QUANTITATIVE STUDIES ON MATING SYSTEMS

I. EVIDENCE FOR THE NON-RANDOMNESS OF OUTCROSSING IN *PHASEOLUS LUNATUS* *

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1. 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 inter-mating in addition to self-fertilisation; genetic models for such populations have been presented by Hayman and Mather (1953), Hayman (1953), Binet *et al.* (1959) and Workman and Allard (1962). Fyfe and Bailey (1951) have presented various procedures for estimating inbreeding coefficients, f , and outcrossing parameters, α , associated with loci scored, where α = frequency of random outbreeding and $(1-\alpha)$ = frequency of selfing (see also Nei and Syaktdo, 1958). Estimates of α and $(1-\alpha)$ have been presented for naturally occurring populations of *Datura meteloides* by Snow and Dunford (1961). Similar results for an experimental population of *Hordeum vulgare* (Composite Cross V of C. A. Suneson) have been presented by Jain and Allard (1960).

The purpose of this investigation is to study in detail the mating systems involved in certain artificial populations of lima beans, *Phaseolus lunatus*. In all the previously mentioned models, outcrossing was assumed to be at random. In view of the results of Guitierrez and Sprague (1959), which showed rather conclusively that outcrossing was not at random in their maize populations, we shall attempt to investigate the randomness of observed outcrossing and possible trends in such cases where outcrossing appears non-random.

2. POPULATIONS

The population which will be designated Population I is a commercial variety known to be segregating for two recognisable loci, D, d and W, w . It was started in 1949 by a commercial lima bean grower in Irvine, California, from breeding stocks which are genotypically $Dd Ww$. Since 1955 the acreage has ranged from 240 to 800 acres; hence, the population size has been more than four million individuals per year. Random samples of plants were taken directly from two fields in 1959 and three fields in 1960. These plants were

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progeny tested for the purpose of estimating genotypic and allelic frequencies at the two marker loci. In addition recessive phenotypes were selected and progeny tested in order to estimate the proportion of outcrossing.

Population II was grown at Davis, California, and originated from an F_1 hybrid between L21 and L48, two homozygous accession stocks differing at seven marker loci, five of which were used to estimate outcrossing. Population size ranged from 1000 to 2000 individuals per year. Each generation was a random sample from the seed produced by the previous generation and estimates of outcrossing were made on the F_2 , F_4 , F_6 , F_7 , F_8 , F_9 and F_{10} . Since seed can be stored over a period of three or four years, in some cases different generations were tested in the same season.

Population III originated from an F_1 hybrid of two homozygous accession stocks, L20 and L185. In 1961 three F_2 populations were grown at Davis and three at Irvine; each population consisted of 500 to 600 individuals. The total seed produced from each population was randomly sampled and F_3 populations were grown in 1962; population size ranged from 1500 to 2000. From the seed which remained after sampling, randomly selected homozygotes for the C , c and S , s loci were tested for the outcrossing estimates.

3. ESTIMATION PROCEDURE

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

$$\hat{T} = \frac{a}{a+b}, \quad (1)$$

$$\text{Var } \hat{T} = \frac{ab}{(a+b)^3}, \quad (2)$$

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

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

where q is the gene frequency associated with the homozygote selected and which results directly if either expectation, $\alpha(1-q)N$, or $(1-\alpha+\alpha q)N$, is equated with the observed value a or b , respectively (Bailey, 1951). If q is known, then using maximum likelihood methods

$$\text{Var}_{\hat{\alpha}} = \frac{\alpha(1-\alpha+\alpha q)}{N(1-q)} = \frac{\alpha(1-\alpha q)}{Np} \quad (4)$$

where $p+q=1$.

Ordinarily q 's true value is unknown and q must also be estimated. If a random sample from the population is available then q can be estimated and its estimate substituted into (3). This procedure of taking two samples, one of recessives and one random, is unnecessary for populations in equilibrium because α could be estimated from the random sample according to Fyfe and Bailey (1951). However, experimental populations are seldom, if ever, in equilibrium. Since the true value of q is unknown in such cases, its estimate, which is subject to sampling error, must be used.

