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POPULATION ECOLOGY OF HETEROSTYLE AND HOMOSTYLE *PRIMULA VULGARIS*: GROWTH, SURVIVAL AND REPRODUCTION IN FIELD POPULATIONS

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SUMMARY

(1) Homostyle primroses are self-fertile and are also able to fertilize heterostyles with the pin morph. In theory these characteristics should give homostyles an advantage over heterostyles and homostyly should spread. In fact primrose populations with significant numbers of homostyles are rare in Britain and the frequency of homostyles in them has not noticeably increased during forty years of observation.

(2) In search of an ecological explanation for the homostyle handicap, growth, survival and reproduction of heterostyle (pin and thrum) and homostyle primroses were compared in natural field populations, and the pollination of heterostyles was tested in a glasshouse experiment.

(3) No significant difference between morphs was found in the size of flowering plants in the field, or in their rate of clonal expansion when grown in pots. In two successive years in the field homostyles survived significantly better than thrums but were similar to pins.

(4) In the glasshouse, heterostyle primroses only set seed when caged with moths. Seed set was significantly lower in thrums than in pins when the pollinators were micromoths. Pins set seed equally in the presence of large or small moths, and homostyles equally in the presence or absence of any moths.

(5) Levels of flower damage in the field were low and did not differ between morphs. In two out of three years thrums had significantly more flowers plant^{-1} than homostyles. Damage to seed capsules occurred but did not differ between morphs.

(6) Homostyles produced more seeds capsule^{-1} and plant^{-1} than heterostyles in 1983 but thrums out-produced pins and homostyles in 1984.

(7) A significant negative relationship between seed number capsule^{-1} and mean seed weight occurred in all three morphs. This relationship could mean that in years of poor pollination when heterostyle seed set is low, heterostyle seeds will be significantly larger than homostyle seeds. If seed size is more important than seed number in determining the fitness of primrose morphs, the trade-off between seed size and seed number could handicap homostyles on the common occasions when heterostyle seed set is pollinator-limited.

INTRODUCTION

Primula vulgaris Huds., the common primrose, is a rosette-forming perennial in the family Primulaceae. Like most species in the genus, *Primula vulgaris* is distylous and has an incompatibility system restricting legitimate pollination to that between pin and thrum flower morphs. 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 the corolla tube or even beyond it, whilst its anthers are near the middle of the corolla tube. In the thrum form the position of stigma and anthers are reversed. Heterostyly is controlled by a supergene complex which effectively comprises the two

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alleles *S* and *s*. The dominant allele *S* gives rise to the thrum morph. The recessive homozygote, *ss* produces pin phenotypes and the heterozygote is thrum in a normal, purely heterostyle population (Lewis 1949, 1954). There are no known 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, known as 'homostyle', has stigma and anthers at the same height in the corolla tube, is self-fertile (Crosby 1949), and is produced by a third allele *s'* which is dominant to *s* and recessive to *S*.

Homostyles have a mating advantage over heterostyles. Seed production in the distylous morphs is pollinator-limited (Piper, Charlesworth & Charlesworth 1984) and consequently self-compatible homostyles sometimes produce more seeds than heterostyles and rarely fewer. Homostyle pollen is able to fertilize pins, all of whose seeds will be homostyle when the pollen parent is a homozygote and half of whose seeds will be homostyle when the pollen parent is a heterozygote. Despite these advantages homostyly has remained localized and rare in the populations where it was first discovered fifty years ago (Curtis & Curtis 1985). Genetic studies (Piper, Charlesworth & Charlesworth 1984, Piper & Charlesworth 1986) have been unable to account for the failure of homostyly to spread as predicted by population genetic models (Fisher 1949; Crosby 1949, 1960; Bodmer 1960). Richards (1986) believes that homostyles probably occur at low frequency ($< 1\%$) in all wild *P. vulgaris* populations, and Ford (1975) and Bodmer (1984) regarded this as certain. If this is so, then the presence of higher frequencies of homostyly in only a few populations cannot be attributed to the rarity of the recombination event within the supergene which produces this phenotype. An hypothesis is needed which will explain both the rarity of homostyly and its local occurrence at unusually high frequency. Heterostyle species appear to have evolved from monomorphic ancestors (Vuilleumeir 1967). Both the origin and the maintenance of heterostyly therefore call for explanation.

