A GENERAL METHOD FOR THE DETECTION AND ESTIMATION OF ADDITIVE, DOMINANCE AND EPISTATIC VARIATION FOR METRICAL TRAITS

IV. TRIPLE TEST CROSS ANALYSIS FOR NORMAL FAMILIES AND THEIR SELFS

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SUMMARY

For plant species in which a single cross pollination yields one or few seed and self pollination is easier, the families of the rather demanding triple test cross design can be replaced by their progeny families produced by selfing. The theoretical consequences of making this substitution on the sensitivity of the tests for epistasis and on the reliability of the estimates of the additive and dominance components of variation in the presence and absence of epistasis are described for F_2 and F_∞ populations. The practical consequences are illustrated by comparative analyses of normal families of a triple test cross and their selfs for the F_∞ population using a population of 80 F_{13} families produced from a cross between varieties 1 and 5 of *Nicotiana rustica*.

Both in theory and in practice the additive genetic component D is detected and estimated with similar reliabilities from the normal families of a triple test cross and their selfs. On the other hand, the dominance component H is detected and estimated with much lower reliability from the selfs and in the presence of epistasis the estimate is more biased. The sensitivity of the standard test for epistasis is also lower for selfs but for an F_∞ population this is partially offset by the additional test for epistasis involving the means of the F_∞ families ($\bar{P}_t$) themselves.

In the comparative analyses family size and replications for the normal families and their selfs have been kept the same to make direct comparisons possible. In normal circumstances, however, one would not devote the time and effort required to produce the selfs unless as a result larger families and more replicates could be raised. Depending on the magnitude of the resulting increase in the number of plants raised the loss of reliability in the estimate of H and of sensitivity in the test for epistasis would be partly or wholly restored.

1. INTRODUCTION

THE triple test cross breeding programme (TTC) which is an extension of the NCM III design of Comstock and Robinson (1952) proposed by Kearsey and Jinks (1968), is the best design currently available for detecting the presence of non-allelic interaction and estimating the additive and dominance effects with equal efficiency when epistasis is absent. It can be applied to any population regardless of its gene and genotypic frequencies or its degree of inbreeding (Jinks, Perkins and Breese, 1969; Perkins and Jinks, 1970; Jinks and Perkins, 1970). The crossing programme however is relatively demanding and it could be particularly laborious when the material under investigation produces only one or few seeds per cross pollination.

This could constrain the experimental size and therefore decrease the reliability of the results. In such circumstances Kearsey and Jinks (1968) suggested using progeny families produced by self-pollinating the individual crosses instead of the crosses themselves.

In this paper we compare the analysis of the normal families of a triple test cross with one in which these families are replaced by their selfed progenies. The theoretical expectations of corresponding items in the two analyses are presented for an F_2 and an F_∞ population. Applications of the latter are illustrated by an experimental investigation of 80 families of the F_{13} generation of a cross between varieties 1 and 5 of *Nicotiana rustica* raised in 1977.

2. THEORY

Following Kearsey and Jinks (1968) individuals from the L_{1i} (crosses with P_1), L_{2i} (crosses with P_2) and L_{3i} (crosses with their F_1) families of a triple test cross can be self-pollinated to produce progeny families that may replace them in the analysis. If however L_{1i} , L_{2i} and L_{3i} are segregating, as they will be for example in a triple test cross of an F_2 , a random sample of individuals in the L_{1i} , L_{2i} and L_{3i} families will have to be self-pollinated to satisfactorily replace them. In fact only the L_{1i} and L_{2i} sets of a triple test cross on a sample of pure breeding families such as F_∞ families, will not be segregating and can therefore be replaced by a progeny family produced by self-pollinating a single plant of the L_{1i} and L_{2i} families. L_{3i} families will always be segregating. Where, however, the population under test is a collection of pure breeding lines, L_{3i} families may be replaced by the selfs of these lines themselves (P_i) in the analysis (Jinks, Perkins and Breese, 1969).

We will refer to the families produced by self-pollinating L_{1i} , L_{2i} , L_{3i} and P_i families as L_{1si} , L_{2si} , L_{3si} and P_i respectively and denote their corresponding means by $\bar{L}_{1si}$, $\bar{L}_{2si}$, $\bar{L}_{3si}$ and $\bar{P}_i$.

If each of the L_{1i} , L_{2i} and L_{3i} families is replaced by the corresponding L_{1si} , L_{2si} and L_{3si} families the analysis of the data will follow the same pattern as that of a normal triple test cross and only the genetical expectations of the components of variance will differ. One can also however raise simultaneously any combination of the original and the selfed test cross families providing that sufficient seed is available.

(i) Test of epistasis

Where L_{1i} , L_{2i} and L_{3i} are replaced by L_{1si} , L_{2si} and L_{3si} the standard test for epistasis still applies. The variance component of $(\bar{L}_{1si} + \bar{L}_{2si} - 2\bar{L}_{3si})$ comparison provides an overall test of epistasis for n degrees of freedom. This can still be partitioned into one degree of freedom for the correction factor, to provide a test of additive $\times$ additive interaction, the $[i]$ component, and the corrected mean squares for $(n-1)$ degrees of freedom which detect the presence of additive $\times$ dominance (Σj_{jk}^2) and dominance $\times$ dominance (Σl_{jk}^2) epistasis together with some of their cross products. Where the original and selfed families are raised simultaneously epistasis may additionally be detected by the $(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3si})$ and $(\bar{L}_{1si} + \bar{L}_{2si} - 2\bar{L}_{3i})$ comparisons as mean squares for $(n-1)$ degrees of freedom (table 1).

All of these tests are equally valid for any kind of population but their

TABLE 1

Genetical expectations of all the components of epistasis that can be tested for by an analysis of variance when the triple test cross families (L_{1i} , L_{2i} and L_{3i}) and their selfs (L_{1si} , L_{2si} and L_{3si}) are raised together. The expected variance components and degrees of freedom for an analysis based upon family means are given for the test crosses on samples of $n F_2$ individuals and $n F_\infty$ families including the additional tests that are only possible if the means of the latter are available

