

An experimental test of frequency-dependent fitness in mixtures of the two seed morphs of *Spergula arvensis*

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Abstract

A ratio cline in the frequency of two seed coat morphs of *Spergula arvensis* occurs in British populations. Experimental mixtures of the two morphs (papillate or smooth) were sown in pots at constant density and a range of frequencies (treatments) to determine whether the polymorphism is maintained by frequency-dependent fitness. Fitness was measured as the ratio of seeds sown to seeds produced by each morph at the end of the experiment. The papillate morph had the higher fitness in all treatments. Germination of papillate seeds was significantly greater than germination of smooth seeds at all frequencies, generating density-differences between treatments. Mortality between emergence and harvest was independent of density and frequency in both morphs, but overall fecundity (seed capsules per pot) was density-dependent. These results are consistent with the predominance of the papillate morph in the source population, but do not help to explain how the polymorphism is maintained.

Keywords: frequency-dependence, density-dependence, ratio cline, germination, fitness, *Spergula arvensis*.

Résumé

On trouve un cline de fréquence relative de deux formes de tégument de graines de *Spergula arvensis* dans des populations britanniques. Des mélanges expérimentaux des deux morphes (à pappus ou lisses) ont été semés dans des pots avec des densités constantes et une variété de fréquences (traitements) pour déterminer si le polymorphisme est maintenu par la valeur sélective dépendant de la fréquence. La valeur sélective est mesurée comme étant le rapport des graines semées aux graines produites par chaque morphe à la fin de l'expérience. Le morphe à pappus présente la valeur sélective la plus élevée dans tous les traitements. La germination des graines à pappus est significativement plus importante que celles des graines lisses à toutes les fréquences, engendrant des différences de densité entre les traitements. La mortalité entre l'émergence et la récolte est indépendante de la densité et de la fréquence chez les deux morphes, mais la fécondité globale (capsules de graines par pot) est dépendante de la densité. Ces résultats sont cohérents avec la prédominance de morphes à pappus dans la population source, mais n'aident pas à expliquer comment le polymorphisme est maintenu.

INTRODUCTION

In plants, genetic differentiation between populations along environmental gradients has frequently been demonstrated for quantitative characters in common garden experiments, or transplants between sites (e.g. BØCHER & LARSEN, 1958; CLAUSEN *et al.*, 1940; MCGRAW & ANTONOVICS, 1983), but Mendelian characters whose frequencies vary clinally have been less extensively studied (DADAY, 1954;

JONES & CRAWFORD, 1977). Ratio clines offer a particular challenge because we must explain two things 1. how a balanced polymorphism is maintained at each station on the cline, and 2. why the equilibrium point changes systematically along the cline. Among the several possible mechanisms that could maintain a stable ratio cline, frequency-dependent fitness is perhaps the most likely and certainly the most testable mechanism, though its presence has rarely been sought in wild plants (ANTONOVICS & KAREIVA, 1988).

In the British Isles *Spergula arvensis*, a widespread calcifuge annual plant, exhibits clinal variation in the frequency of two seed morphs (NEW, 1958, 1978). One morph has a seed coat densely covered in papillae, and the other has a smooth seed coat. Inheritance of these characters is determined by two alleles at a single locus, one homozygote being papillate and the other smooth. The heterozygote has a seed coat with up to half the density of papillae found on the papillate morph, but because selfing rates are high ($s=0.97-1.0$) heterozygotes are rare (NEW, 1959).

The ratio cline was first recorded by New (1958) in a survey of 25 localities in Britain and N. Ireland. This survey showed that the proportion of papillate seeds decreased with increasing distance NNW from Dover. Approximately 90% of plants were papillate at the south-eastern end of the cline and this morph disappeared almost entirely in the extreme north and north west. In 1975-1976 NEW (1978) resampled 16 localities in mainland Britain and found that little change had taken place in morph frequencies during the 20 years since her first survey. Being an annual plant, this timespan potentially represents 20 generations, although seed of *S. arvensis* is capable of longterm dormancy and there is a significant soil seed pool that can considerably lengthen the effective generation time of a plant (TEMPLETON & LEVIN, 1979).

NEW (1958) showed that the proportion of papillate seeds was correlated with mean July temperature along the cline and found that papillate seeds germinated better than smooth ones at high temperature, while the reverse was the case at low temperature. Later experiments by New & HERRIOTT (1981) found that papillate seeds germinated significantly better at low water tensions than did smooth seeds. In summary, all germination experiments to date have provided evidence that papillate seeds are favoured in the warmer, drier climate of the SE, and smooth seeds in the cooler, wetter climate of the N and NW, but no mechanism that might balance the polymorphism has been found. The present paper reports the results of an experiment designed to test for frequency-dependent fitness in mixtures of the two seed morphs.

