SOME ADDITIONAL CONSIDERATIONS ON THE METHOD OF GENETICAL ANALYSIS FOR INDUCED CONTINUOUS VARIATION OF SELF-FERTILISING PLANTS

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SUMMARY

Genetic parameters for describing the induced continuous variation of self-fertilising plants have been defined in terms of mutation rate per allele and the additive and dominance effects of the genes concerned. Estimation and interpretation of these parameters are discussed.

It is shown that no single generation up to the M_3 provides enough statistics to estimate the parameters, unless mutagenic treatment is applied to zygotic cells. As in the case of generations derived from hybridisation between two inbred lines, information on the average degree of dominance and gene association in parental lines can be obtained using these estimates. The mutation rate can be estimated when two parental lines with significantly different phenotypic values are investigated simultaneously.

1. INTRODUCTION

WHILE many experiments have been conducted to assess the amount of induced genetic variation in self-fertilising plants, the genetical interpretation of the variation has received relatively less attention. Further advance awaits assessment of the biometrical method for manipulating induced variation.

Diallel crosses have been tried by some workers (*e.g.* Lawrence, 1965) using inbred lines which have been selected from mutagenically treated populations. The limitation of this method, however, is the time required to produce the pure lines, and in some plant species, the difficulty of making a sufficiently large number of cross combinations and F_1 seeds per cross. Furthermore, selected lines are not suitable for investigating the original genetic effects of mutagens, and they may not meet the assumption of uncorrelated gene distributions (Jinks, 1954), which may cause misinterpretation of the results of diallel crosses.

Aastveit and Gault (1967) have suggested a simpler method by which genetic variances and covariances of the M_2 , M_3 and M_4 populations are partitioned into the components which more directly reflect the genetic effects of individual genes. In this method, all of the M_2 , M_3 and M_4 populations have to be grown in the same experiment in the same year to eliminate any bias due to genotype $\times$ environment interaction.

Noticing this inconvenience, Virk *et al.* (1978) have proposed another method which requires only a single generation of a population with a hierarchical pedigree structure. As will be explained later, however, their idea is not applicable before the M_4 generation unless the mutagenic treat-

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ment is applied to zygotic cells or at a very early stage of embryo development. Neither does this method provide an estimate of the multiplicative component of the additive dominance gene effects, which is necessary not only in predicting the efficiency of artificial selection but also in obtaining some information about the association of dominant genes in the parental lines used.

In this paper a number of methods which are practicable up to the M_3 generation, will be discussed, since the M_3 is regarded as being the best generation to start selection for breeding (Yonezawa, 1975). Interpretation of the estimates of the parameters will also be discussed.

2. GENETIC STRUCTURE OF THE M_1 AND M_2 POPULATIONS

M_1 plants are in general multicellular at the time of mutagenic treatment, mutations occurring independently in each of the constituent cells. For instance, in cereal crops such as rice and wheat, embryos in mature seeds are well differentiated and carry a number of multicellular spike primordia (*e.g.* Osone, 1963). This means that M_1 plants are genetically chimeric at the levels of both spike and whole plant, and therefore, the genetical segregation in M_1 spike and plant progenies is different from that in the progeny of F_1 individuals.

Now, let us consider a single gene locus at which the parental allele G mutates to mutant allele g , the mutation rate of $G \rightarrow g$ being m . Then, assuming independent occurrence of mutations, the expected frequencies of GG , Gg and gg cells in mutagenically treated embryos are

$$(1-m)^2, \quad 2m(1-m) \quad \text{and} \quad m^2$$

respectively. Since mutation rate m is of the order of 10^{-3} or lower (Yonezawa and Yamagata, 1977), these values can be approximated by $1-2m$, $2m$ and zero, respectively. So, if M_1 spike primordia consist of C initial cells at the time of mutagenic treatment, the frequency of the primordia which are of the Gg genotype for i cells among the C is given by

$$\binom{C}{i} (2m)^i (1-2m)^{C-i}.$$

Since the number C is much smaller than $\frac{1}{m}$, this formula can be approximated by

$$\begin{cases} 1-2Cm & \dots & i=0 \\ 2Cm & \dots & i=1 \\ 0 & \dots & i \geq 2. \end{cases}$$

Hence, for this locus, there is no segregation in the $1-2Cm$ of the total M_1 -spiked-derived M_2 lines, and the remaining $2Cm$ lines segregate with the ratio

$$\frac{4C-3}{4C} GG : \frac{1}{2C} Gg : \frac{1}{4C} gg,$$

which indicates that the ratio $\frac{1}{4} : \frac{1}{2} : \frac{1}{4}$ as assumed by Virk *et al.* (1978) does not hold unless $C = 1$. If C is made equal to the total number of the initial cells contained in an embryo, the above ratio represents the segregation in M_1 -plant-derived M_2 lines.

