SENECIO VULGARIS L.

by James Christopher Horrill

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

Department of Biology and Preclinical Medicine.

University of St. Andrews.

March 1989

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Finally, I should like in particular to thank my long-suffering parents for their patience and support. This thesis is dedicated to them.

'Twas brillig and the slithy toves
Did gyre and gimle in the wabe;

LEWIS CARROLL.

- SUPERVISOR'S CERTIFICATE -

I certify that James Christopher Horrill has fulfilled the conditions of the Resolution and Regulations appropriate to the Degree of Ph.D.

Dr. R.J. Abbott

St. Andrews, March 1989

- DECLARATION -

I James Christopher Horrill hereby certify that this thesis has been composed by myself, that it is a record of my own work, and that it has not been accepted in partial or complete fulfilment of any other degree of professional qualification. I was admitted to the Faculty of Science of the University of St. Andrews under Ordinance General No. 12 on 1.10.83 and as a candidate for the degree of Ph.D. on 1.10.84.

J. C. Horrill

St. Andrews, March 1989.

- ABSTRACT -

The primary aim of this project was to examine factors which are likely to provide a mechanism by which the polymorphism for capitulum type and associated outcrossing rate in Senecio vulgaris may be maintained. The majority of studies conducted examined the demographic genetics (i.e. the changes in the number of individuals of the two morphs at different life history stages) in field experiments initiated either in Spring or Autumn. The first series examined the demography of each morph raised from seedlings to senescence in pure stand and mixture. The second series examined the demography of each morph from seed to senescence and thereby investigated the effect on fitness of any difference between morphs in germination behaviour. The importance of inbreeding depression on the maintenance of the polymorphism was examined by comparing the relative fitness of self and open pollinated offspring of each morph under glasshouse conditions. Germination behaviour of seeds of each morph was also investigated in a series of field trials conducted over an extended period. These field studies were complemented by a series of synchronous laboratory studies to examine the effect of temperature on morph germination behaviour.

It was found that inbreeding depression is not an important factor in the maintenance of the polymorphism. No short term advantage of the radiate over the non-radiate morph was evident in the first series of demography experiments. The germination studies showed that differences between morphs in germination behaviour may occur frequently in autumn sown seed. The second series of demography experiments showed that under certain conditions this difference in germination can lead to the radiate morph attaining a greater relative fitness than the non-radiate morph. Temperature was found to be a major factor controlling the initial dormancy of seeds after sowing.

It is concluded that the difference between morphs in germination behaviour is the most likely factor that could maintain the polymorphism for capitulum type in Edinburgh populations of S. vulgaris.

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CHAPTER I. - INTRODUCTION -

Among the flowering plants great variation exists in the level of outcrossing exhibited by self-compatible species (Levin, 1979). Not only does the level of outcrossing vary between species, it can also vary greatly between populations within a species (Allard et al., 1968; Schoen, 1982). For example, in a study of 21 Californian populations of Gilia achilleifolia, Schoen (1982) demonstrated that the outcrossing rate of individual populations varied between 15%-96%. Furthermore, in some species there is additional variation in the outcrossing rate of different genotypes within populations. Such variation is present in gynodioecious species of self-compatible plants e.g. Thymus vulgaris (Valdeyron et al., 1977), where male steriles function as obligate outcrossers and hermaphrodites reproduce by variable amounts of selfing and outcrossing; it has also been demonstrated in Ipomoea purpurea where a dark flowered morph was shown to have a greater outcrossing rate than a white flowered morph (Brown and Clegg, 1984; Schoen and Clegg, 1985). Finally, Marshall and Abbott (1982, 1984a) have reported that within wild populations of Senecio vulgaris that are polymorphic for capitulum type the rayed or radiate morph outcrosses at a higher rate (15%-35%) than the non-rayed or non-radiate morph (usually <1%).

The coexistence within the same population of distinct genotypes of a species which exhibit different rates of outcrossing is of considerable interest to students of mixed mating systems in plants. Single locus theoretical models in which the mating system is controlled by a single gene (Fisher, 1941; Jain, 1976; Maynard-Smith, 1978; Charlesworth and Charlesworth, 1979; Lloyd, 1979; and Wells, 1979) show that a selfing allele or an allele which promotes a lower level of outcrossing in a population, is at a selective advantage compared to an outcrossing allele provided that selfing has no effect on viability or fertility and both high and low outcrossing alleles are present in the "outcrossing" pollen pool.

From these models it has been concluded that there is a "cost to outcrossing" borne by an outcrossing genotype in a population that is polymorphic for outcrossing and selfing genotypes (Williams, 1975; Jain, 1976). If there are no differences between morphs in viability or fertility, the population should in time become monomorphic for the selfing variant. Clearly, if an allele promoting selfing is present in an outcrossing population, there must be benefits which override the genetic cost borne by the outcrossing allele if outcrossing is to be maintained as the common mode of reproduction.

A primary objective of the work reported in this thesis has been to determine the advantages possessed by an outcrossing morph which has enabled the outcrossing radiate morph of S. vulgaris to be maintained in British populations which are polymorphic for the radiate (outcrossing) and non-radiate (selfing) morphs.

I.2 The origin and spread of the polymorphism for capitulum type in Senecio vulgaris.

The two varieties of S. vulgaris L., the non-radiate morph var. vulgaris L. and the radiate morph var. hibernicus Syme, are annuals/ephemerals which colonise open and disturbed habitats. The difference in capitulum type between these two morphs is under the genetic control of a single locus which determines the presence/absence of ray florets borne by the capitula of individual plants (Trow, 1912). Plants homozygous for the rayless allele, Tn, produce capitula containing only hermaphroditic disc florets while individuals homozygous for the rayed allele, Tr, produce in addition to a complement of hermaphroditic disc florets an outer ring of 8-13 pistillate ray florets (lacking anthers). The alleles show incomplete dominance and heterozygotes, TrTn, are distinguishable by their short stubby ray florets. Marshall and Abbott (1982; 1984a) have demonstrated that the radiate morph TrTr outcrosses at a significantly higher rate than the non-radiate, TnTn morph. This higher outcrossing rate of the radiate morph stems partly from the greater outcrossing of male sterile ray compared to hermaphroditic disc florets (Marshall and Abbott, 1984b) and also from the greater attractiveness of radiate capitula to pollinators (Abbott and Irwin, 1988).

There is strong evidence that the radiate morph of S. vulgaris is of recent origin. It was first recorded in Britain in 1866 and before then had not been recorded from elsewhere (Stace, 1977). Stace (1977) has reviewed the evidence for and against two hypotheses concerning the origin of the radiate morph: (1) the radiate morph arose by introgression between S. vulgaris var. vulgaris (2n=40) and the introduced species Senecio squalidus (2n=20); (2) the radiate morph arose through mutation. He concluded that there was insufficient evidence to discriminate between the two hypotheses. However, in a subsequent publication, Ingram et al. (1980) reported the successful and regular synthesis of triploid S. vulgaris var. vulgaris x S. squalidus hybrids from a diverse range of parental S. vulgaris material, and also the production of some tetraploid radiate offspring following the selfing of a backcrossed product of S. vulgaris var. vulgaris x S. squalidus with S. vulgaris var. vulgaris. These radiate offspring bore a close resemblance to S. vulgaris var. hibernicus in overall phenotype. The balance of evidence, therefore, is in favour of the radiate morph of S. vulgaris having originated through introgression rather than mutation.

Despite the greater level of outcrossing exhibited by the radiate morph and the inherent genetic cost that this entails, over the past 100 years the morph has formed large polymorphic populations with the non-radiate variant in many parts of Britain. The rapid spread of the radiate allele suggests that it is likely to eventually become fixed in many populations. Against this, however, is the fact that some British populations are believed to have remained polymorphic for the last 90 years e.g. in the Cardiff area, S. Wales, and, in addition, there have been no reports from anywhere, as yet, of a population that is monomorphic for the radiate morph. Although it is not known whether the polymorphism is transient or stable in British populations, its genetic control and recent origin make it an ideal subject for an investigation of the factors that favour the evolution of outcrossing in natural populations.

I.3 Maintenance of an outcrossing polymorphism

In a population that is polymorphic for outcrossing rate there are a number of factors which, in theory, can counterbalance the inherent advantage of the selfing variant. A model formulated by Charlesworth and Charlesworth (1978) considered a polymorphism for self-compatible gynomonoecious and hermaphroditic plants, a situation which is analogous to that in S. vulgaris. They showed that a polymorphism for outcrossing rate can be maintained provided inbreeding depression in the hermaphrodite (non-radiate) morph is high or the reproductive capacity of the hermaphrodite is innately lower than that of the outcrossing gynomonoecious (radiate) morph.

Inbreeding depression has often been considered as the most likely factor to balance the inherent cost of outcrossing in natural populations (Maynard-Smith, 1978; Lande and Schemske; 1985). It is predicted, however, that a polymorphism for outcrossing rate can only be maintained if the relative fitness of offspring produced by self-fertilisation is equal to 0.5 that of outcrossed progeny. If the relative fitness of progeny of the selfing variant is less than 0.5, then the allele for outcrossing would proceed to fixation. Conversely, if the relative fitness of offspring of the selfing morph is greater than 0.5, then self-fertilisation would become the mode of reproduction in the population. Clearly the conditions for the maintenance of a polymorphism for outcrossing rate due to inbreeding depression are extremely stringent and if no other factors act on such a polymorphism it would be expected that populations would usually become monomorphic either for the selfing or outcrossing morph. Such a prediction is, indeed, broadly supported by empirical surveys of outcrossing rates in plant species. From a survey of the published outcrossing rates of a number of plant species, Schemske and Lande (1985) detected a bi-modal distribution of outcrossing rates with a scarcity of populations/species exhibiting intermediate outcrossing levels. Where intermediate outcrossing rates were observed, Schemske and Lande (1985) considered these to be the result of environmental rather than genetic effects.

The predictions of the "inbreeding depression" model are of interest in the context of the evolution of the capitulum polymorphism in _S. vulgaris_ in that the outcrossing (radiate) variant arose from the selfing (non-radiate) morph. As an established variant of longstanding, the non-radiate morph should have been well adapted to inbreeding and is unlikely, therefore, to have suffered from the high levels of inbreeding depression (0.5) that are required to favour the spread of the radiate morph.

Some information on whether inbreeding depression is exhibited by either morph of _S. vulgaris_ has come from a comparison of open with self-pollinated offspring, and inbred lines with F1 hybrids (Abbott, 1985). In neither instance did Abbott obtain any evidence of an advantage to being outcrossed. What did emerge from these comparisons, however, was the finding that radiate plants derived from a Cardiff population produced more seeds per plant than non-radiate plants. Greater seed production of radiate compared to non-radiate plants had previously been found in Durham material raised under glass (Richards, 1975) and in several natural populations surveyed by Oxford and Andrews, (1977). Marshall and Abbott (1987) have since shown by means of computer simulation that the difference in seed output between morphs found by Richards (1975) and Oxford and Andrews (1977) would be sufficient to overcome the cost of outcrossing and lead to the fixation of the radiate allele in populations.

In contrast, to the findings of Richards (1975), Oxford and Andrews (1977) and Abbott (1985), a detailed examination of seed outputs of morphs in four Edinburgh populations (Marshall and Abbott 1987; Ross and Abbott, 1987) has revealed that in these populations non-radiate plants often hold advantage over radiate plants. From these findings, it is clear that the factors which may enable the radiate morph to override the "cost of outcrossing" may frequently differ between populations. In this context it is of interest that in addition to (or in place of) differences in seed output, several workers have recorded differences between the two morphs for other life history characters e.g. seed germination behaviour, flowering time and growth rate (Richards, 1975; Kadereit and Briggs, 1985; Abbott, 1986). It is possible, therefore, that each of these character differences may significantly affect the relative fitnesses of the morphs in natural populations and in some populations may be more important than in others.

I.4 Demographic analysis of plant polymorphisms.

Demographic studies of plant polymorphisms have revealed that selection frequently acts on both viability and fertility components of the life cycle. In a demographic analysis of allelic variation at the peroxidase allozyme locus in a natural population of rye grass, Lolium multiflorum, Allard et al. (1979) found that although heterozygotes exhibited superior survivorship than homozygotes from seedling to adult stages, interestingly, there was selection against heterozygotes during the reproductive cycle. This study amply demonstrated that selection can occur at one or more distinct stages in the life cycle of different genotypes of a plant species and, moreover, it is necessary to conduct a demographic analysis through all life cycle stages to gain an accurate picture of relative fitness. With this in mind, it is clear that an understanding of the factors involved in the maintenance of the polymorphism for capitulum type in S. vulgaris is unlikely to be achieved unless the demography of each morph is examined in the field under conditions similar to those experienced by plants in the wild. Only by studying the changes in numbers of each morph through all life history stages of one complete generation in a population will a true picture of the relative fitnesses of morphs be obtained.

I.5 Aims of the project

The primary aim of the project was to examine factors which are likely to provide a mechanism by which the polymorphism for capitulum type and associated outcrossing rate in S. vulgaris can be maintained. Most of the studies conducted, examined the demographic genetics of the two morphs in field experiments initiated either in spring or autumn between 1983 and 1985. The aim of these studies was to monitor changes in the number of individuals of each morph in populations, at different life history stages, so as to compare relative fitness. The first series of field experiments examined the demography of each morph from the seedling stage to senescence with reference to plant interactions in pure and mixed stands at two different sites, St. Andrews and Edinburgh. The second series of experiments examined the demography of each morph from seed to senescence and thereby investigated the effect on fitness of any differences between morphs in germination behaviour. To assess the possible importance of inbreeding depression on the maintenance of the capitulum polymorphism a detailed analysis was conducted on data previously obtained by Perryman (1983) from a study of the relative fitness of open and self-pollinated offspring of each morph under glasshouse conditions. This study was of particular interest in that comparisons were made in pure and mixed stands allowing an assessment of fitness differences under competitive conditions. The data had not been subjected previously to any detailed form of analysis. Finally, the germination behaviour of seeds of each morph was investigated in field trials conducted over an extended period at St. Andrews and Edinburgh. These field trials were complemented by a series of synchronous laboratory studies the aim of which was to examine the effect of temperature on morph germination behaviour.

CHAPTER II. DEMOGRAPHIC STUDIES 1: FROM SEEDLING TO SENESCENCE.

1A. AUTUMN 1983 EXPERIMENTS.

II.1 - INTRODUCTION -

In a population that is polymorphic for outcrossing rate the autoselective advantage of the selfing morph may be overcome if: (i) the selfing morph has an inherent lower fitness relative to the outcrossing morph stemming from reduced viability and/or fertility (Charlesworth and Charlesworth, 1978; Marshall and Abbott, 1987); (ii) there is inbreeding depression (Charlesworth and Charlesworth, 1978, Lloyd, 1979), or (iii) the greater variation expected among the progeny of the outcrossing morph (Gouyon and Vernet, 1982), raises fitness above that exhibited by the uniform progeny of the selfing morph (see Ellstrand and Antonovics, 1985). With respect to (i), several previous studies have reported differences in life history traits between the radiate and non-radiate morphs of S. vulgaris which may affect the relative fitness of the morphs in the field. Four of these studies (Richards, 1975; Kadereit and Briggs, 1985; Abbott, 1985; 1986) examined material raised either in controlled environments or under glass, whilst the fifth (Oxford and Andrews, 1977) compared samples collected directly from wild polymorphic populations. The differences recorded between morphs in these studies varied over populations. Richards (1975) using material derived from a Durham population, Oxford and Andrews (1977) with material from Selby (Yorkshire) and Barry (S. Wales), and Abbott (1985) studying material derived from a Cardiff population found that the radiate morph produced more seed per plant. In addition, Abbott (1986) reported that the radiate morph produced a greater dry weight per plant than the non-radiate morph in three out of four populations studied (i.e., from Edinburgh, Anstruther, and Durham).

In contrast, Kadereit and Briggs (1985) showed that when material from a Cambridgeshire population was raised from seed under glass, there was no significant difference in reproductive output between morphs. Also, Abbott (1986) reported that in samples derived from a population at Coleraine (N. Ireland), the non-radiate morph produced a significantly greater dry weight per plant than the radiate morph. Finally, Perryman (1983) examining Edinburgh material cultivated under glass showed that the non-radiate morph produced either the same or significantly greater amounts of seed per plant than the radiate morph.

Since Perryman's study, Marshall and Abbott (1987) have collected samples from four wild polymorphic populations located in Edinburgh, over four sampling dates, and found that in all cases the non-radiate morph produced greater numbers of seed per plant than the radiate morph.

Lack of consistent differences between morphs over populations for seed output and dry weight, based on samples collected from the wild or raised under controlled conditions, suggests that there is a significant morph x population interaction for these characters in Britain. The presence of this interaction makes the analysis of factors affecting the polymorphism more difficult. Instead of being able to extrapolate from the findings of a single population to all populations, it seems that in each population a particular and unique set of factors may operate on the polymorphism, which, in turn, must be studied in detail. Bearing this in mind, it was decided to restrict analysis in the present study to material of the radiate and non-radiate morphs derived from a single, large polymorphic population located in Edinburgh.

Until the present investigation, no study had examined life history differences between the two morphs raised from the seedling state to maturity under conditions similar to those that occur in the wild. To this end, the major aim of the study reported in this Chapter was to determine whether the radiate and non-radiate morphs differ in regard to relative survivorship and fecundity when grown in pure stands and in mixture. The study was conducted under field conditions so that plants of both morphs experienced conditions which approximated to those that prevail in wild polymorphic populations.

II.2 - MATERIALS and METHODS -

Seedlings used in experiments were germinated from seed produced under the same set of environmental conditions and collected shortly before an experiment was initiated. Seeds were collected from radiate and non-radiate plants raised in pure stands on the experimental plot at the University of St. Andrews Botanic Garden. Pure stands of each morph consisted of 500 plants (10 progeny plants of each of 50 parent plants originally sampled from a polymorphic population located on wasteground at Newhaven Road, Edinburgh). Plants within each pure stand were randomised at 0.5m. intervals and grown so as to flower and set open pollinated seeds in September 1983, that is, at a time when seed is produced naturally in the wild. The pure stands of each morph were separated by a distance of at least 30m. to minimise outcrossing between them. Following collection and removal of pappus, seeds were sown on Levington compost contained in growth trays within an unheated glasshouse. After germination, trays were transferred to cold frames so as to 'harden' the seedlings before transplanting.

Seedlings at the 2-3 true leaf stage (2-3 weeks old) were transplanted into plots that contained an upper layer of sterilised top-soil to a depth of 10cms. Within each plot a total of 168 seedlings were arranged individually at points of a 14 by 12 grid design. Seedlings that died within two weeks from planting were replaced from reserve stocks which had been raised under similar conditions to the experimental plants. As a precaution during the initial stages of experiments, seedlings were protected from molluscan herbivores by the use of 'Draza' slug pellets.

Two experiments were initiated in the Autumn of 1983 at the University Botanic Gardens at St. Andrews (planted 19-22.10.83) and at the Royal Botanic Gardens in Edinburgh (planted 23-27.10.83). The Edinburgh site was chosen as it was situated within the area of Edinburgh where natural populations of S. vulgaris occur that are polymorphic for capitulum type. The St.Andrews site was used because of its close proximity to the department. In each study comparisons were made between three treatments: i) a pure stand of the radiate morph, ii) a pure stand of the non radiate morph, and iii) a mixed stand in which both morphs were present in a 1:1 ratio. This represented a traditional de Wit replacement series design (de Wit, 1960). In each stand seedlings were spaced at 3cm. intervals. In the mixture a checkerboard design was employed with radiate and non-radiate seedlings planted in alternate positions.

In each experiment, plots were arranged, one metre apart, in a randomised block design, with replicates of treatments arranged as blocks. Replication was eight-fold. At both St. Andrews and RBG Edinburgh, plots were monitored after planting on a weekly basis to assess mortality throughout the seedling, vegetative and flowering stages. In the experiment at RBGE, a further record was taken of the number of capitula produced per plant (i.e after all plants in a plot had finished fruiting). This record was taken after plants were harvested on 30.6.84.

II.3 - ANALYSIS OF DATA -

Differences between treatments (i.e. pure stand radiate, pure stand non-radiate and the mixture) for plant survivorship throughout the experimental period, number of plants to fruit, and number of capitula produced per replicate, were examined by a two factor analysis of variance. In the ANOVA, a mixed model was assumed with treatments considered as fixed and replicates as random effects. Each main effect was tested for significance against the interaction mean square. To determine the significance of differences between i) the radiate and non-radiate morphs

Table II.1 ANOVA model for determining differences in survivorship and fecundity: (i) between the radiate (R) and non-radiate (N) morphs; and (ii) between the pure stands (PS) and the mixture (Mix), by two orthogonal contrasts extracted from the treatment term.

Source of Variation		d.f
Replication		7
Treatment	(R v N v Mix)	2
Contrast i)	R v N	1
Contrast ii)	P.S v Mix	1
Interaction		14
Total		23

Table II.2 ANOVA model to determine, for a given morph, differences in survivorship and fecundity between plants raised in pure stand (P.S) compared to mixture (Mix).

Source of Variation	d.f
Replication	7
Treatment (P.S v Mix)	1
Interaction	7
Total	15

when raised in pure stands; and ii) the pure stands and the mixture, two orthogonal contrasts were extracted from the overall treatment term of the ANOVA as shown in Table II.1. For the character capitulum number per plant, the analysis was conducted on individual values rather than replicate values. The ANOVA therefore contained an additional within replicates term against which the replicate term and treatment x replicate interaction could be tested.

For plants raised in mixture, paired t-tests were conducted to determine if differences between morphs were significant. Finally, an analysis of differences in the performance of a morph in pure stand and in mixture, was conducted using a two factor mixed effect ANOVA described in Table II.2.

In addition to analysing differences in means, a comparison of morph variances was also conducted. The total phenotypic variance of each morph was calculated from the pooled data of each treatment. Variances were compared using Fisher's variance ratio test. As the greater variance always provided the numerator, significance levels of F were calculated using a one-tailed F-test.

Prior to analysis, the normality of data was tested using the 'nscore' facility in Minitab 6.1 (Minitab 6.1 Reference Manual, 1987; Filliben, 1970; Joiner and Rosenblatt, 1971; Ryan and Joiner, 1972). As frequency distributions of capitulum number per plant for both morphs in all treatments at RBGE were positively skewed, analysis was conducted following a natural log transformation. Highly positively skewed and leptokurtic distributions have been shown to be indicative of competitive stress (Obeid et al., 1967; Ross and Harper, 1972; Wilbur and Collins, 1973; Branch, 1975). Thus, it was of interest to calculate the degree of skewness and kurtosis present in the frequency distributions. To this end, coefficients of skewness and kurtosis were calculated using the FREQUENCY command of the SPSS/X (SPSS/X Users Guide 1986), with levels of significance based on t-tests (Sokal and Rohlf, 1981).

Percentages and proportions were always transformed into angles using an arcsine transformation before analysis. Where within cell variation was present, i.e for capitulum number per plant, treatment variance was tested for homogeneity using the Cochrane C test. Significant heterogeneity of variance indicated proportionality of the mean and variance e.g greater mean values were associated with larger variance about the mean. Consequently, the data set was transformed with a log transformation (Sokal and Rolhff, 1981).

All analyses were conducted using the SPSS/X statistical package (SPSS/X Users Guide 1986), or the Minitab 6.1 statistical package (Minitab 6.1 Reference Manual, 1987) incorporated on a VAX/VMS computer. Unbalanced ANOVAS were analysed using a linear regression model (Neter, Wasserman & Kutner 1985) incorporated into a Minitab program by Turner (1986).

II.4 - RESULTS -

II.4.1 St. Andrews Autumn 1983

(i) Survivorship

Between treatment comparisons

From the time of planting (21-23.10.83) until late December 1983 very little mortality was recorded for either morph raised in pure stand or mixture (Fig II.1a). However, during the first three months of 1984, survivorship was significantly lower amongst radiate plants in pure stand (55%) than amongst plants in the non-radiate stand (90%) or plants (of both morphs) in the mixture (94%). Throughout this period (January - March) all plants were subjected to heavy frosts and strong winds. Damaged plants were typically burnt at the tips and margins of the leaves, and in the most severe cases leaf abscission occurred and death followed.

A second period of severe plant mortality occurred during April and early May, 1984, coinciding with a rise in temperature at the site. Much of this mortality was due to the death of plants which had survived the winter but had suffered significant tissue damage. Many such deaths occurred among plants in the non-radiate pure stand (30% mortality) and the mixture (38% mortality). After mid May no further mortality was recorded in any treatment and by the time plants were harvested on July 4th, 1984, survivorship was recorded as 36% in the radiate pure stand, 60% in the non-radiate pure stand, and 56% in the mixture. By this stage all survivors had succeeded to flower and produce seed, however, no record was taken of the reproductive output of survivors because of the labour demands required for gathering equivalent information from the parallel experiment conducted at RBGE (see below).

Survivorship in mixture compared to pure stand

It was of interest that both morphs exhibited better survivorship when grown in mixture compared to their respective pure stand (Fig. II.1b), although differences were not judged to be significant ($p>.05$). In mixture, survivorship of radiate plants was significantly lower than that of non-radiate plants, with the great majority of deaths occurring during April and early May 1984. By the end of the experiment the mean survivorship of radiate plants in mixture was 47% compared to 66% for non radiate plants.

II.4.2 RBGE Autumn 1983

(i) Survivorship

Between treatment comparisons

From the time of planting (23-27.10.83) until late December 1983 no mortality was recorded either in pure stands or in mixture. However, thereafter until early March 1984, there was a steady decrease in plant numbers in all treatments (Fig II.2a). Throughout this period mortality was much greater in pure stand than in mixture, and, as was the case in the

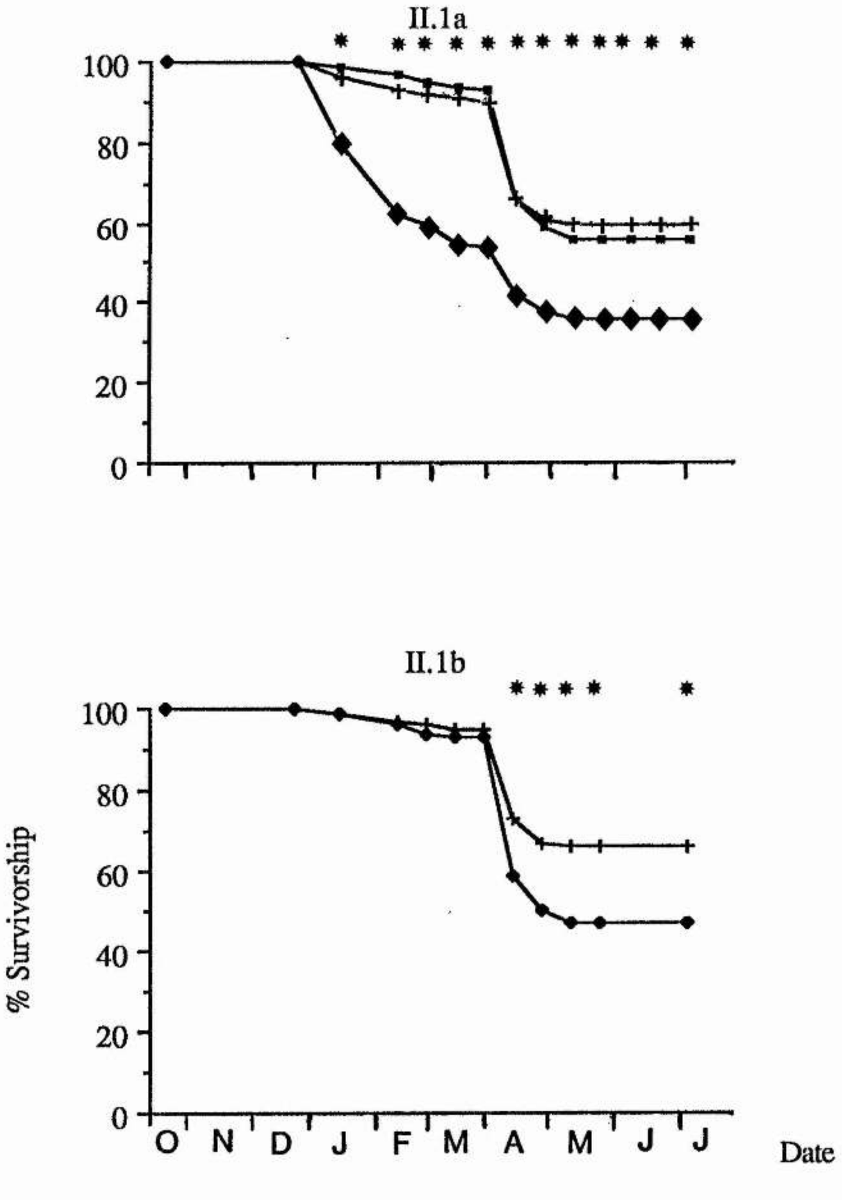


Fig. II.1 Autumn 1983: Percentage survivorship of plants at St. Andrews in: (a) radiate (◆) pure stand, non-radiate pure stand (+) and mixture (■); and (b) within the mixture. Levels of significance between treatments are based on a two factor ANOVA and between morphs within the mixture on paired t-tests.

