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Outcrossing rates and their relationship to phenology in *Triticum dicoccoides*

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Summary. Outcrossing rates (t) were estimated in natural and garden populations of wild emmer wheat, *Triticum dicoccoides*.. The estimated t from the natural populations was 0 with the 0.95 upper confidence limit ranging from 0.102 to 0.925 depending on the genetic variation within the population. Outcrossing rates for two genotype classes of this species grown in a common garden were 0.0077 and 0.0018. The difference in outcrossing rates between the two genotypes is ascribed to phenological differences and hence different available pollen pools. Phenological displacement is discussed as a possible bias in the estimation of outcrossing rates in both predominantly selfing and outcrossing plants.

Key words: Outcrossing – Phenology – Heterozygosity paradox – *Triticum dicoccoides* – Selfers

Introduction

The level of outcrossing and inbreeding has a paramount effect on the genetic organization of plant populations. Highly inbred annual plant species differ from outcrossers in the degree of multilocus asssociations in their populations, their excess of heterozygotes over that expected given the rates of inbreeding, and their tendency towards multiple heterozygosity (Brown 1979; 1984; Brown et al. 1980). Brown (1979) has subsumed the last two characteristics under the term "heterozygosity paradox". Although the forces engendering this paradox are not certain, it has been argued that the apparent dichotomy between populations of outcrossers and inbreeders in these genetic characteristics are not expected under neutrality (Brown 1979, 1984). Schemske and Lande (1985) have questioned this argument submitting that the evidence for heterosis in inbreeders is not sufficient given that its estimation is dependent on the accuracy of estimating outcrossing rates.

Triticum dicoccoides, an annual grass, is the recognized progenitor of cultivated hard wheats and the tetraploid (genome AABB) ancestor of the modern hexaploid (genome AABBDD) bread wheats (Zohary 1969). Natural populations of this species have been studied to determine the range and spatial patterns of genetic variation as detected by isozyme loci (Nevo et al. 1982) and seed protein content (Nevo et al. 1986b), and to identify plants resistant to pathogens (Nevo et al. 1986a, 1985). *Triticum dicoccoides* is distinguished from *Hordeum spontaneum* and *Avena barbata* studied over a similar range by its mosaic or localized patterns of gene distribution (Nevo et al. 1979; Nevo 1983; Kahler et al. 1980). It is also characterized by high levels of gametic phase disequilibrium (Golenberg and Nevo 1987) and low levels and limited distances of gene flow (Golenberg 1987). These findings indicate that outcrossing must be limited in this species. Nevo et al. (1982) reported an estimate of 3% outcrossing in this species based on the observed heterozygosity deficit (F).

This paper reports estimates of outcrossing rates from both natural and common garden populations of wild emmer wheat, *Triticum dicoccoides.* Differences in outcrossing rates between genotypes in this species are further analysed in light of known phenological differentiation. The effects of phenological differentiation

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on the estimation of outcrossing rates in both outcrossers and selfers are discussed.

Material and methods

The collection area was in an open, relatively flat field, with basaltic soil located in the Yehudiyya Nature Preserve northeast of the Sea of Galilee in Israel. Scattered piles of boulders or remains of stone fences were found in the field. The vegetation was dominated by grasses such as *Avena sterilis* and *Hordeum bulbosum*, although *T. dicoccoides* was also common. There was no evidence of recent disturbance, although spring fires are common in this area. Spikes were collected from ten separate plots of about 1.0 to 2.0 m radius. Twenty-five to 30 spikes (one spike per plant) were collected from each plot, and in most cases, all *T. dicoccoides* plants were sampled within the plots.

Single seeds from each spike were germinated and root samples were taken from flooded 5 day old seedlings. Leaf samples were taken after 1 to 3 days. The extraction and electrophoretic techniques are given in Brown et al. (1987a). Seventeen enzyme systems and a coleoptile pigment system were assayed. A total of 13 isozyme loci were used to estimate outcrossing rates.