Genotypic frequencies of the M_2 population as a whole are independent of the cell number, and are given by

$$(1 - \frac{1}{2}m)GG : mGg : \frac{1}{2}m gg.$$

The expected genotypic frequencies in various pedigree units (lines, families or population as a whole) in the following generations are readily obtained by the above ratios.

With k genes concerned with the character at issue, the number of mutations occurring simultaneously in a single cell is expected to be subject to the Poisson distribution with mean $2km$, which is likely to be smaller than unity. This is another characteristic of induced variation, that mutant individuals carrying two or more mutant genes will be very infrequent, indicating that effects of linkage, if any, would be much less than in genetic variation caused by hybridisation.

3. DEFINITION AND ESTIMATION OF GENETIC PARAMETERS

Means and genetic variances of the populations following mutagenic treatment are formulated below in terms of the genetic parameters to be estimated.

Using the additive-dominance model (Mather and Jinks, 1971) and the genotypic frequencies formulated in the preceding section, the mean and genetic variance of M_2 populations are described as

$$\bar{M}_2 = \bar{M}_0 - 2[d]_m + [h]_m,$$

and, by omitting the terms with the second power of the mutation rate m ,

$$V_{M_2} = 3D_m + H_m - 2F_m,$$

respectively, where

$$\bar{M}_0 = \text{mean value of the parent used,}$$

$$[d]_m = \Sigma \pm md, \quad (d \geq 0),$$

$$[h]_m = \Sigma mh,$$

$$D_m = \Sigma md^2,$$

$$H_m = \Sigma mh^2,$$

$$F_m = \Sigma \pm mdh.$$

The symbols d and h are similarly defined as in Mather and Jinks (1971). The plus and minus signs in the summation of $[d]_m$ and F_m correspond to whether the original alleles of the parent have larger or smaller effects than the respective mutant alleles. The summation in this case is over all the k genes affecting the character at issue.

Where the M_2 population is made up of the M_2 lines derived from different M_1 plants or spikes, the variance V_{M_2} can be partitioned into two statistically independent components, *i.e.* between and within M_2 line variances, which are

$$V_{M_2} = \frac{2}{C} D_m + \frac{1}{2C} H_m - \frac{2}{C} F_m$$

and

$$\bar{V}_{M_2} = \left(3 - \frac{2}{C}\right) D_m + \left(1 - \frac{1}{2C}\right) H_m - 2\left(1 - \frac{1}{C}\right) F_m,$$

respectively.

The corresponding statistics of the M_3 population are formulated by

$$\bar{M}_3 = \bar{M}_0 - 2[d]_m + \frac{1}{2}[h]_m,$$

and

$$V_{M_3} = \frac{7}{2}D_m + \frac{1}{2}H_m - F_m.$$

When the M_3 population consists of the M_3 lines derived from different M_2 plants, V_{M_3} is divided into

$$V_{M_3} = 3D_m + \frac{1}{4}H_m - F_m,$$

and

$$\bar{V}_{M_3} = \frac{1}{2}D_m + \frac{1}{4}H_m.$$

If the M_3 population is hierarchically structured by M_1 plants, the variance V_{M_3} can be further partitioned into the two components, *i.e.* the variance between M_3 groups derived from different M_1 plants and the variance between M_3 lines from the same M_1 plants, which are presented by

$$V_{M_{3B}} = \frac{2}{C} D_m + \frac{1}{8C} H_m - \frac{1}{C} F_m,$$

and

$$V_{M_{3W}} = \left(3 - \frac{2}{C}\right) D_m + \left(\frac{1}{4} - \frac{1}{8C}\right) H_m - \left(1 - \frac{1}{C}\right) F_m,$$

respectively.