METHODS

Seeds were collected from a field population of *S. arvensis* at Bragenham, Buckinghamshire (Grid Ref SP 901 290), in July 1988. The papillate morph had a frequency of 77% (95% binomial confidence limits 69%-84%, $n=124$) in this population, and 6.5% (95% binomial confidence limits 3%-13%) of plants were heterozygote. After sorting and cleaning, bulked seeds were kept in dry storage till needed. In November 1990, seeds of each homozygous morph were counted into separate lots of 50, that were then combined to produce six mixtures with the proportions 5/0, 4/1, 3/2, 2/3, 1/4, 0/5 at a total of 250 seeds per mixture. Five replicates of each mixture were sown at an equivalent seed density of 39 298 m⁻² in 9 cm (0.375 L) plastic pots filled with 400 g of a 50:50 mixture by volume of builder's sand and John Innes No. 1 compost. Five pots were filled with soil but left unsown to act as controls against soil contamination. Pots were randomly arranged within an array placed on paving slabs in an unshaded position at an outdoor site 8 km from the source population. For the first five months the

experiment was protected by netting from birds and other disturbance. During each of three dry periods in the spring and summer, pots were watered once with tap water.

Germination is epigeal and over half of all seedlings emerged with the seed coat attached to one of the cotyledons, allowing the seed morph to be identified. When seeds germinated, seedlings were counted and marked with a small plastic, colour-coded ring identifying the seed morph. Unidentifiable seedlings were ringed with a colour to indicate this. The experiment was harvested in the first week of August 1991, by which time plants had ceased to grow and were moribund or dead. At harvest, the maternal phenotype (ring colour) and, where possible, the seed morph of every survivor were recorded, along with the number of capsules/plant. Many capsules had shed their seeds by the time of harvest, but seeds/capsule were recorded from one mature capsule per plant whenever an undehisced capsule was present.

RESULTS

Virtually all seedlings emerged between 20 Nov.-21 Dec. 1990. The unsown, control pots showed the soil mixture was free of any seed contamination. The maternal phenotype of over half of all seedlings was identifiable when they emerged. Proportions of seedlings with/without an attached seed coat did not vary between the two monomorphic treatments (60%, 65%, $G=1.91$, $p>0.05$ [G-test; SOKAL & ROHLF, 1981]), so it was possible to treat identifiable seedlings as an unbiased subsample of the composition of each mixture. Based on total emergence in the monomorphic treatments, germination rates were 33% for papillate seeds and 19% for smooth ($G=61.16$, $p<0.001$). Differential germination between the morphs occurred in all mixtures, regardless of frequency. Because papillate seeds germinated significantly better than smooth seeds, the density of plants in mixtures was correlated with the frequency of papillate seeds sown.

Plants not identified at germination were assumed to belong to the two morphs in proportion to their relative numbers among the identifiable seedlings. The

TABLE I. - Mean germination, survival and fecundity of plants identified as germinating from papillate or smooth seeds. Seeds/plant calculated on the basis of 3.2 seeds per capsule.

		Papillate seeds				
Number sown		250	200	150	100	50
Germinating		49.2	37.2	35	19.2	12
	se	5.7	7.5	6.3	4.3	3.2
Surviving		40	28	27	16.2	11.2
	se	4.4	5.6	6.9	5.0	3.4
Capsules/survivor		2.1	2.4	2.6	2.8	2.7
	se	0.6	0.2	0.6	0.4	0.5
Seeds/plant		6.7	7.8	8.4	9.0	8.6
		Smooth seeds				
Number sown		250	200	150	100	50
Germinating		31.4	23.2	15	12.2	4.6
	se	11	5.6	5.9	2.6	1.4
Surviving		20.8	19.4	12.8	9.8	3.4
	se	2.6	4.5	4.1	3.3	0.5
Capsules/survivor		3.4	3.0	2.7	2.8	1.9
	se	0.3	0.4	1.1	0.5	0.9
Seeds/plant		10.9	9.6	8.6	9.0	6.1

germination, survival and fecundity of identified seedlings (table I) were used to calculate the number of seeds produced by the homozygote morphs in each mixture.

Multiple regression was used to test for the dependence of the mortality, fecundity and fitness of each morph on total density of plants per pot and treatment (frequency). Mortality was measured as arcsin transformed proportions of plants dying between germination and harvest and fecundity as capsules per plant. A mean of 3.2 seeds/capsule were produced, and fitness was calculated as the ratio of seeds produced to seeds sown.

