Mol Breeding
DOI 10.1007/s11032-010-9395-3

Gene frequency-based estimation of natural outcrossing in opium poppy (*Papaver somniferum* L.)

Birendra Kumar · N. K. Patra

Received: 17 July 2009 / Accepted: 21 January 2010

Abstract Mating systems are generally thought to be important factors in determining the amount and nature of genetic variability in a population. Nearly 1,000 individuals at a single location (Lucknow) and over two years were crossed and subsequently scored for selfing versus outcrossing in 9–10 monohybrid populations of opium poppy (*Papaver somniferum*). Three different alleles of two marker loci (two—R/r and P/p—for anthocyanin locus and B/b for capsule size locus) were used to determine the male gametes that had effected fertilizations in F_2 recessives (rr, pp and bb). The estimates of the gene frequency-based outcrossing parameter (α) were found to vary with year, cross and marker locus used (α range: 7.21–71.03%). Study of the two dihybrid crosses concerning the two marker loci simultaneously, further confirmed that outcrossing at the R/r or P/p locus was significantly greater than that at the B/b locus. The nature of the outcrossing was, in general, nonrandom. In this species, in general, selfing predominated, with one exception in respect of monohybrid crosses involving the purple form of anthocyanin locus, in which outcrossing predominated.

B. Kumar (✉) · N. K. Patra
Central Institute of Medicinal and Aromatic Plants (CSIR), CIMAP, Lucknow 226015, India
e-mail: birendrak67@rediffmail.com;
biren_k67@yahoo.com

Keywords Mating system · Nonrandom · Outcrossing · Heterogeneity · Outcrossing rate · *Papaver somniferum* · Selfing

Introduction

Many species have a dual pollination mechanism known as cross/often-cross or self/often-self pollination, among them the opium poppy (*Papaver somniferum*), which usually exhibits self pollination but sometimes exhibit often-cross pollination behaviour depending upon the climatic factors etc. Knowledge of the nature and extent of such genetic variability in these plant species is an essential precursor to determining a suitable breeding method. Fyfe and Bailey (1951) and Nei and Syaktdo (1958) had developed various procedures for estimating breeding coefficients, *f*, and outcrossing parameters, α, that assume no selection or population equilibria. Subsequently Allard and Workman (1963) and Harding and Tucker (1964) introduced methods for estimating an outcrossing parameter. These methods, essentially based on gene frequency in a population and the estimated frequency of dominant individuals appearing in the progeny of recessive individuals taken at random from the population, are valid and relevant at any point in time.

The opium poppy (*Papaver somniferum* L.), a plant of immense pharmaceutical importance for its alkaloids and culinary value, is mainly self pollinating

Published online: 25 February 2010

(Kadereit 1986a, b). Varied results are available in the literature. However, for the varied extent of natural cross pollination in this species, honeybees (*Apis mellifera*) play an important role and the visits of foraging bees are confined only to those flowers that are freshly open and that bear fresh pollen (Srivastava and Singh 2006). There are divergent views from different researchers on the extent of cross pollination. Whereas according to Nyman and Hall (1976), cross pollination may be as low as 9%, Khanna and Shukla (1983), in contrast, reported that it may be as high as even 70%. Subsequently Sharma and Singh (1983) suggested that the extent of outcrossing would be not more than 20–30%. Using the above-mentioned gene frequency-based biometrical methods, Patra et al. (1992) arrived at the conclusion that the observed proportion of total outcrossing in different crosses of opium poppy ranged from 9.8 to 37.0% (i.e. outcrossing parameter, α, ranging from 0.098 to 0.370). Although a similar biometrical approach has been used in the present study, the key difference between this study and the previous ones lies in the number of loci used as markers: in the present study, three dominant and corresponding recessive alleles were used (R/r, P/p for petal color and B/b for capsule size) as against only two alleles for these marker loci used by Patra et al. (1992). The present study aimed essentially at corroborating the earlier study made previously.