Covariance between the M_2 and M_3 generations may provide additional sources for parameter estimation. The genetic covariance between M_2 plants and their M_3 progeny means is given by

$$W_{M_3/M_2} = 3D_m + \frac{1}{2}H_m - \frac{3}{2}F_m,$$

and that between M_2 lines and M_3 groups is

$$W_{M_3/M_2} = \frac{2}{C} D_m + \frac{1}{4C} H_m - \frac{3}{2C} F_m.$$

By using the M_2 and M_3 populations, we have two sources for the estimation of the two first-degree parameters, $[d]_m$ and $[h]_m$. Theoretically, seven sources V_{M_2} , $\bar{V}_{M_2}$, $V_{M_{3B}}$, $V_{M_{3W}}$, $\bar{V}_{M_3}$, W_{M_3/M_2} and $\bar{W}_{M_3/M_2}$ are available for the three second-degree parameters, D_m , H_m and F_m .

Among these seven, however, those statistics that are based on the hierarchical pedigree structure originating from M_1 spikes or plants, namely V_{M_2} , $\bar{V}_{M_2}$, $V_{M_{3B}}$, $V_{M_{3W}}$, and W_{M_3/M_2} , include the cell number C and therefore should not be used unless the value of C is precisely known in advance. In practice, C appears to differ with M_1 spikes (Sarvella *et al.*, 1962), and its estimate is influenced by the haplontic and diplontic selection against mutations (Yonezawa and Yamagata, 1975). The covariance W_{M_3/M_2} would also be unsuitable; its estimate may be much biased by both measure-

ment error and genotype $\times$ year interaction, since M_2 plants can neither be replicated nor grown in the same year as their M_3 progenies.

Hence, only the three variances V_{M_2} , V_{M_3} and $\bar{V}_{M_3}$ are reliable sources of estimates of the three second-degree parameters. Procedures for calculating these variances are outlined in table 1 assuming that the parental, M_2 and M_3 lines are grown with a randomised block design. Table 2 shows an estimation method for the case where the parental and M_2 populations are derived from randomly selected seeds of their preceding populations. Then, applying the perfect fit solution, the estimates of the parameters are obtained by

$$\begin{aligned} [\hat{d}]_m &= \frac{1}{2}(\hat{M}_0 + \hat{M}_2 - 2\hat{M}_3), \\ [\hat{h}]_m &= 2(\hat{M}_2 - \hat{M}_3), \\ \hat{D}_m &= -\frac{1}{4}\hat{V}_{M_2} + \frac{1}{2}\hat{V}_{M_3} + \frac{1}{2}\hat{\bar{V}}_{M_3}, \\ \hat{H}_m &= \frac{1}{2}\hat{V}_{M_2} - \hat{V}_{M_3} + 3\hat{\bar{V}}_{M_3}, \\ \hat{F}_m &= -\frac{5}{8}\hat{V}_{M_2} + \frac{1}{4}\hat{V}_{M_3} + \frac{9}{4}\hat{\bar{V}}_{M_3}, \end{aligned}$$

where $\bar{M}_0$, $\bar{M}_2$ and $\bar{M}_3$ are of course estimated by the total mean of the respective populations.

TABLE 1

A variance analysis for estimating genetic and environmental variance components of the parental, M_2 and M_3 populations

Item	d.f.	M.S.	E.M.S.
Blocks	$r-1$	MS_{B1}	$\sigma_w^2 + b\sigma_{BXL}^2 + ab\sigma_{B1}^2$
Lines*	$a-1$	MS_L	$\sigma_w^2 + b\sigma_{BXL}^2 + br\sigma_L^2$
Block $\times$ line	$(a-1)(r-1)$	MS_{BXL}	$\sigma_w^2 + b\sigma_{BXL}^2$
Within line	$ar(b-1)$	MS_W	σ_w^2

Estimators of the variance components;

$$\begin{aligned} \hat{\sigma}_w^2 &= \begin{cases} MS_W & \dots \\ MS'_W \dagger & \dots \end{cases} \quad \begin{matrix} \sigma_{BXL}^2 \\ \text{Significant} \\ \text{Non-significant} \end{matrix} \\ \hat{\sigma}_L^2 &= \begin{cases} (MS_L - MS_{BXL})/br & \dots \\ (MS_L - MS_W)/br & \dots \end{cases} \quad \begin{matrix} \text{Significant} \\ \text{Non-significant} \end{matrix} \end{aligned}$$