Since the variance of α is propagated from variances in T and q , the problem can be approached in the following manner. If small changes in q and T cause changes in α then the following is approximately true (Kendall and Stuart, 1958):

$$\Delta\alpha \approx \frac{\partial\alpha}{\partial q} \Delta q + \frac{\partial\alpha}{\partial T} \Delta T \quad (5)$$

where $\Delta\alpha = (\alpha - \bar{\alpha})$. $\bar{\alpha}$ results from substituting $\bar{T}$ and $\bar{q}$ into (3), and is the best approximation to $E(\alpha)$. By this definition of $\Delta\alpha$, $\sum \Delta\alpha^2$ divided by N will be the variance of α . If both sides of (5) are squared and summed over i , $i = 1, 2, \dots, N$ then

$$\begin{aligned} \sum_{i=1}^N (\Delta\alpha_i)^2 &\approx \left(\frac{\partial\alpha}{\partial q}\right)^2 \sum_{i=1}^N (\Delta q_i)^2 + \left(\frac{\partial\alpha}{\partial T}\right)^2 \sum_{i=1}^N (\Delta T_i)^2 \\ &\quad + 2 \left(\frac{\partial\alpha}{\partial q}\right) \left(\frac{\partial\alpha}{\partial T}\right) \sum_{i=1}^N (\Delta q_i \Delta T_i). \end{aligned} \quad (6)$$

Dividing both sides by N and substituting the appropriate partial differentials (3)

$$\text{Var}_\alpha \approx \frac{T^2}{(1-q)^4} \text{Var}_q + \frac{1}{(1-q)^2} \text{Var}_T + \frac{2}{(1-q)^2} \text{Cov}(q, T). \quad (7)$$

If errors in q and T are independent, the covariance term can be ignored. Substituting from (3)

$$\text{Var}_\alpha \approx \frac{\text{Var}_T + \alpha^2 \text{Var}_q}{(1-q)^2} \quad (8)$$

where $\text{Var}_q = pq/2N$.

The inbreeding coefficient, f , can be estimated if $\alpha = (1-f)(1+f)$ is substituted into (3)

$$f = \frac{N(1-q) - a}{N(1-q) + a} \quad (9)$$

which was given by Fyfe and Bailey for the case of progeny arising from homozygous parents.

If q 's true value is known then (4) should be used to estimate Var_α . However, (4) is biased if q is an estimate since it ignores any sampling

error in q . This bias can be evaluated by comparing the relative contributions of errors in T and errors in q . From equations (5), (6) and (7) it can be seen that variance in q and T are multiplied by their

FIG. 1.—Partial derivatives showing change in α with respect to change in q and T for varying value of $(1-q)$. Note that $\left(\frac{\partial \alpha}{\partial q}\right)$ for $T = 0.05$ and 0.10 is sufficiently small to ignore when $(1-q)$ is greater than about 0.50 (see text).

respective (derivatives)² and these derivatives, therefore, act as weighting factors. For Var_q and Var_T the weights are $\left(\frac{\partial \alpha}{\partial q}\right)^2$ and $\left(\frac{\partial \alpha}{\partial T}\right)^2$ respectively. Weighting factors can be compared in fig. 1, where $\frac{\partial \alpha}{\partial T}$ is plotted against $(1-q)$ and $\frac{\partial \alpha}{\partial q}$ is plotted against $(1-q)$ for several values of T . For populations with low values of T and high values of $(1-q)$ the contribution of Var_q is relatively very small. Formula (8)

should be used where outcrossing exceeds about 15 per cent. and/or if gene frequency ($1-q$) is low. In the following discussion reference will always be made to the formula which has been used.

