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Quantitative Studies on the Mating System of Jute (*Corchorus olitorius* L.)

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Summary. More than 100,000 individuals of *C. olitorius* were scored for selfing versus outcrossing in various populations, at several locations, over a number of years and seasons. Different marker loci, such as A^D/a^0, *Sh/sh*, *Cr/cr* and *Pl/pl*, were used to determine the male gametes which had effected fertilization. The results showed that the frequency of outcrossing was extremely variable among loci, crosses and samples within a single locus. The outcrossing parameter, α, was found to differ with years, locations and seasons within years. It was also found that outcrossing, in general, was nonrandom. Nonrandomness was also independent of flowering dates. The amount of outcrossing was directly associated with the frequency of F_2 plants flowering at different dates. A recalculated outcrossing parameter from different authors' reported data, representing different years and locations, has been found to be nonrandom. It was observed that the propensity to outcross was not a simple function of changing gene frequency but was associated with the genotype of individual selected.

Introduction

Mating systems are generally thought to be important factors in determining the amount and nature of genetic variability in populations. Many plant species have mating systems which allow partial intermating in addition to self-fertilization. Estimation procedures which assume no selection and population equilibrium gene frequencies have been given by Fyfe and Baily (1951) and Nei and Syaktdo (1958). Allard and Workman (1963) and Harding and Tucker (1964) have given methods for estimating an outcrossing parameter when selection is present and/or equilibrium can not be assumed. These methods are based on estimates of gene frequency in the population and estimates of the frequency of dominant individuals appearing in the progeny of recessive individuals taken at random from the population. Estimates of outcrossing parameters have been given by a number of workers (Snow and Dunford, 1961; Jain and Allard, 1960; Harding and Tucker, 1964).

Corchorus olitorius L., one of the commonly cultivated jute species, is partially cross-pollinated (Fryxell, 1957). The extent of natural cross-pollination in this species, reported by Ghose and Dasgupta (1945), Ray (1960) and Dutt and Ghose (1962), is highly variable. Whether cross-pollination is random or not has not been studied except for the locus A^D/a^0 (Basak and Chaudhuri, 1966). The purpose of the present investigation was to study in detail the mating system involved in certain artificial populations of this species using different loci, several years and locations.

Materials and Methods

F_2 populations of different crosses between two varieties differing in dominant marker and recessive genes were used in this experiment. Outcrossing was estimated for four different loci: red-green anthocyanin pigment (A^D/a^0), nonshiny-shiny seed-coat (*Sh/sh*), normal-crumple leaf (*Cr/cr*) and lanceolate-palmate leaf shape (*Pl/pl*). Outcrossing at the A^D/a^0 locus was studied over two years, 1964 and 1965, at location I (Narendrapur), and over the two years, 1966 and 1969, at location II (Haringhata). Within 1964, at location I, the difference between summer and winter seasons was studied. For the experiment at location II during 1969, three populations, termed Bulk 1, Bulk 2 and Bulk 3, were artificially created by making suitable mixtures of the seeds from the crosses between green and green (632 × GE), green and red (632 × WO, GE × WO, GE × TM, 632 × 620) and red and red (620 × TM, WO × TM) so that the allelic frequencies varied in these populations. For studying the relation between flowering dates and outcrossing, a population similar to Bulk 3 in composition and consisting of all the F_2's of the crosses mentioned above was grown at location II in 1969. The actual gene frequency in each of these populations was subsequently scored. Estimations of outcrossing for other loci were made on F_2's segregating for the character pairs, grown in different numbers of replications. Progenies from the randomly selected phenotypic recessives among the F_2's were tested by scoring heterozygotes, an indicator of outcrossing among the recessive plants. Scoring of phenotypic recessives and heterozygotes for the red-green locus was carried out by growing the seedlings in petri dishes, following the procedure described by Basak (1966). Scoring for other loci was mainly done by growing progenies in the field.