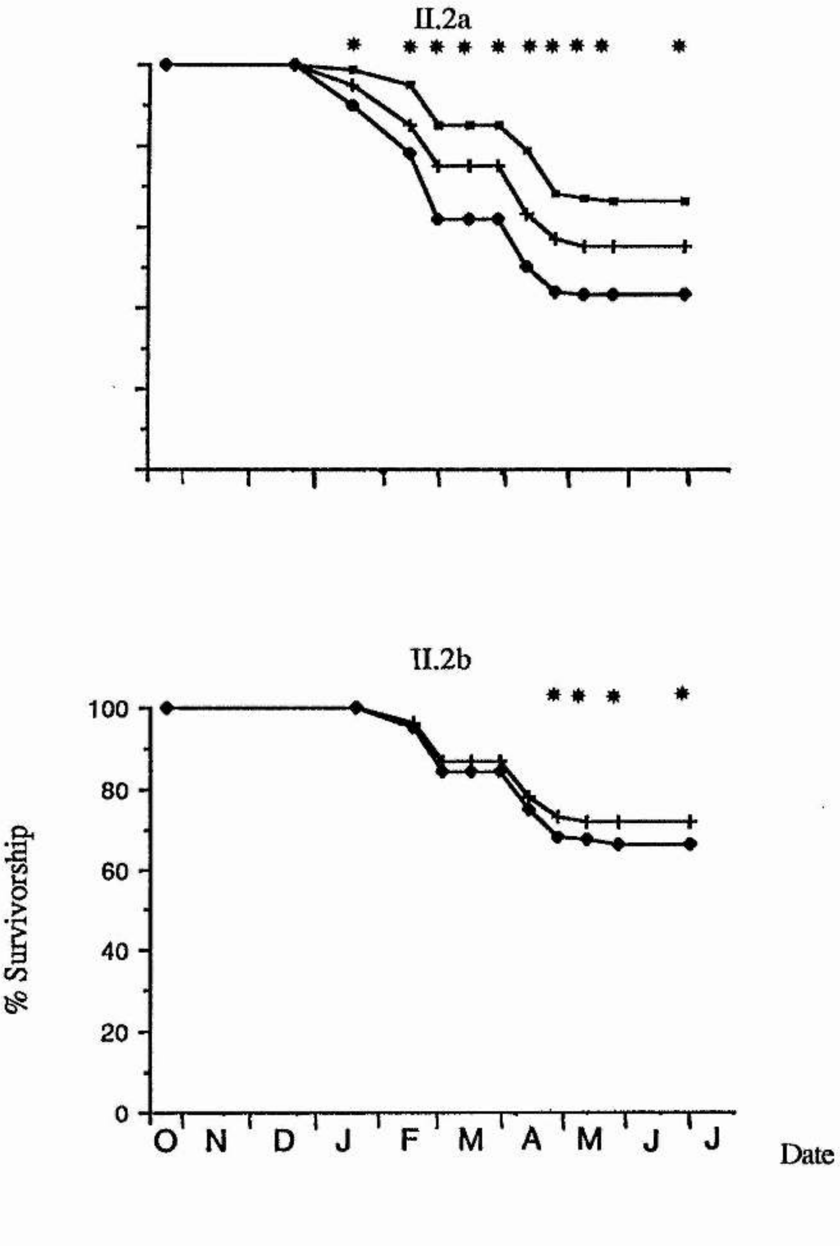


Fig. II.2 Autumn 1983: Percentage survivorship of plants at RBGE in: (a) radiate (◆) pure stand, non-radiate pure stand (+) and mixture (■); and (b) within the mixture. Levels of significance between treatments are based on a two factor ANOVA and between morphs within the mixture on paired t-tests.

experiment at St. Andrews, the radiate morph in pure stands suffered most. Although plants exhibited signs of tissue damage due to heavy frosts, damage to plants was not as severe as at St.Andrews during the same period, and, overall, plant survivorship was higher. During most of March, there was no further mortality, however, a second period of plant mortality coincided with a rise in temperature at the beginning of April and continued until mid May 1984. Plants most affected were those which had incurred tissue damage during the colder first three months of the year. By the end of the experiment, survival was highest among plants raised in mixture (66%), at an intermediate level among non-radiate plants grown in pure stand (55%) and lowest among radiate plants in pure stand (44%).

Survivorship in mixture compared to pure stand

Survivorship of plants of both morphs was again greater in mixture compared to pure stand (Table II.5, Figs. II.2a & II.2b). The difference was significant for the non-radiate morph (p=.009), but not for the radiate morph (p=.094). In mixture, a significantly greater number of non-radiate plants survived compared to radiate plants (Fig II.1b; Table II.4).

In many respects the results recorded for survival at RBGE paralleled those recorded at St. Andrews over the same period. The main difference was that overall survival was somewhat greater at RBGE for plants raised in radiate pure stand and in mixture.

(ii) Fecundity

Between treatment comparisons

Differences between treatments (pure stand radiate, pure stand non-radiate and mixture) for capitulum number per plot and capitulum number per individual were not significant (Table II.3). Nevertheless, both in pure stand and in mixture the non-radiate morph tended to produce greater numbers of capitula per plot than the radiate morph (Tables II.3 & II.4) and in both instances the differences between morphs bordered on being

Table II.3 RBGE 1983-1984: proportion of individuals sown to fruit, number of capitula per plant, and total number of capitula per plot for pure stands and mixtures of the radiate (R) and non-radiate (N) morphs. Levels of significance are presented for two orthogonal contrasts extracted from the ANOVA following partition of the treatment variance.

	Propn. to Fruit				No. Capitula/Plant				Capitula Total			
	R	N	Mix	p	R	N	Mix	p	R	N	Mix	p
Treatment	0.44	0.55	0.66	*	41.7	41.9	34.3	n.s	3056	3867	3850	n.s
Orthogonal Contrasts												
R v N				n.s				n.s				.07 n.s
P.S v Mix				***				n.s				n.s

Table II.4 RBGE 1983-1984 Differences in mixture between the radiate (R) and non-radiate (N) morphs in the proportion of individuals sown to fruit, number of capitula per plant, and total number of capitula per plot. Levels of significance are based on paired t-tests.

Propn. to Fruit			No. Capitula/Plant			Capitula Total		
R	N	p	R	N	p	R	N	p
0.61	0.72	***	31.9	36.7	n.s	1631	2218	.079 n.s

significant $(0.1>p>.05)$.

In pure stand, the difference recorded between morphs for capitulum number was due almost entirely to the significant difference in plant survivorship (Tables II.3 & II.4), there being little difference in capitulum number per survivor. However, in the mixture, the greater capitulum production per plot of the non-radiate compared to the radiate morph was due to both greater survivorship and greater capitulum production per survivor.

Fecundity in mixture compared to pure stand

Capitulum number per survivor of both the radiate and the non-radiate morphs was reduced in the mixture compared to pure stand, although for each morph the difference was not significant (Table II.5). As greater numbers of plants of both morphs survived in mixture compared to pure stand (Table II.5), the reduced production of capitula in mixture was clearly a density dependent effect. To overcome difficulties caused by different initial numbers of each morph in pure stands compared to mixtures, comparisons of morph fitnesses in mixture and pure stands were based on the Net Reproductive Output (N.R.O) of each morph. The latter term was calculated as the mean proportion of seedlings transplanted that survived to fruit multiplied by the mean number of capitula produced per plant. Although, for each morph, individual plant fecundity was reduced in mixture compared to pure stand, the increased survivorship within the mixture resulted in enhanced net reproductive output (N.R.O) in mixture compared to pure stand (Table II.5). However, the differences were judged not to be significant $(p>.05)$.

Frequency distributions of capitulum number per plant.

The frequency distributions for capitulum number per plant of each morph when grown in pure stand and mixture were significantly positively skewed and leptokurtic (Table II.6 & Fig. II.3). The positive skewness

Table II.5 RBGE 1983-1984: Differences in proportion of plants sown to fruit, capitula number per plant and net reproductive output (N.R.O) for the radiate and non-radiate morphs when raised in mixture (Mix) compared to pure stand (P.S). Levels of significance are based on the results from two factor ANOVAS.

	Propn. to Fruit			No. Capitula/Plant			NRO		
	Mix	P.S	p	Mix	P.S	p	Mix	P.S	p
Radiate	0.61	0.44	n.s	31.9	41.7	n.s	19.5	18.3	n.s
Non-Radiate	0.72	0.55	* *	36.7	41.9	n.s	26.4	23.0	n.s

Table II.6 RBGE 1983-1984: Total phenotypic variance and distribution of capitulum number per plant of the radiate (R) and non-radiate (N) morphs when grown in pure stand and mixture. Levels of significance of difference for variance were based on a one tailed F-test and for skewness and kurtosis on t-tests.

Treatment	Morph	N.	Variance	F Ratio	Skewness	Kurtosis
Pure Stand	R	585	3136	1.49 ***	3.04 ***	12.8 ***
	N	737	4669		3.86 ***	18.8 ***
Mixture	R	407	2216	1.10 n.s	3.77 ***	20.7 ***
	N	482	2426		2.99 ***	11.6 ***

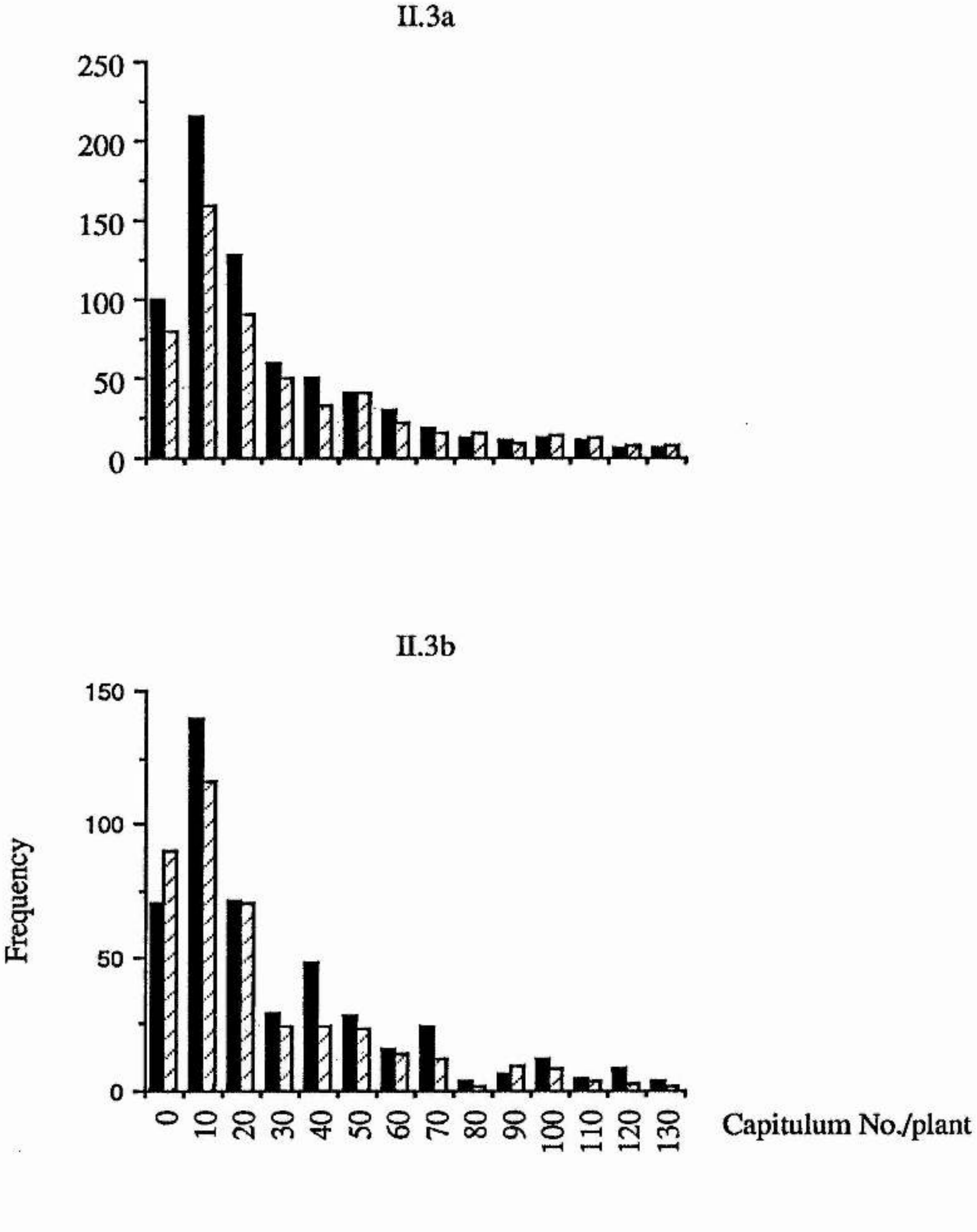


Fig. II.3 RBGE Autumn 1983: Frequency distributions for capitula number per plant of radiate (▨) and non-radiate (■) plants raised in (a) pure stand and (b) mixture.

and kurtosis is evidence that in all treatments the majority of individuals of both morphs produce relatively few capitula per plant and only a few plants produce large numbers of capitula. A comparison of morph variances (Table II.6) showed that the non-radiate morph tended to exhibit more variation than the radiate morph for capitulum number per plant and in pure stands this difference was significant.

II.5 - DISCUSSION -

In each of the two experiments conducted, the non-radiate morph exhibited a greater relative fitness than the radiate morph (Table II.7), through increased survivorship in the experiment at St. Andrews, as well as increased capitulum production per plot in the experiment at RBGE. Greater fitness of the non-radiate morph in material derived from Edinburgh populations had been reported previously by several authors (Perryman, 1983; Marshall and Abbott, 1987; Ross and Abbott, 1987) based on records of capitulum number per plant. This is the first study, however, in which the effect of survival has also been considered in the measurement of fitness.

Table II.7 St. Andrews and RBGE 1983: Relative fitness of the radiate and non-radiate morphs in pure stand and mixture.

	St. Andrews		RBGE	
	Radiate	Non-radiate	Radiate	Non-radiate
Pure stand	0.60	1.0	0.79	1.0
Mixture	0.71	1.0	0.73	1.0

N.B. Relative fitnesses of morphs in St. Andrews experiment are based on final survivorship values. At RBGE they are based on capitulum production per plot.

Differences in survival between morphs were due to differences in susceptibilty to frost damage. Susceptibility of S.vulgaris to frost damage was reported previously by Paul and Ayres (1986a). They found that in affected plants, leaf injury continued to increase for some weeks after severe weather was experienced during late December and January, ultimately leading to plant death. The present results indicate a similar pattern of mortality for both morphs in the studies reported here, however, non-radiate plants were clearly more resistant to frost than radiate plants.

A possible cause of the greater susceptibility of the radiate morph to frost damage might be that genetic material introgressed from S.squalidus along with the ray floret gene (see Chapter I.2), has in some way lowered the frost resistance of the native S.vulgaris. Differential tolerance to frost damage between morphs, coupled with an increased chance of secondary infection in the radiate morph, due to frost damage (Olien and Marchetti, 1976), could be a further factor affecting the maintenance of the capitulum polymorphism in natural populations. In populations where the radiate morph produces more seed (Oxford and Andrews, 1977) a greater susceptibility to frost damage would act to counter this advantage.

In the experiment conducted at the RBGE site, there was little difference between morphs in number of capitula produced per plant when raised in pure stands, however, the non-radiate morph tended to exhibit a greater fecundity in mixture, though the difference was not significant. This trend agrees with the results of previous studies (Perryman, 1983; Marshall and Abbott, 1987) which for Edinburgh material showed that the non-radiate morph normally exhibited greater individual fecundity both in the wild and in material raised in culture.

Analysis of competitive effects

In all treatments, the frequency distributions of capitulum number per plant were significantly positively skewed and leptokurtic. Highly positively skewed and leptokurtic distributions for a given fitness trait indicate that plants have been raised under stress (Obeid et al., 1967; Ross and Harper, 1972; Wilbur and Collins, 1973; Branch, 1975). Given the high density of plants raised in pure stand and mixture in the present studies, stress was most likely the outcome of either intra- and/or intermorph competition.

Competition and its effect on the structuring of natural populations has been considered important by many authors (See refs in Trenbath, 1974; Connell, 1974; Connell, 1975; Connell, 1983; Schoener, 1983; Underwood 1986). Moreover, Schultz et al. (1968), Allard and Adams (1969), Putwain and Harper (1972), Khan et al. (1975) and Antonovics (1978) have all discussed the potential importance of intermorph competition in maintaining polymorphism in plant populations. Ennos (1981) utilised a de Wit replacement series experimental design (de Wit, 1960) to investigate the role of competition in the maintenance of the cyanogenic/acyanogenic polymorphism in Trifolium repens. He reported that plants which possessed the linamarase allele exhibited greater fitness, based on dry weight measurement, when raised in pure stand and mixture than plants lacking the allele. Waller (1984) also investigated the effect of competition on the relative fitnesses of offspring from chasmogamous (outcrossing) and cleistogamous (selfing) flowers of Impatiens capensis, with particular emphasis on the maintenance of outcrossing in that species. Using material from a number of populations, raised in flats at a 1:1 frequency over a range of densities under glass, the author found that plants derived from chasmogamous flowers produced a significantly greater dry weight than plants from cleistogamous flowers. Waller concluded, that this difference in fitness (based on dry weight) might provide a short term advantage to offspring from chasmogamous flowers which would be sufficient to overcome the inherent cost of outcrossing.

The experiments reported in this chapter were designed originally to examine competitive effects between the two morphs of S.vulgaris raised under field conditions using the replacement series design of de Wit (1960). At the time that the experiments were initiated, de Wit's design was considered by many investigators, including Ennos (1981) and Waller (1984), as a most effective method of examining inter- and intramorph competition (see also refs. in Trenbath, 1974; Silvertown, 1982).

Recently, however, there has been increased concern over the interpretation of results from these experiments (Mather and Caligari, 1981; Creese and Underwood 1982; Connell, 1983; Schoener, 1983; Jolliffe, Minjas and Runeckles, 1984; Firbank and Watkinson, 1985; Conolly, 1986; Underwood, 1986; Conolly, 1988). This stems from the fact that the replacement series design is a classic case of an experimental method without controls. As such, it is not possible to interpret unambiguously the mechanisms determining differences in fitness of plants grown in pure stand compared to plants raised in mixture. This is because the density of a morph in pure stand is twice that of its density in mixture. For example, in the present study there were 168 plants of a morph in each pure stand, whereas within the mixture only 84 plants of each morph were present. As all plots were of equal area, clearly the density of each morph in mixture was only half that in the pure stand.

A consequence of this differences in density is that the performance of a morph in mixture relative to its performance in pure stand may reflect: (i) a relaxation of intramorph competition (due to reduction in the morph's density); (ii) greater competitive ability with respect to the other morph; or (iii) a combination of both of these effects. Without suitable controls (which are lacking in the de Wit design) it is not possible to separate the intra- and inter-morph competition effects from each other. A suitable design for a detailed analysis of intra- and inter-morph competition effects on performance is discussed in Chapter IV.

Nevertheless, comparisons of morph performance in mixture compared to pure stand revealed that the fitness of each morph was enhanced in mixture. Moreover, in the St. Andrews study, although the non-radiate morph exhibited greater fitness in mixture compared to the radiate morph, the difference in fitness between morphs was reduced in mixture compared to pure stand (Table II.7). This is of interest in that it implies an effect of frequency dependent competition. The frequency dependent selection hypothesis predicts that rare phenotypes should exhibit greater fitness than common phenotypes because of competitive advantage due to differential niche utilisation and/or avoidance of attack from pests or pathogens through reduced frequency or novel defences. Thus, increased fitness of radiate individuals at low frequency may provide a mechanism which might reduce the rate at which the radiate allele would be lost from a polymorphic population. In contrast, however, in the RBGE study, the fitness difference between morphs was increased in mixture compared to pure stand. This we would expect to increase the rate at which the radiate allele would be lost from a polymorphic population.

Although the studies reported in this chapter have failed to provide detailed information on intra and inter-morph competitive effects they, nevertheless, have shown quite clearly that under the conditions of the experiments, there is no evidence for the radiate morph holding any advantage over the non-radiate morph, in terms of fitness, either in pure stand or in mixture. Indeed, for both components of fitness that were measured, i.e. survival and fecundity, it was the non-radiate morph which appeared to have the edge. The studies, therefore, have failed to detect a factor which may counteract the "cost of outcrossing" incurred by the radiate morph in populations that are polymorphic for capitulum type in the Edinburgh area.

CHAPTER III. DEMOGRAPHIC STUDIES I: FROM SEEDLING TO SENESCENCE.

1B) 1984 EXPERIMENTS

III.1 - INTRODUCTION -

Edinburgh populations of S. vulgaris that are polymorphic for capitulum type exhibit two peak periods of seed production and hence seedling recruitment per year. These occur in late spring/early summer and in autumn (Abbott, pers. comm.). As a consequence, it is feasible that the relative fitnesses of the radiate and non-radiate morphs may differ for plants which grow in populations that develop and reproduce at different times of the year. Abbott (1986) using material of both morphs derived from a large polymorphic Edinburgh population, investigated differences between the radiate and non-radiate morphs of S. vulgaris for a range of life history traits, in experiments initiated under glass in June and November. He found that although the radiate morph produced greater dry weight than the non-radiate morph in both experiments, only in the study initiated in June was the difference between morphs significant.

A difference in relative fitness between morphs might also vary with density. In studies on the relative fitnesses of chasmogamous (open pollinated) and cleistogamous (self pollinated) offspring of Impatiens capensis, Waller (1984) found that although seedlings of chasmogamous offspring always exhibited the greater fitness (based on dry weight), differences in fitness varied significantly at different densities.

In the investigations reported in this chapter, an analysis has been conducted on the possible effects of planting date and density on the relative fitnesses of the radiate and non-radiate morphs grown under conditions which approximate to those in the wild. The effect of planting date on the relative fitnesses of the two morphs was examined by initiating experiments in Spring and Autumn, 1984, at the Botanic Gardens St. Andrews, while the effects of density were investigated by raising plants of each morph in high and low density pure stands and in mixtures. Relative fitnesses were measured in terms of survivorship and fecundity.

III.2 - MATERIALS and METHODS -

Plant material was derived from seeds collected in May 1984 for the Spring 1984 experiment, and September 1984 for the Autumn 1984 experiment. Seedlings were produced from seeds collected from plants raised under conditions previously described in Chapter II section 2.

With the experiment initiated in Spring 1984 (planted 20.5.84), comparisons were made between three treatments previously described in Chapter II, i.e. pure stands of the radiate and non-radiate morphs and a mixed stand in which morphs were present at a 1:1 ratio with individuals spaced at 3cm. intervals (high density). This represented a traditional de Wit replacement series design (de Wit, 1960). In addition, two further treatments were included; these were a pure stand of radiate plants and a pure stand of non-radiate plants in which spacing between individuals was increased to 6cm (low density). These additional plots allowed an assessment of density effects in pure stands.

In Autumn 1984 at the same site at St.Andrews, a second experiment was initiated (planted 7-10.10.84) in which the design of the Spring 1984 experiment was repeated but with the addition of a low density mixed stand in which plants were spaced at 6cm. intervals. Thus, the design of this experiment represented two de Wit replacement series, one series in which plants were raised at high density (3cm. spacing) and the other in which plants were raised at low density (6cm. spacing).

In each experiment, replication was eight-fold and plots were arranged, one metre apart, in a randomised block design, with replicates arranged in blocks. Within each plot, seedlings were planted at points of a 14 x 12 grid. Plots were monitored on a weekly basis to assess mortality throughout the seedling, vegetative and flowering stages. Due to a large "edge" effect noted in plots raised within the 1983 experiments (see Chapter II) the two outer rows of each plot were designated as guard rows (Guard Row Plants). Data were collected separately from guard rows and inner rows (Plot Plants) before analysis.

At harvest a record was taken of the number of plants to set seed, and the number of capitula produced per plant (i.e after all plants in a plot had finished fruiting. Plants which had survived in the Spring 1984 experiment were harvested on 30.8.84 while those from the Autumn 1984 experiment were collected on 30.6.85. In the Spring 1984 experiment, an additional record was taken of ovule and seed number per capitulum plus ovule fertility. This entailed sampling a single capitulum from each of 20 plants in the outer two rows and 30 plants from the inner rows of each plot over a five day period when most plants were fruiting. Capitula were teased open using fine forceps and the number of filled seed recorded. The number of ovules per capitulum was calculated as the total number of filled and aborted seed produced in a capitulum, while ovule fertility was computed as the proportion of ovules to produce filled seed.

III.3 - ANALYSIS OF DATA -

III.3.1 - Spring 1984 Experiment -

Density effects in pure stand

Differences between morphs raised in high and low density pure stands for survivorship, number of plants to fruit and total seed produced per plot, were examined by a three factor analysis of variance. In the ANOVA (see Table III.1), a mixed model was assumed with morphs (treatment term) and density considered as fixed effects, and replication as a random effect. The treatment x density x replicate interaction was used as an estimate of the error term to test the significance of the treatment x density interaction; while the treatment and density main effects were tested for significance against the treatment x replicate and density x

Table III.1 Three factor ANOVA model to examine differences between radiate (R) and non-radiate (N) plants in survivorship and fecundity when raised in high and low density pure stands.

Source of Variation	d.f
Treatment (R v N)	1
Density	1
Replication	7
Treatment x Density	1
Treatment x Replication	7
Density x Replication	7
Treatment x Density x Replication	7
Total	31

replicate interactions respectively.

For seed and capitulum number per plant, and ovule number, seed number and ovule fertility per capitulum, the mean square of the treatment x density x replicate could be tested for significance against the within replicates error term. When the interaction was not significant (with $p > .25$), its mean square was pooled with the within replicates mean square to form a combined error term for testing the significance of the treatment x density interaction (Winer, 1969).

Replacement series analysis

Differences between the high density replacement series treatments (i.e. pure stand radiate, pure stand non-radiate and the mixture) for survivorship and fecundity, were examined by a two factor ANOVA similar to that described in Table II.1 (see Chapter II.3). Subsequent analysis followed the procedure outlined in Chapter II.3.

III.3.2 - Autumn 1984 Experiment -

The structure of the ANOVA used for the analysis of survivorship, number of plants to fruit, capitulum number per plant, and total capitula produced per replicate is described in Table III.2. To determine differences between: (i) morphs raised in pure stand; and (ii) pure stands and the mixture, two orthogonal contrasts were extracted from the treatment term as shown in the table. In addition, an examination of a difference in the effect of each of these contrasts with density was conducted by extracting two further orthogonal contrasts from the treatment x density interaction.

Further analysis of data was conducted in a manner similar to that described in Chapter II.3. However, as the experimental design consisted of both high and low density pure stands and mixtures, differences in survivorship and fecundity for a given morph, raised in pure stand compared

Table III.2 Three factor ANOVA model to examine differences in survivorship and fecundity between pure stands of radiate (R) and non-radiate (N) plants and a 1:1 mixture (Mix) of each morph, raised in high and low density plots. Orthogonal contrasts have been extracted from the treatment main effect and treatment x density interaction to determine differences between: (i) the radiate (R) and non-radiate (N) pure stands; and (ii) pure stands (P.S) and mixture (Mix).

Source of Variation	d.f
Treatment (R v N v Mix)	2
Contrast (i) (R v N)	(1)
Contrast (ii) (PS v Mix)	(1)
Density	1
Replication	7
Treatment x Density	2
Contrast (i) x Density	(1)
Contrast (ii) x Density	(1)
Treatment x Replication	14
Density x Replication	7
Treatment x Density x Replication	14
Total	47

to mixture, utilised a three factor ANOVA with density as the additional factor.

