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Quantitative studies on the mating system of opium poppy (*Papaver somniferum* L.)*

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Summary. Nearly 400 individuals at two locations and over a number of years were crossed and subsequently scored for selfing versus outcrossing in eight monohybrid populations of opium poppy (*Papaver somniferum*). Two different marker loci, petal colour (*R*/*r*) and capsule size (*B*/*b*) were used to determine the male gametes that had effected fertilizations in F_2 recessives (*rr* and *bb*). The estimates of the outcrossing parameter (α) were found to vary with year, location and for the marker locus used (α range: 0.0988–0.3704). Study of two dihybrid crosses involving the two loci simultaneously, further confirmed that outcrossing at the *R*/*r* locus was significantly greater than that at the *B*/*b* locus. The nature of the outcrossing was, in general, nonrandom. Selfing predominated in this species; however, there was a high frequency of natural outcrossing for generating variations in *P. somniferum*.

Key words: *Papaver somniferum* – Outcrossing rate – Selfing rate – Mating system

Introduction

Many plant species allow cross pollination in addition to the usual self fertilization. Natural outcrossing generates genetic variability in populations and hence, a knowledge of its nature and extent is a prerequisite for determining the suitable breeding method in a species. Fyfe and Bailey (1951) and Nei and Syaktdo (1958) have developed various procedures for estimating inbreeding coefficients, *f*, and outcrossing parameters, α, that assume no selection and population equilibria for gene frequencies. Allard and Workman (1963) and subsequently Harding and Tucker (1964) introduced methods for estimating an outcrossing parameter when selection is present and/or equilibrium can not be assumed. These methods are based on gene frequency in a population and estimates of the frequency of dominant individuals appearing in the progeny of recessive individuals taken at random from the population.

Opium poppy (*Papaver somniferum* L.), a plant of immense pharmaceutical importance for its alkaloids and culinary values, is mainly self pollinating. The extent of natural cross pollination in this species is highly variable. It may be as low as 9% (Nyman and Hall, 1976) and as high as 20–30% (Sharma and Singh 1983). Whether cross pollination is random or not has not been studied. The purpose of the investigation presented here was to study in detail the mating system involved in certain artificial populations of this species using different loci, years and locations.

Materials and methods

Red petal colour (*R*) and large capsule size (*B*) in opium poppy are completely dominant over white petal colour (*r*) and small capsule size (*b*), respectively (Patra and Chauhan 1990). Red petal colour and large capsule size were used as markers in detecting cross pollination in recessive white-petalled and small-capsulated plants in F_2 populations of different crosses between two varieties/strains differing at dominant and recessive genes. Outcrossing using the two loci (*B*/*b* and *R*/*r*) was estimated at two locations: Lucknow and Srinagar. At Lucknow, outcrossing was estimated between 1986 and 1990 for locus *R*/*r* and during 1989 and 1990 for locus *B*/*b*. At Srinagar outcrossing for both loci was estimated over 1990–1991. No fewer than 115 dominant plants were randomly drawn from each of the F_2 populations grown at the locations. These plants were progeny tested for the purpose of estimating genotypic and gene frequencies at the two marker loci. The gene frequency for the two loci was approximately 0.5.

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Hence, p or q was taken to be equal to 0.5 in subsequent calculations.

The estimation procedures developed by Harding and Tucker (1964) were followed. If a random sample of recessives is selected from a population, then (a) heterozygotes and (b) recessive homozygotes will be observed in their progeny generation. The observed proportion of outcrossing T and its variance are:

$$\hat{T} = \frac{a}{a+b}$$

and

$$\mathrm{Var}\ \hat{T} = \frac{a\,b}{(a+b)^3}$$

which are the maximum likelihood estimators. However, not all crosses will be observed because homogenous matings result in homozygotes. The maximum likelihood estimate of total outcrossing, α, is

$$\hat{\alpha} = \frac{\hat{T}}{(1-q)}$$

where q is the gene frequency associated with the homozygote selected. If q is known, the using the maximum likelihood method,

$$\mathrm{Var}\ \hat{\alpha} = \frac{(1-\alpha p)}{N p}$$

where $p+q=1$ and $N=a+b$. The expected standard deviation (σ_α) and the observed standard deviation (S_α) were computed following Harding and Tucker (1964).

