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THE EFFICIENCY AND OPTIMAL SIZE OF TRIPLE TEST CROSS DESIGNS FOR DETECTING EPISTATIC VARIATION

H. S. POONI and J. L. JINKS
Department of Genetics, University of Birmingham, Birmingham B15 2TT

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SUMMARY

The triple test cross and two of its associate designs have been compared for their theoretical and practical efficiency in detecting epistatic variation. The comparisons are made on the basis of optimal experimental sizes required for each of these tests to detect a modest level of epistasis significantly ($P \leqq 0{\cdot}05$) and with a reasonable certainty (95 per cent). The experimental sizes are determined for various combinations of heritability, dominance ratio and gene association and for both duplicate and complementary epistasis.

Two versions of the test of epistasis designed by Kearsey and Jinks (1968), Test 1a and Test 1b, do not differ much in their theoretical efficiency for detecting epistasis and the optimal experimental sizes required by them to detect non-allelic interactions significantly are largely impracticable except when dominance and heritability are high and the degree of association is 50 per cent or more. Both the tests require much smaller experiments to detect duplicate epistasis than complementary epistasis of the same magnitude and this difference is more pronounced for lower levels of heritability and dominance. The theoretical efficiency of Test 2 (given by Jinks, Perkins and Breese, 1969), however, does not vary with the type of epistasis but the sensitivity of the test is inversely related to the degree of gene association between the tester parents.

The practical implications of the present investigation are discussed and the validity of some of the most important theoretical predictions and assumptions are tested on a triple test cross involving 80 inbred lines of *Nicotiana rustica*.

1. INTRODUCTION

THE triple test cross (Kearsey and Jinks, 1968) and its various modifications and extensions (Jinks, Perkins and Breese, 1969; Jinks and Perkins, 1970; Perkins and Jinks, 1970) are among the best designs available for the study of the genetical architecture of randomly breeding populations. These designs provide separate tests for, and estimates of the additive, dominance and epistatic components of variability but the presence of additive or dominance components can only be tested for unambiguously and unbiased estimates obtained in the absence of epistasis. Efficient detection of epistasis, therefore, is an important objective of the triple test cross design.

Non-allelic interactions, when large in magnitude, are easily detected by any of the tests available from modestly designed experiments. In general, however, epistasis is not expected to be present on a large scale and will normally be a minor portion of the total variation. (Mather and Jinks, 1971). In these circumstances the detection of epistasis will be dependent upon the size of the experiment conducted and the efficiency of the test applied.

The problems of optimal size and efficiency have been considered by

Kearsey (1970) and Pederson (1971) in respect of the additive and dominance variation in a number of multiple mating designs. They observed that the maximum information could be extracted by allowing family size (m) to vary with the genetical situation and calculating the optimum number of families (n) for the particular experimental design. One objective of this study is to calculate the value of n required to optimise the efficiency of an experiment.

The efficiency of any test to detect epistasis or any other type of gene action will depend on the ratio σ_2^2/σ_1^2 where the magnitudes of these components are direct functions of various genetic and environmental components of variation. The procedure will be to calculate the theoretical expectations of σ_1^2 and σ_2^2 for various tests and their relative magnitudes obtained theoretically for the limiting case of $d_j = d$, $h_j = h$, $i_{jk} = i$, $l_{jk} = l$ and $j_{kj} = j_{jk} = j$ for all the loci involved where d and h are the additive and dominance effects of the genes and i, j and l are the epistatic effects (Mather and Jinks, 1971). To calculate the size of the experiment required to detect a given amount of epistasis with a particular level of statistical reliability it will be necessary to define the inter-relationships between the magnitudes of these various genetical and non-genetical components of variation for a variety of situations. These theoretical predictions will be related to the results of triple test crosses between inbred lines of *Nicotiana rustica*.

2. Tests of epistasis

The first test (Test 1a) is that given by Kearsey and Jinks (1968) and can be presented as a variance ratio (A)

where $$A = \frac{m \times V(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i}) + (\bar{V}L_1 + \bar{V}L_2 + 4\bar{V}L_3)}{(\bar{V}L_1 + \bar{V}L_2 + 4\bar{V}L_3)}.$$

Here, m is the family size; $\bar{L}_{1i}$, $\bar{L}_{2i}$ and $\bar{L}_{3i}$ are the means of families produced by crossing the ith individual of an F_2 to P_1, P_2 and F_1 testers respectively and $\bar{V}L_1$, $\bar{V}L_2$ and $\bar{V}L_3$ are the average within variances of these L_{1i}, L_{2i} and L_{3i} families respectively. For an experiment involving n F_2 parents, the degrees of freedom of this V.R.(A) are n, and $3n(m-1)$.