Materials and methods

The investigation involved a total of 1,000 plants at the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India, for crossing during 2000–2001 and subsequently scoring for selfing versus outcrossing in 9–10 monohybrid populations of opium poppy (*P. somniferum*) for three alleles of two loci (two—R/r and P/p—for anthocyanin locus and B/b for capsule size locus). Red (R) and purple (P) petal colour and large capsule size (B) in opium poppy are completely dominant over their alternative forms white petal colour (r and p) and small capsule size (b) (Patra and Chauhan 1990; Kandpal 2005; Kumar 2007). The three alleles—red and purple forms for petal colour and large capsule for its size—were used as markers in determining cross pollination in recessive white petalled and small capsulated plants in F_2 populations of different crosses grown in 2002–2003 among four varieties (cvs Sanchita, Shyama, Shweta and Vivek) and eight mutant strains (VE01, SG35II, VG20, VG26, VN35I, VG46, RPM and PPH earlier used by Chauhan 1989; Patra and Chauhan 1990; Satpute 2000; Kandpal 2005 and Kumar 2007) differing at dominant and recessive genes. All of these have white petal colour with varying morphology except Red Petalled Mutant (RPM) and Purple Petalled Hybrid (PPH). Outcrossing in the r/r, P/p and b/b genotypes were estimated in the F_3 progeny at CIMAP, Lucknow over 2 years (2003–2004 and 2004–2005) and seeds were sown in a completely randomized block design. During the flowering period the sunshine was bright throughout which enabled the honeybees (*Apis mellifera*) to forage properly. In a separate experiment, no fewer than 109 phenotypically dominant plants were drawn randomly from each of the F_2 populations grown in two successive years (2002–2003 and 2003–2004). These populations at F_3 level were progeny tested during 2003–2004 and 2004–2005 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. Hence, p or q was taken to be equal to 0.5 in subsequent calculations.

The estimation procedures developed by Harding and Tucker (1964) are given as follows:

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

$$\operatorname{var}\hat{T} = \frac{a \cdot b}{(a+b)^3}$$

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

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

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

$$\text{Observed var}\,\hat{\alpha} = \frac{\alpha(1-\alpha q)}{Np}$$

Therefore, the observed standard deviation (So) of $\hat{\alpha} = \sqrt{\text{var}\,\alpha}$ where, $p + q = 1$, $N = a + b$ and $p = q = 0.5$.

The expected standard deviation (Se) was computed following Harding and Tucker (1964) as follows:

$$\text{Expected var}\,\hat{\alpha} = \frac{\text{var of}\,\hat{\mathrm{T}} + \hat{\alpha}^2(\mathrm{p\ q})/2\mathrm{N}}{(1-q)^2}$$

Therefore, the expected standard deviation (Se) of $\hat{\alpha} = \sqrt{\text{var}\,\alpha}$

Results

Petal colour (anthocyanin) locus (R/r)

Table 1 shows the frequencies of heterozygotes, total F_3 offspring, estimates of outcrossing (α) and their standard deviations for different crosses and years. The observed proportion of total outcrossing (T) in different crosses in 2004 ranged from 0.0800 to 0.1743 as against 0.1600 to 0.3486 for the gene frequency-based maximum likelihood estimate of outcrossing, α. The observed proportion of total outcrossing (T) in different crosses in 2005 ranged from 0.1544 to 0.2383 as against 0.3088 to 0.4766 for the gene frequency-based maximum likelihood estimate of outcrossing, α, for 2005. The binomial index χ^2_S for the test of heterogeneity of the estimates of outcrossing revealed that the crosses within years (2004 and 2005) were heterogeneous (pooled $\chi^2_{9\ df}$ values = 40.637 and 22.995 for 2004 and 2005, respectively). Thus, the binomial index χ^2 of the pooled estimate of the outcrossing always revealed heterogeneity and, as in the previous case, the entire variation in outcrossing cannot be attributed to environment (years) alone. All the homozygous recessives thus collected for estimating the extent of cross pollination produced corresponding dominant phenotypes, thus excluding the chance of selfing. Therefore, all the observations over environments (years) and crosses were pooled and a composite estimate was made giving $\alpha = 0.3219$. The observed fluctuation of this composite estimate of α (So) was more than 3 times (So/Se = 3.22), the expected random fluctuation (Se), indicating again the non-random nature of outcrossing.