Mortality was independent of treatment and density at germination in both morphs (table II). Fecundity depended upon treatment and the total density of plants per pot at harvest in both morphs, but there were no density \times treatment interactions (table II). So far as density and frequency could be separated, their effects on fecundity were therefore additive. For both morphs fitness was not dependent on density at harvest, though the fitness of the smooth morph was significantly affected by treatment (table II). Because density and frequency were confounded, this effect was actually indistinguishable from density-dependence. Number of capsules per survivor was negatively correlated with the total density of surviving plants in each pot at harvest ($r^2 = -0.51$, $n = 28$, $p < 0.05$, fig. 1). The two outliers shown in figure 1 were not included in the regression because these were pots from which plant roots managed to enter crevices in the paving on which they were placed and this had a conspicuous effect on their growth near the time of harvest. The mean number of seeds per capsule did not differ significantly between morphs (papillate $\bar{x} = 2.88$, $se = 1.97$, smooth $\bar{x} = 3.56$, $se = 2.21$, $z = 2.06$, $p > 0.05$).

TABLE II. - ANOVA tables for regression models for (a) plants from papillate seeds and (b) plants from smooth seeds. The results for terms are only shown for significant regression models.

(a)	mortality				fecundity				fitness			
	d.f.	m.s	F	<i>p</i>	d.f.	m.s	F	<i>p</i>	d.f.	m.s	F	<i>p</i>
Regression	9	0.0528	1.93	0.124	11	2.6576	10.16	<0.001	9	0.6509	1.62	0.196
Residual	15	0.0273			18	0.2617			15	0.4019		
Total	24	0.0369			29	1.1705			24	0.4953		
Terms:												
+density					1	4.4449	16.99	<0.001				
+treatment					5	4.8036	18.36	<0.001				
+density. \times treat.					5	0.1541	0.59	0.708				
Residual					18	0.2617						
Total					29	1.1705						
(b)												
	mortality				fecundity				fitness			
	d.f.	m.s	F	<i>p</i>	d.f.	m.s	F	<i>p</i>	d.f.	m.s	F	<i>p</i>
Regression	9	0.0773	1.21	0.364	11	3.4666	5.62	<0.001	9	0.4399	7.85	<0.001
Residual	13	0.0637			18	0.6171			15	0.0560		
Total	22	0.0693			29	1.6979			24	0.2000		
Terms:												
+density					1	17.1137	27.73	<0.001	1	0.0597	1.07	0.318
+treatment					5	4.0782	6.61	0.001	4	0.8583	15.32	<0.001
+density. \times treat.					5	0.1256	0.20	0.957	4	0.1166	2.08	0.134
Residual					18	0.6171			15	0.0560		
Total					29				24	0.2000		

The joint abundance diagram in figure 2 shows the relationship between the density and proportions of seeds sown and the mean densities and proportions of

seeds produced in the six treatments. It is clear from the trajectories in figure 2 that the papillate morph had the higher fitness in all four mixtures.

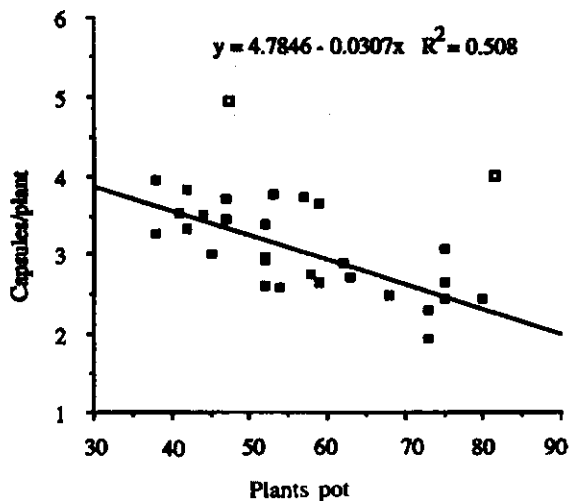


FIG. 1. — Regression of the mean number of capsules per surviving plant on surviving plant numbers per pot. The two outlying points with open symbols were omitted from the regression (see text).

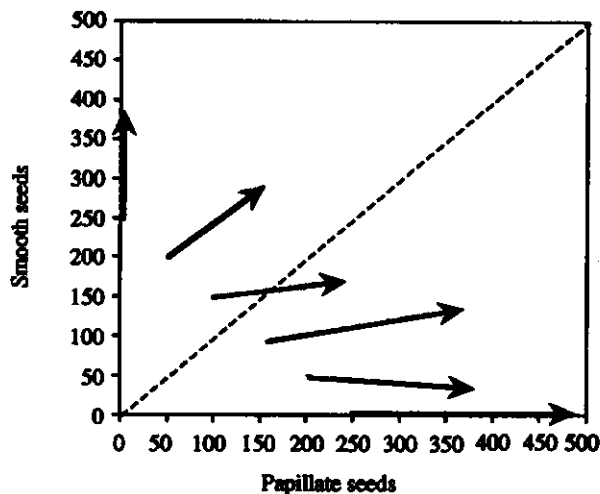


FIG. 2. — A joint-abundance diagram for the two seed morphs based on mean numbers of seeds in each treatment at sowing and harvest. Each arrow originates with the starting composition of a mixture and terminates with its harvest composition.

