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Relationships between flowering phenology, plant size and reproductive success in *Lotus corniculatus* (Fabaceae)

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Abstract

Over three years the flowering phenology of individuals of *Lotus corniculatus* has been studied in relation to fruit set and seed predation to determine the relationships between four components of flowering time, plant size and reproductive success. Timings of first and peak flowering, and duration and synchrony of flowering differed between individuals in the same years. Between years, timing of first flowering was highly correlated for the same individuals, and was closely correlated with plant size and duration of flowering–larger plants flowered earlier and for a longer period. Peak flowering and synchrony were not correlated between-years for individuals.

Fruit production and seed predation were correlated with some of the components of flowering phenology in some years, but not in others. The inconstancy of these relationships suggests that directional or stabilising selection is not acting consistently on the aspects of reproductive success studied in this work. The inconstancy of selection may result in the rather asynchronous flowering phenologies of individuals of *L. corniculatus* observed.

We emphasize the importance of studying different components of flowering phenology in relation to individual plant size over several seasons. This work has shown that plant size not only has a direct effect on individual plant fecundity but also can influence flowering time and hence indirectly affect reproductive output.

Introduction

Plant species' flowering phenologies have ecological relevance at a range of scales–from individual genotypic variation in flowering time within a population (the raw material of natural selection) to interspecific, landscape-wide patterns which may support flower visitor diversity within that landscape (Lack 1976; Fox 1990; Bronstein 1995). Consequently, studies of flowering phenology have been used to address ecological and evolutionary questions concerning intra- and interspecific competition, community structure, keystone relationships, coevolution, animal foraging behaviour, phylogenetic constraints and continent-wide patterns (Waser & Real 1979; Frankie & Haber 1983; Gross & Werner 1983; Kochmer & Handel 1986; Rathcke 1988; Bronstein 1995; Hepburn & Radloff 1995).

There has been some debate as to how significant natural selection has been in shaping the flowering phenologies of species. Rathcke & Lacey (1985) and Primack (1985) reviewed many of the studies on flowering time of individual species and supported and developed explanations for how particular flowering patterns might evolve via selection. On the other hand, Kochmer & Handel (1986) analysed data on global patterns of flowering and demonstrated that flowering time is frequently characteristic of a family or genus and is a conservative, perhaps constrained, trait even between continents. Smaller scale studies, such as Johnson (1993) on the Cape flora, reinforced this view. Of particular interest to this study is the explanation of differences in the flowering times of individuals within plant populations, for example variation in timing of first flowering or flowering synchrony. For instance, a high degree of synchrony within a population has been considered adaptive, through promotion of outcrossing and/or satiation of seed predators (Janzen 1976; Augspurger 1981) though it might equally be the result of a recent population bottleneck reducing genetic variability. A number of adaptive interpretations have been given for flowering asynchrony, for example; as an evolutionary response to intraspecific competition for pollinators; for promoting inter-plant pollinator movement; the advantage of increasing mate availability; for dispersion of seed predators; variation in intensity and timing of seed predation and dispersal; or differential selection in different years depending upon environmental factors (Zimmerman 1980; Bawa 1983; Frankie & Haber 1983; Primack, 1985; Rathcke & Lacey, 1985). Alternatively, within-population flowering asynchrony could be due to relaxed selection on natural genetic variability and/or environmental heterogeneity (Ollerton & Lack 1992). One way to approach this problem is to study how the reproductive success of individual plants varies with the exact flowering times of those plants, utilising either the natural variation in flowering time inherent in most plant populations or by artificially manipulating flowering times.

As well as a lack of consensus in the literature as to the adaptive nature of flowering time, different workers have studied different aspects of flowering phenology when relating it to reproductive success. In this study we deal with four components of flowering phenology:

(1) Timing of first flowering.

(2) Duration of flowering.

(3) Shape of flowering pattern, i.e., how the rate of flower production varies over the flowering period, perhaps resulting in a flowering peak.

(4) Overlap in flowering with other individuals in the population ('synchrony').

The most widely used measure has been timing of first flowering, followed by timing of peak flowering; synchrony is more rarely used. Zimmerman (1988) considered that there is 'ample evidence' that timing of first flowering is heritable and this is borne out by the agricultural literature and by the, albeit limited, studies of wild plants (Pors & Werner 1989; Fox 1990; Widén 1991; Mitchell & Shaw 1993). In our view, measuring only first or peak flowering time is not adequate as selection could be acting on other aspects of the species' flowering phenology (e.g. duration or synchrony) or be counteracted by other facets of the plant's ecology, e.g. individual size (see below). For the majority of self-incompatible species, or those which benefit from maximum outbreeding, one of the most important things for an individual is its flowering behaviour in relation to other plants in the population, i.e. the level of flowering synchrony. The relative flowering synchrony of individuals may well be related to the timing of first flowering and the shape and duration of the flowering curve of the plants in a population. All these are potentially heritable characters. Few studies have addressed the relationships between the different components of an individual's flowering phenology, variation in plant size and individual reproductive success.

Plant size is usually considered a predominantly environmentally influenced characteristic, determined by plant age and growing conditions, though not often tested (Waller 1988). It is normally closely correlated with total flower production and the largest plants in a population are usually the most fecund (Weiner & Thomas 1986; Weiner 1988; Herrera 1993).