Petal colour (anthocyanin) locus (P/p)

Data in Table 2 depicts the frequencies of heterozygotes, total F_3 offspring, estimates of outcrossing (α) and their standard deviations for different crosses and years. The observed proportion of total outcrossing (T) in different crosses ranged from 17.51 to 29.37% in 2004 and 23.47 to 35.51% in 2005, in contrast to the corresponding ranges 35.02–58.74% and 46.94–71.03% for gene frequency-based maximum likelihood estimates of outcrossing for the 2004 and 2005, respectively. The binomial index χ^2_S for the test of heterogeneity of the estimates of outcrossing revealed that the crosses within years (2004 and 2005) were heterogeneous (pooled $\chi^2_{9\ df}$ values = 35.327 and 24.320 for 2004 and 2005, respectively). Thus, the binomial index χ^2 of the pooled estimate of the outcrossing always revealed heterogeneity. As the populations varied genetically, the entire variation in outcrossing can not be attributed to environment (years) alone. Therefore, all the observations over years and crosses were pooled and a composite estimate was made giving $\alpha = 0.5434$. The observed fluctuation of this composite estimate of α (So) was 23-fold greater (So/Se = 23.09) than the expected random fluctuation (Se), indicating again the non-random nature of outcrossing.

Capsule size locus (B/b)

Table 3 reveals the frequencies of heterozygotes, total F_3 offspring, estimates of outcrossing (α) and their standard deviations for different crosses and years. The observed proportion of total outcrossing (T) in different crosses ranged from 3.61 to 7.07% in 2004 and from 7.46 to 11.95% in 2005, as against the corresponding ranges 7.21–14.13% and 14.92–23.90% for gene frequency-based maximum likelihood estimates of outcrossing for the 2 years (2004 and 2005, respectively). The binomial index χ^2_S for the test of heterogeneity of the estimates of outcrossing revealed that the crosses within years (2004 and 2005) were heterogeneous (pooled $\chi^2_{8\ df}$ values = 19.665 and 12.655 for 2004 and 2005, respectively). Thus, the binomial index χ^2 of the pooled estimate of the outcrossing always showed heterogeneity. As the

Table 1 Estimates of outcrossing using R/r locus as marker (female parent having genotype RR and male parent, rr) (data pooled over 2 years: 2004 and 2005)

S.N.	Names of crosses and years		*a*	*n*	$\hat{T}$	$\hat{\alpha}$	$\sigma\hat{\alpha}$
1.	RPM × Shweta	2004	87	650	0.1339	0.2677	0.0267
		2005	110	572	0.1923	0.3846	0.0330
2.	RPM × Shyama	2004	53	435	0.1218	0.2437	0.0314
		2005	48	277	0.1733	0.3466	0.0455
3.	RPM × Vivek	2004	30	375	0.0800	0.1600	0.0280
		2005	23	149	0.1544	0.3087	0.0592
4.	RPM × Sanchita	2004	43	290	0.1483	0.2966	0.0417
		2005	63	329	0.1915	0.3830	0.0434
5.	RPM × VE01	2004	46	296	0.1554	0.3108	0.0421
		2005	23	103	0.2233	0.4466	0.0821
6.	RPM × SG35II	2004	95	545	0.1743	0.3486	0.0325
		2005	45	192	0.2344	0.4688	0.0611
7.	RPM × VG20	2004	32	268	0.1194	0.2388	0.0396
		2005	82	493	0.1663	0.3327	0.0335
8.	RPM × VG26	2004	23	235	0.0979	0.1957	0.0388
		2005	27	171	0.1579	0.3158	0.0558
9.	RPM × VN35I	2004	28	180	0.1556	0.3111	0.0540
		2005	102	428	0.2383	0.4766	0.0412
10.	RPM × VG46	2004	36	286	0.1259	0.2518	0.0392
		2005	65	319	0.2038	0.4075	0.0451
11.	Total	2004	473	3560	0.1329	0.2657	0.0114
		2005	588	3033	0.1939	0.3877	0.0144
12.	Grand total		1061	6593	0.1609	0.3219	0.0009

a = Heterozygotes

n = Total number of F_3 offspring

$\hat{T}$ = Observed proportion of outcrossing

$\hat{\alpha}$ = Gene frequency-based maximum likelihood estimate of outcrossing in the population

$\sigma\hat{\alpha}$ = Observed standard deviation of $\hat{\alpha}$

*Pooled χ^2 value at $9_{df} = \sum \chi^2\, a\, i + \sum \chi^2\, b\, i$

*$\chi^2\, a\, i = 40.637$ at $(r-1)\,(c-1) = 9_{df}$ *$\chi^2\, b\, i = 14.707$ at $(r-1)\,(c-1) = 9_{df}$ (2004)

*$\chi^2\, a\, i = 22.995$ at $(r-1)\,(c-1) = 9_{df}$ *$\chi^2\, b\, i = 14.561$ at $(r-1)\,(c-1) = 9_{df}$ (2005)

populations varied genetically, the entire variation in outcrossing can be attributed to both their genetic nature and environment (years). Therefore, all the observations over environments (years) and crosses were pooled and a composite estimate was made giving $\alpha = 0.1422$. The observed fluctuation of this composite estimate of α (So) was more than 14-fold (So/Se = 14.69) greater than the expected random fluctuation (Se), thereby indicating that outcrossing in the populations is not random.