Population	Comparison	d.f.	Expectation
(a) <i>With progeny families</i>			
F_2	$\frac{1}{6}\sigma^2(\bar{L}_{1si} + \bar{L}_{2si} - 2\bar{L}_{3si})$	1	$\frac{n}{24} [\Sigma i_{jk}]^2$
		$(n-1)$	$\frac{1}{192} \Sigma j_{jk}^2 + \frac{1}{1536} \Sigma l_{jk}^2 + \frac{1}{96} [\Sigma j_{jk} \Sigma j_{sk}]$
F_∞	$\frac{1}{6}\sigma^2(\bar{L}_{1si} + \bar{L}_{2si} - 2\bar{L}_{3si})$	1	$\frac{n}{24} [\Sigma i_{jk}]^2$
		$(n-1)$	$\frac{1}{96} \Sigma j_{jk}^2 + \frac{1}{384} \Sigma l_{jk}^2 + \frac{1}{48} [\Sigma j_{jk} \Sigma j_{sk}]$
	$\frac{1}{3}\sigma^2(\bar{L}_{1si} + \bar{L}_{2si} - \bar{P}_i)$	$(n-1)$	$\frac{1}{12} \Sigma i_{jk}^2 + \frac{1}{24} \Sigma j_{jk}^2 + \frac{1}{192} \Sigma l_{jk}^2 + \frac{1}{24} \Sigma j_{jk} \Sigma j_{js}$ $- \frac{1}{24} [\Sigma i_{jk} (\Sigma l_{js} + \Sigma l_{ks})] - \frac{1}{12} [\Sigma j_{jk} j_{kj}]$ $+ \frac{1}{24} [\Sigma j_{jk} (\Sigma j_{sk} - \Sigma j_{sj} - \Sigma j_{ks})]$
(b) <i>With TTC families</i>			
F_2	$\frac{1}{6}\sigma^2(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$	1	$\frac{n}{24} [\Sigma i_{jk}]^2$
		$(n-1)$	$\frac{1}{48} \Sigma j_{jk}^2 + \frac{1}{96} \Sigma l_{jk}^2 + \frac{1}{24} [\Sigma j_{jk} \Sigma j_{sk}]$
F_∞	$\frac{1}{6}\sigma^2(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$	1	$\frac{n}{24} [\Sigma i_{jk}]^2$
		$(n-1)$	$\frac{1}{24} \Sigma j_{jk}^2 + \frac{1}{24} \Sigma l_{jk}^2 + \frac{1}{12} [\Sigma j_{jk} \Sigma j_{sk}]$
	$\frac{1}{3}\sigma^2(\bar{L}_{1i} + \bar{L}_{2i} - \bar{P}_i)$	$(n-1)$	$\frac{1}{12} \Sigma i_{jk}^2 + \frac{1}{6} \Sigma j_{jk}^2 + \frac{1}{12} \Sigma l_{jk}^2 + \frac{1}{12} \Sigma j_{jk} \Sigma j_{js}$ $- \frac{1}{6} [\Sigma i_{jk} l_{jk} + \Sigma j_{jk} j_{kj}]$ $+ \frac{1}{12} [\Sigma j_{jk} (\Sigma j_{ks} - \Sigma j_{sk} - \Sigma j_{sj})]$
(c) <i>With mixed families</i>			
F_2	$\frac{1}{6}\sigma^2(\bar{L}_{1si} + \bar{L}_{2si} - 2\bar{L}_{3si})$	$(n-1)$	$\frac{1}{96} \Sigma j_{jk}^2 + \frac{1}{1536} \Sigma l_{jk}^2 + \frac{1}{48} [\Sigma j_{jk} j_{kj}]$ $+ \frac{1}{96} [\Sigma j_{jk} (\Sigma j_{js} + \Sigma j_{ks} + \Sigma j_{sj} + \Sigma j_{sk})]$
	$\frac{1}{6}\sigma^2(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3si})$	$(n-1)$	$\frac{5}{192} \Sigma j_{jk}^2 + \frac{1}{96} \Sigma l_{jk}^2 - \frac{1}{24} [\Sigma j_{jk} j_{kj}]$ $+ \frac{1}{12} [\Sigma j_{jk} (\frac{1}{2} \Sigma j_{ks} + \frac{1}{8} \Sigma j_{js} - \frac{1}{4} \Sigma j_{sk} - \frac{1}{4} \Sigma j_{sj})]$
	$\frac{1}{6}\sigma^2(\bar{L}_{1si} + \bar{L}_{2si} - 2\bar{L}_{3i})$	$(n-1)$	$\frac{1}{48} \Sigma j_{jk}^2 + \frac{1}{384} \Sigma l_{jk}^2 + \frac{1}{24} [\Sigma j_{jk} j_{kj}]$ $+ \frac{1}{48} [\Sigma j_{jk} (\Sigma j_{js} + \Sigma j_{ks} + \Sigma j_{sj} + \Sigma j_{sk})]$
F_∞	$\frac{1}{6}\sigma^2(\bar{L}_{1si} + \bar{L}_{2si} - 2\bar{L}_{3si})$	$(n-1)$	$\frac{5}{96} \Sigma j_{jk}^2 + \frac{1}{24} \Sigma l_{jk}^2 - \frac{1}{12} [\Sigma j_{jk} j_{kj}]$ $+ \frac{1}{6} [\Sigma j_{jk} (\frac{1}{2} \Sigma j_{ks} + \frac{1}{8} \Sigma j_{js} - \frac{1}{4} \Sigma j_{sk} - \frac{1}{4} \Sigma j_{sj})]$
	$\frac{1}{6}\sigma^2(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3si})$	$(n-1)$	$\frac{1}{96} \Sigma j_{jk}^2 + \frac{1}{24} \Sigma l_{jk}^2 - \frac{1}{12} [\Sigma j_{jk} j_{kj}]$ $+ \frac{1}{6} [\Sigma j_{jk} (\frac{1}{2} \Sigma j_{ks} + \frac{1}{8} \Sigma j_{js} - \frac{1}{4} \Sigma j_{sk} - \frac{1}{4} \Sigma j_{sj})]$

* The magnitude of the cross products in square brackets ([]) is likely to change with a change in gene association.

powers of detection vary with the type of population. Where the population under study is a collection of pure breeding lines non-allelic interactions can also be detected by the $(\bar{L}_{1si} + \bar{L}_{2si} - \bar{P}_i)$ comparison for $(n-1)$ degrees of freedom. This test, like the $(\bar{L}_{1i} + \bar{L}_{2i} - \bar{P}_i)$ comparison of Jinks *et al.* (1969), is expected to include all the three types of epistatic effects in its expectation (which are subject to internal cancellations depending upon the direction of i_{jk} , j_{jk} , j_{kj} and l_{jk} effects of any pair of loci).