Contents of the variance components:

	σ_w^2	σ_L^2
Parent	$E_1 \ddagger$	E_2
M_2 §	$E_1 + \bar{V}_{M_2}$	$E_2 + V_{M_2}$
M_3	$E_1 + \bar{V}_{M_3}$	$E_2 + V_{M_3}$

* Derived from different parental, M_1 and M_2 individuals for the parental, M_2 and M_3 populations respectively.

† Pooled mean square over the items of block $\times$ line and within line.

‡ E_1 and E_2 are the environmental variance components for within and between plots (Mather and Jinks, 1971).

§ V_{M_2} is estimated by the summation of $\hat{\bar{V}}_{M_2}$ and $\hat{V}_{M_3}$.

TABLE 2

A variance analysis for the parental and M₂ populations derived from randomly sampled seeds

Item	d.f.	M.S.	E.M.S.
Blocks	$r-1$	MS_{B_1}	$\sigma_w^2 + b\sigma_p^2 + ab\sigma_{B_1}^2$
Plots within block	$r(a-1)$	MS_p	$\sigma_w^2 + b\sigma_p^2$
Within plot	$ar(b-1)$	MS_w	σ_w^2

Estimators of the variance components;

		σ_v^2
$\hat{\sigma}_w^2 = \begin{cases} MS_w & \dots \\ MS_w^* & \dots \end{cases}$		Significant Non-significant
$\hat{\sigma}^2 = \begin{cases} (MS_p - MS_w)/b & \dots \\ 0 & \dots \end{cases}$		Significant Non-significant

Contents of the variance components;

	σ_w^2	σ_p^2
Parent	E_1	E_2
M ₂	$E_1 + V_{M_2}$	E_2

* Pooled mean square over the items of plots within block and within plot.

The variances of the above estimates are readily derived using the relationships

$$\hat{V}(\hat{M}_n) = \frac{\hat{\sigma}_{w^2}}{abr} + \frac{\hat{\sigma}_{BXL}^2}{ar} + \frac{\hat{\sigma}_{BI}^2}{b} + \frac{\hat{\sigma}_L^2}{a}, \quad (n = 0, 2, 3),$$

and

$$V(\hat{\sigma}_{w^2}) = \frac{2(MS_w)^2}{ar(b-1)+2},$$
$$V(\hat{\sigma}_L^2) = \frac{2}{(br)^2} \left(\frac{(MS_L)^2}{a+1} + \frac{(MS_{BXL})^2}{(a-1)(r-1)+2} \right),$$

which hold for any of the three populations arranged by the design of table 1. Reduction of $\hat{V}(\hat{\sigma}_{w^2})$ of the parental population from those of the M₂ and M₃ population gives the variances of the estimates $\hat{V}_{M_2}$ and $\hat{V}_{M_3}$, the same reduction for $\hat{V}(\hat{\sigma}_L^2)$ giving the variances of $\hat{V}_{M_2}$ and $\hat{V}_{M_3}$. This procedure applies also the the design of table 2. But in this case, the variance of $\hat{M}_n (n = 0, 2)$ must be modified as

$$\hat{V}(\hat{M}_n) = \frac{\hat{\sigma}_{w^2}}{abr} + \frac{\hat{\sigma}_{p^2}}{ar} + \frac{\hat{\sigma}_{BI^2}}{r}$$

and $V(\hat{\sigma}_L^2)$ does not need to be calculated since the variance of the estimate of the total genetic variance V_{M_2} can be obtained directly by the difference of $\hat{V}(\hat{\sigma}_w^2)$'s.