Two marker loci (R/r or P/p locus vs. B/b locus)

As shown in Tables 4 and 5, outcrossing estimates for locus B/b were consistently lower than those for the two petal colour loci: R/r and P/p (T value for B/b ranged from 3.61 to 11.95% in 2004 and 2005, as against the ranges 8.00–23.83% for R/r locus and 17.51–35.51% for P/p locus in the two years). Consistent with this observation was the result obtained from the two dihybrid crosses of two loci (R/r or P/p

Table 2 Estimates of outcrossing using P/p locus as marker (female parent having genotype PP and male parent, pp) (data pooled over 2 years: 2004 and 2005)

S.N.	Names of crosses and years		a	n	$\hat{T}$	$\hat{\alpha}$	$\sigma\hat{\alpha}$
1.	PPH × Shweta	2004	92	365	0.2521	0.5041	0.0455
		2005	108	361	0.2992	0.5983	0.0482
2.	PPH × Shyama	2004	36	148	0.2432	0.4865	0.0705
		2005	33	118	0.2797	0.5593	0.0826
3.	PPH × Vivek	2004	56	218	0.2569	0.5138	0.0592
		2005	83	266	0.3120	0.6241	0.0565
4.	PPH × Sanchita	2004	88	315	0.2794	0.5587	0.0506
		2005	32	102	0.3137	0.6275	0.0919
5.	PPH × VE01	2004	38	134	0.2836	0.5672	0.0779
		2005	78	233	0.3348	0.6695	0.0618
6.	PPH × SG35II	2004	121	412	0.2937	0.5874	0.0449
		2005	118	340	0.3471	0.6941	0.0516
7.	PPH × VG20	2004	32	176	0.1818	0.3636	0.0581
		2005	28	112	0.2500	0.5000	0.0818
8.	PPH × VG26	2004	38	217	0.1751	0.3502	0.0516
		2005	92	392	0.2347	0.4694	0.0428
9.	PPH × VN35I	2004	84	298	0.2819	0.5638	0.0521
		2005	38	107	0.3551	0.7103	0.0925
10.	PPH × VG46	2004	65	326	0.1994	0.3988	0.0443
		2005	86	314	0.2739	0.5478	0.0503
11.	Total	2004	650	2609	0.2491	0.4983	0.0169
		2005	696	2345	0.2968	0.5936	0.0189
12.	Grand total		1346	4954	0.2717	0.5434	0.0126

a = Heterozygotes

n = Total number of F_3 offspring

$\hat{T}$ = Observed proportion of outcrossing

$\hat{\alpha}$ = Gene frequency-based maximum likelihood estimate of outcrossing in the population

$\sigma\hat{\alpha}$ = Observed standard deviation of $\hat{\alpha}$

*Pooled χ^2 value at 9 $_{df} = \sum \chi^2\ a\ i + \sum \chi^2\ b\ i$

*$\chi^2\ a\ i = 35.3266$ at $(r-1)\ (c-1) = 9_{df}$ *$\chi^2\ b\ i = 35.0848$ at $(r-1)\ (c-1) = 9_{df}$ (2004)

*$\chi^2\ a\ i = 24.3203$ at $(r-1)\ (c-1) = 9_{df}$ *$\chi^2\ b\ i = 35.5255$ at $(r-1)\ (c-1) = 9_{df}$ (2005)

and B/b) (Tables 4 and 5). In these crosses, some F_2 progenies were doubly recessive for petal colour and capsule size (rb/rb or pb/pb) loci. Outcrossings which were effected simultaneously for these two loci by the corresponding dominant genes in the populations were detected and compared in both crosses. The estimates of outcrossing at each of the two loci (R/r and P/p locus) were greater than those at the B/b locus. Whereas T values ranged within 11.03–32.41% for R/r or P/p locus in the two years, the T values for B/b locus in the two years ranged within 4.14–6.94%. This clearly indicated that the R/r or P/p individual is a more effective male parent in cross pollination than the B/b parent. Mean estimates of outcrossing at these two loci over the two crosses in the two years were found to be heterogeneous (pooled χ^2_{1df} of the two years for R/r and B/b locus 9.161; $P = 0.01$ and for P/p and B/b locus 7.16; $P = 0.01$).

