

Volume 1 of 2

The ecology of homostyle and heterostyle primroses

Primula vulgaris Huds.

by

Mark Allen Boyd BSc(Hons) Loughborough

Submitted for the degree of PhD

on 17th December 1986

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ABSTRACT

Genetic theory has predicted the spread of homostyly through heterostyle populations but this has not happened over the last 40 years . This thesis compares the ecology of the three primrose morphs (pin, thrum, homostyle) to determine whether ecological differences between the morphs may limit the spread of homostyles.

Mean individual seed weight decreased with the number of seeds per capsule. Heterostyle seed production was pollinator limited and consequently heterostyles tended to produce larger seeds than homostyles. Seed size appeared to be more important than the phenotype (morph) of the mother in determining germination success. Larger seeds germinated better and may survive better in the soil.

Survival of seedlings in experimental populations and of adults in wild populations was higher for homostyles than for heterostyles with thrums faring worst.

Adult survival was dependent on the number of flowers produced in the preceding flowering season. Survival data are presented as life tables.

A matrix model of population growth is presented showing that homostyles would be expected to increase relative to heterostyles on purely ecological grounds. However, a sensitivity analysis shows that the spread of homostyles may be limited by seed survival in the soil.

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CHAPTER 1

INTRODUCTION

The function and evolution of heterostyly has long been of interest. Darwin (1877) reviewed the subject and was the first to consider its occurrence in the Primulaceae as an outcrossing mechanism. This view of heterostyly has remained throughout the last century with the possible exception of Charnov (1982) who believes that heterostyly can be regarded as a form of sexual dimorphism. There has also been much interest in the different breeding systems found throughout the plant kingdom (Charnov, 1982). However, there have been few studies which consider the implications of the different systems for the ecology of the plants.

Heterostyle species provide an opportunity for the study of the ecology of breeding systems by comparing the ecology of the various morphs. Primroses, *Primula vulgaris* Huds., are particularly suited to the study of this problem.

The common primrose, *Primula vulgaris* Huds., is a rosette-forming perennial in the family Primulaceae. Many species in the genus *Primula* show distyly and an incompatibility system restricting legitimate pollen flow to that between morphs. The morphs and legitimate pollinations of the primrose are shown in Figure 1.1 and the primrose incompatibility system is summarised in Table 1.1.

In most populations there are only the two heterostyle morphs which are readily distinguishable in the field when in flower. The pin form has the stigma extending to the top of

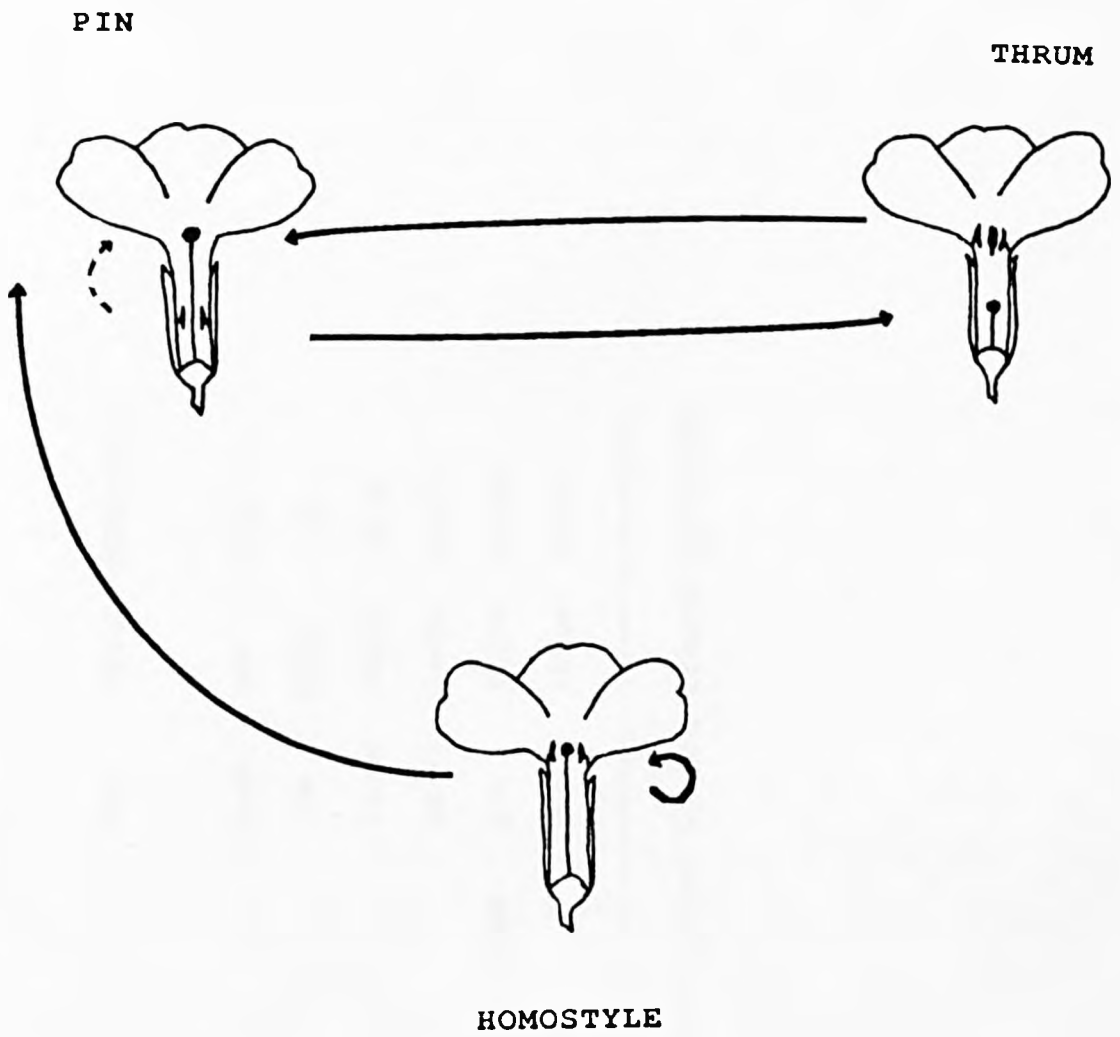


Figure 1.1 Cross-sectional views of the flower types of the three primrose morphs. The arrows show the direction of legitimate pollen flow

	PIN	THRUM	HOMOSTYLE
Position of stigma	high	low	high
Position of anthers	low	high	high
Size of stigmatic papillae	large	small	large
Size of pollen grains	small	large	small
Incompatibility reaction of pollen tube	pin	thrum	thrum
Incompatibility reaction of style	pin	thrum	thrum

Table 1.1. The incompatibility system of the common primrose
modified from Lewis, 1949.

the corolla tube or even beyond it whilst its anthers are at the bottom of the corolla tube. In the thrum form the androecium and gynaecium are in the reverse positions. The corolla tube opening of the thrum morph may be slightly wider than that of pins but this difference is not necessarily consistent (Bell, 1902; Christy, 1922). There are no vegetative markers by which the morphs may be separated when they are not in flower.

In two areas of Britain, a third morph occurs which combines the male characters of the thrum flower with the female characters of the pin flower. This morph is known as the 'long homostyle' or simply the homostyle and is self-fertile (Crosby, 1949). It is generally believed that heterostyle species evolved from monomorphic ancestors and so it is of interest to compare the ecology of the homostyle morph with that of the two heterostyle morphs. On simple genetic grounds, a homostyle morph which is self-fertile and also capable of outcrossing should rapidly spread to fixation in heterostyle populations. Crosby (1940, 1949, 1960) considered the different frequencies of homostyles found in Somerset populations to indicate that homostyly had only recently evolved and that it was in the process of spreading through those populations at the expense of the two heterostyle morphs. This does not appear to have happened during the last 40 years (Curtis and Curtis, 1984a) raising the possibility that homostyles suffer some, as yet unknown, ecological disadvantage with respect to heterostyles.

This thesis represents the first attempts to analyse survival and reproductive differences between the three morphs from an ecological stand-point. The main questions

which will be considered are "are there ecological differences between primrose morphs ?" and "what implications does the ecology of the morphs have for the spread of homostyles through heterostyle populations?".

To answer these questions I compared the performance of the three morphs at different stages of the life cycle under natural conditions and in experiments. Figure 1.2 illustrates the life cycle and indicates the section of the thesis which deals with each stage.

Ecological differences between morphs might be expected to be most obvious during flowering when the morphs are readily discernable. However, mortality tends to be greatest when plants are at the earliest stages of growth and so the problem of determining the mortality of the morphs before they could be identified was tackled.

The aim of this thesis was to tie together any differences in the ecology of the three morphs into a population model based on their ecology rather than on population genetics as has been previously attempted (Crosby, 1949; Bodmer, 1960). In this way a measure of selection for the different primrose phenotypes could be constructed which would be of value both in ecology and in relation to the problems faced by population geneticists concerned with the spread of homostyly.

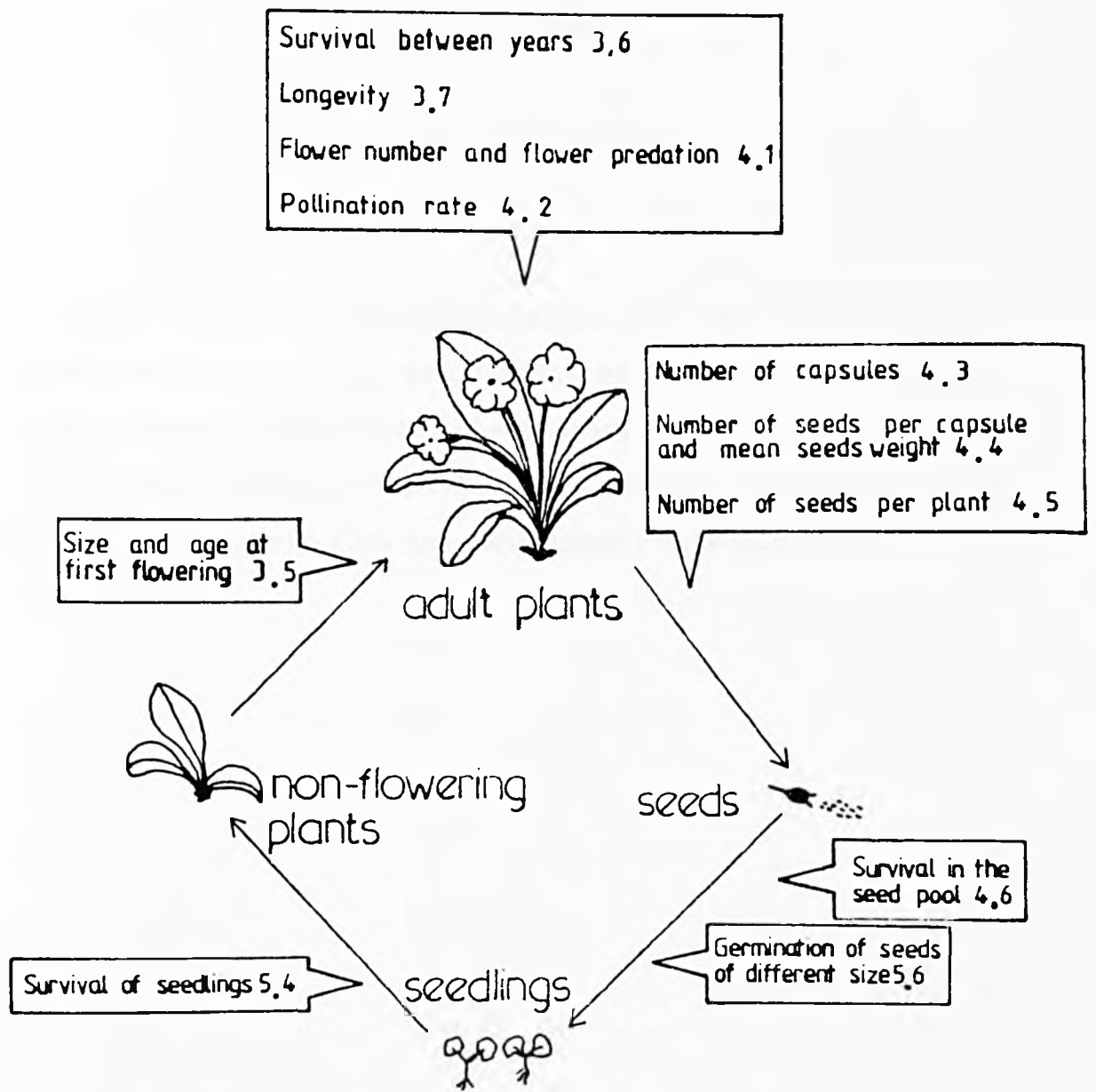


Figure 1.2 The life cycle of the primrose showing the stages where selection may occur and the sections in this thesis where they are discussed.

CHAPTER 2

LITERATURE REVIEW

This review will concentrate on the evolution of heterostyly and on genetical studies of homostyly, particularly in primroses. The history of primrose ecology is considered briefly but pollination biology is dealt with in chapter 4.2 along with the experiments on pollination.

THE EVOLUTION OF HETEROSTYLY. 2.1

Heterostyly is characterised by the existence in a population of two or more cross-compatible morphs which differ visually in the separation of the flower parts. Heterostyly is widespread and occurs in 23 families of angiosperms (Vuillemeir, 1967). In all but one of these families the morphological aspects of heterostyly are associated with a diallelic incompatibility system operating in one or more genes. Most of the 130 genera which display heterostylous species are comprised solely of distylous species (which have two morphs) and only 11 genera contain tristylous species (which have three morphs). With a distribution across so many angiosperm families it is apparent that heterostyly has evolved many times independently.

There have been many reviews which deal with the different aspects of the origin and evolution of heterostyly (Crowe, 1964; Vuillemeir, 1967; Jain, 1976; Weller, 1978; Charlesworth and Charlesworth, 1979; Ganders, 1979; Lloyd, 1979:). Tristyly and tristylous species have occupied a relatively small part of the literature and as they are not directly relevant to the current study they will be largely excluded from this review.

This section of the review can be broadly separated into the evolution of heterostyly in functional terms and the mechanism by which it developed. In addition I will consider the evolution of species from the heterostyle state and also the breakdown of heterostyle systems towards homostyly.