As flowering is the only stage at which the three morphs visibly differ it is possible that the primary differences in flower structure create differences in fitness between the morphs. 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 *P. vulgaris* flowers and so might be expected to render a pin flower functionally male, a thrum flower functionally female and to neuter homostyles altogether.

In this paper the reproduction, growth, survival and spatial distribution of adults of the three morphs in the field, and clonal growth of plants grown in experimental conditions are analysed to determine if there are ecological differences which could explain the failure of homostyles to spread through heterostyle populations.

There has been a long debate about which insects pollinate primroses (Darwin 1888; Christy 1922; Woodell 1960; Proctor & Yeo 1973). This issue is of renewed interest now that it is known that seed set in *P. vulgaris* is often limited by pollination (Piper, Charlesworth & Charlesworth 1984). Several species have been seen to pollinate *P. vulgaris*, 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, these are only rarely reported and are more common visitors to other species such as *Viola* spp., which are in flower at the same time as *P. vulgaris*. It is possible to watch a population for long periods of time without witnessing pollination (M. Boyd, personal observation).

Although direct evidence is scarce, the possibility has been suggested that *P. vulgaris* is pollinated by moths (Darwin 1877; Christy 1922; Proctor & Yeo 1975), and this has been observed by Ford (1975). The scent of *P. vulgaris* is particularly strong at night (M. Boyd, personal observation) and the pale yellow colour of the flowers is highly visible at night. Both of these floral characteristics would attract night-flying moths which are present in the early spring after overwintering as adults. A number of smaller species of moth are also present at the time of flowering, but species with a short proboscis may effect pollination of pin stigmas only. This paper reports the results of field studies on flower predation and of glasshouse studies on flower pollination by moths of differing size.

Differences in the seed output of the three morphs have been reported (Crosby 1960; Piper, Charlesworth & Charlesworth 1984; Piper & Charlesworth 1986) and differences in the components of yield are likely. In heterostyle plants, seed set is pollinator-limited, while the number of seeds capsule^{-1} in homostyles is likely to be limited by the number of ovules flower^{-1}. Differences in seed number capsule^{-1} could trade-off with seed size. To investigate these possibilities number of flowers plant^{-1}, number of capsules plant^{-1} and capsule loss, number of seeds capsule^{-1} and mean seed weight, and number of seeds plant^{-1} were studied. Lastly, there seems to be no published information indicating the presence of a persistent pool of *P. vulgaris* seeds in the soil, although it germinates throughout the year in some populations. A preliminary survey of the extent of the seed pool in the two Somerset populations is also reported.

METHODS

Field sites

Crosby (1940) found homostyles in two main areas of Britain in the 1940s to 1960s, in an area in the Chiltern Hills, Buckinghamshire and in 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. Two Somerset populations, first located by Crosby (1949), were used in this study.

Wyke Champflower, Somerset (ST 656339)

This population covered an area approximately 100 m long by 9 m wide and on the west-facing slope of a bank running along a field boundary. At the top of the slope was a hedge of *Rubus fruticosus* agg. and *Crataegus monogyna* Jacq. and the main part of the field was a *Lolium perenne* L. meadow. *P. vulgaris* was confined to the bank, presumably because of the poor drainage and agricultural activity in the main part of the field. The slope is an embankment for a roadbridge over a railway and so the population may not have been present before 1883 when the bank was created. If a population was present before that date it must have been heavily disturbed during construction work.

Other vegetation within the *P. vulgaris* population included *Lolium perenne*, *Urtica dioica* L. and arable weeds. There was a reasonable amount of bare ground available for colonization as the result of landslips and the activities of rabbits, badgers and rodents.

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 found by Crosby. 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 was similar to Wyke with the exception that the bank was north-facing. A drainage ditch separated this population from the rest of the field which was a *Lolium perenne* meadow. The population measured approximately 60 m × 8 m and had slightly more bare ground than Wyke. This is probably because it lay on a steeper slope. The other vegetation at the site was similar to that at Wyke.

Throughout this study the population at Batcombe proved to be entirely heterostyle, although Crosby (1949) found a small number of homostyles in it; he believed this population to consist of approximately fifty plants in the proportion 45:45:10 (pins:thrums:homostyles, respectively). In the present study homostyles were absent from this population and the number of flowering plants varied between 148 and 207 during the three-year study.