Results

Petal colour (anthocyanin) locus (R/r)

Table 1 presents the frequencies of heterozygotes, total F_3 offspring, estimates of outcrossing (α) and their standard deviations for different crosses, years and locations. Binomial index χ^2's for the test of heterogeneity of the estimates of outcrossing revealed that the crosses within Lucknow in 1986, 1989 and 1990 and within Srinagar in 1990 were heterogeneous (χ^2_{3df} = 24.34, 36.03, 199.38 for 1986, 1989 and 1990, respectively, at Lucknow and χ^2_{3df} = 53.39 at Srinagar). An almost similar trend in results was marked in pooled analyses of heterogeneity between years within Lucknow and between the two locations:

	Heterogeneity source	*df*	Heterogeneity χ^2 value	*P*
1.	Between years within location:			
	Lucknow:			
	1986 versus 1989	1	2.10	0.20–0.01
	1986 versus 1990	1	45.34	0.001
	1989 versus 1990	1	72.04	0.001
2.	Between locations:			
	Lucknow versus Srinagar	1	46.31	0.001

Table 1. Estimates of outcrossing for different years and locations using the *R*/*r* locus as marker (Female parent having genotype *RR* and male parent, *rr*)

Location (year)	Cross	a[a]	*n*[b]	$\hat{\alpha}$	$\sigma_{\hat{\alpha}}$
Lucknow (1986)	PLB × Shweta	98	780	0.2513	0.0473
	PLB × Shyama	86	665	0.2586	0.0512
	PLB × Ranjhatak	42	462	0.1818	0.0627
	PLB × IC-42	30	162	0.3704	0.1003
	Total	256	2,069	0.2475	0.0291
Lucknow (1989)	PLB × Shweta	126	1,132	0.2226	0.0396
	PLB × Shyama	42	340	0.2471	0.0718
	PLB × Ranjhatak	73	418	0.3493	0.0628
	PLB × IC-42	48	275	0.3491	0.0775
	Total	289	2,165	0.267	0.0283
Lucknow (1990)	PLB × Shweta	64	1,295	0.0988	0.0383
	PLB × Shyama	72	960	0.1500	0.0439
	PLB × Ranjhatak	38	496	0.1532	0.0610
	PLB × IC-42	132	788	0.3350	0.0460
	Total	306	3,539	0.1729	0.0227
Srinagar (1990)	PLB × Shweta	82	826	0.1985	0.0467
	PLB × Shyama	106	846	0.2506	0.0455
	PLB × Ranjhatak	75	979	0.1532	0.0434
	PLB × IC-42	74	638	0.2947	0.0517
	Total	357	3,289	0.2171	0.0233
	Grand total	1,373	11,062	0.2482	0.0126

[a] Heterozygotes

[b] Total number of F_3 offspring

Table 2. Estimates of outcrossing for different years using the *B*/*b* locus as marker (female parent having genotype *BB* and male parent *bb*)

Location (year)	Cross	a[a]	*n*[b]	$\hat{\alpha}$	$\sigma_{\hat{\alpha}}$
Lucknow (1989)	BM × Shweta	68	779	0.1745	0.0484
	BM × Shyama	72	770	0.1870	0.0485
	BM × Ranjhatak	120	875	0.2743	0.0444
	BM × IC-42	23	338	0.1361	0.0743
	Total	283	2,763	0.2048	0.0255
Lucknow (1990)	BM × Shweta	66	554	0.2381	0.0564
	BM × Shyama	50	358	0.2793	0.0693
	BM × Ranjhatak	122	956	0.2552	0.0427
	BM × IC-42	35	290	0.2414	0.0779
	Total	273	2,158	0.2530	0.0285
Srinagar (1990)	BM × Shweta	64	778	0.1645	0.0486
	BM × Shyama	32	544	0.1176	0.0588
	BM × Ranjhatak	89	392	0.1995	0.0449
	BM × IC-42	70	568	0.2465	0.0556
	Total	185	2,782	0.1330	0.0259
	Grand total	741	7,703	0.1924	0.025

