PREDICTING THE PROPERTIES OF PURE BREEDING LINES EXTRACTABLE FROM A CROSS IN THE PRESENCE OF LINKAGE

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SUMMARY

The quantitative consequences of using estimates of additive genetic components of variation from the early generations of a cross to predict the properties of the pure breeding lines extractable from it when there is a linkage disequilibrium are investigated theoretically and by examples drawn from the cross of varieties 1 and 5 of *Nicotiana rustica*. In the presence of a linkage disequilibrium very good predictions can be obtained by combining estimates of the rank 1 and 2 forms of the additive genetic component of variation which can be estimated from F_3 and S_3 families of a cross. These predictions can be marginally improved by also estimating the rank 3 form but this requires the addition of F_4 families.

1. INTRODUCTION

THE methods currently used to predict the properties of the pure breeding lines extractable from a cross by pedigree breeding, single seed descent and haploidy assume no linkage although they can detect its presence and direction and allow for its effects qualitatively (Jinks and Perkins, 1972; Jinks and Pooni, 1976, 1980; Pooni and Jinks, 1978, 1979). A linkage disequilibrium after summing over all loci, although not linkage *per se*, will lead to an underestimate or overestimate of the expected variation among the extractable pure breeding lines for an excess of repulsion and coupling linkages, respectively. In this paper we specify in more detail than previously given the effects of a linkage disequilibrium on the predicted and observed properties of the pure breeding lines and show how these effects may be allowed for to improve prediction in its presence.

2. THEORY

The total additive genetic variance in the n th generation of inbreeding by self-pollination (${}_D V_{F_n}$) from a cross between a pair of pure breeding lines is given by the summation $\sum_{r=1}^{n-1} (\frac{1}{2})^r D_r$, where r is rank (Mather and Jinks, 1971). Each of the $r = 1$ to $n - 1$ ranks in the n th generation has the general expectation

$$D_r = \sum_{i=1}^k d_{i-R}^{2+C} \sum_{i=1}^{k-1} \sum_{j=i+1}^k 2(1-2p_{ij})^r d_i d_j$$

where the linkage component, $2(1-2p_{ij})^r d_i d_j$ is added for each pair of genes linked in coupling (C) and subtracted for each pair linked in repulsion (R). For example, in the F_4 the total additive genetic variance (${}_D V_{F_4}$) is

$$\frac{1}{2}D_1 + \frac{1}{4}D_2 + \frac{1}{8}D_3$$

where

$$D_1 = \sum_{i=1}^k d_{i-R}^{2+C} \sum_{i=1}^{k-1} \sum_{j=i+1}^k 2(1-2p_{ij})d_i d_j$$

$$D_2 = \sum_{i=1}^k d_{i-R}^{2+C} \sum_{i=1}^{k-1} \sum_{j=i+1}^k 2(1-2p_{ij})^2 d_i d_j$$

and

$$D_3 = \sum_{i=1}^k d_{i-R}^{2+C} \sum_{i=1}^{k-1} \sum_{j=i+1}^k 2(1-2p_{ij})^3 d_i d_j.$$

When n is large (symbolised as $n = \infty$) that is, the inbred families extracted from the cross are pure breeding, the total additive genetic variance among them (${}_D V_{F\infty}$) is found as the sum from $r = 1$ to ∞ . The sum of this series

$${}_D V_{F\infty} = \frac{1}{2}D_1 + \frac{1}{4}D_2 + \frac{1}{8}D_3 + \frac{1}{16}D_4 + \frac{1}{32}D_5 + \cdots + \left(\frac{1}{2}\right)^\infty D_\infty$$

reduces to

$$\sum d_{i-R}^{2+C} \sum_{i=1}^{k-1} \sum_{j=i+1}^k \frac{2(1-2p_{ij})d_i d_j}{1+2p_{ij}}.$$

Because the coefficients of D_r follow the geometric series $(\frac{1}{2})^r$, successive terms make a rapidly decreasing contribution to the total. Furthermore, because the coefficients of the linkage components of D_r also follow the geometric series $\pm \sum_{i=1}^{k-1} \sum_{j=i+1}^k 2(1-2p_{ij})^r d_i d_j$, the successive D_r 's in the series show rapidly falling differences. Hence $|D_1 - D_2| > |D_2 - D_3| > |D_3 - D_4| > |D_4 - D_5| > \cdots$. Not surprisingly, therefore, $\frac{1}{2}D_1 + \frac{1}{4}D_2 + \frac{1}{4}D_3$ which assumes that $D_3 = D_4 = D_5 \dots$, from which it follows that $\frac{1}{8}D_3 = \frac{1}{16}D_4 + \frac{1}{32}D_5 + \cdots$ is a very close approximation to ${}_D V_{F\infty}$ for all values of p , the recombination frequency. Indeed $\frac{1}{2}D_1 + \frac{1}{2}D_2$, which assumes that $D_2 = D_3 = D_4 = D_5 = \cdots$, from which it follows that $\frac{1}{4}D_2 = \frac{1}{8}D_3 + \frac{1}{16}D_4 + \frac{1}{32}D_5 + \cdots$ is an acceptable approximation in practice to ${}_D V_{F\infty}$.