Census of field populations

The two populations were mapped during the height of the flowering season in May in 1982, 1983 and 1984, by recording the rectangular coordinates of all plants in eleven 3-m-wide permanent transects. These covered 40% by area of the population at Wyke and 55% of the population at Batcombe. The accuracy of mapping was ±5 cm, which was found to be sufficient to relocate the smallest flowering plants. Where smaller rosettes and seedlings formed tight aggregations, the number of individuals in each clump was counted and recorded with the coordinates of the whole clump. The survival of individuals in these clumps was determined from counts of the number of flowering adults or larger vegetative individuals present in them at subsequent censuses.

For each mapped plant the rosette diameter, number of flowers and morph type was noted. Maps made in successive years were superimposed to determine survival between years. If a plant was not present in the maps for successive years, it was assumed to have died. These data also provided information on population density, morph ratios, size distribution of the morphs, and spatial pattern analysed by nearest-neighbour analysis.

Age-specific mortality of adult plants cannot be assessed directly in so short a study, but the importance of age-related mortality can be gauged from a comparison of the survival of those plants which were adult at the start of the study with those which only matured during it. The former group was expected to contain a proportion of senescent plants whilst all of the latter were less than two years old.

In a short study longevity estimates (the mean age at death of plants reaching adulthood) of a long-lived perennial can only be made if it is assumed that the population is at equilibrium and that population turnover is constant. Longevity estimates for each of the three morphs were calculated from the equation:

$$L=(N/D)+A,$$

Where L = longevity, N = total numbers of a morph present in a year (Table 1a), D = a number of deaths in a year and A = age at first flowering, estimated to be two years (see Results, below).

Clonal growth in experimental conditions

In gardens *P. vulgaris* spreads clonally by rhizomes. It probably also does so in wild populations where differences in the rate of rhizome production between morphs could affect the morph frequency. Clonal growth was examined by growing ten glasshouse-grown seedlings (raised from seeds collected in Somerset) in each of sixteen 3-cm pots to

maturity outdoors. Levington's potting compost was used and the plants were fed and watered regularly. Plants were raised to flowering and then the number of rosette centres on each was counted.

Moth-pollination experiment

A Robinson pattern moth trap using a mercury-vapour lamp (Southwood 1978) was used to trap moths that were in or near the Wyke population during the second week of May 1984. Pollination of *P. vulgaris* by the captured moths was investigated in a glasshouse. Moths were caged with virgin plants reared from seed. Twelve homostyle plants, or 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 plants were then removed from the cage, their flowers were covered and their seed capsules were allowed to develop. There were five trials with each moth type for heterostyles and homostyles. Plants growing in pots on the open benches of the glasshouse were used as controls.

Flower number and flower predation

Flower number plant^{-1} was counted at both sites in 1982 and 1983 and at Wyke only in 1984. Predation on flowers was sampled 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. Missing stigmas and missing anthers were recorded separately.

Seed production, capsule predation and seed weight

Capsule number plant^{-1}, number of seeds capsule^{-1} and number of seeds plant^{-1} were recorded in 1982 and 1983 at Batcombe and in 1983 and 1984 at Wyke. 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 also noted in 1984. 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 mainly of small-mammal activity.

Seed capsules collected from the Somerset populations were sampled at random and seed number and mean seed weight capsule^{-1} were recorded.

Seeds in the soil

The seed pool was sampled in May, just before the annual seed crop of *P. vulgaris* was 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. Twenty soil cores, 15 cm in diameter, were taken from each of the Somerset populations in the third week of May 1983. These cores were washed in a solution of 125 g l^{-1} sodium hexametaphosphate 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 binocular microscope and any *P. vulgaris* seeds were identified, removed and sown in a glasshouse where the numbers of each morph were scored when the plants flowered.

RESULTS

Census of field populations

Density and proportions of morphs

The numbers and proportions of the three morphs are given in Table 1a.

TABLE 1. Comparisons between morphs of *Primula vulgaris* in field and experimental conditions: (a) proportions of each morph and total number of flowering plants; (b) mean (S.D.) density m^{-2} of each morph and of all flowering *P. vulgaris*; (c) mean diameter (cm) of flowering plants. There were no significant differences between morphs (*t*-test, $P > 0{\cdot}05$); (d) mean (S.D.) distance (m) between nearest neighbours of the three morphs. Values marked * are significantly lower than expected (*t*-test, $P < 0{\cdot}05$); (e) survival probabilities of flowering adults. Values in the same row, sharing the same superscript letter differ significantly (*t*-test, $P < 0{\cdot}05$); (f) longevity estimates in years ($\pm 95\%$ confidence limits); (g) mean (S.D.) number of rosettes $plant^{-1}$, and number of plants in each sample raised from seed under experimental conditions. The number of rosettes $plant^{-1}$ did not differ significantly between morphs (χ^2, $P > 0{\cdot}1$).