In this study we consider individual plant size and flowering phenology of *Lotus corniculatus* L. (Fabaceae). Initial observations showed that this species has a long flowering season, but individuals differed in size and flowering pattern. Our aim was to see whether plant size affected flowering phenology, how size and phenology in turn influenced maternal reproductive success, and to assess whether selection is likely to be causing the observed patterns through the differential reproductive success of individuals in relation to flowering phenology. More specifically, the objectives of this study were as follows:

(1) To determine how variable the following four components of flowering time are, between individuals within years and between years for the same individuals: timing of first flowering, timing of peak flowering, duration of flowering time and flowering synchrony.

(2) To examine how these components of flowering phenology are related and how they in turn relate to plant size.

(3) To determine whether female reproductive success (measured by fruit-set and seed predation) is correlated with flowering phenology in a pattern consistent with an adaptive explanation for the pattern of flowering in this species.

Only female reproductive success was studied in this hermaphroditic species; the less tractable male component was not considered, though male function may have important implications for the evolution of flowering phenologies (see Discussion).

The species and study sites

Lotus corniculatus L. (Birdsfoot trefoil) [Fabaceae] is an iteroparous perennial with a deep tap root, growing mainly in grasslands and heaths in Europe, Asia and Northern Africa (Jones & Turkington 1986). Regeneration is mostly by seeds; vegetative spread may occur in places, but stems rarely root into the soil (personal observation). It has inflorescences of 1 to 5 (occasionally up to 9) yellow flowers. The pollinators are *Bombus* spp. (Hymenoptera: Apidae) (Proctor et al. 1996; Ollerton 1993). Flowers are often visited by species of Lepidoptera, but they do not contact the reproductive parts. A late-acting self-incompatibility system appears to be present (Seavey & Bawa 1986) and selfing rates under normal circumstances are extremely low (Darwin 1876; Brødsgaard & Rasmussen 1990; Ollerton 1993). The fruit is a pod containing between 1 and 30+ brown, sometimes mottled, seeds; it is dehiscent, splitting longitudinally and catapulting the seeds to distances of over five metres (Rasmussen & Brødsgaard 1992). General reviews of the biology of *L. corniculatus* are given by Turkington & Franko (1980) and Jones & Turkington (1986).

A genetic basis for timing of first flowering in a cultivar of *L. corniculatus* was reported by Sandha, Twamley & Christie (1977) who found that there was a high heritability for the number of days from germination to flowering, using either seedlings (h^2=84%) or cuttings (h^2=87%). *L. corniculatus* is an important forage crop in some parts of the world and early and late flowering varieties have been bred (Buzzell & Wilsie 1964). Also under genetic control are a number of characteristics which conceivably could influence plant size, e.g., stem length and leaf size (Jones and Turkington 1986).

Compton (1983) surveyed the insects which feed on *L. corniculatus* and described some aspects of their ecology. He found that the larvae of three insect species were responsible for most pre-dispersal seed predation: *Cydia compositella* (Lepidoptera: Tortricidae); *Eurytoma (Bruchophagus) platyptera* (Hymenoptera: Chalcidoidea) and *Apion loti* (Coleoptera: Curculionidae). Seed predation by the latter weevil can result in damaged, viable seeds in which seed dormancy has been broken (Ollerton & Lack 1996).

The work described here used plants from two parts of the Wytham Estate, Oxfordshire, U.K. - Upper Seeds and Lower Seeds Reserve (see Gibson 1986). The sites are situated adjacent to one another on the top of Wytham Hill, and have thin soils overlying Jurassic corallian limestone. Upper Seeds was abandoned as an arable field in 1981; Lower Seeds Reserve has not been cultivated since at least 1960, and perhaps not since 1945. Different levels of grazing by deer, other mammals (e.g., rabbits) and sheep exist; the plants used in this study were generally not grazed over the summer. Both sites are open grassland, somewhat variable in composition, with scattered tree saplings. They are partially separated by a strip of woodland some 50 m wide, which is unlikely to be a barrier to the *Bombus* pollinators of *L. corniculatus* and, since it is also possible that seed dispersal via deer is occurring between the sites, we have treated plants from Upper Seeds and Lower Seeds Reserve as a single population.

Methods

Individual, randomly chosen marked plants of *Lotus corniculatus* were used in this work. Plants were chosen according to the following criteria:

(1) Size: a range of plant sizes was selected to enable the relationship between size and reproductive output to be established. This range encompassed most of the variation in plant size found at the site except for some of the very largest patches where it was unclear whether they were single plants or several coalesced individuals.

(2) Position: plants were chosen which as far as possible reflected the distribution of this species in the two sites, though avoiding those parts of Upper Seeds grazed by sheep in summer.

(3) Discreteness: *L. corniculatus* grows from a central perennating point and single individuals on these sites tended to be more or less circular in shape and usually well defined. These were the plants chosen, as opposed to plants which could not be clearly distinguished as single individuals (see (1), above).

The number of marked plants varied between years: 1990 = 19; 1991 = 41; 1992 = 34. Where possible the same plants were followed from year to year, though losses due to plant death, occasional deer grazing and ambiguous identification of individuals, etc. has reduced to 9 the number of plants which spanned the full three years. When sample sizes deviate from these numbers (e.g. some small plants produced no fruit in some years) the sample sizes are indicated by the stated degrees of freedom, i.e. for correlation and regression n = df + 2.