The closeness of these approximations to the true value of ${}_D V_{F\infty}$ is illustrated in table 1. The upper half of the table contains the values of D_r for $r = 1$ to 5 for a pair of genes linked in coupling and in repulsion each with an additive effect $d = 1$ and recombination frequencies of $p = 0.1$, 0.25 and 0.4 . The lower half of the table contains the true values of ${}_D V_{F\infty}$ corresponding with these parameter values and approximate predictions of ${}_D V_{F\infty}$ based upon the first few terms in the summation series, ${}_D V_{F\infty} = \sum_{r=1}^{\infty} (\frac{1}{2})^r D_r$.

Reference to table 1 shows that irrespective of the linkage phase or of the recombination frequency, very close approximations to the total genetic variance among the means of the pure breeding families of the F_∞ generation (${}_D V_{F\infty}$) can be obtained without going beyond D_3 or even D_2 in the summation series. It follows, therefore, that any breeding programme that will yield estimates of D_1 , D_2 and possibly D_3 will allow acceptable predictions of the distribution of the means of the pure breeding families extractable from a cross.

TABLE 1

The values of D_r for $r=1$ to 5 for $p=0.1, 0.25$ and 0.4 , the true values of ${}_D V_{F\infty}$ and approximate predictions based upon the first few terms in the summation series ${}_D V_{F\infty} = \sum_{r=1}^{\infty} (\frac{1}{2})^r D_r$

D_r	$p =$	Coupling			Repulsion		
		0.1	0.25	0.4	0.1	0.25	0.4
D_1		3.6	3.0	2.4	0.4	1.0	1.6
D_2		3.28	2.5	2.08	0.72	1.5	1.92
D_3		3.024	2.25	2.016	0.976	1.75	1.984
D_4		2.819	2.125	2.0032	1.181	1.875	1.9968
D_5		2.656	2.0625	2.00064	1.344	1.9375	1.99936
Estimators of ${}_D V_{F\infty}$							
D_1		3.6	3.0	2.4	0.4	1.0	1.6
$\frac{1}{2}D_1 + \frac{1}{2}D_2$		3.44	2.75	2.24	0.56	1.25	1.76
$\frac{1}{2}D_1 + \frac{1}{4}D_2 + \frac{1}{4}D_3$		3.376	2.688	2.224	0.624	1.312	1.776
$\frac{1}{2}D_1 + \frac{1}{4}D_2 + \frac{1}{8}D_3 + \frac{1}{8}D_4$		3.350	2.674	2.2224	0.65	1.326	1.7776
$\frac{1}{2}D_1 + \frac{1}{4}D_2 + \frac{1}{8}D_3 + \frac{1}{16}D_4 + \frac{1}{16}D_5$		3.340	2.668	2.22224	0.66	1.332	1.77776
${}_D V_{F\infty}$		3.333	2.666	2.222	0.666	1.333	1.777

3. EXPERIMENTAL DESIGNS

Estimation of the additive genetic components of variance of different rank, that is D_1 , D_2 and D_3 is described by Mather and Jinks (1971, 1982). The usual approach is a pedigree inbreeding design using self-pollination (Mather, 1949, Virk *et al.*, 1981) or sib-mating (Cooke and Mather, 1962). Unless the non-additive genetic sources of variation are non-significant the inbreeding series must be taken as far as the fourth generation (F_4) to obtain estimates of D_1 and D_2 and to the fifth generation to estimate D_3 also. Because of the relatively small contribution that non-additive sources such as dominance make to the variation following inbreeding they can be non-significant even when the dominance ratio is high. Hence it will often be correct to assume that the non-additive sources of variation make no significant contributions so that D_1 and D_2 can be estimated by the F_3 or S_3 and D_3 also by the F_4 and S_4 .

Other designs will estimate D_1 , the best estimates being provided by an F_2 triple test cross (Kearsey and Jinks, 1968) but D_2 cannot be obtained earlier than the F_3 or S_3 . By combining the two inbreeding series, selfing and sib mating, D_1 and D_2 can be estimated even in the presence of a significant dominance component of variation from the F_3 and S_3 . Thus from a random sample of F_2 individuals we can simultaneously produce F_3 progeny families by selfing and S_3 progeny families by sib mating pairs at random. From an appropriately randomised and replicated experiment the F_3 and S_3 progeny families will yield estimates of between and within family components of variance with the expectations (Mather and Jinks, 1982):

$$\text{Between family means } V_{1F3} = \frac{1}{2}D_1 + \frac{1}{16}H_1, \quad V_{1S3} = \frac{1}{4}D_1 + \frac{1}{16}H_1$$

$$\text{Within families } V_{2F3} = \frac{1}{4}D_2 + \frac{1}{8}H_2 + E_w, \quad V_{2S3} = \frac{1}{4}D_2 + \frac{3}{16}H_2 + E_w.$$