Heterostyly enforces outcrossing. This outcrossing mechanism is considered to be primarily the result of selection to promote pollen exchange (Darwin, 1877) or as the result of selection to reduce selfing (Charlesworth and Charlesworth, 1979). In both cases the assumption is that selfing lowers fitness because it increases the frequency of homozygotes of recessive deleterious genes.

The reported disadvantages of selfing are that if deleterious recessive genes accumulate they will be expressed more frequently under selfing (Muller, 1960). This has been demonstrated to occur in many species including humans (Neel and Schull, 1962). Selfing may also be disadvantageous in changing environments where the greater variability of outcrossed offspring may be advantageous (Tax and Callender, 1959; Fisher, 1930). Jain and Allard (1960) also suggest that there may be a heterozygote advantage over and above that resulting from the build up of deleterious recessive genes mentioned above. The frequency of heterozygotes is greater in an outcrossing system.

The variation in plant breeding systems which encompasses such extremes as dioecy and cleistogamy suggests that selfing is not a universally disadvantageous strategy. Baker (1955, 1967) has suggested that the prevalence of selfing and asexual species in many island floras is the result of the fact that establishment of a population from a single individual is only possible under these circumstances. Stebbins (1950) and Solbrig and Rollins (1977) have suggested that selfing species would be at an advantage when pollinators were scarce and outcrossing was not possible.

Stebbins (1950) also considered that selfing would be advantageous when the homozygous recessive state had highest fitness. He believed that selfing was favoured in weedy species as a result. Levin (1975) suggested that selfing could be advantageous if two or more closely related taxa lived in the same area and produced sterile hybrids. Wells (1979) presented a model showing that under some circumstances a gene for selfing would be expected to spread through a population if a selfing plant could transfer pollen to outcrossing individuals. This differs from the other advantages of selfing described above in that it showed that selfing could spread in situations other than when the alternatives were only asexual reproduction or extinction.

It has also been suggested that inbreeding depression of fitness through the accumulation of deleterious recessive genes is less important for species which regularly self (Mather, 1973) because deleterious genes are purged from the population through their expression in homozygous phenotypes. Conversely one might expect an outcrossing mechanism to be strengthened through time as the build up of deleterious recessive genes which are not expressed continues.

The heteromorphic incompatibility system of primroses described in Chapter 1 is typical of distylous species. Distyly is considered to have evolved relatively recently and is present in advanced taxa (Vuilleumeir, 1967). The precursors are generally believed to be monomorphic compatible species possibly with separated flower parts (Ganders, 1979).

The physiological and morphological elements in the

distylous system are generally believed to have evolved independently but some authors (Mather and de Winton, 1941) believed that they evolved simultaneously. This assumption was based on the belief that diallelic incompatibility and the separation of flower parts are always found together. This assumption is not, however, correct. Dulberger (1964) showed that the variations in style length in the genus *Narcissus* are not linked to the multiallelic incompatibility system also present in that genus.

Most authors believe that the physiological incompatibility system evolved prior to the morphological separation of flower parts (Darwin, 1877; Riley, 1936; Yeo, 1975). Species which only show a displacement of flower parts also show high levels of selfing (Yeo, 1975). Ornduff (1979) showed the pollen loads of primrose stigmas to be mainly of own-type pollen and concluded that heteromorphy as such is not a good outcrossing mechanism.

The nature of the physiological incompatibility system is largely unknown but is thought to be the result of either differential pollen tube growth, stylic inhibition of pollen tube growth or both (Lewis, 1949). There are several theories regarding the evolution of the physiological incompatibility barrier and these will now be discussed.

It is possible that diallelic incompatibility evolved through the degeneration of a multiallelic system (Crowe, 1964). All but two alleles would have to be lost and one of these two alleles would have to be dominant to the other. It is unlikely that this would occur with the regularity with which the classical diallelic system is found

for two reasons. Firstly a multiallelic system would be a more efficient outcrossing mechanism than a diallelic one in that an increased number of morphs increases the probability of legitimate pollen transfer. Secondly, Lewis (1954) showed that the elimination of incompatibility alleles resulted in a breakdown of the system towards self-fertility rather than towards a refinement of the incompatibility system.

Thrum phenotypes have the genotype Ss and pins are of the genotype ss. Crosses between these two genotypes will normally result in equal numbers of the two morphs if there is no selfing. The dominant homozygote SS does not occur in nature but would be phenotypically thrum. This system was first described by Bateson and Gregory (1905) for *Primula sinensis* and has since been shown to be true for all cases examined (Vuilleumeir, 1967) . The thrum allele S is dominant in the majority of cases except in the *Plumbaginaceae* (Baker, 1966) and there is a clear dominance relationship between the two alleles in all cases.

Mather and de Winton (1941) believed that the evolutionary pathway towards distyly started with the inviability of the SS. They believed that the s allele arose as a mutant of S and that as time elapsed outcrossing was selected for with the resulting increase in ss individuals. At the same time they believed that deleterious recessive genes built up near the S locus because of its sheltering effect during outcrossing. This continued to such an extent that SS individuals were no longer viable and so are not found in nature. Their pathway assumed that there was no difference between the sterility of S pollen on the Ss pistil in comparison with s pollen on the

Ss pistil. This has since proved to be untrue (Ganders, 1979)

Of the more recent authors, Charlesworth and Charlesworth (1979) and Ganders (1979) have developed the most convincing models for the evolution of distyly. Charlesworth and Charlesworth (1979) consider the first stage towards the evolution of heterostyly to be a mutation creating a new pollen type. This pollen mutant would reduce selfing by being inviable on its own flower's stigma and could spread only if inbreeding depression was sufficiently great to promote outcrossing through this new pollen type. Once the new pollen type had become established within a population, a stigmatic mutation which resulted in stigmas more responsive to the new pollen type could spread. Charlesworth and Charlesworth (1979) believe that such a stigmatic mutation would only be successful if it was at a locus linked to that producing the new pollen type. This differs from other theories in which linkage between the two mutations is selected for later.

Ganders (1979) believed that distylous species evolved from outcrossing but self-compatible ancestors which showed some displacement of the flower parts that reduced selfing. The evolution of distyly could be initiated in such a species by a change in pollinator behaviour or availability which resulted in increasing levels of selfing. This would then cause the expression of deleterious recessive genes and would create conditions in which a mutant pollen type which reduced selfing would be favoured. This would result in a functionally gynodioecious stage which would then favour the spread of a new stigmatic mutation. Ganders (1979) considers that selection for tighter linkage of these characters would then

result in the relative constancy of the physiological incompatibility system.

The morphological characters of distyly are likely to have evolved because they increase the number of legitimate pollinations rather than because they reduce selfing. Charlesworth and Charlesworth (1979) believe that selfing would be reduced by the separation of flower parts. If this were so, separation of flower parts may have evolved but it is not a sufficient explanation for the dimorphism seen.

Pin stigmas capture more pollen than thrum stigmas (Ganders, 1979) and Ornduff (1979) found more own-type pollen on primrose stigmas than other-type pollen. He interpreted this as evidence that distyly does not increase pollination between morphs. Darwin (1877) was the first of several authors (eg Crosby, 1959) to suggest that the separation of flower parts of the morphs of distylous species could actually increase the number of legitimate pollinations. This is not irreconcilable with Ornduff's (1979) observation that primrose pollen loads are mainly of own-type. The increase in legitimate pollinations resulting from the separation of flower parts may be slight but it could be important particularly if pollination is relatively incomplete. Piper et al (1984) showed that pollination is not always complete in primroses and incomplete pollination is often regarded as an important event in the evolution of distyly (Ganders, 1979; Charlesworth and Charlesworth 1979).

The importance of differential positioning of the flower parts is further supported by the work of Rosov and Serebtsova (1958) and Oleson (1979) who both found

differential deposition of pollen grains of the two morphs on the bodies of insects which pollinate heterostyle species. This all supports Darwin's (1877) view that the number of legitimate pollinations is increased by dimorphy.

Another route by which distyly has evolved is through the loss of a morph from a tristylous species. Ornduff (1964) has shown that the mid-style form of *Oxalis suksdorfii* has a lower fitness than the other two forms and so should be selected against. *Oxalis violacea* may have evolved distyly by a similar route (Mulcahy, 1964). It is unlikely that the degeneration of tristyly has been important for the evolution of distyly except in a few cases and there is no evidence that suggests tristyly is much less common today than previously.

So far in this review I have been concerned with the evolution of distyly and have almost assumed that distyly marks a stable evolutionary end-point. This may not always be so. Lloyd (1979) has suggested that dioecious *Cordia* spp. have passed through a distylous stage in their evolution. This evolution depends on the morphs becoming specialised in the functions of a single sex. The selective forces involved in such a specialisation may be a change in the pollinator species. Robertson (1892) noted that the butterflies visiting *Houstonia purpurea* L. var *calycosa* Gr. are only able to pollinate the short-styled form. This would make that species functionally dioecious. Charnov (1982) has also suggested that heterostyly may have evolved as a response to the morphs differing in their abilities to gain reproductive success through male and female function. The suggestion is that thrum plants may be more successful as males than pins and

that pins may be more successful as females than thrums. A change in the relative contributions to fitness of the two sexes in the two morphs may result in the evolution of dioecy. Beach and Bawa (1980) and Opler et al (1975) have shown that in all cases where dioecy has evolved from heterostyly it is the thrum form which has become male.

Crosby (1959) believes heterostyly to be an unstable state in primroses with homostyly being a more stable evolutionary end-point. Other authors (Ernst, 1955; Ray and Chisaki, 1957; Charlesworth and Charlesworth, 1979) believe that homostyly results from a breakdown of heterostyly as a result of rare crossovers within the supergene controlling distyly rather than as a selected change.

In contrast to the evolution of heterostyly, its breakdown is fairly well understood. A rare split in the supergene controlling distyly can result in plants which display the gynoecium of pin plants with the androecium of thrum plants in the same flower. These 'long homostyles' are self-compatible and are dominant to pins but recessive to thrums. Pollen from long homostyles is compatible with long homostyle and pin stigmas only and so progeny from pins can express the homostyle phenotype. By contrast 'short homostyles' (thrum gynoecium combined with pin androecium) would produce pollen compatible with thrum stigmas and, as thrum is dominant, fewer progeny would express that phenotype. Other breakdown forms rarely occur as they can prove incompatible with everything. Long homostyle is the most prevalent breakdown form reported from nature (Ernst, 1955; Ray and Chisaki, 1957) except in the

Plumbaginaceae in which the dominance relationships are reversed. The event which leads to the maintenance of breakdown forms of heterostyle species appears to be the absence or unpredictability of the usual pollen vectors.

THE HISTORY OF PRIMROSE ECOLOGY.2.2

The literature on primrose ecology is sparse. There is more work on primrose cultivation but this is not relevant to the current study.

Darwin (1877) presented the first and most thorough analysis of primrose heterostyly and provided the first data which show it to be an outcrossing mechanism. This work has been the basis of all ecological studies on this species to date with the exception of Whale (1983).

Darwin's (1877) hypothesis that heterostyly in primroses was an outcrossing mechanism was accepted without question and other workers have been concerned with the discovery of the pollinator species responsible for the outcrossing (Bell, 1902; Christy, 1922). These authors were concerned with the problem of why very few insect visitors were seen on primrose flowers in relation to other early-flowering species such as *Viola* spp. They provided no new data on primrose ecology and their discussions on the problems of the pollinator species will be considered in chapter 4.3.

Whale (1983) looked at the distribution of British *Primula* spp. in relation to their water tolerances. He showed that they had sufficiently different tolerances to water-logging for it to be regarded as a niche difference between the three species concerned. Whale did not consider differences between morphs or any aspects of the survival of primroses.

The study of primroses moved away from ecology towards population genetics with the studies of Crosby (1940-1960) and their ecology has been largely ignored since.

THE GENETICS OF PRIMROSES 2.3

The inheritance and genetics of heterostyly was first described by Bateson and Gregory (1905) for *Primula sinensis*. This was followed by Ernst (1933,1936) and Lewis (1949,1954) who demonstrated that the inheritance of heterostyly was caused by a supergene complex which could be described as comprising the two alleles \underline{S} and \underline{s} . The dominant allele \underline{S} gives rise to the thrum morph. The recessive homozygote, \underline{ss} produces pin phenotypes and the heterozygote is thrum in a normal, purely heterostyle population.

Ernst (1936) showed that although the inheritance of heterostyly appears to be under the control of a single diallelic gene this is not the case. He was able to split the supergene to produce long homostyles in *Primula sinensis*. Lewis (1949) showed that the heterostyle system could be divided into six separate elements which were chromosomally linked and so were normally inherited as a complete unit. This corresponded with the elements of the incompatibility system shown in Table 1.1.

Crosby (1940) was the first to study homostyle primroses in the field and he showed that they were self-fertile. He considered the fate of the gene-complex responsible for the homostyle phenotype to be dependent on the viability of the homostyle homozygote and thought that the range of frequencies of homostyles in the Somerset populations he found to be indicative of a spread of homostyles through the populations at the expense of first thrums and then pins.

Crosby (1949) considered the linkage of the heterostyle

PIN	THRUM	HOMOSTYLE
<u>ss</u>	<u>Ss</u> or	<u>s's</u> or
	<u>SS*</u> or	<u>s's'</u>
	<u>Ss'</u>	

Table 2.1. The genotypes of the three primrose morphs. The genotype marked * is not found in nature.

gene-complex to be incomplete allowing homostyles to occur through a rare crossover. The crossover which represents homostyly is characterised by the recessive pin genes for female characteristics and the dominant thrum genes for male characteristics. It is called s'. This notation is in accord with homostyle forms of other species (Ganders, 1975). The genotype of the three primrose morphs are given in Table 2.1.

Crosby (1949) considered homostyles to be totally self-fertile and to self entirely. He felt that homostyle pollen would exclude thrum pollen from homostyle stigmas by sheer weight of numbers and that homostyle pollen would be in direct competition with thrum pollen for pin stigmas.