In the case where the M₄ and later populations are also available some least square method (*e.g.* Mather, 1949) or maximum likelihood method (*e.g.* Hayman, 1960) can be used, which will increase the reliability of the estimates and provide a test of the goodness-of-fit of the model. These populations should be made up of hierarchial pedigrees originating from

M_2 plants, because sampling errors in the estimates of the parameters will be minimised by this procedure and the amount of information will be increased. The method of Virk *et al.* (1978) is applicable in this case, their mathematical formulae being modified to fit the above system of pedigree (they assumed a hierarchical system starting from M_1 plants). For future convenience, the variances and covariances of different generations and ranks are formulated in table 3 in terms of the parameters D_m , H_m and F_m . Mean of the M_n population is given by

$$\bar{M}_n = \bar{M}_0 - 2[d]_m + (\frac{1}{2})^{n-2}[h]_m.$$

TABLE 3

Components of genetic variances of the M_n population and covariances between the M_{n-1} and M_n populations

	D_m	H_m	F_m	Pedigree system
Variances;				
M_n population as a whole	$4 - (\frac{1}{2})^{n-2}$	$(\frac{1}{2})^{n-2}$	$-(\frac{1}{2})^{n-3}$	$(n \geq 2)$
Between M_n families of rank 0	3	$(\frac{1}{2})^{2(n-2)}$	$-(\frac{1}{2})^{n-3}$	} Hierarchical* ($n \geq 2$)
Within M_n family of rank 0	$1 - (\frac{1}{2})^{n-2}$	$(\frac{1}{2})^{n-2} - (\frac{1}{2})^{2(n-2)}$		
Between M_n families of rank r	$(\frac{1}{2})^r$	$(\frac{1}{2})^{2n-r-3}$		
Between M_n lines	$4 - (\frac{1}{2})^{n-3}$	$(\frac{1}{2})^{n-1}$	$-(\frac{1}{2})^{n-3}$	} Non-hierarchical† ($n \geq 3$)
Within M_n line	$(\frac{1}{2})^{n-2}$	$(\frac{1}{2})^{n-1}$		
Covariances;				
M_{n-1} and M_n families of rank 0	3	$(\frac{1}{2})^{2n-5}$	$-3(\frac{1}{2})^{n-2}$	} Hierarchical ($n \geq 3$)
M_{n-1} and M_n families of rank r	$(\frac{1}{2})^r$	$(\frac{1}{2})^{2n-2r-3}$		
M_{n-1} individuals and their M_n progenies	$4 - (\frac{1}{2})^{n-3}$	$(\frac{1}{2})^{n-2}$	$-3(\frac{1}{2})^{n-2}$	Non-hierarchical ($n \geq 3$)

* The rank r starts from the M_2 generation, assuming 0 for between M_2 plants or their progeny families (cf. Virk *et al.* (1978) for the rank notation).

† Applicable whether the M_n population is hierarchially structured or not, but should be used for the latter case, *e.g.* for the case where the $M_2 \sim M_{n-1}$ populations have been kept by bulk seed sampling.

4. CROSSING IN THE M_1 AND M_2 GENERATION

Some populations which are made through hybridisation between parent, M_1 and M_2 genotypes were expected to provide additional sources for the parameter estimation. The use of some of these populations will be discussed below.

(i) *Backcrosses and pairwise crosses of M_1 and M_2 plants*

In the case where M_1 plants are backcrossed to the parental line, the mean and variance of the progeny population, say M'_2 populations are represented by

$$\bar{M}'_2 = M_0 - [d]_m + [h]_m,$$

and

$$V_{M'_2} = D_m + H_m - 2F_m,$$

respectively. This variance may be divided into

$$V_{M'_2} = \frac{1}{2C} D_m + \frac{1}{2C} H_m - \frac{1}{C} F_m = \frac{1}{2C} V_{M'_2},$$

$$\bar{V}_{M'_2} = \left(1 - \frac{1}{2C}\right) V_{M'_2}$$

which are of course the between and within variance components of the M'_2 families.

