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Mating system in rye: variability in relation to the population and plant density

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The amount of outcrossing was estimated using seven enzyme loci assayed in seven populations of rye (*Secale cereale* L.). Single-locus outcrossing values fluctuated widely from locus to locus in each population. The weighted mean single-locus estimates ranged from 0·716 to 0·946, and multilocus estimates ranged from 0·701 to 0·910. The analysis showed that self-pollination occurred in the rye populations, and, as a result of selfing, populations contained homozygotes in excess of random mating expectations at the seedling stage of development. Low plant density, which causes low pollen density during fertilization, seems to weaken the self-incompatibility system; at low plant density, the outcrossing estimate was significantly lower than was obtained at high plant density.

INTRODUCTION

The mating system has important effects on the genetic structure of plant populations because of its importance in determining the amount, distribution, and transmission of genetic variation from generation to generation (Allard *et al.*, 1975).

It is generally admitted that the pollination pattern in most species fits the "mixed mating model". This model states that a certain proportion of zygotes arises from self-fertilization and the remainder is a product of random mating with other plants of the population (Ritland, 1983).

Cultivated rye (*Secale cereale* L.) is a predominantly allogamous species. Outcrossing is favoured by a gametophytic incompatibility system which is controlled by two multiallelic loci (Lundqvist, 1956; Trang *et al.*, 1982). However, a certain amount of selfing has been detected in natural and experimental populations (Bailey *et al.*, 1978; Pérez de la Vega and Allard, 1984). Furthermore, the effectiveness of the incompatibility system can be decreased by the effect of high temperature for a few days during flowering (Wricke, 1979). In addition it has been demonstrated that several environmental factors can disturb the mating system of plant populations, such as humidity, population density, etc. (Brown *et al.*, 1978; Farris and Mitton, 1984; Neale and Adams, 1985). So, it is possible to find in a given plant species some variability in outcrossing rate between populations as well as from year to year (Allard and Workman, 1963; Marshall and Abbott, 1982; 1984; Cheliack *et al.*, 1985). Likewise mating system can be subjected to evolutionary change, and some authors have been able to detect directional variation of the outcrossing rate over generations (Kahler *et al.*, 1975).

In the present work, we have studied seven populations of cultivated rye by means of isozyme electrophoresis with the purposes of analyzing the genetic structure of rye cultivars collected in different countries and grown in natural field conditions, and of estimating the outcrossing rate of these populations, as well as the possible variations between them. The influence of plant density on mating system is also examined.

MATERIAL AND METHODS

The material used in this study consisted of seven populations of *S. cereale* L. cultivated under natural field conditions, and therefore subjected to free pollination. Table 1 shows the country of origin of each cultivar, the locality where populations were grown and the year of collection. Each population belongs to a different cultivar, except

Table 1 Rye populations

Population	Country of origin	Grown at	Year
Ailes	Spain	Zaragoza (Spain)	1981
Insave	Argentina	Córdoba (Argentina)	1983
Merced	California, U.S.A.	Zaragoza (Spain)	1981
MM	Spain	León (Spain)	1981
MMCA	Spain	León (Spain)	1981
Polycross	California, U.S.A.	Davis (California)	1979
Zaragoza	Spain	Zaragoza (Spain)	1981

MM and MMCA: MM, like the other samples except MMCA, was raised as a normal (high) density population (*i.e.*, 180-210 plants per m^2 in experimental fields of 100 m^2), while MMCA grew under conditions of low density (1-2 plants per m^2), as a contaminant of an 1 ha oat field.