Flower censusing

The number of flowers produced by each of the plants in each of the three years was counted every 6 or 7 days, with occasionally up to 12 days between counts. In 1990, all of the flowers were marked with ink as they were counted; from this, it was found that the flowers usually last about 7 days, depending to some extent upon weather conditions and perhaps whether they had been pollinated. A negligible number of flowers opened and withered between censuses, or lasted in good condition to the next census. For this reason flowers were not marked in 1991 and 1992.

In 1990 all of the flowers produced by all of the plants were counted; in 1991 and 1992 samples were taken of large plants at times of peak flowering. The data were recorded as numbers of flowers per inflorescence. Inflorescences in which some of the flowers were open and others closed were included in that day's census.

Rate of flower production, i.e., the number of new flowers produced per day by a plant, was calculated from the total number of new flowers censused on a particular day divided by the number of days since the last census of that plant.

Flowering synchrony was calculated using the method of Augspurger (1983, modified from Primack 1980). Each plant was assessed for the number of days of flowering overlap with all other individuals. The index of synchrony (X) for an individual plant (i) is given by:

$$X_i = \left(\frac{1}{n-1}\right)\left(\frac{1}{f_i}\right)\sum_{j=i}^{n} e_{j\neq i}$$

where: $e_{j\neq i}$ is the number of days individuals i and j overlap in their flowering; f_i is the total number of days individual i is flowering; and n is the number of individuals in the sample. When the flowering time of an individual overlaps completely with all other individuals $X = 1$. When there is no overlap in an individual's flowering time $X = 0$. A measure of the overall synchrony of the population is gained by averaging the individual synchronies.

This index regards all flowering days as equivalent independent of the number of flowers produced on each day, i.e., a single flower has equal weight to a flowering peak. However periods of low flower production are unlikely to contribute much to the reproductive output of an individual plant. Because of this Primack (1980) included only those days on which his plants were 'flowering strongly', which he interpreted as having 50% or more of their flowers open. Using a different approach, Augspurger (1983) included in the index only the days over which 90% of all flowers were produced, i.e., excluding the tail ends of the flowering period, but this did include days of low flower production within the 90% period. In order to compare the effect of different flower production cut-off points we have used three thresholds of 0.1%, 10% and 20% of the total flower production of each plant; any days with flower output at or below this level are counted as days when no flowers were produced. The aim of this was to assess how these different thresholds affected the calculation of flowering synchrony.

Fruit set and seed predation

In 1990 all of the fruits of the census plants were collected. In 1991 and 1992, except for the smallest plants, samples of fruits from each plant were collected using random 10 cm $\times$ 10 cm quadrats. These were used to calculate total fruit production per plant, by relating mean number of fruits per unit area in samples to the total area encompassed by the plant. Each pod was subsequently dissected, the number of undamaged seeds counted, and the identity and numbers of any seed predators noted. For most plants, the majority (up to 100% in smaller individuals) of pods contained seed predators and estimating total numbers of seeds per pod prior to any predation proved impossible. In 1991 and 1992, therefore, the lengths of mature, undamaged pods were measured; this proved to be closely correlated with number of seeds per pod (Pearson's Correlations 1991: 0.78; df=37; p <0.001. 1992: 0.83; df=35; p <0.001). In the following analyses mean pod length is used as a proxy measure of mean number of original seeds per pod, prior to any predation.

Because of these problems of high seed predation it was decided to score overall seed predation as the numbers of pods showing evidence of seed predation, rather than number of seeds destroyed.

Summary of methods

Using the above methods, for each individual plant, in each of the three years, the following information was obtained:

(1) Timing of first flowering.
(2) Shape of flowering pattern.
(3) Duration of flowering.

(4) Flowering synchrony.
(5) Total flower production.
(6) Total fruit-set.
(7) Mean pod length (1991 and 1992 only).
(8) Total number of pods suffering seed predation.

Statistical analyses

Formal analyses of selection coefficients have not been attempted with these data because such analyses require that the total seed output of a plant is known. Seed predation for most plants at our site was very high and hence accurate estimates of seed production were not possible. Instead we have correlated phenological and size components with other measures of reproductive success, e.g. percentage fruit set, proportional fruits suffering seed predation, etc. Pearson correlations have been used when data were normally distributed; Spearman rank correlations were used when data were non-normally distributed and could not be transformed. We were searching for correlations consistent across three years, so the likelihood of Type I errors occurring is much reduced.

Sample sizes varied depending upon the analysis performed. For example, some very small plants did not have a well-defined flowering peak, and so had to be excluded from analyses involving that particular component of flowering phenology. Also, quite a number of plants did not produce sufficient mature, undamaged fruits to allow an accurate estimate of mean pod length. As we have already stated (see above) the degrees of freedom cited for each test indicates the sample size.

Over the three years of this study, larger plants produced more flowers, fruits and seeds than smaller plants (Ollerton 1993; see also ‘The effect of plant size on reproductive output and seed predation’ in the Results section) and so total reproductive output may not be a good indicator of the likelihood of selection acting on particular plant traits. For this reason proportional (percentage) measures of reproductive output have mostly been used, except for mean pod length and percentage pod predation, which are not correlated with plant size. The three measures of potential reproductive success which have been used are: percentage fruit set, mean pod length and percentage pod predation. Mean pod length may be taken as a measure of potential reproductive success if it is determined by frequency of pollinator visits, as is possible in a highly self-incompatible plant such as *L. corniculatus*, or by resource availability.