For the population, say M''_2 population, which is made by the pairwise crosses between randomly selected M_1 plants, the corresponding statistics are formulated by

$$\bar{M}''_2 = M_0 - 2[d]_m + 2[h]_m,$$

and

$$V_{M''_2} = 2D_m + 2H_m - 4F_m = 2V_{M'_2},$$

which may be partitioned into the two components,

$$V_{M''_2} = \frac{1}{C} D_m - \frac{1}{C} H_m - \frac{2}{C} F_m = 2V_{M'_2},$$

$$\bar{V}_{M''_2} = 2\bar{V}_{M'_2}.$$

It is known from the above formulae that the M'_2 population adds one source for the estimation of the first-degree parameters, and one reliable source, i.e. $V_{M'_2}$, for the second-degree parameters. The between and within M'_2 family variance components ($V_{M'_2}$ and $\bar{V}_{M'_2}$) cannot be used, since both of them carry the cell number C and are mathematically homogeneous with the total genetic variance $V_{M'_2}$. Pairwise crosses provide no new information; mean and variances of the M''_2 population are homogeneous with those of the M'_2 population. All other methods including diallel crosses among M_1 plants may be difficult to carry out on a sufficiently large scale due to the physiological damage of M_1 plants, and even if practicable, they add no more than the above methods.

Thus, only two statistics for each of the first- and second-degree parameters, namely, M_2 and M'_2 for the former parameters and V_{M_2} and $V_{M'_2}$ for the latter, can be used within two generations following mutagenic treatment. Hence, there is no way of estimating D_m , H_m and F_m within these two generations.

Backcrosses between the parental line and M_2 plants give the population, say M'_3 population, the statistics of which are represented by

$$\bar{M}'_3 = \bar{M}_0 - [d]_m + [h]_m,$$

$$V_{M'_3} = \frac{3}{4}D_m + \frac{5}{4}H_m - \frac{3}{2}F_m,$$

$$\bar{V}_{M'_3} = \frac{1}{4}D_m + \frac{1}{4}H_m - \frac{1}{2}F_m,$$

For the population (say M''_3 population) which is made by pairwise crosses between M_2 plants, the corresponding statistics are

$$\bar{M}''_3 = \bar{M}_0 - 2[d] + [h]_m = \bar{M}_2,$$

$$V_{M''_3} = \frac{3}{2}D_m + \frac{3}{2}H_m - F_m$$

$$\bar{V}_{M''_3} = \frac{1}{2}D_m + \frac{1}{2}H_m - F_m = 2\bar{V}_{M'_3}$$

The M'_3 population provides one source for the estimation of the first-degree parameters and two for the second-degree parameters. The M''_3 population adds no more than the M_2 population for the first-degree parameters, but one new source *i.e.* $V_{M''_3}$ for the second-degree ones. Neither the M'_3 nor M''_3 populations alone can provide enough statistics, but they would greatly increase the reliability of the estimates, when grown together with the M_3 population.

(ii) *Diallel crosses among M_2 plants*

The generalised diallel cross method of Dickinson and Jinks (1956) may be applicable to the M_2 population of some plant species where crossing among individuals can be easily made. Substituting the M_2 genotypic frequencies $1 - \frac{3}{2}m$, m and $\frac{1}{2}m$ for α , β and γ of Dickinson and Jinks, and neglecting the terms with the second power of the mutation rate, we obtain the relationships,

$$\bar{M}_D = \bar{M}_0 - 2[d]_m + 2[h]_m,$$

and

$$V_{P_1} = 3D_m + H_m - 2F_m$$

$$V_{P_2} = 3D_m + \frac{1}{4}H_m - F_m$$

$$\bar{V}_r = V_r = \frac{3}{4}D_m + \frac{3}{4}H_m - \frac{3}{2}F_m,$$

$$\bar{W}_{P_1/r} = \frac{3}{2}D_m + \frac{1}{2}H_m - 2F_m$$

$$\bar{W}_{P_2/r} = \frac{3}{2}D_m + \frac{1}{4}H_m - \frac{7}{4}F_m$$

$$W_{P_1/P_2} = 3D_m + \frac{1}{2}H_m - \frac{3}{2}F_m$$

where M_D stands for the overall mean of progeny families (see Dickinson and Jinks, 1956, for other symbols). Among these six statistics, the ones involving the parent P_1 should not be used for the reason given for the covariance between M_2 plants and their M_3 progenies.

This leaves one and three statistics for the estimation of the first- and second-degree parameters, respectively. From the latter, parameters D_m ,

H_m and F_m can therefore be estimated without any other populations. This may be the most advantageous features of the diallel cross method.