		Morph			
		Pin	Thrum	Homostyle	Total
(a)					
Batcombe	1982	0·51	0·49	0	148
	1983	0·61	0·39	0	170
	1984	0·51	0·49	0	207
Wyke	1982	0·48	0·31	0·21	249
	1983	0·45	0·26	0·29	343
	1984	0·48	0·23	0·29	315
(b)					
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)
(c)					
Batcombe		16·1 (4·6)	15·0 (4·9)	—	108
Wyke		14·7 (3·9)	15·5 (3·5)	15·9 (4·6)	99
(d)					
Pin		0·175 (0·19)*	0·674 (0·75)	0·671 (0·44)	
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)	
(e)					
Batcombe	1982–83	0·942 (0·09)^{a}	0·893 (0·08)^{a}	—	
	1983–84	0·930 (0·13)	0·910 (0·11)	—	
Wyke	1982–83	0·931 (0·08)^{a}	0·823 (0·13)^{ab}	0·950 (0·07)^{b}	
	1983–84	0·940 (0·12)^{a}	0·857 (0·09)^{ab}	0·962 (0·10)^{b}	
(f)					
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)	
(g)					
Rosettes $plant^{-1}$		7·6 (2·9)	8·4 (3·4)	9·0 (4·1)	
Plants		39	62	44	

The total plant densities were similar in the two populations over the three years of the study and there were no significant differences between years or sites (*t* test, $P > 0{\cdot}05$). The total plant densities given in Table 2 include a large number of non-flowering individuals. There was considerable variation from year to year in the flowering plant densities of the two populations (Table 1a, b).

TABLE 2. (a) Total plant density in each of the two Somerset *Primula vulgaris* populations in the second week of May 1982–84. Figures are individuals m^{-2} with standard deviations in parentheses (b) Survival over the subsequent two years of *P. vulgaris* seedlings first recorded in 1982.

Year	1982	1983	1984
(a)			
Batcombe	12·45 (4·12)	15·63 (4·47)	16·99 (5·08)
Wyke	14·87 (3·85)	15·99 (4·68)	18·04 (5·36)
(b)			
Batcombe	179	17	5
Wyke	143	11	4

Rosette size of flowering plants

Mean rosette diameters for the three morphs in the two populations are shown in Table 1c. Flowering plant size was normally distributed with no significant differences between the morphs.

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 1984, and so an estimate of the age at which flowering first occurs is possible. The mean age at which flowering first occurred was twenty 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 *modal* value of two years is a more useful estimate of the age at first flowering than the mean.

The sizes of a sample of 108 flowering rosettes and of fifty-five non-flowering rosettes measured at Wyke in 1984 were compared. Rosette diameters were significantly different between flowering ($15{\cdot}2 \pm 4{\cdot}2$ cm) and non-flowering plants ($9{\cdot}1 \pm 3{\cdot}9$ cm, $t = 15{\cdot}2$, $P < 0{\cdot}001$). It would appear that the size of a rosette was a reasonable indicator of whether or not it would flower in a given season, although there was overlap between the two size distributions.

Spatial pattern

A nearest-neighbour analysis was conducted on the maps for Wyke in 1984. The mean distances between each flowering plant and its nearest flowering neighbour of each morph were measured and used to calculate the mean intermorph distances shown in Table 1d.

Survival and longevity of morphs

The survival of plants present as seedlings in 1982 is shown in Table 2b. The pattern of survivorship was similar in the two populations and the total mortality over two years was large at both sites. Survival of both old and young flowering plants between 1983 and 1984 was high. At Batcombe and Wyke, respectively, survivorship was $0{\cdot}95 \pm 0{\cdot}15$ and $0{\cdot}90 \pm 0{\cdot}09$ for young adults and $0{\cdot}90 \pm 0{\cdot}17$ and $0{\cdot}94 \pm 0{\cdot}21$ for old adults. There were no significant differences between the survival of flowering plants in the two age groups ($P > 0{\cdot}05$, t-test), but comparing the survival of the different morphs, thrums suffered the greatest mortality and had the shortest longevity in both populations (Table 1e, f).

Clonal growth in experimental conditions

Morphs did not differ in clonal spread under experimental conditions (Table 1g).

