

ASSORTATIVE MATING IN PHLOX

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SUMMARY

Experimental populations of *P. drummondii* were established in which pink-flowered and red-flowered variants were distributed in a chequerboard design. The mating patterns therein were estimated from the percentage hybrid progeny. Moderate positive assortative mating occurred when the variants were established at the same height. When stature differentials were introduced, the level of assortative mating increased. Thus pollinators of *P. drummondii* forage in an assortative manner with regard to flower colour and plant stature. The distribution of pollinators on the two phloxes indicated that the pink was not preferred over the red.

1. INTRODUCTION

Mating systems and the selective forces that have moulded them have been prime subjects of evolutionary thought for over a century. Systems of mating determine the pattern in which gametes are brought together, and thus exert primary control on the relatedness of gametes being joined during fertilisation, the genetic structure of zygote populations, the spatial organisation of variation and levels of gene pool homogenisation. Plant evolutionists have sought to characterise the mating system from pollen and seed dispersal patterns (Levin and Kerster, 1974) and from genotype distributions within and among families (Clegg, 1980). These approaches provide information on the potential for or the actual level of inbreeding within populations. Other systems of mating also may have a substantial impact on the genetic structure of populations, yet have received little attention. Positive assortative mating is the most important in this respect and the most likely deviation from random mating other than inbreeding.

There is considerable potential for assortative mating based upon polymorphic reproductive characteristics in animal-pollinated plant populations. Pollinators such as bees, lepidopterans and hummingbirds can discriminate between colours, odours, corolla sizes and corolla configurations (Faegri and van der Pijl, 1979; Waddington, 1983; Waser, 1983), and such pollinators often remain constant to a single food image in spite of the presence of suitable alternative images (Grant, 1949, 1963; Levin, 1978a). Discontinuous variation in flower colour is known in *Linanthus parryae* (Epling, Lewis and Ball, 1960), *Encelia farnosa* (Kyhos, 1971), *Leavenworthia crassa* (Lloyd, 1969), *Lupinus nanus* (Harding, 1970), *Delphinium nelsonii* (Waser and Price, 1981), *Cirsium palustre* (Mogford, 1974), *Raphanus raphanistrum* (Kay, 1976), and *Aquilegia caerulea* (Miller, 1981). Polymorphisms also are known for floral scent in *Gilia* species (Grant, 1949), *Polemonium viscosum* (Galen and Kevan, 1980), and *Medicago sativa* (Waller *et al.*, 1974), stature in *Potentilla erecta* (Watson, 1969), flowering time in *Nicotiana rustica* (Breese, 1956) and *Epacris impressa* (Stace and

Fripp, 1977), and for floral form in *Dactylorhiza fuchsii* (Heslop-Harrison, 1958).

In this paper, we describe assortative mating in response to alternative corolla colours and stature differentials in *Phlox drummondii* Hook. *Phlox drummondii* is pollinated by a variety of lepidopterans (*Battus philenor*, *Papilio* spp., skippers and hawkmoths) (Levin, 1972, 1975). The species is a self-incompatible, spring-flowering annual endemic to the prairies of central Texas. It is composed of pink- and red-flowered population systems which hybridise along zones of secondary contact. Populations in these zones contain pink-flowered and red-flowered plants.

2. MATERIALS AND METHODS

Experiments on the mating pattern in *P. drummondii* were conducted at the Brackenridge Field Laboratory of the University of Texas. Twenty-four plants each of a red-flowered and a pink-flowered variant were placed eighteen inches apart in a chequerboard pattern in garden plots cleared of other vegetation. Each plot was arranged into one of three height treatments. In the first two treatments, the two phloxes were displaced vertically eight and sixteen inches, respectively, by raising all pink-flowered plants on inverted pots. All plants were placed at the same level in the third treatment, thus serving as a control. Four plots were established for each of the three height treatments, giving a total of twelve plots. A reciprocal experiment with elevated red-flowered plants was attempted after the first, but it is excluded from the analysis because of poor seed production and germination. As a result, this is not a complete factorial design. However, we show that hybridisation is similar for the high and low varieties.

Seeds from each variant within each plot were collected at approximately bi-weekly intervals. Seeds from each plot were then planted, and progeny were scored as pure or hybrid. Hybrids are easily distinguished by their magenta flowers in contrast to the pink or red flowers of the parental types.

Differences in the percentage of hybrid progeny were investigated after arcsine square root transformation by a split-plot analysis of variance with height differential and corolla colour as the main effects.

Pollinator preference for corolla colour in *P. drummondii* was estimated from observations on approximately one hundred pollinations for each height treatment. These were tabulated and tested for equality using a G-test (Sokal and Rohlf, 1981).

The red-flowered variety was grown from seed collected near Bastrop, Bastrop Co., Texas; and the pink-flowered variety was from seed collected near Austin, Travis Co., Texas. The possibility that hybrid production might be limited due to crossing barriers was investigated using dual pollinations and scoring the frequencies of hybrids among the progeny. Approximately equal amounts of pollen of both varieties were mixed together and placed on stigmas of each variety in a greenhouse. Progeny were identified as hybrids on the basis of flower colour.