The second test (Test 1b) is a modification of Test 1a in which F_2 individuals are replaced by a random sample of F_∞ inbreds in the crossing programme. Test 1a and Test 1b will therefore use the same analytical procedures but are expected to have different genetical expectations.

The third test (Test 2), as described by Jinks, Perkins and Breese (1969), is only applicable to F_∞ inbreds and is based on their L_{1i} and L_{2i} families. No L_{3i} families are required and the test takes the form:

$$A = \frac{m \times V(\bar{L}_{1i} + \bar{L}_{2i} - \bar{P}_i) + (\bar{V}L_1 + \bar{V}L_2 + \bar{V}P)}{(\bar{V}L_1 + \bar{V}L_2 + \bar{V}P)}.$$

Here, $\bar{P}_i$ is the mean of ith inbred and $\bar{V}P$ the average variation within inbred families. The degrees of freedom in this case are $(n-1)$ and $3n(m-1)$.

The theoretical expectations of various statistics involved in these tests are given in table 1. Where E stands for the environmental component of variation and the additive, dominance and epistatic gene effects are defined according to the F_∞ metric discussed by Van der Veen (1959). The number of loci segregating for a character are denoted by K and the symbol 'r'

represents the coefficient of gene association in the parental genotypes (Jinks and Jones, 1958).

3. Estimation of experimental sizes

The tests of epistasis can take the form $A = 1 + m\sigma_2^2/\sigma_1^2$ where σ_1^2 and σ_2^2 are $(\bar{V}L_1 + \bar{V}L_2 + 4\bar{V}L_3)$ and $V(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$ respectively for Test 1a and Test 1b and $(\bar{V}L_1 + \bar{V}L_2 + \bar{V}P)$ and $V(\bar{L}_{1i} + \bar{L}_{2i} - \bar{P}_i)$ for Test 2. The degrees of freedom for each of these tests will depend upon the number of families to be included in the experiment and the number of sibs to be raised for each of these families. For most plant breeding and biometrical genetical experiments the total number of individuals raised runs into several hundred.

Table 1

*Genetical and environmental expectations of various statistics which have been used in various tests of epistasis**

Statistic F_2 population	Expectation
$V(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$	$\frac{1}{4}[i]^2 + \frac{1}{8}\Sigma j_{jk}^2 + \frac{1}{16}\Sigma l_{jk}^2 + \frac{1}{8}[\Sigma j_{jk}\Sigma j_{sk}]$
$\bar{V}L_1 + \bar{V}L_2$	$\frac{1}{4}\Sigma d_j^2 + \frac{1}{4}\Sigma h_j^2 + \frac{7}{32}\{\Sigma i_{jk}^2 + \Sigma j_{jk}^2 + \Sigma l_{jk}^2\} \pm \frac{1}{4}\Sigma d_j j_{jk}$ $\pm \frac{1}{4}[\Sigma d_j j_{kj}] \pm \frac{1}{4}\Sigma l_{jk}(h_j + h_k) \mp \frac{1}{4}[\Sigma i_{jk}(h_j + h_k)]$ $+ \frac{1}{8}\Sigma l_{jk}(\Sigma l_{js} + \Sigma l_{sj} + \Sigma l_{sk} + \Sigma l_{ks}) - \frac{1}{16}[\Sigma i_{jk} l_{jk}]$ $- \frac{1}{8}[\Sigma i_{jk}(\Sigma l_{js} + \Sigma l_{sj} + \Sigma l_{sk} + \Sigma l_{ks})] + \frac{1}{8}[\Sigma j_{jk}(\Sigma j_{js} + \Sigma j_{sk})]$ $+ \frac{1}{8}[\Sigma i_{jk}(\Sigma i_{js} + \Sigma i_{sj} + \Sigma i_{sk} + \Sigma i_{ks})] - \frac{1}{16}[\Sigma j_{jk} j_{kj}]$ $- \frac{1}{8}[\Sigma j_{jk}(\Sigma j_{sj} + \Sigma j_{ks})] + 2E$
$\bar{V}L_3$	$\frac{3}{8}\Sigma d_j^2 + \frac{1}{4}\Sigma h_j^2 + \frac{15}{64}\Sigma i_{jk}^2 + \frac{7}{32}\Sigma j_{jk}^2 + \frac{3}{16}\Sigma l_{jk}^2$ $\pm \frac{3}{8}\Sigma d_j j_{jk} \pm \frac{1}{4}\Sigma l_{jk}(h_j + h_k) + \frac{3}{32}\Sigma j_{jk}\Sigma j_{js}$ $+ \frac{1}{16}\Sigma l_{jk}(\Sigma l_{js} + \Sigma l_{sj} + \Sigma l_{ks} + \Sigma l_{sk}) + E$
F∞ inbreds $V(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$	$\frac{1}{4}\{[i]^2 + \Sigma j_{jk}^2 + \Sigma l_{jk}^2\} + \frac{1}{4}[\Sigma j_{jk}\Sigma j_{sk}]$
$V(\bar{L}_{1i} + \bar{L}_{2i} - \bar{P}_i)$	$\frac{1}{4}\Sigma i_{jk}^2 + \frac{1}{2}\Sigma j_{jk}^2 + \frac{1}{4}\Sigma l_{jk}^2 - \frac{1}{2}[\Sigma i_{jk} l_{jk} + \Sigma j_{jk} j_{kj}]$ $+ \frac{1}{4}[\Sigma j_{jk}(\Sigma j_{js} + \Sigma j_{sj} + \Sigma j_{ks} + \Sigma j_{sk})]$
$\bar{V}P$, $\bar{V}L_1$, $\bar{V}L_2$	E
$\bar{V}L_3$	$\frac{1}{4}\Sigma d_j^2 + \frac{1}{4}\Sigma h_j^2 + \frac{3}{16}\{\Sigma i_{jk}^2 + \Sigma j_{jk}^2 + \Sigma l_{jk}^2\} \pm \frac{1}{4}\Sigma l_{jk}(h_j + h_k)$ $\pm \frac{1}{4}\Sigma d_j j_{jk} + \frac{1}{16}\Sigma j_{jk} j_{js} + \frac{1}{16}\Sigma l_{jk}(\Sigma l_{js} + \Sigma l_{sj} + \Sigma l_{ks} + \Sigma l_{sk}) + E$