Moth-pollination experiment

The number of capsules that set seed on plants of each morph after being caged with each of the two moth types is given in Table 3a. Large moths pollinated pin and thrum equally and resulted in similar values of seed set for all three morphs. Micromoths pollinated pins much more effectively than thrums. Homostyle seed set in the cages was similar to that on the open glasshouse benches. There was no seed set by heterostyle morphs on the glasshouse benches.

Flower number and flower predation

Mean flower number for the three morphs in the two populations is given in Table 3b. Thrums had significantly more flowers than homostyles at Wyke in 1983 and 1984 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. There was more variation in flower numbers between years but within years thrums consistently had the greatest number of flowers.

The rates of flower predation were extremely low. Total rates of damage did not differ between morphs (Table 3c). These measurements probably over-estimate the effect of flower predation on male function because some of the anthers may have dehisced before predation occurred.

Seed production, capsule predation and seed weight

Table 3d shows the percentage of flowers that set seed at Wyke. The homostyle flowers that did not set seed were generally buried low in the vegetation and were therefore more prone to rotting than other flowers. Pin seed set was also high but it was lower than that for homostyles whilst thrum seed set shows the greatest variation and was the lowest of all three morphs in two of the three years studied (Table 3d).

A comparison of seed output of the three morphs is shown in Table 3e. Homostyles produced significantly more flowers than pins in Batcombe in 1983 (Table 3b) but produced fewer capsules containing seeds in that year (Table 3g). There was no evidence of differential pre-dispersal seed predation between morphs (Table 3h), nor was there any evidence to suggest that certain categories of plant, such as those which produced a large number of seed capsules, were more at risk than others.

There was a significant negative relationship between the mean seed weight in a capsule and the number of seeds in the capsule for all three morphs. Only the results for 1983 are presented here (Fig. 1), but the data for the other years of the study are similar. Capsules containing fewer than six seeds were excluded from the regression analysis because these pushed the balance used to weigh them to the lower extreme of its scale.

Linear regression provides an adequate description of the relationship between mean seed weight and the number of seeds capsule^{-1} (Fig. 1). The regression coefficients are significant for all morphs ($P < 0{\cdot}05$) and the gradients of the three lines do not differ significantly. The intercept of the thrum line is significantly lower than that of the pin and homostyle lines ($P < 0{\cdot}05$, analysis of covariance).

TABLE 3. Comparisons of reproductive variables for *Primula vulgaris* morphs. Values are means, with standard deviations shown in parentheses unless stated otherwise. Values in the same row, sharing the same superscript letter differ significantly from each other ($P < 0.05$) using the tests shown in parentheses below: (a) the mean number of capsules per plant^{-1} which set seed after exposure to moths of two size categories (χ^2); (b) the mean number of flowers (*t*-test); (c) number of stigmas and anthers lost from flowers at Wyke in 1984. The proportion of intact flowers did not vary significantly between morphs (*G*-test of heterogeneity); (d) the percentage of flowers which produced capsules with seeds at Wyke (χ^2); (e) the mean number of seeds plant^{-1} (χ^2); (f) the mean number of seeds capsule^{-1} (χ^2); (g) the mean number of capsules plant^{-1}. Capsule numbers did not differ significantly between morphs within years (χ^2); (h) the percentage of seed capsules which suffered predation by small mammals. The morphs were not attacked differentially (χ^2).

		Morph		
		Pin	Thrum	Homostyle
(a)				
Micromoths		3·8 (0·3)^{a}	0·8 (1·1)^{ab}	4·1 (0·8)^{b}
Macromoths		4·4 (0·4)	4·6 (1·3)	4·3 (0·4)
(b)				
Batcombe	1982	3·7 (1·1)	4·1 (1·2)	—
	1983	3·9 (0·4)^{a}	4·3 (0·74)^{a}	—
Wyke	1982	6·2 (2·1)	8·0 (1·7)	6·4 (1·6)
	1983	8·6 (1·9)	9·1 (2·1)^{a}	8·2 (1·8)^{a}
	1984	8·4 (1·9)	8·6 (1·8)^{a}	8·0 (2·0)^{a}
(c)				
Stigmas lost		5	0	8
Anthers lost		7	12	0
Both lost		8	0	8
% damaged		7·4 (270)	4·6 (262)	6·0 (266)
(d)				
Wyke	1982	88·8 (232)^{a}	81·7 (202)^{a}	98·4 (187)^{a}
	1983	83·3 (204)^{a}	59·5 (247)^{a}	94·8 (210)^{a}
	1984	89·5 (200)	92·6 (175)	93·8 (243)
(e)				
Batcombe	1982	103	90	—
	1983	74^{a}	41^{a}	—
Wyke	1983	78^{a}	75^{b}	143^{ab}
	1984	214^{a}	324^{ab}	269^{b}
(f)				
Batcombe	1982	25·1 (4·9)	29·0 (5·2)	—
	1983	21·5 (2·2)^{a}	14·9 (2·6)^{a}	—
Wyke	1983	19·9 (1·6)^{a}	23·4 (4·1)^{b}	39·7 (2·8)^{ab}
	1984	48·8 (3·9)^{a}	63·5 (7·9)^{a}	57·2 (2·2)^{a}
(g)				
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)
(h)				
% Attacked		16·9 (248)	21·5 (274)	14·5 (325)

