A MODIFIED TRIPLE TEST-CROSS ANALYSIS TO TEST AND ALLOW FOR INADEQUATE TESTERS

J. L. JINKS and D. S. VIRK

Department of Genetics, University of Birmingham

Received 24.i.77

SUMMARY

When the population under investigation consists of highly inbred lines the full triple test-cross of Kearsey and Jinks (1968) supplemented by the selfed progenies of the population allows unambiguous and independent tests for epistasis and the adequacy of the pure-breeding testers, L_1 and L_2 . This can also be achieved by supplementing the simplified triple test-cross of Jinks, Perkins and Breese (1969) with the selfed progenies of the L_{1i} and L_{2i} families.

If the L_1 and L_2 testers prove to be inadequate due to the presence of common loci, modifications of the analyses are proposed which correct the resulting biases in the genetical components of variation.

1. INTRODUCTION

For populations of pure-breeding lines the triple test-cross design of Kearsey and Jinks (1968) can be simplified by omitting the F_1 tester (L_3) and hence the L_{3i} families and substituting the pure-breeding lines (P_i) themselves in the test for epistasis (Jinks, Perkins and Breese, 1969). This is a perfectly satisfactory alternative if the pure-breeding testers (L_1 and L_2) differ at all k loci at which the population of inbred differs. If, however, L_1 and L_2 differ only at $k_2 = k - k_1$ loci where $k_2 < k$ and are homozygous at k_1 loci for the same alleles, the test for epistasis is no longer unambiguous and the estimates of the additive and dominance components of variation are biased (Virk and Jinks, 1977). In order to test and allow for such biases a modification of the simplified triple test-cross analysis is suggested in this paper.

2. EXPERIMENTAL DESIGNS

The following two experimental designs provide independent tests for epistasis and for the adequacy of the L_1 and L_2 testers and also allow the biases due to any inadequacy of L_1 and L_2 to be removed and their magnitudes estimated.

- (a) The full triple test-cross design in which two extreme pure-breeding lines and the F_1 (L_3) are used as testers to produce $3n$ progeny families (L_{1i} , L_{2i} and L_{3i}) along with the n parental pure-breeding lines grown in a replicated block design.
- (b) Alternatively, for naturally self-fertilising crops it may be more convenient to raise progenies produced by selfing the L_{1i} and L_{2i} crosses rather than producing the L_{3i} crosses so that the L_{1i} and L_{2i} crosses and their selfs along with the n parental lines and the two testers L_1 and L_2 are raised in a replicated block design.

3. ANALYSIS

It is convenient to divide the analysis into two parts, the one concerned with the tests for epistasis and the adequacy of the testers and the other with the estimation of unbiased genetical components when the testers are inadequate.

(i) *Tests for epistasis and adequacy of testers*

The simple additive-dominance model can be unsatisfactory either because of the presence of non-allelic interactions or because the testers are inadequate. For each of the (a) and (b) designs we now have two separate tests for the simple model which when applied simultaneously can differentiate between these causes. For design (a) the two tests are the same as those described by Kearsey and Jinks (1968) and Jinks *et al.* (1969), based on the significance of the variances of (i) $L_{1i} + L_{2i} - 2L_{3i}$ and (ii) $L_{1i} + L_{2i} - \bar{P}_i$ over the $i = 1$ to n inbred lines. If design (b) is used, test (i) is equivalent to the C scaling test of Mather, namely $4L_{1is} - 2L_{1i} - \bar{P}_i - L_{1s}$ or $4L_{2is} - 2L_{2i} - \bar{P}_i - L_{2s}$ for the L_1 and L_2 testers, respectively, where s denotes the family produced by selfing the $i = 1$ to n L_{1i} and L_{2i} families. This test can be applied to each set of progeny families separately and the deviations tested against the pooled error variances of the family means (see Mather and Jinks, 1971). Alternatively an analysis of variance may be performed for the following statistical comparisons:

Test for	L_{1is}	L_{2is}	L_{1i}	L_{2i}	$\bar{P}_i$	L_{1s}	L_{2s}
L_{1i} progenies	4	0	-2	0	-1	-1	0
L_{2i} progenies	0	4	0	-2	-1	0	-1

The block $\times$ comparison item or the pooled within-family variances may provide the estimate of the corresponding error variance depending upon the field design. For each pair of loci displaying non-allelic interactions, the C scaling test will equal $-[I] - 2[i]$ in a quarter of the progeny families of each tester.