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 offspring. The observed proportion of outcrosses, $\hat{T}$, and its variance are

$$\hat{T} = \frac{a}{a+b} \tag{1}$$

and

$$\operatorname{Var} \hat{T} = \frac{a\,b}{(a+b)^3} \tag{2}$$

which are the maximum likelihood estimators. However, not all crosses will be observed because homogeneous matings result in homozygotes. The maximum likelihood estimate of total outcrossing, $\hat{\alpha}$, is

$$\hat{\alpha} = \frac{a}{(a+b)\,(1-q)} = \frac{T}{(1-q)} \tag{3}$$

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

$$\mathrm{Var}\,\hat{\alpha} = \frac{(1-\alpha+\alpha q)}{N\,(1-q)} = \frac{(1-\alpha p)}{N p} \tag{4}$$

where $p + q = 1$ and $\mathrm{N} = a + b$.

When outcrossing exceeds 15 per cent and/or gene frequency $(1-q)$ is low, then the following formula for estimating the variance of α should be used:

$$\mathrm{Var}\,\hat{\alpha} \approx \frac{\mathrm{Var\,T} + \alpha^2\,\mathrm{Var}\,q}{(1-q)^2} \tag{5}$$

where $\mathrm{Var}\,q = p\,q/2\,\mathrm{N}$. In the results, reference is made to the above formulae. The approximate expected range of binomial variation ($\pm\,2\,\sigma_\alpha$) and the observed fluctuation of α were computed following Harding and Tucker (1964).

The same method of estimation of α was followed for the data presented by Ghose and Dasgupta (1945), Ray (1960) and Dutt and Ghose (1962). These authors studied cross-pollination in populations where homozygous dominant and homozygous recessive plants were equally frequent; hence q was taken to be 0.50 when estimating α from the published data.

Results

A^{D}/a^0 locus:

Table 1 presents frequencies of heterozygotes, total F_3 offspring, estimates of outcrossing and their standard deviations for the different years, locations and seasons. At location I, during 1964, the estimates of outcrossing for different crosses were heterogeneous and the difference in outcrossing between summer and winter was significant as revealed by a binomial χ^2 test ($\chi^2_{1\,\mathrm{df}} = 36.8$; $P = < 0.001$). The crosses within the year 1965 at location I and the same within the year 1966 at location II were heterogeneous. Within the year 1969, at location II, the different populations varied in gene frequencies, and Bulk 1, 2 and 3 differed in outcrossing estimates ($\chi^2_{2\,\mathrm{df}} = 1369.3$; $P = < 0.001$). Comparisons between years or locations could not be made because the contrasts were not orthogonal, so that it was not possible to determine definitely whether the environmental difference was due to year or location effect. As the genetic populations varied in every case the entire variation cannot possibly be attributed to environment alone. However, all the observations over years, locations and seasons were pooled and a composite estimate was made, giving $\bar{\alpha} = 0.1512$. The approximate expected range ($\pm\,2\,\sigma_\alpha$) due to random fluctuation was 0.1476–0.1548, but if the observed fluctuation of α for the different samples was used, the approximate range ($\pm\,2\,s_\alpha$) became 0.1166 to 0.1858. The observed fluctuation in the estimate of α was approximately nine times the fluctuation expected on the basis of chance alone. The heterogeneity of outcrossing appeared to be large enough, so the binomial sampling error alone can not explain the variation. The correlation between $\hat{\alpha}$ and q for different samples of A^{D}/a^0 locus was significantly negative ($r = -0.599$; $P = < 0.001$). Standard deviations of α were calculated according to formulae numbers 4 and 5, respectively, for Bulk 1 and the CG × DRE × CG cross, where gene frequency