Results

Reproductive behaviour of the Wytham population

In each of the three years, individual plants exhibited a wide range of flower and fruit production, proportional fruit set, fruit length and pod predation (Table 1). For example, in 1991 flower production spanned five orders of magnitude whilst the percentage of fruit set from these flowers varied from 0% to 100%. Between years differences are also apparent in all of these reproductive parameters. Thus there appears to be ample variation in reproductive output between individual plants which, if consistently correlated with flowering time, could result in natural selection occurring.

The effect of plant size on reproductive output and seed predation

Plants with more flowers consistently produced greater numbers of fruit, though the proportion of flowers setting fruit was only correlated with total flower production in 1990 (Table 2). Total flower production of individuals was closely correlated between years (1990 v 1991: $r = 0.87$; df $= 9$; $p = 0.0002$. 1991 v 1992: $r = 0.83$; df $= 32$; $p < 0.0001$). Percentage pod predation was negatively correlated with total flower production (Table 2) – larger plants suffer proportionately less seed predation. There was no significant relationship between plant size and mean pod length (Table 2).

Quantifying flowering patterns

Flowering patterns of individual plants varied considerably both within one season and between years for the same plant (Figure 1). Individual plants usually had a well-defined flowering peak or peaks except for some small plants in Lower Seeds Reserve, which were consequently excluded from analyses involving peak flowering time. Between individuals in any one year, there was variation in the timing of first flowering and flowering peak, duration of flowering and degree of flowering synchrony (Figure 2a and b). The amount of variation and the shapes of the frequency distributions for each component of flowering phenology varied considerably between years; for example, there was a much broader spread of first flowering dates in 1991 than in 1990 and 1992, and a more right-skewed synchrony distribution in 1992 compared with the other two years.

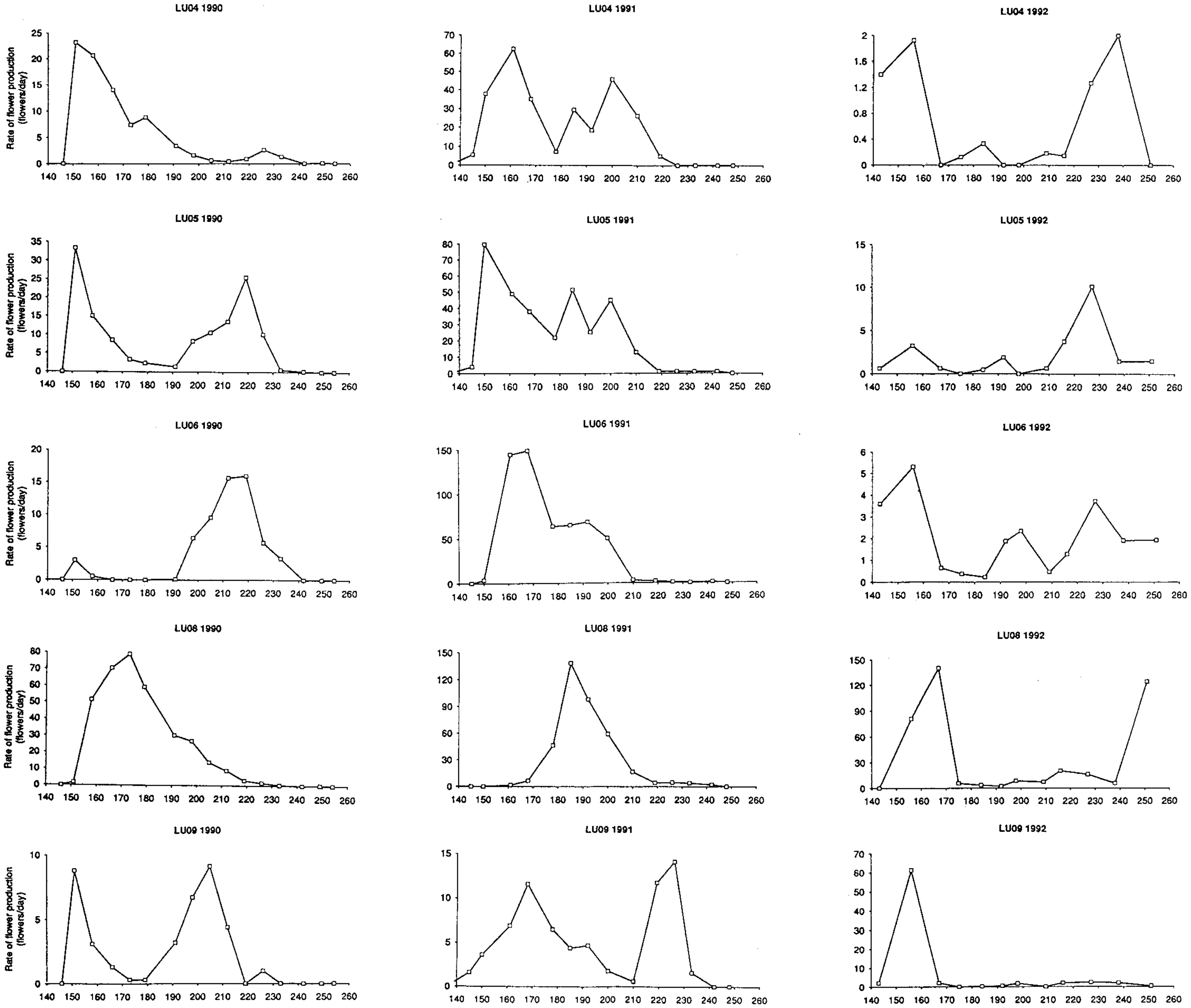

Figure 1. Representative examples of the flowering phenology of *Lotus corniculatus* in Upper Seeds. Five individual plants in the three years of the study are shown. Letter/number combinations at the top of each graph (e.g. LU04) are individual plant identification codes. Dates are given by day of the year, with 1st January = 1.