Test (ii) can be applied independently on L_{1i} and L_{1is} , and L_{2i} and L_{2is} progeny families. They can, however, be combined by employing an extension of Cavalli's scaling test (Mather and Jinks, 1971).

On the basis of tests (i) and (ii) four situations can arise in practice each with its own interpretation. These are:

(a) *Neither (i) nor (ii) are significant*

This means that there is no detectable epistasis and the testers are adequate. Alternatively the testers may be inadequate but there is no dominance and hence the estimates of the parameters are unbiased. Therefore the standard triple test-cross analysis of Jinks *et al.* (1969) can be used to test the significance and to obtain the estimates of the various parameters.

(b) *(i) is non-significant and (ii) is significant*

There is no epistasis but the testers are inadequate because they share common alleles. The biases arising from the latter must then be removed by the procedure described later.

(c) *(i) is significant and (ii) is non-significant*

This is trivial in that it could only arise because the two tests differ in their sensitivities to epistasis. There is also the possibility, though highly

unlikely, of the two causes of failure of the simple model, namely epistasis and the inadequacy of the testers, having effects which cancel out in test (ii).

(d) *Both (i) and (ii) are significant*

Epistasis is present and we cannot therefore be sure whether the testers are or are not adequate. Any estimates of the genetical parameters will be biased by non-allelic interactions and possibly by inadequate testers also. We could remove any bias from the latter by proceeding with the estimation of the parameters as for (b).

(ii) *Estimation of unbiased components*

For situation (b) where failure of the simple model is attributable solely to inadequate testers the following procedure is proposed for obtaining unbiased estimates of the genetical components.

(i) *Design (a)*

For each set of $\bar{L}_{1i}$, $\bar{L}_{2i}$, $\bar{L}_{3i}$ and $\bar{P}_i$ families we can make the following orthogonal comparisons:

Comparison	$\bar{L}_{1i}$	$\bar{L}_{2i}$	$\bar{L}_{3i}$	$\bar{P}_i$	Component
1	1	1	1	0	σ_s^2
2	1	-1	0	0	σ_d^2
3	0	0	0	1	σ_p^2

The first comparison replaces the variance of $\bar{L}_{1i} + \bar{L}_{2i}$ as suggested by Jinks and Perkins (1970) for estimating σ_s^2 while comparison 2 is that originally suggested by Kearsey and Jinks (1968) for computing σ_d^2 . The third comparison which estimates σ_p^2 will always give unbiased estimates of additive genetic component (D) since it is independent of the testers used. The genetical expectations of the components of variance obtainable from these comparisons for any number of loci with arbitrary allele frequencies but with alleles independently distributed among the inbred lines will be:

$$\begin{aligned}\sigma_s^2 &= \sum_{i=1}^{k_1} uv d_i^2 + \sum_{i=1}^{k_1} uv h_i^2 - 2r_1 \sum_{i=1}^{k_1} uv d_i h_i \\ &= \frac{1}{4}D_k + \frac{1}{4}H_{k_1} - \frac{1}{2}F_{k_1} \\ \sigma_d^2 &= \sum_{i=1}^{k_2} uv h_i^2 = \frac{1}{4}H_{k_2} \\ \sigma_p^2 &= \sum_{i=1}^k uv d_i^2 = D_k\end{aligned}$$

Two further statistics which are not independent of these three are essential for the estimation of the unbiased parameters. These are:

$$\sigma^2(\bar{L}_{1i} + \bar{L}_{2i} - \bar{P}_i) = \frac{1}{9} \left[\sum_{i=1}^{k_1} 4uv h_i^2 \right] = \frac{1}{9}H_{k_1}$$

$$\text{Cov}(\bar{L}_{1i} + \bar{L}_{2i})(\bar{L}_{1i} - \bar{L}_{2i}) = -r_2 \sum_{i=1}^{k_2} uv d_i h_i = -\frac{1}{4}F_{k_2}$$

where r_1 and r_2 are the degrees of association of alleles of like effect (see Virk

and Jinks, 1977 for details). The only other statistic $\sigma^2 (\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$, which is orthogonal to the first three is, of course, an estimate of error variation only, in the situation of no epistasis under discussion.