Table 1. *Estimation of outcrossing using A^{D}/a^0 locus as marker*

Year/location/season	Cross	a	N	q	$\hat{\alpha}$	$\sigma_{\hat{\alpha}}$ (4)
1964, Location I,	632 × WO	86	1031	0.4379	0.1484	0.0153
	CG × WO	862	10827	0.5000	0.1592	0.0052
Summer	LRE × PM	318	2818	0.5000	0.2028	0.0108
Winter	DRE × PM	524	6393	0.5000	0.1516	0.0064
Total		1790	21069	0.4970*	0.1690	0.0038
1965, Location II	CG × DRE × CG	201	3753	0.8000	0.2680	0.0183 0.0166(5)
	DRE × CG	716	11400	0.5000	0.1256	0.0045
	CG × WO	86	1290	0.4748	0.1270	0.0132
Total		1003	16443	0.5665*	0.1407	0.0043
1966, Location II	WO × 632	516	6658	0.4517	0.1413	0.0060
	WO × CG	496	5938	0.5094	0.1702	0.0073
Total		1012	12596	0.4789*	0.1541	0.0046
1969, Location II	Bulk $F_2(1)$	513	8888	0.7857	0.2692	0.0115 0.0121(5)
	Bulk $F_2(2)$	596	11207	0.2857	0.0746	0.0030
	Bulk $F_2(3)$	1260	11010	0.4000	0.1907	0.0051
Grand Total		6174	81213	0.4975*	0.1512	0.0018

* Mean gene frequency weighted to N of crossing sample.

Table 2. *Estimation of α's for different years and locations in A^D/a^0 locus*

Authors	Year	Location	a	N	$\hat{\alpha}$	$\sigma_{\hat{\alpha}}$
Ghose and Dasgupta (1945)	1940	Dacca	365	1444	0.5056	0.0229
Ray (1960)	1958	Barrackpore	3112	31238	0.1992	0.0034
Dutt and Ghose (1962)	1961–1962	Barrackpore	959	18384	0.1043	0.0052
Present study	1964	Narendrapur	1704	20038	0.1701	0.0038
	1965	Narendrapur	716	11400	0.1256	0.0045
	1966	Haringhata	496	5938	0.1702	0.0081
Total			7352	88442	0.1663	0.0019

Frequency of both the alleles has been taken to be the same, that is, $p = q = 0.50$.

$(1 - q)$ was very low. Estimates of the standard deviation using these formulae for both cases were practically the same.

α's for the A^D/a^0 locus, estimated from the data of Ghose and Dasgupta (1945), Ray (1960) and Dutt and Ghose (1962), and from the data of the present study with gene frequency $p = q = 0.50$, are presented in Table 2. The estimates were highly heterogeneous. A χ^2 index of dispersion test ($\chi^2_{5\text{df}} = 2090.0$; $P = <0.001$) shows that the estimates of α are highly heterogeneous. Since year and location effects are confounded it can not be determined whether one or both factors caused the heterogeneity.

Table 3 presents the number of progenies from the plants flowering on five different dates, their corresponding $\hat{\alpha}$'s and their standard deviations. A comparison for randomness among the dates revealed that there was a deviation from random chance in general ($s_\alpha/\sigma_\alpha = 8.7$ times) among the dates. A binomial index χ^2 testing the heterogeneity of $\hat{\alpha}$'s between these dates of flowering was highly significant, which showed that the amount of outcrossing on these dates was different ($\chi^2_{4\text{df}} = 152.5$; $P = <0.001$). Another binomial χ^2-test was made between the amount of outcrossing in this population and in Bulk 3, both of which were grown in the same year and at the same location and with the same gene frequency ($q = 0.40$). The test ($\chi^2_{1\text{df}} = 290.0$; $P = <0.001$) indicated that these two populations were highly heterogeneous. The different dates of flowering, their corresponding estimates of α and percentage of plants flowered are given in Table 4. The amount of $\hat{\alpha}$ varied directly with the percentage of plants flowered except for the last date (5. 9. 69). The value of $\hat{\alpha}$ varied inversely with the date of flowering from 10. 8. 69 to 5. 9. 69.