Crosby (1949) looked at the potential for homostyle spread through heterostyle populations given the possible advantages of their mating system and the possibility of inbreeding depression of fitness affecting homozygotes. Crosby constructed a model using different assumptions about the level of homozygous homostyle viability. Mather and de Winton (1941) showed that homozygote thrums in *Primula sinensis* were less viable than heterozygotes. Crosby used this to justify his assumption of inbreeding depression of homostyle fitness. If deleterious recessive genes had accumulated near the thrum locus, their homozygotes would be expected to be less viable. If this linkage were maintained during the crossover event resulting in homostyles, homozygous homostyles may be expected to have reduced viability in comparison with heterozygous homostyles. Fisher (1949), however, saw no reason to suppose that homozygous homostyles would be at a physiological disadvantage given that there was no

experimental evidence in this species.

Crosby (1949) constructed a model showing the spread of homostyles through heterostyle populations but he had to make assumptions regarding the ecology of the three morphs. For example he assumed that, with the exception of homozygous homostyles, all three morphs had similar viability and survival. He assumed that seed yield of the three morphs was equal but this was corrected in a later version of the model (Crosby, 1960). These assumptions are explored experimentally in the remainder of this thesis (Chapters 3-7).

Crosby's (1949) model used curves of population change in heterostyle populations to which homostyles had been added at the rate of one homostyle in 499 heterostyles. He showed that if homozygous homostyle viability was 100% then the population would attain 100% homostyly. In this version of the model, after 28 generations, the population would consist of 97% homostyles and of those, 92% would be homozygous. Under assumptions of homozygous viability of 65%, the population change results in 80% homostyles and 20% pins at equilibrium with thrums being excluded entirely. If homozygous homostyle viability was greater than 81.5%, homostyles would entirely displace heterostyles. He also showed that homostyles would reach an equilibrium level of 55.5% of the population or greater if homostyle viability was greater than zero.

Comparing the frequency of homostyles in Somerset populations with those from his model, Crosby (1949) suggested that a viability of 65% for the homozygous homostyle was found in nature. Updating this model, Crosby (1960) was

able to gain even greater accord between his predictions and the observed range of homostyle frequencies in Somerset populations. This later version of the model incorporated seed yield differences between the three morphs and had a stochastic element.

Bodmer (1958) presented data on protogyny in homostyles which suggested that they were by no means certain to be selfing. He suggested that this would buffer a trend towards homostyly in the populations in Somerset. Bodmer (1960) showed that only relatively small changes in the viability of homozygous homostyles are required to prevent the spread of homostyles if they are principally outcrossers. He showed that homostyles had not spread with the rapidity with which Crosby had suggested and that homostyles had actually decreased over a period of twelve years in some populations. He suggested that homostyles were on the threshold of zero increase in many populations.

Bodmer suggested that homostyles were not generally selfing because of their protogyny and suggested that this would tend to prevent their spread through a heterostyle population because heterostyly was more effective in promoting outcrossing. That is to say, he believed homostyles to be atavistic and thought that they would not be in a position to eliminate heterostyles.

It was clear that the differences between Crosby and Bodmer needed field studies for their resolution both from a genetical and ecological stance.

Curtis and Curtis (1984a) surveyed the Somerset primrose populations between 1978 and 1984 and so were able to assess

changes which had occurred since Crosby's (1949) original censuses. They found that little change in the proportions of the morphs had occurred. In addition Curtis and Curtis (1984b) and Piper et al (1984) provided data showing the outcrossing rate of homostyles at less than 10%.

Piper et al (1984) provided data showing that the potential seed output of the three morphs is similar and suggested pollinator limitation of seed yield in heterostyles. More data on the pollination of primroses are presented in Chapter 4.3.

Curtis and Curtis (1984a) showed that the relative stability of the Somerset primrose populations could be interpreted as the result of frequency-dependent selection but once again the possibility of ecological differences between the morphs was ignored.

The stability of primrose morph frequencies over the last 40 years is similar to that found in *Amsinckia spectabilis* (Ganders, 1975) where populations have remained composed of mixed heterostyle and homostyle members for over 100 years. Ganders suggests that homostyles have an advantage in small or new populations because of the limitation of pollinator activity for the heterostyle morphs. However, pollinators are not limiting in large populations and the advantage of a greater seed set by homostyles is lost above certain levels of population size. Ganders regards this as sufficient explanation for the stability of homostyly in this species.

Richards (1984) follows Crosby (1949) in suggesting that homostyle spread may be limited by the predation of flower parts and this is discussed in Chapter 4 of the current work.

Bodmer, (1984) and Piper *et al* (1984) both remain equivocal about the nature of the limits of the spread of homostyles in primroses and so it would appear that more data are required. This thesis provides ecological data which may assist in the solution of this problem.

DESCRIPTION OF FIELD SITES. 2.4

Crosby found homostyles in two main areas of Britain in the 1940's to 1960's. They are an area in the Chiltern Hills, Buckinghamshire and part of Somerset and North Dorset between Shepton Mallett and Shaftesbury. The size of the latter area has been extended by C.F. and J. Curtis but no new homostyle areas have been found. In the current study, two Somerset populations were observed for the work on the survival and reproduction of the three primrose morphs under natural conditions and one Buckinghamshire and one Bedfordshire population were used as sites for experiments on juvenile survival. Although the sites do have environmental differences, homostyles were observed over a wide range of sites, differing considerably in their environmental characteristics. The sites are described below.

WYKE CHAMPFLOWER, SOMERSET (ST 656339)

This population covers an area approximately 100m long by 9m wide and is on the west-facing slope of a bank running along a field boundary. At the top of the slope is a bramble, *Rubus fruticosus* ag. and hawthorn, *Crataegus monogyna*, hedge and the main part of the field is a ryegrass, *Lolium perenne*, meadow. Primroses are confined to the bank presumably because of the poor drainage and agricultural activity of the main part of the field. The slope is an embankment for a roadbridge over a railway and so the population can have been present no earlier than 1883 when the bank was created.

Other vegetation within the primrose population includes

ryegrass, nettles, *Urtica dioica*, and arable weeds. There is a reasonable amount of bare ground available for colonisation as the result of landslips and the activities of rabbits, badgers and many small rodents.

The site at Wyke Champflower (henceforth referred to as Wyke) was chosen for this work for several reasons. Access was good, there are reasonable numbers of all three morphs present and there is a history of censuses of the proportions of morphs there.

Crosby (1949) found the three morphs present at Wyke in approximately equal numbers. C.F. and J. Curtis have surveyed this population over the last ten years and have found the three morphs present in the same proportions as Crosby but a direct comparison of the numbers in the populations at the two times is not possible. The ratio of the morphs does not seem to have changed towards the increased levels of homostyly predicted by Crosby (1949).

BATCOMBE, SOMERSET. (ST 685398)

This site lies in a similar position to that at Wyke with the exception that it is north-facing. A drainage ditch separates this population from the rest of the field which is a ryegrass meadow. This population measures approximately 60m by 8m and has slightly more bare ground than Wyke. This is probably because it lies on a steeper slope. The other vegetation at the site is similar to that at Wyke.

Throughout this study Batcombe proved to be entirely heterostyle but Crosby (1949) found a small number of homostyles in it. Crosby (1949) believed this population to

consist of approximately 50 plants in the proportion 45:45:10, pins:thrums:homostyles respectively. In the present study homostyles were absent from this population and there were 147-247 adult plants.

The similarity between this site and the one at Wyke coupled with the contrast between Crosby's (1949) findings and my own made this an interesting heterostyle population.

WITHERIDGE WOOD, BUCKINGHAMSHIRE. (SU 926933)

For the two experimental sites I wanted two ecologically similar sites with different morph ratios. This was to compare the survival of the different morphs under conditions which were known to allow homostyle growth with those from an area from which homostyles were unknown.

The Buckinghamshire and Bedfordshire sites differ from the Somerset ones in that they are woodland sites with relatively heavy and poorly-drained soils.

Witheridge is a mature beech wood. The ground flora is poor consisting mainly of bramble and ivy, *Hedera helix*, but a dense covering of beech leaves covers the whole of the site and this may prevent the emergence of many seedlings (Sydes and Grime, 1979).

Crosby (1949) found that the population at Witheridge consisted of over 100 plants in the proportion 25:75, pins:homostyles with thrum plants completely absent but thrums are now present with the proportion of morphs being 22:7:71, pins:thrums:homostyles. The number of adult plants present in 1982 was 147.

WOBURN ESTATE, BEDFORDSHIRE. (SP 972315).

This primrose population is broadly similar to that at Witheridge but is outside either of the two main homostyle areas of Britain. It is a completely heterostyle population of approximately 120 adults in the proportion 51:49,pins:thrums. Homostyle primroses have not been reported growing wild within 30 miles of this site.

The population at Woburn is shaded by trees and a wall and has a poor ground cover of grasses, nettles and ivy on poorly-drained soils. There is an extensive ground covering of leaves in autumn and winter but the ground is mainly bare from March onwards.

CHAPTER 3

GROWTH AND SURVIVAL IN PRIMROSE POPULATIONS.

With the exception of the surveys of Crosby (1949) and Curtis and Curtis (1984a), there have been few attempts to assess the changes in the proportions of the three primrose morphs over time in natural populations. In this chapter I will describe the growth, survival and recruitment into the breeding population of primroses between 1982 and 1984 in two populations. This study follows individual plants over time rather than simply assessing the proportions of morphs present in a population at different times.

The two Somerset populations described in Chapter 2 were compared and this chapter is divided into the following sections;

- 1) Methods
- 2) Population composition
- 3) Population density
- 4) Population pattern
- 5) Size and age at first flowering
- 6) Adult plant size
- 7) Survival between years
- 8) Vegetative reproduction
- 9) Longevity
- 10) Causes of death

METHODS 3.1

The aim of this work was to assess adult survival, overall juvenile survival and recruitment into the breeding population at Wyke and Batcombe. Only the adult survival is compared between morphs in this chapter with juvenile mortality being assessed experimentally for the three morphs in Chapter 5.

The two populations were mapped during the height of the flowering season in May in each of the three years 1982-1984. Maps were superimposed to determine survival between years.

The mapping of adults and the larger non-flowering rosettes was relatively simple but the mapping of smaller rosettes and seedlings was more difficult at times because of their close aggregation. However, this was not a serious problem because it was possible to count the number of adults or larger vegetative individuals at subsequent censuses and so infer the level of mortality in the initial patch of seedlings.

Both Somerset populations are at field boundaries and are linear and interspersed with other species such as bramble, hawthorn and nettles which made the mapping process more difficult. The method of mapping both populations is shown in Figure 3.1a.

The method used for mapping the Somerset populations (Figure 3.1a) was found to be sufficiently accurate to pinpoint the smallest flowering plants. A check on the accuracy was maintained by mapping easily recognised markers such as fenceposts or a pin plant with purple flowers. From these markers it is possible to be sure that the accuracy of the mapping was $\pm 5\text{cm}$ around each plant.

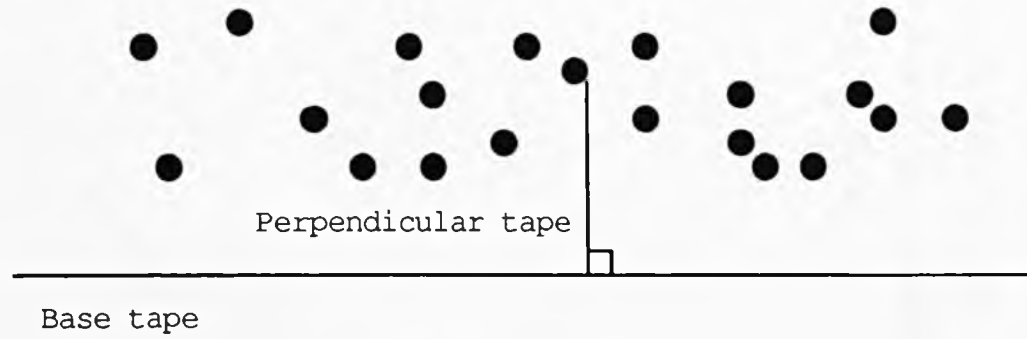


Figure 3.1a The method used to map the Somerset primrose populations

For each plant the rosette diameter, number of flowers and morph type was noted as well as its coordinates. These coordinates and the other plant information were then transferred to computer files. The computer was programmed to draw and compare maps of the populations over successive years.

The two populations were too large to be mapped in their entirety and so eleven 3m strips were mapped in each population (Figure 3.1b).

40% of the population at Wyke and 55% of the population at Batcombe were mapped by area. The mapping scheme was used to see whether there was a gradient in the number of homostyles along the bank at Wyke.

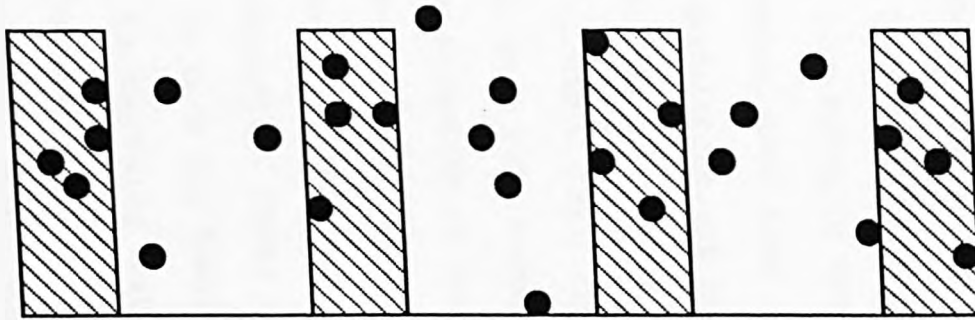


Figure 3.1b Diagrammatic representation of the mapped areas in the Somerset primrose populations. Shaded areas were mapped.

POPULATION COMPOSITION 3.2

This section describes the numbers and ratios of the morphs in the two Somerset populations both from the mapping of the present study and from Crosby (1949). Table 3.2a shows the numbers and proportions of the three morphs as reported by Crosby (1949).

Crosby (1949) found homostyles at both of the Somerset populations considered in this thesis. The numbers and proportions of the three morphs found in the present study are given in Table 3.2b.

At Batcombe, no homostyles were found in the present study even though Crosby reported low levels of homostyly in this population. According to Crosby's (1949) model homostyles should increase once they have become established in a heterostyle population but this could possibly be explained by the random changes in gene frequencies introduced into his later model (Crosby, 1960). The absence of homostyles in the present study also fits Bodmer's (1960) view that homostyles may be on the threshold of zero increase in some populations.