Table 1. Summary of reproductive behaviour of the Wytham population of *Lotus corniculatus* in the three years of the study.

	1990		1991		1992	
	Range	Mean (s.e.)	Range	Mean (s.e.)	Range	Mean (s.e.)
Total flower production	6–210	371.0(150.3)	1–13014	1782.2(409.4)	0–6321	830.6(272.1)
Total fruit-set	0–1994	255.1(111.6)	0–5560	480.4(167.9)	0–817	119.6(37.1)
% fruit-set	0–85	43.6(7.1)	0–100	26.7(3.8)	0–57.1	22.6(2.6)
Mean fruit length (mm)	–	–	14.9–21.7	17.6(0.6)	13.7–18.5	15.4(0.6)
% pods suffering predation	0–100	60.8(6.0)	34–100	82.4(3.1)	50–100	92.3(2.4)

Table 2. Spearman Rank correlations between total flower production and female reproductive success for the Wytham plants in 1990, 1991 and 1992. Correlations significant at $p \leq 0.05$ are in **bold** type.

Total flower production v	1990	1991	1992
Total fruit	**0.92; df=17; $p<0.0001$**	**0.89; df=38; $p<0.0001$**	**0.95; df=31; $p<0.0001$**
% fruit set	**0.69; df=17; $p=0.0005$**	0.008; df=38; $p=0.48$	0.075; df=31; $p=0.34$
Pod length	–	-0.18; df=11; $p=0.59$	0.42; df=7; $p=0.30$
% pod predation	**-0.48; df=17; $p<0.02$**	**-0.58; df=33; $p=0.0001$**	**-0.40;df=25; $p<0.02$**

The flowering synchronies of individual plants were calculated using three flowering thresholds - 0.1%, 10% and 20% of total flower production. At higher threshold values, the mean population synchrony is reduced, as expected (Table 3), i.e. individuals become more asynchronous because their effective flowering period is shortened, decreasing the number of days in which their flowering overlaps with other individuals. The synchronies of individual plants are highly correlated across the flowering thresholds used (Table 3) and only the results obtained using the 10% threshold are presented for correlations involving this component of flowering phenology.

For any one plant timing of first flowering was consistently correlated between years, as was duration of flowering (Table 4). Thus early flowering individuals in one year also flowered early the next and plants with long flowering periods in one year had long flowering periods in the next. There was no consistent between-years correlation for timing of peak flowering and synchrony (Table 4) – individual plants varied from year to year in the exact timing of their peak flowering period and their relative synchrony with the rest of the population.

Within-year correlations between the components of flowering phenology for each individual plant are generally inconsistent (Table 5) with the following exceptions: (i) in two out of three years (1991 and 1992) timing of first flowering is positively correlated with timing of peak flowering – plants which started flowering earlier tended to have an earlier peak of flowering intensity; (ii) there is a negative relationship between flowering synchrony and duration which is significant in 1990 and marginally significant in 1992 – in some years at least, plants which flowered for a longer period had a smaller overall synchrony; (iii) there is a consistent negative relationship between date of first flowering and duration of flowering - plants which flowered earlier tended to flower for a longer period. Of particular interest is the fact that no single aspect of flowering phenology determines an individual plant's relative flowering synchrony. As individual plants' synchronies are inconsistent between years but timing of first flowering and duration of flowering are consistently correlated (Table 4), it is likely that the relative importance of the three other components of flowering phenology varies between years. This is backed up by our finding that synchrony is correlated with timing of first flowering in 1991, with timing of peak flowering in 1990 and with duration in 1990 (and perhaps 1992) (Table 5).

Total flower production was consistently correlated with only two components of flowering phenology: timing of first flowering and duration of flowering – plants which produced more flowers had an earlier flowering date and flowered for a longer period (Ta-

Table 3. Flowering synchrony data for the Wytham plants in 1990, 1991 and 1992. Values given are Pearson Correlations except those superscripted^{S} which are Spearman Rank Correlations. Correlations significant at $p \leq 0.05$ are in **bold** type.

Flowering threshold(%)		Mean index of synchrony ±95% c.l.	Range
0.1	1990	0.77±0.20	0.62–0.93
	1991	0.76±0.18	0.35–0.92
	1992	0.68±0.10	0.60–0.81
10.0	1990	0.66±0.18	0.39–0.78
	1991	0.67±0.26	0.23–0.91
	1992	0.49±0.10	0.36–0.75
20.0	1990	0.56±0.19	0.26–0.72
	1991	0.59±0.27	0.17–0.87
	1992	0.42±0.22	0.22–0.72

Correlations between flowering thresholds	1990	1991	1992
0.1% v 10%	**0.67; df=17; $p < 0.001$**	**0.75; df=36; $p < 0.0001^{S}$**	**0.67; df=29; $p < 0.0001$**
0.1% v 20%	**0.62; df=17; $p < 0.003$**	**0.76; df=36; $p < 0.0001^{S}$**	**0.54; df=29; $p < 0.001$**
10% v 20%	**0.98; df=17; $p < 0.0001$**	**0.97; df=36; $p < 0.0001$**	**0.96; df=29; $p < 0.0001$**

Table 4. Between-years correlations for the four components of flowering phenology of the same individual Wytham plants in 1990, 1991 and 1992. Values given are Pearson Correlations except those superscripted^{S} which are Spearman Rank Correlations. Correlations significant at $p \leq 0.05$ are in **bold** type.