* Lower signs are for duplicate genes, and each value in a square bracket is a function of the coefficient of gene association. Subscript s equals 1 to K but not j or k.

The value of $3n(m-1)$ is usually very large and much larger than n or $n-1$. Thus following Kearsey (1970), variance ratios can be replaced by χ^2 such that

$$\chi^2_{(\alpha_1)}/\chi^2_{(\alpha_2)} = 1 + m\sigma_2^2/\sigma_1^2 \ldots (1).$$

Where α_1 and α_2 have the probability values of 0·05 and 0·95 respectively, the $\chi^2_{(0\cdot05)}$ and $\chi^2_{(0\cdot95)}$ values for any number of degrees of freedom can be obtained from the statistical tables.

Following equation (1) and for a given number of families the optimal family size for, say, Test 1a will be

$$m = \{(\chi^2_{(0\cdot05)}/\chi^2_{(0\cdot95)} \text{ for } n \text{ d.f.}) - 1\} \times (\sigma_1^2/\sigma_2^2).$$

The total experimental size (3 nm) will, then, be given by

$$3n\{(\chi^2_{(0\cdot05)}/\chi^2_{(0\cdot95)} \text{ for } n \text{ d.f.}) - 1\}(\sigma_1^2/\sigma_2^2).$$

Thus the size of the experiment is theoretically linked to the magnitudes of three quantities, namely, $3n$, $(\chi^2_{(0\cdot05)}/\chi^2_{(0\cdot95)} - 1)$ and σ_1^2/σ_2^2. Quantity $3n$ is linearly related to n whereas $(\chi^2_{(0\cdot05)}/\chi^2_{(0\cdot95)} - 1)$ has a negative but curvilinear relationship with the number of families. The product

$$3n(\chi^2_{(0\cdot05)}/\chi^2_{(0\cdot95)} - 1)$$

in fact has a minimum and at an intermediate value of n.

The magnitudes of

$$T_1 (= 3n(\chi^2_{(0\cdot05)}/\chi^2_{(0\cdot95)} \text{ (for } n \text{ d.f.}) - 1))$$

and

$$T_2 (= 3n(\chi^2_{(0\cdot05)}/\chi^2_{(0\cdot95)} \text{ (for } n-1 \text{ d.f.}) - 1)$$

have, therefore, been worked out for various tests and for n equals 3 to 80. The results obtained are listed in table 2. For Test 1a and Test 1b $n = 12$ while for Test 2 $n = 15$ for the smallest values of T_1 and T_2 respectively.

The third unknown quantity required to calculate the experimental sizes is σ_1^2/σ_2^2. Our interest lies in this ratio rather than in the absolute magnitudes of σ_1^2 and σ_2^2. The relative magnitudes of genetic and non-genetic components involved in the expectations of σ_1^2 and σ_2^2 can be presented therefore as proportions which can be derived from the interrelationships of these components. The commonest relationships in biometrical genetics are heritability (h_n^2) and dominance ratio. The proportionate values of the additive, dominance and environmental components can thus be obtained for varying genetic situations by changing h_n^2 and the dominance ratio while keeping the total phenotypic variance as unity in the absence of epistasis.