Outcrossing rates, t, and their confidence limits were estimated using the methods of Allard et al. (1972b) and Brown et al. (1978b). Outcrossing events were verified by analysis of siblings within a spike. Free assortment within a locus was taken as evidence that the parent plant was heterozygous and no outcrossing was assumed to have occurred in the progeny generation. The values of the upper confidence limits of the outcrossing rates for these sites were determined using the multilocus technique outlined in Brown et al. (1978b). The likelihood function for no outcrosses observed is

$$L = (1 - H_e t)^N,$$

where H_e is the diversity parameter, t is the outcrossing rate, and N is the sample size. The sampling estimate of H_e is

$$H_e = (1 - \Sigma g_i^2)\, N/(N - 1),$$

where g_i are the frequencies of the multilocus genotypes in the sample. When there are h heterozygotes from maternal heterozygotes,

$$L = (1 - H_e t)^{N-h} \text{ and } H_e = (1 - \Sigma g_i^2) N^2/(N(N - 1) - h/2).$$

The approximate upper confidence limit is determined by setting $L = 0.05$. This value provides a maximum estimate of t given the number of outcrosses observed (zero) and the number of plants tested.

In order to obtain an upper estimate of potential outcrossing rates, plants were grown in a controlled garden experiment where plant density was high and there were no intervening interspecific plants. (Low density and potential interspecific pollen traps may decrease outcrossing (Antonovics and Levin 1980; Campbell 1985). Seeds from parent plants originated from two collection sites, Yehudiyya and Qazrin (Description of these sites and maps may be found in Golenberg and Nevo 1987). These two populations may be differentiated by two alternate eight-locus genotypes. Two plant densities were used in the garden plots, 36 plants per 0.09 m² and 20 plants per 0.20 m². Genotype proportions within plots established as 75:25, 50:50, and 25:75. Pooled estimates of outcrossing rates were calculated by summing the number of outcrosses weighted by the potential pollen pool within the plot. The potential pollen pool was estimated by assuming equal pollen contribution from each plant regardless of genotype.

A second estimate of outcrossing was made by assuming equal contributions of pollen per available spike. Spike availability was estimated by the overlap of third or later spikes of one genotype with all other first and second spikes within a two day period. Later spikes appeared to contain flowers with exposed unpollinated stigmas, apparently due to pollen sterility within the flower (personal observation). These flowers are expected to be preferential targets for outcrossing. The period of two day overlap takes into account the potential pollen viability which was estimated to be about 2 days in grasses (Johri and Vasil 1961), although this may be an overestimate for *T. dicoccoides* (A. Grama, personal communication). The proportion of overlap was estimated previously on plants of the two genotypes grown under controlled greenhouse conditions (Golenberg 1986a).

Results

Thirteen of the 42 loci tested in plants from the 10 collection sites were polymorphic in at least one site. The allele frequencies of these polymorphic loci are listed by population in Table 1. Six of 287 seeds tested were heterozygous in at least one locus. Sibling tests from the same spike indicated that all of these heterozygotes were derived from heterozygous parent plants. Thus, there were no detectable outcrosses, and the estimates of outcrossing for all populations were zero (Table 2). The upper 95% confidence limits ranged from 0.102 to 0.925 and are inversely related to the degree of diversity within the sample sites, H_e, which ranged from 0.110 to 0.929 (Table 2).

The results of the common garden experiment are summarized in Table 3. Because within-population genotype variation in these garden plots was minimal, outcrosses were detected only between Yehudiyya and Qazrin individuals and not between individuals from the same population. Outcrosses could be verified over several loci. Seedlings from Yehudiyya seed parents, which normally lack coleoptile anthocyanin, were tested for the presence of the red coleoptile color which was dominant over the green coleoptile of the Yehudiyya genotype, and thus signified an outcross with Qazrin-originated pollen. Endosperm of seeds from Qazrin seed parents was analyzed for the MDH-1 phenotype. The fast allele from Yehudiyya appears to be phenotypically dominant over the modal allele of the Qazrin genotype (Golenberg 1986b). Suspected heterozygotes also were tested for EST-5 phenotypes. Heterozygotes could be identified as the alleles are codominant at this isozyme locus. Although several loci were used to test the heterozygosity of offspring, the estimation of t in the common garden experiment is based on single locus data. Because disequilibria exists

Table 1. Allele frequencies at 13 polymorphic loci of 10 collection sites of *Triticum dicoccoides*. Sample sizes from each site are also listed