Up to sixty spikes, each one from a different maternal plant, were collected from each population; and nine or ten seedlings from each spike were assayed by horizontal starch gel electrophoresis. The isozyme systems surveyed were: glutamic oxaloacetic transaminase (GOT, EC 2.6.1.1), phosphoglucose mutase (PGM, EC 2.7.5.1), phosphoglucose isomerase (PGI, EC 5.3.1.9), acid phosphatase (ACPH, EC 3.1.3.2), malic dehydrogenase (MDH, EC 1.1.1.37), and 6-phosphogluconic dehydrogenase (6PGD, EC 1.1.1.44). Electrophoretic procedures have been given by Vences *et al.* (1987), and the inheritance and nomenclature follow that mainly described by Pérez de la Vega and Allard (1984). Locus *Got*3 was previously named *Got*2 (Pérez de la Vega and Allard, 1984), however, it is now known that there are three different GOT zones and four loci code for the GOT isozymes in rye (Vaquero, 1987; Rebordinos and Pérez de la Vega, 1988). Locus *Acph*2 refers to the *Phos*2 of Pérez de la Vega and Allard (1984); this new name responds to the generally used nomenclature suggested by Schlegel *et al.* (1986). 6PGD is controlled at least by two loci (Vaquero, 1987), but only one of them (6*Pgd*2) has been scored in this work because of the very low genetic variability observed in 6PGD1.

When some alleles are infrequent, especially in multiallelic loci, a large number of the observational classes are empty, causing difficulties in estimation procedures. In these cases the data were reduced to a two or three-allele model. Such reductions were made by pooling the infrequent alleles with the second less frequent one (two-allele model) or with the third one (three-allele model) thus creating a synthetic allele. Single-locus adult plant gene and genotype frequencies, pollen genotype frequencies, and outcrossing rates (t) were estimated following the methods of Clegg *et al.* (1978). The variances of the single-locus outcrossing estimates were calculated by applying the "bootstrap" method of Schoen and Clegg (1986), and the weighted mean of single locus estimates was calculated after Kahler *et al.* (1984). Multilocus outcrossing rates were determined using the "method of moments" estimator of Shaw *et al.* (1981).

RESULTS

The observed numbers of seedling genotypes and the expected ones under Hardy-Weinberg equilibrium are given in table 2. In general significant departures from the expectation were found, and in most cases the departures were due to a deficiency of heterozygotes. This fact can be also seen from the heterozygosity values (table 3): the heterozygosity obtained by direct count is, in general, lower than that computed by the method of Nei (1975), which assumes Hardy-Weinberg equilibrium.

Table 4 gives the observed numbers of single locus adult mother plant genotypes inferred from the progeny genotype arrays by means of the method of Clegg *et al.* (1978), and the expected ones under Hardy-Weinberg equilibrium. As opposed to the seedling populations, χ^2 goodness of fit values for adult plant genotypes (observed vs. expected) were always non-significant. Differences between the number of heterozygous individuals and the expected ones were in general in the direction of excess of heterozygotes. Thus, of a total of 48 tests, only three cases of deficiency of heterozygotes (*Got*3 and *Pgm* in Polycross and *Acph*2 in Zaragoza) were found, while nineteen loci showed excess of heterozygotes. Therefore the values of heterozygosity obtained by direct counts were, in general, higher than calculated by Nei's method (1978) (table 5).

When the inferred genotypic frequencies of both maternal and paternal (pollinator) plant samples of each population were compared non-significant differences were observed between samples, since samples in each population had very similar frequencies.

Outcrossing estimates are given in table 6. Single-locus outcrossing estimates ranged from 0·441 (*Pgi*2 of MMCA) to 1·137 (6*Pgd*2 of Merced). The weighted mean per population ranged from 0·716 of Polycross to 0·946 of MM. Every weighted mean was significantly different from 1, when they were compared by a χ^2 test (Rao, 1973), except Ailes. Heterogeneity χ^2 tests were always significant, indicating that the single-locus estimates differed from locus to locus. The two populations which belong to the same cultivar, MM and MMCA, showed significantly different t values: 0·946 and 0·790, respectively.

The multilocus estimates (t_m) were made from the same data set as the single locus estimates, and ranged from 0·701 (Zaragoza) to 0·910 (Ailes and Insave). The multilocus estimates for MM and MMCA were respectively 0·909 and 0·775, thus, like single-locus estimates, t_m is lower in the population MMCA which grew at a low density of plants.

DISCUSSION

The seedling populations showed genotypic frequencies that did not fit the Hardy-Weinberg expectations, and deviations from equilibrium were generally in the direction of a deficiency of heterozygotes.