There are no corresponding relationships involving epistasis. One reason is the large number of possible relationships and another is the non-availability of proper estimates of various epistatic components of variance. However, some useful relationships between epistatic and non-epistatic effects are theoretically possible because of the way the various epistatic components are defined, but for our present purposes we are less interested in defining these relationships than obtaining a realistic range of relative values for the epistatic components. We therefore, chose the relationships:

$$\Sigma i_{jk}^2 = \tfrac{1}{10}\Sigma d_j^2, \quad \Sigma j_{jk}^2 = \tfrac{1}{10}(\Sigma d_j^2 + \Sigma h_j^2)$$

and

$$\Sigma l_{jk}^2 = \tfrac{1}{10}\Sigma h_j^2.$$

These epistatic components together with Σd_j^2, Σh_j^2 and E were calculated for the following combinations of heritability and dominance ratio.

$$h_{(n)}^2 = 0\cdot25,\ 0\cdot50,\ 0\cdot75$$
$$\text{dominance ratio} = 0\cdot25,\ 0\cdot50,\ 0\cdot75,\ 1\cdot00$$

K, the number of genes segregating for a character was taken to be 10 and 'r' the coefficient of gene association, was allotted the values of 0·0, 0·2, 0·4, 0·6, 0·8 and 1·0.

TABLE 2

The expected values of T_1* *and* T_2† *for different number* (n) *of families to be raised in an experiment*

n	*T_1	†T_2	n	T_1	T_2	n	T_1	T_2
3	190·82	514·49	11	108·92	120·33	19	112·83	118·24
4	148·13	254·42	12	*108·84*	118·83	20	113·68	118·77
5	130·02	185·17	13	109·02	117·91	30	123·03	127·13
6	120·63	156·03	14	109·38	117·40	40	132·40	135·71
7	115·32	140·73	15	109·91	*117·20*	50	141·27	144·10
8	112·17	131·79	16	110·52	117·24	60	149·60	152·09
9	110·39	126·20	17	111·24	117·43	70	157·44	159·69
10	109·39	122·65	18	112·02	117·78	80	164·90	166·94

$$* \quad T_1 = 3n\left(\frac{\chi^2(0{\cdot}05)}{\chi^2(0{\cdot}95)} \text{ (for } n \text{ d.f.)} - 1\right)$$

$$\dagger \quad T_2 = 3n\left(\frac{\chi^2(0{\cdot}05)}{\chi^2(0{\cdot}95)} \text{ (for } n-1 \text{ d.f.)} - 1\right)$$

TABLE 3

Minimum experimental sizes for detecting epistasis from a TTC involving F_2 *individuals*

h_n^2	Dominance ratio	Degree of gene association in the tester parents: 0·00	0·20	0·40	0·60	0·80	1·00
0·25	0·25	91380	124100	55655	10311	2932	1125‡
		65792	89581	40487	7598	2200	864
	0·50	87745	108686	49103	10102	2957	1144
		58328	72488	33077	6919	2073	826
	0·75	82688	91625	41746	9808	3003	1179
		48454	53911	24865	5962	1879	765
	1·00	77185	77004	35339	9466	3061	1228
		38258	38355	17861	4902	1640	687
0·50	0·25	49931	67748	30299	5587	1578	600
		24342	33229	15132	2875	846	339
	0·50	49750	61549	27707	5665	1644	628
		20332	25351	11680	2482	759	311
	0·75	49326	54583	24767	5778	1752	679
		15093	16870	7887	1933	627	265
	1·00	48689	48508	22168	5896	1887	746
		9762	9859	4691	1332	466	206
0·75	0·25	36115	48963	21847	4013	1127	425
		10526	14444	6670	1300	395	—
	0·50	37085	45837	20574	4186	1206	456
		7667	9639	4548	1003	322	—

— The expected minimal experimental size is of less than 200 individuals.

‡ Upper and lower figures represent the experimental sizes required to detect complementary and duplicate epistasis respectively.

The minimal experimental sizes were only computed for the relationship between i, j and l consistent with duplicate and complementary epistasis since non-allelic interactions in general can only be classified into these two types for quantitative traits (Jinks and Jones, 1958). The figures obtained have been tabulated in tables 3, 4 and 5. Before we draw any conclusions from these theoretical results we shall test the applicability of some of the assumptions in a practical solution.

TABLE 4

Minimum experimental sizes for detecting epistasis from a TTC involving F∞ inbreds

h_n^2	Dominance ratio	Degree of gene association in the tester parents: 0·00	0·20	0·40	0·60	0·80	1·00
0·25	0·25	56402	54750	21948	5660	1851	760‡
		53263	51702	20726	5344	1748	718
	0·50	43428	39692	17730	5036	1731	726
		40210	36751	16046	4663	1602	672
	0·75	31093	26924	12716	4231	1555	674
		27823	24092	11379	3786	1392	603
	1·00	21908	18256	9128	3423	1352	609
		18622	15518	7759	2910	1150	517
0·50	0·25	20916	20303	8139	2099	687	282
		17777	17256	6917	1784	584	240
	0·50	15769	14413	6293	1829	628	264
		12552	11472	5009	1456	500	210
	0·75	10870	9413	4446	1479	544	236
		7590	6581	3108	1034	380	—
	1·00	7214	6012	3006	1127	445	200
		3929	3274	1637	614	243	—
0·75	0·25	9088	8821	3536	912	298	—
		5948	5774	2315	597	—	—
	0·50	6550	5987	2614	760	261	—
		3332	3046	1330	386	—	—

— and ‡ as for Table 3.