It is also interesting to note that the total numbers have at least doubled at Batcombe since Crosby's (1949) estimates of morph frequencies. Under these circumstances it would be of interest to know why homostyles have apparently had more difficulty in becoming established from seed than the heterostyle morphs.

Pins were always more common than thrums in the Batcombe population (Table 3.2b), particularly in 1983. This may be the result of selfing by pins (Crosby, 1949; Ganders, 1979). However, such a difference in the proportion of adult plants could be the result of survival differences between the

	PIN %	THRUM %	HOMOSTYLE %	NUMBERS
BATCOMBE	ND	ND	4-10	90+
WYKE	ND	<10	>30	90+

Table 3.2a. The numbers and ratios of morphs at Batcombe and Wyke (Crosby, 1949). ND = no data given.

	PIN %	THRUM %	HOMOSTYLE%	NUMBERS
BATCOMBE				
1982	51	49	0	148
1983	61	39	0	170
1984	51	49	0	207
WYKE				
1982	48	31	21	249
1983	45	26.5	28.5	343
1984	48	23	29	315

Table 3.2b. The numbers and ratios of morphs found at Batcombe and Wyke in the present study between 1982-1984.

morphs (Charnov, 1982).

The population composition at Wyke changed over the course of this study. The proportion of homostyles increased at the apparent expense of thrums (Table 3.2b). In terms of actual numbers, it is apparent that this change was the result of a higher recruitment of homostyles into the adult population between 1982-1983 and increased mortality of thrums in relation to homostyles between 1983-1984. Such a replacement of thrums by homostyles is predicted over a period of several generations rather than a few years (Crosby 1949, 1960). The detection of such a change has not occurred in other studies (Curtis and Curtis, 1984a) presumably as the result of differences in the sampling routine.

POPULATION DENSITY 3.3

Population densities for each of the three morphs were obtained from the maps of the two populations. The total plant densities in the two populations over the three years of this study are given in Table 3.3a

Total density of primroses at Wyke was greater than at Batcombe but this difference is not significant. These consistent differences could simply be the effect of site aspect. The total plant densities given in Table 3.3a include a large number of non-flowering individuals. The adult plant densities of the three morphs is given in Table 3.3b.

It can be seen from Table 3.3b that there is considerable variation in the adult plant densities in the two populations and that the total adult flowering plant density is greatest at Wyke in all three years. These figures are slightly higher than those found by Cahalan (1983) for heterostyle populations in North Wales but there is some overlap between the two groups of estimates.

If only the two heterostyle morphs are considered in Table 3.3b, then it will be seen that Batcombe has a higher density of flowering adults than Wyke. This is an indication that the homostyle plants at Wyke are occupying sites which may otherwise hold heterostyle plants rather than occupying a completely separate set of sites. This is not surprising but it suggests that homostyles may be in direct competition with heterostyles for sites suitable for growth, rather than their being a simple addition to the population.

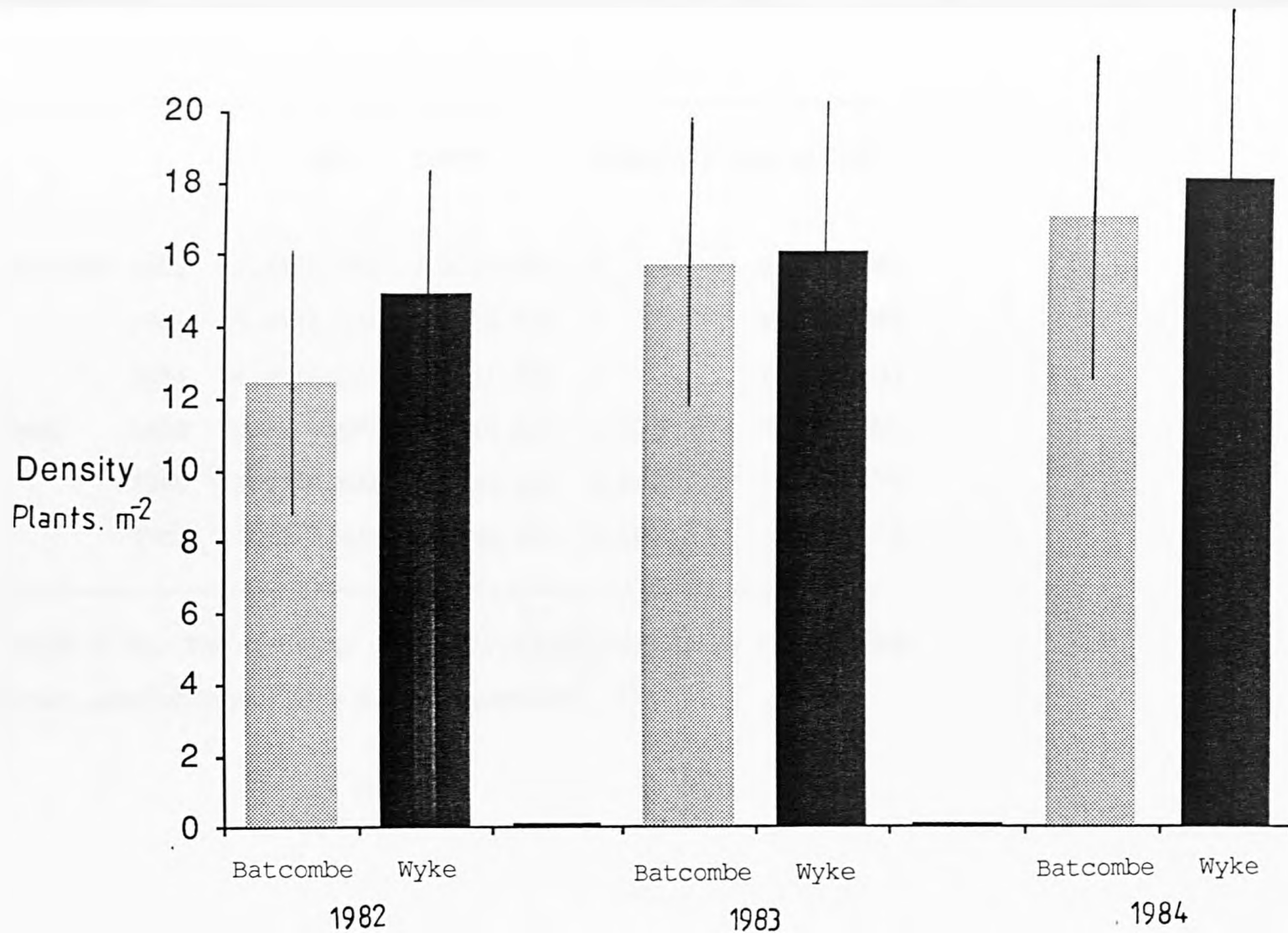


Figure 3.3a. Total plant density in the two Somerset populations (individuals.m⁻²). There are no significant differences between years or sites (t test, p > 0.05).

		PIN	THRUM	HOMOSTYLE	ALL ADULTS
BATCOMBE	1982	2.68 (0.79)	2.33 (0.84)	0	5.01 (1.48)
	1983	3.44 (1.37)	2.34 (0.69)	0	5.78 (1.68)
	1984	4.45 (1.24)	4.35 (1.28)	0	8.80 (2.61)
WYKE	1982	2.61 (0.68)	1.99 (0.59)	1.30 (0.57)	5.90 (1.81)
	1983	2.57 (0.82)	1.52 (0.47)	2.24 (0.74)	6.33 (1.79)
	1984	3.98 (1.15)	2.33 (0.67)	3.10 (0.91)	9.41 (2.70)

TABLE 3.3b. The density (/m²) of adult primroses in the two Somerset populations. S.D's are in brackets.

POPULATION PATTERN 3.4

The pattern of plants population could be important for the spread of homostyles within heterostyle populations. For example, if the mean distance between pin and homostyle was less than that between pin and thrum, homostyle pollen might be expected to outcompete thrum pollen for pin stigmas. Cahalan (1983) and Cahalan and Glidden (1985) have shown that pollen dispersal distances are relatively short in heterostyle primrose populations in North Wales and so a measure of the distance between plants of different morphs in the Somerset populations may, at the least, provide an interesting pointer to further work on pollen flow.

A nearest neighbour analysis was conducted on the maps for Wyke 1984 to determine whether any morph tended to be associated with another within the population. The distances between each flowering plant and its nearest adult neighbour of a specified morph were found and so the mean intermorph distances could be calculated (Table 3.4a).

It is apparent from Table 3.4a that for any given plant, its nearest neighbour is most likely to be of its own morph. This could be for several reasons. The site could be composed of a mosaic of microsites which would favour one morph over another. This explanation requires that there are ecological differences between morphs which affect their distribution.

A more likely explanation for the tendency for plants of the same morph to grow together is that they are the products of the same clone. Clonal growth does occur in all three morphs under controlled conditions (chapter 3.7) and so it is likely to occur in the wild.

		SPECIFIED PLANT		
		PIN	THRUM	HOMOSTYLE
	PIN	0.175 (0.19) *	0.674 (0.75)	0.671 (0.44)
NEIGHBOUR	THRUM	0.549 (0.55)	0.271 (0.22) *	0.553 (0.38)
	HOMOSTYLE	0.440 (0.36)	0.603 (0.52)	0.400 (0.36)

Table 3.4a. The mean distance between nearest neighbours of the three morphs (m). Standard errors are given in brackets. Values marked * are significantly lower than expected (t test, $p < 0.05$).

SIZE AND AGE AT FIRST FLOWERING.3.5

By following plants from their seedling stage through to flowering it is possible to determine the age at which they first flower. This age is used in the models of primrose population growth presented in Chapter 6.

For some species it has been shown that the age of a plant is not as good a predictor of its probability of flowering at a particular time as the size of its rosette (eg Werner and Caswell, 1977). This is a reflection of the plasticity of plant growth.

The sizes of a sample of the rosettes of both flowering (adult) and non-flowering (juvenile) plants were measured at Wyke in 1984. These diameters are given in Table 3.5a.

Above-ground rosette diameter is significantly different between adult and juvenile plants (Table 3.5a). It would appear that the size of a rosette is a reasonable indicator of whether it will flower in a given season but there is overlap between the two size distributions.

The age at which a plant first flowers was estimated by comparing the maps between years from Wyke. Most plants found either as seedlings in 1982 or as new plants in 1983 had either died or flowered for the first time by the end of this study and so an estimate of the age at which flowering first occurs is possible. The mean age at which flowering first occurs in this species is 20 months (± 5 months S.D.).

This mean is a composite of those plants which flowered in their first year and the majority of plants which did not flower until they were two years old. For this reason, the

ADULT ROSETTE DIAMETER (cm)	15.2 (4.2)	n = 108
JUVENILE ROSETTE DIAMETER (cm)	9.1 (3.9)	n = 55

Table 3.5a. The rosette sizes of adult and non-flowering rosettes at Wyke, May 1984. The sizes differ significantly ($t = 15.2, p < 0.05$).

modal value of 2 years is a more useful estimate of the age at first flowering than the mean and it is this value which is used in the models of Chapter 6.

ADULT PLANT SIZE 3.6

Plants of different size may be expected to differ in their ecology and reproductive outputs. It is possible that differences between the morphs are simply the result of differences in plant size between the morphs.

Adult plant size was measured by taking the mean of the longest rosette diameter and that at right angles to the longest diameter through the plant centre.

Mean rosette diameters for the three morphs in the two populations are shown in Table 3.6a.

Adult plant size was normally distributed with no significant differences between the morphs (Table 3.6a). The size distribution would suggest that there was no general increase in size with age during adult life and that ecological differences between the morphs could not be explained in terms of size differences between morphs.

	PIN	THRUM	HOMOSTYLE	N
BATCOMBE 1984	16.1(4.6)	15.0(4.9)	-	108
WYKE 1984	14.7(3.9)	15.5(3.5)	15.9(4.6)	99

Table 3.6a. The mean plant diameters (cm) of primroses in two Somerset populations. There are no significant differences between morphs (t test, $p > 0.05$).

SURVIVAL BETWEEN YEARS 3.7

This section describes mortality at Batcombe and Wyke in relation to plant age and flowering activity. If a plant was not present in the maps for successive years, it was assumed to have died. The survival of plants present as seedlings in 1982 is shown in Figures 3.7a and 3.7b.

The pattern of survivorship is similar in the two populations (Figures 3.7a,b). Juvenile mortality is massive in both populations and is under-estimated in this analysis. There would undoubtedly have been considerable mortality between seed dispersal and germination as well as between germination and detection in the first census.

It is of interest that plants which flower after two years are likely to survive for many years but those that do not flower then are unlikely to persist as vegetative plants.

It is difficult to assess age-related mortality of adult plants in so short a study but it is of interest to discover whether a plant which survives for, say, 15 years is likely to die of senescence or the same mortality factors which could have affected it at the age of 4.

In order to assess the importance of senescence in flowering adults, a comparison was made of the survival of those plants which were adult at the start of this study with those which only matured within the timespan of this study. The former category may be expected to contain a proportion of senescent plants whilst all of the latter will be less than two years old. The survival between 1983 and 1984 of old and young adults is presented in Table 3.7a.