The significance of each of these mean squares can be tested against an appropriate error mean square which for a completely randomised design is obtained by adding the pooled within variances (adjusted for the fact that the analysis is based on family means) of sets of families involved in the comparison premultiplied by the square of their coefficients (Kearsey and Jinks, 1968). In a normal analysis of variance, however, any orthogonal comparison contributes only $1/\Sigma k^2$ of its variance to the total variation (Snedecor and Cochran, 1967) and this is the fraction of the mean square

TABLE 2

Genetical expectations of σ_b^2 for additive genetic variation when it is estimated from the selfed (as $\frac{1}{4}\sigma^2(\bar{L}_{1s1} + \bar{L}_{2s1})$ and $\frac{1}{9}\sigma^2(\bar{L}_{1s1} + \bar{L}_{2s1} + \bar{L}_{3s1})$) and the triple test cross (as $\frac{1}{4}\sigma^2(\bar{L}_{11} + \bar{L}_{21})$ and $\frac{1}{9}\sigma^2(\bar{L}_{11} + \bar{L}_{21} + \bar{L}_{31})$) sets of families in the absence and presence of epistasis for test crosses of F_2 and F_∞ populations

Population	σ_b^2	Expectations	
		Epistasis absent	Epistasis present
(a) <i>From selfed families</i>			
F_2	$\frac{1}{4}\sigma^2(\bar{L}_{1si} + \bar{L}_{2si})$	$\frac{1}{8}\Sigma d_j^2$	$\frac{1}{8}\Sigma d_j^2 + \frac{1}{64}\Sigma i_{jk}^2 + \frac{1}{256}\Sigma l_{jk}^2 + \frac{1}{64}\Sigma j_{jk}^2$ $+ \frac{1}{16}\Sigma d_j \Sigma j_{jk}^* - \frac{1}{16}[\Sigma d_j \Sigma j_{kj}]^* + \frac{1}{128}[\Sigma i_{jk} l_{jk}]$ $- \frac{1}{32}[\Sigma j_{jk} j_{kj}] + \frac{1}{64}[\Sigma j_{jk}(\Sigma j_{sk} - \Sigma j_{sj} - \Sigma j_{ks})]$ $+ \frac{1}{64}\Sigma j_{jk} \Sigma j_{js}$
	$\frac{1}{9}\sigma^2(\bar{L}_{1si} + \bar{L}_{2si} + \bar{L}_{3si})$	$\frac{1}{8}\Sigma d_j^2$	$\frac{1}{8}\Sigma d_j^2 + \frac{1}{64}\Sigma i_{jk}^2 + \frac{1}{2304}\Sigma l_{jk}^2 + \frac{2}{2304}\Sigma j_{jk}^2$ $+ \frac{1}{16}\Sigma d_j \Sigma j_{jk}^* - \frac{1}{24}[\Sigma d_j \Sigma j_{kj}]^* + \frac{1}{192}[\Sigma i_{jk} l_{jk}]$ $- \frac{1}{96}[\Sigma j_{jk} j_{kj}] + [\Sigma j_{jk}(\frac{1}{144}\Sigma j_{sk} - \frac{1}{64}\Sigma j_{sj}$ $- \frac{1}{64}\Sigma j_{ks})] + \frac{3}{256}\Sigma j_{jk} \Sigma j_{js}$
F_∞	$\frac{1}{4}\sigma^2(\bar{L}_{1si} + \bar{L}_{2si})$	$\frac{1}{4}\Sigma d_j^2$	$\frac{1}{4}\Sigma d_j^2 + \frac{1}{16}\Sigma i_{jk}^2 + \frac{1}{64}\Sigma l_{jk}^2 + \frac{1}{32}\Sigma j_{jk}^2$ $+ \frac{1}{8}\Sigma d_j \Sigma j_{jk}^* - \frac{1}{8}[\Sigma d_j \Sigma j_{kj}]^* + \frac{1}{32}[\Sigma i_{jk} l_{jk}]$ $- \frac{1}{16}[\Sigma j_{jk} j_{kj}] + \frac{1}{32}[\Sigma j_{jk}(\Sigma j_{sk} - \Sigma j_{sj} - \Sigma j_{ks})]$ $+ \frac{1}{32}\Sigma j_{jk} \Sigma j_{js}$
	$\frac{1}{9}\sigma^2(\bar{L}_{1si} + \bar{L}_{2si} + \bar{L}_{3si})$	$\frac{1}{4}\Sigma d_j^2$	$\frac{1}{4}\Sigma d_j^2 + \frac{1}{16}\Sigma i_{jk}^2 + \frac{1}{576}\Sigma l_{jk}^2 + \frac{2}{1152}\Sigma j_{jk}^2$ $+ \frac{1}{8}\Sigma d_j \Sigma j_{jk}^* - \frac{1}{12}[\Sigma d_j \Sigma j_{kj}]^* + \frac{1}{48}[\Sigma i_{jk} l_{jk}]$ $- \frac{1}{48}[\Sigma j_{jk} j_{kj}] + [\Sigma j_{jk}(\frac{1}{72}\Sigma j_{sk} - \frac{1}{32}\Sigma j_{sj}$ $- \frac{1}{32}\Sigma j_{ks})] + \frac{3}{128}\Sigma j_{jk} \Sigma j_{js}$

TABLE 2—continued

Population	σ_b^2	Expectations	
		Epistasis absent	Epistasis present
(b) <i>From TTC families</i>			
F_2	$\frac{1}{4}\sigma^2(\bar{L}_{1i} + \bar{L}_{2i})$	$\frac{1}{8}\Sigma d_j^2$	$\frac{1}{8}\Sigma d_j^2 + \frac{1}{64}\Sigma i_{jk}^2 + \frac{1}{64}\Sigma l_{jk}^2 + \frac{1}{16}\Sigma j_{jk}^2$ $+ \frac{1}{8}\Sigma d_j \Sigma j_{jk}^* - \frac{1}{8}[\Sigma d_j \Sigma j_{kj}]^* + \frac{1}{32}[\Sigma i_{jk} l_{jk}]$ $- \frac{1}{8}[\Sigma j_{jk} j_{kj}] + \frac{1}{16}[\Sigma j_{jk}(\Sigma j_{sk} - \Sigma j_{sj} - \Sigma j_{ks})]$ $+ \frac{1}{16}\Sigma j_{jk} \Sigma j_{js}$
	$\frac{1}{9}\sigma^2(\bar{L}_{1i} + \bar{L}_{2i} + \bar{L}_{3i})$	$\frac{1}{8}\Sigma d_j^2$	$\frac{1}{8}\Sigma d_j^2 + \frac{1}{64}\Sigma i_{jk}^2 + \frac{1}{144}\Sigma l_{jk}^2 + \frac{1}{288}\Sigma j_{jk}^2$ $+ \frac{1}{8}\Sigma d_j \Sigma j_{jk}^* - \frac{1}{12}[\Sigma d_j \Sigma j_{kj}]^* + \frac{1}{48}[\Sigma i_{jk} l_{jk}]$ $- \frac{1}{12}[\Sigma j_{jk} j_{kj}] + [\Sigma j_{jk}(\frac{1}{36}\Sigma j_{sk} - \frac{1}{24}\Sigma j_{ks}$ $- \frac{1}{24}\Sigma j_{sj})] + \frac{1}{16}\Sigma j_{jk} \Sigma j_{js}$
F_∞	$\frac{1}{4}\sigma^2(\bar{L}_{1i} + \bar{L}_{2i})$	$\frac{1}{4}\Sigma d_j^2$	$\frac{1}{4}\Sigma d_j^2 + \frac{1}{16}\Sigma i_{jk}^2 + \frac{1}{16}\Sigma l_{jk}^2 + \frac{1}{8}\Sigma j_{jk}^2$ $+ \frac{1}{4}\Sigma d_j \Sigma j_{jk}^* - \frac{1}{4}[\Sigma d_j \Sigma j_{kj}]^* + \frac{1}{8}[\Sigma i_{jk} l_{jk}]$ $- \frac{1}{4}[\Sigma j_{jk} j_{kj}] + \frac{1}{8}[\Sigma j_{jk}(\Sigma j_{sk} - \Sigma j_{sj} - \Sigma j_{ks})]$ $+ \frac{1}{8}\Sigma j_{jk} \Sigma j_{js}$
	$\frac{1}{9}\sigma^2(\bar{L}_{1i} + \bar{L}_{2i} + \bar{L}_{3i})$	$\frac{1}{4}\Sigma d_j^2$	$\frac{1}{4}\Sigma d_j^2 + \frac{1}{16}\Sigma i_{jk}^2 + \frac{1}{36}\Sigma l_{jk}^2 + \frac{1}{144}\Sigma j_{jk}^2$ $+ \frac{1}{4}\Sigma d_j \Sigma j_{jk}^* - \frac{1}{6}[\Sigma d_j \Sigma j_{kj}]^* + \frac{1}{12}[\Sigma i_{jk} l_{jk}]$ $- \frac{1}{6}[\Sigma j_{jk} j_{kj}] + [\Sigma j_{jk}(\frac{1}{18}\Sigma j_{sk} - \frac{1}{12}\Sigma j_{ks}$ $- \frac{1}{12}\Sigma j_{sj})] + \frac{1}{8}\Sigma j_{jk} \Sigma j_{js}$