III.4 - RESULTS -

III.4.1 St. ANDREWS SPRING 1984

Analysis of variance of the 'pure stand' data (Table III.3) revealed that the treatment x density interaction was not significant for any of the characters recorded. Attention below is, therefore, focused on differences in the main effects i.e. morphs raised in pure stands (radiate v non-radiate) and density (high v low), when examining the results of the pure stand treatments.

III.4.1.1 Survivorship

Plot plants

Plant survivorship in high and low density pure stand remained high until flowering with little difference occurring between morphs (Table III.3i). At the high density (3cm. spacing), plant survivorship in mixture was reduced compared to pure stand (Table III.4i), however, the difference was not significant and within the mixture both morphs exhibited identical survivorships (Table III.5i). Subsequent analysis showed that survivorship of each morph was reduced in mixture compared to pure stand, although again the differences were not significant (Table III.6i).

Guard row plants

As found for plot plants, survivorship of guard row plants in pure stand remained high until flowering with no difference occurring between morphs or densities (Table III.7i). At the high density, plant survivorship was again reduced in mixture compared to pure stand (Table III.8i); however, the difference was not significant, and both morphs in mixture exhibited a similar level of survivorship (Table III.9).

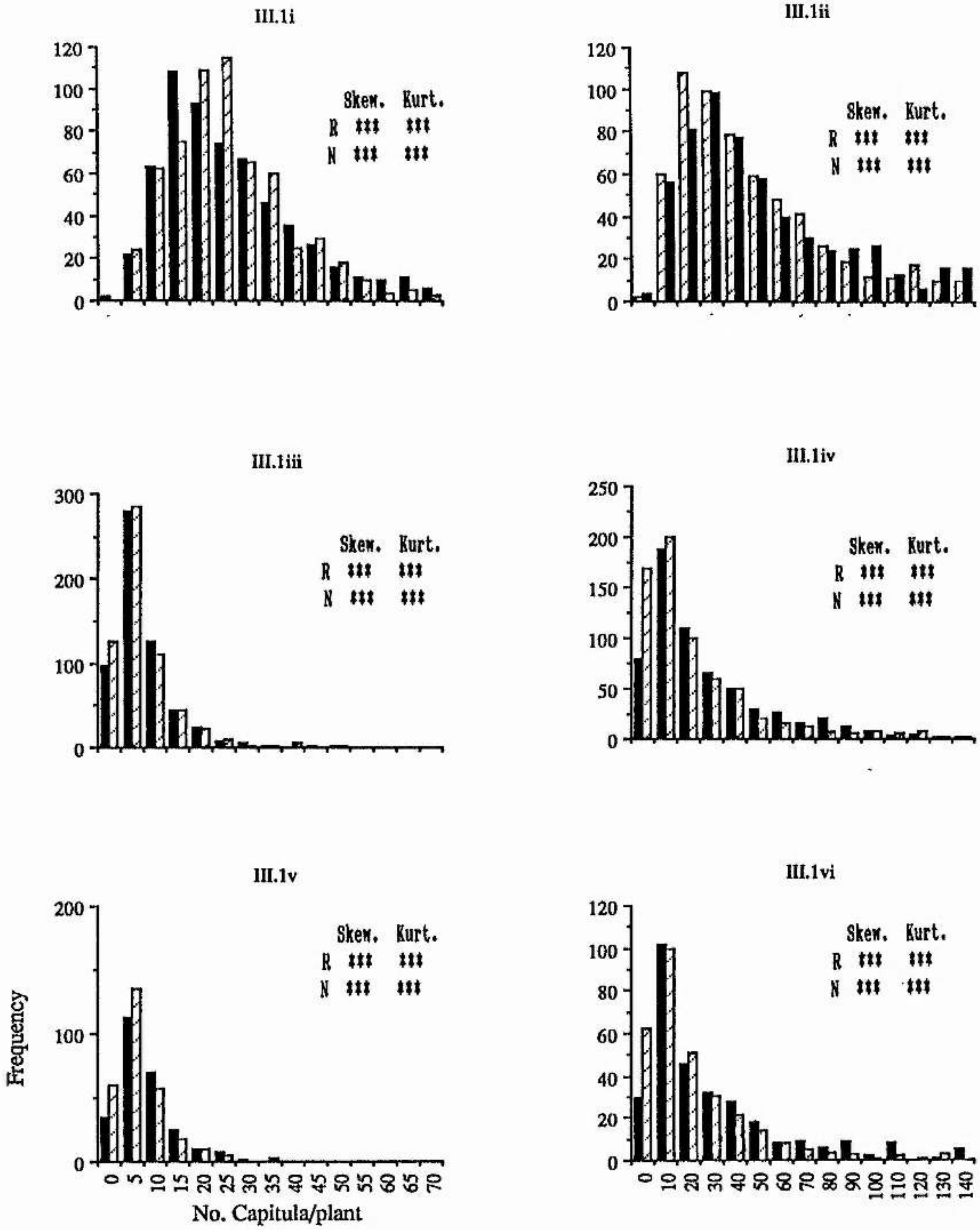


Figure III.1 Spring 1984: Frequency distributions for capitulum number per plant of plot (i, iii, & v) and guard row (ii, iv & vi) plants of the radiate (▨) and non-radiate morphs (■) when raised at: low density in pure stand (i & ii); high density in pure stand (iii & iv); and high density in mixture (v & vi). Levels of significance for coefficients of skewness and leptokurtosis are based on t-tests.

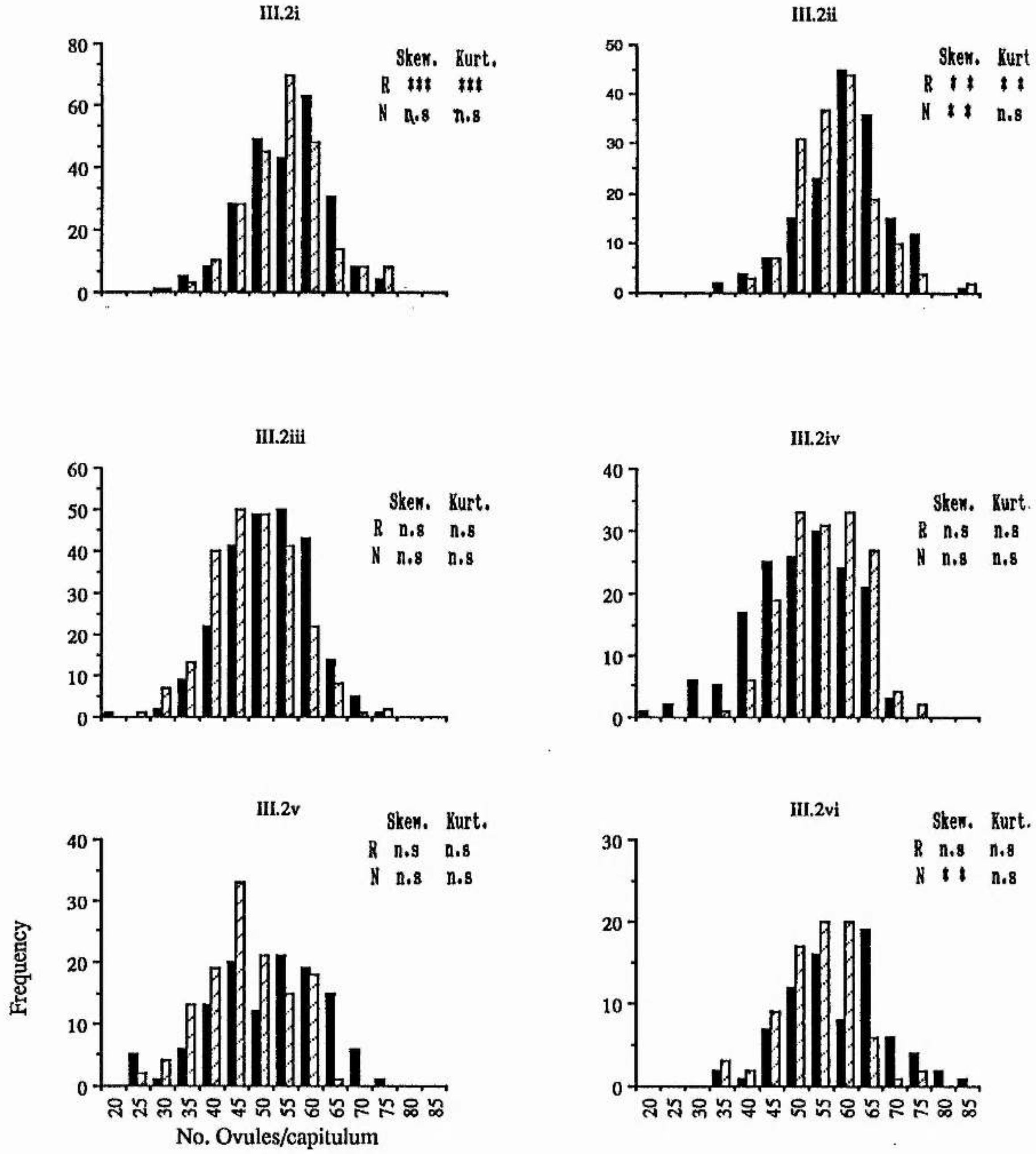


Figure III.2 Spring 1984: Frequency distributions for ovule number per plant of plot (i, iii, & v) and guard row (ii, iv & vi) plants of the radiate (▨) and non-radiate morphs (■) when raised at: low density in pure stand (i & ii); high density in pure stand (iii & iv); and high density in mixture (v & vi). Levels of significance for coefficients for skewness and leptokurtosis are based on t-tests.

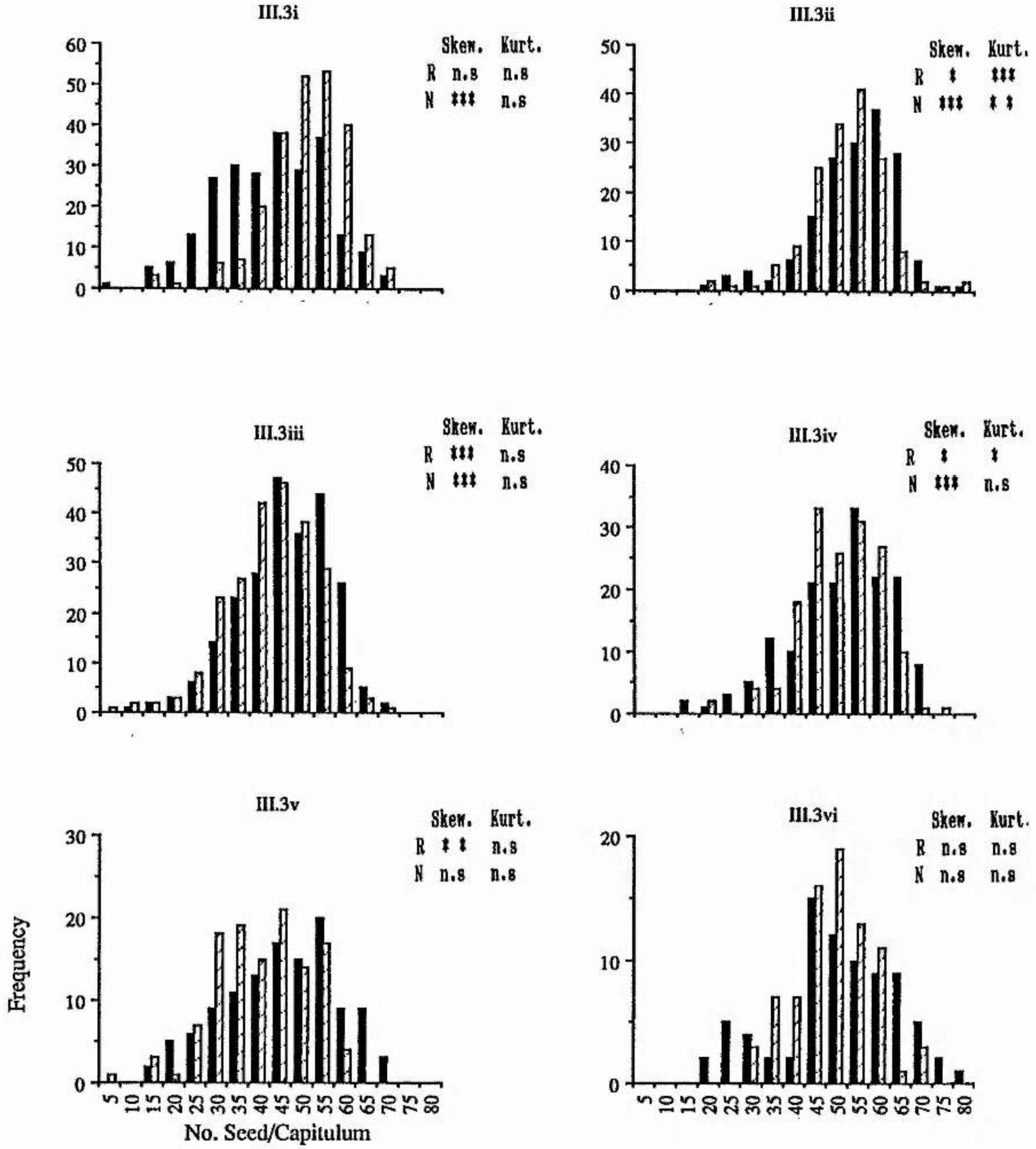


Figure III.3 Spring 1984: Frequency distributions for seed number per plant of plot (i, iii, & v) and guard row (ii, iv & vi) plants of the radiate (▨) and non-radiate morphs (■) when raised at: low density in pure stand (i & ii); high density in pure stand (iii & iv); and high density in mixture (v & vi). Levels of significance for coefficients for skewness and leptokurtosis are based on t-tests.

III.4.1.2 Fecundity

Before subjecting data to analysis of variance the frequency distributions of capitulum number per plant, and ovule and seed number per capitulum were examined. For capitulum number per plant frequency distributions were positively skewed and leptokurtic in all treatments (Fig. III.1). In contrast, the distributions for ovule number per capitulum were normal, while those for seed number per capitulum were negatively skewed (Figs. III.2 & III.3).

Plot plants

Analysis of the plot plant data revealed no differences between morphs for seed number per plant or total seed produced per plot in pure stand at either density (Table III.3i).

Each low density plot originally contained the same number of plants as was contained in each high density plot (i.e. 168 plants per plot) and so occupied four times the area of a high density plot. Comparisons of seed totals per plot between high and low density pure stands were therefore made between the seed totals of high density plots and one quarter of the seed total of low density plots. As such, seed totals were compared as seed totals per unit area.

It was evident that for each morph, there was little effect of density on total seed number per unit area per plot (Table 111.3i). In contrast, there was a marked difference between densities for seed produced per plant with plants raised at low density producing approximately four times as many seed as plants raised at high density. Analysis of the fertility component characters (Table III.3ii), i.e capitulum number per plant, and ovule and seed number per capitulum, showed that each of these characters contributed to the difference between densities for seed number per plant, however, capitulum number per plant had, by far, the greater effect.

Table III.3 St. Andrews Spring 1984 Plot Plants: (i) Mean proportion of plants sown to fruit, seed number per plant and seed total per unit area per plot; and (ii) capitulum number per plant, ovule and seed number per capitulum, and percentage ovule fertility for high (3cm.) and low (6cm.) density pure stands of the radiate (R) and non-radiate (N) morphs. Levels of significance are based on the results of a three factor ANOVA.

(i)	Propn. to Fruit		Seed/Plant		Seed Total	
	R	N	R	N	R	N
3cm.	0.94	0.92	309	342	23285	25169
6cm.	0.97	0.92	1324	1348	25778	24974
Treatment (R v N)	n.s		n.s		n.s	
Density	n.s		***		n.s	
Treat. x Density	n.s		n.s		n.s	

(ii)	Capitulum No. per Plant		Ovule No./ Capitulum		Seed No./ Capitulum		% Ovule Fertility	
	R	N	R	N	R	N	R	N
3cm.	7.3	7.4	48.2	53.1	42.6	46.1	89.0	89.0
6cm.	25.9	26.4	54.9	55.2	51.1	50.9	93.0	92.0
Treatment (R v N)	n.s		n.s		n.s		n.s	
Density	***		***		***		* *	
Treat x Density	n.s		n.s		n.s		n.s	

Table III.4 St. Andrews Spring 1984 Plot Plants: i) Proportion of individuals sown to fruit, number of seed per plant, and total number of seed per unit area per plot; and (ii) number of capitula per plant, ovule and seed per capitulum and ovule fertility for the radiate (R) and non-radiate (N) morphs raised in high density pure stands and mixture.

(i)	Prop. Fruit	Seed/Plant	Seed Total
R	0.94	309	23285
N	0.92 n.s	342 n.s	25169 n.s
Mix	0.89	306	21838

(ii)	Capitulum No. per Plant	Ovule No./ Capitulum	Seed No./ Capitulum	Ovule Fertility (%)
R	7.3	48.2	42.6	89.0
N	7.4 n.s	53.1 n.s	46.1 n.s	89.0 n.s
Mix	7.1	49.1	42.8	86.5

At the high density, a comparison of treatment means (R v N v Mix) for each fecundity character (Table III.4) revealed no significant differences between treatments, although there were indications that seed number per plant, and per plot, was greater for non-radiate plants raised in pure stand. Moreover, it was apparent that in the mixture the non-radiate morph produced more ovules and seed per capitulum, more capitula per plant and more seed per plant and per plot (Table III.5). Only for ovule and seed number per capitulum was the greater production significant; however, the overall trend was that the non-radiate morph exhibited the greater fecundity.

Comparisons of the fecundity character means for each morph raised in pure stand and in mixture (Table III.6), indicate that each morph performed slightly better in pure stand than in mixture although in no instance was the difference significant.

Guard row plants

In the low density plots (6cm. spacing) guard row plants were clearly raised at a lower density than equivalent plants in the high density plots (3cm. spacing). However, as plants raised in the outer row of each plot were only bounded by neighbouring plants on three sides, it cannot be assumed that the area available for growth at low density was four times greater than that at the high density pure stands. It was not possible, therefore, to quantify the change in density of guard row plants between the high and low density plots, and examine density effects as was done for plot plants.

At both densities, non-radiate guard row plants in pure stand produced greater numbers of seeds per survivor than radiate plants, due to increased capitulum numbers per plant coupled with greater numbers of seeds per capitulum (Tables III.7ii & III.8ii). Within the mixture, the non-radiate

Table III.5 St. Andrews Spring 1984 Plot Plants: i) Mean proportion of individuals sown to fruit, number of seed per plant, and seed total per plot; and (ii) number of capitula per plant, ovule and seed per capitulum, and ovule fertility, of the radiate (R) and non-radiate morphs (N) raised in mixture. Levels of significance are based on paired t-tests.

i)	Prop. Fruit	Seed/Plant	Seed Total
R	0.89 n.s	273 n.s	9687 n.s
N	0.89	339	12152

ii)	Capitulum No. per Plant	Ovule No./ Capitulum	Seed No./ Capitulum	Ovule Fertility (%)
R	6.7 n.s	46.6 **	40.3 *	86.0 n/s
N	7.5	51.7	45.3	87.0

Table III.6 St. Andrews Spring 1984 Plot Plants: i) Mean proportion of plants to fruit, number of seed per plant, and net reproductive output (NRO); and (ii) number of capitula per plant, ovule and seed per capitulum and percentage ovule fertility for the radiate (R) and the non-radiate (N) morphs when raised in mixture compared to pure stand.

(i)	Propn. to Fruit			No. Seed/Plant			NRO		
	Mix	P.S	p	Mix	P.S	p	Mix	P.S	p
R	0.89	0.94	n.s	273	309	n.s	242	291	n.s
N	0.89	0.92	n.s	339	342	n.s	302	314	n.s

(ii)	Capitulum/Plant			Ovules/Capitulum			Seeds/Capitulum			Ovule Fertility		
	Mix	P.S	p	Mix	P.S	p	Mix	P.S	p	Mix	P.S	p
R	6.7	7.3	n.s	46.6	48.2	n.s	40.3	42.6	n.s	86.0	89.0	n.s
N	7.5	7.4	n.s	51.7	53.1	n.s	45.3	46.1	n.s	87.0	89.0	

Table III.7 St. Andrews Spring 1984 Guard Row Plants: (i) Mean proportion of plants sown to fruit, seed number per plant and seed total per plot; and (ii) capitulum number per plant, number of ovules and seed per capitulum, and percentage ovule fertility for high (3cm.) and low (6cm.) density pure stands of the radiate (R) and non-radiate (N) morphs.

(i)	Propn. to Fruit		Seed/Plant		Seed Total	
	R	N	R	N	R	N
3cm.	0.90	0.90	1213	1561	96458	123710
6cm.	0.91	0.92	2992	3772	240838	300554
Treatment (R v N)		n.s		***		* *
Density		n.s		***		-
Treat. x Density		n.s		n.s		-

(ii)	Capitulum No. per Plant		Ovule No./ Capitulum		Seed No./ Capitulum		Ovule Fertility (%)	
	R	N	R	N	R	N	R	N
3cm.	23.9	30.6	55.6	56.2	50.5	51.1	91.0	91.0
6cm.	57.2	67.8	57.7	60.1	52.1	54.8	90.0	91.0
Treatment (R v N)		***		n.s		n.s		n.s
Density		***		* *	.056	n.s		n.s
Treat x Density		n.s		n.s		n.s		n.s

Table III.8 St. Andrews Spring 1984 Guard Row Plants: i) Proportion of individuals sown to fruit, number of seed per plant, and total number of seed per unit area per plot; and (ii) number of capitula per plant, ovule and seed per capitulum and ovule fertility for the radiate (R) and non-radiate (N) morphs raised in high density pure stands and mixture. Levels of significance are based on the results of a two factor ANOVA.

(i)	Prop. Fruit	Seed/Plant	Seed Total
R	0.90	1213	96458
N	0.90 n.s	1561 ***	123710 n.s
Mix	0.87	1625	123942

(ii)	Capitulum No. per Plant	Ovule No./ Capitulum	Seed No./ Capitulum	% Ovule Fertility
R	23.9	55.6	50.5	91.0
N	30.6 ***	56.2 n.s	51.1 n.s	91.0 n.s
Mix	31.8	56.5	50.1	88.5

Table III.9 St. Andrews Spring 1984 Guard Row Plants: i) Mean proportion of individuals sown to fruit, number of seed per plant, and seed total per plot; and (ii) number of capitula per plant, ovule and seed per capitulum, and ovule fertility, of the radiate (R) and non-radiate morphs (N) raised in mixture. Levels of significance are based on paired t-tests.

(i)	Prop. Fruit	No. Seed/Plant	Seed Total
R	0.84 n.s	1367 n.s	50561 n.s
N	0.89	1870	73381

(ii)	Capitulum No. per Plant	Ovule No./ Capitulum	Seed No./ Capitulum	% Ovule Fertility
R	27.3 n.s	54.4 .09 n.s	49.3 n.s	91.0 n.s
N	36.1	58.7	50.9	86.0

morph again held advantage for nearly all fecundity characters (Table III.9), although in no instances were differences significant.

Comparison of morph performance in mixture and in pure stand (Table III.10), indicated that each morph performed somewhat better in mixture although differences were not significant.

III.4.1.3 Relative morph fitness.

From the values for the number of seed produced per plot by each morph in pure stand or mixture it is possible to derive measures of relative morph fitness for "plot plants" and "guard row plants". It is noteworthy that for all comparisons bar one, the non-radiate morph exhibited the greater relative fitness (Table III.11).

III.4.2 St. ANDREWS AUTUMN 1984.

III.4.2.1 Survivorship.

Plot plants.

Effect of treatment

There was little difference in mean survivorship between treatments (R v N v Mix) for plants raised at either high or low density (Table III.12, Fig. III.4i & iv). However, in mixture, survivorship of the non-radiate morph was greater than that of the radiate morph throughout the latter part of the experiment, that is from late February onwards.

Of particular interest was the finding that plant survivorship was greatly reduced at high density compared to low density. In fact, approximately four times as many plants survived in low density treatments than in high density treatments. As the initial density in high density plots was four times that within low density plots, the fourfold difference in survivorship at the two densities was a clear indication that mortality was density dependent.

Table III.10 St. Andrews Spring 1984 Guard Row Plants: i) Mean proportion of plants to fruit, number of seed per plant, and net reproductive output (NRO); and (ii) number of capitula per plant, ovule and seed per capitulum and percentage ovule fertility for the radiate (R) and the non-radiate (N) morphs when raised in mixture compared to pure stand.

(i)	Propn. to Fruit			No. Seed/Plant			NRO		
	Mix	P.S	p	Mix	P.S	p	Mix	P.S	p
R	0.84	0.90	n.s	1367	1213	n.s	1147	1086	n.s
N	0.89	0.90	n.s	1870	1561	n.s	1667	1401	n.s

(ii)	Capitulum/Plant			Ovules/Capitulum			Seeds/Capitulum			Ovule Fertility		
	Mix	P.S	p	Mix	P.S	p	Mix	P.S	p	Mix	P.S	p
R	27.3	23.9	n.s	54.4	55.6	n.s	49.3	50.5	n.s	91.0	91.0	n.s
N	36.1	30.6	n.s	58.7	56.2	n.s	50.9	51.1	n.s	86.0	91.0	n.s

Table III.11 St. Andrews Spring 1984: Relative fitness of the radiate (R) and non-radiate (N) morphs in high and low density pure stands (P.S) and mixture (Mix).

	Relative Fitness			
	Plot Plants		Outer Row Plants	
	R	N	R	N
High Density P.S	0.92	1.0	0.78	1.0
High Density Mix.	0.80	1.0	0.69	1.0
Low Density P.S	1.0	0.97	0.80	1.0

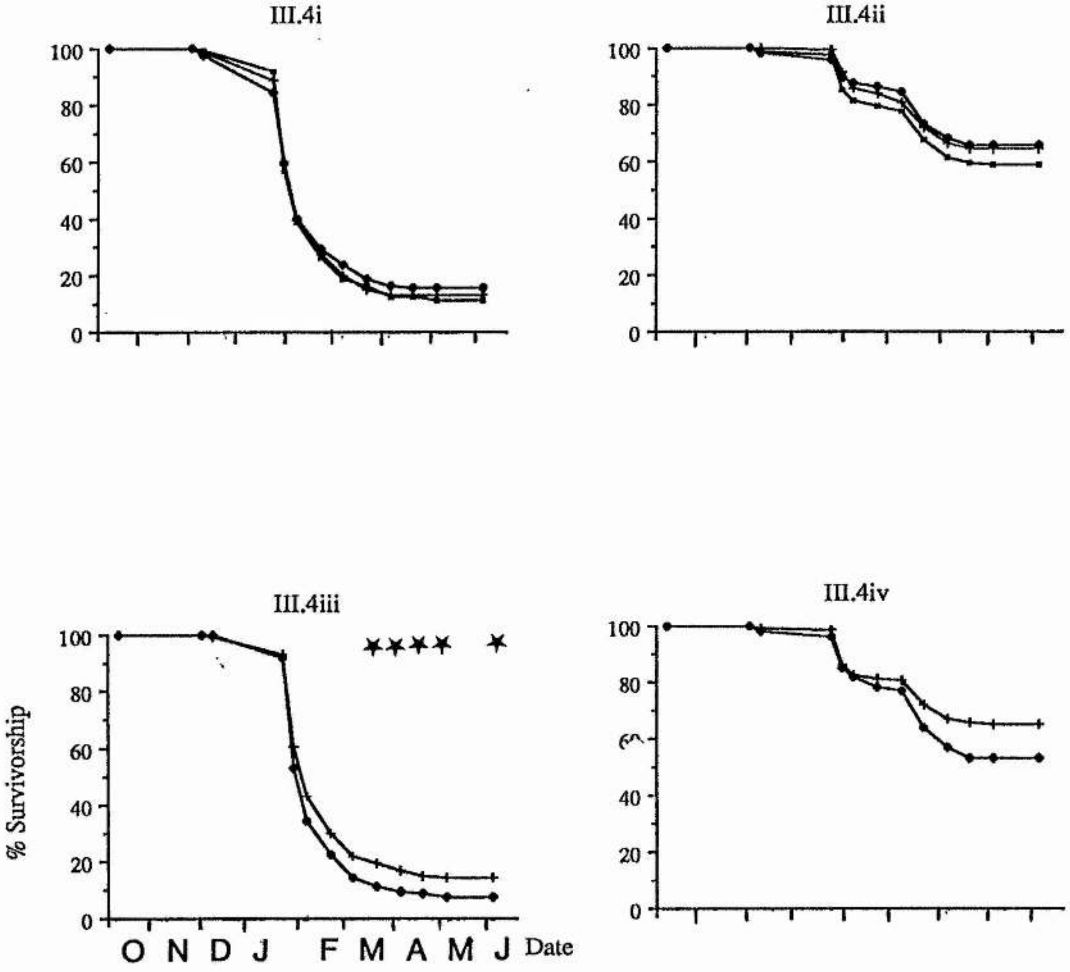


Figure III.4 Autumn 1984 Percentage Survivorship of Plot Plants: (i) in high density treatments, i.e. pure stand radiate (◆), pure stand non-radiate (+), and mixture (■); (ii) in low density treatments; (iii) morph survivorship within the high density mixture; and (iv) within the low density mixture. Levels of significance between treatments are based on a three factor ANOVA and between morphs within the mixture on paired t-tests.

