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Variability and restrictions against inbreeding and unequal family size in control populations of *Tribolium*

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1. INTRODUCTION

In recent years the problem of optimum design of control populations for genetic studies has received some attention. Designs involving restrictions against inbreeding and unequal family size have been utilized by Gowe, Robertson & Latter (1959) and by King, Carson & Doolittle (1959). An experiment designed to test the general suitability of many types of controls was reported by Bray, Bell & King (1962).

These studies drew on the theoretical expectations developed by Wright (1921, 1931) and Crow (1954). The non-significant effects of restrictions against inbreeding and unequal family size obtained by Bray *et al.* (1962) prompted the author to continue experiments of this type. The results of a second experiment, reported by Bray (1961), suggested that the effect of the restriction against unequal family size was greater than the effect of the inbreeding restriction. While this result was anticipated from the work of Wright, theoretical expectations concerning these two restrictions applied simultaneously seemed obscure. This situation stimulated Robinson & Bray (1965) to investigate the theory as it relates to control populations more fully. A paper by Robertson (1964) has appeared since these studies were initiated. Some of the theoretical results are similar to those suggested by Robinson & Bray (1965).

In maintaining a single control population, the two restrictions may not be applied at all, may be applied singly, or may be applied simultaneously so that the four mating systems considered here result. Following the notation of Robinson & Bray (1965), the four mating systems may be designated: $R:R$, pupae were randomly chosen and randomly mated so that neither restriction was applied; $2:R$, two pupae, chosen from each family, were randomly mated so that the restriction against unequal family size only was imposed; $R:S$, randomly chosen pupae were subsequently mated so that no sib matings occurred; $2:S$, two pupae chosen from each family were subsequently mated so that no full sib matings occurred. The factorial nature of the two restrictions is illustrated in Fig. 1.

The ideal control population is one in which no change in gene frequency or genotypic frequency occurs from generation to generation and which is genetically

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related to the lines under treatment (see Bray *et al.*, 1962). Robinson & Bray (1965) suggested that such a situation can be most easily obtained when a group of inbred lines is used to form a base population and the base is reconstituted at intervals. For some types of quantitative studies it may be considered desirable to random-mate the base population and its reconstituted replicates for a few generations in order to permit equilibrium proportions to be established before estimates of genetic variability are made. When it is not possible or convenient to use such a population, some type of random mating control is required. If an ideal control were measured in a constant environment it would give identical estimates of mean performance, phenotypic variance, and genetic variance from generation to generation.

The relative effect of the restrictions considered here will, no doubt, vary with the number of loci affecting the character, the degree of dominance, and the degree of epistasis expressed by these loci. The theory concerned with the rate of gene

Method of choosing individuals	Method of mating individuals	
	Random	No sib mating
Random	$R:R$	$R:S$
Two from each family	$2:R$	$2:S$

Fig. 1. Four mating systems illustrating the use of two restrictions which may be applied to the methods of choosing and mating breeding individuals in a control population.

loss and changes in the inbreeding coefficient does not permit precise predictions when the levels of these factors are unknown. However, some general expectations do follow from the work of Robinson & Bray (1965).

When a gene is lost, or conversely when another is fixed, at least two genotypic classes are lost and a reduction in phenotypic variance occurs. Using the criterion of rate of gene loss, we can expect the restriction against unequal numbers to be more powerful than the inbreeding restriction in preserving phenotypic variability when either is used alone, and the latter to be a hindrance when both are used together.

Alterations in the genotypic proportions will also affect estimates of variance. Under random mating, with or without the use of the restriction against unequal numbers, the usual Hardy-Weinberg equilibrium proportions are expected. When matings between relatives are avoided, the genotypic proportions in the next generation are altered in the direction of fewer homozygotes. A more uniform population is therefore expected, having smaller phenotypic variance than that in a random-mating population. This effect is expected regardless of whether the restriction against unequal numbers has been imposed or not. Since the expected number of full sib matings is only one regardless of the total number of single pair matings (Robinson & Bray, 1965), this additional effect must become small as the size of the mating population increases.

A complex situation exists in which the restriction against unequal numbers

affects the sampling procedure to preserve variability while the inbreeding restriction only affects the sampling procedure when used alone and always acts to reduce variability by preventing the formation of some identical homozygotes. In the absence of a complete model specifying the number and effect of all genes in a given quantitative genetic system it is impossible to predict in advance the relative effects of the two restrictions on phenotypic variability when small numbers of mating pairs are used.

The experiments reported here were planned to permit estimates of the generation to generation variability of mating system means without benefit of the theory now available. Nevertheless, it seems worthwhile to record the results of these experiments since they bear directly on the effect of two restrictions commonly in use.

2. MATERIALS AND METHODS

Three separate experiments were conducted, one at each of three different institutions, in which *Tribolium castaneum* served as the experimental animal and pupa weight was the measured variable. Four lines of *Tribolium* were reproduced from single pair matings in each replicate of each experiment to provide a test of the two restrictions.

When a family did not produce at least two progeny, additional pupae were drawn from the remaining families within that mating system.