* The asterisked co-products are likely to change sign with the type of epistasis present while components within square brackets ([]) are affected by the gene association.

(due to that comparison) which is generally tested for its significance against the pooled error. Genetical expectations of the tests of epistasis referred to above for the special cases of F_2 and F_∞ populations have therefore been tabulated in table 1 in the form of these fractions. For example the epistatic variance of comparison $(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$ is given as a 1/6th of its total variance because $\Sigma k^2 = 6$ in this case.

(ii) Additive and dominance components

In the absence of epistasis we can simply substitute $\bar{L}_{1si}$ for $\bar{L}_{1i}$, etc. in the standard comparison for estimating the additive and dominance components. Comparisons $(\bar{L}_{1si} + \bar{L}_{2si})$ and $(\bar{L}_{1si} + \bar{L}_{2si} + \bar{L}_{3si})$ provide estimates of the additive genetic variance $D = \Sigma d_j^2$ and the σ_b^2 for either of them (which are equal to one-fourth and one-ninth of the respective variances) estimate $\frac{1}{8}D$ for F_2 population and $\frac{1}{4}D$ for F_∞ inbred lines. Similarly the

TABLE 3

Genetical expectations of σ_b^2 for dominance variation when it is estimated from selfed (as $\frac{1}{4}\sigma^2(\bar{L}_{1si} - \bar{L}_{2si})$) and triple test cross (as $\frac{1}{4}\sigma^2(\bar{L}_{1i} - \bar{L}_{2i})$) sets of families in the absence and presence of epistasis for test crosses on F_2 and F_∞ populations

Population	σ_b^2	Expectation	
		Epistasis absent	Epistasis present
(a) <i>From Selfed Families</i>			
F_2	$\frac{1}{4}\sigma^2(\bar{L}_{1si} - \bar{L}_{2si})$	$\frac{1}{32}\Sigma h_j^2$	$\frac{1}{32}\Sigma h_j^2 + \frac{1}{16}\Sigma i_{jk}^2 + \frac{1}{256}\Sigma l_{jk}^2 + \frac{1}{256}\Sigma j_{jk}^2$ $+ \frac{1}{128}[\Sigma j_{jk}j_{kj}] - \frac{1}{32}[\Sigma i_{jk}l_{jk}]$ $+ \frac{1}{256}\Sigma l_{jk}(\Sigma l_{js} + \Sigma l_{ks}) + \frac{1}{16}[\Sigma i_{jk}(\Sigma i_{js}$ $+ \Sigma i_{ks})] + \frac{1}{64}\Sigma l_{jk}(h_j + h_k)^* - \frac{1}{16}[\Sigma i_{jk}(h_j$ $+ h_k)]^* - \frac{1}{128}[\Sigma i_{jk}(\Sigma l_{js} + \Sigma l_{ks})]$
F_∞	$\frac{1}{4}\sigma^2(\bar{L}_{1si} - \bar{L}_{2si})$	$\frac{1}{16}\Sigma h_j^2$	$\frac{1}{16}\Sigma h_j^2 + \frac{1}{8}\Sigma i_{jk}^2 + \frac{1}{128}\Sigma l_{jk}^2 + \frac{1}{64}\Sigma j_{jk}^2$ $+ \frac{1}{32}[\Sigma j_{jk}j_{kj}] - \frac{1}{16}[\Sigma i_{jk}l_{jk}]$ $+ \frac{1}{128}\Sigma l_{jk}(\Sigma l_{ks} + \Sigma l_{js}) + \frac{1}{8}[\Sigma i_{jk}(\Sigma i_{js} + \Sigma i_{ks})]$ $+ \frac{1}{32}\Sigma l_{jk}(h_j + h_k)^* - \frac{1}{8}[\Sigma i_{jk}(h_j + h_k)]^*$ $- \frac{1}{64}[\Sigma i_{jk}(\Sigma l_{js} + \Sigma l_{ks})]$
(b) <i>From TTC families</i>			
F_2	$\frac{1}{4}\sigma^2(\bar{L}_{1i} - \bar{L}_{2i})$	$\frac{1}{8}\Sigma h_j^2$	$\frac{1}{8}\Sigma h_j^2 + \frac{1}{16}\Sigma i_{jk}^2 + \frac{1}{16}\Sigma l_{jk}^2 + \frac{1}{64}\Sigma j_{jk}^2$ $+ \frac{1}{8}\Sigma l_{jk}(h_j + h_k)^* - \frac{1}{8}[\Sigma i_{jk}(h_j + h_k)]^*$ $+ \frac{1}{32}[\Sigma j_{jk}j_{kj}] - \frac{1}{8}[\Sigma i_{jk}l_{jk}] + \frac{1}{16}[\Sigma i_{jk}(\Sigma i_{js}$ $+ \Sigma i_{ks})] + \frac{1}{16}\Sigma l_{jk}(\Sigma l_{js} + \Sigma l_{ks}) - \frac{1}{16}[\Sigma i_{jk}(\Sigma l_{js}$ $+ \Sigma l_{ks})]$
F_∞	$\frac{1}{4}\sigma^2(\bar{L}_{1i} - \bar{L}_{2i})$	$\frac{1}{4}\Sigma h_j^2$	$\frac{1}{4}\Sigma h_j^2 + \frac{1}{8}\Sigma i_{jk}^2 + \frac{1}{8}\Sigma l_{jk}^2 + \frac{1}{16}\Sigma j_{jk}^2$ $+ \frac{1}{4}\Sigma l_{jk}(h_j + h_k)^* - \frac{1}{4}[\Sigma i_{jk}(h_j + h_k)]^*$ $+ \frac{1}{8}[\Sigma j_{jk}j_{kj}] - \frac{1}{4}[\Sigma i_{jk}l_{jk}] + \frac{1}{8}\Sigma l_{jk}(\Sigma l_{js}$ $+ \Sigma l_{ks}) + \frac{1}{8}[\Sigma i_{jk}(\Sigma i_{js} + \Sigma i_{ks})] - \frac{1}{8}[\Sigma i_{jk}(\Sigma l_{js}$ $+ \Sigma l_{ks})]$

* The asterisked co-products are likely to change sign with the type of epistasis present while components within square brackets ([]) are affected by the gene association.