4. THE EXPERIMENT

The experiment involved 80 inbreds each produced by consecutive selfing to F_{11} of a single randomly chosen F_2 individual from a cross between varieties 1 and 5 of *Nicotiana rustica* (Mather and Vines, 1952). Individual plants from each of these inbred families were selfed and crossed to P_1, P_5 and their F_1 to produce P_i, L_{1i}, L_{2i} and L_{3i} families. Ten replicates were raised for each of these 80×4 families and the material was grown as a part of a larger experiment conducted during the summer of 1973. Single plant randomisation was practised and all the plants were scored individually for the following morphological characters.

1. Height (cm) of individual plants; 2 weeks (H_1), 4 weeks (H_2) and 6 weeks (H_3) after planting in the field.
2. Number of days taken to flower from 1st June (FT).

3. Height (cm) at flowering time (HFT).
4. Corolla length (cm) at the time of flowering (CL).
5. Stamen stigma heights (relative) of the first (open) flower (SSP).
6. Leaf length (cm) of the largest leaf (blade) at the time of flowering (LL).
7. Leaf width (cm) of the largest leaf (blade) at the time of flowering (LW).
8. Plant diameter (cm) across the pair of leaves involving the largest leaf (LS).
9. Final height (cm) at the end of the season (FH).

TABLE 5

Minimum experimental sizes for detecting complementary or duplicate epistasis from an experiment using L_{1i}, L_{2i} and inbred scores

h_n^2	Dominance ratio	Degree of gene association in the tester parents					
		0·00	0·20	0·40	0·60	0·80	1·00
0·25	0·25	1615	1680	1907	2464	4165	37111
	0·50	1326	1380	1573	2052	3578	80862
	0·75	1001	1043	1191	1562	2766	*
	1·00	719	749	856	1124	1998	*
0·50	0·25	527	548	622	804	1359	12110
	0·50	403	420	479	625	1089	24610
	0·75	265	276	315	413	731	80862
	1·00	—	—	—	225	400	*
0·75	0·25	—	—	—	251	424	3776
	0·50	—	—	—	—	259	5860

— As for Table 3.

* Situations for which the experimental sizes cannot be estimated because, theoretically, the epistatic component of variation will be 0·0 with $h_j \simeq d_j$ at each locus.

The data were processed through the university's 1906A Computer to test for the presence of epistasis using Test 1b and Test 2. The results are tabulated in table 6.

We noted earlier from table 2 that Test 1b and Test 2 demand n to be 12 and 15 respectively to give the smallest possible experiment for detecting the presence of epistasis. In practice, however, the total experimental sizes may not vary much even if n varies between 9 and 16 for the first case and between 13 and 18 for the second. But an experiment which involves raising L_{1i}, L_{2i} and L_{3i} or P_i families from 80 inbreds is expected to require a much larger experimental size to detect epistasis with the same precision as an experiment based on 16 inbreds.

The inbreds involved in this experiment, as explained previously, are a random sample of F_{11} inbred lines which could be produced from an F_2 cross 1×5 (Perkins and Jinks, 1973). These 80 families are numbered in the order of the random field positions occupied by the F_2 plants from which they were derived. Their numbering is, therefore, at random with respect to their origin and performance. Successive sets of 16 inbreds can, therefore, be regarded as independent random samples of pure breeding lines drawn from

all possible inbreds extractable from the 1×5 cross. In this way, the experiment can be split into five small experiments of equal size each with $n = 16$ and $m = 10$. Similarly two successive replicates can be allocated from each of the families raised from all 80 inbreds to give five independent experiments with $n = 80$ and $m = 2$. This random sub-division of the total