In all treatments, only a few plant deaths occurred between the time of planting and mid-January 1985. However, from late January until early March, 1985, there was a massive amount of mortality in the high density plots (Fig. III.4i & iii). Thereafter, during the remainder of March and throughout April, survivorship improved and after flowering no further mortality occurred. In the low density plots, not as many plants died during late January and survivorship remained relatively high during February before a further bout of mortality occurred in March and early April. At both densities plants exhibited symptoms of frost damage; similar to those witnessed in the Autumn 1983 studies (Chapter II), however, the symptoms were much more extreme at the higher density in 1984.

Morph survivorship in mixture compared to pure stand

In marked contrast to the trend found in the Autumn 1983 studies, survivorship of the radiate morph was greater in pure stand than in mixture with the difference bordering on significance (Table III.14). For the non-radiate morph there was no apparent difference in performance over the two treatments.

Guard row plants.

Effect of treatment

As was found for plot plants, little difference occurred between treatments in guard row plant survivorship at either high or low density (Table III.15, Fig. III.5i & ii). Again there was a significant effect of density, with survivorship at low density twice that at high density. In the mixture, survivorship of non-radiate plants was again greater than that of radiate plants (Figs. III.5iii & iv) and the difference was significant at the higher density (Table III.16).

Survivorship in mixture compared to pure stand

In keeping with the results for the plot plants, survivorship of radiate plants was greater in pure stand than in mixture, although

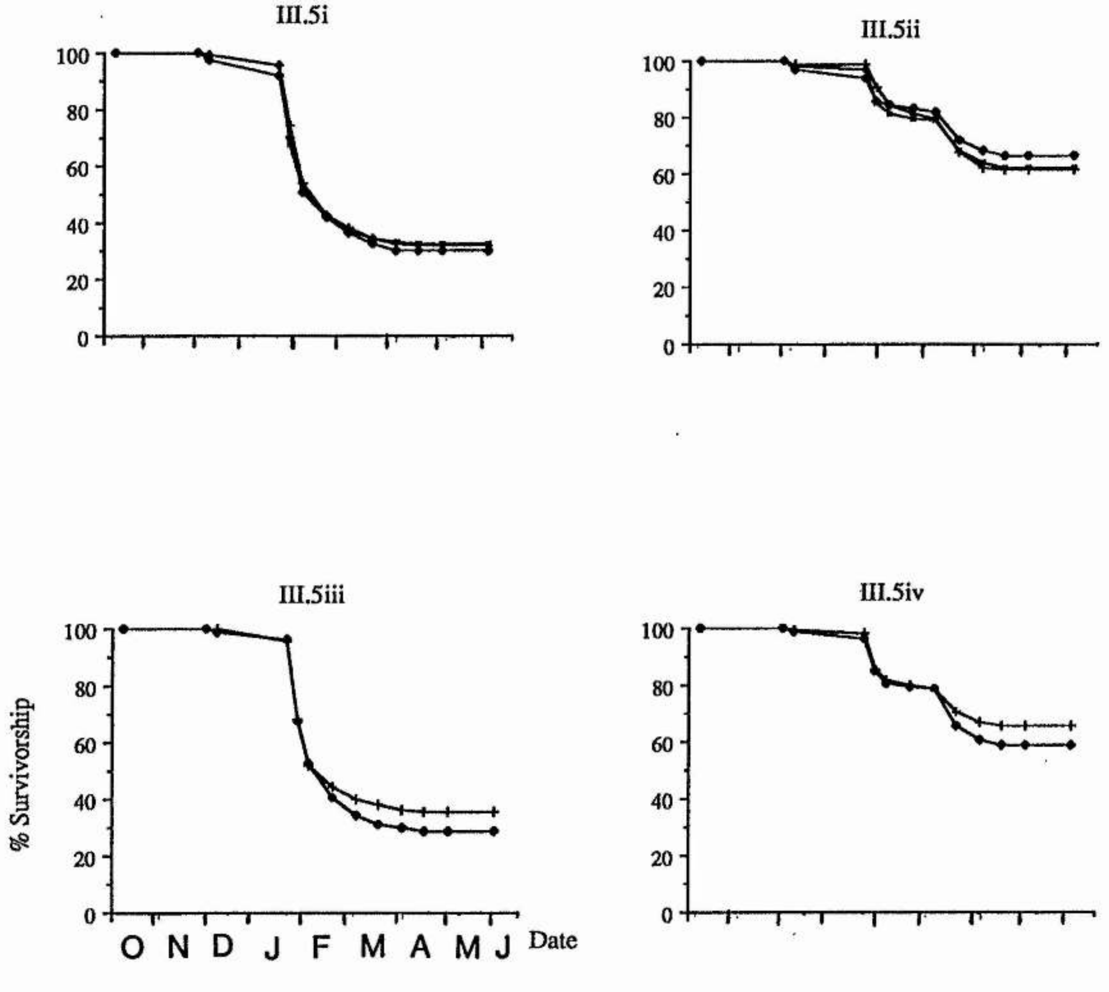


Figure III.5 Autumn 1984 Percentage Survivorship of Guard Row Plants: (i) in high density treatments, i.e. pure stand radiate (◆), pure stand non-radiate (+), and mixture (■); (ii) in low density treatments; (iii) morph survivorship within the high density mixture; and (iv) within the low density mixture. Levels of significance between treatments are based on a three factor ANOVA and between morphs within the mixture on paired t-tests.

differences were not significant (Table III.17i). Non-radiate plants exhibited no difference in survivorship between the two treatments (Table III.17ii).

III.4.2.2 Fecundity.

Frequency distributions of capitulum number per plant were positively skewed in all treatments (Figs. III.6 & III.7), indicating that competition had occurred in the plots.

Plot plants.

Effect of treatment

At both densities non-radiate plants produced greater numbers of capitula per plot than radiate plants in pure stand and in mixture (Tables III.12 & III.13). This advantage stemmed mainly from a greater number of capitula produced per non-radiate plant.

Mean values for capitulum number per plot once more were corrected for unit area to allow for examination of density effects (see above p.42). In contrast to what was found in the spring experiment, a significantly greater number of capitula per unit area was produced in high density pure stands than at low density. Again this stemmed from greater numbers of capitula produced per survivor. In mixture no such difference between densities was evident (Table III.13).

Morph fecundity in mixture compared to pure stand

In contrast to the trend noted in the Autumn 1983 studies, plants of each morph raised in pure stand tended to exhibit greater net reproductive outputs (N.R.O) than plants in mixture (Table III.14). For the radiate morph raised at high density this difference was highly significant. The greater N.R.O. of the radiate morph stemmed from greater survivorship and, in particular greater capitulum production per survivor.

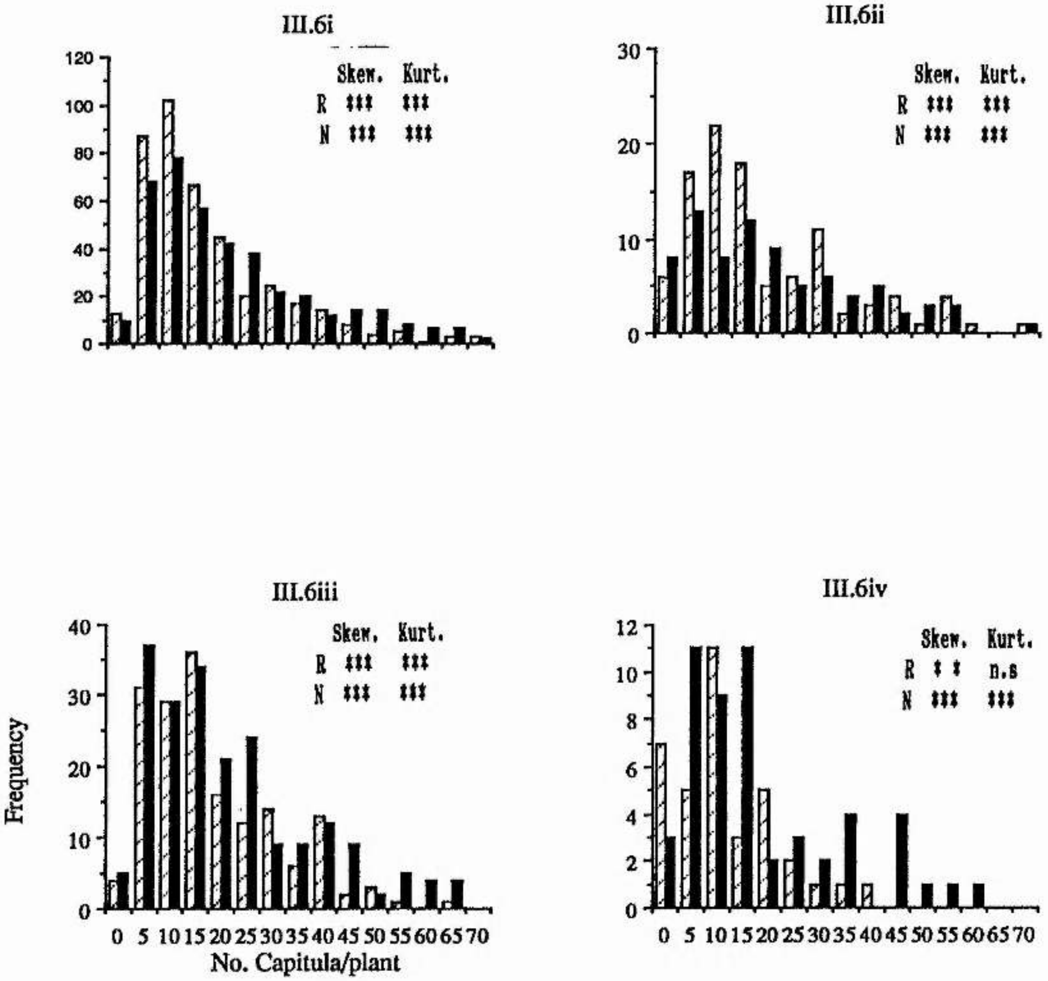


Figure III.6 Autumn 1984: Frequency distributions for capitulum number per plant of plot plants of the radiate (▨) and non-radiate morphs (■) when raised in: (i) low density pure stand; (ii) high density pure stand; (iii) low density mixture and (iv) high density. Levels of significance for coefficients of skewness and leptokurtosis are based on t-tests.

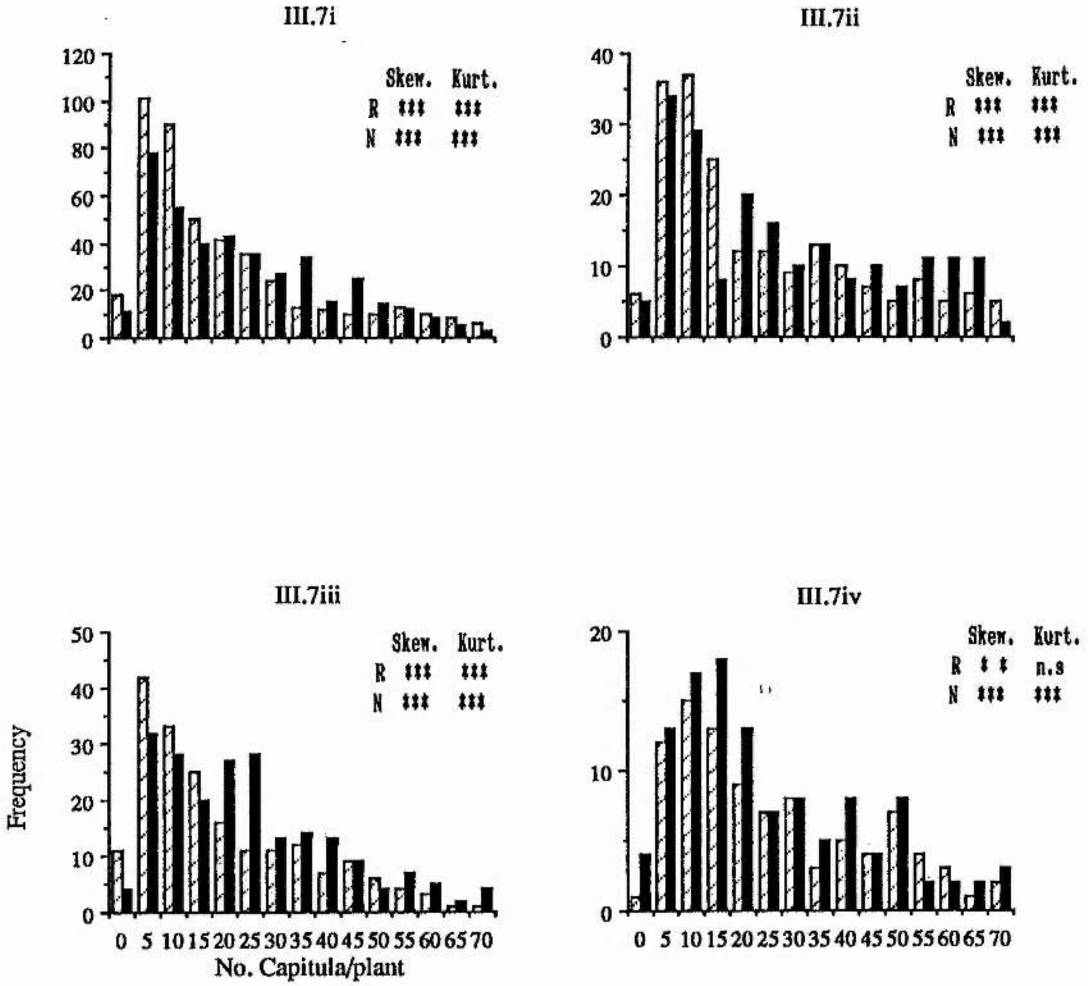


Figure III.7 Autumn 1984: Frequency distributions for capitulum number per plant of guard row plants of the radiate (▨) and non-radiate morphs (■) when raised in: (i) low density pure stand; (ii) high density pure stand; (iii) low density mixture and (iv) high density mixture. Levels of significance for coefficients of skewness and leptokurtosis are based on t-tests.

Table III.12 St. Andrews Autumn 1984 Plot Plants: Proportion of individuals sown to fruit, number of capitula per plant, and capitulum number per unit area per plot for low and high density pure stands and mixtures of the radiate (R) and non-radiate (N) morphs. Levels of significance are based on results of a three factor ANOVA with two orthogonal contrasts (i & ii) extracted from the treatment variance.

	Prop. Fruit			No. Capitula/Plant			Capitulum Total		
Treatment	R	N	Mix	R	N	Mix	R	N	Mix
6cm.	0.65	0.64	0.59	17.4	23.1	20.7	226	295	245
3cm.	0.17	0.14	0.14	25.9	31.1	18.3	347	349	218
Treatment (R v N v Mix)			n.s			n.s			*
i) R v N			n.s			n.s			*
ii) P.S v Mix			n.s			.054 n.s			.055 n.s
Density			***			.078 n.s			***
Treatment x Density			n.s			n.s			n.s
i) R v N x Density			n.s			n.s			.052 n.s
ii) P.S v Mix x Density			n.s			.058 n.s			n.s

Table III.13 St. Andrews Autumn 1984 Plot Plants: Differences in mixture between the radiate (R) and non-radiate (N) morphs for proportion individuals sown to fruit, number of capitula per plant, and capitula total per plot. Levels of significance are based on result of paired t-tests.

Treatment	Prop. Fruit			No. Capitula/Plant			Capitula Total		
	R	N	p	R	N	p	R	N	p
6cm.	0.53	0.65	n.s	19.2	22.2	*	101	143	*
3cm.	0.11	0.17	*	13.0	23.7	*	55.4	163	* *

Table III.14 St. Andrews Autumn 1984 Plot Plants: Differences in the proportion of plants to fruit, number of capitula per plant and net reproductive output for (i) the radiate and (ii) non-radiate morphs when grown in high (3cm.) and low (6cm.) density mixture compared to pure stand.

(i) Radiate

	Propn. to Fruit		Capitula/plant		NRO	
	Mix	P.S	Mix	P.S	Mix	P.S
3 cm.	0.11	0.17	13.0	25.9	1.38	4.33
6 cm.	0.53	0.65	19.2	17.4	10.10	11.30
Treatment (P.S v Mix)	.068 n.s		*		***	
Density	***		n.s		***	
Treat x Density	n.s		*		n.s	

(ii) Non-radiate

	Propn. to Fruit		Capitula/plant		NRO	
	Mix	P.S	Mix	P.S	Mix	P.S
3 cm.	0.17	0.14	23.7	31.1	4.1	4.4
6 cm.	0.65	0.65	22.2	23.1	14.4	14.8
Treatment (P.S v Mix)	n.s		.07 n.s		n.s	
Density	***		.09 n.s		***	
Treat x Density	n.s		.07 n.s		n.s	

Guard row plants.

Effect of treatment

Although differences between treatments (R v N v Mix) for capitulum number per plot were not significant at either density, a partitioning of the treatment term revealed that in pure stand, the non-radiate morph again produced significantly more capitula than the radiate morph (Table III.15). This difference was almost entirely due to greater capitulum production per survivor. The advantage of the non-radiate morph was also apparent in mixture (Table III.16), due this time to both better survivorship and greater individual fecundity.

The effect of density on capitulum totals per plot was not examined for guard row plants for the reasons given earlier (p. 42). However, in contrast to what was found for plot plants, guard row plants raised at low density produced significantly greater numbers of capitula per plant than plants at high density (Table III.14).

Morph fecundity in mixture compared to pure stand

The Net Reproductive Output of the radiate morph was greater in mixture than in pure stand at high density, however, the opposite was the case at low density (Table III.17i). In contrast, N.R.O. of the non-radiate morph showed little difference between treatments at high density, but at low density, was greater in mixture (Table III.17ii).

III.4.2.4 Relative morph fitness.

A comparison of the relative fitnesses of each morph in both plot and guard row fractions of all treatments, once again revealed a clear advantage of the non-radiate morph over the radiate morph (Table III.18). Amongst the plot plants in the high density mixture, this advantage was particularly striking with the fitness of the radiate morph being approximately one third that of the non-radiate morph.

Table III.15 St. Andrews Autumn 1984 Guard Row Plants: Proportion of individuals sown to fruit, number of capitula per plant, and capitula number per unit area per plot for low and high density pure stands and mixtures of the radiate (R) and non-radiate (N) morphs. Levels of significance are based on results of a three factor ANOVA with two orthogonal contrasts extracted from the treatment variance.

	Prop. Fruit			No. Capitula/Plant			Capitula Total		
Treatment	R	N	Mix	R	N	Mix	R	N	Mix
6cm.	0.67	0.61	0.62	45.0	54.1	53.6	2654	2900	2877
3cm.	0.31	0.32	0.33	61.8	79.6	70.1	1662	2268	2008
Treatment (R v N v Mix)			n.s			n.s			n.s
i) R v N			n.s			n.s			*
ii) P.S v Mix			n.s			n.s			n.s
Density			***			*			* *
Treatment x Density			n.s			n.s			n.s
i) R v N x Density			n.s			n.s			n.s
ii) P.S v Mix x Density			n.s		.09	n.s			n.s

Table III.16 St. Andrews Autumn 1984 Guard Row Plants: Differences in mixture between the radiate (R) and non-radiate (N) morphs for proportion individuals sown to fruit, number of capitula per plant, and capitula total per plot. Levels of significance are based on result of paired t-tests.

Treatment	Prop. Fruit			No. Capitula/Plant			Capitula Total		
	R	N	*p*	R	N	*p*	R	N	*p*
6cm.	0.59	0.65	n/s	47.4	59.7	n/s	1198	1679	*
3cm.	0.29	0.36	*	72.7	67.5	n/s	936	1072	n/s

Table III.17 St. Andrews Autumn 1984 Guard Row Plants: Differences in the proportion of plants to fruit, number of capitula per plant and net reproductive output for (i) the radiate and (ii) non-radiate morphs when grown in high (3cm.) and low (6cm.) mixture compared to pure stand. Levels of significance are based on three factor ANOVAS.

(i) Radiate

	Propn. to Fruit		Capitula/plant		NRO	
	Mix	P.S	Mix	P.S	Mix	P.S
3 cm.	0.29	0.31	72.7	61.8	21.3	18.9
6 cm.	0.59	0.67	47.4	45.0	27.9	29.3
Treatment (P.S v Mix)	n.s		n.s		*	
Density	***		* *		n.s	
Treat x Density	n.s		.09 n.s		* *	

(ii) Non-radiate

	Propn. to Fruit		Capitula/plant		NRO	
	Mix	P.S	Mix	P.S	Mix	P.S
3 cm.	0.36	0.32	67.5	79.6	24.4	25.8
6 cm.	0.65	0.61	59.7	54.1	38.7	33.0
Treatment (P.S v Mix)	n.s		n.s		n.s	
Density	***		.06 n.s		*	
Treat x Density	n.s		n.s		n.s	

Table III.18 St. Andrews Autumn 1984: Relative fitness of the radiate (R) and non-radiate (N) morphs when raised in high and low density pure stand and mixture.

	Relative Fitness			
	Inner Row Plants		Outer Row Plants	
	R	N	R	N
3cm. Pure Stand	0.99	1.0	0.73	1.0
3cm. Mixture	0.34	1.0	0.87	1.0
6cm. Pure Stand	0.77	1.0	0.91	1.0
6cm. Mixture	0.70	1.0	0.71	1.0

III.5 - DISCUSSION -

Morph differences in fitness

Although differences in fitness between morphs were reduced in the spring compared to the autumn study, it was clear that in each experiment the non-radiate morph tended to exhibit a greater relative fitness than the radiate morph, through increased seed production in the spring experiment and capitulum production in the autumn study (Tables III.12 & III.18). The greater recorded fitness of the non-radiate morph is consistent with the findings of the Autumn 1983 experiments reported in Chapter II, and those of previous studies which have investigated fitness differences between morphs in Edinburgh populations based on seed number per plant (Marshall and Abbott, 1987; Ross and Abbott; 1987).

In contrast to the findings of Waller (1984) for siblings derived from chasmogamous and cleistogamous seed of I. capensis (see Introduction), the differences in fitness observed between morphs in the present study did not vary significantly with density. In both the spring and autumn experiments there was no significant effect of density on differences between morphs for any character examined.

Another interesting feature to emerge from the results was the tendency for there to be no difference between morphs for survivorship in either experiment. This stood in marked contrast to what had been found in the Autumn 1983 studies (see Chapter II). The absence of differential mortality in the Autumn 1984 experiment was most likely the result of the extremely severe frosts of the 1984-1985 winter. It is possible that the severity of these frosts overcame the environmental tolerance of the non-radiate as well as that of the radiate morph. By contrast, in the spring experiment, environmental conditions were considerably less severe than those experienced by individuals planted in Autumn 1983 or 1984 and this enabled both morphs to exhibit very high levels of survivorship. One notable exception to the general rule of no difference in mortality between morphs occurred in the mixture raised at high density in Autumn 1984. In this treatment non-radiate plot plants exhibited significantly better survivorship than radiate plants.

In the absence of a general difference in survivorship between morphs in each experiment, differences in relative fitness between morphs were due almost entirely to differences in individual fecundity. Within the Spring 1984 experiment, these were based on differences in seed number per capitulum, and/or capitulum number per plant with the latter character being of primary importance. The considerable effect of capitulum number per plant on overall fitness of S. vulgaris has been reported previously by Paul and Ayres (1986). In a study which examined the relative fitness (based on seed number per plant) of healthy and rust infected groundsel, they noted that seed production by infected plants was reduced primarily because of reductions in capitulum number. A further point to emerge from the Spring 1984 results was that when a difference between morphs occurred for both seed number per capitulum and capitulum number per plant, the character differences were correlated. For this reason, therefore, capitulum number per plant was considered as an accurate indicator of any difference in morph fecundity and was used alone as a measure of fecundity in the Autumn 1984 experiment.

Density effects

As previously discussed (p. 42) it was not possible to quantitatively appraise the effect of density on the fitness of guard row plants. The following discussion of density, therefore, relates to plot plants only. In the Spring 1984 experiment, there was little difference between densities for seed number produced per plot by each morph raised in pure stand (Table III.3). Similar results have been found for other organisms (Donald, 1951; Palmbad, 1968; Kays and Harper, 1974; Branch, 1975; Watkinson, 1984), due to what has been termed "the law of constant final yield" (Kira et al., 1953). As there was no difference in survivorship between low and high density pure stands, the equivalent numbers of seed produced by low and high density pure stands of each morph must have resulted from compensating density dependent fecundity (Silvertown, 1982; Begon, Harper and Townsend, 1987). Thus, although final plant density in low density plots was only one quarter of that at high density, each individual plant produced on average four times as many seed as produced by individuals at high density. This change in individual plant fecundity, therefore, compensated for the change in density.

In the Autumn 1984 study, far fewer capitula were produced per plot in low density than in high density pure stands (Table III.12). This rather surprising result may be explained as an outcome of two effects. The first concerns compensating density dependent mortality occurring within the high density pure stands of both morphs. This resulted in final densities of survivors which were equivalent in both the "high" and "low" density plots. Such compensating density dependent mortality has been reported often in many plant species (see Silvertown, 1982; and Begon, Harper and Townsend, 1987). In turn, the greater mortality which occurred in the high density plots may have created a second effect which ultimately led to the greater individual fecundity that was recorded in these plots. Two possibilities come to mind. First, because a greater number of seedlings suffered mortality in the high compared to the low density pure stands, the effect of intramorph competition on survivorship would have been considerably greater in the high density pure stands. It might follow that a greater proportion of survivors in the high density plots were strong competitors. Subsequent competition during later stages of plant growth, therefore, would reduce the individual fitness of surviving plants to a lesser extent in the high compared to the low density pure stands. The second possibility is based on the uneven spatial distribution of survivors in "high" density plots following the severe mortality that occurred within them. This may have led to a high proportion of survivors in these plots to grow at what was effectively a more reduced density than was experienced by the more uniformly distributed survivors in the "low" density plots. As a consequence intramorph competition between many survivors in "high" density plots would be considerably reduced which, in turn, may have resulted in greater individual fitness of these plants.

Performance in mixture

Comparisons of morph performance in mixture relative to performance in pure stand revealed a marked difference between the results of the Autumn 1983 experiment at St. Andrews and the experiments conducted in 1984. In the St. Andrews Autumn 1983 study, the fitness of each morph was increased in mixture compared to pure stand along with a decrease in the difference in fitness between morphs in mixture. This result was of interest in that it might be thought to provide a mechanism which could slow the loss of the radiate allele from a population polymorphic for capitulum type (see Chapter II.5). In contrast, the results of the 1984 studies show that the fitness of each morph was reduced in mixture. Moreover, the difference in fitness between morphs tended to increase in mixture compared to pure stand. This we would expect to select for a population monomorphic for the morph which exhibits the greater fitness. As in each 1984 study it was the non-radiate morph which exhibited the greater fitness in mixture, it would be expected that populations should ultimately become monomorphic for the non-radiate morph.

Conclusions

In conclusion, the present studies once again failed to provide any evidence of a short term advantage that the radiate morph may hold over the non-radiate morph. Irrespective of planting date or initial density it was the non-radiate morph which tended to exhibit the greater fitness. It should be pointed out, however, that each of these studies used seedlings rather than seed as the starting material and did not, therefore, take into account any morph differences in germination behaviour. Recently it has been emphasised that the value of these types of experiment may be limited if, in fact, differences are present between morphs in their respective germination behaviours (Law and Watkinson, 1987). Field studies that were conducted to detect differences between morphs in seed germination behaviour are reported in Chapter V.