σ_b^2 for $(\bar{L}_{1si} - \bar{L}_{2si})$ detects and estimates the dominance component $H = \Sigma h_j^2$. It estimates however only $\frac{1}{3}H$ for F_2 and $\frac{1}{6}H$ for F_∞ populations which are one-quarter and one-half of the corresponding estimates from the normal triple test cross families respectively.

In the presence of epistasis these estimates will be biased and the level and magnitude of these biases may differ from those incurred by the corresponding estimates from the normal triple test cross families. The expectations of $\sigma^2(\bar{L}_{1si} + \bar{L}_{2si})$, $\sigma^2(\bar{L}_{1si} + \bar{L}_{2si} + \bar{L}_{3si})$ and $\sigma^2(\bar{L}_{1si} - \bar{L}_{2si})$ components have therefore been derived for genes which show digenic interactions. These are given in tables 2 and 3 (table 2 for additive and table 3 for dominance components) in the form of their σ_b^2 values for F_2 and F_∞ inbred populations. The corresponding expectations for $(\bar{L}_{1i} + \bar{L}_{2i})$, $(\bar{L}_{1i} + \bar{L}_{2i} + \bar{L}_{3i})$ and $(\bar{L}_{1i} - \bar{L}_{2i})$ are also given for comparison. These together with the expectations for $(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$ and $(\bar{L}_{2i} + \bar{L}_{2i} - \bar{P}_i)$ comparisons have already been published by Pooni and Jinks (1976, 1979) and Pooni, Jinks and Jayasekara (1978).

3. EXPERIMENTAL DESIGN

The experimental material consisted of 80 F_{13} families (P_i) each derived by single seed descent from an individual of the F_2 generation following a cross between pure breeding varieties 1 and 5 of the Birmingham collection of *Nicotiana rustica* varieties; their crosses to $V_1(L_{1i})$, $V_5(L_{2i})$ and their $F_1(L_{3i})$, the progeny families of self-pollinated individuals of the non-segregating L_{1i} and L_{2i} families (L_{1si} and L_{2si}) and the original parental varieties, V_1 and V_5 . Ten individually randomised plants were raised for each of these 482 families during the summer of 1977. The material was scored for plant height (in cm) 4 weeks (H_2) and six weeks (H_3) after planting in the field; number of days taken for the opening of the first flower (FT); height (in cm) at flowering time (HFT) and final height (FH) at the end of the season. The data were processed through the Birmingham University ICL 1906A Computer.

4. RESULTS

(i) Detection of epistasis

The results of applying the only test for epistasis available for the selfed families in our data, namely, that based on the comparison $(\bar{L}_{1si} + \bar{L}_{2si} - \bar{P}_i)$ are given in table 4. Also tabulated are the results of the corresponding comparison for the normal triple test cross families $(\bar{L}_{1i} + \bar{L}_{2i} - \bar{P}_i)$ and of the standard test for epistasis $(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$. Normal and selfed families have been combined in the comparison $(\bar{L}_{1si} + \bar{L}_{2si} - 2\bar{L}_{3i})$ to provide an additional test.

The results of these tests are presented in the form of an analysis of variance consisting of two items, the mean square between the sets for 79 degrees of freedom which is tested for significance as a $\chi^2_{(79)}$ against the other item, the mean square based on the variation within families. The standard test for epistasis provides a third item, the correction factor for $(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$ for one degree of freedom, which is used to test the significance of the $[i]$ component (Jinks and Perkins, 1970).

TABLE 4

Tests for the significance of epistatic variation for the five characters. The bracketed numbers are the χ^2 values rounded off to integers while the CF mean square for 1 d.f. was tested as a variance ratio against the corresponding between-sets mean square whenever the latter was significantly greater than the error

Comparison	d.f.	Character				
		H ₂ m.s.	H ₃ m.s.	FT m.s.	HFT m.s.	FH m.s.
$(\bar{L}_{1st} + L_{2st} - \bar{P}_t)$	Between sets	3.20 (216)***	(a) <i>Selfs</i> 21.41 (190)***	11.00 (342)***	44.29 (197)***	42.67 (224)***
	Within	1.17	8.92	2.54	17.73	15.04
$(\bar{L}_{2t} + L_{2st} - \bar{P}_t)$	Between sets	5.24 (382)***	(b) <i>TTC amilies</i> 38.42 (444)***	10.17 (382)***	50.12 (328)***	41.31 (291)***
	Within	1.08	6.84	2.10	12.07	11.22
$(\bar{L}_{1st} + \bar{L}_{2st} - 2\bar{L}_{3st})$	C.F.	1	34.74 ^{NS}	22.36*	61.17 ^{NS}	0.00 (0.0) ^{NS}
	Between sets	2.19 (117)**	16.75 (130)***	4.26 (131)***	21.78 (111)**	17.73 (100) ^{NS}
	Within	1.48	10.20	2.56	15.48	14.05
$(\bar{L}_{1st} + \bar{L}_{2st} - 2\bar{L}_{3st})$	Between sets	3.19 (162)***	(c) <i>Mixed families</i> 22.98 (176)***	5.67 (141)***	24.11 (104)*	22.54 (112)*
	Within	1.55	10.34	2.17	18.31	15.96

*** $P \leq 0.001$; ** $0.001 < P \leq 0.01$; * $0.01 < P \leq 0.05$; NS $P > 0.05$

Independently of the triple test cross analyses, the V_1 , V_5 , L_1 , L_2 , L_3 , L_{1s} , L_{2s} and F_{13} generations which make up the experiment together provide a powerful test for the presence of non-allelic interactions. Using weighted least squares procedures we can fit models to the eight means which will estimate and test the significance of the components of the model and simultaneously test the adequacy of the model itself. In the presence of non-allelic interactions a model which excludes them will be inadequate while estimates of their contributions when an adequate model is fitted will be significant.