It should be noted, however, that the merit of diallel crosses is less than in the case of F_2 and later populations which have been made by artificial crossing between two different genotypes. Firstly, no parameters other than D_m , H_m and F_m are considered to be meaningful components of genetic variance. This lessens the amount of information from diallel crosses; for instance, the number of loci showing dominance as calculated by Dickinson and Jinks (1956) cannot be estimated. Secondly, the V_r , W_r relationship cannot be used to detect failure of the simple additive-dominance model, because the values of $W_r - V_r$ differ with parental genotypes even if neither linkage disequilibrium nor non-allelic interaction exist. The geometrical interpretation of diallel crosses would be even more complicated in the presence of multiple allelism and variation due to chromosomal aberrations and cytoplasmic mutations.

5. INTERPRETATION OF THE PARAMETERS

The genetic nature and selection efficiency in later generations can be predicted using the estimates of the first- and second-degree parameters defined above. Table 3 gives the coefficients of the constituent parameters of the genetic variances and covariances in various generations. The use of the parameters is not confined to this.

Some rough information on the degree of dominance and gene distribution can be obtained by the use of the estimates. Supposing that each gene is alike in both genetic effect and mutation rate, the parameters may then be written as

$$[d]_m = mkr_1d$$

$$[h]_m = mkr_2 |h|$$

$$D_m = mkd^2,$$

$$H_m = mkh^2,$$

$$F_m = mkr_3d |h|,$$

where the coefficient r_1 corresponds to the degree of gene association of Mather and Jinks (1971), which in this case assumes +1, 0 and -1 according to whether all, half and none of the parental genes have larger contributions to the phenotype than mutant genes. The coefficient r_2 measures the isodirectionality of dominance, taking the values of +1, 0 and -1 if all, half and none of the alleles with increasing effect are dominant to the ones with decreasing effect, respectively. The coefficient r_3 shows the degree of association of dominant genes, the value of which is +1, 0 and -1 when all, half and none of the parental alleles are dominant to mutant ones.

As in case of the populations obtained by crossing between two inbred lines, the ratio

$$\sqrt{\frac{H_m}{D_m}} = \frac{|h|}{d}$$

gives the average dominance.

The coefficients r_1 and r_2 cannot be estimated uniquely, but their signs, *i.e.* the signs of $[d]_m$ and $[h]_m$, together with the ratio

$$\left(\frac{[h]_m}{\sqrt{H_m}}\right) / \left(\frac{[d]_m}{\sqrt{D_m}}\right) = \frac{r_2}{r_1}$$

may in some cases give some idea on the balance sheet of gene association and isodirectionality of dominance. For instance, the situation of $[h]_m > 0$ and $|r_2/r_1| \gg 1$ strongly suggests that $r_1 \approx 0$ and $r_2 \approx 1$, namely, about half of the parental genes have increasing effect compared to mutant genes, dominance being positively directed for most of the genes concerned. The validity of this speculation would be confirmed if the value of r_3 calculated as

$$\frac{F_m}{\sqrt{D_m \cdot H_m}} = r_3,$$

is around zero.

Estimation of m and k has a great importance for both genetical and breeding research. There is no way of obtaining these values by the use of a single parental line. Theoretically, m could be estimated if additional parameters were incorporated and estimated which included the second or higher power of m . To obtain reliable estimates of such parameters, however, unpracticably large-scale experiments with highly homogeneous environmental conditions would be required. The number k cannot be estimated unless the values of r_1 and/or r_2 are obtained from some other source of information.

In the case where two parental lines with significantly different phenotypes, say P_1 and P_2 , are available, the mutation rate can be estimated by

$$\frac{[d]_{m1} - [d]_{m2}}{\bar{P}_1 - \bar{P}_2} = \frac{2 \sum_{k'} \pm md}{2 \sum_{k'} \pm d} = m,$$

where

$\bar{P}_1$ and $\bar{P}_2$ = means of P_1 and P_2 ,

$[d]_{m1}$ and $[d]_{m2} = [d]_m$ of p_1 and P_2 ,

k' = number of genes for which the two parents carry different alleles.

Further, using the populations derived from hybridisation between the two parents, useful information on the ratio k'/k could be obtained by the relationship,

$$\frac{D}{D_m} = \frac{\sum_{k'} d^2}{\sum_k md^2} = \frac{k'd^2}{kmd^2},$$

$$\therefore \frac{k'}{k} = \frac{m \cdot D}{D_m},$$

where the parameter D (variance component due to additive gene effect) is estimated by the method described in Mather and Jinks (1971). The calculation of the absolute value of k is straightforward if two parental lines for which k' is known approximately are used.