3. RESULTS

Assortative mating is quite evident in our experimental populations. With no height differential between variants, an average of 37 per cent of the progeny were hybrid compared to 50 per cent expected from random

mating (table 1). A stature differential between the variants increased the level of assortment beyond that due to colour alone. With an eight inch differential, an average of 26 per cent of the progeny were hybrid, while with a sixteen inch differential only 16 per cent of the progeny were hybrid.

TABLE 1
Percentage hybrid progeny in relation to height differential and corolla color in P. drummondii

Plot	Colour	Height differential (inches)		
		Zero	Eight	Sixteen
1	pink	38.0 (150)*	30.8 (104)	16.1 (63)
1	red	36.0 (86)	13.6 (107)	21.2 (67)
2	pink	22.2 (18)	24.0 (75)	10.0 (45)
2	red	38.6 (41)	22.0 (72)	20.8 (49)
3	pink	43.9 (106)	27.6 (81)	13.9 (61)
3	red	41.8 (106)	42.5 (81)	17.9 (104)
4	pink	32.0 (110)	28.3 (158)	13.1 (85)
4	red	33.1 (163)	28.5 (88)	17.4 (119)
MEAN		36.6	26.1	16.1

* Sample size.

The complete analysis of variance for the colour-height experiment is shown in table 2. The main effect height is very highly significant, and thus the null hypothesis of no differences between the height treatments may be rejected. The effect of the height differentials is clearly very powerful.

TABLE 2
The analysis of variance of the data shown in table 1

Item	d.f.	MS
Heights	2	336.8052***
Plots, within heights	9	18.5137
Colours	1	22.6010
Colours × Heights	2	17.0686
Colours × Plots	9	18.6650
Total	23	—
Theoretical variance	∞	11.6439

*** = $P < 0.001$.

Overall, there is no significant difference in the percentage of hybrid progeny produced by the two variants, indicating a lack of pollinator preference (table 2). Were one variant favoured, it would have a substantially lower proportion of hybrid progeny than the ill-favoured variant, because pollinators would forage primarily among plants of the former, and only occasionally switch to the latter. The interaction of corolla colour and height

differential shows the degree of assortment at each height level does not differ between the variants. In fact, their responses are highly concordant.

If pollinators were moving from plant to plant in a random fashion when no height differences were present, the percentage hybrid progeny should have been proportional to the ratio of the variants, 1:1. A G-test of the frequency of hybrid vs. non-hybrid progeny against the expected 1:1 ratio revealed a highly significant overall deviation in both variants ($P < 0.001$, table 3). This consistently was always in the direction of fewer hybrid progeny, and consistently so as indicated by the lack of a significant heterogeneity G. Therefore, positive assortative mating is occurring at a statistically significant level.

TABLE 3
Test for random outcrossing in populations of P. drummondii

Plot	Parental	Progeny of pink-flowered variants			df	
		Hybrid	Expected	G		
One	92	58	75	7.7741	1	$p < 0.01$
Two	14	4	9	5.8839	1	$p < 0.025$
Three	59	47	53	1.3614	1	ns
Four	75	35	55	14.8843	1	$p < 0.001$
Total	240	144	192	29.9036	4	$p < 0.001$
Pooled G				24.2565	1	$p < 0.001$
Heterogeneity G				5.6471	3	ns
Plot	Parental	Progeny of red-flowered variants			df	
		Hybrid	Expected	G		
One	55	31	43.0	6.7874	1	$p < 0.01$
Two	23	18	20.5	0.6113	1	ns
Three	62	44	53.0	3.0715	1	ns
Four	108	55	81.5	17.5504	1	$p < 0.001$
Total	248	148	198.0	28.0206	4	$p < 0.001$
Pooled G				25.5280	1	$p < 0.001$
Heterogeneity G				2.4926	3	ns

Observations of pollinators showed that the number of plants pollinated of each variant was similar, even when there was a height differential (table 4). Accordingly, neither flower colour is favoured over the other, nor are tall plants more or less attractive than short ones.

TABLE 4
Number of plants pollinated in P. drummondii plots

Height differential	Flower colour		Total	Expected	G	df	
	Red	Pink					
Zero	40	49	89	44.5	0.911670	1	ns
Eight	56	46	102	51.0	0.981969	1	ns
Sixteen	56	54	110	55.0	0.036366	1	ns
Pooled	152	149	301	150.0	0.029901	1	ns

The dual pollination experiment demonstrated that the pink and red-flowered variants are cross-compatible and cross-fertile. Dual pollinations with the pink-flowered variant as the female parent yielded 48 per cent hybrids as compared to the expected 50 per cent; such pollinations on the red-flowered variant yielded 46 per cent hybrids (table 5). Although there was a small deficiency of hybrids, parental types and hybrids did not deviate significantly from equality. Accordingly, we propose that the substantial deficiencies of hybrids produced in the experimental populations are due in very large measure if not exclusively to pollinator foraging behaviour.