The generation means and their standard errors for the five characters are given in table 5. In the absence of linkage between interacting genes the expected means of the L_{1i} , L_{2i} , L_{3i} , L_{1si} and L_{2si} generations are identical with those of the B_1 , B_2 , F_2 , B_{1s} and B_{2s} families, respectively, of a cross between a pair of pure breeding lines (Jinks and Perkins 1969, Jinks 1978). The theoretical expectations of each of the generations for a model which allows for the additive, dominance and digenic non-allelic interactions effects of the genes are given in the lower half of table 5.

A model which excluded non-allelic interactions was inadequate for all characters except final height where the $\chi^2_{(5)}$ testing the goodness of fit

TABLE 5
Generation means for the five characters with their standard errors and their genetical expectations in a standard six-parameter epistatic model

Generation	Character					
	H ₂	H ₃	FT	HFT	FH	
(a) <i>Parents</i>						
P ₁	15.34 ± 1.236	54.00 ± 1.074	40.60 ± 2.486	64.50 ± 2.714	120.11 ± 3.672	
P ₂	13.95 ± 1.053	40.45 ± 1.484	34.78 ± 0.803	58.35 ± 4.554	104.10 ± 3.332	
(b) <i>F₁₃ inbreds</i>						
Inbreds	11.49 ± 0.344	38.07 ± 1.026	43.11 ± 0.841	71.76 ± 1.795	117.85 ± 1.606	
(c) <i>TTC families</i>						
L ₁	14.22 ± 0.279	48.73 ± 0.810	36.73 ± 0.466	67.28 ± 1.340	122.10 ± 0.806	
L ₂	16.62 ± 0.328	50.78 ± 0.960	37.31 ± 0.478	65.04 ± 0.873	117.70 ± 0.800	
L ₃	15.27 ± 0.288	49.07 ± 0.807	37.66 ± 0.413	67.47 ± 0.901	120.20 ± 0.847	
(d) <i>Selves</i>						
L _{1s}	13.25 ± 0.252	45.44 ± 0.746	39.21 ± 0.489	68.08 ± 0.971	119.32 ± 0.950	
L _{2s}	14.74 ± 0.258	44.85 ± 0.699	38.74 ± 0.406	65.81 ± 0.873	113.36 ± 0.850	
		<i>m</i>	<i>[d]</i>	<i>[h]</i>	<i>[i]</i>	<i>[j]</i>
P ₁		1	1	0	1	0
P ₂		1	-1	0	1	0
Inbreds F _∞		1	0	0	0	0
L ₁ (= B ₁)		1	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{4}$
L ₂ (= B ₂)		1	$-\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$-\frac{1}{2}$
L ₃ (= F ₂)		1	0	$\frac{1}{2}$	0	$\frac{1}{4}$
L _{1s} (= B _{1s})		1	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{8}$
L _{2s} (= B _{2s})		1	$-\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$	$-\frac{1}{8}$

had a borderline probability of six per cent. The six-parameter model which included digenic interactions, on the other hand, was adequate for all characters. However $[l]$ was non-significant for all characters, $[i]$ was non-significant for HFT and FH and $[d]$ was non-significant for H_2 . The results of fitting models which exclude those non-significant parameters are given in table 6. All models are adequate as judged by the χ^2 test of goodness of fit.

TABLE 6

Weighted least squares estimates of the components of generation means, their significances and standard errors and a χ^2 (d.f.) value for the goodness of fit of the model for the five characters

Parameter	Character				
	H_2	H_3	FT	HFT	FH
m	11.65*** ± 0.30	38.21*** ± 0.87	42.60*** ± 0.63	70.69*** ± 1.34	115.76*** ± 1.21
$[d]$	—	6.45*** ± 0.85	2.60** ± 0.90	2.26* ± 0.89	5.45*** ± 0.80
$[h]$	6.69*** ± 0.67	19.77*** ± 1.91	-9.22*** ± 1.29	-5.57* ± 2.73	9.62*** ± 2.41
$[i]$	2.47** ± 0.72	8.77*** ± 1.27	-4.89*** ± 1.16	-8.51** ± 2.48	-4.50 ^{NS} ± 2.39
$[j]$	-5.19*** ± 0.74	-18.97*** ± 3.03	-6.79** ± 2.47	—	—
χ^2 (d.f.)	3.40(4) ^{NS}	4.20(3) ^{NS}	2.36(3) ^{NS}	1.13(4) ^{NS}	5.99(4) ^{NS}

*** $P \leq 0.001$; ** $0.001 < P \leq 0.01$; * $0.01 < P \leq 0.05$; NS $P > 0.05$

(ii) Additive and dominance effects

The mean squares for detecting and estimating the additive D and dominance H components of variation from orthogonal comparisons $(\bar{L}_{1si} + \bar{L}_{2si})$ and $(\bar{L}_{1si} - \bar{L}_{2si})$ between the means of the L_{1si} and L_{2si} families are presented in table 7 and the estimates of D and H derived from them are presented in table 8. The corresponding mean squares and estimates from the conventional L_{1i} and L_{2i} families of a triple test cross are also given in the same tables. Additionally, are included two comparisons which detect and estimate D only, one $(\bar{L}_{1i} + \bar{L}_{2i} + \bar{L}_{3i})$ being the most widely used source of D in triple test cross analysis and the other a direct estimate from comparing the means of the P_i (in this case F_{13}) families. For each comparison the mean square between sets for 79 degrees of freedom is tested for significance as a $\chi^2_{(79)}$ against the mean square based on the variation within the families included in the comparison. All the mean squares are on the basis of family means.

Because of the significant non-allelic interactions the estimates of D and H are not only biased (tables 2 and 3) but the magnitude of the bias is expected to differ depending on the comparison from which they were estimated.

Whether or not the estimates of D and of H from the different sources differ significantly can be tested by standard maximum likelihood model

TABLE 7

Analysis of variance for the four comparisons ($L_{1st} + L_{2st}$), ($L_{1t} + L_{2t}$), ($L_{1t} + L_{2t} + L_{3t}$) and ($\bar{P}_i$) which estimate D , and for the two comparisons ($L_{1st} - L_{2st}$) and ($L_{1t} - L_{2t}$) which estimate H for each of the five characters