4. RESULTS

(i) Population I

The data and estimates of outcrossing with standard deviations from Population I are given in table 1. Two fields were sampled in

TABLE 1

Estimates of outcrossing in Population I using D, d and W, w loci

Population I <i>D, d</i> Locus								
Gen.	Year	Field	<i>a</i>	N	<i>q</i>	$\hat{\alpha}$	$\sigma_{\hat{\alpha}}(4)$	$\sigma_{\hat{\alpha}}(8)$
F ₁₁	1959	—	105	1213	0.0301	0.0892	0.0083	0.0083
F ₁₂	1960	I	9	313	0.0173	0.0293	0.0096	0.0096
		II	17	144	0.0309	0.1218	0.0277	0.0278
		III	67	1006	0.0221	0.0681	0.0080	0.0081
		Totals	198	2676	0.0256*	0.0759	0.0052	0.0052

Population I <i>W, w</i> Locus								
F ₁₁	1959	—	25	1173	0.8811	0.1792	0.0355	0.0388
F ₁₂	1960	I	11	372	0.9462	0.5496	0.1632	0.1921
		II	1	101	0.9498	0.1972	0.1962	0.1998
		III	4	411	0.9338	0.1470	0.0731	0.0768
		Totals	41	2057	0.9068*	0.2139	0.0331	0.0356

* Mean gene frequency weighted to N of crossing sample.

1959, but they were not kept separate; hence, the estimates in 1959 represent averages over two fields. However, the samples from fields I, II and III in 1960 were kept separate and a comparison of field differences can be made.

The most striking result of Population I is the fact that α estimated from *ww* samples is 0.2139, whereas α estimated from *dd* samples is only 0.0759. This difference would seem significant in view of their expected standard deviations of 0.035 and 0.005, respectively. However, the observed standard deviations between samples for the same locus are much larger than their expected values, *viz.* $s_{\hat{\alpha}} = 0.039$ for *dd*, $s_{\hat{\alpha}} = 0.189$ for *ww*. Consequently the mean estimates for the two loci are not significantly different using their empirical standard deviations based on 3 d.f. (they might be different since $p \sim 0.10$), but the variability observed between samples is significantly greater than expected in both loci.

Inspection of table 1 shows that unusual estimates were obtained from field I (1960), *viz.* $\alpha = 0.029$ and $\alpha = 0.549$ for D, d and W, w respectively. The high estimate of α using $w w$ is subject to a sampling error of nearly 0.20, but σ_α using dd is less than 0.01. Such sporadic results can only be expected to occur in a variable mating system.

In the last two columns of table 1, expected variances from (4) and from (8) have been presented for comparison. For the case of D, d (4) is essentially unbiased, resulting from the fact that q is quite small. To the contrary, q is quite large in the case of W, w and (8) indicates that (4) is a significant underestimate.

(ii) Population II

The estimates which were obtained from Population II are arranged into tables 2, 3 and 4 according to the locus scored. If the individual

TABLE 2

Estimation of outcrossing in Population II using D, d and V, v loci as markers

Population II D, d Locus							
Gen.	Year	a	N	q	$\hat{a}$	$\sigma_\alpha^2(4)$	σ_α
F_2	1960	11	352	0.5000*	0.0625	3.44×10^{-4}	0.0185
F_8	1958	4	223	0.2945	0.0254	1.59×10^{-4}	0.0126
F_{10}	1960	6	213	0.1546	0.0333	1.80×10^{-4}	0.0134
Totals		21	788	0.3485†	0.0409	7.75×10^{-5}	0.0088
Population II V, v Locus							
F_8	1958	7	346	0.5767	0.0478	3.20×10^{-4}	0.0179
F_9	1959	1	112	0.5047	0.0180	3.22×10^{-4}	0.0179
F_{10}	1960	15	903	0.4327	0.0293	5.62×10^{-5}	0.0075
Totals		23	1361	0.4752†	0.0322	4.43×10^{-5}	0.0067

* q assumed to be 0.5 in F_2 generation.

† Mean gene frequency weighted to N of crossing sample.

samples are totalled for each locus, average values for each locus (except S, s , where SS and ss are kept separate) are as follows:

For Vv , $\hat{a} = 0.0322$, $N = 1361$,
 Dd , $\hat{a} = 0.0409$, $N = 788$,
 Cc , $\hat{a} = 0.0845$, $N = 804$,
 $Sr sr$, $\hat{a} = 0.1281$, $N = 228$,
 ss , $\hat{a} = 0.1173$, $N = 359$,
 SS , $\hat{a} = 0.2416$, $N = 381$.