Locus	Allele	Locality									
		A	B	C	D	E	F	G	H	I	J
IPORA	M	1.00	1.00	1.00	1.00	1.00	0.92	1.00	1.00	1.00	1.00
	S						0.08				
IPORB	M	1.00	1.00	0.87	0.93	1.00	1.00	1.00	1.00	0.97	1.00
	S			0.13	0.07					0.03	
PGMA	F						0.60		0.14	0.41	0.20
	M	1.00	1.00	1.00	1.00	1.00	0.40	1.00	0.86	0.59	0.80
GDHB	F	0.03	0.11	0.54	0.13	0.50	0.64		0.21	0.43	0.10
	M	0.97	0.89	0.46	0.87	0.50	0.36	1.00	0.79	0.57	0.90
PGIA	F		0.04			0.43			0.03	0.09	
	M	0.97	0.93	0.61	0.90	0.50	1.00	1.00	0.97	0.91	1.00
	S	0.03	0.03	0.39	0.10	0.07					
ACPH3	F					0.10		0.07	0.03		
	M	1.00	1.00	1.00	1.00	0.90	1.00	0.93	0.97	0.68	1.00
	S									0.32	
ESTDA	M	0.13	0.36	0.45	0.17	1.00	0.64		0.02	0.93	0.47
	S	0.87	0.64	0.55	0.83		0.36	1.00	0.98	0.07	0.50
	S–										0.03
NADH1A	F		0.09	0.48	0.12	0.08	0.20			0.21	0.16
	M	1.00	0.91	0.52	0.88	0.92	0.80	1.00	1.00	0.79	0.84
NADH1B	M		0.09	0.04		1.00	0.70			1.00	0.52
	S	1.00	0.91	0.96	1.00		0.30	1.00	1.00		0.48
PEPT1B	F										0.20
	M	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.80
ESTAA	M	0.90	0.64	0.50	0.90		0.32	1.00	0.86		
	N	0.10	0.36	0.50	0.10	1.00	0.68		0.14	1.00	1.00
β-GLUA	M	1.00	0.96	0.61	0.92	0.90	1.00	1.00	1.00	0.86	0.77
	N		0.04	0.39	0.08	0.10				0.14	0.23
β-GLUB	F		0.04			0.07				0.04	0.33
	M	1.00	0.96	1.00	1.00	0.93	1.00	1.00	1.00	0.96	0.67
N		30	28	28	30	30	25	28	29	29	30

Table 2. Estimation of outcrossing rate from ten localized sampling sites. Listed are the number of seeds tested, N: number of heterozygotes from heterozygous parent plants, h: number of new heterozygotes, O: maximum diversity parameter, $He^{(o)}$: estimated outcrossing rate, t and its lower and upper confidence limits at the 0.95 level of probability

Site	N	h	O	$He^{(o)}$	t	t_{min}	t_{max}
A	30	0	0	0.308	0	0	0.308
B	28	0	0	0.540	0	0	0.188
C	28	2	0	0.873	0	0	0.125
D	30	0	0	0.414	0	0	0.230
E	30	0	0	0.772	0	0	0.123
F	25	0	0	0.817	0	0	0.138
G	28	1	0	0.110	0	0	0.925
H	29	1	0	0.612	0	0	0.160
I	29	2	0	0.926	0	0	0.113
J	30	0	0	0.929	0	0	0.102

Table 3. Estimation of outcrossing rates between Yehudiyya and Qazrin genotypes when grown in proximity. Listed are the number of seeds tested, N: number of new heterozygotes, O: maximum diversity parameter, $He^{(o)}$: estimated outcrossing rate, t and its upper and lower confidence limits at the 0.95 and 0.90 (listed below) levels of probability. Estimates are made based on equal pollen pools based on parental plant frequencies, and on weighted pollen pools based on spike overlap

Genotype	N	O	$He^{(o)}$	t	t_{min}	t_{max}
Yehudiyya	924	4	0.562	0.0077	0.0030	0.0158
					0.0041	0.0130
Qazrin	996	1	0.558	0.0018	0.0001	0.0079
					0.0002	0.0063
Yehudiyya (wt)	924	4	0.734	0.0059	0.0023	0.0122
					0.0031	0.0100
Qazrin (wt)	996	1	0.2574	0.0039	0.0002	0.0174
					0.0004	0.0139

between the variable loci in these particular test populations (Golenberg and Nevo 1987), no additional information was available from multilocus analyses. One outcross was detected from the Qazrin material, and four from the Yehudiyya material.