	1990 v 1991	1991 v 1992
First	**0.79; df=9; $p < 0.002$**	**0.78; df=30; $p < 0.0001^{S}$**
Peak	−0.16; df=9; p=0.32	**0.53; df=26; $p < 0.002^{S}$**
Duration	**0.59; df=9; $p < 0.03$**	**0.75; df=23; $p < 0.0001$**
Synchrony	−0.20; df=9; p=0.28	**−0.50; df=24; $p < 0.005$**

Table 5. Within-years correlations between the four components of flowering phenology for Wytham plants in 1990, 1991 and 1992. Values given are Pearson Correlations except those superscripted^{S} which are Spearman Rank Correlations. Correlations significant at $p \leq 0.05$ are in **bold** type.

		First	Peak	Duration
Peak	1990	−0.01; df=16; p=0.48		
	1991	**0.53; df=37; $p < 0.001$**		
	1992	**0.54; df=26; $p < 0.002^{S}$**		
Duration	1990	−0.35; df=16; p=0.076	0.21; df=16; p=0.21	
	1991	**−0.64; df=37; $p < 0.0001$**	**−0.53; df=34; $p < 0.01$**	
	1992	**−0.57; df=27; $p < 0.001^{S}$**	0.12; df=25; p=0.28^{S}	
Synchrony	1990	0.15; df=17; p=0.48	**−0.58; df=16; $p < 0.01$**	**−0.56; df=16; $p < 0.01$**
	1991	**0.28; df=36; $p < 0.05$**	0.06; df=34; p=0.37	−0.21; df=35; p=0.11
	1992	−0.08; df=27; p=0.34	−0.14; df=26; p=0.24	−0.25; df=27; p=0.09