There is no significant difference between the survival of

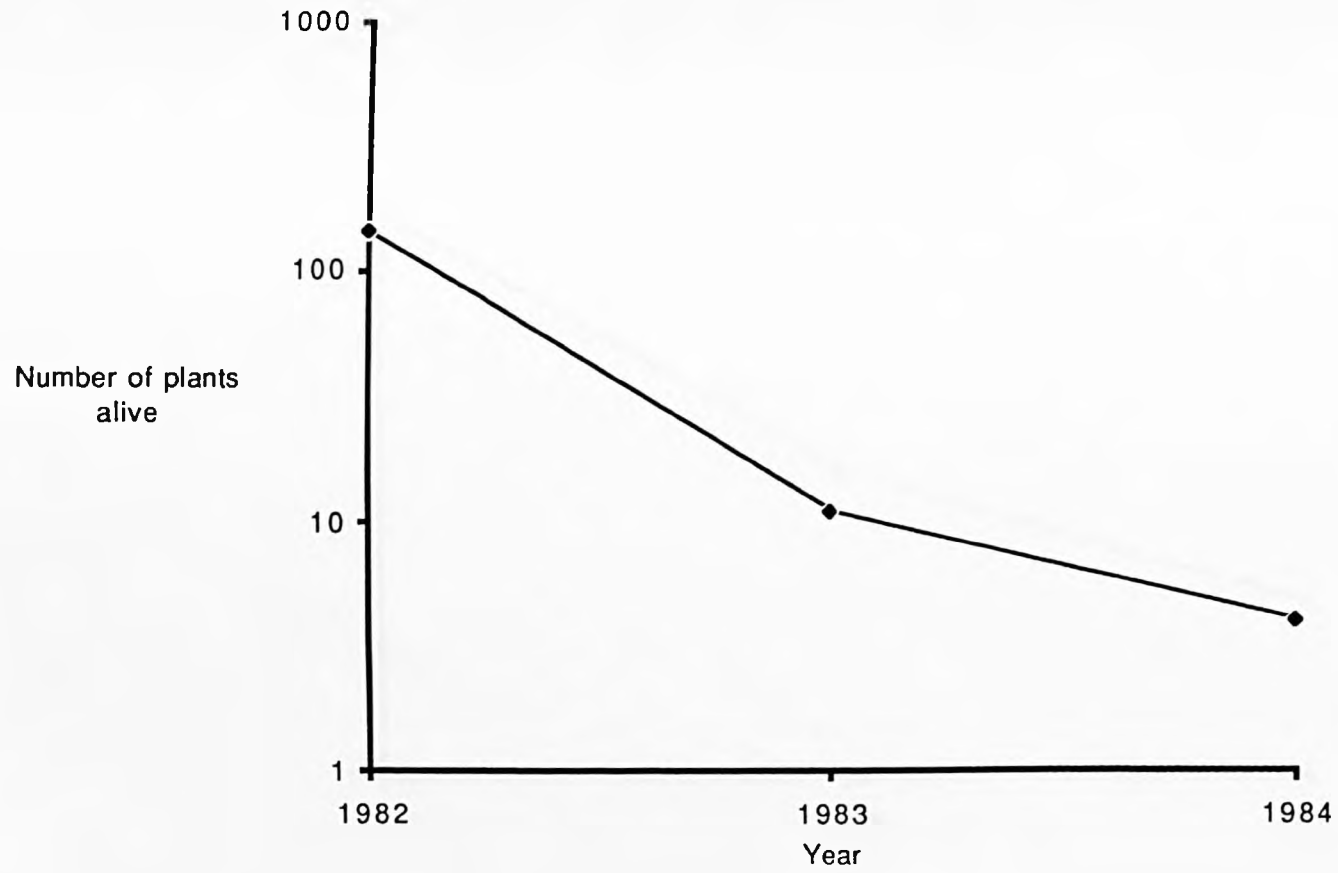


Figure 3.7a. Survival of primroses present as seedlings at Wyke in 1982

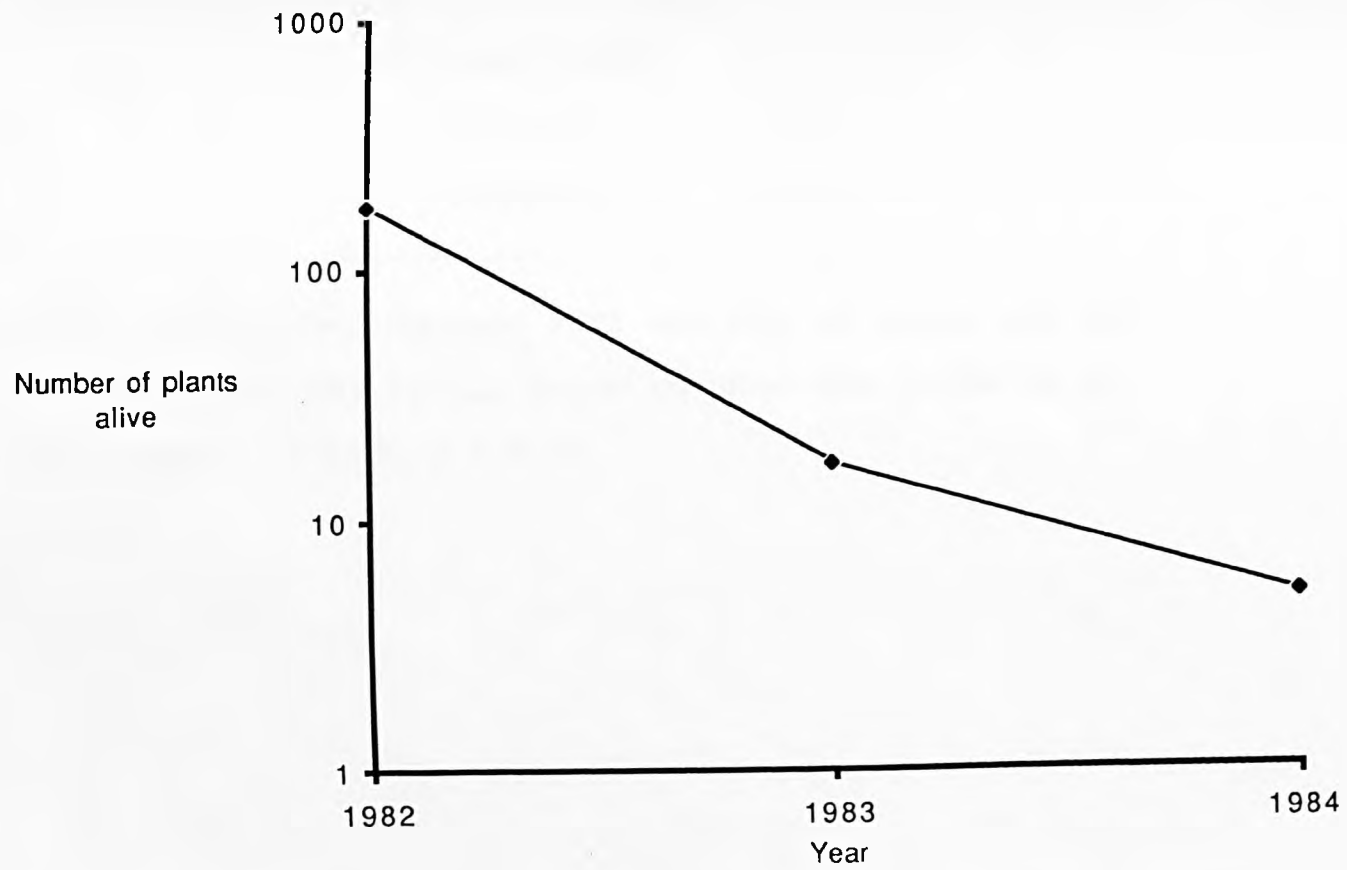


Figure 3.7b. Survival of primroses present as seedlings at Batcombe in 1982

	YOUNG ADULTS	OLD ADULTS
BATCOMBE	0.95 (0.15)	0.90 (0.17)
WYKE	0.90 (0.09)	0.94 (0.21)

Table 3.7a. The survival between 1983 and 1984 of young and old adults at Batcombe and Wyke for all morphs combined (The values do not differ significantly . T test, $p > 0.05$)

young and old adults (Table 3.7a) and this is an indication that adult mortality is random with regards age. It would appear from this rather limited analysis that senescence is not important in this species.

Adult survival of the three morphs is shown in Table 3.7b.

Thruns suffer the greatest mortality in both populations (Table 3.7b). This can provide an alternative explanation for the skew towards pins reported in some heterostyle populations (Crosby, 1949; Ganders, 1979). Differential survival of morphs has not been demonstrated before but it has been proposed as a possible explanation for biased morph ratios (Charnov, 1982).

Reproduction in the three morphs will be compared in Chapter 4. However, I would like to discuss the consequences of flowering for adult mortality here. Thrums tend to produce more flowers than the other two morphs (Table 4.1a) and have a greater variance in seed output (Table 4.5a). The number of flowers produced by a plant would appear to affect its chances of surviving to the following year. This is illustrated in Figures 3.7c,d,e.

It is apparent from Figures 3.6c,d,e that a plant producing a large number of flowers in a given year has a lower probability of surviving to the following year. The possible explanations for this relationship are several. If plants continue to grow throughout their lifetime, then older plants would be expected to produce more flowers. Another possibility is that the production of a large number of flowers is a heavy physiological burden for the plant the cost of which may be death in extreme cases. There is no evidence from the present study to suggest that flower number

	PIN	THRUM	HOMOSTYLE
BATCOMBE			
1982-1983	<u>0.942 (0.09)</u>	<u>0.893 (0.08)</u>	-
1983-1984	0.930 (0.13)	0.910 (0.11)	-
WYKE			
1982-1983	<u>0.931 (0.08)</u>	<u>0.823 (0.13)</u>	<u>0.950 (0.07)</u>
1983-1984	<u>0.940 (0.12)</u>	<u>0.857 (0.09)</u>	<u>0.962 (0.10)</u>

Table 3.7b. The survival of adult primroses at Batcombe and Wyke. Pairs of figures underlined differ significantly (t test, $p < 0.05$).

Pin

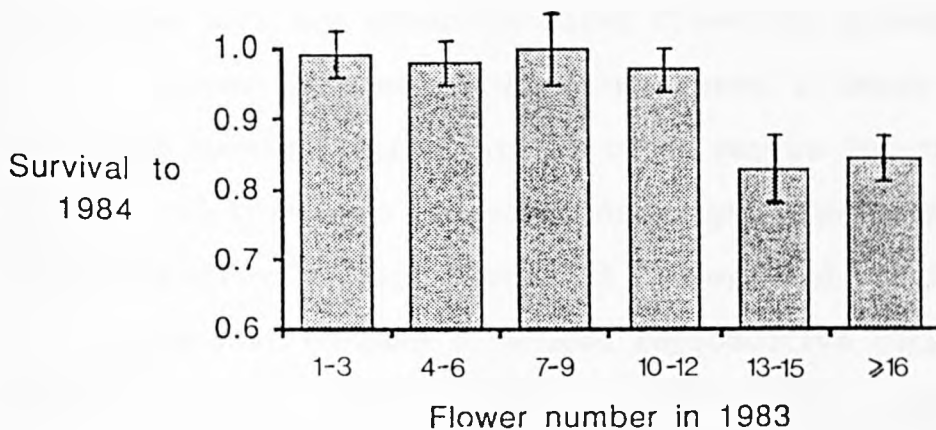


Figure 3.7c The relationship between flower number and survival in pins.

Thrum

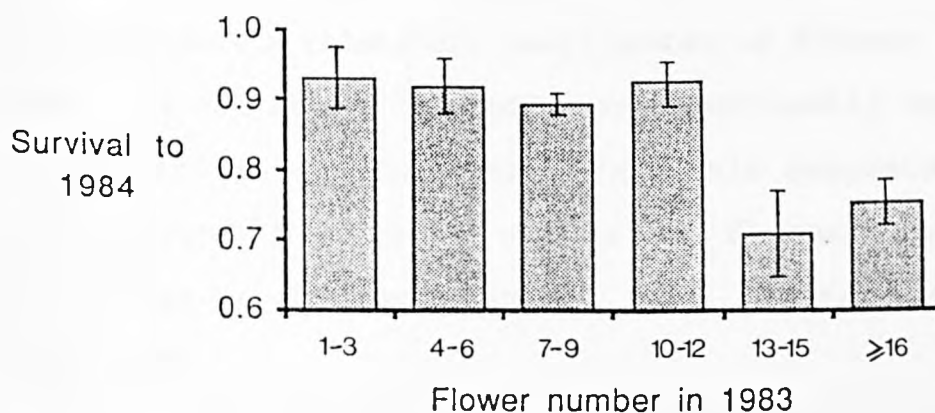


Figure 3.7d The relationship between flower number and survival in thrums.

Homostyle

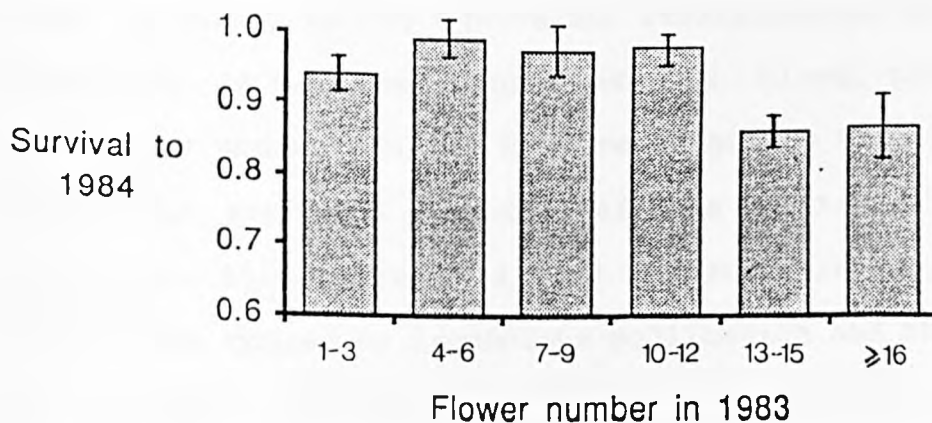


Figure 3.7e The relationship between flower number and survival in homostyles.

increases with age after the first flowering episode.

If flower production does represent a heavy cost to a plant in survival terms, lesser costs may be incurred by some plants. If this were the case, one might expect those plants which produced a large number of flowers and survived to the following year to have a reduced reproductive output in that second year. The relationship between flower number in one year and the next is shown in Figure 3.7f.

Figure 3.7 f shows that plants which produce 10 or more flowers in a year are likely to produce less than three flowers in the following year. It is also clear that plants which produce a relatively small number of flowers in a given year are not likely to produce an exceptionally large number of flowers in the following year. This suggests that the production of a large number of flowers may not be advantageous if reproduction over several seasons is considered.

It is more usual to consider seed production as a cost rather than flower production. However, the relationship between seed production in a given year and mortality in the following year or between seed production in successive years is not as strong as the equivalent relationships for flower production. It has been suggested (D.G. Lloyd, pers comm) that flower production may be more expensive than has been previously supposed because of the costs of nectar production. Also there is a high variation in seed output (Table 4.5a) caused by incomplete pollination and this would tend to improve the flower production relationship relative to that for seed production.