[a] Heterozygote

[b] Total number of F_3 offspring

Thus, the binomial index χ^2 of the pooled estimate of outcrossing always, barring only one case with respect to the comparison of pooled data of 1986 and 1989, revealed heterogeneity. The lack of homogeneity, however, does not explain whether the differences in the estimates of outcrossing were due to a variation in locations or years, or both. Since the populations varied genetically, the en-

Table 3. Estimates of outcrossing in two crosses using *R*/*r* and *B*/*b* loci simultaneously, with the parents having genotypes *bbRR* and *BBrr*

Location (year)	Crosses	a[a]		n[b]	$\hat{\alpha}$		$\sigma_{\hat{\alpha}}$	
		R/*r*	*B*/*b*		*R*/*r*	*B*/*b*	*R*/*r*	*B*/*b*
Lucknow (1989)	PLB × BM	32	18	260	0.2461	0.1385	0.0821	0.0846
	RPM × BM	40	15	248	0.3226	0.1210	0.0822	0.0870
	Total	72	33	508	0.2835	0.1299	0.0581	0.0607

[a] Heterozygotes

[b] Total number of F_3 offspring

tire variation can not be attributed to environment alone. Therefore, all of the observations over locations and years were pooled, and a composite estimate was made, giving $\hat{\alpha} = 0.2482$. The observed fluctuation of this composite estimate (S_α) was more than 6 times ($S_\alpha/\sigma_\alpha = 6.54$) the expected random fluctuation (σ_α), indicating the nonrandom nature of outcrossing.

Capsule size locus (B/b)

The frequencies of heterozygotes, total F_3 offspring and their standard deviations for the *B*/*b* locus are presented in Table 2. The binomial index chi-square test revealed that outcrossing in different crosses at Lucknow in 1989 ($\chi^2_{3df} = 41.65$; $P = <0.001$) and at Srinagar in 1990 ($\chi^2_{3df} = 34.24$; $P = <0.001$) were heterogeneous, while the crosses in 1990 within Lucknow, ($\chi^2_{3df} = 2.19$; $P = 0.70$ to 0.50) were homogenous. The individual pooled estimates of outcrossing in the two locations were compared and found to be heterogeneous ($\chi^2_{1df} = 34.24$; $P = <0.001$). All the observations for this locus were pooled and a composite estimate was made, giving $\hat{\alpha} = 0.1924$. The observed fluctuation of the outcrossing was more than 2 times the fluctuations expected on the basis of change alone ($S_\alpha/\sigma_\alpha = 2.16$), indicating the nonrandom nature of the outcrossing.

As shown in Table 1 and 2, outcrossing estimates for locus *B*/*b* were consistently lower than those for the *R*/*r* locus. Consistent with this observation was the result obtained from two dihybrid crosses of these two loci (Table 3). In these crosses some F_2 progenies were doubly recessive for petal colour and capsule size (i.e. *rb*/*rb*) loci. Outcrossings effected simultaneously for these loci by the corresponding dominant genes in the populations were detected and compared. In both crosses the estimates of outcrossing at the *R*/*r* locus were greater than those at the *B*/*b* locus. It appears likely that the *R*/*r* individual is more effective male parent in cross pollination than the *B*/*b* parent. Mean estimates of outcrossing at these two loci over all crosses and locations were found to be heterogeneous ($\chi^2_{1df} = 80.83$; $P = <0.001$).

Discussion

From our quantitative studies involving different loci, crosses, years and locations it is clear that the amount of outcrossing in opium poppy is, in general, heterogeneous and nonrandom. There were, however, two exceptions. First, outcrossings involving the *B*/*b* locus in four crosses at Lucknow in 1990 were homogenous. Second, notwithstanding the highly significant outcrossing heterogeneity at locus *R*/*r* among the four crosses in each of the years 1986 and 1989 at Lucknow, the individual pooled estimates of outcrossing in 1986 and 1989 at Lucknow were similar. At both locations for the two loci, the females in all of the crosses were the same; therefore, the observed heterogeneity in outcrossing estimates in different crosses may be due to the differing male parents. Heterogeneity and nonrandomness, in outcrossing have been documented in many crop plants, namely *Phaseolus lunatus* (Harding and Tucker 1964), *Phaseolus aureus* (Dana 1969), barley (Jain 1961), maize (Gutierrez and Sprague 1959) and jute (Basak and Chaudhury 1966; Basak and Gupta 1972; Basak and Paria 1975).