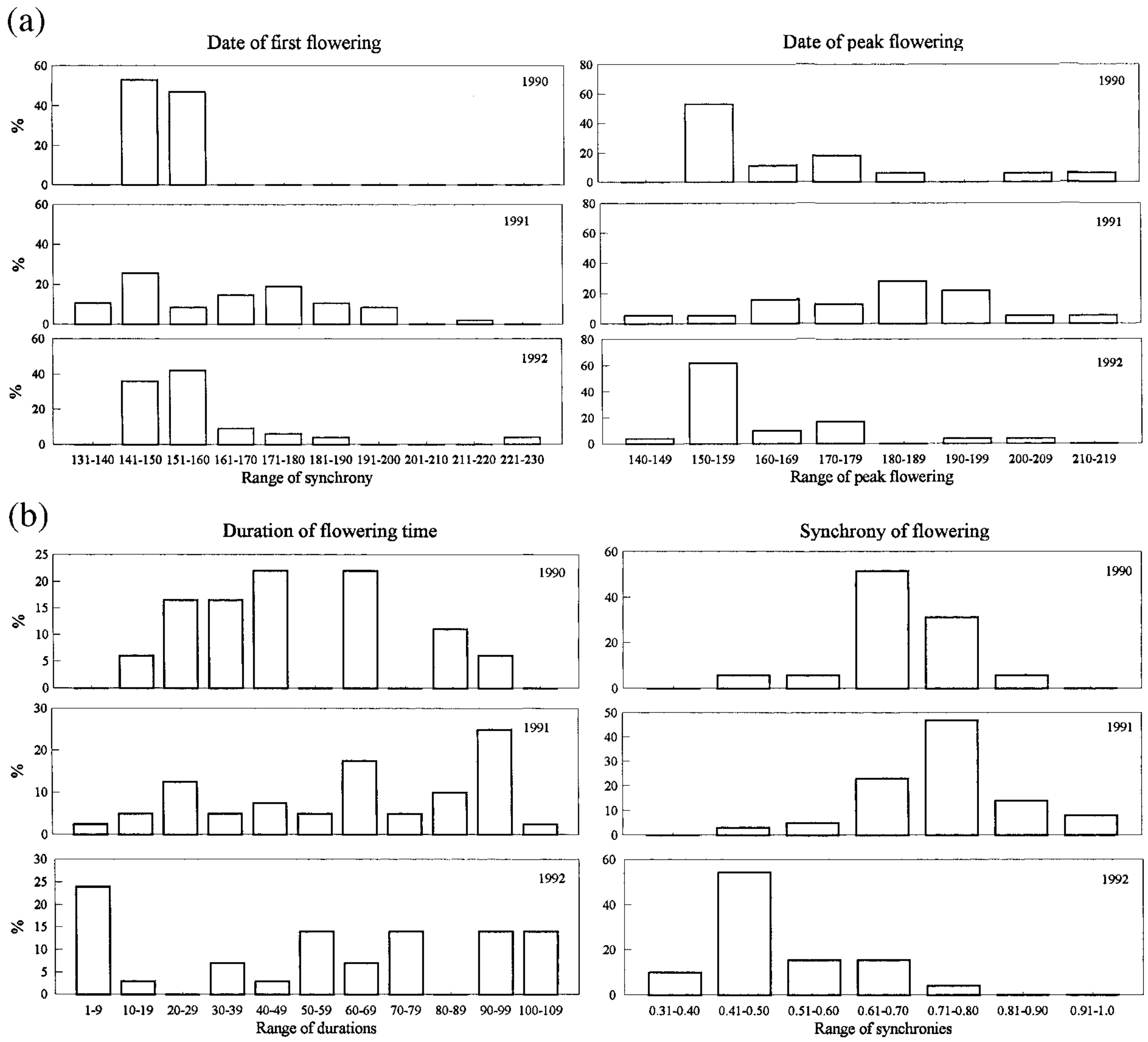

Figure 2. Frequency distributions of the four components of flowering phenology for plants at Wytham in 1990, 1991 and 1992. Dates are given by day of the year, with 1st January = 1. (a) Dates of first and peak flowering. (b) Duration and synchrony of flowering.

Table 6. Correlations between total flower production and the four components of flowering phenology for individual Wytham plants in 1990, 1991 and 1992. Values given are Pearson Correlations except those superscripted^S which are Spearman Rank Correlations. Correlations significant at $p \leq 0.05$ are in **bold** type.

	First	Total flower production v		
		Peak	Duration	Synchrony
1990	**−0.58; df=17;** $p < 0.01^S$	**0.46; df=16;** $p < 0.03^S$	**0.88; df=16;** $p < 0.0001^S$	**−0.65; df=17;** $p < 0.002^S$
1991	**−0.66; df=39;** $p < 0.0001^S$	**−0.56; df=37;** $p < 0.0001^S$	**0.88; df=35;** $p < 0.0001^S$	−0.27; df=36; $p = 0.56^S$
1992	**−0.65; df=31;** $p < 0.0001^S$	−0.01; df=26; $p = 0.48^S$	**0.81; df=27;** $p < 0.0001^S$	0.02; df=28; $p = 0.47^S$

ble 6). The link between total flower production and duration of flowering probably explains why the duration of flowering of individual plants is consistently correlated between-years, as total flower production is correlated between-years (Table 2).

Flowering phenology and reproductive success

Individual plants which have their first or peak flowering dates significantly *before* or *after* the majority of the population could be at a reproductive advantage or disadvantage if reproductive success (measured by fruit-set, seed number per pod or seed predation) is affected by the timing of first or peak flowering. One must therefore look for non-linear relationships between these data, e.g., reduced reproductive success in early and late flowering individuals if there is stabilising selection on flowering time or increased reproductive success in early flowering individuals if there is directional selection favouring earlier flowering. These analyses have been performed and yielded no significant relationships between timing of first or peak flowering and percentage fruit-set, mean pod length or percentage pod predation in any of the three years of this study. For the sake of brevity these results are not presented here, but are available on request from the first author.

Duration and synchrony of flowering, in contrast, would be expected to show linear relationships with reproductive success if selection were acting on these components of flowering phenology, e.g., a positive correlation between percentage fruit set and synchrony would suggest that selection was favouring the most synchronous individuals in the population. Duration of flowering is consistently negatively correlated with percentage pod predation (Table 7) – longer-flowering plants suffer less seed predation. This is probably because duration of flowering is positively correlated with plant size (Table 6) and larger plants suffer proportionately less seed predation (Table 2). Flowering synchrony was positively correlated with percentage pod predation in 1991 and marginally significantly correlated with this in 1992 (Table 7). Thus, there was an inconsistent trend for the most synchronous plants to suffer reduced reproductive output through enhanced seed predation.

Discussion

Larger plants of *Lotus corniculatus* produced more fruit and suffered a lower proportion of seed predation than smaller plants and are at a reproductive advantage in this population, at least from the perspective of maternal reproduction. They also flowered earlier and, although there is a genetic component to flowering time, it seems that environmental influences complicate the underlying genetic factors, e.g., are earlier flowering plants larger because they start growing earlier in the season and thus have a longer period in which to produce flowers or do they flower earlier because they are larger and have greater stored resources? If first flowering time was simply an environmentally determined function of plant size, then as smaller plants grew, we might expect their date of first flowering to become progressively earlier. There is no evidence for this - for the 9 plants which were tracked over all three years, the mean (±95% confidence interval) first flowering times are: $1990 = 148.2 \pm 1.6$; $1991 = 156.0 \pm 10.9$; $1992 = 151.9 \pm 7.1$. These mean first flowering dates are not significantly different from one another (one-way ANOVA on log-transformed data: $F_{2,24} = 0.90$; p=0.42) indicating that there is no progression of flowering time. Though individuals do vary between-years in their exact timing, overall mean date of first flowering appeared to be quite constant in this population of *L. corniculatus*, despite significant variation in the total flower production of individuals between years, often spanning an order of magnitude (Ollerton 1993). However, changes in flower production of individuals do not result in predictable changes in the rank order of flowering of those plants - there is no significant relationship between difference in flower production and difference in rank order of flowering between adjacent years (Spearman Rank Correlations: 1990 to 1991 = 0.02; df=6; p=0.9; 1991 to 1992 = -0.26; df=31; p=0.15). Both of these suggest that underlying genetic controls on flowering time can override some of the influence of environmental factors. Large plants certainly seem to be at a reproductive advantage and there is a link between plant size and flowering time, but the overall relative effects of heritability and environmental conditions are not clear.