Estimates of E_w , the within family environmental component of variation, can be obtained in the usual way by including the parental, P_1 and P_2 and their F_1 families in the experiment. A perfect fit solution is then

possible in which

$$\begin{aligned}D_1 &= 4(V_{1F3} - V_{1S3}) \\D_2 &= 12V_{2F3} - 8V_{2S3} - 4E_w \\H_1 &= 32V_{1S3} - 16V_{1F3} \\H_2 &= 16(V_{2S3} - V_{2F3}).\end{aligned}$$

We can now predict ${}_DV_{F\infty}$ as

$$\frac{1}{2}D_1 + \frac{1}{2}D_2 = 2V_{1F3} + 6V_{2F3} - 2V_{1S3} - 4V_{2S3} - 2E_w.$$

4. EXAMPLES

An example of the estimation of D_1 , D_2 and D_3 from the F_3 and F_4 families of a pedigree selfing series is provided by Virk *et al.* (1981) and Ingram and Jinks (1982). The cross was between varieties 1 and 5 of *Nicotiana rustica* and two characters, flowering time and plant height at flowering time showed a significant linkage disequilibrium attributable to an excess of the repulsion phase. Because there was no significant non-additive variation (section 3) D_1 , D_2 and D_3 could be estimated by iterative weighted least squares procedures. For both characters D_1 was significantly smaller than D_2 and D_3 but D_2 and D_3 did not differ significantly. We can, therefore, predict ${}_DV_{F\infty}$ as $\frac{1}{2}D_1 + \frac{1}{2}D_2 = \frac{1}{2}D_1 + \frac{1}{4}D_2 + \frac{1}{4}D_3$ (table 1).

The estimates of D_1 , $D_2 = D_3$, the predictions of ${}_DV_{F\infty}$ based upon them and two direct estimates of it obtained from a random sample of 82 F_{15} families produced by single seed descent and a random sample of 188 F_6 families produced by pedigree inbreeding by self-pollination are presented in table 2. The predictions, which allow for the linkage disequilibrium, are closer to the observed additive genetic variances in the F_6 and F_{15} than F_2 triple test cross estimates of 29.9 for FT and 193.6 for HFT obtained from the same experiment, which do not. The latter estimates because of the repulsion linkages are, as expected, smaller.

TABLE 2

Estimates of the rank 1, 2 and 3 additive genetic component of variation (D) and the predicted and observed additive genetic component of variation (${}_DV_{F\infty}$) among highly inbred families produced by single seed descent (F_{15}) and pedigree inbreeding (F_6)

Character	Estimates		Predicted ${}_DV_{F\infty}$	Observed ${}_DV_{F\infty}$	
	D_1	$D_2 = D_3$	$\frac{1}{2}D_1 + \frac{1}{4}D_2 + \frac{1}{4}D_3$	F_6	F_{15}
Flowering time	21.0	67.9	44.45	39.4	38.4
Height at flowering time	92.5	384.9	238.7	271.0	275.2

5. DISCUSSION

In the presence of a linkage disequilibrium the genetical variation in the population of pure breeding lines extractable from a cross by self-pollination is specifiable as the sum of a geometric series in which the terms are in ascending order the rank 1 to ∞ forms of the additive genetic component of variation D . A very good approximation, however, can be

obtained from the first three rank forms D_1 , D_2 and D_3 because D_1 and D_2 make by far the largest contributions to the sum of the series and D_3 can be substituted for D_4 to D_∞ because they are unlikely to differ significantly in magnitude (table 1). If as is often the case in a selfing series, the non-additive sources of variation such as dominance are making a non-significant contribution, D_1 , D_2 and D_3 can be estimated from the F_3 and F_4 families. If, however, there is a significant dominance contribution F_3 and F_4 families or F_3 and S_3 families are required to estimate D_1 and D_2 .

Most of these theoretical points are confirmed by flowering time and plant height at flowering time in the cross of varieties 1 and 5 of *Nicotiana rustica* (table 2). Although these two characters display dominance (Pooni *et al.*, 1978), in the F_3 and F_4 generations its contribution to the variation is too small because of the low level of heterozygosity to be significant, hence D_1 , D_2 and D_3 can be estimated. But whereas, because of the excess of repulsion linkages, D_1 is significantly smaller than D_2 and D_3 , D_2 and D_3 do not differ significantly. The estimate of $D_2 = D_3$ can, therefore, be substituted for D_4 to D_∞ in predicting the expected additive genetic variance among the pure breeding lines extracted from the cross. This prediction is more accurate than the best estimate obtained from an F_2 triple test cross which makes no allowance for linkage. Nevertheless, it should be noted that while there is this improvement in accuracy in the *N. rustica* examples the F_2 triple test cross prediction is close enough for all practical purposes.

Where pure breeding lines are extracted by dihaploidy as opposed to single seed descent or pedigree inbreeding the extraction usually takes place from the earliest generations of a cross. There can be no contribution of recombination in generations subsequent to the one chosen for extraction to the genetical variation among the pure breeding lines. Hence D_1 , D_2 and occasionally D_3 are the only contributors to this variation (Jinks and Pooni, 1981) and the only rank forms of D that need to be estimated in order to predict its magnitude.

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