Table 3. *Estimation of outcrossing at different dates of flowering using A^D/a^0 as marker*

Year/ location	Dates of flowering	a	N	$\hat{\alpha}$	$\sigma_{\hat{\alpha}}$
1969, Location II	10. 8. 69	92	739	0.2075	0.0253
	18. 8. 69	99	1549	0.1065	0.0128
	25. 8. 69	136	2912	0.0778	0.0080
	31. 8. 69	49	1065	0.0767	0.0132
	5. 9. 69	10	546	0.0305	0.0117
Total		386	6811	0.0945	0.0057

Table 4. *Association between $\hat{\alpha}$ and percentage of plants flowered*

Dates	$\hat{\alpha}$	Percentage of plants flowered*
10. 8. 69	0.2075	32.75
18. 8. 69	0.1065	26.75
25. 8. 69	0.0778	13.75
31. 8. 69	0.0767	7.25
5. 9. 69	0.0305	19.50

* Total number of plants for these dates is 322.

The frequencies of the estimates of outcrossing for the A^D/a^0 locus over locations, years and seasons followed a positively skewed distribution (see Table 8).

Sh/sh locus:

The data and the estimates of outcrossing with standard deviations for the *Sh/sh* locus are given in Table 5. The estimates of α for different blocks varied significantly and, therefore, the $\hat{\alpha}$'s were inconsistent ($\chi^2_{3\text{df}} = 298.0$; $P = <0.001$). All the data for this locus were pooled and one composite estimate was obtained, giving $\bar{\alpha} = 0.1456$. The approximate expected range due to random fluctuation was 0.1348–0.1528, but the observed fluctuation of $\hat{\alpha}$ for the four blocks ranged from 0.1068 to 0.1844. The observed fluctuation in α was approximately 5.4 times the fluctuation expected on the basis of random chance ($s^2/\sigma^2 = 29$). The frequency distribution of the outcrossing estimates for this locus, given in Table 8, shows that it was positively skewed.

Cr/cr locus:

The data and the estimates of outcrossing with standard deviations for this locus appear in Table 6. The estimates of α for these two blocks were highly variable. A binomial index χ^2 test indicated that the heterogeneity between the two blocks was significant and thus the $\hat{\alpha}$'s were inconsistent ($\chi^2_{1\text{df}} = 570.9$; $P = <0.001$).

All the data for this locus were pooled and a composite estimate was obtained to show $\bar{\alpha} = 0.1977$. The approximate expected range due to random fluctuation was 0.1851–0.2103; when the observed fluctuation of $\hat{\alpha}$ for these two samples was used, the approximate range became 0.1153–0.2901. The observed fluctuation in $\hat{\alpha}$ was approximately 6.5 times the fluctation expected on the basis of random chance ($s^2/\sigma^2 = 44$). The frequency distribution of the outcrossing estimates for this locus, given in Table 8, showed a bimodal type of distribution.

Pl/pl locus:

Table 7 gives the data and the estimates of outcrossing with standard deviations for the *Pl/pl* locus. Binomial index χ^2 testing of the heterogeneity in four different blocks was significant, indicating the inconsistency of the estimates ($\chi^2_{3\,\mathrm{df}} = 30.5$; $P = < 0.001$).

All the data in the estimation of outcrossing for this locus were pooled and one composite estimate was obtained to give $\bar{\alpha} = 0.1203$. The approximate expected range due to random fluctuation was 0.1131 to 0.1275; the observed fluctuation of $\hat{\alpha}$ for these four blocks, which ranged from 0.1003 to 0.1403, was approximately three times the fluctuation expected on the basis of random chance ($s^2/\sigma^2 = 9$). The estimates of outcrossing for this locus followed a positively skewed distribution (see Table 8).