Seeds in the soil

A mean of $3·0 \pm 8·2$ *P. vulgaris* seeds sample^{-1} was found at Batcombe and $3·7 \pm 7·9$ at Wyke. There was therefore a persistent seed pool in these populations, although it was

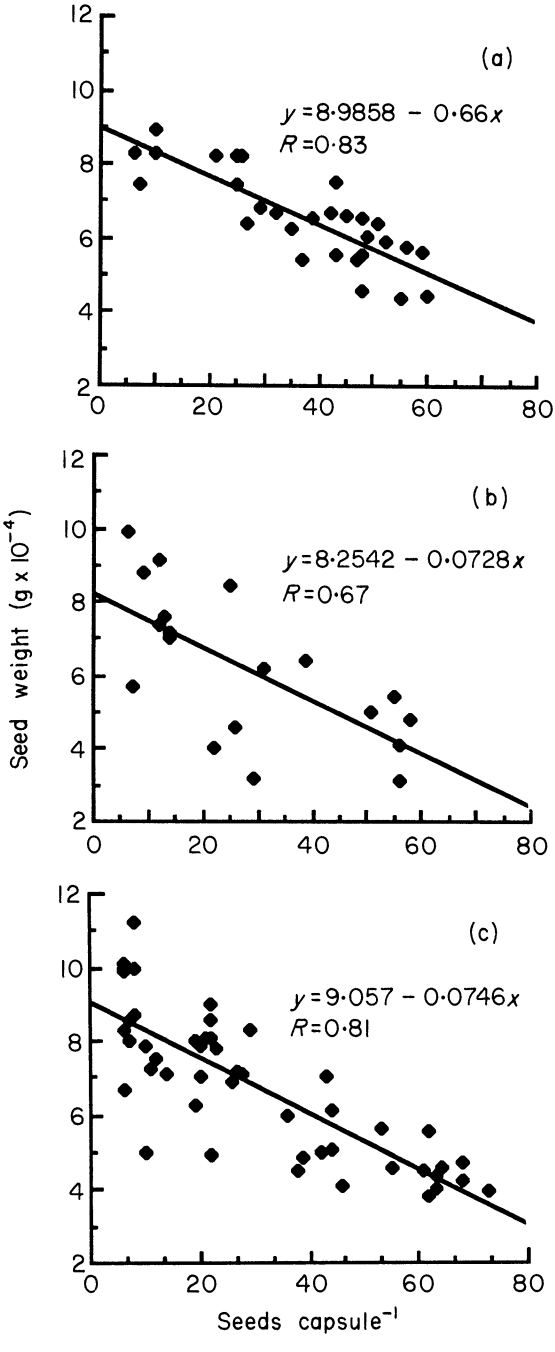

FIG. 1. Relationships between seed number capsule $^{-1}$ and mean weight ($g \times 10^{-4}$) of individual seeds of *Primula vulgaris* in capsules for (a) homostyle, (b) thrum and (c) pin morphs harvested at Wyke, Somerset in 1983.

very patchily distributed as can be seen from the large standard errors. These samples correspond to mean densities of over 1000 seeds m^{-2}.

The seeds separated from the seed pool looked as though they were from the larger end of the range noted in Fig. 1, but could not be weighed individually. They could have swelled either in the soil or during their extraction. Alternatively, smaller *P. vulgaris* seeds could have escaped detection but this is not likely as smaller seeds of other species were found in profusion.