Comparison		d.f.	Character				
			H ₂ m.s.	H ₃ m.s.	FT m.s.	HFT m.s.	FH m.s.
I. <i>Analysis of additive genetic variance (D)</i>							
(a) <i>Selfs</i>							
(L _{1st} + L _{2st})	Between sets	79	7.95***	66.47***	27.24***	119.73***	114.81***
	Within	1478	1.31	10.04	3.84	19.30	16.71
(b) <i>TTC families</i>							
(L _{1t} + L _{2t})	Between sets	79	13.76***	117.40***	32.73***	151.29***	95.29***
	Within	1475	1.08	6.91	1.36	10.80	10.98
(L _{1t} + L _{2t} + L _{3t})	Between sets	79	18.73***	155.62***	42.90***	205.56***	149.38***
	Within	2207	1.28	8.26	1.96	13.13	12.51
(c) <i>Inbred (P_i) families</i>							
(P _i)	Between sets	79	9.51***	85.09***	90.46***	271.77***	213.45***
	Within	725	1.09	6.69	3.59	14.62	11.71
II. <i>Analysis of dominance variance (H)</i>							
(a) <i>Selfs</i>							
(L _{1st} - L _{2st})	Between sets	79	3.65***	25.06***	6.61***	41.28***	30.89***
	Within	1478	1.31	10.04	3.84	19.30	16.71
(b) <i>TTC families</i>							
(L _{1t} - L _{2t})	Between sets	79	2.59***	20.17***	3.96***	34.57***	23.30***
	Within	1475	1.08	6.91	1.36	10.80	10.98

*** $P \leq 0.001$

fitting procedures. For example, we can test whether the estimates of D obtained from $\bar{P}_i$ and ($L_{1st} + L_{2st}$) differ by fitting a model to the between sets and within family mean squares from these two sources (table 7) assuming a single value for D but allowing the two within family mean squares, which on these data are the non-heritable components, to take their own values. Since we are then effectively fitting three components to four mean squares there is one degree of freedom for testing the goodness of fit of the model. The model cannot fail unless the D 's from the two sources differ significantly. We could, of course, compare all possible pairs of sources of estimates of D in this way or extend the comparisons to three or more sources

TABLE 8
Estimates of additive genetic variance (D) and dominance variance (H) as obtained from $(L_{3st} + L_{1st})$, $(L_{11} + L_{21})$, $(L_{11} + L_{21} + L_{31})$ and $(\bar{P}_1)$ and $(L_{1st} - L_{2st})$ and $(L_{11} - L_{21})$ comparisons, respectively

From	Comparison	Character			
		H ₂	H ₃	FT	FH
		(a) Estimates of additive genetic variance (D)			
Selfs	(L _{1st} + L _{2st})	13.28 ± 2.54	112.86 ± 21.16	46.80 ± 8.68	200.86 ± 38.12
TTC	(L _{1st} + L _{2st})	25.36 ± 4.38	220.98 ± 37.36	62.74 ± 10.42	280.98 ± 48.15
TTC	(L _{1st} + L _{2st} + L _{3st})	23.27 ± 3.97	196.08 ± 33.02	54.59 ± 9.10	256.57 ± 43.61
F ₁₃ inbreds	(P _t)	8.42 ± 1.51	78.40 ± 13.54	86.87 ± 14.39	257.15 ± 43.25
		(b) Estimates of dominance variance (H)			
Selfs	(L _{1st} - L _{2st})	18.64 ± 4.66	120.16 ± 32.04	22.16 ± 8.49	175.84 ± 52.85
TTC	(L _{1st} - L _{2st})	3.02 ± 0.83	26.48 ± 6.44	5.20 ± 1.26	67.54 ± 11.10
					24.62 ± 7.46

simultaneously. Since, however, the estimates from the TTC families and from the progeny families produced by selfing them are attempts to estimate the genetic variation expected between F_∞ family means the most appropriate comparisons are between the three estimates from these sources and that of the P_i (F_{13}) families. These along with the $\chi^2_{(1)}$ for the single comparison of the two sources of estimates of H are given in table 9.

TABLE 9

Tests of goodness of fit of a model which assumes a single D component for the mean squares from the $\bar{P}_i$ and $(\bar{L}_{1st} + \bar{L}_{2st})$ comparisons, the $\bar{P}_i$ and $(\bar{L}_{1i} + \bar{L}_{2i})$ comparisons and the $\bar{P}_i$ and $(\bar{L}_{1i} + \bar{L}_{2i} + \bar{L}_{3i})$ comparisons and of a model which assumes a single H component for the mean squares from the $(\bar{L}_{1st} - \bar{L}_{2st})$ and $(\bar{L}_{1i} - \bar{L}_{2i})$ comparisons when their corresponding within-comparison mean squares are allowed to vary independently

Comparison		Character				
		H ₂	H ₃	FT	HFT	FH
(a) <i>Single additive genetic component (D)</i>						
(1) $\bar{P}_i$ and $(\bar{L}_{1i} + \bar{L}_{2i})$	$\chi^2(1)$	3.06 ^{NS}	2.07 ^{NS}	5.88*	0.94 ^{NS}	0.01 ^{NS}
(2) $\bar{P}_i$ and $(\bar{L}_{1i} + \bar{L}_{2i})$	$\chi^2(1)$	17.45***	16.34***	1.90 ^{NS}	0.14 ^{NS}	0.53 ^{NS}
(3) $\bar{P}_i$ and $(\bar{L}_{1i} + \bar{L}_{2i} + \bar{L}_{3i})$	$\chi^2(1)$	15.46***	13.42***	3.82 ^{NS}	0.00 ^{NS}	0.17 ^{NS}
(b) <i>Single dominance component (H)</i>						
$(\bar{L}_{1si} - \bar{L}_{2si})$ and $(\bar{L}_{1i} - \bar{L}_{2i})$	$\chi^2(1)$	27.65***	18.35***	7.45**	11.30***	9.54**

*** $P \leq 0.001$; ** $0.001 \leq P \leq 0.01$; * $0.01 < P \leq 0.05$; NS $P > 0.05$

5. DISCUSSION

(i) Epistasis

Table 1 shows that the tests for epistasis based on selfed $(\bar{L}_{1st} + \bar{L}_{2st} - 2\bar{L}_{3st})$ and normal triple test cross $(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$ families involve identical components of epistasis. They differ however in the smaller coefficients for the additive $\times$ dominance (j) and dominance $\times$ dominance (l) components and their cross products in the comparison based on the selfs. Consequently the latter test will be less efficient in detecting j and l types of interaction but both tests will be equally efficient in detecting additive $\times$ additive components in the form of $[i]$. The comparison based on the selfs will however be subject to a larger error variance because of the release of genetical variability following selfing.

The tests which combine triple test cross families and their selfs (table 1(c)) differ from those based on one kind of family only (table 1(a) and (b)) in that they do not provide an independent test for $[i]$ interaction or indeed include in their expectations any additive $\times$ additive (i) components at all. On the other hand, they contain additional cross product terms involving additive $\times$ dominance interaction and have larger coefficients for some of the other components than the corresponding expectation for $(\bar{L}_{1st} + \bar{L}_{2st} - 2\bar{L}_{3st})$. Consequently the efficiency of the combined tests for epistasis is

largely dependent upon the magnitude of additive $\times$ dominance interaction and the direction and magnitude of their cross products. Of the two combined tests, however, $(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$ is likely to be more efficient because it has larger coefficients for both the additive $\times$ dominance and dominance $\times$ dominance components.