The χ^2 binomial index of dispersion associated with these estimates indicates that they are heterogeneous ($p < 0.01$). In fact if all the data

are pooled and one composite estimate made, $\bar{\alpha} = 0.0784$. The approximate expected range ($\pm 2\sigma_a$) due to random fluctuations is 0.0672-0.0896; but, if the observed fluctuation of $\hat{\alpha}$ over the 6 genotypic samples is used the approximate range ($\pm 2s_a$) becomes 0.0226-0.1342. The observed fluctuation in α is, approximately five times the fluctuation expected on the basis of random chance ($s^2/\sigma^2 = 24.8$).

Except for the *S, s* locus all estimates are based on recessive samples and hence measure the amount of crossing of dominant males on to

TABLE 3

Estimation of outcrossing in Population II using S, s and Sr, sr loci as markers

Population II <i>S, s</i> Locus— <i>SS</i> selected							
Gen.	Year	<i>a</i>	N	<i>q</i>	$\hat{\alpha}$	$\sigma_{\alpha}(4)$	$\sigma_{\alpha}(8)$
F_9 F_{10}	1959	10	81	0.7647	0.5247	0.1553	0.1645
	1960	12	300	0.7600	0.1667	0.0461	0.0497
	SS Totals	22	381	0.7610*	0.2416	0.0500	0.0528
<i>ss</i> selected							
F_{10}	1960	32	359	0.2400	0.1173	0.0198	0.0201
Population II <i>Sr, sr</i> Locus							
F_9 F_{10}	1959	9	81	0.4240	0.1929	0.0606	0.0620
	1960	8	147	0.4145	0.0929	0.0319	0.0326
	Totals	17	228	0.4179*	0.1281	0.0299	0.0305

* Mean gene frequency weighted to N of crossing sample.

recessive females. In the case of the *S, s* locus, heterozygotes are distinguishable from either homozygote which makes possible reciprocal estimates, *i.e.* $S\hat{S} \times ss$ and $s\hat{S} \times SS$.

Estimates of outcrossing using *vv* appear in table 2. These estimates from F_8 , F_9 and F_{10} do not appear heterogeneous ($p \sim 0.18$) and consistently give rather low estimates for outcrossing.

Samples of *dd* phenotypes were taken from Population II in the F_2 , F_8 and F_{10} generations (table 2). The F_2 estimate of α appears larger than the estimates from later generations. The question of whether the three estimates are heterogeneous remains unanswered because the binomial index $\chi^2 = 5.27$ (d.f. = 2) and $p \approx 0.075$. Thus, *D, d* locus estimates result in rather low values for α that are probably heterogeneous. If a trend does exist, it appears to be for decreasing α as generations advance.

TABLE 4
Estimation of outcrossing in Population II using C, c locus as the marker

Population II C, c Locus							
Gen.	Year	a	N	q	$\hat{a}$	$\sigma_a^2(4)$	σ_a
F ₄	1958	4	162	0.3209	0.0364	3.23×10^{-4}	0.0180
F ₆	1958	6	139	0.2733	0.0594	5.63×10^{-4}	0.0237
F ₇	1957	13	187	0.1361	0.0806	4.65×10^{-4}	0.0216
F ₈	1958	14	172	0.1322	0.0938	5.77×10^{-4}	0.0240
F ₉	1959	3	44	0.0556	0.0722	1.62×10^{-3}	0.0402
F ₁₀	1960	15	100	0.1227	0.1710	1.66×10^{-3}	0.0407
Totals		55	804	0.1906*	0.0845	1.21×10^{-4}	0.0110

* Mean gene frequency weighted to N of crossing sample.

FIG. 2.—Estimates of outcrossing, $\hat{a}$, using *cc* from Population II plotted against gene frequency, *q*, of *c*.

Since heterozygotes at the *S, s* locus are distinguishable, both *ss* and *SS* phenotypes were sampled and the results are presented in table 3. The two *SS* estimates (*F*₉ and *F*₁₀) are heterogeneous ($p < 0.01$), even though the sample size was small in *F*₉ and $\hat{a} = 0.5247$ was subject to

large sampling error. If the average value for the two *SS* estimates (0.2416) is compared to the estimate from the *ss* sample (0.1173), they are found to be significantly different ($p < 0.01$). Therefore, reciprocal differences in outcrossing can occur at the *S, s* locus.