The first experiment in which two replications of all lines were maintained using 50 single pair matings has already been reported in full (Bray, Bell & King, 1962). The two replicates were initiated by collecting large samples of eggs from the same mass matings in two successive weeks so that Replication I proceeded with generations cycling one week ahead of Replication II. The lines designated R_o, R_s, R_n, R_{sn} in that paper are those which are reconsidered in the present paper, here designated as $R:R$, $R:S$, $2:R$, $2:S$ respectively. Mass matings of the Purdue Foundation stock were maintained in subsequent years at the University of British Columbia and at the Animal Research Institute, Ottawa. All experimental lines were formed from this same base population.

The second experiment followed the same general plan as the first except that two mating population sizes were used, 8 pairs and 16 pairs. Preliminary results of this experiment, completed at the University of British Columbia, have been reported (Bray, 1961). Culture conditions relative to media, temperature and humidity were the same as in the first experiment. As in Experiment I, all lines were grown for two generations in a wet environment (70% relative humidity) followed by two generations in a dry environment (40% relative humidity). The second experiment was conducted over a period of six generations.

The third experiment was conducted at the Animal Research Institute, Ottawa. Mating population size was reduced to four pairs, and three replications were reared through four generations. In this experiment all generations were reared in the 70% relative humidity environment whereas all other culture conditions remained the same as in the first experiment.

The number of pupae measured varied among experiments. In Experiment I approximately 12 pupae per full sib family were weighted. Group weights were obtained as described by Bray *et al.* (1962). In Experiment II approximately 6 pupae were measured per family. One group weight was taken for each method in each replication on a less sensitive balance than that used for Experiments I and III. In Experiment III, 4 pupae (2 males and 2 females where possible) were individually weighed from each full sib group on the same type of balance as was used in Experiment I.

As will be indicated in the Results and Discussion it was considered desirable to obtain an estimate of variability due to the use of the restriction which was as free as possible from gross generation to generation variation. A difference statistic was therefore employed which was computed in the following manner.

For each of the experiments, the mean of all four mating systems was computed within each generation in each replication. The difference (sign included) of each mating system from each appropriate generation mean within replicate was then computed. The generation to generation within replicate variance of these differences for each mating system provided the basic data reported here for all three experiments.

In order to take full advantage of the data, the 2×2 factorial arrangement of the treatments was utilized. Difficulties encountered when testing for differences among variances using an analysis of variance have been discussed by Bartlett & Kendall (1946) who recommended the use of the logarithmic transformation. This transformation was carried out, and all tests of significance were performed on the transformed data. The usual analysis of variance models were used with each restriction considered a fixed effect. The significance of each restriction was then tested by the residual variance which was made up of replicate $\times$ treatment interactions. In the case of Experiment II, an additional fixed factor, number of mating pairs, was introduced and that analysis took the form of a $2 \times 2 \times 2$ factorial where the fixed effects were again tested by the residual replicate $\times$ treatment interactions.

3. RESULTS AND DISCUSSION

It is clear that a good control population is one which is not subject to drift. Regressions of mean pupa weight on generation were computed for all lines as previously reported by Bray *et al.* (1962) for Experiment I. None of the regression coefficients were significantly different from zero and no clear pattern was discernible. One would expect that some drift would be observed if data were collected over more generations.

A second criterion of stability is that the mean phenotypic values should exhibit minimum variability from generation to generation if the line is reared in a constant environment. A constant environment, however, proves to be a difficult thing to produce. In nearly every experiment disease, accident, chance, and variability in materials, machines, instruments and technicians combine to produce different

conditions in each generation. If, however, one minimizes generation to generation variability by utilizing differences from generation means within replicates, then the remaining variability in the differences from generation to generation is an estimate of what could be expected in a constant environment.

The phenotypic standard deviations of the differences for each mating system are given in Table 1. An analysis of variance within each of Experiments I and III separately failed to illustrate significant effects (Table 2). An anaylsis of variance within Experiment II showed that only the number of pairs was significant (Table 3).

Table 1. *Phenotypic standard deviations of the differences *for each mating system in micrograms*

		Number of pair matings			
		Exp. III†	*Exp. II*‡		*Exp. I*§
Method	Replication	4	8	16	50
R:*R*	1	38·7	73·0	41·0	40.6
	2	66·8	46·5	100·0	28·6
	3	44·7	120·1	61·6	—
	Mean	50·1	79·9	67·5	34·6
R:*S*	1	25·1	59·7	34·2	36·4
	2	43·1	71·5	66·2	22·8
	3	30·6	97·2	82·5	—
	Mean	32·9	76·1	61·0	29·6
2:*R*	1	18·4	79·6	41·1	29·0
	2	59·3	86·7	62·5	21·5
	3	39·4	63·3	62·7	—
	Mean	39·0	76·5	55·4	25·3
2:*S*	1	19·7	42·6	46·4	21·3
	2	20·5	65·7	41·8	27·7
	3	50·5	107·5	43·5	—
	Mean	30·2	71·9	43·9	24·5

* See text for description of the differences.

† Data collected at the Animal Research Institute, Canada Department of Agriculture, Ottawa, Canada, 1962–3.