The additional tests $(\bar{L}_{1si} + \bar{L}_{2si} - \bar{P}_i)$ and $(\bar{L}_{1i} + \bar{L}_{2i} - \bar{P}_i)$ which are applicable to F_∞ populations only (table 1) are likely to be the most sensitive of all in most circumstances. This is partly because of the relatively larger contributions that the epistatic components make to their expectations and partly because the contribution of the additive $\times$ additive interaction is a sum of squares Σi_{jk}^2 whereas in the other expectations it is the square of the net directional component of the means $[\bar{i}]^2$.

Of these two additional tests $(\bar{L}_{1si} + \bar{L}_{2si} - \bar{P}_i)$ has the smaller coefficients for all of the epistatic components except the additive $\times$ additive interaction and the larger error variance because of the release of genetical variability following selfing. It will therefore be less efficient than $(\bar{L}_{1i} + \bar{L}_{2i} - \bar{P}_i)$ particularly when j and l type interactions are relatively large.

The results in table 4 support these conclusions in respect of F_∞ populations. The greater $\chi^2_{(79)}$ values for $(\bar{L}_{1si} + \bar{L}_{2si} - \bar{P}_i)$ and $(\bar{L}_{1i} + \bar{L}_{2i} - \bar{P}_i)$ show that they detect epistasis more efficiently than the other tests. On the same criterion $(\bar{L}_{1i} + \bar{L}_{2i} - \bar{P}_i)$ is the more sensitive for all characters. This is partly due to its smaller error variance for characters H_2 , H_3 and HFT, and entirely due to this cause for the other two characters FT and FH. For H_2 , H_3 and HFT, therefore, a high level of j and l types of interactions must be partly responsible.

Of the other two tests $(\bar{L}_{1si} + \bar{L}_{2si} - 2\bar{L}_{3i})$ seems to be the more sensitive in detecting j and l types of interaction for all characters except HFT, where its larger error variance makes it less sensitive. For these data, therefore, the standard triple test cross test for epistasis $(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$ is the least sensitive although it detects significant epistasis for all characters except FH. However, it is the only test which separates i from j and l types of interaction and shows unambiguously that the former is significant for FT only while the latter are significant for all except FH.

Taken together these tests provide strong evidence for j type interactions. The larger $\chi^2_{(79)}$ values observed for the $(\bar{L}_{1si} + \bar{L}_{2si} - 2\bar{L}_{3i})$ test relative to the $(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$ test are expected only when Σj_{jk}^2 is large and significant and the individual j contributions are predominantly of the same sign. The greater sensitivity of the $(\bar{L}_{1i} + \bar{L}_{2i} - \bar{P}_i)$ test relative to the $(\bar{L}_{1si} + \bar{L}_{2si} - \bar{P}_i)$ test is also consistent with a large contribution from j type interactions. And, of course, the model fitting to the generation means (table 6) shows highly significant net directional $[j]$ components for H_2 , H_3 and FT.

There are no indications that l type interactions are contributing to the significances in table 4, and the model fitting to generation means, which because of the small coefficients (table 5) provides a relatively weak test for these interactions, fails to detect their presence (table 6).

While in the absence of j type interactions i type interactions are the most likely cause of the difference in the $\chi^2_{(79)}$ values between the tests for epistasis based on P_i and those based on L_{3i} , where, as in these data, there is a large contribution from j type interactions this conclusion cannot be drawn with any confidence (tables 1 and 4). In these circumstances we

can rely only on the one unambiguous test for i type interactions provided by the correction term of $(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$ for one degree of freedom which detects $[i]$ interactions for FT only (table 4). In contrast the model fitting to the generation means detects significant $[i]$ interactions for all characters except FH.

(ii) *Additive and dominance effects*

The genetical expectations in table 2 show that in the absence of epistasis the additive genetic component D should be detected and estimated with the same efficiency from normal triple test cross families and from their progeny families produced by selfing. Because of the smaller coefficient the dominance component H will be detected and estimated from the latter families much less efficiently than from the normal triple test cross families (table 3).

In the presence of epistasis the expected magnitudes of the estimates may be substantially different between the different sources of estimates. The estimates of D from all sources are equally affected by i type interactions but the estimates from the normal triple test cross families will be more affected by j type interactions than the estimates from their selfs. In contrast, the two sources of estimates of H will be equally affected by j type interactions but the estimates from the normal families will be less affected by i and more by l interactions than those from their selfs. It is extremely unlikely, however, because of their very small coefficients that the l type interaction will have any effect in practice.

The estimates of D in table 8 confirm these theoretical expectations. Because of the presence of j type interactions the estimates from the normal triple test cross families are all inflated relative to those from their selfs except for FH where the evidence for these interactions is weakest (tables 4 and 6). In only two characters, H_2 and H_3 , has this inflation made the normal triple test cross estimates significantly larger than the genetical variation between F_∞ (F_{13}) family means (tables 8 and 9). In no case is the estimate from the selfs significantly larger than the latter although for FT it is just significantly smaller.

For every character the estimate of H from the normal triple test cross families is significantly smaller and has as expected a smaller error variance than the estimate from their selfs (tables 8 and 9). This is presumably because of the presence of i type interactions for which we have unambiguous evidence for all except FH (tables 4 and 6).

6. CONCLUSIONS

For the additive genetic variance D the estimate from the selfs is almost as good as from the normal triple test cross families. In the circumstances encountered in the *N. rustica* data the estimate from the selfs is the better predictor of the genetical variation among F_{13} families.

For the dominance variance H the estimate from the selfs is much poorer and in the *N. rustica* data it is much more biased by the presence of epistasis.

In general the selfs also provide a less sensitive test for epistasis. Where, however, we are analysing an F_∞ population (as in the *N. rustica* data) with the additional test for epistasis involving the F_∞ family means ($\bar{P}_i$) this loss of sensitivity is much reduced.

In the *N. rustica* data the corresponding tests and estimates from the normal families and from their selfs were based on the same number of plants in order to make them directly comparable. In practice, however, one would substitute selfs for the normal families only if by so doing more seed would be readily produced and hence larger families and more replications could be raised for each cross. If this were not so the extra time and effort required to produce the selfs would never be justified. In practice, therefore, the loss of information about the dominance component H and the loss of sensitivity of the test for epistasis that result from the use of selfs will be partly or wholly restored by the greater replication they make possible. This, however, will not overcome the greater bias on the estimate of H in the presence of epistasis or restore the reliability of H to that of D since these are irrevocably determined by the relative coefficients of the additive, dominance and epistatic components in the expectations.

Where, as in *N. rustica*, cross and self-pollinations are equally productive both normal triple test cross families and their selfs can be raised simultaneously and their analyses combined. This can throw additional light on the nature of the epistatic contributions and the biases they lead to, but in general, and particularly in the absence of epistasis, the additional time and effort required to produce selfs is not going to be compensated for by additional information.

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