This result is only relatively unexpected as, in spite of the reported outbreeding habit of rye, outbreeding species often show a tendency to have lower heterozygosity than expected under panmixia (Brown, 1979); this phenomenon has been called the "heterozygosity paradox". Moreover, herbaceous outbreeders tend to show larger deficits than tree species (Brown, 1979). Among the various factors that might cause this bias the most important are probably: (i) family structure, (ii) the Wahlund effect, and (iii) partial selfing.

The seed samples we used were taken and planted at random from the complete seed-bulk of the preceding generation, so they are expected to be free of family structure. The Wahlund effect, due to non-random spatial distribution of genotypes, is also rejected for the same reason. In fact, as recorded in table 6, there was a measurable selfing rate in these rye populations.

In contrast with the results for seedling populations, χ^2 values for observed vs. expected adult frequencies were non-significant, and heterozygotes were generally in excess. The frequent excess of heterozygotes in the adult populations and the general deficiency of them amongst seedlings suggests that heterozygous seedlings are at an advantage and show better survival to adulthood than homozygotes. Such heterozygote advantage has been reported in several plant species (Clegg and Allard, 1973; Kahler *et al.*, 1975; Farris and Mitton, 1984; Pollack *et al.*, 1984). Thus although inbreeding causes a deficit of heterozygotes, selection favours them to become adult plants; therefore the adult populations fit Hardy-Weinberg expectation perhaps only because the effects of nonrandom mating and selection cancel each other. It seems that selection of this type must act after germination since germination rates were nearly always 100 per cent (*i.e.*, greater than 97 per cent for the worst case).

With the exception of the Ailes single locus weighted mean, every analyzed population had an outcrossing rate which was significantly different from one, the means over populations being closely similar: $0{\cdot}834 \pm 0{\cdot}033$ and $0{\cdot}829 \pm 0{\cdot}031$ estimated respectively by the single-locus or the multilocus methods.

Single-locus estimates differed from locus to locus, as reported previously for other species: *e.g.*, in *Zea mays* (Kahler *et al.*, 1984), *Pseudotsuga menziessi* (Ritland and El-Kassaby, 1985), *Abies balsamea* (Neale and Adams, 1985), *Hordeum* (Clegg *et al.*, 1978; Brown *et al.*, 1978) and *Secale cereale* (Pérez de la Vega and Allard, 1984). This variability has been attributed to different causes, including chance, (Shaw *et al.*, 1981) statistical aberrations (Clegg, 1980), and the violation of some of the assumptions of the mixed mating model. Failure of any of these assumptions will affect both single-locus and multilocus estimates, but multilocus estimates are affected to a much lesser extent (Shaw *et al.*, 1981; Ritland, 1983).

The assumption of homogeneity of pollen distribution can be violated by the existence of family structure or Wahlund effect. In our case, as we previously discussed, we did not expect any of these two effects because of the way in which seeds were planted. Additionally, heterogeneity is expected to cause underestimation of outcrossing rates when single-locus estimates are used. The mean values of single-locus estimates (0·834) and multilocus estimates (0·829) are very similar, supporting the expectation of homogeneity in the pollen pool.