CHAPTER IV: RELATIVE FITNESS OF SELF AND OPEN POLLINATED OFFSPRING OF THE RADIATE AND NON-RADIATE MORPHS

IV.I - INTRODUCTION -

As previously discussed (Chapter I.3), inbreeding depression has been cited as one of the most important factors which might balance the inherent genetic cost of outcrossing in a population that is polymorphic for outcrossing rate (Charlesworth and Charlesworth, 1979; Lloyd, 1980; Brown and Marshall, 1982; Lande and Schemske, 1985). Inbreeding depression amongst selfed offspring will occur if there is overdominance or dominance effects at loci affecting fitness traits (Wright, 1977). With overdominance, heterozygotes at a given locus are always superior to the corresponding homozygotes. With dominance, deleterious recessive alleles will be retained in populations at low frequencies due to masking by dominant alleles in the heterozygote. In both cases, selfing will reduce fitness due to the increase of homozygosity within individuals. It has been shown, however, that to balance the cost of outcrossing in a polymorphic population, the relative fitness of selfed offspring must be less than or equal to 0.5 compared to outcrossed progeny (Maynard-Smith, 1978; Lloyd, 1979). This point is important in that such high levels of inbreeding depression are not expected in natural populations of selfing plants (Grant, 1975; Wright, 1977).

Several workers have investigated the importance of inbreeding depression in natural populations of a variety of plant species by comparing the relative fitness of outcrossed and selfed progeny (e.g., Jain, 1979; Schemske, 1983; Schoen, 1983; Levin, 1984; Kesseli and Jain, 1984; Waller, 1984; Harder et al, 1985; Mitchell-Olds and Waller, 1985). In all of these studies, there was a significant effect of inbreeding depression with outcrossed progeny exhibiting greater fitness than inbred progeny. Interestingly, Waller (1984) investigated the relative fitnesses of progeny from chasmogamous (outcrossing) and cleistogamous (selfing) inflorescences of Impatiens capensis and concluded that the greater fitness of the chasmogamous offspring was sufficient to counter the inherent cost of outcrossing. In a similar manner it was of interest to investigate whether selfed progeny of non-radiate and radiate parents exhibit large reductions in fitness due to inbreeding depression. If inbreeding depression is high in S.vulgaris, this would favour the spread of the radiate 'outcrossing' morph.

In a preliminary study of inbreeding depression within the two morphs of S. vulgaris, Moncrieff (unpub.) compared the relative fitness of open and second generation self pollinated progenies derived from each of 23 parent plants of each morph from two Welsh populations. In this study, progeny were raised under glass as single plants in 8cm. pots containing 'Levington' compost. Fitness was measured as seed output per plant and flowering time. The results (published in Abbott, 1985) showed that there was no effect of inbreeding on either character for the material from Cardiff, but, inbreeding appeared to delay flowering in material from Rhosllanerchrugog.

In Moncrieff's study, plants were raised singly in pots and thus were not subject to competition. Fitness differentials between progeny of inbred and open pollinated parents may be lower in an experiment conducted under relatively "benign" conditions (e.g. single plants per pot) than if measured under "stressful" conditions (e.g. competition between progeny) (Antonovics, 1968; Parsons, 1971; Schemske, 1983). Until the study presented here, there had been no detailed analysis of the possible effects of inbreeding depression on the fitness of open and self pollinated progenies of the radiate and non-radiate morphs of S. vulgaris when grown in competition with each other. A recent study by Schmitt and Erhardt (1987) used a de Wit replacement series experimental design (de Wit, 1960) to examine competition between open pollinated and selfed progenies of Impatiens capensis. Their results revealed no difference in fitness between the two offspring types. However, as the effect of competition was confounded with the effect of density in the de Wit experimental design (see Chapter II.5), the results obtained by the authors may not be as conclusive as first reported. A more suitable design for the investigation of competition between different plant types is one developed by Mather and Caligari (1981) and Underwood (1986). This design, as it relates to the analysis of competition between selfed and open pollinated progenies, is best described with reference to Table IV.1.

Open pollinated and self pollinated offspring from the same parent plant are grown as monocultures, i.e. single plants per pot (treatments 1 & 5), or as dual cultures, i.e. two plants per pot (treatments 2-4). In dual cultures, both plants are either (i) of the same breeding type, that is both "open-pollinated" offspring (treatment 2) or both "self-pollinated" offspring (treatment 4); or (ii) represent one plant of each breeding type (treatment 3). The design therefore, incorporates two densities; a low density of 1 plant per pot, treatments 1 & 5, which act as controls; and a high density of 2 plants per pot, treatments 2-4, in which plants are subject to competition. The only difference between the controls and the "competition" treatments is of course, the presence of the "competing" plant. Using this design, the level of competition between progeny of parents of the same breeding type, i.e open or selfed pollinated progeny raised in pure stand (termed Type A competition), can be compared to the level of competition occurring between offspring from parents of different breeding types, i.e open v self pollinated progeny raised in mixture (termed Type B competition). By comparing the treatments in which two plants are grown (dual cultures) with the controls (mono cultures), it is possible to detect, in turn, the effects of Type A and Type B competition. The required comparisons for detecting Type A and Type B competition on selfed and open pollinated progeny are given below.

Table IV.1 An experimental design to detect and investigate the effects of competition on progeny of open (O) and self (S) pollinated parents.

	Treatment				
	1	2	3	4	5
Progeny from Open Pollinated Parents (O)	1	2 (1 O + 1 O)	1	-	-
Progeny from Self Pollinated Parents (S)	-	-	1	2 (1 S + 1 S)	1

Comparison	Type A Competition	Type B Competition
Effect of competition on open-pollinated offspring	2 vs 1 (O on O)	O in 3 vs 1 (S on O)
Effect of competition on self-pollinated offspring	4 vs 5 (S on S)	S in 3 vs 5 (O on S)

N.B. Numbers in table refer to treatments given in Table IV.1.

Effects of Type A and Type B competition within each breeding type are measured as the reduction in mean of a given fitness trait e.g. seed output per plant, when performance in dual culture (two plants per pot) is compared with that in monoculture. If selfed progeny are at a disadvantage (due to inbreeding depression) compared to open pollinated offspring this should be reflected in a greater reduction in the mean of a given fitness trait, e.g. seed output per plant.

In addition to the comparisons just described, the design also allows comparisons between the performance of open and self-pollinated offspring raised in <u>pure stand</u>, either as mono- or dual cultures. If it is assumed that inbreeding depression effects are magnified under stress, we might anticipate that any difference between selfed and open pollinated offspring in fitness would be greater in dual culture than in mono culture.

The analysis of competition presented in this Chapter has been conducted on data obtained from an experiment reported by Perryman (1983). Previous analysis of the data by Perryman did not examine competition in any detailed manner as described above. The objective of the analysis was to answer the following question: (i) is the fitness of selfed offspring lowered when selfed offspring are raised in competition with open pollinated offspring? (ii) is the reverse true for open pollinated offspring, i.e. their fitness is increased when raised in competition with selfed offspring? (iii) is the relative fitness of selfed offspring raised in pure stand lower than that of open pollinated offspring grown in pure stand and, if so, is the reduction in fitness of selfed offspring more evident in dual than in mono-culture pure stands?

As comparisons of fitness between selfed and open pollinated progeny were conducted on both the radiate and non-radiate morphs, the study also enabled a comparison of the fitnesses of the two morphs in mono and dual cultures.

IV.2 - MATERIALS and METHODS -

Open pollinated seeds (O1) were collected separately from a sample of parent plants of the radiate and non-radiate morphs from a wild polymorphic population located at Newhaven Rd. Edinburgh in May 1980. Several O1 seeds of each parent plant were germinated to yield offspring that were raised to produce selfed seed (S1) in a glasshouse. The O1 and S1 seeds so produced were, in turn, germinated on filter paper in petri dishes, in an unheated glass house. Once large enough to handle, these seedlings were transplanted into small 3.5 cm. pots containing a soil-based compost. For offspring of each of 20 radiate and 20 non-radiate parent plants the following treatments (see Table IV.1) were set up:

1. One open pollinated seedling per pot (Treatment 1).
2. Two open pollinated seedlings per pot (Treatment 2).
3. One open pollinated seedling and one self pollinated seedling per pot (Treatment 3).
4. Two self pollinated seedlings per pot (Treatment 4)
5. One self pollinated seedling per pot (Treatment 5).

In pots that contained two seedlings, care was taken to ensure that seedlings were transplanted at a similar growth stage. Each treatment was replicated twice giving a total of 400 pots (i.e. 2 replicates x 2 morphs x 20 parents x 5 treatments). Pots were completely randomised on a bench in an unheated glass house at the University Botanic Garden. Throughout the experiment plants were kept free of disease and pests by the use of appropriate chemical applications. Pots were planted on 1.11.82 and after 91 days of growth plants were harvested by clipping the stems at soil level.

For each plant harvested a record was taken of the following fitness characters: (i) capitulum number per plant; (ii) seed number per capitulum; and (iii) ovule number per capitulum. From these records an estimate of the total number of seed produced per plant was obtained by multiplying the capitulum number per plant by the mean number of seed produced per capitulum. A measure of ovule fertility was also estimated in terms of the percentage of ovules to set seed.

IV.3 - ANALYSIS OF DATA -

Pots in which plant death occurred before harvest were excluded from the analysis. Where this created an unbalanced design (i.e. missing cells in the analysis of variance) all plants of the family affected were excluded. Consequently, the analysis was restricted to twelve lines of each morph.

The total data set was subjected to a four factor analysis of variance with replication (Table IV.2). The four factors were morphs (radiate v non-radiate), Breeding types (Open v Self pollinated), Treatments (Control v Type A competition v Type B competition), and Lines within Morphs. Because lines (a random effect) were nested within morphs (a fixed effect), the term "lines within morphs" is also taken as a fixed effect (Sokal and Rolhff, 1981). The ANOVA, therefore, is of the model I type, and all sources of variation are tested against the error mean square (Sokal and Rohlff, 1981; p.374).

Table IV.2 ANOVA model to examine possible effects of inbreeding depression and of type A and type B competition on the fitness of open and selfed offspring of each morph.

Source of Variation		d.f
Morph (Mo)		1
Breeding Type (BT)		1
Treatment (Tr)	(Control v type A v type B)	2
Line within Morph (L w Mo)		22
Morph x Breeding Type		1
Morph x Treatment		2
Breeding Type x Treatment		2
Line within Morph x Treatment		44
Line within Morph x Breeding Type		22
Treatment x Morph x Breeding Type		2
Line within Morph x Treatment x Breeding Type		44
Error		144
Total		287

In the analysis, particular interest centres on the breeding type x treatment interaction. This interaction will be significant if: (i) the performance of open pollinated offspring is increased in mixed dual culture (O v S1) relative to performance in pure dual culture (O v O), while the reverse is true for self pollinated offspring. If this occurs, it can be concluded that selfed offspring exhibit significant inbreeding depression in competition with open pollinated offspring due to the greater competitive ability of open pollinated offspring; (ii) any difference in performance between open and selfed progeny is significantly less in mono culture than in pure stand dual culture; (iii) the interaction is due to both effects ((i) & (ii)) given above.

In the absence of a significant interaction between breeding types and treatments it would be possible to rule out each of the above effects in the analysis of inbreeding depression and instead focus entirely on differences between breeding types (Open v Self-pollinated) as measured over all treatments. Only if the interaction is significant does it become necessary to examine the individual effects of Type A and Type B competition as outlined in the Introduction.

Before analysis all data sets were tested to examine whether the assumptions of the analysis of variance (i.e. normality of distribution and homogeneity of variance) were satisfied. These tests were conducted as previously described in Chapter II.3.

IV.4 - RESULTS -

The results of the analysis of variance conducted on the total data set of each character are presented in Table IV.3. From these results it is evident that neither the breeding type x treatment interaction, nor differences between breeding types were significant for any of the five characters investigated. It can be safely concluded, therefore, that selfing does not result in a reduction of fitness (inbreeding depression)

Table IV.3 Results from the analysis of variance for: seed number per plant; capitulum number per plant; seed and ovule number per capitulum; and, ovule fertility.

Source of Variation	Seed/ plant	Capitulum/ plant	Seed/ capitulum	Ovule/ capitulum	Ovule fertility
Morph (Mo)	***	*	***	***	***
Breeding Type (BT)	n.s	n.s	n.s	n.s	n.s
Treatment (Tr)	***	***	***	***	***
Lines within morphs (L w Mo)	***	***	***	***	***
Mo x Bt	n.s	n.s	n.s	n.s	n.s
Mo x Tr	n.s	n.s	n.s	n.s	n.s
BT x Tr	n.s	n.s	n.s	n.s	n.s
L w Mo x BT	n.s	n.s	n.s	n.s	n.s
L w Mo x Tr	n.s	*	*	* *	n.s
Mo x BT x Tr	n.s	n.s	n.s	n.s	n.s
L w Mo x BT x Tr	n.s	n.s	n.s	n.s	n.s

in either the radiate or non-radiate morphs of S. vulgaris. This is so whether the comparison is made between open and self pollinated offspring raised as single plants per pot, as two plants per pot or as a mixed stand (O v S1).

Although there is no difference between breeding types, highly significant differences are present between morphs, treatments, and lines within morphs. From an examination of the treatment means for open and selfed offspring within each morph (Table IV.4), and for each morph over both breeding types (Table IV.5), it is clear that: (i) plants in dual culture (Type A and Type B treatments) were greatly affected by competition - note the reduction in mean expression for each character relative to the controls (monoculture); and (ii) for each character the non-radiate morph exhibited the greater mean.

IV.5 - DISCUSSION -

From the results of the experiment reported in this chapter it is clear that selfing of the radiate or non-radiate morphs of S. vulgaris does not lead to any reduction in mean expression of a range of fitness characters. This finding suggests, therefore, that inbreeding depression does not occur in S. vulgaris and consequently is not a factor of importance in countering the cost of outcrossing incurred by the radiate morph in populations that are polymorphic for capitulum type and associated outcrossing rate.

It is recognised that several characters which may affect the relative fitness of open versus self pollinated offspring were not measured in the experiment, therefore, possible effects of inbreeding depression on the fitness of selfed progeny can not be entirely ruled out. For example, Steven and Bougard (1988) found that in Allium shoenoprasum (an outcrossing species), selfing adversely affected seed germination. In the present study neither the viability nor germination of seed produced by open or self pollination was assessed for either morph of S. vulgaris. Similarly

Table IV.4 Mean number of seed per plant, capitula per plant, ovule and seed per capitulum, and ovule fertility for progeny of open (O) and self (S1) pollinated radiate and non-radiate parents in the control (monoculture), Type A and Type B competition (dual cultures) treatments.

Character		Radiate			Non-radiate		
		Control	Type A	Type B	Control	Type A	Type B
Seed/plant	(O)	524	265	234	548	305	293
	(S)	483	270	260	606	319	295
Capitula/plant	(O)	16.1	10.0	9.4	15.4	10.9	10.1
	(S)	14.9	10.5	10.0	16.8	10.9	10.5
Seed/capitulum	(O)	32.2	26.6	24.6	35.4	28.1	28.9
	(S)	32.4	25.5	26.1	35.5	28.9	28.2
Ovule/capitulum	(O)	35.5	30.4	29.1	38.6	31.7	32.5
	(S)	36.0	29.7	29.9	38.2	32.5	32.3
Ovule fertility	(O)	90.3	87.2	84.2	91.6	88.2	88.6
	(S)	89.6	85.3	86.5	92.9	88.7	87.5

Table IV.5 Mean number of seed per plant, capitula per plant, ovule and seed per capitulum, and ovule fertility for progeny of radiate (R) and non-radiate (N) parents in the control (monoculture), Type A and Type B competition (dual culture) treatments.

Character		Control	Type A	Type B
Seed/plant	(R)	503.6	267.5	247.1
	(N)	577.0	311.8	293.9
Capitula/plant	(R)	15.5	10.1	9.7
	(N)	16.1	10.9	10.3
Seed/capitulum	(R)	32.3	26.0	25.4
	(N)	35.4	28.5	28.5
Ovule/capitulum	(R)	35.8	30.0	29.5
	(N)	38.4	32.1	32.4
Ovule fertility	(R)	89.9	86.2	85.3
	(N)	92.2	88.5	88.1

because the study was conducted under glass in the absence of pests and pathogens it is not possible to conclude that selfed progeny suffer no more than open pollinated offspring from infection by certain pests and pathogens. Clearly a more comprehensive study would need to be conducted on selfed and open pollinated offspring of S. vulgaris before the possibility of inbreeding depression resulting from selfing could be entirely ruled out.

On the other hand, it is not surprising that for those characters investigated, no evidence of inbreeding depression was forthcoming. Although the radiate morph tends to show significantly greater outcrossing rates than the non-radiate morph, both morphs reproduce mainly by selfing. Estimates of outcrossing for the parents of the open-pollinated seeds used in the present study were 7.2% and 0.9% for the radiate and non-radiate morphs respectively (Perryman, 1983; from Marshall and Abbott, 1984). Data reviewed by Wright (1975) suggests that although inbreeding depression may occur in predominately selfing species it is usually much less severe than in species that are normally outbred. Therefore, in a plant species such as S. vulgaris in which all individuals exhibit a relatively high selfing rate, it is to be expected that inbreeding depression will be minimal if it occurs at all.

In contrast to the absence of inbreeding effects, there were clear differences between morph means for each character recorded in each treatment (Table IV.5). Indeed, for each character recorded the non-radiate morph exhibited the greater mean. It is to be concluded, therefore, from this study, as for those reported in chapters II and III, that the radiate morph in Edinburgh populations holds no advantage over the non-radiate morph of S. vulgaris which might counter the cost of outcrossing. Instead, the non-radiate morph appears to exhibit a greater inherent fitness based on fecundity characters and this should cause it to proceed rapidly to fixation within populations in the Edinburgh area.

CHAPTER V. GERMINATION BEHAVIOUR OF THE RADIATE AND NON-RADIATE MORPHS OF S. VULGARIS I: FIELD TRIALS.

V.1 - INTRODUCTION -

Differences in seed germination behaviour between the radiate and non-radiate morphs of *S. vulgaris* were first reported by Richards (1975) following a study of material from a polymorphic Durham population. More recently, in an investigation of life history variation in material from four geographically distinct polymorphic populations in Britain, Abbott (1986) showed that character differences between morphs were common, however, only for seed germination behaviour were differences consistent over populations. In the studies conducted by Richards and Abbott, seeds of the non-radiate morph tended to germinate more quickly and in greater numbers than seed of the radiate morph. However, both studies were preliminary in nature in that experiments were conducted under glass or in growth cabinets, over relatively short periods of time after sowing, and on seed that had been stored under laboratory conditions for at least 5 months.

In a follow up study conducted under field conditions, Noble (1984) also detected differences between morphs in seed germination behaviour based on an examination of fresh seeds from an Edinburgh population. His results showed that although differences between morphs were not consistent over years and sowing dates, seeds of the non-radiate morph often germinated more rapidly and in greater numbers than seed of the radiate morph. Noble's experiments were run for up to 19 weeks from sowing; however, after the initial flush of seed germination very little or no further germination occurred. Despite this lack of germination late on in the experiment most of the seed that remained dormant was viable and germinated in the laboratory after treatment with 1mM giberellic acid.

In view of the results obtained by Noble (1984) it seemed advisable to repeat the type of germination tests that he had conducted in the field but allow them to continue for much longer periods of time, i.e. until most seed had had an opportunity to germinate. To this end, therefore, a series of germination experiments using freshly collected seed was conducted over an extended period (up to 35 weeks) under field conditions. The aim of each experiment was to compare the seed germination behaviour of the non-radiate and radiate morphs under conditions which seeds were likely to experience in the wild.

The seeds tested for each morph were collected at different times of year in 1983, 1984 and 1985, and tested at two different sites (St. Andrews and Edinburgh). This enabled an analysis of the effects of date of seed production and site of sowing in the comparison of germination behaviour between morphs.

V.2 - MATERIALS AND METHODS -

Seeds used in all experiments were collected from plants that were raised in the manner previously described in Chapter II, section 2. The plants that produced the seed were the offspring of material originally sampled from a polymorphic population at Newhaven Rd. in Edinburgh.

At intervals of 1m., 30 open ended PVC drainage pipes, 15 cm. in diameter and 25 cm. in length, were sunk vertically into a previously prepared experimental plot within the nursery areas of the Royal Botanic Garden Edinburgh (RBGE) and the Botanic Garden, St. Andrews. The rim of each pipe was kept approximately 5 cm. above the surrounding ground level. The bottom of each pipe had been previously netted with a fine gauge nylon mesh to exclude potential soil borne seed predators. Sterilised soil was then added to the pipes so that after settling, the level of soil within the pipe was the same as ground level. Twelve replicates of 500 seed of the radiate and non-radiate morphs were sown as pure stands on the soil surface within pipes. Replicates were completely randomised over pipes. Six pipes that were not sown acted as controls.

After sowing, pipes were netted to exclude birds and thereafter the experimental plot and surrounding area was regularly weeded to prevent seed immigration into the pipes. Experiments were initiated at both RBGE and St. Andrews in Autumn 1983 and Spring 1984; at RBGE in Autumn 1984 and Spring 1985, and at St.Andrews in Autumn 1985. Spring sown experiments were conducted over a 16 week period while autumn sown experiments were run for between 32-35 weeks. At weekly intervals, emerging seedlings were recorded and removed. Controls remained clear of germinated seedlings throughout the experimental period.

V.3 - ANALYSIS OF DATA -

In all experiments the significance of a difference between morphs in cumulative percentage germination was determined by a t-test after transforming percentages into angles using an arcsin transformation (Sokal and Rohlf, 1969).

Records of daily maximum and minimum air temperatures and rainfall for St. Andrews and the RBGE throughout the relevant experimental periods were obtained from the Meteorological Office, Edinburgh. From these records weekly maximum and minimum temperature means, and weekly rainfall totals were calculated.

V.4 - RESULTS

V.4.1 Autumn Experiments

For seed sown at RBGE in Autumn 1983 and 1984, significantly greater numbers of non-radiate compared to radiate seed germinated in the initial flush of germination after sowing (Figs. V.1 & V.3). In contrast, for seed sown at St. Andrews in Autumn 1983 and 1985, there was little difference between morphs in the number of seed to germinate in the initial flush of germination (Figs. V.2 & V.4). In each experiment, the first flush of germination occurred during the first 4-5 weeks from sowing after which very little germination occurred over a 11-12 week period i.e. between late November and mid February, when maximum weekly temperature failed to rise above 10 °C.

At both sites and at each sowing date further flushes of germination of seeds of both morphs occurred during late February-early March, and late March-early April. These flushes which were particularly marked in the Autumn 1984 sown plots at RBGE (Fig. V.3) and the Autumn 1985 sown plots at St. Andrews (Fig. V.4) tended to parallel a general increase in weekly temperature that followed a period of low temperatures in January. It was of interest that in the autumn sown 1983 and 1984 experiments at RBGE more radiate compared to non-radiate seed germinated during the spring flushes. Consequently, at the end of each of these experiments, there was little difference recorded between morphs in respective final cumulative germination percentage. In contrast, few seed of either morph germinated during the spring flush of the autumn sown 1983 St. Andrews study (Fig. V.2), while in the 1985 sown St. Andrews experiment, more non-radiate than radiate seed germinated. (Fig. V.4). The effect of the germination patterns at St. Andrews was such that by the end of the autumn sown 1983 experiment, there was no difference between morphs in final cumulative percentage while by the end of the 1985 experiment, a significantly greater percentage of non-radiate seed had germinated.

V.4.2 Spring Sown Experiments

Despite favourable conditions little or no germination occurred for 5-8 weeks after sowing in the spring sown 1984 studies at RBGE and St. Andrews (Figs V.5 & V.6). At RBGE, approximately 80% of the seed of each morph germinated during weeks 9 and 10 after 11mm. of rain had fallen in week 8 (Fig V.5). Thereafter, despite favourable conditions, almost no

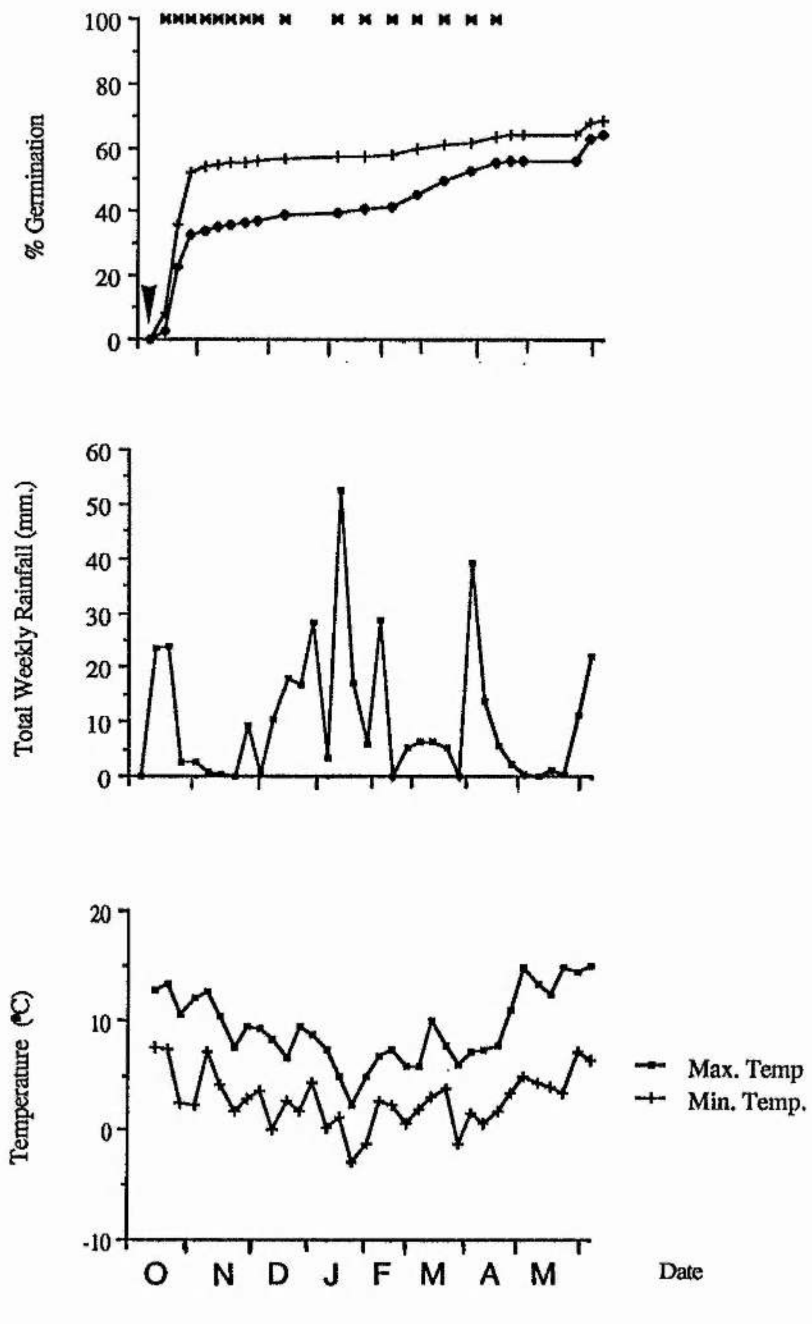


Figure V.1 RBGE Autumn 1983: Cumulative percentage germination of seeds of the radiate (◆) and non-radiate (+) morphs of *S. vulgaris*. (▼) marks sowing date and (*) where differences between morph means were significant ($p<.05$). Also presented are the weekly rainfall values and weekly mean maximum and minimum air temperatures recorded at RBGE throughout the experimental period.