TABLE 6

Tests of significance for the presence of epistatic variation

		Test 1b			Test 2		
Character	Item	d.f.	M.S.	$\chi^2_{(80)}$	d.f.	M.S.	$\chi^2_{(79)}$
H_1	Epistasis	80	0·71	150·0***	79	0·97	201·7***
	Error	2155	0·36		2155	0·38	
H_2	Epistasis	80	5·35	147·2***	79	7·97	225·1***
	Error	2155	2·91		2155	2·80	
H_3	Epistasis	80	32·35	141·0***	79	47·04	221·5***
	Error	2155	18·36		2155	16·78	
FT	Epistasis	80	4·35	119·2**	79	7·08	194·2***
	Error	2155	2·92		2155	2·88	
HFT	Epistasis	80	22·18	91·7 N.S.	79	30·10	137·1**
	Error	2155	19·34		2155	17·32	
CL	Epistasis	80	0·0013	117·6**	79	0·0017	193·4***
	Error	2155	0·0009		2155	0·0007	
SSP	Epistasis	80	0·0412	76·6 N.S.	79	0·0474	103·3*
	Error	2155	0·0430		2155	0·0363	
LL	Epistasis	80	0·73	81·1 N.S.	79	1·24	155·7***
	Error	2155	0·94		2155	0·63	
LW	Epistasis	80	0·97	82·4 N.S.	79	1·52	151·9***
	Error	2155	0·94		2155	0·79	
LS	Epistasis	80	4·66	81·4 N.S.	79	7·73	153·8***
	Error	2155	4·58		2155	3·97	
FH	Epistasis	80	27·72	91·0 N.S.	79	43·70	174·5***
	Error	2155	24·38		2155	19·78	

N.S. $P > 0{\cdot}05$; * $P = 0{\cdot}05$-$0{\cdot}01$; ** $P = 0{\cdot}001$-$0{\cdot}01$; *** $P < 0{\cdot}001$.

data allows us to compare the results obtained from an experiment with $n = 16$ with those from the one with $n = 80$ within a constant total experimental size.

The analyses were carried out for all the characters but the full details will only be reported for characters H_2 (table 7a)and FT (table 7b) as these characters are known to take higher (nearer to 0·75) h_n^2 values (Eaves and Brumpton, 1972) and show a highly significant epistatic component (table 6) of variation on both tests.

The results for the remaining characters are summarised in table 8. The three probability classes represent significant ($P \leqq 0{\cdot}05$), near significant ($0{\cdot}09 \geqq P \geqq 0{\cdot}06$) and non-significant ($1{\cdot}0 \geqq P \geqq 0{\cdot}1$) contribution of epistasis to the genetical variation (based on the calculated χ^2 value for a test) and the figures given against each of these classes describe the number of experiments (out of a total of five) falling within that probability class for that particular character.

5. Discussion

Experimental sizes given in tables 3, 4 and 5 are generally large and they are not only linked to the changing magnitude of epistasis but also to its (classical) type and the degree of gene association in the tester parents. So closely is the detection of epistasis (by Test 1a and Test 1b) tied to gene association that most of the experiments are impractically large except when 'r' is greater than 0·6 or unless the heritability is exceptionally high.

Table 7

Results from tests for epistasis after splitting the data into 5 experiments

(*a*) *Developmental Height* H_2

	Set 1		Set 2	
Expt. no.	Test 1b $\chi^2_{(80)}$	Test 2 $\chi^2_{(79)}$	Test 1b $\chi^2_{(16)}$	Test 2 $\chi^2_{(15)}$
I	102·45*	135·45***	19·30 N.S.	28·44*
II	76·03 N.S.	98·23 N.S.	48·35***	59·33***
III	138·25***	108·15*	17·94 N.S.	31·56 **
IV	100·56 N.S.	124·32***	21·04 N.S.	49·97***
V	101·40*	137·06***	30·65*	43·16***

(*b*) *Flowering time FT*

	Set 1		Set 2	
Expt. no.	Test 1b $\chi^2_{(80)}$	Test 2 $\chi^2_{(79)}$	Test 1b $\chi^2_{(16)}$	Test 2 $\chi^2_{(15)}$
I	90·87 N.S.	118·31**	20·95 N.S.	26·47*
II	92·89 N.S.	117·60**	39·97***	49·71***
III	118·56**	101·11*	23·87 N.S.	32·36**
IV	86·63 N.S.	114·81**	11·02 N.S.	39·38***
V	105·76*	111·25*	18·39 N.S.	34·91**

N.S. P > 0·05; * P = 0·05-0·01; ** P = 0·01-0·001; *** P < 0·001.

The abrupt change in the efficiency of these tests even when heritability is low can be attributed largely to the change in the magnitude of σ_2^2 component which includes $\frac{1}{4}[i]^2$ as a part of its theoretical expectation. With 'r' less than 0·5, $\frac{1}{4}[i]^2$ $\left(\text{which when expanded is } \frac{1}{4}\left[\frac{Kr^2-1}{K-1}\Sigma i_{jk}\right]^2\right)$ theoretically takes a value nearer to zero. With increasing values of 'r' above 0·5 any increase in the linear value of $[i]$ increases the σ_2^2 value quadratically. On the other hand, no corresponding changes in the magnitudes of Σl_{jk}^2 and Σj_{jk}^2 occur because they are not influenced in the same way by alteration in the 'r' value. It is therefore quite evident that larger experiments would be required to pick up these effects particularly when Test 1a or Test 1b is applied. Furthermore Σl_{jk}^2 has little chance of influencing the outcome of the results from Test 1a because of its relatively small coefficient until and unless a disproportionately large portion of the epistatic variation is of this

kind. But this is not so for Test 1b where its coefficient takes the same value as those of the other components.