‡ Data collected at the University of British Columbia, Vancouver, Canada, 1960–1.

§ Data collected at Purdue University, Lafayette, Indiana, U.S.A., 1958–9.

Table 2. *Analysis of variance of data from* 50 *and* 4 *mating pairs*

	Exp. I (50 pairs)		Exp. III (4 pairs)	
Source	d.f.	M.S.	d.f.	M.S.
Replications	1	0·0914	2	0·3987
Sib mating (*S*)	1	0·0189	1	0·3317
Equal numbers (*N*)	1	0·1154	1	0·1818
S × *N*	1	0·0096	1	0·0214
Residual	3	0·0528	6	0·1315

Table 3. *Analysis of variance of data from Experiment II* (8 *and* 16 *pairs*)

Source	Degrees of freedom	Mean squares
Replications	2	0·3545
Number of pairs (P)	1	0·5245*
Sib mating (S)	1	0·0704
$P \times S$	1	0·0146
Equal numbers (N)	1	0·0928
$P \times N$	1	0·0410
$S \times N$	1	0·0228
$P \times S \times N$	1	0·0001
Residual	14	0·0920

* Significant at $P = 0{\cdot}05$.

In spite of the fact that it was not possible to demonstrate statistically significant effects within each experiment, Table 1 does indicate that general expectations have been realized. The percent reductions in standard deviation due to the mating systems have been computed from the mean values in Table 1 and are given in Table 4. In each case the effect of applying the restriction is compared to the R:R system. Comparisons within population size indicate that the full sib restriction alone produced greater reductions than the restriction against equal numbers alone in the smaller populations (34·3% > 22·2%, 4·8% > 4·3%) while the reverse was true for the larger populations (17·9% > 9·6%, 27·5% > 14·4%). It is also clear that the effect of avoiding sib mating in addition to drawing an equal number from each family is considerably less for the largest number of mating pairs than for the smallest (27·5/29·2 versus 22·2/39·7).

Table 4. *Percent reduction in phenotypic standard deviation relative to* R:R

Method	Number of mating pairs			
	4	8	16	50
R:S	34·3	4·8	9·6	14·4
2:R	22·2	4·3	17·9	27·5
2:S	39·7	10·0	35·0	29·2

Since conditions of measurement in Experiment II differed so markedly from those in Experiments I and III, and since environmental rearing conditions of Experiment III are different from the other two experiments, there is little point in attempting to combine these data to assess the overall effects of population size. If, however, each of the four population size levels is considered as a separate 'experiment' in which information was obtained on each of the four methods, the analysis shown in Table 5 may be obtained. Since Experiment I had only two replications while the others all had three, mean values of each cell were entered as the third value in Experiment I to permit an equal subclass analysis and the

Table 5. *Analysis of variance over all experiments*

Source	Degrees of freedom	Mean squares
'Experiments'	3	2·2414
Sib mating (S)	1	0·3135*
Equal numbers (N)	1	0·4054*
$S \times N$	1	0·0007
Residual	37	0·1110

* Significant at P approximately 0·10.

degrees of freedom were appropriately adjusted. In this analysis there is a fairly strong suggestion that the two restrictions are having an effect (P approximately 0·10).

In interpreting these results, it must be remembered that minimum variability of the control mean is not a sufficient criterion to establish the superiority of a particular system. Additional information on the within generation phenotypic variability is required and this must be separated into effects due to more representative sampling when parents are chosen as well as effects due to altering the genotypic frequencies when they are mated. Partitioning of variances from Experiment III within replicate and generation was attempted, but the results from sets of 16 observations were extremely variable and are not reported here.

At present, one is in the difficult position of suggesting, for example, that in the case of 50 mating pairs the reduction in standard deviation from 34·6 to 25·3 is good since it should be due to more representative sampling, whereas the reduction from 25·3 to 24·5 is bad since it should be due to alterations of the genotypic frequencies which would effect estimates of phenotypic and genotypic variance. As untenable as this position may appear, it is what the theory implies, and more information is clearly needed.

In future experiments, two types of phenotypic variance should be estimated. That studied here, which is an indicator of the stability of the mean of the mating system, should be studied in greater detail. The variability within generations should also be studied for each mating system. An attempt should be made to estimate the relative importance of the two restrictions in preserving the same gene frequencies and the same genotypic frequencies as in the initial population. Perhaps more simulated computer studies will be helpful in estimating expected effects under varying conditions of dominance and epistasis.

In the absence of more complete experimental data it can only be stated that the use of the restriction against unequal numbers alone should produce the most desirable control population.

SUMMARY

Data from three experiments bearing on the relative stability of the four mating systems required to test the restrictions against inbreeding and unequal family

size were examined in relation to the results given by Robinson & Bray (1965). *Tribolium castaneum* was the experimental animal used in these experiments.

An analysis of variance indicated that both restrictions were probably effective (P approximately 0·10) in reducing the phenotypic variability of control population means. It seems likely that the apparent gain in stability obtained when the inbreeding restriction was used in addition to the restriction against unequal numbers is due to non-random genotypic proportions which would affect estimates of genetic variability based on assumptions of random mating.

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