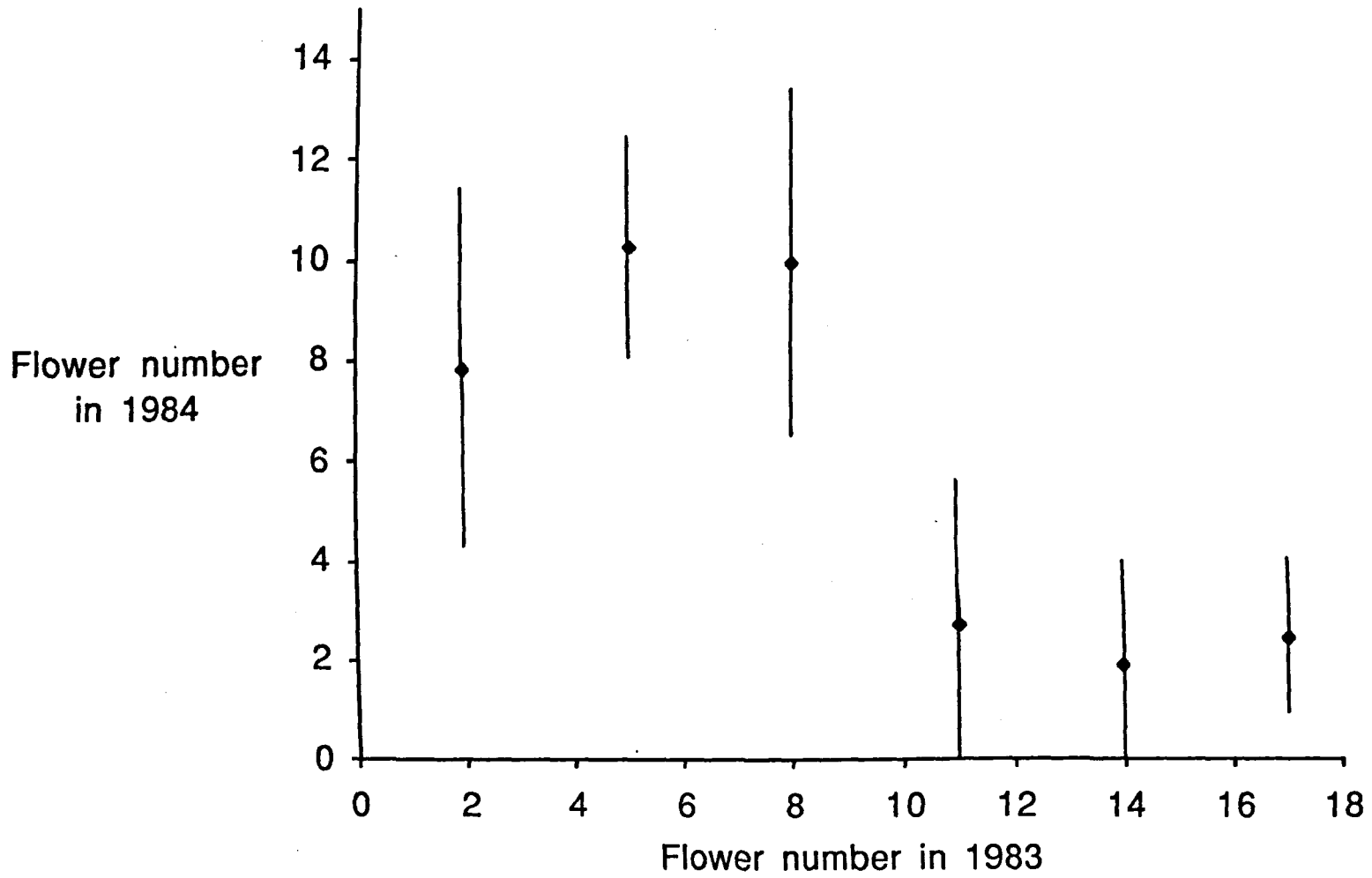


Figure 3.7f Flower number in successive years

VEGETATIVE REPRODUCTION 3.8

Primroses spread clonally in gardens and probably also in natural populations by rhizome growth. This spread could facilitate the maintenance of a morph in a population in spite of any differences in sexual reproduction between them.

Clonal spread was examined by growing 10 greenhouse-grown seedlings (raised from the same batch of seeds from the Somerset populations as used in the other experiments) in each of sixteen 30cm pots to maturity outside. Levington's potting compost was used and the plants were fed and watered regularly. The plants were raised to flowering and then the number of rosette centres on each was counted as a measure of potential spread. These data are given below in Table 3.8a.

Morphs did not differ in terms of their clonal spread under experimental conditions. The number of plant centres may seem rather high for all three morphs but this could be an effect of the light soil and lack of competition within the pots. It would not be possible to extrapolate to the field from these results but the fact that there was no morph difference under these conditions is perhaps encouraging. It is possible that any differences between the morphs would show after sexual maturity rather than before it. For example, if the morphs allocate differing quantities of resources to sexual reproduction they may, as a result, have differing levels available for clonal growth. However, without detailed field comparisons of intra-plant performances over several years this must remain purely speculative.

	Mean number of rosettes		Number of plants
Pin	7.6	(2.9)	39
Thrum	8.4	(3.4)	62
Homostyle	9.0	(4.1)	44

Table 3.8a. The mean number of rosettes per plant. There are no significant differences between morphs (paired T test, $P > 0.05$).

LONGEVITY 3.9

There is no evidence from the present study that plants survive in a vegetative state after they have left the breeding population but they may occasionally skip a year of flower production. This means that longevity (the mean age at death of those plants that survive to adulthood) minus the age at which a plant first flowers represents the active breeding lifespan of the plants.

Longevity estimates of a long-lived perennial like primroses can only be made in so short a study if it is assumed that the the population is at equilibrium and that the population turnover is constant. Longevity estimates for the three morphs are calculated from the equation;

$$L = (T/M) + A \quad \text{eqn 3.9}$$

Where L = Longevity; T = Total numbers of a morph present in a year (Table 3.2b); M = Number of deaths in a year (from data for Table 3.6b); A = Age at first flowering (Chapter 3.5).

The longevity estimates for the three morphs (Table 3.9a) show that thrums have the lowest life expectancy in all three years. Plants which produce a large number of flowers are the least likely to survive to the following year (Chapter 3.7). This may be expected to adversely affect thrums relative to the other morphs because of the relatively high frequency of thrums with a large number of flowers. An analysis of variance was performed to separate the effects of morph type and flower number in the previous year on the longevity estimates. This is presented in Table 3.9b.

		PIN	THRUM	HOMOSTYLE
BATCOMBE	1983	16.4 (2.9)	13.2 (3.4)	-
	1984	17.3 (4.1)	14.7 (2.8)	-
WYKE	1983	18.9 (3.7)	15.1 (3.2)	24.1 (6.1)
	1984	18.7 (4.1)	9.0 (5.7)	28.3 (4.9)

Table 3.9a. Longevity estimates in years for primroses in the two Somerset populations (\pm 95% confidence limits).

Sources of variation	Sum of squares	Degrees of freedom	Variance
Flower number in previous year	137.25	5	27.45
Morph	74.58	2	37.39
Residual variance	272.6	10	27.26
TOTAL	484.43		

Table 3.9b. Analysis of variance showing the effects of morph type and flower number in the previous year on longevity estimates of primroses. Neither flower number in the previous year ($F = 1.007$, $p > 0.05$) nor morph type ($F = 1.37$, $p > 0.05$) have a significant effect on longevity.

The number of flowers in the previous year accounted for only 29.8% of the variation in the longevity estimates with morph type accounting for a further 40.6% of the variance. However, neither is significantly greater than the residual variance (F test, $p > 0.05$). This is probably because the calculation of longevity is very sensitive to small differences in survival in those categories which suffer the least mortality.

CAUSES OF DEATH 3.10

Most of the mortality noted in this work has been in the form 'missing presumed dead', particularly for seedlings but two specific causes of death have been identified. Cattle trample the populations and may chew the rosettes on occasions. Primrose leaves do seem, however, to be left alone by most predators possibly because of their covering of irritant hairs. Another apparent cause of death in these populations is from landslips caused by the undermining of the slopes by rabbits and small rodents. Similar earth movements at Batcombe both resulted in the death of adult plants and the creation of gaps in which seedlings may become established.

REPRODUCTION IN NATURAL POPULATIONS.

In this chapter I will present data from the Somerset populations which were taken concurrently with data presented in Chapter 3. Differences in the seed output of the three morphs have been reported (Crosby, 1960) and there has been some controversy over the pollination of the species (Christy, 1922; Woodell, 1960) but a detailed analysis of the flowering episodes of the three morphs has not been attempted. The components of yield (Abrahamson and Gadgil, 1973) were analysed to investigate the causes behind possible differences in the seed output of the three morphs. The elements of the flowering episode considered in this chapter are;

- 1) Flower number and flower predation
- 2) Pollination rate and the species of pollinator
- 3) Number of capsules per plant and capsule loss
- 4) Number of seeds per capsule and mean seed weight
- 5) Number of seeds per plant

The separation of these components facilitates, for example, a comparison of the effects of an increase in the number of pollinators on the three morphs in a way that simple comparisons between seed outputs could not.

The extent of the seed pool in the two Somerset populations is also considered in this chapter.

FLOWER NUMBER AND FLOWER PREDATION 4.1

Flowering is the only stage where the three morphs differ visibly and so it is appropriate to consider the number of flowers produced by each of them. Mean flower number for the three morphs in the two populations is given in Table 4.1a.

Thrums had significantly more flowers than homostyles at Wyke in 1983 and 1984 (Table 4.1a) and they had significantly more flowers than pins at Batcombe in 1983. In addition, thrums had more flowers than pins at Batcombe in 1982 and also more at Wyke in 1983 but these differences were not significant ($p > 0.1$ at Batcombe 1982 and $0.1 > p > 0.05$ at Wyke 1983). There is more variation in flower numbers between years but within years thrums consistently have the greatest number of flowers. This suggests that the differences between flower numbers in the three morphs are real biological differences but that environmental conditions determine the total level of flower production.

It has been suggested (Crosby, 1949; Richards, 1984) that differential flower predation between morphs may provide a check on homostyle spread. Slugs and snails graze the tops of primrose flowers and so might be expected to render a pin flower functionally male, a thrum flower functionally female and homostyles may be expected to be neutered. The level and nature of this predation was examined by counting the number of flowers of the three morphs at different stages of predation at Wyke in 1984 until 250 intact flowers had been recorded. These data are presented in Table 4.1b.

The rates of flower predation are extremely low but the three morphs are grazed differentially (Table 4.1b). These

	PIN	THRUM	HOMOSTYLE
BATCOMBE 1982	3.7 (1.1)	4.1 (1.2)	-
1983	<u>3.9 (0.4)</u>	<u>4.3 (0.74)</u>	-
WYKE 1982	6.2 (2.1)	8.0 (1.7)	6.4 (1.6)
1983	8.6 (1.9)	<u>9.1 (2.1)</u>	<u>8.2 (1.8)</u>
1984	8.4 (1.9)	<u>8.6 (1.8)</u>	<u>8.0 (2.0)</u>

Table 4.1a. The mean number of flowers in the three morphs at the two Somerset populations. Figures in brackets are standard deviations. Pairs underlined are significantly different from each other. (t test, $p < 0.05$).

	STIGMAS LOST	ANTHERS LOST	BOTH LOST	INTACT
PIN	5	7	8	250
THRUM	0	12	0	250
HOMOSTYLE	8	0	8	250

Table 4.1b. Number of stigmas and anthers lost from flowers at Wyke in 1984. Predation of flower parts differs significantly between morphs. Pin and thrum, $\chi^2 = 16.8$, $p < 0.05$; Pin and homostyle, $\chi^2 = 7.3$, $0.1 > p > 0.05$, Thrum and homostyle, $\chi^2 = 28$, $p < 0.05$.

levels of flower predation are an over-estimate because it is probable that some of the anthers dehisced before predation and so their pollen would have been transported without interference. It would seem, then, that the predation of flower parts is not widespread enough to seriously disadvantage homostyles in general even though it may be of importance to individual plants.

To conclude this section, it appears that thrum primroses generally have more flowers than either pins or homostyles. The differential predation of flower parts does not significantly alter this and so a straight count of flower number should be a reasonable indication of the potential opportunities for pollination regardless of predation.

POLLINATION RATE AND SPECIES OF POLLINATOR 4.2

There has been a long debate about which insects pollinate primroses (Darwin, 1877; Christy, 1922; Woodell, 1960; Proctor and Yeo, 1973). Several species have been seen to pollinate primroses such as bee-flies *Bombylius* spp., the bumble bee *Bombus hortorum*, five species of butterfly of which the brimstone *Gonepteryx rhamni* is the most important and the mullein moth *Cucullia verbasci* (Christy, 1922). However, all of these species are only rarely reported and are more common visitors to other species such as violets *Viola* spp., which are in flower at the same time as primroses. It is possible to watch a primrose population for long periods of time without witnessing pollination. These observations are of interest because primrose seed set is sometimes limited by pollination (Piper et al, 1984). Table 4.2a shows the number of flowers which set seed at Wyke .

The proportion of flowers which produce seed is a reflection of the number of flowers which have been pollinated but does not take into account the number of legitimate pollinations which are required to fertilise all the ovules in each flower.

Homostyle pollination is consistently high in all three years (Table 4.2a). This is expected because they are self-compatible and so are not dependent on the vagaries of pollinating insects. The homostyle flowers which did not set seed were generally buried low in the vegetation and so were more prone to rotting than their other flowers. Pin pollination is also high but it is lower than that for homostyles whilst thrum pollination shows the greatest

	PIN	THRUM	HOMOSTYLE
1982			
SEEDED CAPSULES	208	165	184
UNSEEDED CAPSULES	<u>26</u>	<u>37</u>	<u>3</u>
1983			
SEEDED CAPSULES	171	147	199
UNSEEDED CAPSULES	<u>33</u>	<u>100</u>	<u>11</u>
1984			
SEEDED CAPSULES	179	162	228
UNSEEDED CAPSULES	21	13	15

Table 4.2a. The numbers of flowers which produced seeds at Wyke.
 Pairs of morphs underlined are significantly different. χ^2 , $p < 0.05$.

variation and is the lowest of all three morphs in two of the three years considered (Table 4.2a).