Several other studies have looked at the effect of plant size on flowering phenology. Primack (1980) found no correlation between total flower production and timing of peak flowering for any of the three New Zealand shrubs that he examined. Schmitt (1983) found that for the annual *Linanthus androsaceus* (Polemoniaceae), larger plants flowered for a longer period, as for *Lotus corniculatus* in this study. In the studies of *Agalinis strictifolia* (Scrophulariaceae), *Lobelia inflata* (Lobeliaceae) and *Silene* *uniflora* (Caryophyllaceae) larger plants flowered earlier (Dieringer 1991, Kelly 1992, Pettersson 1994) whilst Widén (1991) found the opposite to be true in *Senecio integrifolius* (Asteraceae). In *Arum maculatum* (Araceae) larger plants flowered earlier in one population in 1995, but not in a second population in 1996 and 1997 (Ollerton, unpublished). It appears that the effect of plant size on flowering time varies between species and can be inconsistent between years and populations.

The effect of using different flowering thresholds when calculating synchrony indices was as expected: at higher thresholds the apparent synchrony is reduced as the amount of overlap in flowering time between individuals is decreased. This comparison identifies a source of ambiguity in population studies of flowering synchrony – exactly what is a ‘synchronously’ or ‘asynchronously’ flowering population? Direct comparison of the population synchrony values obtained for *L. corniculatus* at Wytham with those obtained by Primack (1980), Augspurger (1983) and Gómez (1993) is unwise, as slightly different techniques were used in each case. Nonetheless, individual plants of *Lotus corniculatus* at Wytham flowered quite asynchronously and were inconsistent in their relative synchrony between years. The other three components of flowering phenology varied in their influence on flowering synchrony. For *Lotus corniculatus* at Wytham the only evidence we have that this ecologically important component of flowering time is under selection is the positive correlation with percentage pod predation in two out of three years. Though this relationship is inconsistent, it may be resulting in selection against flowering synchrony, by favouring less synchronous individuals. This might explain why the population of *Lotus corniculatus* at Wytham has a rather asynchronous flowering phenology. Other studies have also found that the most asynchronous plants in a population are at a reproductive advantage (see Gómez 1993).

To summarise, it is our contention that, for *Lotus corniculatus* at Wytham, there is some evidence that selection may be acting on duration of flowering time and on flowering synchrony (albeit inconsistently) and that it is seed predation that is the factor affecting reproductive output, not seed set. The importance of predispersal seed predation in plant demography and the evolution of plant traits has been debated for some time (e.g., Louda & Potvin 1995; Brody 1992) and this is further evidence that seed predators can affect traits

Table 7. Correlations between duration and synchrony of flowering with four measures of potential reproductive success for Wytham plants in 1990, 1991 and 1992. Values given are Pearson Correlations except those superscripted^{S} which are Spearman Rank Correlations. Correlations significant at $p \leq 0.05$ are in **bold** type.

	Duration			Synchrony		
	1990	1991	1992	1990	1991	1992
% fruit set	**0.60; df=16; p <0.005**	−0.25; df=35; p=0.07	−016; df=25; p=0.21	−0.34; df=17; p=0.08	−0.02; df=35; p=0.45	−0.19; df=26; p=0.16
Pod length	–	**−0.79; df=12; p=0.002**	−0.002; df=8; p=1.0	–	0.21; df=11; p=0.54	0.20; df=4; p=0.8
% pod predation	**−0.42; df=16; p <0.05**	**−0.28; df=33; p=0.05**	**−0.4; df=24; p <0.03^{S}**	0.19; df=17; p=0.22	**0.29; df=32; p <0.05**	0.28; df=25; p=0.08^{S}

such as flowering time which are normally associated with the pollination environment.

A few studies of flowering phenology have demonstrated consistent selection over two or more years on flowering phenology; these are mainly studies of timing of first or peak flowering in relation to seed set or (less often) seed predation (e.g., Schemske 1977; Gross & Werner 1983; Flanagan & Moser 1985; Carthew 1993; Kudo 1993; Louda & Potvin 1995; Eriksson 1995). A majority of workers have only looked at a single year and results from these must be treated with considerable caution since variation between years in the relationship between flowering time and reproductive success has been found by us and other studies longer than a single year (e.g., Zimmerman & Gross 1984; Primack 1980; Galen & Stanton 1991; Pettersson 1992; Gómez 1993; Dominguez & Dirzo 1995). This is consistent with variation in climate and, particularly, abundance and identity of seed predators and pollinators (see references in Ollerton 1996). It follows that the type, direction and intensity of selection on flowering time may differ between years, populations and species.

Plant size was the dominant influence on individual reproductive success of *Lotus corniculatus* in this study. Despite large variations in the four components of flowering time, some of which influence reproductive output, the largest plants in the population were the most fecund and any selection on flowering time is likely to be mitigated by the effect of plant size. Although timing of first flowering and duration of flowering were correlated with plant size, only duration of flowering affected any measure of reproductive success, with longer flowering individuals suffering proportionately less seed predation. It is unclear whether this was a result of the longer flowering period per se, or is an effect of plant size, and in fact it is hard to see how one could untangle these two factors. What is clear is that the interactions between plant size and flowering phenology are complex and any future studies should take into account variation in plant size when studying the effect of flowering time on reproductive success.

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