The outcrossing rate for each genotypic class was determined by the method of Allard et al. (1972 b). This equation gives the probability distribution for the offspring of a population where no parental heterozygotes are present, which is appropriate for the present analysis. As outcrosses were rare, the effects of population density or genotype composition could not be ascertained, and the data were pooled. To combine data from separate plots, the individual likelihood equations were multiplied together, and the t which gave the maximum value of the products of the likelihood equations was determined. As the model assumes that the pollen pool equals the maternal pool in allele frequencies, the expected heterozygotes among the progeny were calculated using the composition ratios of each plot for the allele frequencies. Confidence limits were determined by setting $L=0.05$ or 0.10 and solving for t for the 0.95 and 0.90 limits, respectively. The outcrossing rate of the Yehudiyya genotypes was estimated at $t=0.0077$, whereas that of the Qazrin genotypes was $t=0.0018$. These values are well within the range of the upper 0.95 limit for the field samples. The estimate of outcrossing for the Qazrin genotypes in the garden plots was below the minimum 0.95 limit of the Yehudiyya genotypes (0.0030), whereas the Yehudiyya estimate was within the upper limit of the Qazrin genotypes (0.0079). When the confidence limits are set to 0.90 limits, each population estimate is out of the range of the other (Table 3).

As mentioned earlier, the above estimates assume an equal pollen pool and equal outcrossing rates for the two genotypes. Brown et al. (1975) developed an estimate in which outcrossing rates are assumed to be equal but pollen gene frequency may differ. In the case where no heterozygous maternal plants are present,

$$\hat{X} = O_i/N_i \quad \text{and} \quad \hat{Y} = O_j/N_j,$$

where O and N are the number of heterozygote progeny and maternal homozygotes for each genotype *i* and *j*, respectively. X and Y are estimates of the expected frequencies of detectable outcrosses,

$$X = qt \quad \text{and} \quad Y = pt,$$

where q and p are pollen pool frequencies of haploid genotypes *i* and *j*. The variances of these estimates are

$$\text{var}(\hat{X}) = \hat{X}(1-\hat{X})/N_i, \quad \text{var}(\hat{Y}) = \hat{Y}(1-\hat{Y})/N_j,$$
$$\text{and cov}(\hat{X}, \hat{Y}) = O.$$

The estimates of t and p and their variances are

$$\hat{t} = \hat{X} + \hat{Y}$$
$$p = \hat{Y}/(\hat{X} + \hat{Y})$$
$$\text{var}(\hat{t}) \approx \text{var}(\hat{X}) + \text{var}(\hat{Y}) + 2\,\text{cov}(\hat{X}, \hat{Y}), \quad \text{and}$$
$$\text{var}(\hat{p}) \approx (\hat{X}^2\,\text{var}(\hat{Y}) + \hat{Y}^2\,\text{var}(\hat{X}) - 2\hat{X}\hat{Y}\,\text{cov}(\hat{X}, \hat{Y}))/(\hat{X} + \hat{Y})^4.$$

Accordingly, X was estimated to be 0.0043 (var(X)= 0.0000047) and $Y=0.0010$ (var(Y)=0.0000010). The estimated outcrossing was $t=0.0054$ (var(T)= 0.0000057), while p and q were estimated to be 0.1878 and 0.8122, respectively (var(p)=0.0288). These results suggest a predominance of Qazrin pollen in the outcross pool, far in excess of the maternal frequencies.

Male sterile flowers in *Triticum dicoccoides* are usually open with the unpollinated stigma exposed. It was observed in greenhouse populations that spikes with open flowers tended to be the later appearing spikes. As a working hypothesis, it was assumed that these later appearing spikes would preferentially be the agents for outcrossing as seed parents in these populations. Complementarily, it was also assumed that the earlier spikes would be the potential pollen donors. Under these assumptions, the pollen pool available to the potential outcrossed flowers would neither equal the maternal genetic pool nor be equal for the two populations because of the disjunction of the flowering phenology described in Golenberg (1986a). To estimate a potential pollen pool for each genotype, data obtained from greenhouse populations were used to divide the spikes into two classes for each genotype: class 1 included the first and second spikes, and class 2 included all following spikes. Their distributions are shown in Fig. 1. The pollen pool for each genotype was determined by the frequency of class 1 spikes from each genotype that overlapped with class 2 spikes. A lag time of $\pm$ two days was included in the overlap to account for maximum pollen life and stigma receptivity (Johri and Vasil 1961). The Qazrin frequencies were weighted downward to allow for the greater number of Qazrin plants compared to Yehudiyya in the original greenhouse data set. These estimates are, of course, only a first approximation as environmental variance in the greenhouse is expected to be much lower than in the field which may influence flowering time differentially for the genotypes. Furthermore, equal pollen contribution by the spikes was assumed.