The experimental sizes required for Test 1a and Test 1b to detect duplicate epistasis are much smaller than those needed to detect complementary interactions even though the magnitude of the epistasis was kept theoretically

TABLE 8

Summary of the results from the tests of epistasis for the remaining characters in a five-experiment situation

Character	Probability of $\chi^2_{(n)}$ value	Number of experiments: Set 1, Test 1b	Set 1, Test 2	Set 2, Test 1b	Set 2, Test 2
H_1	1·00 – 0·10	2	1	2	0
	0·09 – 0·06	0	1	1	0
	≦ 0·05	3	3	2	5
H_3	1·00 – 0·10	3	0	2	1
	0·09 – 0·06	0	1	2	0
	≦ 0·05	2	4	1	4
HFT	1·00 – 0·10	4	3	4	2
	0·09 – 0·06	0	0	1	1
	≦ 0·05	1	2	0	2
CL	1·00 – 0·10	4	3	3	1
	0·09 – 0·06	1	0	0	0
	≦ 0·05	0	2	2	4
SSP	1·00 – 0·10	4	4	4	2
	0·09 – 0·06	1	0	1	1
	≦ 0·05	0	1	0	2
LL	1·00 – 0·10	4	4	4	2
	0·09 – 0·06	0	0	1	0
	≦ 0·05	1	1	0	3
LW	1·00 – 0·10	4	3	4	1
	0·09 – 0·06	0	0	0	1
	≦ 0·05	1	2	1	3
LS	1·00 – 0·10	3	1	4	2
	0·09 – 0·06	0	2	1	0
	≦ 0·05	2	2	0	3
FH	1·00 – 0·10	3	2	4	0
	0·09 – 0·06	1	0	1	1
	≦ 0·05	1	3	0	4

the same. This is as expected since complementation is expected to increase the variance of the population in such a way that the σ_1^2/σ_2^2 ratio is increased and hence the total experimental size required to detect epistasis is also increased. Duplicate genes, on the contrary, reduce the differences between genotypes making the population curve kurtotic and therefore σ_1^2/σ_2^2 takes a smaller value leading to a smaller experimental size.

Experiments required to achieve the same level of precision by Test 2 are not so large and are practicable except for low heritabilities and very high 'r' values. The decrease in the sensitivity of this test for 'r' = 1·0 can be

attributed to the coefficient $\left(-\frac{Kr^2-1}{K-1}\right)$ of the cross products $[\frac{1}{2}\Sigma i_{jk}l_{jk}+\Sigma j_{jk}j_{jk}]$ which will take a value nearer to -1 and hence effectively neutralise the contributions of the main epistatic effects to σ_2^2. This test however does not require different experimental sizes to detect complementary and duplicate types of epistasis.

Both tests (Test 1b and Test 2) consistently detect the presence of epistasis for only five of the eleven characters studied. These characters include flowering time and various height measurements which have already been comfirmed as having epistasis as a minor component of their genetic variation (Jinks and Perkins, 1969; Jinks and Perkins, 1970). There is, therefore, little doubt that epistasis is a part of the genetical architecture of these characters and that in this experiment it has been correctly detected by both the tests. The situation is however complicated for the remaining characters for which Test 2 detects significant epistasis but Test 1b is not sensitive enough to detect them. For most of these characters, however, there is no independent evidence which can be used to either support or reject the above conclusion. But for Final Height, which has been extensively studied in the 1×5 cross, there is previous evidence of a low level of predominantly duplicate epistasis (Jinks and Perkins, 1969). The failure of Test 1b to detect non-allelic interactions for these characters is, therefore, probably due to the experimental size being too small. The size of experiment required to pick up epistasis for final height when Test 1b is applied can be estimated from its heritability, dominance ratio and 'r' value.

Final height, together with other height measurements and flowering time, is a highly heritable character (Eaves and Brumpton, 1972). Most of the genes controlling this character ($K \simeq 9$) are dispersed between P_1 and P_5 ('r' $\simeq 0{\cdot}2$; Jinks and Perkins, 1972; Eaves and Brumpton, 1972) and the increasing alleles at most of the loci are partially dominant ($\sqrt{H/D} \simeq 0{\cdot}25$) to the corresponding decreasing alleles. On the basis of this information, it can be readily seen from table 4 that an experiment with 5774 individuals would be required for Test 1b to detect epistasis of the level specified in Section 3. Test 2 however would require less than 200 individuals to detect epistasis of similar magnitude under these conditions. The present experiment incorporates 2400 individuals, a number considerably smaller than 5774 and this is most probably the main reason for the failure of Test 1b to detect significant epistasis for some of the characters. On the other hand, the present experiment is at least twelve times larger than the one required by Test 2 and that is presumably why it has detected highly significant epistasis for all except one of the characters studied.