Table 2 Observed and expected[1] numbers of single-locus seedling genotypes

		Ailes		Insave		Merced		MM		MMCA		Polycross		Zaragoza	
Got3	*11*	333	342·22	673	673.16	489	482·31	438	434·95	484	480·33	301	281·75	353	330·20
	12	234	213·56	21	20·68	95	106·78	144	150·10	102	109·43	221	259·50	170	215·61
	13					2	3·59			1	0·90				
	22	23	33·22		0·16	12	5·91	16	12·95	10	6·23	79	59·75	58	35·20
	23						0·10								
	33					1	0·01								
	D		0·096				−0·124		−0·041		−0·067		−0·148		−0·212
	χ^2		5·405*		0·164		156·525***		0·988		2·924		13·288***		29·997***
Pgm	*11*	436	439·98	576	564·74	386	379·85	484	479·72	442	443·78	502	496·86	577	577·06
	12	147	139·03	99	103·51	157	170·41	103	109·98	141	138·42	87	97·37	10	9·90
	13					22	21·50					1	0·91	2	1·98
	14					3	2·39	2	3·58	5	6·02				
	22	7	10·98	7	4·74	26	19·11	9	6·30	9	10·82	10	4·77		0·42
	23					5	4.82								0.02
	24						0.53	2	0·41	2	0·94				
	33						0·30								0·00
	34						0·07								
	44						0·01		0·01		0·02				
	D		0·057		−0.044		−0.064		−0·061		0·018		−0·105		
	χ^2		1·937		1·279		4·723		8·503		1·741		6·989		0·062
Pgi2	*11*	460	442,58			291	265·33	211	183·71	322	262·23	157	146·01	219	198·43
	12	39	68·42			139	190·67	197	257·30	113	229·79	50	71·51	78	105·91
	13	63	68·42			75	74·67	45	39·29	34	37·08	119	194·87	166	179·23
	14									1	0·66	29	33·56		
	22	14	2·64			61	34·25	123	90·09	109	50·34	17	8·76	28	14·13
	23	12	5·29			25	26·83	22	27·51	16	16·25	52	47·72	48	47·83
	24											9	8·22		
	33	2	2·64			6	5·25	2	2·10	3	1·31	65	65·02	47	40·47
	34										0·47	14	22·40		
	44										0·00	8	1·93		
	D		−0·198				−0·182		−0·0185		−0·423		−0·067		−0·124
	χ^2		71·200***				37·602***		32·146***		144·284***		38·481***		25·127***
Acph2	*11*	578	578·06	661	658·12	597	597·01	587	587·06	588	588·03	600		506	497·83
	12	12	11·88	12	17·76	2	1·99	12	11·88	9	8·93			71	87·34
	22		0·06	3	0·12				0·06		0·03			12	3·83
	D				−0·324										−0·188
	χ^2		0·062		71·112***		0·002		0·061		0·034				20·613***
Mdh1	*11*	543	543·94	670	670·01	597	597·00	587	587·07	587	587·04	600		589	
	12	47	45·13	2	1·99	1	0·99	8	7·91	10	9·92				
	13			1	1·00	1	0·99	4	3·96						
	14							1	0·99						
	22		0·94						0·03		0·04				
	D		0·041												
	χ^2		1·015		0·003		0·002		0·072		0·043				

Mdh2	*11*	493	489·67	607	606·72	514	509·61	492	491·41	523	524·36	523	514·44	588	588·00
	12	89	95·66	64	64·56	77	85·78	92	94·12	48	46·86	67	84·12	1	1·00
	13							10	9·05	25	23·43				
	22	8	4·67	2	1·72	8	3·61	6	4·51	1	1·05	12	3·44		
	23								0·87		1·05				
	33								0·04		0·26				
	D		−0·070				−0·102		−0·020		−0·023		−0·204		
	χ^2		2·875		0·051		6·276*		1·551		1·447		24·940***		
6*Pdg2*	*11*	361	355·53	499	474·33	316	326·89	384	378·42	344	339·40	509	500·59	423	405·14
	12	187	197·95	120	138·52	253	231·22	184	193·78	208	216·94	76	89·44	125	160·89
	13	1	0·78	12	42·82			1	2·38	2	2·27	2	3·65	1	0·83
	14	6	6·21									1	2·74		
	22	33	27·50	21	10·11	30	40·89	29	24·81	39	34·67	11	3·99	34	15·97
	23			3	6·25			2	0·61	1	0·73		0·33		0·17
	24	2	1·73										0·24		
	33			18	0·97							1	0·01		
	34												0·01		
	44											1	0·00		
	D		−0·053		−0·280		0·094		−0·050		−0·041		−0·181		−0·222
	χ^2		2·131		339·561***		5·313*		5·258		1·112		405·600***		29·337***

[1] Expected under Hardy-Weinberg equilibrium.
D = (Observed heterozygotes − Expected heterozygotes)/Expected heterozygotes. D is only scored when the numerator is higher than 1.
* $P < 0.05$; ** $P < 0.01$; $P < 0.001$.