There are several reasons why pins should be more frequently pollinated than thrums. Pins may self more than thrums and so might not be so dependent on the presence of pollinating insects. In a population which contains homostyles, there may be more opportunity for pins to receive legitimate pollinations than thrums because both thrum and homostyle pollen is compatible with pin stigmas whereas only pin pollen is compatible with thrum stigmas. Alternatively, the difference in the number of pollinated flowers between the heterostyle morphs may be the result of differences in the activity of pollinator species.

The first of these explanations could be tested experimentally but is outside the scope of this thesis. The second possibility is not borne out by the fact that the difference in pollinated flowers can be seen in purely heterostyle populations such as Batcombe.

An investigation of the pollinator species may prove informative. Despite the fact that relatively few species of insects have been noted pollinating primroses, it is generally believed that moths do so (Darwin, 1877; Christy, 1922; Proctor and Yeo, 1975) though the direct evidence for this is slight. Darwin (1877) reported that *Cucullia verbasci* had been caught in the act of pollinating primroses but the other authors have assumed night-flying insects to be responsible for pollination by a process of elimination.

On the island of St Kilda there are very few insects and butterflies, bees, wasps and possibly ants are completely absent (Christy, 1922) though there are several species of

large moths. Primroses grow on this island and set seed (Gibson, 1893). It is further supported by the fact that the scent of primroses is particularly strong at night (pers. obs.) and the fact that the pale yellow flower colour is highly visible at night. Christy (1922) suggests that all the pale yellow *Primula* spp. are early-flowering and so may be adapted to pollination by night-flying moths which are present then after overwintering as adults.

In addition to the larger moths which are present at the time of flowering, a number of smaller species are also present. Whilst it may be reasonable to assume that the larger moth species normally effect pollination, the smaller species will also be attracted to the primrose flowers. If only the larger species have probosces long enough to effect pollination in both heterostyle morphs, smaller species may effect pollination of pin stigmas only.

Differential pollination of the morphs of heterostyle species has been noted before. Robertson (1892) showed that the butterflies that pollinate *Houstonia purpurea* L var *calycosa* Gr. were only able to pollinate the short-styled form and Ornduff (1975) has shown that the pollination of the morphs of *Jepsonia heterandra* is effected by different species.

To test whether primrose morphs suffer differential pollination resulting from their floral architecture, I decided to try and trap insect visitors to the flowers and to test the pollination of primrose flowers experimentally.

Attempts to trap insect visitors to primrose flowers were made using funnel traps and 'Tangletrap'. Tangletrap is a sticky aerosol which can be used to coat flowers and so trap

insect visitors in a manner similar to bird-lime. Neither the Tangletrap nor the funnel traps over individual flowers were successful in trapping insect visitors at Batcombe in 1984. This was probably because both techniques reduced the attractiveness of the flowers to potential insect visitors and also there were many more flowers available with no kind of trap set.

A Robinson pattern moth trap using a mercury-vapour lamp was used to trap moths which were in or near the Wyke population during the 2nd week of May 1984. Light traps are known to vary in their effectiveness for different species and under different environmental conditions (Southwood, 1978) but they are useful in the present study because they can provide good numbers of a variety of species intact.

To investigate the pollination of primroses by moths, moths were caged with virgin primroses. Twelve primroses, six each of pin and thrum, were placed in a muslin cage with either 'macromoths' or 'micromoths' and each trial was left for a week. The primroses were then removed from the cage, their flowers were covered and their seed capsules were allowed to develop. There were five trials for each moth type (Table 4.2b). Homostyle pollination in the muslin cage with the two moth types is also given.

Homostyle pollination in the cages was similar to that on the open greenhouse benches whereas there was no heterostyle pollination in the greenhouse other than when caged with moths. Moths present in the Wyke population at the time of peak flowering are capable of pollinating primroses under experimental conditions and they may be responsible for the differences in seed set seen in the heterostyle morphs. If

	PIN	THRUM	HOMOSTYLE
MICROMOTHS	3.8 (0.3)	<u>0.8 (1.1)</u>	<u>4.1 (0.8)</u>
MACROMOTHS	4.4 (0.4)	4.6 (1.3)	4.3 (0.4)

Table 4.2b. The number of primrose capsules per plant which contained seed after exposure to moths of two size categories. Figures are means (\pm S.D) with pairs underlined being significantly different from each other (χ^2 , $p < 0.05$).

these results resemble the field situation, then it would appear that the pollinator species may limit the seed set in thrums in some years at least.

There is no evidence to suggest that the pollination of primroses is less complete now than in the past but it is interesting to note that Ganders (1975) showed that the level of pollination determined whether homostyle *Amsinckia spectabilis* would be at an advantage over heterostyle plants. The same author, (Ganders, 1979) also suggests that a change in the pollination efficiency may have been responsible for the evolution of heterostyly as well as its breakdown to homostyly.

To conclude this section, then, it appears that thrums may be pollinated by macrolepidoptera whilst pin are likely to be pollinated by microlepidoptera also. Interestingly, 1984 was a much better year for moths than 1983 and this was, perhaps by coincidence, the best year for the pollination of primroses in this study. This suggests that the conditions which favour a high level of over-winter survival by larger moths is crucial to the pollination of heterostyle primroses.

NUMBER OF CAPSULES PER PLANT AND CAPSULE LOSS 4.3

Hand pollination of primrose flowers has shown that all three morphs have similar potential seed outputs per flower (Piper *et al*, 1984). This supports the view that pollination limits seed set in primroses. One may expect the number of capsules produced by a plant to be more closely linked to the pollination rate (Table 4.2a) than the number of flowers (Table 4.1a) as a result. The number of seed capsules produced by the three morphs is given in Table 4.3a.

Thrums produced significantly more flowers than pins in Batcombe in 1983 (Table 4.1a) but produced fewer capsules containing seeds in that year (Table 4.3a). The data above (Table 4.3a) suggest that the possible advantage in terms of maximum potential seed output that thrums have by producing large numbers of flowers is lost because of their lower pollination rate (Table 4.2a). The fact that thrums produced most seed capsules in 1984 is probably a reflection of the high level of pollinator availability in that year.

Capsule loss was noted at Wyke in 1984. The number of seed capsules that had been broken open or removed (if stalks were still present) from individual plants was noted. In this way it was possible to tell whether pre-dispersal seed predation was correlated with morph or whether certain categories of plant were more at risk. Seed predation appeared to be the result of small mammal activity and the data for capsule loss are presented in Table 4.3b.

There was no evidence of differential pre-dispersal seed predation between primrose morphs (Table 4.3b). Nor was there any evidence to suggest that certain categories of plant,

		PIN	THRUM	HOMOSTYLE
BATCOMBE	1982	4.1 (0.42)	3.1 (0.71)	-
	1983	3.5 (0.39)	2.6 (0.30)	-
WYKE	1983	3.9 (0.80)	3.2 (0.73)	3.6 (0.64)
	1984	4.4 (1.30)	5.1 (2.1)	4.7 (1.7)

Table 4.3a. The mean number of capsules produced per plant in two Somerset populations. Standard deviations are in brackets. Capsule numbers do not differ significantly between morphs within years (χ^2 , $p < 0.05$).

	PIN	THRUM	HOMOSTYLE
NUMBERS NOT ATTACKED	206	215	278
NUMBERS ATTACKED	42	59	47

Table 4.3b. The number of seed capsules of the three morphs which suffered predation. The morphs are not attacked differentially ($\chi^2 = 5.21, p > 0.05$).

such as those which produced a large number of seed capsules,
were more at risk than others.

NUMBER OF SEEDS PER CAPSULE AND MEAN SEED WEIGHT. 4.4

The number of seeds per plant is limited by the number of ovules per flower and the number of those ovules which are fertilised. For heterostyle plants this suggests that seed set is pollinator limited and for homostyles the limit to seed set is likely to be the number of ovules per flower. Seed capsules collected from the Somerset populations were sampled at random and the mean number of seeds per capsule for the three morphs is given in Table 4.4a.

Homostyles produced more seeds per capsule than the two heterostyle morphs in 1983 and significantly more seeds per capsule than pins in 1984 (Table 4.4a). The standard deviations of seed number per capsule are greatest for thrums in both populations. These two results are in accord with the likelihood of pollination of the three morphs.

Looking at seeds from a large number of capsules, it became clear that there was variation in the size of seeds as well as the number of seeds per capsule. Seeds within a capsule were of similar size but the seeds from one capsule could often be visually distinguished from those of another by size alone. I decided to investigate the nature of this variation because until recently seed size has been considered to be relatively invariable in plants (Salisbury, 1942; Harper, Lovell and Moore, 1970). Variation in seed size within capsules was not investigated because individual seeds were too small to weigh and there was little visual variation in this factor. There was a relationship between the mean seed weight per capsule and the number of seeds in that capsule for all three morphs. Only the data for 1983 are

		PIN	THRUM	HOMOSTYLE
BATCOMBE	1982	25.1 (4.9)	29.0 (5.2)	-
	1983	<u>21.5 (2.2)</u>	<u>14.9 (2.6)</u>	-
WYKE	1983	<u>19.9 (1.6)</u>	<u>23.4 (4.1)</u>	<u>39.7 (2.8)</u>
	1984	<u>48.8 (3.9)</u>	<u>63.5 (7.9)</u>	<u>57.2 (2.2)</u>

Table 4.4a. The mean (S.D.) number of seeds per capsule in the three morphs. Pairs underlined differ significantly (χ^2 , $p < 0.05$).

presented below (Figures 4.4a, b, c), the data for the other years of the study are similar.

Capsules containing five or fewer seeds are included in the graphs, but are excluded from the regression analysis. It was felt that the larger range of individual seed weights from capsules with few seeds was an artifact of the lower sensitivity of the balance used at the extremes of its scale. Open circles represent points which are included in the regression analysis.

Linear regression provides an adequate description of the relationship between mean seed weight and the number of seeds per capsule (Figures 4.4a, b, c). The regression coefficients are significant for all morphs ($p < 0.05$) and the gradients of the three lines do not differ significantly. An analysis of covariance reveals that the intercept of the thrum line is significantly lower than that of the pin and homostyle lines.

The regression analysis indicates that the three morphs are very similar in terms of this relationship but this masks the differences in distribution of the points between the morphs. Homostyles produce more capsules with larger numbers of seed than the other two morphs and so many of the points in figure 4.4c are found in the bottom right hand side of the graph. The variation in seed number per capsule found in the homostyles is possibly the result of environmental factors. Thrums in particular show a wide range of seed numbers and have a higher proportion of points in the top left of the graph (Figure 4.4b). Possible causes and consequences of the differences between the three relationships are discussed below.

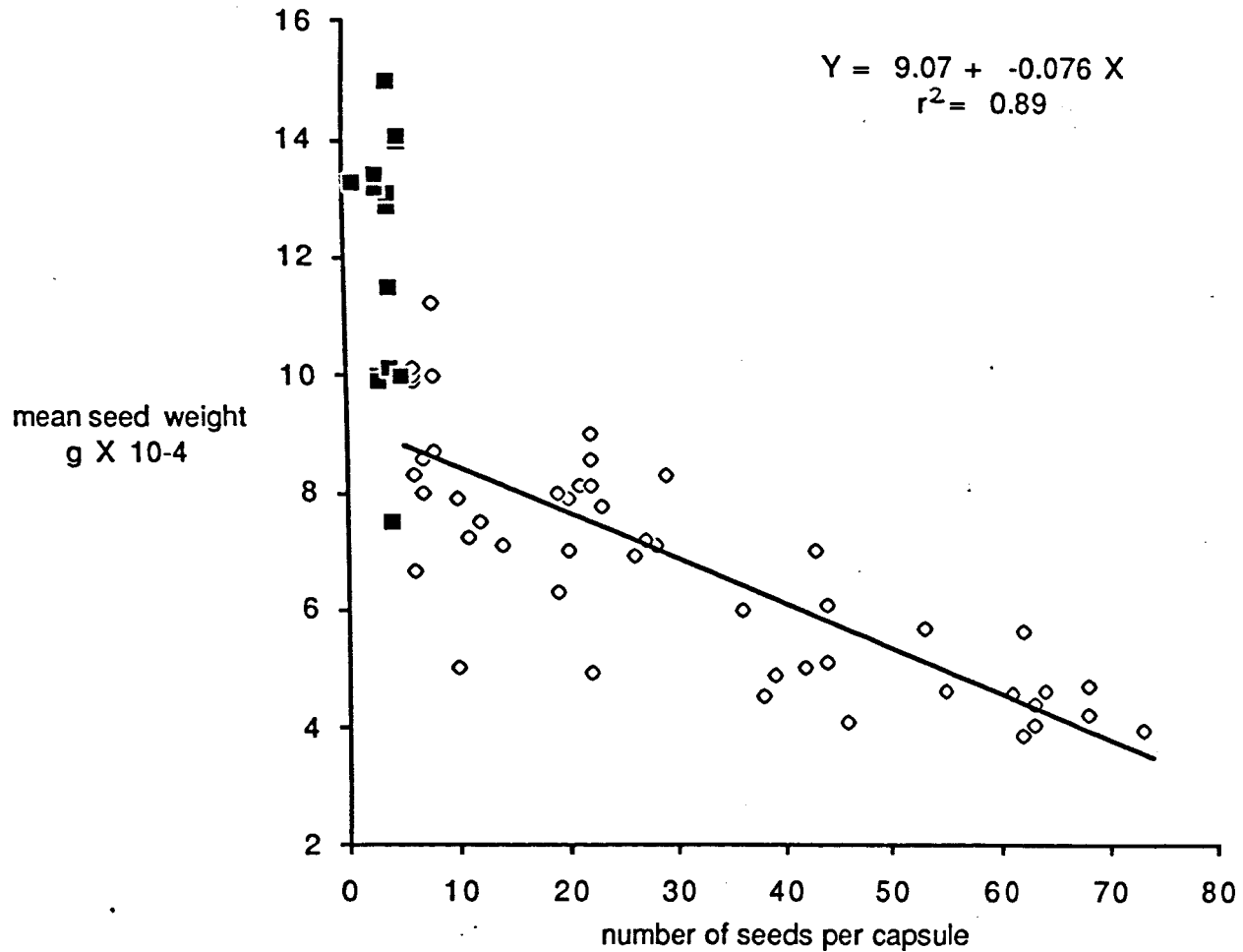


Figure 4.4a Mean individual seed weight versus number of seeds per capsule for pins (1983)

Solid points(■) represent capsules containing fewer than five seeds, and these are excluded from the regression analysis.