A total of 531 plants were scored for seed phenotype. Twenty-seven (5.1%) were heterozygotes, a very similar proportion to that observed in the parental field population. All the rest bore their maternal phenotype. Since heterozygotes could only be identified when mature seeds were present, capsule production was compared with smooth and papillate plant identified under the same restriction. Heterozygotes produced a mean of 4.88 capsules per plant (se 4.05), which was not significantly different from either homozygote ($z_{26,70}$, het vs pap=0.31, $p < 0.05$; $z_{26,70}$, het vs smo=1.67, $p > 0.05$).

The outcrossing rate t in the field population may be calculated from the proportion of heterozygotes and the frequency of the two alleles if three assumptions are made: 1. t does not vary from one generation to the next, 2. there is no heterozygote advantage, 3. such outcrossing as does occur is random with respect to seedcoat morph. On these assumptions:

$$t = \frac{0.5 H}{2 ab}$$

where H is the proportion of heterozygotes, and a , b are the observed allele frequencies (New, 1959). This gives an outcrossing rate of 0.1.

DISCUSSION

This study has shown that the papillate morph of *Spergula arvensis* had a consistent, frequency-independent fitness advantage over the smooth morph due to a higher rate of seed germination. Since the trait differentiating the two morphs is one that has been shown to affect germination, there can be little doubt that this difference in fitness can be attributed to the trait itself. We are not dealing only with a marker. Although the result is consistent with the high frequency of the papillate morph near the experimental site, it is not consistent with the existence of a balanced polymorphism since, if this pattern occurs under natural conditions, it is difficult to see why populations should not become entirely papillate. Plants did interfere with each other sufficiently to induce density-dependent fecundity (fig. 1), so the opportunity for some form of specific interaction between morphs in competition for resources was present, although no frequency dependence occurred.

In plant competition experiments where frequency-dependence has been shown, the mechanism often seems to involve temporal partitioning of space. For example KAHN *et al.* (1975) found frequency-dependent fitness in mixtures of flax cultivars where one genotype was early- and the other late-maturing. A similar mechanism led to frequency-dependent competition between male and female plants in *Rumex acetosella* (PUTWAIN & HARPER, 1972) and in *Spinaca oleracea* (ONYEKWELU & HARPER, 1979). There was no evidence of phenological differences between *S. arvensis* morphs in the current experiment, though the existence of seasonal seed dormancy (KARSSSEN *et al.*, 1988) and the differences in germination behaviour between morphs found by NEW (1958; NEW & HERRIOTT, 1981) suggest phenological differences might occur in the field. Temporal partitioning between genotypes will only lead to frequency-dependent fitness if the resource being divided is a renewable one such as light. The two outliers in figure 1 suggest that plants in the experiment were competing for soil resources, which tend to be non-renewable. By comparison,

competition for light was probably very weak because plants were small and the species has thin, bristle-like leaves that do not shade neighbours very heavily. It is possible that frequency dependence would have been observed in different experimental conditions, for example at higher density, where competition for light was more important.

In theory heterozygote advantage might maintain this polymorphism, but there was no evidence of this in the number of capsules per plant, and in any case outcrossing rates were low. NEW (1958), who found even lower outcrossing rates between zero and 3%, also came to this conclusion. An alternative explanation for the cline is that the papillate allele is in fact currently spreading (ENDLER, 1977), and that populations, at least in the south, will ultimately become monomorphic at this locus—as populations appear to be in S. Europe (NEW, 1958). Since germination of the papillate morph is favoured by higher temperatures and the climate has been getting warmer this century (PARKER, 1988) this is a possible hypothesis. Against the hypothesis is the fact that NEW (1978) found the cline to be stable over a period of twenty years. Nevertheless, because the seed pool would retard elimination of the smooth allele to an unknown degree, it is not clear whether twenty years is sufficient time over which to judge stability.

Finally, as with all short-term ecological experiments, there is the likelihood that the observed equilibrium is the product of selection that varies in strength and direction from year to year (e.g. KALISZ, 1986). This is especially important in plants with a long-lived seed pool (SILVERTOWN, 1988), and requires detailed demographic studies to test the idea in the field. Field experiments are planned.

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