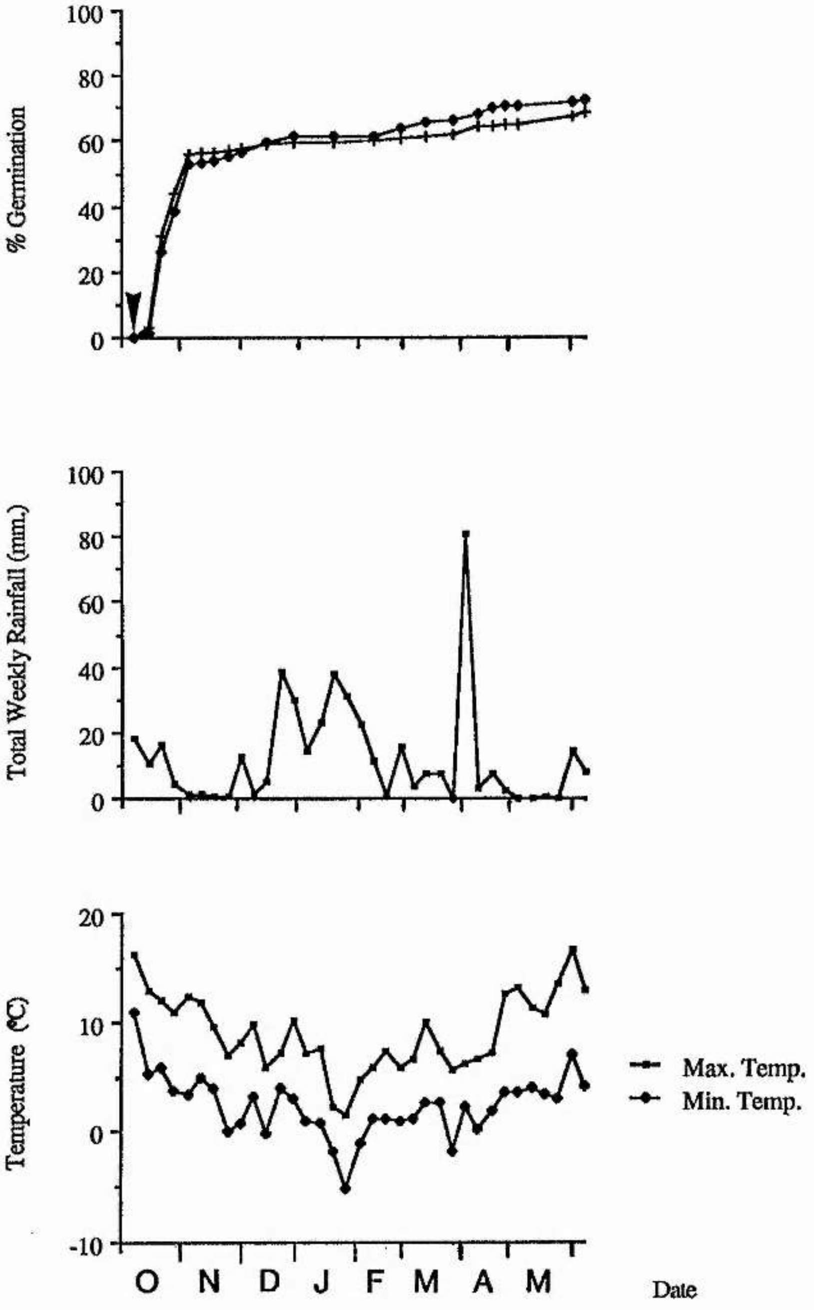


Figure V.2 St. Andrews Autumn 1983: Cumulative percentage germination of seeds of the radiate (◆) and non-radiate (+) morphs of <u>S. vulgaris</u>. (▼) marks sowing date and (*) where differences between morph means were significant (p<.05). Also presented are the weekly rainfall values and weekly mean maximum and minimum air temperatures recorded at St. Andrews throughout the experimental period.

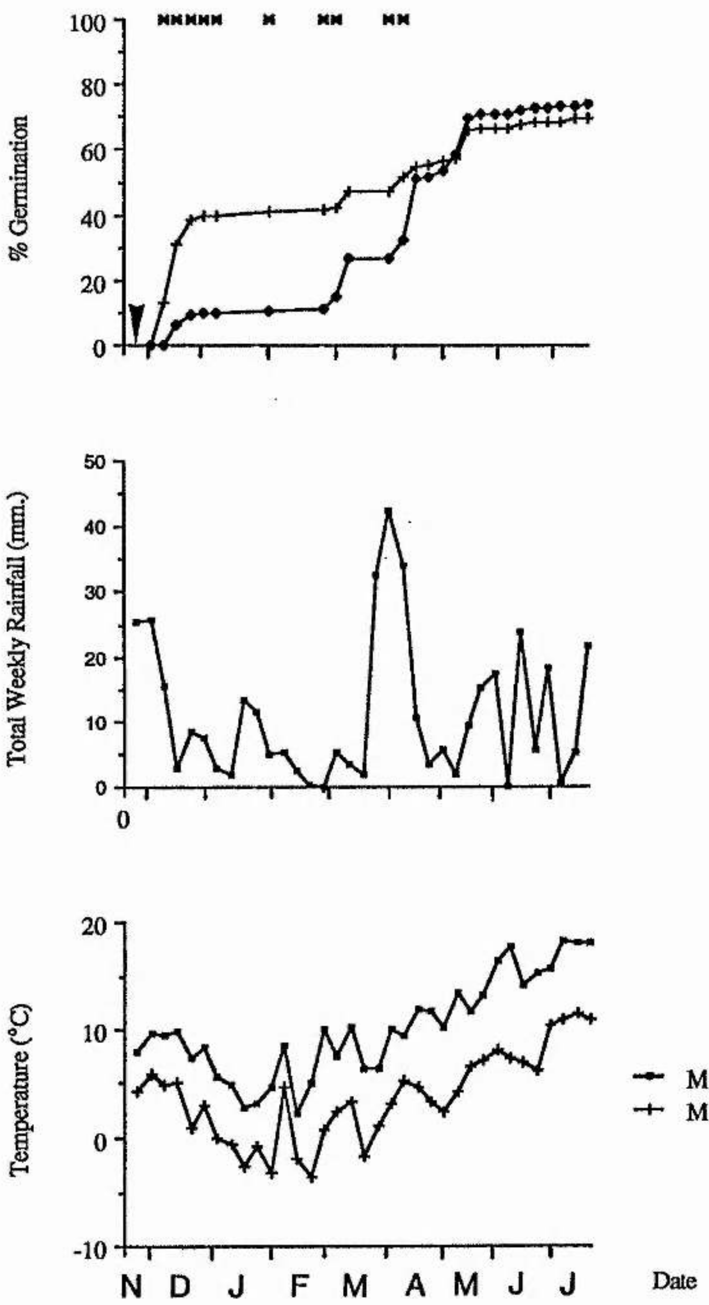


Figure V.3 RBGE Autumn 1984: Cumulative percentage germination of seeds of the radiate (◆) and non-radiate (+) morphs of *S. vulgaris*. (▼) marks sowing date and (*) where differences between morph means were significant ($p<.05$). Also presented are the weekly rainfall values and weekly mean maximum and minimum air temperatures recorded at RBGE throughout the experimental period.

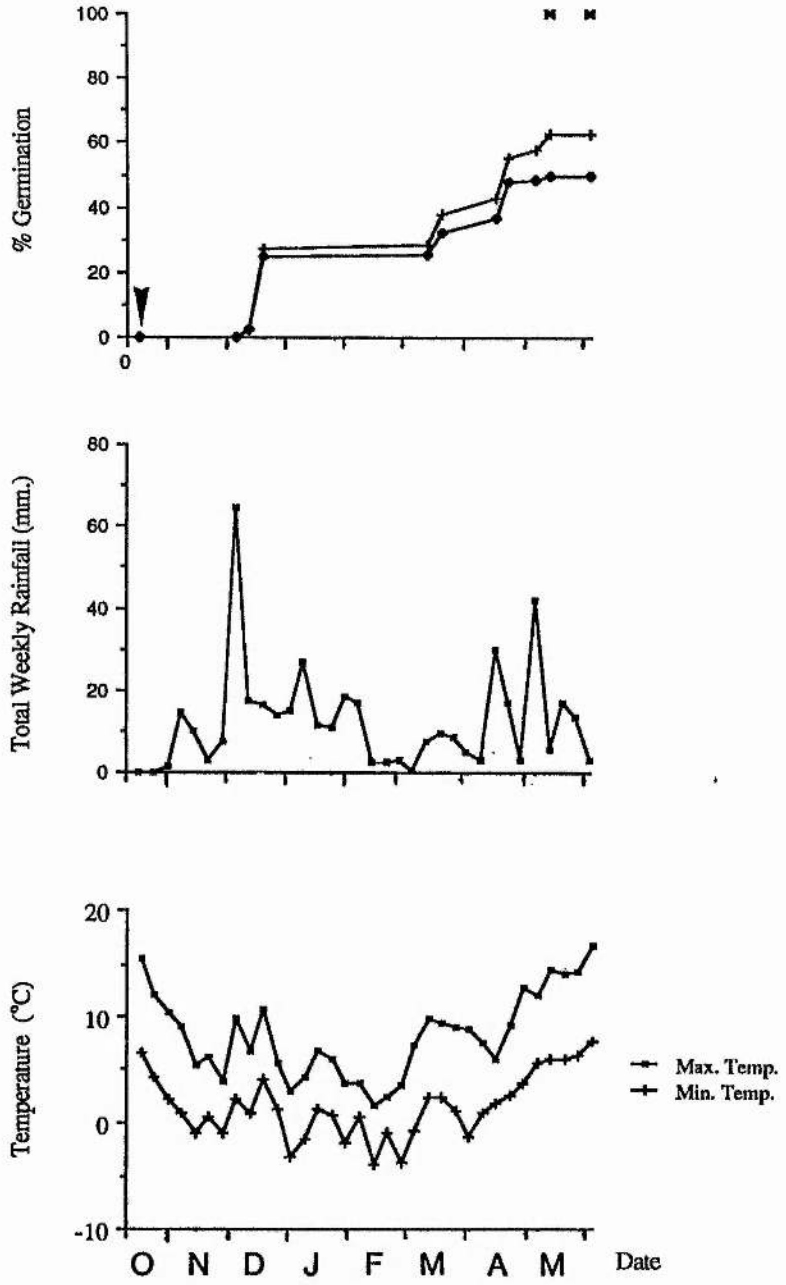


Figure V.4 St. Andrews Autumn 1985: Cumulative percentage germination of seeds of the radiate (◆) and non-radiate (+) morphs of S. vulgaris. (▼) marks sowing date and (*) where differences between morph means were significant ($p<.05$). Also presented are the weekly rainfall values and weekly mean maximum and minimum air temperatures recorded at St. Andrews throughout the experimental period.

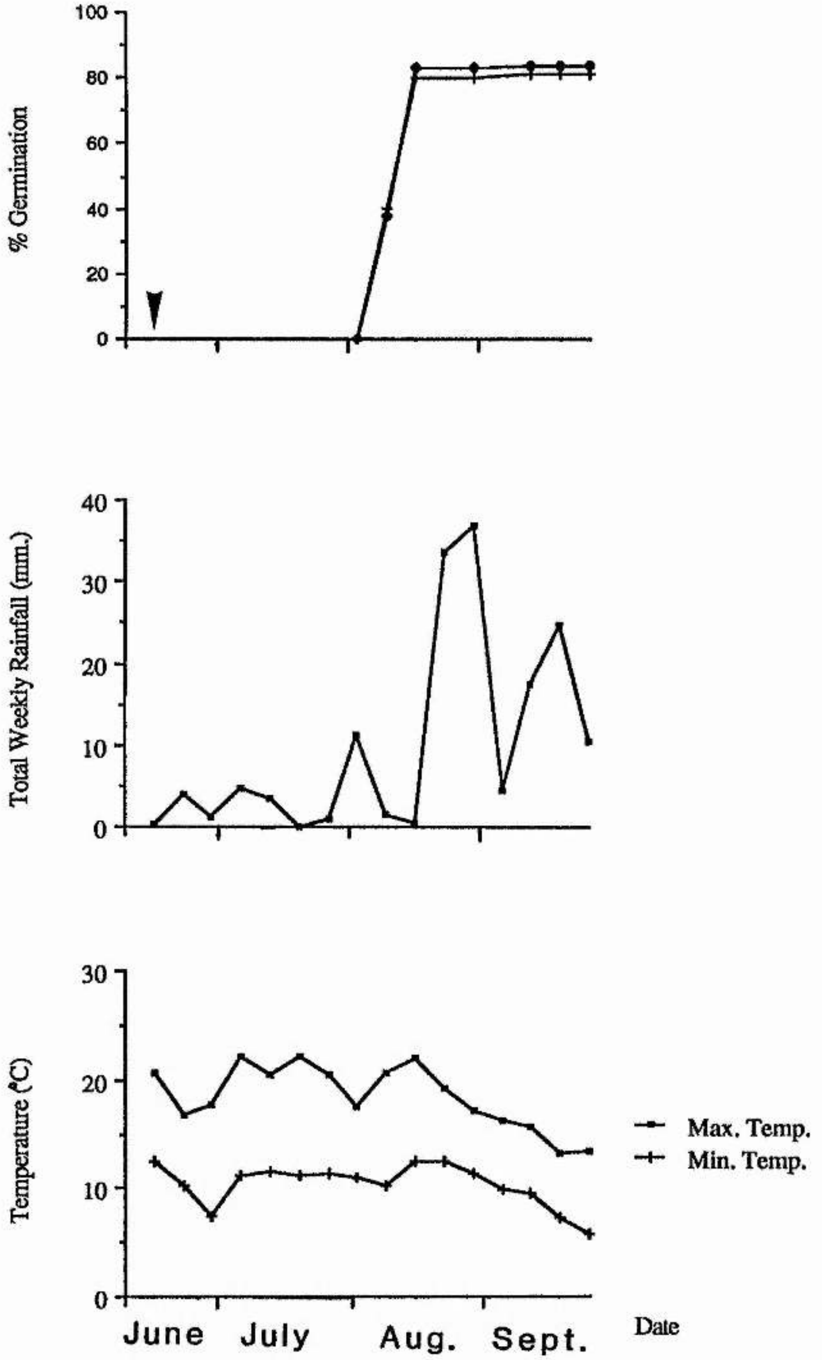


Figure V.5 RBGE Spring 1984: Cumulative percentage germination of seeds of the radiate (◆) and non-radiate (+) morphs of S. vulgaris. (▼) marks sowing date and (*) where differences between morph means were significant ($p<.05$). Also presented are the weekly rainfall values and weekly mean maximum and minimum air temperatures recorded at RBGE throughout the experimental period.

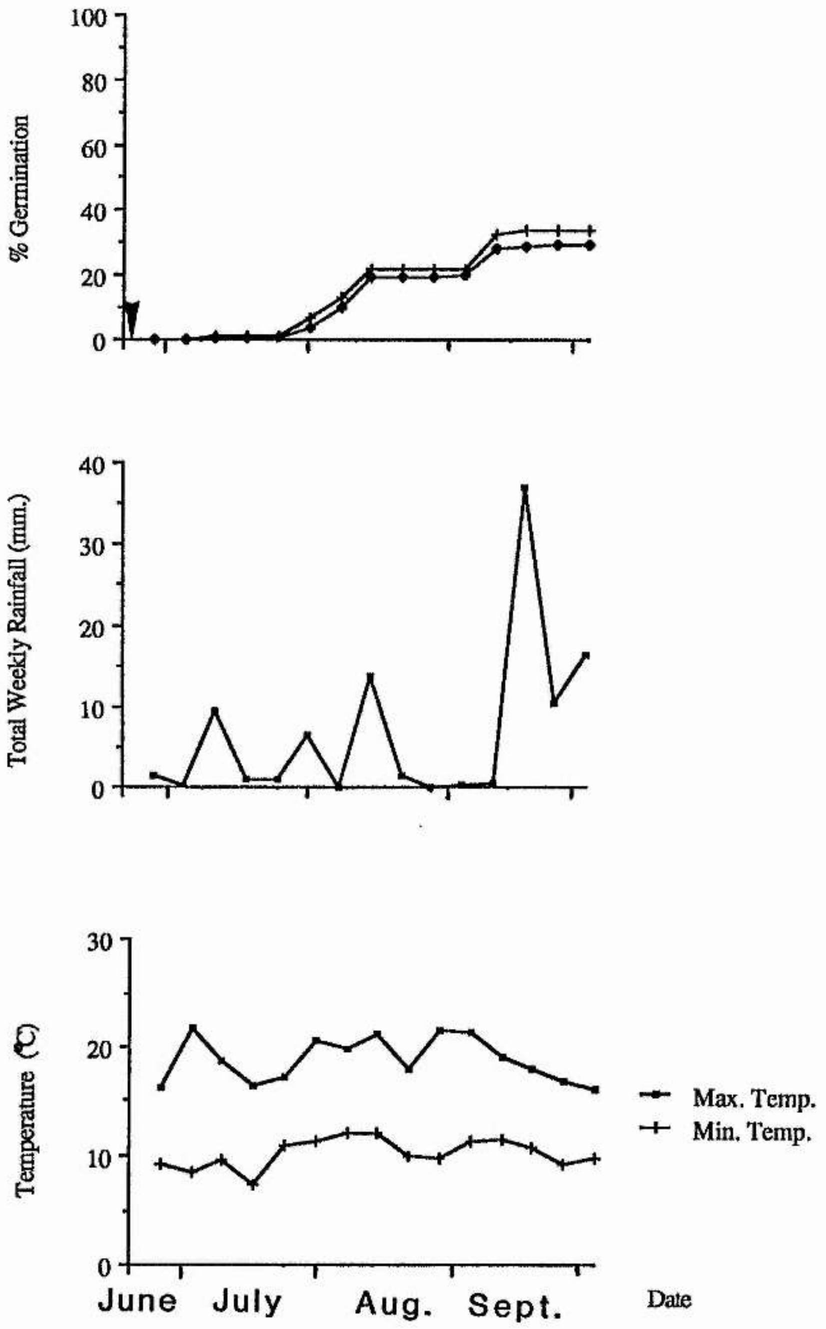


Figure V.6 St. Andrews Spring 1984: Cumulative percentage germination of seeds of the radiate (◆) and non-radiate (+) morphs of *S. vulgaris*. (▼) marks sowing date and (*) where differences between morph means were significant (p<.05). Also presented are the weekly rainfall values and weekly mean maximum and minimum air temperatures recorded at St. Andrews throughout the experimental period.

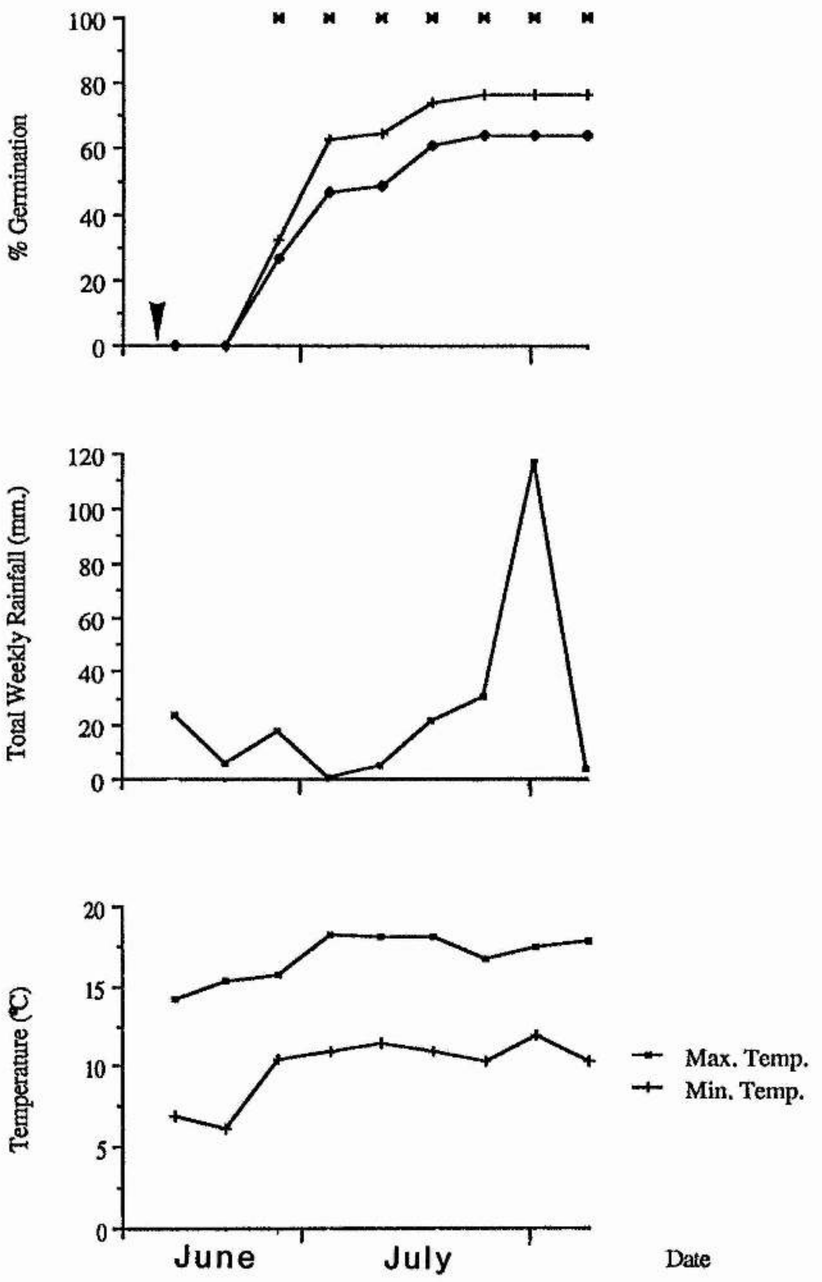


Figure V.7 RBGE Spring 1985: Cumulative percentage germination of seeds of the radiate (◆) and non-radiate (+) morphs of <u>S. vulgaris</u>. (▼) marks sowing date and (*) where differences between morph means were significant (p<.05). Also presented are the weekly rainfall values and weekly mean maximum and minimum air temperatures recorded at RBGE throughout the experimental period.

further germination occurred up to the time the experiment was terminated 16 weeks from sowing. In contrast, at St. Andrews two flushes of germination were recorded after two periods of substantial rainfall (Fig V.6). In each flush both morphs exhibited equivalent patterns of germination. However, throughout the experiment cumulative percentage germination remained low rising to only 30-35% by the time the study was terminated 15 weeks from sowing.

In the spring sown 1985 study at RBGE (Fig V.7), significantly greater numbers of seed of the non-radiate morph germinated in the initial flush after sowing. Germination started after a week in which 19 mm. of rain fell. Following a week when little germination occurred due to dry conditions, a further 20 mm. of rain triggered a second flush of germination in week 6 after sowing. In this flush, greater numbers of seed of the radiate morph than the non-radiate morph germinated. However, the cumulative percentage germination of the radiate morph (69%) remained significantly lower than that of the non-radiate morph (76%) by the time the experiment was terminated 9 weeks from sowing.

V.5 - DISCUSSION -

The results of the studies reported in this chapter demonstrate that when a difference between morphs in germination behaviour occurs, fresh seed of the non-radiate morph germinates more readily during the first flush of germination that follows sowing. This finding is consistent with the results of previous studies on the germination behaviour of the two morphs of S. vulgaris (Richards, 1975; Noble, 1984; Abbott, 1986). Despite the tendency for a difference, both morphs on occasion exhibit a similar pattern of germination over the initial period. The presence or absence of a difference is likely to depend on: (i) the season and conditions under which seeds were produced; and (ii) the conditions at the site where comparisons were made. In a previous study of non-radiate seeds of S. vulgaris produced during the summer months, Popay and Roberts (1970b) found that dormancy of fresh seed varied according to the date of collection. In another study, Roberts and Feast (1973) found that after sowing seed in the field in late July, the emergence of non-radiate seedlings before the end of December of the same year, varied greatly between years. In the present study the differences recorded between sites for germination behaviour in both the autumn sown 1983 experiment (Figs. V.1 & V.2) and the spring sown 1984 experiment (Figs. V.5 & V.6) must have been due to differences in local conditions at the test sites as in each case the seed tested was from the same source.

For seeds sown in autumn, a long period of dormancy followed the initial flush of germination, after which further flushes of germination occurred in the spring. At the RBGE site many more seed of the radiate morph than the non-radiate morph germinated during these spring flushes so that by the time that the studies were terminated the early advantage of the non-radiate had been cancelled out and no difference was present between the two morphs in cumulative percentage germination. One possible cause of the disjunct flushes of germination which occurred in autumn and spring is the presence of a seed dormancy polymorphism (Harper, 1957) among seed produced by plants that reproduce in late summer and early autumn. Although such a polymorphism may be present in the seed of both morphs it would seem to be of a more extreme form in the radiate morph which appears to produce a greater proportion of dormant seed that overwinters before germinating in the spring.

Based on the results of the autumn sown experiments at RBGE, fresh seeds of the non-radiate morph produced and dispersed in late summer/early autumn tend to exhibit a pattern of germination which is skewed toward early germination, whereas fresh autumn sown seeds of the radiate morph tend to exhibit a germination pattern skewed towards late germination. Although such results were not found in the autumn sown experiments conducted at St. Andrews it is instructive that they did occur at the site (in Edinburgh) close to where natural populations of S. vulgaris are polymorphic for capitulum type. This difference between morphs in germination behaviour may, therefore, have important consequences on the relative frequencies of the two morphs, as timing of germination can affect seedling survival and subsequent fecundity of survivors in populations (see Rathke and Lacey, 1985). Indeed, it is possible, that a difference between morphs in germination behaviour will have a direct effect on relative morph survivorship and fecundity and, in turn, the demographic genetics and maintenance of the ray floret polymorphism in Edinburgh populations. This possibility is investigated in the following chapter.

CHAPTER VI. DEMOGRAPHIC STUDIES II: FROM SEED TO SENESCENCE.

VI.1 - INTRODUCTION -

Field experiments initiated with even aged seedlings of the radiate and non-radiate morphs failed to reveal a mechanism by which the polymorphism for capitulum type in Edinburgh populations of S. vulgaris could be maintained (see Chapters II & III). However, these studies only examined the relative performance of plants from the seedling stage through to senescence and, therefore, did not take into account possible effects of morph differences in seed germination behaviour. Law and Watkinson (1987) have stated that conclusions based on experiments on even aged stands may be of limited value if differences between species/morphs occur with respect to germination behaviour. In this context, therefore, it is of interest that a comparison of germination behaviour between the radiate and non-radiate morphs of S. vulgaris (Chapter V) clearly demonstrated that a difference frequently occurs. Results showed that autumn sown non-radiate seeds will often exhibit a germination pattern skewed towards early germination, whereas the germination behaviour of radiate seeds may often be skewed towards late germination.

The importance of the timing of seed germination after dispersal on the subsequent survivorship and fecundity of seedlings has been recognised in many plant species (see Rathke and Lacey, 1985). Moreover, the timing of germination is especially critical in an annual colonising species such as S. vulgaris, as such species cannot afford to risk total reproductive failure (Harper, 1977). Studies with Papaver dubium (Arthur, Gale and Lawrence, 1973), Leavenworthia stylosa (Baskin and Baskin, 1972), and Lactuca serriola (Marks and Prince, 1981) have demonstrated that although early germinated seedlings encounter a greater risk of mortality compared to later germinated seedlings, they tend to capitalise on a longer growing season to produce plants of higher fecundity (see also Venable, 1984). This has led Rathke and Lacey (1985, p. 181) to suggest that for many species "

...time of germination reflects a trade-off between selection for high seed set and selection for high survivorship". It is feasible, therefore, that the difference between morphs in germination behaviour, may have a significant effect on the demographic genetics of the radiate and non-radiate morphs, which, in turn, could be of considerable importance in the maintenance of the polymorphism for capitulum type in Edinburgh populations.

To examine the effects of a difference in germination behaviour between morphs on relative morph fitness in S. vulgaris, two experiments were initiated in the nursery area of the Royal Botanic Gardens in Edinburgh in autumn, 1984, and spring, 1985. Within each experiment plants of each morph were raised in 50:50 mixtures from seed to senescence. A comparison between "early" and "late" sown plots was also made in each experiment to determine the effect of sowing date within season on germination behaviour and subsequent survival and fecundity.

VI.2 - MATERIALS AND METHODS -

Prior to sowing, seeds were collected from radiate and non-radiate plants raised under the conditions previously described in Chapter II section 2. After collection and removal of pappus, seeds were sown onto plots that contained an upper layer of sterilised top-soil to a depth of 10cms. In Autumn 1984 at the Royal Botanic gardens, Edinburgh, a series of "early sown plots" were sown between 7-11.11.84 with a second series of "late sown plots" being sown two weeks later between 21-24.11.84. This experimental proceedure was repeated the following spring (1985) with early sown plots sown between 9-11.6.85 and late sown plots between 23-25.6.85. In each experiment comparisons were made between morphs raised in a mixed stand in which both morphs were sown in a 1:1 ratio. In each plot 200 seeds of each morph were sown individually at points of a 20 x 20 grid design. seeds were sown at 1cm intervals in a checkerboard design with radiate and non-radiate seeds placed in alternate positions. The positions of seeds of each morph within a plot were marked with colour coded cocktail sticks. Replication in each experiment for each sowing date was eight fold. Following germination emerged seedlings were protected from molluscan herbivores by the use of "Draza" slug pellets.