An important theoretical prediction which it would be useful to verify in practice is whether we require smaller experiments to detect epistasis when n is kept between say 10 and 18. It looks as though it is true for Test 2 which detects significant epistasis for H_2 and FT although the experimental size is down to 480 individuals only. It also detects significant or nearly significant epistasis for 34 out of a total of 45 tests carried out on the rest of the characters. The only cases where it fails to detect non-allelic interactions is where the experiment is smaller than is required theoretically.

Equally, the sensitivity of the test for detecting epistasis should decrease

with the increase in n value within a fixed experimental size. And this is shown when we compare the results described previously with those obtained by applying Test 2 (table 7, Set 1) for $n = 80$. Here, it is quite apparent that the average probability of $\chi^2_{(79)}$ has increased and hence the significance of epistasis has decreased as compared to the average probability of $\chi^2_{(15)}$ and for one experiment the $\chi^2_{(79)}$ is not significant. Overall, the $\chi^2_{(79)}$ test is able to pick up significant or near significant epistasis on only 24 out of 45 occasions for the remaining characters.

The results obtained from Test 1b show epistasis to be relatively unimportant for all the characters. This is expected because 480 is a much smaller experimental size than the one theoretically required for this test to detect non-allelic interactions at the level specified (Section 3). Hence the expected decrease in the sensitivity of the test is observed. Furthermore, there is not much difference between the results obtained from the $n = 16$ and $n = 80$ samples and it looks, therefore, as though the $n = 16$ sample is not appreciably more sensitive when experimental size is too small. In general, however, it can be concluded that Test 2 is more efficient than Test 1b under the present circumstances.

6. Practical implications

It is indeed significant that the optimal experimental sizes required to detect epistasis depend largely on the gene dispersion in the tester parents. This makes the sensitivity of a test conditional on the 'r' value and therefore it is possible to lower the minimum limit of the total experimental size by deliberately selecting the tester genotypes. In this way the presence of epistasis can possibly be tested with some certainty, even for the least heritable characters, without conducting particularly large experiments. However, the reduction in experimental sizes for Test 1a and Test 1b can only be achieved if Σi_{jk} is neither absent nor completely ambidirectional. For Test 2, the absence or complete ambidirectional nature of any but not all of the four epistatic components is expected to reduce the experimental size required because, as a consequence, the total effects of the cross-product terms will be considerably reduced (see table 1).

Another major factor which influences the optimal size of the experiment is heritability. The lower the heritability of a character the larger is the size of the experiment required to detect epistasis for that trait. This is because the statistical reliability of the estimates of various genetic parameters is reduced as a result of the masking effects of environmental variation. Therefore, more individuals will be required to restore the accuracy of these estimates. The opposite is of course true for highly heritable traits because the information required to detect epistasis can be easily obtained from relatively few individuals. It will, therefore, be of some help to know the heritability of a character for which the test of epistasis is being planned and such information is sometimes readily available if the material under investigation was extensively studied previously. If, however, a direct estimate of heritability is unavailable, a conservative test of epistasis can be planned and the required experimental size can be obtained by assigning values at the lower end of the range of heritability, dominance ratio and epistasis.

The size of experiment required ultimately depends on the magnitude

and the type of epistasis prevailing in the material. Most of the experimental sizes given in tables 3, 4 and 5 are impracticable and would be unjustified by the level of epistasis present and its importance as a source of variation. However, it is known that relatively smaller experiments would be required to detect epistasis of larger magnitude and if dominance and epistasis are equally important, the experimental sizes required to detect dominance are adequate to detect epistasis as well (see Kearsey, 1970).

Complementary epistasis generally requires larger experiments for its significant detection than duplicate epistasis and the differences are more prominent in low than in medium or high heritability situations. It would be better, therefore, to plan an experiment for the detection of complementary epistasis as this will also be adequate to detect duplicate epistasis, if present.

None of the tests for epistasis is preferable to all others in all circumstances. Test 1b and Test 2 cannot be applied to an F_2 population while biometrical geneticists and practical breeders working with diallel populations will be tempted to use Test 2 rather than Text 1b because of the extra work involved with the latter. However, Test 1b always requires smaller optimal experiments as compared to Test 1a and therefore should be preferred over the latter wherever possible. If 'r' $< 0{\cdot}8$, it will always be advantageous to use Test 2 whereas with extreme genotypes as tester parents, maximum information about the epistasis can be extracted by using either Test 1a or Test 1b.

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