Table 3 Heterozygosity frequencies of the seedling populations

Population		*Got3*	*Pgm*	*Pgi2*	*Acph2*	*Mdh1*	*Mdh2*	*6Pgd2*	Mean
Ailes	O	0·397	0·249	0·193	0·020	0·080	0·151	0·322	0·203 ± 0·051
	E	0·362	0·236	0·241	0·020	0·076	0·162	0·351	0·207 ± 0·050
Insave	O	0·030	0·147		0·017	0·004	0·095	0·201	0·082 ± 0·027
	E	0·029	0·154		0·026	0·004	0·096	0·279	0·098 ± 0·039
Merced	O	0·162	0·312	0·400	0·003	0·003	0·129	0·422	0·204 ± 0·066
	E	0·185	0·333	0·489	0·003	0·003	0·143	0·386	0·220 ± 0·071
MM	O	0·241	0·178	0·440	0·020	0·022	0·170	0·312	0·198 ± 0·057
	E	0·251	0·190	0·540	0·020	0·021	0·173	0·328	0·218 ± 0·069
MMCA	O	0·173	0·247	0·273	0·015	0·017	0·122	0·355	0·172 ± 0·049
	E	0·185	0·243	0·474	0·015	0·017	0·119	0·370	0·203 ± 0·066
Polycross	O	0·368	0·147	0·450	0·000	0·000	0·111	0·131	0·172 ± 0·066
	E	0·432	0·164	0·588	0·000	0·000	0·140	0·160	0·212 ± 0·083
Zaragoza	O	0·293	0·020	0·498	0·121	0·000	0·002	0·216	0·164 ± 0·070
	E	0·371	0·020	0·568	0·148	0·000	0·002	0·278	0·198 ± 0·082

O: observed frequency; E: estimated frequency by Nei's method (1975).

A second assumption is the independence between probability of outcrossing and maternal genotype. Out data do not demonstrate the accomplishment of this assumption but, otherwise, we have no evidence of preferential mating between specific gametic genotypes or differential probability of selfing for different maternal genotypes as it has been reported in several species (Clegg, 1980; Ritland and El-Kassaby, 1985; Bijlsma *et al.*, 1986).

Finally, the model assumes that selection does not intervene between mating and the time of census. Our results support this assumption because of the high fertility of plants (spikes were normally and uniformly seedy) and the low mortality percentages of seedlings (less than 3 per cent for the worst case).

Consequently in our case, although we cannot eliminate the possibility of a maternal effect on mating system, perhaps the variability among the single-locus estimates could be due to the statistical procedure. Any other effect which could be disturbing the mating system did not appear to be strong enough to bias the single-locus estimates significantly in a specific direction. So, while some populations had lower t than t_m, and other populations had lower t_m than t, the overall population average t and t_m are nearly identical.

Although the present data do not exclude the possibility that inbreeding other than selfing occurs in natural populations of rye, they indicate that most of the inbreeding observed must be due to self-fertilization, because, in general, the means of single locus estimates are equal to or higher than the multilocus estimates (Shaw and Allard, 1982).

The averaged outcrossing rate is approximately 0·83 for the rye populations analyzed in this work. This rate is lower than the previously reported value of 0·92 by Pérez de la Vega and Allard (1984) in a population of Ailes. However, the sample of the Ailes cultivar analyzed in the present study gave a single-locus estimate of 0·902 and a multilocus estimate of 0·910, values which are not significantly different from the value reported by Pérez de la Vega and Allard. Significant differences in outcrossing rates were found among the rye cultivars (table 6), ranging from 0·95 to 0·70.

In spite of the existence in rye of a two-locus incompatibility system (one having at least six alleles, and the other at least twelve, Trang *et al.*, 1982), a relatively high number of self-pollinations were detected in this species. The self-incompatibility system can be overcome by genes of self-compatibility, allowing selfing rates up to 40 per cent in autocompatible populations (Wricke, 1979; Schmidt-Stohn *et al.*, 1986), and also in synthetic populations obtained by hybridization with *Secale vavilovii* (Bailey *et al.*, 1978). We currently have no information on the presence of self-compatibility genes in our populations, however it is not possible to reject their presence.