Table 5. *Estimation of outcrossing using Sh/sh locus as marker*

Year/location/ cross	Material collected from	a	N	q	$\hat{\alpha}$	$\sigma_{\hat{\alpha}}$
1967, Location II, CG × DRE	Block I	565	6235	0.5394	0.1967	0.0079
	Block II	205	5154	0.5154	0.0821	0.0056
	Block III	443	5872	0.5366	0.1627	0.0074
	Block IV	272	4665	0.5488	0.1292	0.0076
Total		1485	21926	0.5350*	0.1456	0.0036

* Mean gene frequency weighted to N of crossing sample.

Table 6. *Estimation of outcrossing using Cr/cr locus as marker*

Year/location/ cross	Material collected from	a	N	q	$\hat{\alpha}$	$\sigma_{\hat{\alpha}}$
1969, Location II, Narrow × Crumple	Block I	445	3345	0.5610	0.3030	0.0134
	Block II	325	6362	0.5115	0.1046	0.0057
Total		770	9707	0.5286*	0.1977	0.0063

* Mean gene frequency weighted to N of crossing sample.

Table 7. *Estimation of outcrossing using Pl/pl locus as marker*

Year/location/ cross	Material collected from	a	N	q	$\hat{\alpha}$	$\sigma_{\hat{\alpha}}$
1969, Location II, CG × Palmate	Block I	225	3738	0.5287	0.1277	0.0082
	Block II	191	3519	0.5337	0.1164	0.0082
	Block III	469	6589	0.3565	0.1106	0.0049
	Block IV	150	1938	0.5021	0.1555	0.0122
Total		1035	15787	0.4547*	0.1203	0.0036

* Mean gene frequency weighted to N of crossing sample.

Table 8. *Frequency distribution of the estimates of α for different loci*

	Frequency												
Classes	<.009	.010 .059	.060 .109	.110 .159	.160– .209	.210– .259	.260– .309	.310– .359	.360– .409	.410– .459	.460– .509	.510– .559	>.560
Locus													
A^D/a^0 (pooled)		18	42	38	23	12	6	2	5	2	—	2	1
Sh/sh		11	19	13	9	5	3	1					1
Cr/cr	2	5	6	2	2	5	3	3	1	2			
Pl/pl	2	9	14	11	3	3	2	—	1	—	1		

Discussion

A study involving different loci, locations, years and seasons has shown that the amount of outcrossing, in general, was heterogeneous and nonrandom. The estimates of outcrossing for the A^D/a^0 locus as marker, within locations, years and seasons, were significantly heterogeneous. The observed heterogeneity among the estimates for years or locations cannot be attributed definitely to year or location effect, since these effects were nonorthogonal. The recalculated estimates of outcrossing from published data of different authors also showed heterogeneity in the amount of outcrossing. The observed fluctuations in $\hat{\alpha}$ for the locus A^D/a^0 were found to be much larger than the fluctuations expected on the basis of chance. For the other loci, *Sh/sh*, *Cr/cr* and *Pl/pl*, outcrossing was also highly heterogeneous and the fluctuations of $\hat{\alpha}$ were nonrandom. Thus, all these results show that the assumption that outcrossing occurs at random is not tenable for jute (*C. olitorius*). Earlier, Basak and Chaudhuri (1966) reached a similar conclusion for outcrossing at the A^D/a^0 locus. In many other species, such as *Phaseolus lunatus* (Harding and Tucker, 1964), *Phaseolus aureus* (Dana, 1969), barley (Jain, 1961), and maize (Gutierrez and Sprague, 1959), outcrossing has also been found to be nonrandom.