Discussion

From the results of quantitative studies on the mating system of the opium poppy involving different loci,

Table 3 Estimates of outcrossing using B/b locus as marker (female parent having genotype BB and male parent, bb) (data pooled over 2 years: 2004 and 2005)

S.N.	Names of crosses and years		a	n	$\hat{T}$	$\hat{\alpha}$	$\sigma\hat{\alpha}$
1.	BM (Vivek) × Shweta	2004	26	368	0.0707	0.1413	0.0267
		2005	38	318	0.1195	0.2390	0.0364
2.	BM (Vivek) × Shyama	2004	14	345	0.0406	0.0812	0.0213
		2005	18	220	0.0818	0.1636	0.0370
3.	BM (Vivek) × Sanchita	2004	9	172	0.0523	0.1047	0.0340
		2005	10	102	0.0980	0.1961	0.0589
4.	BM (Vivek) × VE01	2004	20	286	0.0699	0.1399	0.0302
		2005	23	205	0.1122	0.2244	0.0441
5.	BM (Vivek) × SG35II	2004	34	548	0.0620	0.1241	0.0206
		2005	40	390	0.1026	0.2051	0.0307
6.	BM (Vivek) × VG20	2004	23	516	0.0446	0.0892	0.0182
		2005	16	187	0.0856	0.1711	0.0409
7.	BM (Vivek) × VG26	2004	15	416	0.0361	0.0721	0.0183
		2005	42	563	0.0746	0.1492	0.0221
8.	BM (Vivek) × VN35I	2004	35	552	0.0634	0.1268	0.0207
		2005	15	140	0.1071	0.2143	0.0523
9.	BM (Vivek) × VG46	2004	16	356	0.0449	0.0899	0.0220
		2005	48	533	0.0901	0.1801	0.0248
10.	Total	2004	192	3559	0.0539	0.1079	0.0008
		2005	250	2658	0.0940	0.1881	0.0113
11.	Grand total		442	6217	0.0711	0.1422	0.0007

a = Heterozygotes

n = Total number of F_3 offspring

$\hat{T}$ = Observed proportion of outcrossing

$\hat{\alpha}$ = Gene frequency-based maximum likelihood estimate of outcrossing in the population

$\sigma\hat{\alpha}$ = Observed standard deviation of $\hat{\alpha}$

*Pooled χ^2 value at $8_{df} = \sum \chi^2 a\ i + \sum \chi^2 b\ i$

*$\chi^2 a\ i = 19.665$ at $(r-1)\ (c-1) = 8_{df}$ *$\chi^2 b\ i = 2.378$ at $(r-1)\ (c-1) = 8_{\mathrm{d}}$ (2004)

*$\chi^2 a\ i = 12.6553$ at $(r-1)\ (c-1) = 8_{df}$ *$\chi^2 b\ i = 2.9319$ at $(r-1)\ (c-1) = 8_{\mathrm{d}}$ (2005)

crosses and years, it is clear that the amount of outcrossing in this species is, in general, heterogeneous and nonrandom for a specific locus, with the gene frequency-based outcrossing parameter, α, ranging between as low as 7.21% (for B/b locus) and as high as 71.03% (for P/p locus) depending on the specific cross and environment (years). Such a high frequency of natural outcrossing (i.e. 71.03%) for the anthocyanin allele was not observed earlier by Patra et al. (1992) who, however, only studied the single anthocyanin allele (Red form) but not the new allele (Purple form) with a high frequency of outcrossing. The graphical representation of the pooled data over the years of the observed and expected frequency of three alleles against monohybrid crosses proves the nonrandom nature of outcrossing (Fig. 1). The results of two marker loci (R/r or P/p and B/b) in the two dihybrid crosses have demonstrated that the R/r or P/p individual is a more effective male parent in cross pollination than the B/b parent. In both years for the two loci at homozygous level, 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. All in all, the direct results of outcrossing heterogeneity, nonrandomness and locus specificity for degree

Table 4 Estimates of outcrossing in two crosses (in 2 years: 2004 and 2005) using colour locus (R/r) and capsule size locus (B/b) simultaneously with the parents having genotypes bbRR and BBrr