After sowing, plots were monitored on a weekly basis to assess seed germination and subsequent mortality throughout the seedling, rosette and flowering stages. A further record was taken of the number of plants to set seed per plot, and the number of capitula produced per plant (i.e after all plants in a plot had finished fruiting). Ovule and seed number per capitulum were also recorded. This entailed sampling a single capitulum from each plant as plants initiated seed production. The capitula were teased open using fine forceps and the number of filled seed recorded. The number of ovules per capitulum and ovule fertility were calculated as given in Chapter III section 2. An estimate of the total number of seeds produced per plant was obtained by multiplying capitulum number per plant by the number of seeds per capitulum.

Analysis of data

Differences between morphs for seed germination, survivorship and fecundity were tested using paired t-tests on plot means. Prior to analysis all data were tested for 'normality' as described in Chapter II section 3.

VI.3 - RESULTS -

VI.3.1 Autumn 1984 Experiment

Seed Germination

In both the early and late sown plots a significantly greater number of non-radiate compared to radiate seeds germinated in the initial flush of germination following sowing (Figs VI.1a & VI.1b, Table VI.1). After this first flush of germination, which lasted for 4-5 weeks, no further seed

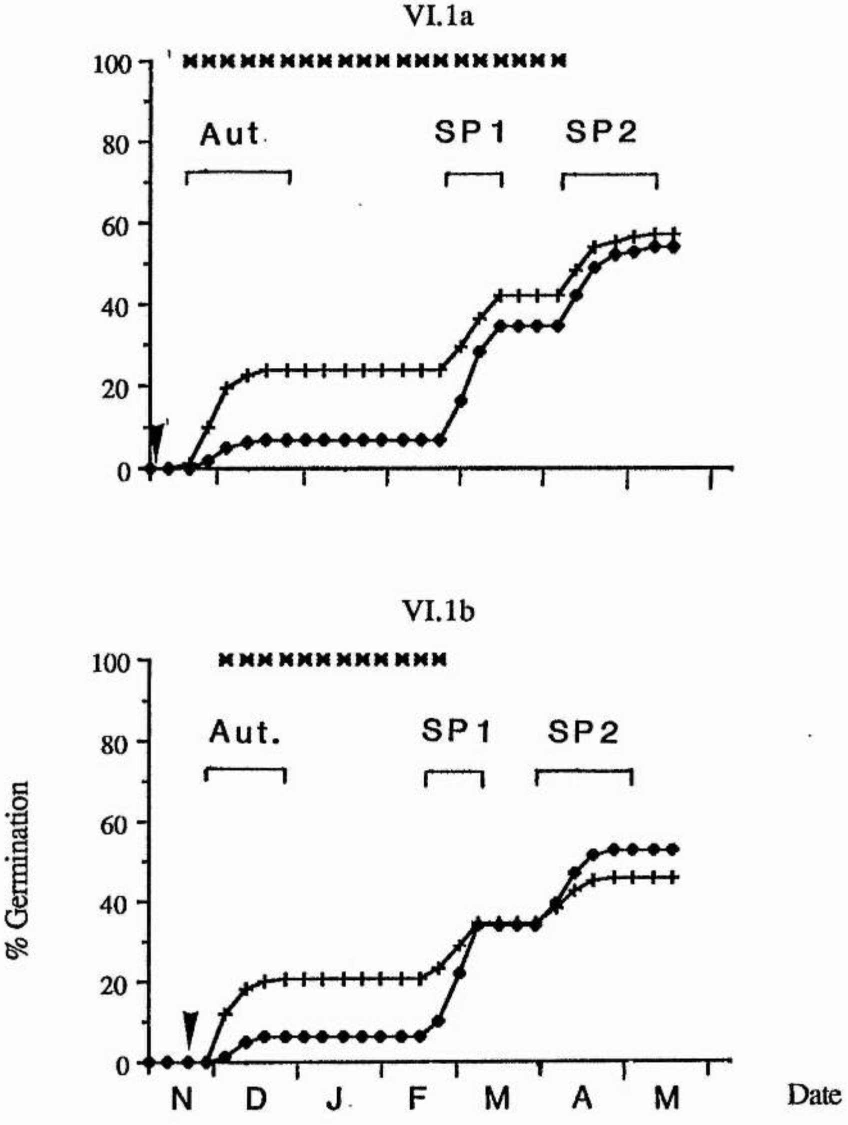


Figure VI.1 Autumn 1984: Cumulative percentage germination of seeds of the radiate (◆) and non-radiate (+) morphs of S. vulgaris in a) the early and b) the late sown plots. (▼) marks the sowing date and (*) where differences between means were significant (p<.05).

germination occurred between early December through to late February. However, two further flushes of seed germination occurred in spring 1985. The first flush occurred in early spring (late February-mid March), while the second occurred in late spring/early summer (late April-early May). In each of the spring flushes a significantly greater number of radiate compared to non-radiate seed germinated. Hence, when the experiment was terminated there was little difference between morphs for cumulative percentage germination in either early or late sown plots.

As a result of the three distinct flushes of seed germination, three distinct phases of plant recruitment (cohorts) were incorporated into the experimental populations. For ease of presentation and interpretation these three cohorts have been named the autumn cohort, the early spring cohort (SP1), and the late spring cohort (SP2).

Survivorship

After germination, individuals within each cohort were subject to varying levels of mortality before fruiting (Tables VI.1a,b). In the early sown plots (Table VI.1a) mortality was high in the autumn cohort, particularly for radiate plants. As a result of the greater germination and survivorship of non-radiate individuals, many more non-radiate than radiate plants survived to fruit. Considerable mortality also occurred within the spring cohorts. However, in each of these cohorts survivorship of the radiate morph was greater than that of the non-radiate morph and as a consequence of this, plus the fact that a greater number of radiate seed had germinated in these cohorts, many more radiate than non-radiate plants survived to fruit. When the data were summed over all cohorts, it was apparent that in the early sown plots the total number of radiate plants per plot to have survived and fruited was greater than that for non-radiate plants although the difference was not judged as significant.

Table VI.1 Autumn 1984: Mean number of seeds to germinate, number of plants to fruit, mean percentage of seedlings (seedl) to fruit (Fr), mean percentage of seedlings to form rosettes (Ros) and mean percentage of rosettes to flower for Autumn, Spring I and Spring II cohort plants of the radiate (R) and non-radiate (N) morphs.

a) Early Sown Plots.

	Autumn			Spring I			Spring II			Total		
Character	R	N	p	R	N	p	R	N	p	R	N	p
No. to germ.	14.2	48.4	***	55.0	36.0	***	37.4	30.0	***	107	114	n.s
No. to fruit	3.6	18.2	***	25.5	12.0	***	14.0	5.9	***	43.1	36.1	n.s
% Seedl-Fr.	25.7	37.3	n.s	70.6	56.3	*	47.2	32.0	n.s	53.8	39.6	n.s
% Seedl-Ros	32.5	42.8	n.s	89.0	76.7	*	75.6	71.3	n.s	74.1	56.3	* *
% Ros-Fruit	81.2	86.6	n.s	78.6	75.2	n.s	60.0	45.7	*	72.0	71.0	n.s

b) Late Sown Plots.

	Autumn			Spring I			Spring II			Total		
Character	R	N	p	R	N	p	R	N	p	R	N	p
No. to germ.	12.6	42.0	***	55.0	26.6	***	38.0	23.0	***	106	91.2	n.s
No.to fruit	2.4	5.4	*	48.9	22.4	***	20.4	12.5	* *	71.0	40.2	***
% Seedl-Fruit	18.6	13.7	n.s	88.9	83.7	n.s	54.7	53.3	n.s	68.0	44.4	***
% Seedl-Ros	19.5	15.5	n.s	95.3	94.5	n.s	64.1	61.5	n.s	74.8	50.9	***
% Ros-Fruit	97.0	92.5	n.s	93.2	88.7	n.s	84.8	88.6	n.s	90.8	87.1	n.s

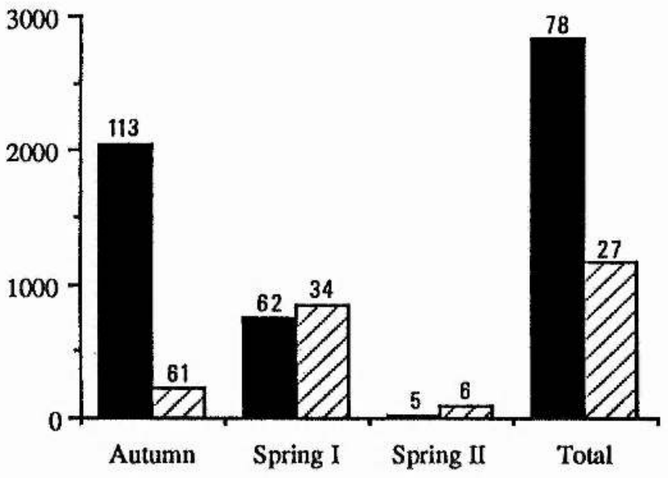
In the late sown plots (Table VI.1b) very few plants of either morph survived to flower in the autumn cohort and mortality of the non-radiate morph was particularly severe. In contrast, most individuals of both morphs survived to fruit in the early spring (SP1) cohort while survivorship in the late spring cohort (SP2) was approximately 50%. Taken over all cohorts, many more radiate than non-radiate plants fruited per plot and the difference between totals was highly significant.

Fecundity

Measures of morph fecundity in each cohort are presented in terms of mean capitulum number per plant and per plot (Fig. VI.2, Table VI.2a,b). Estimates of seed and ovule production per capitulum were made without distinguishing between cohorts. However, an analysis of the relationship between capitulum number per plant and seed number per capitulum showed that the two characters were not correlated (Radiate, $r= 0.37$, $p>.05$; Non-radiate, $r= 0.24$, $p>.05$) providing strong evidence that seed number per capitulum did not vary between cohorts. Because cohorts were not distinguished, values for seed per capitulum, per plant and per plot (Table VI.3a,b) are presented only for the total number of plants to fruit in each plot.

In the early sown plots the non-radiate morph produced many more capitula per plot than the radiate morph (Fig. VI.2). This large and highly significant difference stemmed mainly from the great difference in relative performance between morphs in the autumn cohort. Plants of this cohort that survived the winter developed into large plants which produced large numbers of capitula. As most survivors were non-radiate, (Table VI.Ia) and because non-radiate survivors produced more capitula per plant than radiate survivors, there was a great difference in capitulum production per plot between morphs in the autumn cohort. In contrast, in the early spring (SP1) cohort there was little difference between the two morphs in capitulum production per plot (Fig. VI.2) due to the greater

a) Early Sown Plots



b) Late Sown Plots

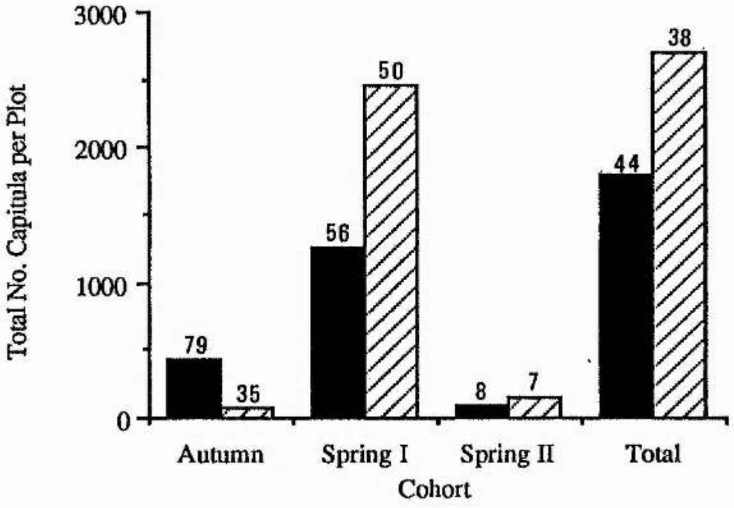


Figure VI.2 Autumn 1984: Total number of capitula produced per plot with contributions of autumn, spring I, spring II cohort radiate (▨) and non-radiate (■) plants in the (a) early sown and (b) late sown plots. Numbers at the head of each column represent the mean capitulum number per plant.

Table VI.2 Autumn 1984: Mean number of ovules and seeds per capitulum, seeds produced per plant and total seed production per plot for plants of the radiate (R) and non-radiate (N) morphs raised in (a) the early and (b) the late sown plots.

	a) Early Sown Plots.			b) Late Sown Plots.		
Character	R	N	p	R	N	p
Ovule number per capitulum	49.4	50.6	n.s	53.1	54.2	n.s
Seed number per capitulum	46.0	47.3	n.s	48.7	47.7	n.s
Seed number per plant	1238	3701	***	1826	2122	n.s
Seed total per plot	53620	134326	*	133027	87302	*
Relative morph fitness	0.4	1.0	-	1.0	0.66	-

numbers of radiate plants in the cohort (Table VI.1a) which offset the higher capitulum production per non-radiate individual. Only in the late spring (SP2) cohort which contained more radiate than non-radiate individuals, and in which there was no difference between morphs in the number of capitula per plant, was capitulum production per plot greater for the radiate morph. The contribution of plants of this late spring cohort to the total number of capitula produced per plot, however, was so small that it hardly affected the large advantage of the non-radiate morph which had been established in the autumn cohort.

Turning to the late sown plots, a very different set of results was recorded (Fig. VI.2). In the autumn cohort, very few seedlings that germinated survived to fruit and, as a result, contributed little to the total capitula production of each morph in these plots. This had the effect of improving greatly the ultimate performance of the radiate morph. The early spring seedlings (SP1) developed into the most successful plants. As most of these were radiate, and also, because in this cohort there was no real difference in mean capitulum number between morphs, the capitulum production per plot of the radiate morph was considerably greater. Examination of the total capitulum production per plot of each morph shows that in the late sown plots, in marked contrast with what had been recorded in the early sown plots, the radiate morph produced a far greater number of capitula.

Examination of mean seed numbers per capitulum in both early and late sown plots (Table VI.2b) revealed no significant differences between morphs. Morph differences in seed production per plant and per plot (Table VI.2b) were, therefore, determined mainly by the number of capitula produced per plant and the number of plants to fruit in each plot. Consequently differences in total seed production per plot paralleled those for capitulum production illustrated in Fig. VI.2.

Table VI.3 Autumn 1984: Pooled results over all cohorts and plots in the early and late sown plots.

Character	Early sown plots.		Late sown plots.	
	R	N	R	N
Total seed sown	1600	1600	1600	1600
Total germinated	653	711	849	740
Total survivors	335	289	574	323
Total capitula no.	9286	22618	21524	14327
Capitulum no. /survivor	26.9	78.3	37.5	44.5
Seed no. /capitulum	46.0	47.3	48.7	47.7
Total seed no.	428941	1083402	1048219	683398
Relative fitness	0.39	1.0	1.0	0.65

A summary of the results of the experiment in which data have been pooled over all cohorts and plots is presented in Table VI.3. It is evident that the relative fitnesses of the two morphs differ markedly in the early and late sown plots with the non-radiate morph holding a significant advantage in the early plots, but suffering a marked disadvantage in tha late sown plots.

VI.3.2 Spring 1985

Heavy rain washed away considerable quantities of soil and seeds from the late sown plots which were therefore abandoned during the course of the experiment. Consequently only the results obtained from the early sown plots are presented in this section.

Seed Germination

Following sowing, large numbers of seed of both the radiate and non-radiate morphs germinated rapidly over a three week period (mid June-early July), after which little germination of seed of either morph occurred (Fig VI.3). In this one flush of germination there was little difference between morphs for cumulative percentage germination with 69.5% of radiate and 71% of non-radiate seeds germinating respectively. As only one flush of germination was recorded, all plants in the plots are considered as part of a single cohort and results are presented accordingly.

Survivorship and Fecundity

Although there was little difference between morphs in the number of seed to germinate, many more non-radiate than radiate plants survived to the fruiting stage (Table VI.4). In marked contrast to what had been found in the Autumn 1984 experiment, very little mortality occurred between the seedling to rosette stages of the life cycle. Instead, nearly all the mortality occurred between the rosette to fruiting stages. In addition to

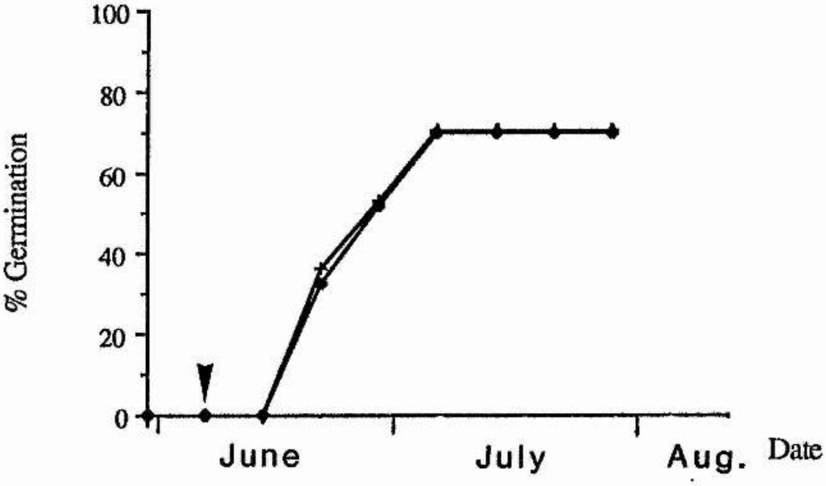


Figure VI.3 Spring 1985: Cumulative percentage germination of seeds of the radiate (◆) and non-radiate (+) morphs of <u>S. vulgaris</u>. (▼) marks the sowing date and (*) where differences between means were significant (p<.05).

Table V1.4 Spring 1985: Mean number of seeds to germinate, number of plants to fruit, percentage of seedlings to flower, percentage of seedlings to form rosettes and percentage of rosettes to flower for each morph.

Character	Radiate	Non-radiate	*p*
No. Seed to germinate	139	141	n.s
No. plants to fruit	45.5	57.4	*
% Seedlings-flower	32.7	40.2	*
% Seedlings-rosette	97.4	97.9	n.s
% Rosettes-flower	33.8	41.4	*

its greater survivorship, the non-radiate morph also produced a significantly greater number of capitula per plant. Thus the net effect of these two advantages of the non-radiate morph was that its reproductive output in terms of capitulum production per plot, was more than twice that of the radiate morph (Table VI.5). As both morphs produced the same number of seed per capitulum (Table VI.5), morph differences in reproductive output were not altered when transformed into seed output.

Again it is of interest to examine a summary of the results after data are pooled over all plots (Table VI.6). Based on the total number of seed produced by non-radiate and radiate plants, the fitness of the non-radiate morph is nearly twice that of the radiate morph. The considerable advantage held by the non-radiate morph in the experiment appeared to stem from its more rapid development following germination. Non-radiate plants tended to flower earlier than radiate plants due to rapid development from the rosette to flowering stage. When non-radiate plants reached the flowering stage, rosettes of the radiate morph remained at a lower level in the plots and are likely to have suffered from reduced light which, in turn, may have further affected their ablility to survive and reduced capitulum production during flowering.

VI.4 - DISCUSSION -

A major finding to emerge from the results reported in this chapter was the clear effect that sowing date had on the relative fitnesses of the two morphs in the autumn 1984 study. In the late sown plots the fitness of the radiate morph was considerably greater than that of the non-radiate morph. This was a notable finding in that it represents the only occasion that the radiate morph has exhibited a greater fitness than the non-radiate morph in all the experiments that have been reported in this thesis. Though this result is of interest its importance must be viewed in relation to the findings obtained from the early sown plots of the autumn 1984

Table VI.5 Spring 1985: Mean number of seeds and ovules per capitulum, capitula per plant and per plot, seeds per plant, total seed produced per plot and relative fitness of each morph.

	Radiate	Non-radiate	p
Ovules/capitulum	47.6	48.1	n.s
Seed/capitulum	44.4	44.8	n.s
Capitula/plant	9.0	12.8	* *
Capitula/plot	395	682	* *
Seeds/plant	391	546	*
Total seed/plot	18648	50714	* *
Relative Fitness	0.37	1.0	-

Table VI.6 Spring 1985: Results pooled over all plots recorded in the spring sown experiment.

Character	Radiate	Non-radiate
Total seed sown	1600	1600
Total seed germinated	1113	1131
Total number survivors	364	459
Total no. of capitula	3160	5454
No. capitula /survivor	8.7	11.9
Seed no. per capitulum	44.4	44.8
Seed total per plot	136166	229962
Relative fitness	0.59	1.0

experiment and also those of the spring 1985 experiment in which the non-radiate morph exhibited a considerably greater fitness than the radiate morph. These results are, of course, consistent with those of experiments reported previously in this thesis (see Chapters II-IV) plus those of other studies that have investigated fitness differences between morphs in material derived from polymorphic Edinburgh populations (Abbott, 1986; Marshall and Abbott, 1987).

Variation in relative morph fitness with sowing date in the autumn study was a direct consequence of the different germination behaviours of the two morphs plus the effect of winter mortality. As was the case in the germination studies reported in Chapter V., seeds of the non-radiate morph tended to exhibit a pattern skewed toward early germination, that is before the onset of winter. This resulted in a greater risk of seedling mortality; however, those that survived developed into large flowering plants of high individual fecundity. In contrast, radiate seed tended to exhibit a pattern skewed toward late germination with most seed germinating after winter had passed. This resulted in the survival of a large number of radiate flowering plants which exhibited lower individual fecundity compared to autumn germinated plants. The effect of early and late germination on survival and fecundity reported in this study parallels the pattern found in other annuals (see Ross and Harper, 1972) and is also similar in kind to those found in colonising populations of the perennials Plantago coronopus (Waite, 1980; Hutchings and Waite, 1985) and Agrostis curtisii (Gray, 1988).

Although winter mortality was high in both early and late sown plots of the Autumn experiment, in the early plots a sufficient number of non-radiate plants survived in the autumn cohort to develop into highly fecund plants which, together, contributed disproportionately to the total capitulum and seed production in these plots (Fig. VI.2, Table VI.2a). In contrast, in the late sown plots, increased winter mortality considerably reduced the number of early germinated non-radiate individuals in the autumn cohort and consequently greatly reduced the contribution of these plants to the total reproductive output in the plots. In the late sown plots, therefore, by far the greatest contribution to reproductive output was made by the relatively large number of radiate plants derived from seed which germinated during the early spring (SP1) flush (Fig. VI.2, Table VI.2b).

From the results obtained in the early and late sown plots of the autumn 1984 study, a possible mechanism for the maintenance of the ray floret polymorphism in Edinburgh populations may be inferred. Results of this autumn study, plus those of the demographic studies reported previously in this thesis (see Chapters II & III), have demonstrated that winter mortality of autumn recruited plants can be extremely high. As it is frequent for greater numbers of non-radiate plants to be recruited into populations in autumn, severe winter mortality may often significantly reduce the fitness of this morph. Such a situation occurred in the late sown plots in which the relative fitness of the non-radiate morph by the end of the experiment was estimated as only two thirds that of the radiate morph. Should such a difference in fitness commonly occur between morphs, it would be sufficient to enable the radiate morph to overcome the inherent cost of outcrossing which it incurs in natural populations (Marshall and Abbott; 1987). On the other hand, during mild winters survivorship of autumn germinated plants would be much improved and under such circumstances a situation similar to that which occurred in the early sown plots would prevail. Under these conditions the fitness of the non-radiate morph would be greater than that of the radiate morph due to the greater fecundity of the early germinated non-radiate individuals.

In conclusion, the results of the autumn sown study have revealed a possible mechanism by which the inherent cost of outcrossing incurred by the radiate morph in Edinburgh populations polymorphic for capitulum type may be overcome in certain seasons and in certain years. It is unfortunate that the effect of sowing date on the fitness of each morph in the spring sown experiment could not be examined as the late sown plots were destroyed by flooding. Finally, it needs pointing out that although the studies reported in this chapter have investigated the physical factors which may affect a polymorphic population over a single generation, no attempt was made to examine the effect of biotic factors. It is feasible that the differential effects of pathogen and/or pest attack and herbivory on morphs may also cause differences in the relative fitnesses of the radiate and non-radiate morphs. Pathogen attacks by the orange rust, *Puccinia lagenophora*, (Paul and Ayres, 1986a & b) and the powdery mildew, *Erysiphe fischeri* (Ben-Kalio and Clarke, 1979; Harry and Clarke, 1986) have been shown to cause a reduction in the survivorship and fecundity of plants of the non-radiate morph of *S. vulgaris*. A greater resistance of radiate plants to attack by these pathogens would enhance the fitness of the radiate compared to the non-radiate morph. Indeed, on this point, it is noteworthy that Campbell (pers. comm.) has recently found that the radiate morph is less susceptible than the non-radiate morph to infection by *E. fischeri*. It is clear that to gain a full understanding of the maintenance of the ray floret polymorphism in Edinburgh populations, all aspects of the biology of the two morphs, including the effects of pathogens and herbivores require further investigation.

CHAPTER VII GERMINATION BEHAVIOUR OF THE RADIATE AND NON-RADIATE MORPHS OF S. VULGARIS

THE ROLE OF TEMPERATURE IN THE CONTROL OF GERMINATION

VII.1 - INTRODUCTION -

The initiation of seed germination in plants is triggered by a remarkable diversity of environmental factors which include temperature, moisture, light intensity, light quality, photoperiod, gas levels (e.g carbon dioxide), and minerals (Williams and Harper, 1965; Koller, 1972; Heydecker, 1973; Angevine and Chabot, 1979; Mayer, 1980/81; Karssen, 1982). In a recent review of the germination requirements of a large number of plant species Baskin and Baskin (1988) concluded that: "Temperature, through its influence on dormancy and germination, is the primary environmental factor regulating germination, and light and soil moisture are of secondary importance". A previous study of the effect of temperature on the germination behaviour of radiate and non-radiate S. vulgaris by Abbott (now published in Abbott, Horrill and Noble, 1988), revealed that for seed collected from an Edinburgh population in Spring 1982 (aged one month before testing) the cumulative percentage germination of the non-radiate morph was significantly greater than that of the radiate morph over all temperatures investigated, and at lower temperatures the difference between morphs was enhanced.

Field tests (see Chapter V), have since revealed that the number of non-radiate and radiate seeds to germinate in the initial flush of germination after sowing varies according to the date of seed production and the site at which tests are carried out. This observed variation in seed dormancy during the initial period after sowing may be due to one or any combination of three types of seed dormancy classified by Harper (1977) as innate, induced and enforced dormancy. Innate or primary seed dormancy prevents germination during seed development and ripening on the parent plant, and, also, for some time after dispersal even in periods when conditions are favourable for germination. Induced or secondary dormancy is acquired after dispersal if the seed is unable to germinate, e.g through burial, and then acts to prevent germination at a later stage when conditions become more favourable (see Karssen, 1980/81a). Seeds with enforced or imposed dormancy fail to germinate due to the presence of unfavourable conditions after dispersal, e.g. low temperatures, but will germinate as soon as conditions become favourable again. The persistence of dormancy following exposure to conditions favourable for germination distinguishes induced seed dormancy from enforced dormancy (Roberts, 1972).

To determine which dormancy mechanisms was/were responsible for the seed dormancy observed in the field trials reported in Chapter V, fresh seeds of both morphs (collected from the same population at the same date as seed used in the field trials) were sown at a range of constant temperatures in a controlled environment immediately after the initiation of a field experiment. If greater numbers of seeds of a morph germinate at any temperature under controlled conditions compared to in the field, this would indicate that failure of seeds of that morph to germinate in the field was due to enforced dormancy. If, however, seed germination is similar under controlled conditions to that in the field, innate and/or induced dormancy would be the cause of the dormancy exhibited in the field.

VII.2 - MATERIALS -

Seeds of both morphs were collected from pure stands of plants raised on the experimental plot at St. Andrews Botanic Garden in the manner described in Chapter II section 2. All germination tests were conducted using a thermogradient bar similar in design to that described by Grime (1982).