One of the important features of the variability in inbreeding species is the genetic differentiation between the populations within the species. Clonal variation is frequently observed in association with progressive changes in rainfall, temperature and other factors of the physical environment and such local differentiation appears to provide massive storage of genetic variability (Allard, Jain and Workman, 1968). It is true that the population structure in inbreeding species is much more complicated than has been commonly supposed and probably it does not take the same form in all inbreeding species or even in different populations of the same species. The situation becomes further complicated when various factors which are more ecological than genetic are taken into account. For example, the heterozygotes and homozygotes of the *S/s* locus of lima bean were equal in fitness when all three genotypes were frequent in the population (Harding, Allard and Smeltzer, 1966). However, when heterozygotes were rare in the population, their fitness increased relative to homozygotes. Hence, the maintenance of a stable nontrivial polymorphism depends on a complex set of interactions between genetic factors, mating system and ecological factors. The observed variability of the mating system of jute under different ecogeographical conditions for different loci may well contribute to a complex population structure as stated above.

Another factor which may affect nonrandomness is flower structure. In jute, style length and stigmatic surface are variable among the varieties and may cause differential cross pollination (Basak and Chaudhuri, 1967). At anthesis, the stigma remains above the anthers in about 6 per cent of the flowers (Basak, unpublished), which precludes complete selfing. Gutierrez and Sprague (1959) also observed significant variation in the dates of anthesis or silking of nine stocks of maize, resulting in partial or complete physiological isolation. Even the stocks having similar dates of pollen shedding and of silking revealed nonrandom mating, which obviously indicated the existence of some type or degree of selective fertilization. Barnes and Cleveland (1963) also reported a competitive difference between long and short pollen tubes in an alfalfa variety, resulting in nonrandom fertilization.

The proportions of plants flowering on different dates had a positive relation with their outcrossing estimates. As the proportion of plants flowering simultaneously gradually declined, the amount of outcrossing also declined, probably due to the gradually diminishing supply of pollen to effect nonassortative mating. The number of plants shedding pollen and the date of tasselling and date of silking have been observed to be interrelated with the overall performance of the stocks of maize as male parents (Gutierrez and Sprague, 1959).

As q decreased, $\hat{\alpha}$ increased in the Bulk 1, 2, and 3 materials tested in 1969 using the A^D/a^0 locus. In lima beans Harding and Tucker (1964) did not find significant correlations between $\hat{\alpha}$ and q, and concluded that the tendency of α to increase with generations was not a simple function of its changing gene frequency.

Not only the marker locus but also the genotypic background as a whole apparently affects the rate of outcrossing. This was evident from the wide variation of $\hat{\alpha}$ in different crosses with a common locus, particularly for the A^D/a^0 locus. Harding and Tucker (1964) observed in lima beans that the estimates of outcrossing for the loci *W/w*, *D/d*, *C/c* and *S/s* varied and the viny genotype (*D-*) outcrossed at a higher rate than the bushy genotype (*d/d*). Similar differences in outcrossing due to growth habit were reported earlier in common beans, *Phaseolus vulgaris* (Barrons, 1938).

In the present study, distribution of outcrossing for A^D/a^0, *Sh/sh* and *Pl/pl* was positively skewed while the distribution of outcrossing for *Cr/cr* was bimodal in nature. Considering the limitations of the number of outcrossing estimates for each of these loci, particularly for *Sh/sh*, *Cr/cr* and *Pl/pl*, and the high amount of inseparable environmental variation in outcrossing, only a tentative assumption can be made for the genetic basis of the control of selfing in jute. As the type of distribution of outcrossing indicates the number of loci controlling the selfing (Allard, Jain and Workman, 1968), the continuous nature of the distributions, with skewedness or bimodality, suggests that selfing might be under the control of one or two pairs of major genes modified by a large number of minor genes. In view of the sporadic results in outcrossings in lima bean, Harding and Tucker (1964) concluded that such results could only be expected to occur in a variable mating system which was under quantitative genetic control.

The mating system is commonly considered to be the chief factor determining the genetic structure and evolutionary potential in a population. Evidence did not suggest that jute populations followed models of complete random mating or complete inbreeding. The mating system of the present populations was partial inbreeding, i.e. a mixed system of random mating and self-fertilization. Workman (1964) and Allard, Jain and Workman (1968) have discussed in detail the evolutionary consequences and significance of such a mating system.

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