The number of seeds from the seed pool that could be germinated was relatively small but this may have been because they were oven-dried rather than that they were inviable in the first place. The samples produced eight pin and seventeen thrum morphs from Batcombe and four pin, thirteen thrum and one homostyle from Wyke. These numbers are too small to analyse statistically or to interpret further.

DISCUSSION

Growth and survival in the field

The comparisons of relative morph densities, survival, rosette size and clonal growth made in this study did not reveal any ecological disadvantage affecting homostyles—indeed, quite the reverse. Total plant numbers and the number of flowering adults increased at both sites during the course of the study and at Wyke, where there were homostyles present, this morph increased more than the other two.

On the basis that homostyle pollen is of the same incompatibility type as thrum pollen, it is expected that thrums will decrease in frequency faster than pins in a population where homostyly is spreading. This process would take several generations of *P. vulgaris* to observe. An unexpected short-term decline in thrums was observed, which must have had an ecological rather than a genetic cause. Thrums did increase in number at Wyke, but because this increase was smaller than the increase of pins or homostyles, their frequency fell. The survival of thrums was also consistently and significantly lower (Table 1e). This differential mortality appears not to have been size-related, as there was no significant size difference between the morphs (Table 1c).

In fact, thrums suffered the greatest mortality in both populations (Table 1e) and had the lowest life expectancy in all three years (Table 1f). Differential survival of morphs has not been demonstrated before, although it has been proposed as a possible explanation for biased morph ratios (Charnov 1982) which have been found in some heterostyle populations (Crosby 1949, Ganders 1979). In purely heterostyle populations the mating system should stabilize the morph ratio at 50:50, because half the progeny of every legitimate cross are of each flower morph. This imposes a limit on how far the relatively poorer survival of thrums can bias morph ratios in a heterostyle population. The situation is different in a population containing homostyles, and here the poorer survival of thrums should accelerate the spread of homostyly because it would reduce the competition faced by homostyle pollen for pin stigmas. If the survival disadvantage of thrums varies from population to population because of ecological differences between them, this could render invasion by homostyles easier in some populations than in others. From the disappearance of homostyles at Batcombe it seems that differences in invasibility between populations do exist.

Although there were 21–29% homostyles at Wyke and none at Batcombe, total plant densities were similar at the two sites. Total numbers *P. vulgaris* appeared to have at least

doubled at Batcombe since Crosby (1949) found a low frequency of homostyles there. Despite this, no homostyles were found at Batcombe in the present study.

According to Crosby's (1949) model, homostyles should increase once they have become established in a heterostyle population but their extinction at this site could possibly be explained by random changes in gene frequency of a kind which he allowed for in his later model (Crosby 1960).

Pins were always more common than thrums in the Batcombe population (Table 1a), particularly in 1983. This may be the result of a low level of selfing by pins (Crosby 1949; Ganders 1979) or of survival differences between the morphs (Table 1e).

For any given plant, its nearest neighbour was most often another plant of the same morph (Table 1d). There could be for several reasons for this. The site could be composed of a mosaic of microsites which would favour one morph over another. This explanation requires that there should be 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 was vigorous in all three morphs when they were grown in fertile compost (Table 1g) and it is likely to occur, although less prolifically, in the wild. There was no difference in clonal growth between morphs grown in experimental conditions, but it cannot be ruled out that such differences would show up after more flowering seasons, or in less nutrient-rich soil. If the morphs allocate differing quantities of resources to sexual reproduction they may, as a result, have differing levels available for clonal growth after several seasons of reproduction.

Ecological differences between the sexes of dioecious plants are common (Lloyd & Webb 1977), but have not been reported for the polymorphic mating types of cosexual plants. Since homostyles are highly selfing (C. Curtis & J. Curtis unpublished) and heterostyles obligately out-cross, homostyles produced by selfing might be expected to suffer some inbreeding depression. If this occurs, it was not great enough to prevent the increase in homostyles at Wyke.

There is a close parallel between the problem of explaining the maintenance of heterostyly in *Primula vulgaris* and that of explaining the maintenance of sex itself (Piper, Charlesworth & Charlesworth 1984). Both homostyles and parthenogens have a twofold advantage over obligately out-crossing individuals, but both sexual reproduction and heterostyly persist, the latter having evolved many times independently (Vuilleumeir 1967). A general answer to both problems may lie in the distinction between long-term and short-term advantage. Parthenogenetic lineages tend to be short and of recent origin, suggesting that parthenogens run a higher risk of extinction than sexual taxa. The disappearance of homostyles from Batcombe remains unexplained, but suggests that there may be a parallel long-term hazard of extinction in these plants.