TABLE 5
Progeny of dual pollinations involving two P. drummondii variants

Parental	Progeny of pink-flowered variants			df	
	Hybrid	Expected	G		
47	43	45	0.1778	1	ns
	Progeny of red-flowered variants				
56	50	53	0.3398	1	ns
	Progeny of both variants				
103	93	98	0.5104	2	ns

4. DISCUSSION

Positive assortative pollination within a polymorphic population is the population counterpart of flower constancy, *i.e.*, the tendency of single pollinators to restrict their foraging to flowers of a single species although suitable flowers of other species are present. Both assortative pollination and flower constancy are the consequences of pollinator response to differences in flower colour, form, size and fragrance (Grant, 1949; Free, 1970; Levin, 1978*a*; Waser, 1983). In contrast to floral constancy, assortative pollination has been documented in only a few instances (Kay, 1978, 1982). In addition to providing an additional example of assortative pollination, the present study is the first to document assortment in a red-pink mixture by any class of pollinator, the first to document assortment for stature in a lepidopteran pollinated species, and the first to use progeny tests to describe the cumulative effect of pollen flow.

Pollinators of *P. drummondii* can discriminate among a broad range of flower colour variants (Levin, 1972), and forage assortatively as seen in this study. In plots where two variants had the same stature, the frequency of hybrids was 0.37 vs the expected 0.50. Whereas we have demonstrated a significant departure from random mating, we do not wish to imply that the mating system within a given mixture is invariant. Indeed, the frequency of hybrids probably would have been quite different were the experiment conducted at another plant density or population size because pollinator response to divergent floral signals is dependent upon these variables (Levin, 1978*b*). In synthetic populations of *P. drummondii* cultivars, the level of assortment among colour variants is positively correlated with plant density (Watkins and Levin, unpubl.). This relationship also has been observed in bee-pollinated systems (Marden and Waddington, 1981). In natural dimorphic populations of *P. pilosa*, the level of assortment is less in small

populations than in large ones (Levin, unpubl.). These observations are consistent with the relationship between flower constancy, and population size and density in multispecies assemblages (Levin, 1978*b*), and indicate the dynamic nature of assortative mating.

In a dense array of plants heterogeneous in stature, the average distance between flowers of neighbouring plants is least if plants are similar in stature. Since pollinators forage economically (Heinrich, 1983; Waddington, 1983), we would expect them to pollinate assortatively for stature, the greater the variance in the latter the greater the degree of assortment. Assortative pollination for stature has been observed in the bee-pollinated *Lythrum salicaria* (Levin and Kerster, 1973) and *Brassica oleracea* (Faulkner, 1976). The present study is the first to demonstrate that assortment for stature is dependent on the height differential.

Assortative mating in plants probably is much more common than one would judge from the literature. Assortative mating will occur if one group of pollinators prefers one morph while another group prefers an alternative morph as has been shown in several species (Kay, 1982; Miller, 1981). Assortative mating will also occur in the absence of morph preference if pollinators distinguish between alternate morphs and display some level of flower constancy. This pollination pattern has been observed in *Phlox drummondii* and in *Lantana camara* (Dronamraju, 1960) and *Antirrhinum* (Mather, 1947). Assortative mating also will ensue if alternate morphs were clumped in space even if pollinators did not discriminate between them, provided that pollinator flights were principally between neighbouring plants. This pollination pattern is the norm in most plant populations (Levin and Kerster, 1974; Levin, 1981). The patchy distribution of alternate morphs has been documented in *Linanthus parryae* (Epling and Dobzhansky, 1942; Epling, Lewis and Ball, 1960), *Cirsium palustre* (Mogford, 1974), and *Leavenworthia crassa* (Lloyd, 1969).

It is important to know the extent and magnitude of assortative mating in plant populations, because this mating pattern may promote the maintenance of floral polymorphisms through its effects on the genetic constitution of populations. In addition to increasing the incidence of the recessive phenotype, assortative mating may reduce the influx of neutral or weakly beneficial alleles (Moore, 1979) or increase the genetic load (Lande, 1977). For quantitative characters, assortative mating inflates the total phenotypic variance, but disproportionately inflates the additive genetic variance so that the heritability of the character, and hence the effect of selection is potentially increased (Breese, 1956; Crow and Felsenstein, 1968; Crow and Kimura, 1970). Assortative mating also increases the patchiness of phenotypes in space beyond that achieved by restricted gene flow (Stephen, Turner and Anderson, unpubl.).

Given the potential for assortative mating in plants and its manifold effect, it is clear that this form of non-random mating warrants closer scrutiny. Our preoccupation with inbreeding has diverted attention from this form of non-random mating to the point where it has been ignored.

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