S.N.	Names of crosses and years		a		N	$\hat{T}$		$\hat{\alpha}$		$\sigma\hat{\alpha}$	
			R/r	B/b		R/r	B/b	R/r	B/b	R/r	B/b
1.	RPM × BM (Vivek)	2004	16	6	145	0.1103	0.0414	0.2207	0.0828	0.0520	0.0331
		2005	46	18	268	0.1716	0.0672	0.3433	0.1343	0.0461	0.0306
2.	Grand total		62	24	413	0.1501	0.0581	0.3002	0.1162	0.0352	0.0230

a = Heterozygotes; N = Total number of F_3 offspring; $\hat{T}$ = Observed proportion of outcrossing

$\hat{\alpha}$ = Gene frequency-based maximum likelihood estimate of outcrossing in the population; $\sigma\hat{\alpha}$ = Observed standard deviation of $\hat{\alpha}$

*Pooled χ^2 value at 1 $_{df} = \sum \chi^2\ a\ i + \sum \chi^2\ b\ i$

*$\chi^2\ a\ i = 4.714$ at $(r-1)\ (c-1) = 1_{df}$ (R/r); 2.142 at $(r-1)\ (c-1) = 1\ _{df}$ (B/b) (2004)

*$\chi^2\ b\ i = 2.023$ at $(r-1)(c-1) = 1\ _{df}$ (R/r); 0.282 at $(r-1)\ (c-1) = 1\ _{df}$ (B/b) (2005)

Table 5 Estimates of outcrossing in two crosses (in 2 years 2004 and 2005) using colour locus (P/p) and capsule size locus (B/b) simultaneously with the parents having genotypes bbPP and BBpp

S.N.	Names of crosses and years		a		N	$\hat{T}$		$\hat{\alpha}$		$\sigma\hat{\alpha}$	
			P/p	B/b		P/p	B/b	P/p	B/b	P/p	B/b
1.	PPH × BM (Vivek)	2004	30	6	118	0.2542	0.0508	0.5085	0.1017	0.0802	0.0404
		2005	70	15	216	0.3241	0.0694	0.6482	0.1389	0.0637	0.0346
2.	Grand total		100	21	334	0.2994	0.0629	0.5988	0.1258	0.0501	0.0266

a = Heterozygotes; N = Total number of F_3 offspring; $\hat{T}$ = Observed proportion of outcrossing

$\hat{\alpha}$ = Gene frequency-based maximum likelihood estimate of outcrossing in the population; $\sigma\hat{\alpha}$ = Observed standard deviation of $\hat{\alpha}$

*Pooled χ^2 value at 1 $_{df} = \sum \chi^2\ a\ i + \sum \chi^2\ b\ i$

*$\chi^2\ a\ i$ 2.487 at $(r-1)\ (c-1) = 1_{df}$ (P/p); 0.841 at $(r-1)\ (c-1) = 1\ _{df}$ (B/b) (2004)

*$\chi^2\ b\ i = 3.711$ at $(r-1)(c-1) = 1\ _{df}$ (P/p); 0.121 at $(r-1)\ (c-1) = 1\ _{df}$ (B/b) (2005)

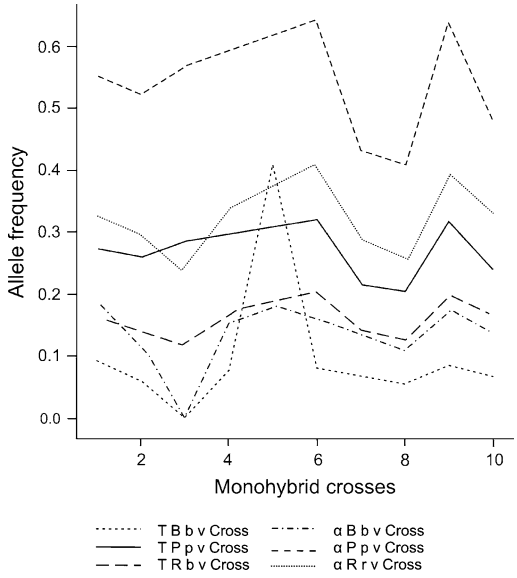

Fig. 1 Observed (T) and gene frequency (α)-based outcrossing for three alleles of two loci 9–10 monohybrid crosses of opium poppy (polled date over the years: 2004 and 2005)

of outcrossing are in conformity with the results obtained by Patra et al. (1992) and Kumar (2007). Heterogeneity and nonrandomness in outcrossing have also been documented in many other crop plants such as *Phaseolus lunatus* (Harding and Tucker 1964), *Phaseolus aureus* (Dana 1969), barley (Jain 1961), maize (Gutierrez and Sprague 1959), jute (Basak and Chaudhuri 1966; Basak and Gupta 1972; Basak and Paria 1975), *Lathyrus sativus* (Chowdhury and Slinkard 1997), *Hibiscus sabdariffa* (Vaidya 2000), *Vernonia galamensis* (Baye and Becker 2004) and barley (Parzies et al. 2008).