6. DISCUSSION

It has been assumed in deriving the formulae in previous sections that there are only two alleles for each gene, mutations occurring from one allele to the other. This may not be true in many cases. In the case of multiple allelism where parental genes mutate multidirectionally, the parameters must be somewhat differently defined.

If the original allele G mutates to r different alleles with mutation rates m_i ($i = 1, 2, \dots, r$), the mutation rate m in the previously given formulae stands for the total mutation rate summed over all of the different mutant

alleles, i.e. $\sum^r m_i$. The genetic effect d now becomes half the difference between parental genotypic value and weighted average of the r different mutant homozygotes, the dominance effect h being measured by the deviation from the mid-point of these two values.

Mathematical formulation of the genetic parameters need not be changed by this generalisation, but interpretation of them must be modified according to the above redefinition. The estimation of the mutation rate m and the ratio k'/k as described in the preceding section is invalid in this case, because the value of d in mutagenically treated populations may be different from that of the populations derived from hybridisation between inbred lines.

Extragenic variations due to chromosomal aberrations and cytoplasmic mutations impose complications on the interpretation of the parameter estimates. The hereditary behaviour of extragenic mutations and their genetic contributions to quantitative characters have not yet been well defined, and therefore, no biometrical formulation can be made to separate extragenic components from other genetic variations. Following Oka *et al.* (1958), one way to counter this would be to remove so-called macro-mutants from the population to be analysed, since most macro- or visible mutants for morphological and physiological characters are expected to arise from chromosomal, cytoplasmic, or major gene mutations.

This method, however, is by no means perfect. The M_n populations seldom show clearly discontinuous distribution, and therefore the distinction of macro-mutants has been arbitrary. In addition, some extragenic mutations may have relatively small genetic effects. The fact that aberrant mutants such as steriles, dwarfs and chlorophyll deficient often segregate in the progeny of seemingly normal plants (*e.g.* Yamagata and Syakudo, 1963) suggests that some chromosomal mutations are partly or completely recessive. About half of such mutations will remain uneliminated each generation, even if they are unambiguously removed in the homozygous state. To minimise the influence of extragenic mutations, late generations should be used, taking the greatest care to minimise the bias in gene frequency due to natural selection and random drift during generation advance. Comparison of the parameter estimates for early and late generations would give some useful information on the nature of non-genic variations.

Epistatic gene interaction is another factor causing a great complication in interpretation, although its contribution to the total variation is in general not very large. Methods for detecting epistatic variation in induced variants may be developed based on the principle adopted by Perkins and Jinks (1970), which will be discussed elsewhere.

Heritabilities have often been calculated to predict the efficiency of selection on induced continuous variation (*e.g.* Rao and Siddig, 1976). The predictive power of these heritabilities, however, has not been satisfactory in general; large differences exist between expected and realised heritabilities, the latter being much smaller than the former in most cases. This may be explained partly by dominance and epistatic gene actions and by unpredictable genotype $\times$ environment interaction. But, the main reason seems to be ascribable to inadequate design of experiment.

Control or non-treated populations have not been used at all, or used only on a much smaller scale than mutagenically treated populations. This inevitably causes overestimation of some genetic variance components, since environmental variances such as between-plot variance cannot be unambiguously separated. Heritability estimates will be more or less inflated if an overestimated variance component is used as a numerator in calculating heritability.

Apart from the various sources of experimental error, heritability should be equal or sufficiently close to a regression coefficient of certain pedigree units (individuals, lines etc.) on their progeny means, if it is to be used as a predictor of the genetic improvement achievable by each cycle of artificial selection. In most cases in mutation researches, heritabilities have been calculated as the ratios of genetic variance components of certain pedigree units to the total phenotypic variance. Heritabilities of this type may be useful for measuring the relative contribution of each genetic variance component to the whole and for identifying the major sources of the genetic variation, but they do not meet the above requirement. Estimates of the parameters D_m , H_m and F_m together with appropriate environmental variance components allow us to calculate any type of heritability for any generation.

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