In addition to genetic factors it seems that at least some environmental factors could exert an influence on the autoincompatibility system efficiency. Thus, some cases of pseudocompatibility have been described in rye following exposure to high temperature (≅30°C) during flowering (Wricke, 1979). At the localities where our populations were grown (Spain, Argentina and

Table 4 Inferred[1] and expected[2] numbers of adult plant genotypes

		Ailes		Insave		Merced		MM		MMCA		Polycross		Zaragoza	
Locus		I	E	I	E	I	E	I	E	I	E	I	E	I	E
*Got*3	*11*	32	34·47	65	65·30	46	46·79	46	46·78	48	48·60	31	30·07	32	33·03
	12	27	22·01	3	2·93	13	11·45	14	11·70	12	10·08	23	24·81	25	22·97
	13					1	0·91								
	22	1	3·51				0·71		0·82		0·60	6	5·12	3	3·99
	23				0·03		0·12								
	33						0·01								
		D = 0·227				D = 0·124		D = 0·197		D = 0·111		D = −0·073		D = 0·088	
Pgm	*11*	41	42·54	58	58·37	36	37·64	47	47·74	44	45·10	51	50·45	60	60·00
	12	19	15·96	10	9·26	19	19·63	12	10·70	16	13·84	8	9·13		
	13					4	3·14								
	14							1	0·86						
	22		1·50		0·37	1	1·84		0·60		1·06	1	0·41		
	23						0·69								
	24								0·10						
	33						0·06								
		D = 0·191				D = 0·124		D = 0·115		D = 0·156		D = −0·124			
*Pgi*2	11	41	41·73			20	23·44	13	17·04	22	24·73	11	11·25	16	17·04
	12	9	8·31			26	21·23	35	27·18	30	25·04	8	7·38	12	10·68
	13	9	8·31			9	6·90	3	2·69	3	2·54	18	18·60	20	19·19
	14											4	3·48		
	22		0·41			3	4·81	7	10·83	4	6·34	1	1·21	1	1·67
	23	1	0·83			2	3·12	2	2·14	1	1·28	7	6·10	6	6·01
	24												1·14		
	33		0·41				0·51		0·11			8	7·69	5	5·40
	34											2	2·88		
	44											1	0·27		
		D = 0·089				D = 0·184		D = 0·250		D = 0·178		D = 0·059			
*Acph*2	*11*	59	59·00	65	65·03	60	60·00	60	60·00	59	59·00	60	60·00	51	50·45
	12	1	0·99	3	2·93					1	0·99			8	9·13
	22		0·01		0·03						0·01			1	0·40
														D = −0·124	
*Mdh*1	*11*	56	56·11	68	68·00	60	60·00	60	60·00	59	59·04	60	60·00	60	60·00
	12										0·95				
	13	4	3·83												
	22									1	0·01				
	33		0·07												
*Mdh*2	*11*	48	48·60	64	64·06	50	50·45	49	48·60	53	53·21	51	51·34	60	60.00
	12	12	10·80	4	3·88	9	8·25	10	10·80	5	4·71	9	8·32		
	13					1	0·88			2	1·88				
	22		0·60		0·06		0·34	1	0·60		0·10		0·34		
	23				0·07				0·08						
	33				0·01				0·02						
		D = 0.111													
6*Pgd*2	*11*	34	35·29	38	41·3	33	34·47	40	41·60	36	36·78	50	50·45	42	41·63
	12	23	20·71	18	14·03	25	22·01	20	16·69	22	20·39	8	7·37	16	16·69
	13			12	9·35							1	0·91		
	14	1	0·74									1	0·91		
	22	2	3·04		1·19	2	3·51		1·67	2	2·82		0·27	2	1·67
	23				1·59								0·07		
	24		0·22										0·07		
	33				0·53								0·01		
	34												0·01		
	44												0·01		
		D = 0·108		D = 0·201		D = 0·136		D = 0·198		D = 0·079					

[1] I: inferred by the method of Clegg *et al* (1978).
[2] E: expected under H–W equilibrium.
D = (Observed heterozygotes − Expected heterozygotes)/Expected heterozygotes, D is scored only when the numerator is higher than 1.