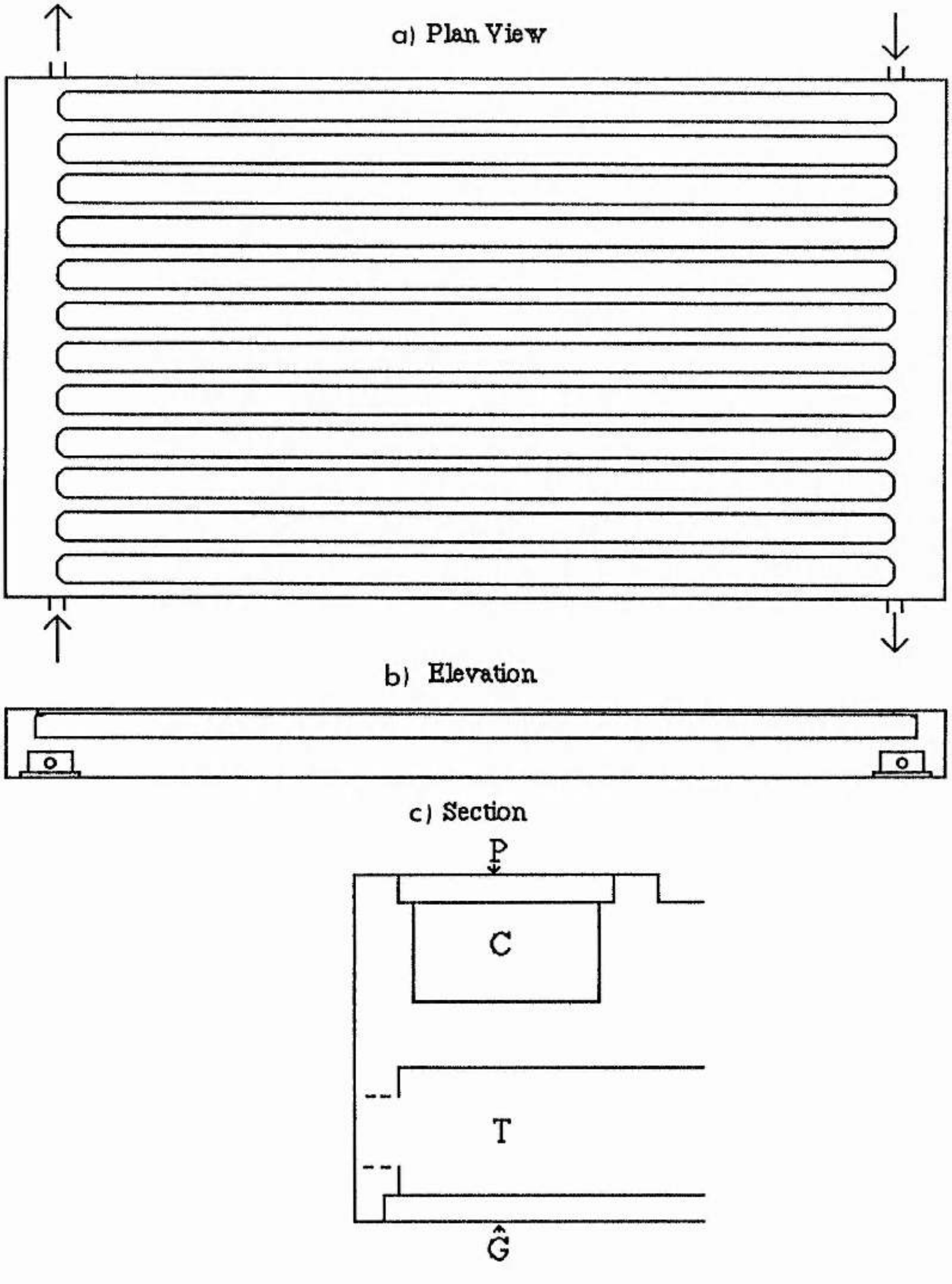


Figure VII.1 Diagram of thermogradient bar. (a) Plan showing route of circulating water (arrows). (b) Side view showing route of air through germination chambers. (c) Section of thermogradient bar. C, Channels to contain seeds cut in upper surface, covered by perspex lid, P. T, water-conducting channels cut in lower surface and sealed with sheet aluminium screwed onto rubber gaskets (G).

The bar was subdivided into twelve germination chambers which were constructed by cutting parallel channels (C) each 83 cm. long, 2.6 cm. wide and 1.7 cm. deep, into the upper surface of a solid block of aluminium of dimensions 91.4 cm. x 45.7 cm. x 5.8 cm. (Fig VII.1). Around the edge of each chamber a 3mm. rebate was cut for the location of a perspex lid (P). On the underside of the block and at right angles to the chambers were cut two channels (T), each 43cm. x 5cm.x 2.5 cm. positioned 5cm. from the respective ends of the block. Holes were drilled at each end of these channels and fitted with connectors for attaching hoses. The channels were covered by 45 cm. x 7 cm. lengths of aluminium sheet screwed down firmly onto rubber gaskets. These channels, therefore, were formed into water-tight compartments which acted as heating/cooling jackets for the thermogradient bar. To facilitate the monitoring of the end temperatures of the bar, four holes (H) were drilled 3cm. from the top and 2cm. from the ends of the bar for the placement of rod thermistors. Holes were also drilled at each end of the perspex lids so that air could be pumped through each germination chamber at intervals throughout the duration of the experiment.

The thermogradient bar was supported within an angle iron frame and all exposed surfaces were insulated with 6cm. thick expanded polystyrene. Heating/cooling was achieved using two Grant FH 15-A flow heaters and a Grant FC 25 flow cooler. Water flowed to and from the bar in lagged pipes. Temperatures were recorded using a 9 channel, intermittent, Grant (D89-U) chart recorder. The end temperatures of the bar were monitored using rod thermistors, and disc thermistors were employed for the germination chamber temperatures. Lighting was supplied by 6 x 40 watt Thorn "warm white" fluorescent tubes via a water/glass infra-red filter.

VII.3 - METHODS -

Calibration

Calibration of the thermogradient bar was conducted with end temperatures of 6 °C and 33 °C. Seven disc thermistors were placed at known distances along the length of the bar which was subjected to a 16 hour photoperiod with air pumped through the germination chambers for 1 hour twice daily. Temperatures along the bar were recorded once every hour over a 48 hour period. The temperature gradient along the bar was found to be linear (Fig. VII.2) with only a slight fluctuation across the cold end of the bar (±1/2 C). As a consequence of this fluctuation, each channel was calibrated individually before experiments were conducted.

Experimental procedure

A single layer of 3 mm. diameter polypropylene granules was placed at the bottom of each chamber and covered with distilled water. A strip of chromatography paper was placed on top of the granules onto which batches of seed were sown. For each experiment batches of 50 seed of each morph were sown along the length of the bar in positions chosen to represent precise constant temperatures. Experiments were conducted on seed of both morphs collected during October 1984 (at temperatures of 8, 12, 17, 21, 25, and 29 °C), June 1985 (at temperatures of 5, 9, 15, 18, 21, 25, and 28 °C), and October 1985 (at temperatures of 1, 3, 6, 9, 12, 15, 18, and 21 °C). In each experiment replication was sixfold. After sowing, seeds that germinated were recorded and removed at daily intervals. Germination was defined as the emergence of approximately 1 mm. of radicle through the seed coat.

In each experiment the significance of a difference between morphs in cumulative germination percentage was determined by a t-test after transformation of percentages into angles using an arcsin transformation.

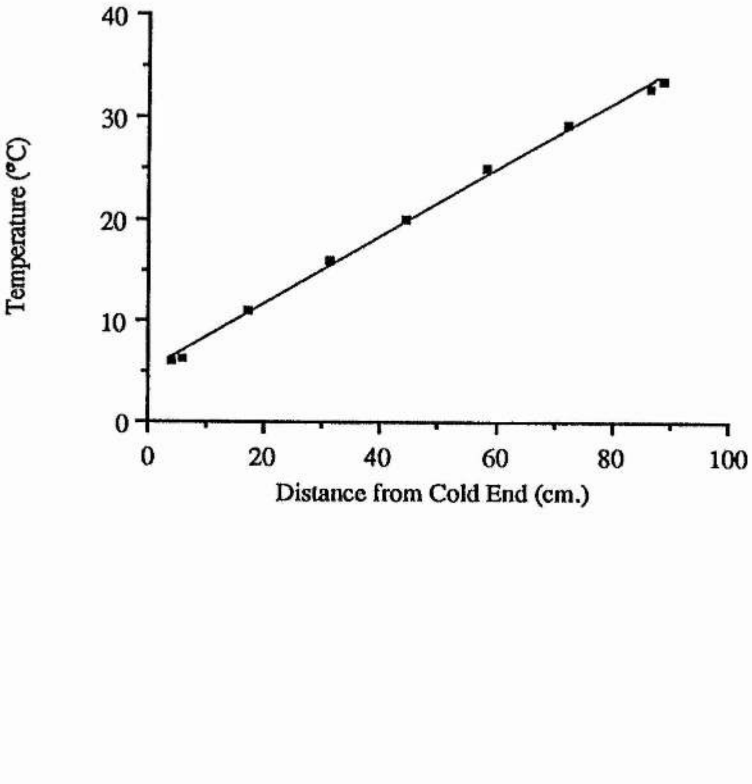


Figure VII.2 Calibration of thermogradient bar showing increasing temperature with increasing distance from the cold end of the bar.

VII.4 - RESULTS -

The major point of interest to emerge from the results was that differences between morphs in germination behaviour varied according to temperature. For seed collected and tested in October 1984 and June 1985 significantly greater numbers of seed of the radiate than the non-radiate morph germinated at a temperature of 25 °C; whereas at temperatures below 12 °C more non-radiate seed than radiate seed germinated (Figs. VII.3 & VII.4). In contrast, for seed collected and tested in October 1985, there was little difference between morphs with regard to the final cumulative percentage germination of seed at all temperatures (Fig. VII.5). However, there was a tendency for radiate seed to germinate faster than non-radiate seed at high temperatures (12 °C - 21 °C), while non-radiate seed germinated faster than radiate seed at low temperatures (1 °C - 9 °C).

At temperatures of 28 C and above very few seed of either morph germinated. In contrast, both morphs exhibited good germination at temperatures as low as 1 C (Fig. VII.5). The latter result was surprising in view of the lack of germination in the field at low temperatures (see Chapter V.

- DISCUSSION -

The results of the studies reported in this chapter indicate that the failure of seeds to germinate during the initial flush of germination that followed sowing in field tests (Chapter V) was primarily due to the effects of enforced dormancy. Enforced dormancy in seeds of each morph can be overcome by exposure to favourable temperatures. The temperatures at which enforced dormancy is broken varies between morphs. Seeds of the radiate morph tend to germinate more readily at higher temperatures; whereas seeds of the non-radiate morph germinate more frequently at lower temperatures. Reduced germination of radiate seed at low temperatures was also reported by Abbott (see Abbott, Horrill, and Noble, 1988). The difference between

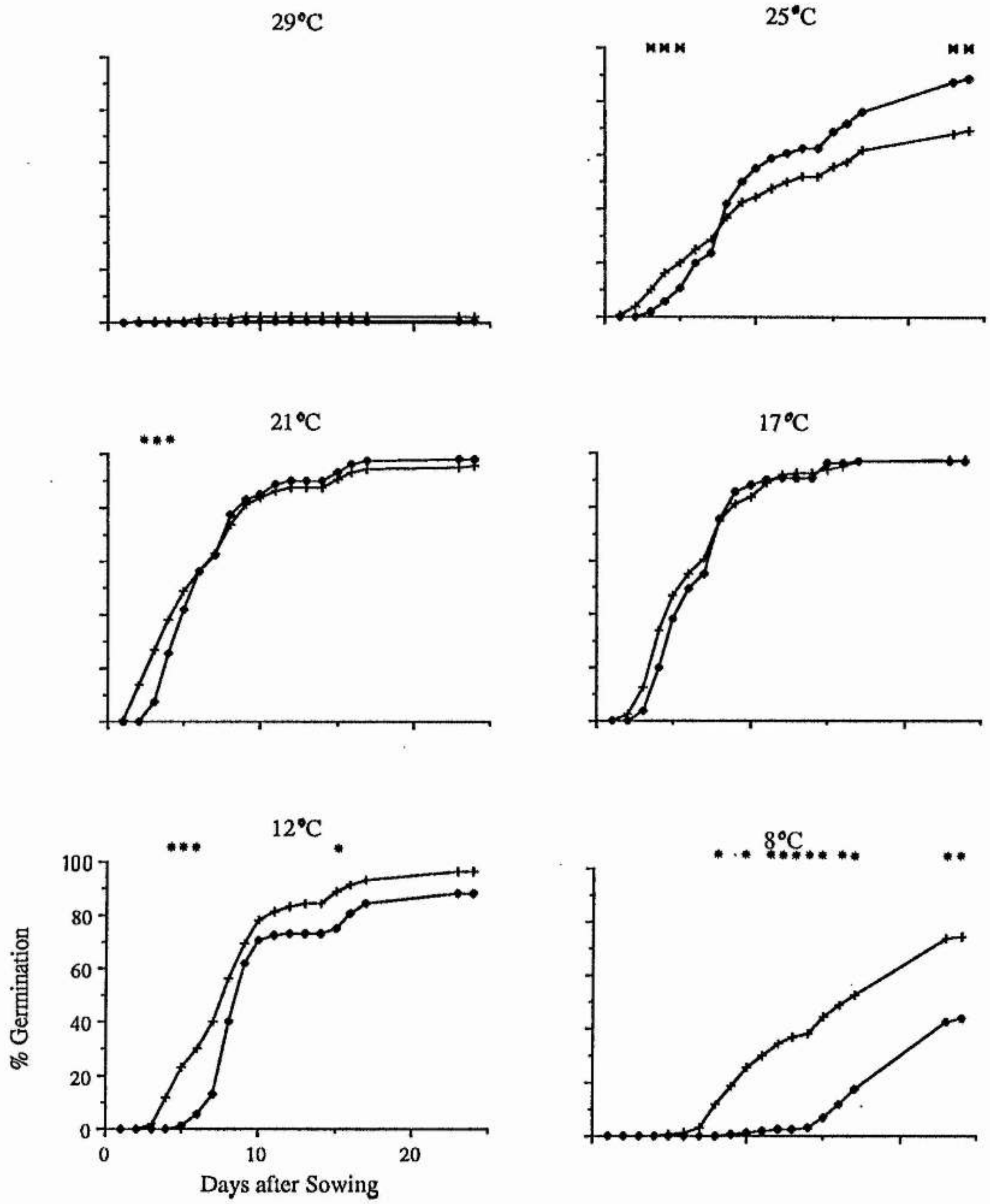


Figure VII.3 Seed Collected October 1984: Cumulative percentage germination of seeds of the radiate (◆) and non-radiate (+) morphs of S. vulgaris at 29 °C, 25 °C, 21 °C, 17 °C, 12 °C, and 8 °C. (*) marks where differences between morph means were significant (p<.05).

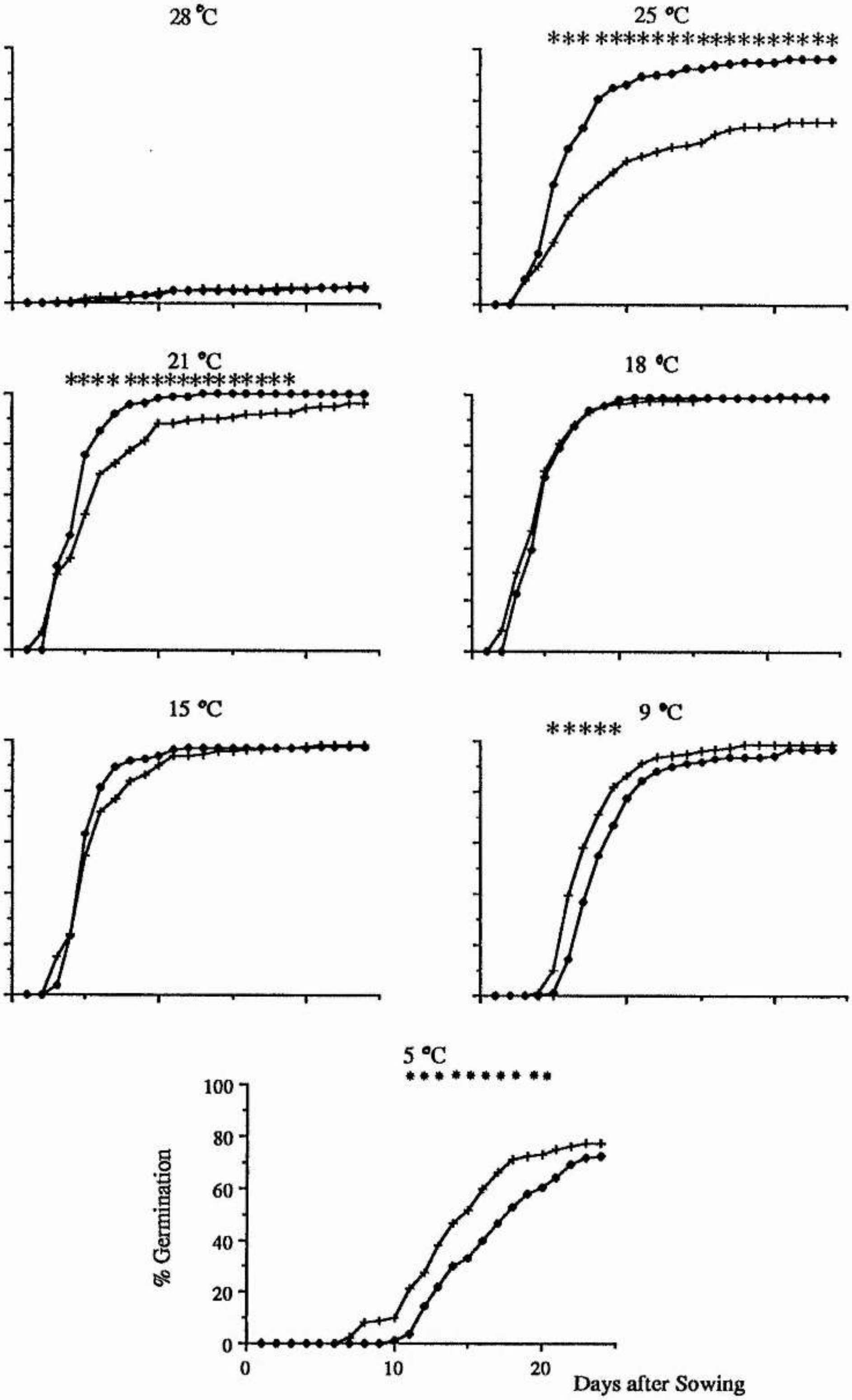


Figure VII.4 Seed Collected June 1985: Cumulative percentage germination of seeds of the radiate (◆) and non-radiate (+) morphs of S. vulgaris at 28 °C, 25 °C, 21 °C, 18 °C, 15 °C, 9 °C, and 5 °C. (*) marks where differences between morph means were significant (p<.05).

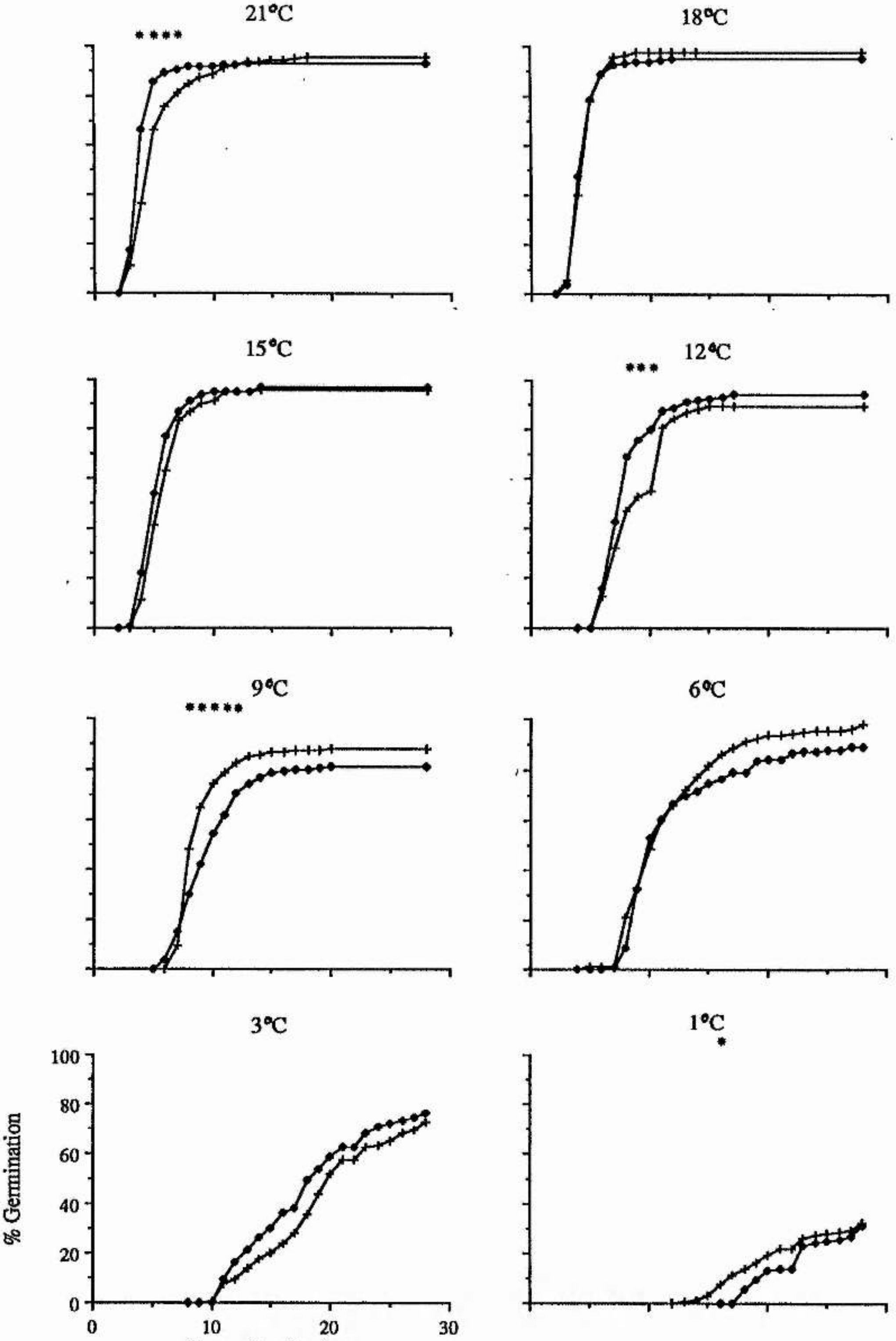


Figure VII.5 Seed Collected October 1985: Cumulative percentage germination of seeds of the radiate (◆) and non-radiate (+) morphs of S. vulgaris at 21 °C, 18 °C, 15 °C, 12 °C, 9 °C, 6 °C, 3 °C, and 1°C. (*) marks where differences between morph means were significant ($p<.05$).

morphs in the temperatures required to overcome enforced dormancy appears to be influenced by the conditions experienced by seeds during ripening on the mother plant. This would explain the finding that seeds of the radiate morph collected in October 1984 exhibited considerably poorer germination at low temperatures (below 12 °C) than did seed collected in October 1985.

There seem to be two possible explanations for the differences between morphs in germination behaviour with respect to temperature. First, if the radiate morph arose through introgression with Senecio squalidus (see Chapter I. section 4), then the difference may be due to a pleiotropic effect of the ray floret gene or to genes introgressed along with the ray floret allele. Alternatively, the difference between morphs may be the product of selection. Currently there is no evidence that enables us to distinguish between these two possibilities. Clearly, the cause of the difference between morphs in temperature requirements for germination is an aspect requiring further investigation.

CHAPTER VIII - DISCUSSION -

The aim of the project reported in this thesis was to investigate possible factors which could favour the radiate morph of Senecio vulgaris and hence maintain the polymorphism for capitulum type and associated outcrossing rate in Edinburgh populations of the species. Although much importance has been attached to the role of inbreeding depression in the maintenance of outcrossing polymorphisms (Charlesworth and Charlesworth, 1978; Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987), the results of the analysis presented in Chapter IV indicated that inbreeding depression is not an important factor in the maintenance of the polymorphism for capitulum type in S. vulgaris. It was also clear that, despite extensive experimentation, no short term advantage of the radiate over the non-radiate morph was evident either in pure stand or mixture when plants were raised from seedlings of equivalent age (Chapters II & III). Indeed, in these studies it was the non-radiate morph which showed the greater fitness through either increased viability (Chapter II) or both increased viabiltiy and fecundity (Chapters II & III). However, as mentioned previously, results gained from trials using even aged stands may be of limited value if differences occur between species/morphs in germination behaviour (Law and Watkinson, 1987). It was of interest, therefore, that a comparison of the germination behaviours of the two morphs (Chapter V) clearly showed that differences may be of frequent occurrence in seed sown in autumn, and that under certain conditions these differences can lead to the radiate morph attaining a greater fitness than that of the non-radiate morph (Chapter VI).

In an r-selected species such as S. vulgaris which colonises disturbed habitats where population crashes are likely, random processes are expected to be of great importance in determining morph frequencies in populations. Under such conditions it is to be expected that populations will often become fixed for one or the other of the two morphs. As yet, however, no population of S. vulgaris which is monomorphic for the radiate allele has been reported in the wild. This may indicate that genetic drift is not as important in affecting the maintenance of the ray floret polymorphism as may have been anticipated. Alternatively, since the non-radiate morph of S. vulgaris is extremely common and produces large numbers of seeds, it is feasible that it colonises monomorphic populations of radiate groundsel so rapidly that such populations are never in fact observed.

The frequent occurrence of dramatic falls in population size coupled with the difference between morphs for germination behaviour should often act to maintain the polymorphism for capitulum type in Edinburgh populations. In autumn, the majority of seeds of the non-radiate morph tend to germinate during the initial flush of germination which follows dispersal, whereas seeds of the radiate morph tend to exhibit a germination pattern skewed toward late germination (i.e. spring). This later germination of radiate seeds will favour the radiate over the non-radiate morph when levels of winter mortality are high (Chapter VI). Thus, if a population crash occurs during winter because of heavy mortality, then, due to the greater numbers of radiate seed present in the seed bank, greater numbers of radiate compared to non-radiate seedlings will emerge in the subsequent flush of germination. This will increase the relative frequency of the radiate allele in the population. However, if no further disturbance occurs during subsequent generations then the non-radiate allele will increase in frequency through the greater viability and fecundity of non-radiate plants (Chapters II & III) plus the advantage which stems from greater selfing. The increase in frequency of the non-radiate allele will continue until the next population crash after which the cycle would be repeated. The role of this mechanism in the maintenance of the polymorphism for capitulum type is clearly dependent on reductions in population size occurring on a regular but not continuous basis. In view of the likely importance of differences between morphs in seed germination behaviour on relative morph fitness, it is of interest that Abbott (1986) has reported that differences between morphs in seed germination occur in all populations which he examined. Thus, it is feasible that this particular difference in the biology of the two morphs will have an important effect on the maintenance of the capitulum polymorphism in all polymorphic British populations, not just those located in Edinburgh.

Considerable emphasis has so far been placed on the possible effects of the difference between morphs in germination behaviour. It is of interest, therefore, to reiterate the possible reasons as to why the morphs sometimes differ in their germination behaviour. The results of Chapter VII showed that temperature is a major factor controlling the initial dormancy of seeds of both morphs after sowing. Seeds of the radiate morph tend to germinate more readily at higher temperatures, whereas seeds of the non-radiate morph germinate more frequently at lower temperatures. As pointed out previously, differences between morphs in their respective germination behaviour may be due to either a pleiotropic effect of the ray floret allele or to genes introgressed along with the ray floret allele, and/or, to the effect of selection. With S. vulgaris it is difficult to discriminate between the relative importance of these two effects as selection may have accentuated a difference between morphs originally caused by the ray floret gene or genes introgressed along with it.

In conclusion, it needs pointing out that there are two major problems in trying to identify the factors which favour the evolution and maintenance of the polymorphism for capitulum type and associated outcrossing rate in natural populations of S. vulgaris. Firstly, plant populations are dynamic (Harper, 1977) but the investigations undertaken in this thesis were conducted over a relatively short time period in evolutionary terms and, therefore, can not accurately assess the effects of small differences in fitness between morphs which can accrue over longer periods of time. Secondly, the polymorphism is of recent origin and it is not yet known whether it is still in the transient phase or has reached equilibrium. If a considerable amount of gene exchange has occurred between the two morphs since the origin of the polymorphism, the factors originally favouring the radiate morph may not be present in modern populations and the radiate morph may now be slowly heading towards extinction. It would seem, therefore, that to gain an accurate picture of the factors that have favoured increased outcrossing in the past, it might be necessary to re-synthesise radiate plants from native non-radiate populations which have never been subject to introgressed genes from S. squalidus. A long term investigation aimed at comparing the demography of the newly synthesised plants and their non-radiate parents in the field over many years would likely yield important information on the factors responsible for the evolution of the polymorphism for capitulum type and associated outcrossing rate in S. vulgaris.

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