Smaller samples are available for the *Sr, sr* locus (table 3), yet they are still significantly heterogeneous ($p < 0.05$). A complication associated with the *Sr, sr* locus results because it is hypostatic to *cc* and *ss* and can only be scored in a minority of individuals. Estimates from

FIG. 3.—Estimates of outcrossing, $\hat{\alpha}$, using *cc* from Population II plotted against generation from which estimate was made. Solid line is least squares linear model.

sr sr samples are therefore not representative of the entire population of genetically *sr sr* individuals.

Estimates of α from *cc* samples were made on the F_4 , F_6 , F_7 , F_8 , F_9 and F_{10} generations and are presented in table 4. A trend for $\hat{\alpha}$ to increase as generations advance is found at the *C, c* locus. This trend in $\hat{\alpha}$ for the 6 generations is plotted against gene frequency, q (fig. 2), and against generations (fig. 3). The correlation of $\hat{\alpha}$ and q is non-significant ($p \approx 0.2$), but the correlation between $\hat{\alpha}$ and generations is significant ($r = 0.825$; $p < 0.05$). The best fitting linear equation is

$$E(\alpha) = -0.0434 + 0.0176N,$$

where N is the filial generation, (F_N). The slope of the equation, 0.0176, is significantly greater than zero ($p < 0.05$). The tendency for $\hat{\alpha}_{cc}$ to increase is therefore not a simple function of its changing gene

frequency but probably results from the forces of natural selection which are changing the population genotype.

Tables 2, 3 and 4 show that high and low estimates appear in all years. It does not appear that seasonal fluctuations, considered alone, can account for a large part of the variability observed.

(iii) Population III

Estimates from replicated Population III at Davis and Irvine are presented in table 5 (*C, c* locus) and table 6 (*S, s* locus). Recessives at

TABLE 5
Estimation of outcrossing in Population III using C, c locus as the marker

Population III <i>C, c</i> Locus							
Gen.	Year	Loc.	Rep.	<i>a</i>	N	<i>a</i> *	$\sigma_a(4)$
F ₂	1961	Davis	I	12	447	0.0537	0.0153
			II	14	434	0.0645	0.0170
			III	10	454	0.0441	0.0138
			Total	36	1335	0.0539	0.0089
F ₂	1961	Irvine	I	12	347	0.0692	0.0196
			II	10	321	0.0629	0.0195
			III	7	275	0.0509	0.0190
			Total	29	943	0.0615	0.0112
F ₃	1962	Davis	I	24	552	0.0870	0.0174
			II	14	468	0.0598	0.0157
			III	14	441	0.0635	0.0167
			Total	52	1461	0.0712	0.0097
F ₃	1962	Irvine	I	1	580	0.0029	0.0029
			II	2	537	0.0062	0.0044
			III	2	463	0.0072	0.0051
			Total	5	1580	0.0053	0.0024
Summation of totals				122	5319	0.0433	0.0039

* q is assumed to be 0.5 in all F₂ populations and F₃ populations at Davis, but q for F₃ at Irvine populations seems to have been reduced to 0.4 in one generation; hence $q = 0.4$ is assumed for F₃ at Irvine.

the *C, c* locus were sampled in 1961 and 1962, whereas *SS* and *ss* homozygotes were sampled only in 1962. The results from the *C, c* locus were, with one exception, quite homogeneous over replications, locations and years (*viz.* $\hat{a} \approx 0.06$). The exception was at the Irvine location in 1962 where outcrossing was one-tenth to one-fifteenth the frequency of other cases for Population III (*viz.* $\hat{a} = 0.005$).