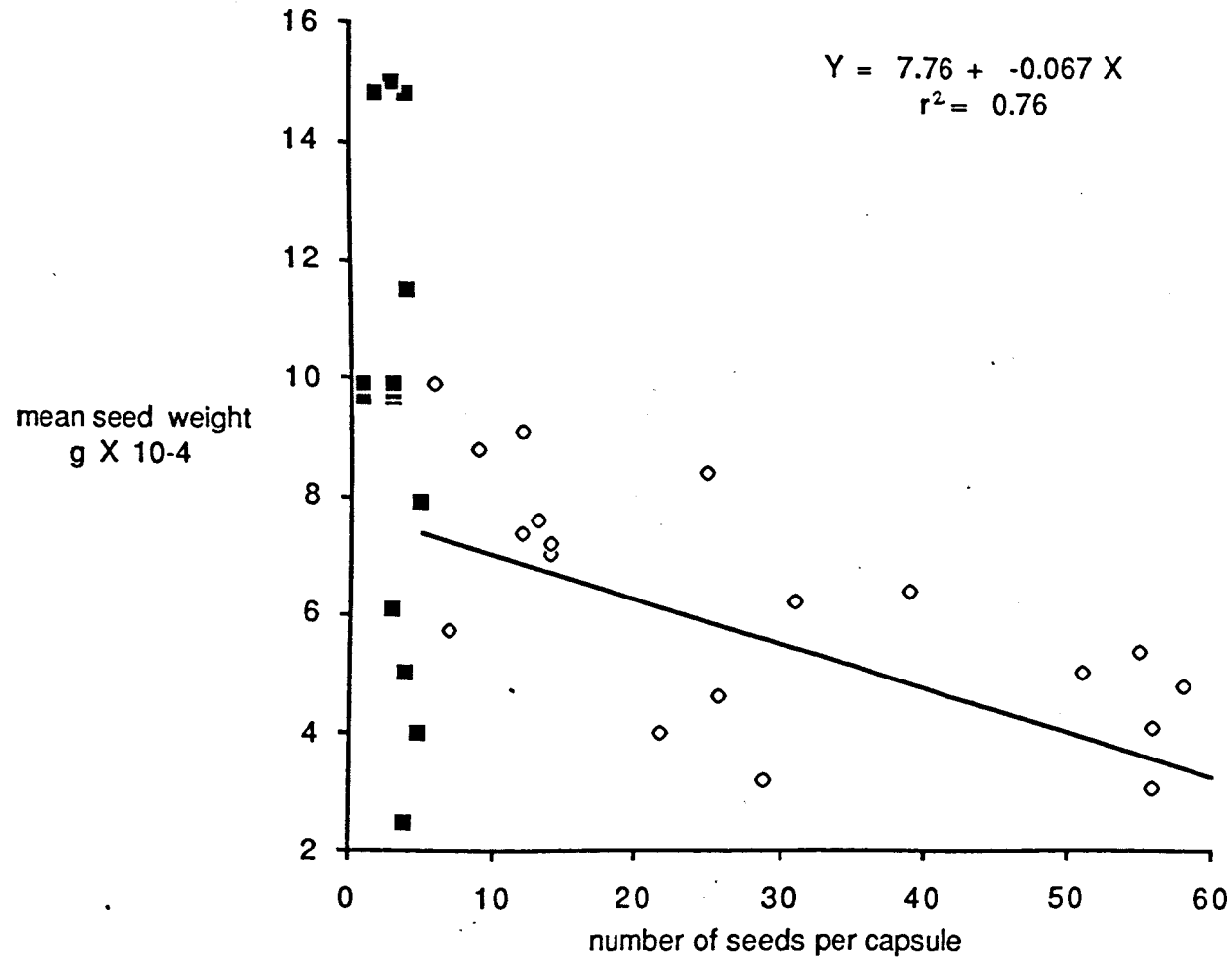


Figure 4.4b Mean individual seed weight versus number of seeds per capsule for thrums (1983)
 Solid points(■) represent capsules containing fewer than five seeds, and these are excluded from the regression analysis.

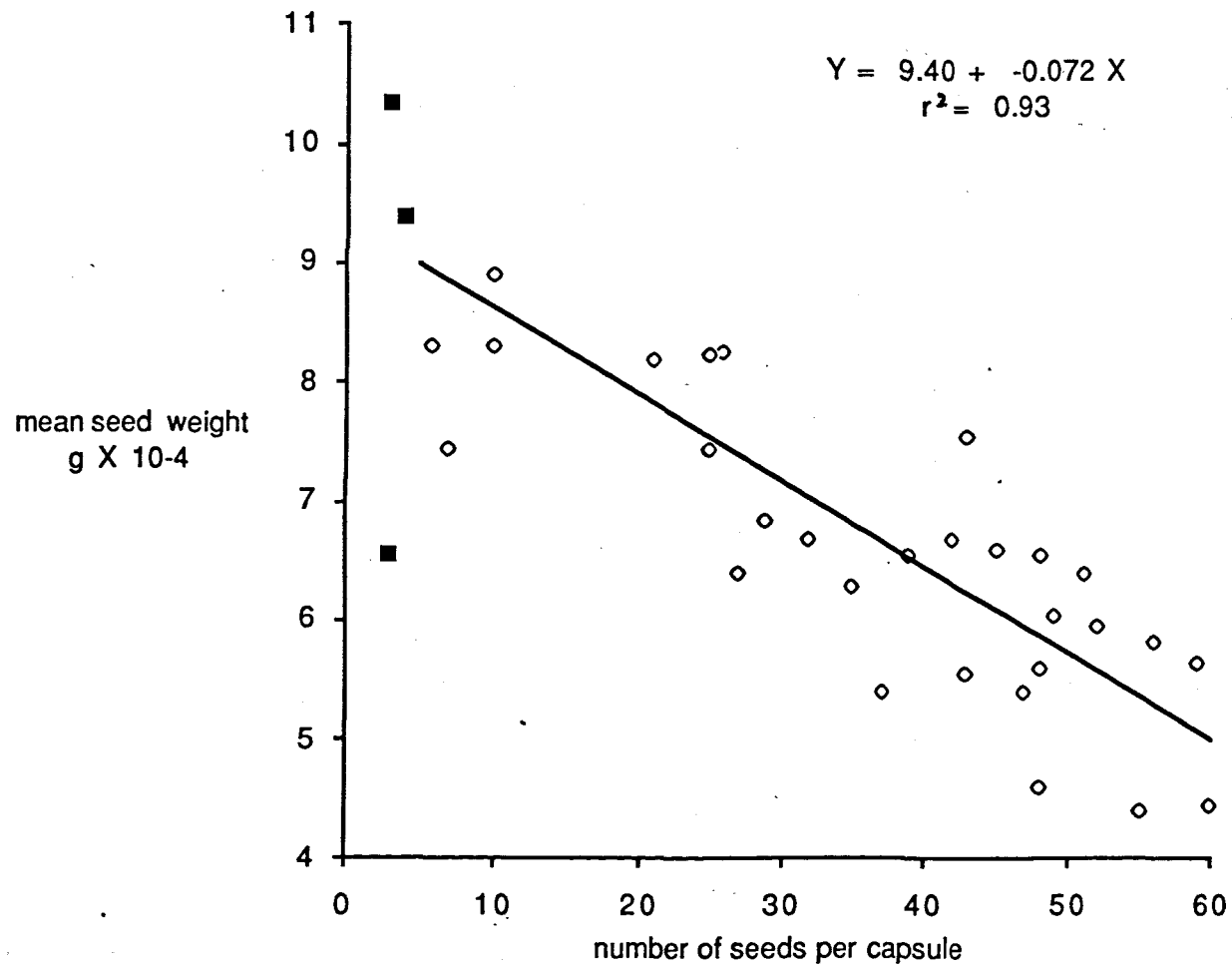


Figure 4.4c Mean individual seed weight versus number of seeds per capsule for homostyles (1983)
 Solid points(■) represent capsules containing fewer than five seeds, and these are excluded from the regression analysis.

Pin and Thrum	1.536
Pin and Homostyle	0.989
Thrum and Homostyle	2.197**

Table 4.4b Comparison of the regression lines of Figures 4.4a, b, c (Clarke and Cooke, 1983). The thrum and homostyle lines differ significantly ($p < 0.05$).

DISCUSSION

The lower intercept of the thrum line (Figure 4.4b) suggests that for a given number of seeds per capsule, thrum seeds will be lighter than those of the other two morphs. The suggestion is that the allocation of resources available for filling seeds is similar for all three morphs but that thrums have fewer resources available in the first place. If all three morphs expend similar amounts on reproduction each year, it is possible that thrums allocate relatively more of their resources to flower production (cf Chapter 4.1) with the result that each thrum flower has more difficulty in filling its seeds.

The result of the grouping of homostyle capsules in the bottom right hand side of the graphs is that in years where pollination is poor, thrums may produce the heaviest seeds but if pollination is relatively complete thrums will have the lowest seed weight. For example, the mean number of seeds per capsule in 1983 for the three morphs was 19.9 pin, 23.4 thrum and 39.7 homostyle (Table 4.4a). The mean individual seed weights from figures 4.4a, b, c would then be 0.96mg for pins, 0.733g for thrums and 0.68g for homostyles despite the lower intercept of the thrum line.

The canalisation of seed weight is presumably the result of natural selection for a particular, optimal seed size but there have been several recent examples of seed size variation within species (Waller, 1982; Stanton, M.L. 1984; Hendrix, 1984). In primroses there appears to be a wide range of possible seed sizes. This variation is probably not in itself advantageous or the product of selection for two reasons. If pollination is relatively complete, the variation in seed sizes would be relatively small. That an advantageous variability in seed size could be countered by efficient

pollination seems unlikely. Seed size would perhaps be expected to vary within seed capsules if it were an advantage as this would then bypass the effects of variations in the pollinators.

NUMBER OF SEEDS PER PLANT 4.5

The number of seeds produced by a plant in one season is a component of that plant's fitness. A comparison of seed output of the three morphs is shown in Table 4.5a.

Homostyles produced more seeds than the heterostyle morphs in 1983 but not in 1984. Homostyles would be expected to produce a large number of seeds in all years because they do not rely on pollinators. Thrums did well at Wyke in 1984 presumably because pollinators were plentiful and thrums have more flowers than pins. It would appear that the effects of flower number and pollinator availability are both very important for determining primrose seed set. From this it would appear that homostyles should spread through heterostyle populations most rapidly when there is a paucity of insects.

	PIN	THRUM	HOMOSTYLE
BATCOMBE 1982	103	90	-
1983	<u>74</u>	<u>41</u>	-
1984	-	-	-
WYKE 1982	-	-	-
1983	<u>78</u>	<u>75</u>	<u>143</u>
1984	<u>214</u>	<u>324</u>	<u>269</u>

Table 4.5a. The mean number of seeds per plant in two Somerset primrose populations. Pairs underlined are significantly different from each other (χ^2 , $p < 0.05$).

THE NATURE OF THE SEED POOL 4.6

There seems to be no published information indicating the presence of a persistent pool of primrose seeds in the soil. This is surprising since primroses germinate throughout the year in some populations. The methods used and the time of the year of the investigation determine whether primrose seeds are detected in the soil. The best single time of the year to look for a persistent seed pool is in May, just before the annual seed crop is shed. Any seeds found at this time must have persisted for at least an entire year. Seeds detected at other times of the year may only represent a transient seed population.

20 Soil cores, 15cm in diameter, were taken from each of the Somerset populations in the 3rd week of May 1983. These cores were washed in a solution of 125g/l sodium hexametasulphite to break up the soil particles. Seeds were separated from this solution by filtration and the samples were dried. The seeds were sorted under a microscope and any primrose seeds were identified, removed and sown in a greenhouse. The number of seeds found in the seed pool is given in Table 4.6a.

There is a persistent seed pool in these populations, though it is very patchily distributed as can be seen from the high sample variance.

The seeds separated from the seed pool looked as though they were from the larger end of the range noted in Figures 4.4a, b, c. They could have swelled either in the soil or during their extraction. Alternatively, smaller primrose seeds could have escaped detection but this is not likely as

	n	mean/sample	mean/m ²
BATCOMBE	20	3 (8.2)	1061
WYKE	20	3.7 (7.9)	1305

Table 4.6a. The mean number (S.D.) of seeds in the soil cores taken at the two Somerset populations in May 1983.

smaller seed of other species were found in profusion.

The possibility that the seeds from the soil pool were from the larger end of the primrose seed size range raises interesting ecological questions. It could be explained if larger seeds survive better in the soil than smaller seeds. Alternatively, smaller seeds may germinate immediately on leaving the parent plant leaving only large seeds in the seed pool.

It is not known whether either of these two explanations for the predominance of large primrose seeds is correct. If it is assumed that the larger seeds from the seed pool are the same size as when they were shed, reference to Figures 4.4a, b, c will show that they are most likely to be of heterostyle origin. The number of seeds from the seed pool which could be germinated was relatively small but this is probably because they were oven-dried rather than they were inviable in the first place. The seeds were grown to flowering in the greenhouse and the numbers of plants which flowered is shown in Table 4.6b.

The ratios of the adult population to the seed population are different in both Batcombe and Wyke. However, the sample sizes are not sufficiently large for these differences to be relied upon. The excess of thrums in both populations is unexpected but the predominance of heterostyle plants grown from the seed pool may be a consequence of their larger seed size.

Homostyles may be under-represented in the seed pool in comparison to the adult population. It is unclear whether this would be a great disadvantage for homostyles because most of the recruitment into the population would appear to

	PIN	THRUM	HOMOSTYLE
BATCOMBE	8	17	-
WYKE	4	13	1

Table 4.6b. The numbers of primroses flowering from the seed pool at Wyke and Batcombe.

come from fresh seed (see Chapter 5). Also, very few of the seeds shed appear in the seed pool.

Whilst the seed pool may be unimportant in most circumstances, some plants do become established from it. This could be important if a population re-establishes itself from seed following extinction by, for example, disease or human disturbance. Under such conditions, homostyles could suffer extinctions whereas heterostyles may recover.