Reproduction and pollination

The results in Table 3 indicate the relative importance of different stages of the reproductive process in producing differential fecundity between morphs. Homostyles start with the advantage of not requiring cross-pollination, which the glasshouse experiment demonstrated can be accomplished by large moths. Although thrums at Wyke had significantly more flowers than homostyles in two of three years (Table 3b) rates of fertile capsule production were usually lower (Table 3d), so that equivalent numbers of capsules were set by all three morphs (Table 3g). The number of seeds capsule^{-1} varied between morphs (Table 3f), with homostyle > thrum = pin in 1983 when pollination was

poor, and thrum > homostyle > pin in 1984 when pollination was good. These relationships were almost exactly reproduced in the numbers of seeds plant^{-1} in the respective years. Neither flower nor capsule predation (Table 3c, h) affected morphs differentially and both can thus be ignored for present purposes.

Thus far, these results agree well with the findings of Piper, Charlesworth & Charlesworth (1984), Piper & Charlesworth (1986) and M. Wilson (in Richards 1986) in showing that homostyles in Somerset populations have a fecundity which is higher than or equivalent to that of heterostyles, depending upon the activity of pollinators. Like Piper & Charlesworth (1986) this study measured one more component of seed yield–seed weight. They concluded that self-fertilization did not lead to reduced mean seed weight, but this was based upon data for 1984 when pollination was good and naturally pollinated heterostyles and homostyles produced equivalent numbers of seeds capsule^{-1}. That result showed that inbreeding depression did not affect seed mass. However, Piper & Charlesworth (1986) did not report a regression of mean seed weight on seeds capsule^{-1}, and this study found a consistent and significant negative relationship between these variables in all three morphs (Fig. 1).

A negative correlation between seed size and seed number capsule^{-1} has been found in *Ipomopsis aggregata*, which like *Primula vulgaris* is pollinator-limited. Wolf *et al.* (1986) demonstrated experimentally that the number of seeds set in a capsule was positively correlated with the number of pollen grains received on the stigma, thus confirming that pollinator activity can influence seed weight.

The trade-off between seed size and number in *P. vulgaris* suggests a potentially significant ecological handicap of homostyly. Although this results from selfing it is not a consequence of inbreeding depression. In years of poor pollination homostyle seeds will be smaller than heterostyle seeds (Fig. 1). For example, the mean number of seeds capsule^{-1} in 1983 for the three morphs was 19·9 pin, 23·4 thrum and 39·7 homostyle (Table 3f). The mean individual seed weights from Fig. 1 would then be 0·96 mg for pins, 0·733 mg for thrums and 0·68 mg for homostyles despite the lower intercept of the thrum line.

In other species size of seed has been shown to be a variable which strongly affects plant fitness when seedlings grow in dense stands (e.g. Black 1958). In such situations small differences in initial seed weight can be transformed into large differences in plant size and survival because larger seeds capture a strongly disproportionate share of available light. Although homostyles may be handicapped by relatively small seeds they will simultaneously have the advantage over heterostyles of greater seed number. Whether or not the seed size–seed number trade-off has a negative effect upon the spread of homostyly must depend upon the relative contribution to homostyle fitness of seed size compared to the contribution of seed number.

It is interesting that the potential seed size handicap of homostyles will operate only when pollinators limit seed set in the heterostyle morphs, for only then will homostyles have more and therefore smaller seeds than heterostyles. In effect the trade-off between seed size and seed number in *P. vulgaris* capsules may operate as an ecological constraint on the evolutionary spread of homostyly. According to this hypothesis homostyly should occur only in populations where heterostyle seed set is not usually limited by pollinators. This is the reverse of the usual expectation that selfing is favoured when pollinators are scarce (Richards 1986).

Ganders (1975) showed that the level of pollination determined whether or not 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 for its breakdown to homostyly. The pollination experiment showed that pollinator type and availability affect primrose fecundity. Thrums can be pollinated by macrolepidoptera whilst pins are likely to be pollinated by microlepidoptera also. It is notable that 1984 was a much better year for moths than 1983 and this was, perhaps by coincidence, the best year for the pollination of *P. vulgaris* 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 *P. vulgaris*. In conclusion, the seed size–number trade-off is potentially the source of a handicap to homostyly but its actual significance depends upon pollinator activity and must be evaluated in the context of the whole life cycle.

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