It can be seen from table 6 that ss and SS estimates from Irvine consistently support the hypothesis of a rather low frequency of outcrossing for that location (*viz.* $\alpha = 0.03$). While α appears the same for reciprocal crosses at Irvine, reciprocal differences appear at Davis. However, a large increase in heterogeneity (significant at $P = 0.05$)

TABLE 6

Estimates of outcrossing for Population III in 1962 using S, s locus as the marker

Population III S, s Locus							
Loc.	♀	Rep.	a	N	q	â	σâ(4)
Davis	ss	I	78	552	0.5104	0.2886	0.0303
		II	34	485	0.4753	0.1336	0.0221
		III	23	542	0.5185	0.0881	0.0180
		Total	135	1579	0.5024*	0.1718	0.0141
Irvine	ss	I	12	558	0.5155	0.0444	0.0127
		II	3	407	0.4394	0.0131	0.0075
		III	8	535	0.4732	0.0284	0.0100
		Total	23	1500	0.4798*	0.0295	0.0061
Davis	SS	I	19	553	0.4896	0.0673	0.0152
		II	23	502	0.5247	0.0964	0.0196
		III	23	499	0.4815	0.0889	0.0181
		Total	65	1554	0.4984*	0.0834	0.0101
Irvine	SS	I	5	444	0.4845	0.0218	0.0097
		II	11	517	0.5606	0.0484	0.0144
		III	4	482	0.5268	0.0175	0.0087
		Total	20	1443	0.5259*	0.0292	0.0065
Summation of totals			243	6076	0.5014*	0.0802	0.0050

* Mean gene frequency weighted to N of crossing sample.

has accompanied the increase in $\hat{\alpha}$ and has considerably increased the empirical standard deviations of the means, *i.e.* of 0.1718 and 0.0834, and the difference is, therefore, not significant ($p \approx 0.3$). However, the S, s locus gives significantly greater estimates ($p < 0.01$) for the Davis location than for the Irvine location. This is in agreement with the C, c locus estimates, *i.e.* Davis populations outcross more than Irvine populations in 1962.

5. CONCLUSIONS

In general estimates from Population III are homogeneous but with two striking exceptions. In one case (Irvine; C, c) an exceptionally low

estimate was obtained and in another case (Davis; *ss*) an exceptionally high estimate was obtained, which was also highly heterogeneous. Estimates from Populations I and II were generally heterogeneous and large discrepancies have been observed within years and within populations. The most "discordant" result occurred in Population I, in one field, and in one year, when the estimate from one locus (*D, d*) was less than 0.03 and the estimate from another locus (*W, w*) was greater than 0.50.

Seasonal effects seem equally inconsistent. Selecting the *C, c* locus in Population III (1961) indicates that Davis and Irvine environments lead to similar amounts of outcrossing, but the opposite is true in 1962 when Davis populations outcrossed more. Samples of either *ss* or *SS* phenotypes also indicate that there is more outcrossing in the Davis environment. There is, on the other hand, evidence from Population I that outcrossing is high at Irvine, so it is quite clear that generalisations are not at all obvious.

Reciprocal differences appear in Population II and III using both homozygotes at the *S, s* locus. However, results from Population II (Davis), Population III (Irvine) and Population III (Davis) are not in agreement. Reciprocal differences, therefore, may or may not occur, but depend upon the locus, population, location or year involved.

The difficulty in generalising from these results infers that the heterogeneity in outcrossing is sporadic variation. An exception to this inference is the steady trend for α (*C, c*) to increase in Population II. This suggests the possibility that the mating system is under quantitative genetic control and hence subject to the pressures of natural selection.

All the results combine to warn of the dangers in assuming constant values for outcrossing parameters in genetic models. They also suggest that randomness of outcrossing should not be assumed and that reciprocal as well as more complex differences should be considered. Additional experiments are being conducted which are designed to study reciprocal differences and frequency dependency of outcrossing in *P. lunatus*.

6. SUMMARY

Approximately 20,000 individuals were scored for selfing versus outcrossing in three different populations, in two locations, and over a number of seasons. Different marker loci were used to determine the male gamete which had effected fertilisation. The results show that frequency of outcrossing is extremely variable and, furthermore, that propensity to outcross is associated with the genotype of individuals selected for study. In one case, outcrossing was found to be associated very closely with the generation under study. In conclusion the mating systems of populations of *P. lunatus* must be considered to be quite complex.

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