The present study was not able to differentiate the roles of year and location on outcrossing estimates. Either only year or location or year and location together may be responsible for causing the observed outcrossing variations. The differences among the crosses within a particular locus, or more specifically, the wide outcrossing variations in the crosses with common female parent and common locus, are suggestive of the fact that outcrossing variation in opium poppy has an ingrained genetic control. The two marker loci used in this study were observed to have different rates of outcrossing: the *Rr* locus as male effected a higher rate of outcrossing than did the *Bb* locus. Basak and Paria (1975) observed in jute (*Corchorus capsularis*) that estimates of outcrossing for the *C*/*c*, *Sr*/*sr* and *Fs*/*fs* loci were different.

Mating systems are generally thought to be important factors in determining the amount and nature of genetic variability in populations. The observed variability in the outcrossing of opium poppy under the influence of genetic and environmental factors is very likely to contribute to a complex population structure. Thus, the mating systems of opium poppy populations must be considered to be quite complex. The present finding upholds the earlier suggestion made by Allard et al. (1968) that the population structure in inbreeding species is much more complicated than commonly supposed and probably does not take the same form in all inbreeding species or even in different populations of the same species.

In conclusion, *P. somniferum* populations neither follow models of complete random mating nor those of complete inbreeding; instead, the mating system of the instant populations is partial inbreeding. A considerably high amount of natural cross pollination in opium poppy seems to be potentially capable of enhancing genetic variation. Elegant discussions on the evolutionary consequences and significance of such a mating system as the one observed here have been put forward by Workman (1964) and Allard et al. (1968).

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References

Allard RW, Workman PL (1963) Population studies in predominantly self-pollinated species. IV. Seasonal fluctuations in estimated values of genetic parameters in lima bean populations. Evolution 18:470–480

Allard RW, Jain SK, Workman PL (1968) The genetics of inbreeding species. Adv Genet 14:55–131

Basak SL, Chaudhuri BB (1966) Extent and nature of natural cross-pollination in 'Tossa' Jute (*Corchorus olitorius* L.). Indian J Agric Sci 36:267–272

Basak SL, Gupta S (1972) Quantitative studies on the mating system of jute (*Corchorus olitorius* L.). Theor Appl Genet 42: 319–324

Basak SL, Paria P (1975) Quantitative studies on the mating system of jute (*Corchorus capsularis* L.). Theor Appl Genet 46:347–351

Dana S (1969) Non-random outcrossing in mung bean. Indian J Genet Plant Breed 29:142–146

Fyfe JL, Bailey NTJ (1951) Plant breeding studies in leguminous forage crop. I. Natural crossing in winter beans. J Agric Sci 41:371–378

Gutierrez MG, Sprague GF (1959) Randomness of mating in isolated polycross planting of maize. Genetics 44:1075–1082

Harding J, Tucker CL (1964) Quantitative studies on mating systems. I. Evidence for nonrandomness of outcrossing in *Phaseolus lunatus.* Heredity 19:369–381

Jain SK (1961) A note on estimation of natural crossing by maximum likelihood method. Indian J Genet 21:146–148

Nei M, Syaktdo K (1958) The estimation of outcrossing in natural population. Jpn J Genet 33:46–51

Nyman V, Hall O (1976) Some varieties of *P. somniferum* L. with changed morphine alkaloid. Heriditas 84:69

Patra NK, Chauhan SP (1990) Morphophysiology and genetics of induced mutants expressed in the M_1 generation in Opium poppies. J Heredity 81:347–350

Sharma JR, Singh OP (1983) Genetics and genetic improvement. In: Husain A, Sharma JR (eds.). The opium poppy. Central Institute of Medicinal and Aromatic Plants, Lucknow, pp 39–68

Workman PL (1964) The maintenance of heterozygosity by partial negative assortative mating. Genetics 50:1369–1382