Mating systems are usually thought to be an important factor in determining the amount and nature of genetic variability in plant populations. The recorded variability in the outcrossing rate of opium poppy under the influence of genetic and environmental factors should very likely be considered a complex one. Thus, the mating systems of opium poppy populations should be considered as

complex ones. The present finding is consonant with the earlier suggestion made by Allard et al. (1968) that the population structure in inbreeding species is much more complicated than commonly supposed and, perhaps, does not take the same form in all inbreeding species or even in different populations of the same species.

Taken together, the results in the present study lead to the conclusion that *P. somniferum* populations follow neither models of complete random mating nor those of complete inbreeding; instead, the mating system of the studied opium poppy populations is partial inbreeding. The high amount of natural cross pollination in the opium poppy appears to be potentially capable of enhancing genetic variability. In this context, elegant discussions on the evolutionary consequences and significance of such a mating system as the one observed in the present study have been put forward by Workman (1964) and Allard et al. (1968).

Acknowledgments The authors are thankful to Dr. S.P.S. Khanuja, Director, CIMAP, Lucknow, for providing the necessary facilities during the investigation. They are also very grateful to Prof. (Dr.) S. L. Basak, Ex-Director of C.R.I.J.A.F. (ICAR), West Bengal, for giving valuable suggestions on up-grading the paper, and to P. Christou and anonymous reviewers for valuable comments and improving the manuscript.

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

Baye T, Becker HC (2004) Natural outcrossing rate in *Vernonia galamensis*. Plant Breed 123:398–399

Chauhan SP (1989) Studies on induced mutagenesis in opium poppy (*Papaver somniferum* L.) in relation to genetic improvement and cytogenetic analysis. PhD thesis, Kanpur University, India

Chowdhury MA, Slinkard AE (1997) Natural outcrossing in grasspea. J Hered 88:154–156

Dana S (1969) Non-random out-crossing 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 the non-randomness 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

Kadereit JW (1986a) *Papaver somniferum* L. (Papaveraceae): a triploid hybrid? Bot Jahrb Syst 106:221–244

Kadereit JW (1986b) A reuision of *Papaver* L. Sect. *Papaver* (Papaveraceae). Bot Jahrb Syst 108:1–16

Kandpal A (2005) Cytogenetical investigations in induced mutants and inter-specific hybrids of *Papaver* species. PhD thesis, Lucknow University, India

Khanna KR, Shukla S (1983) Degree of out-crossing in opium poppy. New Botanist 10:65–67

Kumar B (2007) Study on genetic architecture of opium poppy (*Papaver somniferum* L.) in relevance to yield improvement. PhD thesis, Lucknow University, India

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

Nyman V, Hall O (1976) Some varieties of *P. somniferum* L. with changed morphine alkaloid. Hereditas 84:69–76

Parzies HK, Fosung Nke C, Abdel-Ghani AH, Geiger HH (2008) Outcrossing rate of barley genotypes with different floral characteristics in drought-stressed environments in Jordan. Plant Breed 127:536–538

Patra NK, Chauhan SP (1990) Morpho-physiology and genetics of induced mutants expressed in the M_1 generation in opium poppies. J Hered 81:347–350

Patra NK, Ram RS, Chauhan SP, Singh AK (1992) Quantitative studies on the mating system of opium poppy (*Papaver somniferum* L.). Theor Appl Genet 84:299–302

Satpute GK (2000) Mutation breeding for genetic enhancement of desirable alkaloids in opium poppy (*Papaver somniferum* L.). PhD thesis, Lucknow University, India

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, India, pp 39–68

Srivastava HK, Singh D (2006) Honeybees foraging response in genetically diversified opium poppy. Bioresour Technol 97:1578–1581

Vaidya KR (2000) Natural cross-pollination in roselle, *Hibiscus sabdariffa* L. (Malvaceae). Genet Mol Biol 23: 667–669

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