The unbiased estimate of the dominance component of variation (H) and covariance between additive and dominance effects can be obtained by adding the respective effects of the k_1 and k_2 loci as follows:

$$\text{Dominance component, } H_k = H_{k_1} + H_{k_2} = 4\sigma_d^2 + 9\sigma^2 (\bar{L}_{1i} + \bar{L}_{2i} - \bar{P}_i)$$

$$\text{Covariance, } F_k = F_{k_1} + F_{k_2} = \sigma_p^2 + 9\sigma^2 (\bar{L}_{1i} + \bar{L}_{2i} - \bar{P}_i) - 4 \text{ Cov sums/diffs} - 4\sigma_s^2$$

The standard errors of these parameters can be computed from the linear relationships of the statistics involved but since there are tests of significance for all the basic σ^2 's and the covariance on which they are based there will in general be little necessity for computing them.

Alternatively, weighted least square estimates of the components of variation can be obtained by using the reciprocals of the expected variances of these statistics as weights (see Mather and Jinks, 1971). If this procedure is used, additional statistics can be obtained from the pooled within family variance (σ_w^2) of L_{3i} progenies and the variance between L_{3i} family means. These have the expectations:

$$\sigma_{wL_{3i}}^2 = \sum_{i=1}^{k_2} uv(d_i^2 + h_i^2) = \frac{1}{4}D_{k_2} + \frac{1}{4}H_{k_2}$$

and

$$\begin{aligned} \sigma_{L_{3i}}^2 &= \sum_{i=1}^k uv d_i^2 + \sum_{i=1}^k uv h_i^2 - 2r_1 \sum_{i=1}^{k_1} uv d_i h_i \\ &= \frac{1}{4}D_k + \frac{1}{4}H_k - \frac{1}{2}F_{k_1} \end{aligned}$$

(ii) Design (b)

The expectations of σ_s^2 , σ_d^2 and the covariance of sums and differences computed from the selfed L_{1i} and L_{2i} progenies remain unchanged for the additive (D) gene effects while the coefficients of the dominance (H) and F components are reduced by $\frac{1}{4}$ and $\frac{1}{2}$, respectively for each generation of selfing. After making allowance for these changes we can obtain unbiased estimates of the genetical components by utilising the comparisons outlined for design (a) which do not use L_{3i} families. The analysis of sums, differences and the covariance between them, in this situation, remains unaltered from that described by Jinks *et al.* (1969). We can therefore have two independent estimates of the parameters from the L_{1i} and L_{2i} and L_{1is} and L_{2is} sets of families.

For the weighted least square estimation the pooled within-family (σ_{w0}^2) variances of the L_{1is} and L_{2is} families yields an additional statistic which equals $\frac{1}{4}D_k + \frac{1}{8}H_k$. This statistic also provides for the direct estimation of the unbiased dominance component (H_k) since D_k is obtainable from σ_p^2 .

Both designs also provide for the estimation of the dominance (H_k) component of variation by computing the deviations of each L_{1i} or L_{2i}

family mean from its parental mean and performing an analysis of variance for the following statistical comparisons:

Comparison	L_{1i}	L_{2i}	$\bar{P}_i$	L_{1s}	L_{2s}	expected value of σ^2
1. For L_{1i} progenies	2	0	+1	-1	0	$\frac{1}{36}H_k$
2. For L_{2i} progenies	0	2	-1	0	-1	$\frac{1}{36}H_k$

The corresponding error variance for these comparisons can be obtained from the block interactions or from the pooled within-family variances depending upon the experimental design. A joint comparison of 1 and 2 will only estimate the dominance effects of the k_1 common loci between the L_1 and L_2 testers and it is therefore not recommended. Because of its small coefficient this estimate of H_k is relatively unreliable.