The distribution of overlap between spike classes is given in Table 4. After correcting for number of plants, the Qazrin population had more than twice the number of first and second spikes (class 1) overlapping Yehudiyya class 2 spikes than did Yehudiyya (55.9 to 25, or $p_{Yehudiyya}=0.309$). This preponderance of available Qazrin pollen was even more striking for Qazrin class 2 spikes, where $p_{Yehudiyya}=0.211$. The proportion of the

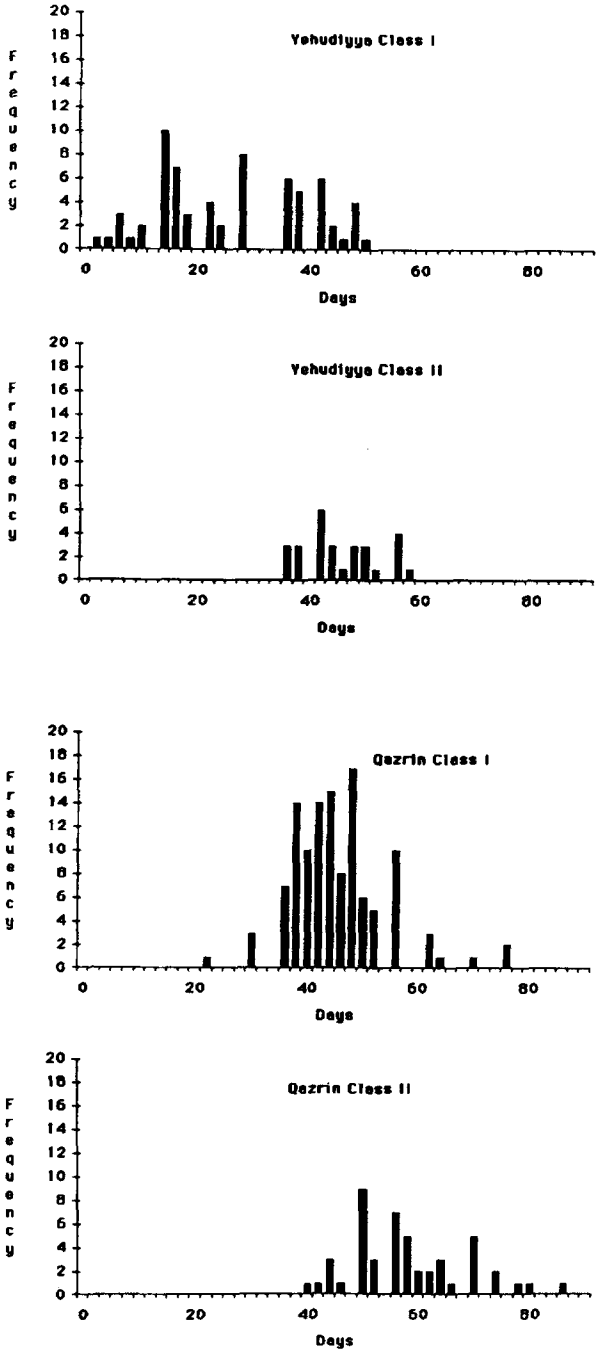

Fig. 1. The distribution of spikes by genotype according to classes. Class 1 are first and second spikes of a parental plant. Class 2 are all subsequent spikes. Plants were grown in a greenhouse (Golenberg 1986a)

total effective pollen pool available to class 2 spikes was $p_{Yehudiyya}=0.265$. These approximate estimates derived from the greenhouse populations showed a trend of reduced availability of Yehudiyya pollen similar to that indicated by estimates from the methods of Brown et al. (1975). New estimates of outcrossing rate, t, and confidence limits derived using pollen frequencies adjusted for spike overlap are listed in Table 3. The Qazrin outcrossing rate was increased, and that of Yehudiyya reduced (0.0039 and 0.0059, respectively). Both fall well within the confidence limits of the other population even at the 0.90 level.