Table 5 Heterozygosity frequencies of the maternal populations

Population		*Got*3	*Pgm*	*Pgi*2	*Acph*2	*Mdh*1	*Mdh*2	6*Pgd*2	Mean
Ailes	O	0·450	0·317	0·317	0·017	0·067	0·200	0·400	0·253 ± 0·062
	E	0·370	0·291	0·294	0·016	0·064	0·181	0·364	0·226 ± 0·054
Merced	O	0·233	0·383	0·617	0·000	0·000	0·167	0·417	0·260 ± 0·086
	E	0·223	0·344	0·525	0·000	0·000	0·155	0·370	0·231 ± 0·074
MM	O	0·233	0·217	0·667	0·000	0·000	0·167	0·333	0·231 ± 0·086
	E	0·208	0·208	0·538	0·000	0·000	0·181	0·281	0·202 ± 0·069
MMCA	O	0·200	0·267	0·567	0·017	0·000	0·117	0·367	0·219 ± 0·076
	E	0·181	0·232	0·485	0·016	0·034	0·140	0·343	0·204 ± 0·063
Polycross	O	0·389	0·133	0·650	0·000	0·000	0·150	0·167	0·213 ± 0·088
	E	0·417	0·153	0·665	0·000	0·000	0·136	0·156	0·218 ± 0·091
Zaragoza	O	0·417	0·000	0·633	0·133	0·000	0·000	0·267	0·207 ± 0·093
	E	0·386	0·000	0·603	0·153	0·000	0·000	0·280	0·203 ± 0·088

O: observed frequencies; E: estimated frequencies by Nei's method (1978).

Table 6 Single-locus and multilocus estimates of outcrossing rates

Locus	Ailes	Insave	Merced	MM	MMCA	Polycross	Zaragoza
*Got*3							
t	1·119	0·903	0·807	0·957	0·849	0·666	0·577
S.D.	0·013	0·011	0·015	0·014	0·017	0·011	0·013
Pgm							
t	1·135	0·926	0·873	0·919	0·991	0·842	0·771
S.D.	0·036	0·016	0·008	0·011	0·009	0·031	0·002
*Pgi*2							
t	0·806		0·788	0·942	0·441	0·800	0·755
S.D.	0·009		0·011	0·008	0·009	0·013	0·008
*Acph*2							
t	0·556	0·752					0·743
S.D.	0·037	0·021					0·014
*Mdh*1							
t	0·920						
S.D.	0·016						
*Mdh*2							
t	0·923		0·911	0·951	0·733	0·678	
S.D.	0·015		0·013	0·008	0·017	0·016	
6*Pdg*2							
t	0·908	0·774	1·137	0·968	0·906	0·708	0·696
S.D.	0·015	0·006	0·011	0·012	0·009	0·026	0·016
Weighted mean							
t	0·902	0·812	0·911	0·946	0·790	0·716	0·763
S.D.	0·060	0·029	0·027	0·024	0·029	0·046	0·026
S.E.	0·023	0·015	0·012	0·011	0·013	0·021	0·012
Multilocus estimates							
t_m	0·910	0·910	0·799	0·909	0·775	0·802	0·701
S.D.	0·040	0·060	0·036	0·041	0·039	0·032	0·034

S.D. = Standard deviation; S.E. = Standard error.

California) temperature frequently rises above 30°C in daylight during late spring, when most pollination occurs. Such conditions could have favoured high rates of selfing.

Other factors may also affect the mating system, for example, pollen density. The data obtained from the MM and MMCA samples suggest that a greater proportion of selfed seeds is obtained when pollen density is low, *i.e.*, at low plant density in the MMCA sample. The comparison between MM and MMCA is also useful to test the hypothesis that selfing rate in rye is due to disruption of the self-incompatibility system (pseudocompatibility) and not to the presence of self-compatible plants in the populations. If the latter was true it might be expected that at low density self-compatible plants would show high fertility, while self-incompatible genotypes would have much reduced seed set. In fact, MMCA spikes were uniformly seeded.

It would seem, therefore, that selfing in rye is due mainly to pseudocompatibility possibly caused by environmental factors, such as high temperatures during flowering time. Under such conditions of pseudocompatibility low plant densities and, therefore, low pollen densities may increase the selfing rate due to an increased self-pollination.

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