4. DISCUSSION

If we combine the $3n$ progeny families of a full triple test-cross investigation of a population of n pure-breeding lines with the P_i selfed families of the n pure-breeding lines themselves, two tests for the presence of epistasis can be carried out. One of these, the test of Kearsey and Jinks (1968), is unambiguous and applicable to any population. It is not invalidated if the common loci $k_1 > 0$ and $k_2 < k$ and it will always detect epistasis for the non-common k_2 loci. The other test (Jinks *et al.*, 1969) applies solely to pure-breeding populations and it is an unambiguous test for epistasis only if $k_1 = 0$ and $k_2 = k$. Under all other circumstances this test may be significant even when no epistasis is present because it is sensitive to the presence of common loci. Discrepancies between these two types of tests can, therefore, be used to detect the presence of common loci between the L_1 and L_2 testers.

Any test which unambiguously detects epistasis in all circumstances can be substituted for the test of Kearsey and Jinks (1968). For self-fertilising species in which it is easier to produce progenies by selfing L_{1i} and L_{2i} families than to produce L_{3i} families it can be replaced by the C scaling test of Mather (see Mather and Jinks, 1971).

Having demonstrated the inadequacy of the L_1 and L_2 testers due to the presence of common alleles at some loci the biases to which this normally leads (Virk and Jinks, 1977) can be avoided by following alternative procedures for estimating the genetical parameters. These procedures provide estimates of the dominance (H) and F components separately for the k_1 and k_2 loci at which L_1 and L_2 have common and non-common alleles respectively. Unfortunately, the price we pay for these estimates is the loss of one of the major advantages of the triple test-cross analysis, namely, uncorrelated estimates which are known with equal precision. Nevertheless, because most of the statistics used in these alternative procedures are still the same as those of the standard triple test-cross analysis the estimates vary less in precision and are less correlated than those that would be obtained from most, if not all, alternative designs.

Because it is a relatively simple analysis and requires fewer crosses than the original triple test-cross the simplified version of the triple test-cross design is becoming increasingly popular and in many instances detailed data are being collected and analysed for several quantitative traits simultaneously. The basic requirement of phenotypically extreme testers is

being violated for some of the traits since the frequency of adequate testers is only 2^{k-1} out of $[2^k(2^k+1)]/2$ possible combinations of randomly paired pure-breeding testers. Of these 2^{k-1} pairs only one will be completely associated and therefore, recognisable as phenotypic extremes, while all others will vary in their degree of gene dispersion. It follows, therefore, that there is quite a high probability of the testers being inadequate for any traits recorded in the experiment other than that for which the testers were selected as the phenotypic extremes. How high this probability is will depend largely upon the extent of the correlation between the additional traits and that used for choosing the extreme testers. The proposed modified analysis permits the use of data from the additional traits without leading to biased estimates and misleading conclusions and therefore adds to the general range of situations in which the triple test-cross may be used. This modification, however, should not be regarded as a satisfactory alternative to the selection of adequate testers before the commencement of the experiment.

Acknowledgments.—These are due to the Royal Commission for the Exhibition of 1851 for financial support to D. S. V. during these investigations.

5. REFERENCES

- JINKS, J. L., AND PERKINS, J. M. 1970. A general method for the detection of additive, dominance and epistatic components of variation. III. F_2 and backcross populations. *Heredity*, 25, 419-429.
- JINKS, J. L., PERKINS, J. M., AND BREESE, E. L. 1969. A general method of detecting additive, dominance and epistatic variation for metrical traits. II. Application to inbred lines. *Heredity*, 24, 45-57.
- KEARSEY, M. J., AND JINKS, J. L. 1968. A general method of detecting additive, dominance and epistatic variation for metrical traits. I. Theory. *Heredity*, 22, 97-103.
- MATHER, K., AND JINKS, J. L. 1971. *Biometrical Genetics*, 2nd edition. Chapman and Hall, London.
- VIRK, D. S., AND JINKS, J. L. 1977. The consequences of using inadequate testers in the simplified triple test-cross. *Heredity*, 38, 237-251.