Table 4. Distribution of the number of overlapping spikes. Class 1 spikes are the first and second appearing spikes of an individual. Class 2 spikes are the third and following spikes. Data are taken from raw data of the greenhouse populations reported in Golenberg (1986a). The second column of Qazrin Class 1 spikes is a weighted frequency to account for differences in original number of parental plants. Sample size of plants are listed in parenthesis

Class 2 spikes	Class 1 spikes		
	Yehudiyya	Qazrin	Qazrin (weighted)
Yehudiyya (n=35)	25	107	55.9
Qazrin (n=67)	14	100	52.2

Discussion

Outcrossing rates are influenced by plant density (Antonovics and Levin 1980; Bateman 1947; Ellstrand and Marshall 1985; Ellstrand et al. 1978; Levin and Kerster 1969; Farris and Mitton 1984), environmental variation (Sanders and Hamrick 1980) and genotypic composition (Schoen 1982; Brown and Clegg 1984; Handel and Mishken 1984; Schoen and Clegg 1985; Ritland and Ganders 1985, and discussion above), so any estimate of t must be local both in time and space. Nevo et al. (1982) reported an outcrossing rate in *Triticum dicoccoides* of about 0.03. This was based, however, on F statistics assuming equilibrium conditions and not on actual outcrossing. Such estimates may be greatly affected by gametic phase disequilibrium, genetic variation, and selection within a population, and may not be reliable. The levels of outcrossing reported for the populations presented in this paper are well below the earlier reported estimate. The extremely low estimates are consistent, however, with previously published outcrossing rates for other natural populations of selfing grasses. Brown et al. (1978b) reported a mean outcrossing rate for 28 populations of *Hordeum spontaneum* to be 0.016. Fifteen of these populations had an estimated t=O. Kahler et al. (1980) reported an outcrossing rate for *Avena barbata* in Israel of much less than 1%, although rates for the same species in California reached values greater than 7% (Marshall and

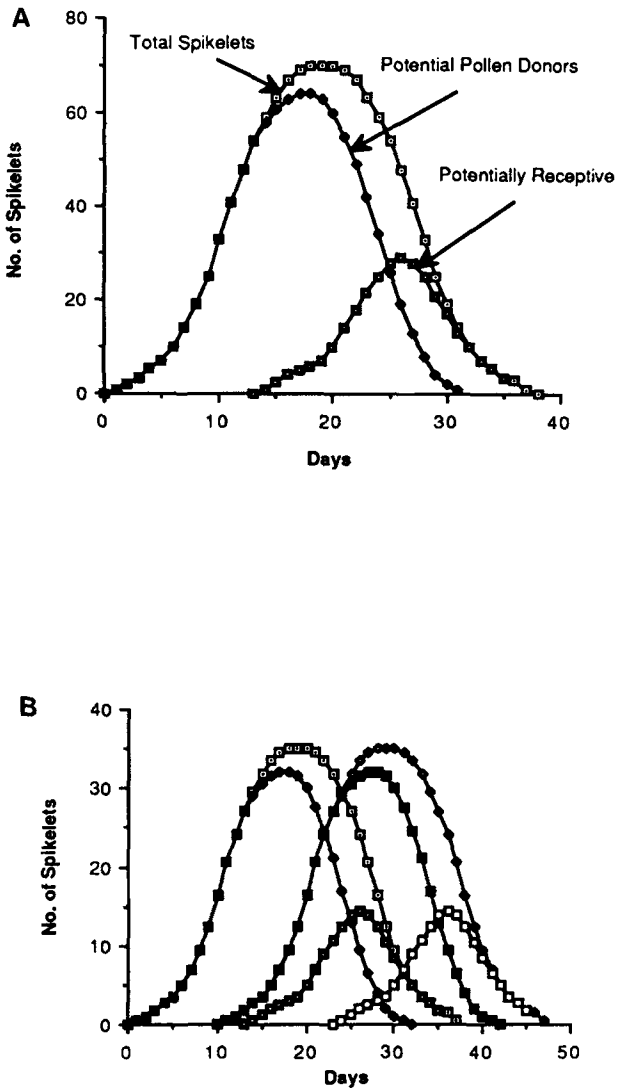

Fig. 2. A schematic diagram showing the potential pollen overlap with receptive stigma. The uppermost curve (*dotted squares*) gives the appearance of spikes over time for a genotype. The *solid squares* are the number of early occurring spikes presumed to be the primary pollen donors for outcrossing. The *open squares* are the later occurring spikes presumed to have male sterile flowers and, therefore, to be potential targets for outcrossing. The overlap of these last two curves indicates the potential non-selfing male–female overlap. As can be seen in the second set of curves, a displacement in flowering time increases the proportion non-selfing male–female overlap

Allard 1970; Allard et al. 1972a; Hamrick and Allard 1972).

An important feature of the results presented in this paper is the indication that flowering phenology may bias the estimation of the outcrossing rate by changing the available pollen pool. The idea that changes in phenology influence outcrossing, in general, and genetic isolation, in particular, is not new (eg., McNeilly 1968). However, the present data suggest that phenological displacement will tend to increase average effective outcrossing in a population of predominant selfers. When a displacement in flowering causes a differential overlap between receptive stigmas and a heterogeneous pollen pool, outcrossing will increase and inbreeding (either through geitonogamy or through outcrossing with genetically related individuals) will decrease (Fig. 2). In the data reported herein, this can be seen when earlier spikes of the Qazrin genotype overlap with the later spikes of the Yehudiyya genotype.

There are two preconditions for the increase of outcrossing rate and the decrease of inbreeding with flowering displacement. The first is that flowering phenology is genetically controlled and that within a population flowering synchrony is related to genetic relatedness. The second is that pollen production and the production of potentially outcrossing stigmas be largely non-overlapping. The first precondition may be met in many highly inbreeding species in which populations are made up of genetically differentiated clones. Significant genetic variation was found among families within populations of *T. dicoccoides* in flowering times (Golenberg 1986a). The second precondition is met in *T. dicoccoides* because the receptive stigmas are produced in male sterile flowers which occur late in the flowering of the plant. The effects of this displacement on outcrossing are analogous to the effects of changes in protandry on outcrossing in predominant outcrossers (Schoen 1982), or general flower overlap as discussed in Smyth and Hamrick (1984) and Stephanson (1982).

In outbreeders for which the distributions of pollen production and of receptive stigmas largely overlap, displacement in flowering will have the opposite effect; inbreeding will increase with increased displacement. This is caused by an increase of the proportion of genetically related pollen in the pollen pool sampled by the stigma. Bijlsma et al. (1986) and Gutierrez and Sprague (1959) reported that asynchronous flowering between cultivated varieties of corn, *Zea mays*, led to assortative mating and increased inbreeding. Ennos and Dodson (1987) report similar findings in *Cynosuras cristatus*.

One consequence of such a phenology-influenced model is that outcrossing may be expected to increase with increased genetic differentiation in predominantly inbreeding species. If general genetic differentiation is associated with phenological differentiation within a population, then one would expect that overlapping receptive stigmas and viable pollen would not be closely related. This would be especially true in populations for which seed dispersal is very limited. In such a situation, localized neighborhoods which would be expected to show less environmental variance would

also show little genetic diversity. Flowering synchrony would then be greatest within genotypes and overlap of receptive stigmas and viable pollen from plants of the same genotype would be low. Most viable pollen available to the receptive stigmas would be from different genotypes. Accordingly, populations with high genetic diversity would be expected to have greater heterogeneous overlap, and thus greater outcrossing, than would populations with low diversity. A correlation between genetic diversity and outcrossing rate has been found in the highly inbreeding grasses *Hordeum spontaneum* (Brown et al. 1978 b), *Avena barbata* (Hamrick and Allard 1972; Allard et al. 1972 a), and *Hordeum jubatum* (Babbel and Wain 1977).

Ennos and Clegg (1982) have argued that localized genetic differentiation for outcrossers would be expected to increase effective inbreeding, as near neighbor pollen donors would tend to be closely related. Spatial differentiation then has an effect similar to temporal differentiation on the outcrossing rate. Disjunction or displacement of pollen availability may be expected to reduce effective outcrossing in predominant outcrossers, and increase effective outcrossing in predominant inbreeders with asynchronous male and female gamete availability.

Brown (1979) demonstrated that inbreeders tended to be more heterozygous than would be expected by their outcrossing rates alone. Outcrossing rates are estimated using mean genetic diversity of the pollen pool. Furthermore, outcrossing rates are assumed to be independent of genetic variation within a population. The distribution of outcrosses, however, is not independent of genetic diversity and so estimates of outcrossing based on sample events must weight the observed outcrosses by diversity. Thus, a sampled outcrossing event in a genetically diverse population is weighted down when compared to a similar event in a more homogeneous population. If, however, outcrossing is not independent of genetic diversity, but is causally, positively correlated with it, estimated outcrossing rates in genetically diverse populations may be systematically biased downward. This would produce an excess heterozygosity in some populations of predominantly inbreeding species, and may be a partial explanation for the heterozygosity paradox.

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