COMPARATIVE ANALYSIS OF ASSOCIATION AND DISPERSION CROSSES TO DETECT LINKAGE AND EPISTATIC COMPONENTS OF VARIATION

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SUMMARY

In theory, a matched pair of crosses in which the pure breeding parents display association and dispersion for the same alleles at the same loci provides sensitive tests for the presence of complex sources of variation. Thus comparison of the genetical component of variation within the F_2 and first backcross families of such a pair of crosses can detect and distinguish between non-allelic interaction, a linkage disequilibrium of non-interacting genes and a linkage disequilibrium of interacting genes.

These theoretical expectations are confirmed by analyses of two sets of matched association and dispersion pairs of crosses involving pure breeding varieties 1 and 5, and 2 and 12 of *Nicotiana rustica* and pure breeding selections for final height extracted from crosses between them by single seed descent. Furthermore, these analyses show that comparison of the genetical components of the variation within F_2 , B_1 and B_2 families of matched association and dispersion crosses are more sensitive to non-allelic interaction and linkage disequilibrium than much larger and more complex experiments based on a single cross and analysed by conventional model fitting procedures.

1. INTRODUCTION

ALTHOUGH the means of the parents, P_1 and P_2 and of the first backcrosses, B_1 and B_2 are dependent on whether the genes are associated ($r_d > 0$) or dispersed ($r_d = 0$) in the parents, the F_1 mean, the F_2 mean and the heritable variation within F_2 families (V_{1F_2}) and first backcross families after summing ($V_{1B_1} + V_{1B_2}$) are not (Mather, 1949). This is still true for the F_1 mean and the F_2 mean and variance in the presence of non-allelic interaction (Mather and Jinks, 1971). If, however, the association and dispersion lead to linkage disequilibrium in the segregating generations, in the former because of an excess of coupling linkages and in the latter because of an excess of repulsion linkages, V_{1F_2} and ($V_{1B_1} + V_{1B_2}$) will differ between the two kinds of crosses even in the absence of non-allelic interaction. Both V_{1F_2} and ($V_{1B_1} + V_{1B_2}$) will then be greater for the association than for the dispersion cross (Mather and Jinks, 1971).

Although these relationships are well established in theory only recently have crosses become available in which the two pairs of parents display association and dispersion for the same alleles at the same loci (Jayasekara and Jinks, 1976; Pooni and Jinks, 1981). Furthermore, the joint effects of linkage and non-allelic interaction have been theoretically explored and demonstrated in practice only in respect of family means (Jinks and Perkins, 1969; Jinks, 1978). In this paper we extend these theoretical expectations to the variances, and analyse data from the two sets of association and dispersion crosses between pure breeding lines of *Nicotiana rustica*

previously reported, to show how they may be used to detect non-allelic interaction and a linkage component of variation and to distinguish between them.

2. THEORY AND METHOD

We shall illustrate the theory with two loci each with two alleles A, a ; B, b . The associated pair of pure breeding parents are then $P_1 AABB$ and $P_2 aabb$, while the corresponding dispersed pair are $P_1 AA bb$ and $P_2 aa BB$. In table 1 are summarised the expectations for V_{1F2} and $(V_{1B1} + V_{1B2})$ for the association and the dispersion crosses for four situations:

1. Linkage equilibrium and no non-allelic interaction (Mather 1949)
2. Linkage disequilibrium and no non-allelic interaction (Mather 1949)
3. Linkage equilibrium and non-allelic interaction (Mather 1967, Mather and Jinks 1971)

and

4. Linkage disequilibrium and non-allelic interaction derived by the authors.

By comparing the corresponding variances of the association and dispersion crosses we can recognise the following relationships:

(i) V_{1F2} and $(V_{1B1} + V_{1B2})$ do not differ between the association and dispersion crosses, in which case neither a linkage disequilibrium nor non-allelic interaction is making a significant contribution.

(ii) V_{1F2} does not differ but $(V_{1B1} + V_{1B2})$ does differ between the association and dispersion crosses, in which case non-allelic interaction is making a significant contribution but there is no evidence of a linkage disequilibrium.

(iii) Both V_{1F2} and $(V_{1B1} + V_{1B2})$ differ between the association and dispersion crosses in which case there is a linkage disequilibrium. If both V_{1F2} and $(V_{1B1} + V_{1B2})$ are greater for the association cross than for the dispersion cross and by an equal amount, there is no evidence for an involvement of non-allelic interaction. If, however, for one or both variances the association cross is smaller than the dispersion cross or V_{1F2} and $(V_{1B1} + V_{1B2})$ differ by unequal amounts there is evidence for the presence of non-allelic interaction also.

In theory, therefore, we can distinguish between all four situations in table 1 simply by comparing the heritable components of V_{1F2} and $(V_{1B1} + V_{1B2})$ from corresponding association and dispersion crosses. Potentially it is a powerful experimental design that can in theory separate contributions to variation that much more complex experimental designs have failed to resolve unambiguously (Opsahl, 1956; Perkins and Jinks, 1970; Mather and Jinks, 1971, 1982). Furthermore, with the growing availability of large random samples of pure breeding lines extracted from crosses by single seed descent or dihaploidy the opportunities for using the design are now becoming available.

Where non-allelic interactions have a net directional effect they can be detected by very sensitive tests applied to the family means of the basic generations (Jinks, 1956; Jinks and Jones, 1958, Mather and Jinks, 1971). Linkage disequilibrium, however, can only be detected by an analysis of means if they involve interacting genes which are themselves making a net directional contribution and their detection requires in addition to those

TABLE 1

Genetical expectations of the F_2 variance and the pooled backcross variances for a pair of alleles at each of two loci which are associated in one cross and dispersed in the other in the absence and presence of non-allelic interactions and linkage disequilibrium

	Pair of genes in dispersion		Pair of genes in association	
	(a) F_2 variance (V_{1F2})			
1. Linkage equilibrium and epistasis absent	$\frac{1}{2}d_a^2 + \frac{1}{2}d_b^2 + \frac{1}{4}h_a^2 + \frac{1}{4}h_b^2$		$\frac{1}{2}d_a^2 + \frac{1}{2}d_b^2 + \frac{1}{4}h_a^2 + \frac{1}{4}h_b^2$	
2. Linkage disequilibrium and epistasis absent	$(1) - (1 - 2p)d_ad_b + \frac{1}{2}(1 - 2p)^2h_ah_b$		$(1) + (1 - 2p)d_ad_b + \frac{1}{2}(1 - 2p)^2h_ah_b$	
3. Linkage equilibrium and epistasis present	$(1) + \frac{1}{4}i_{ab}^2 + \frac{1}{4}(j_{ab}^2 + j_{ba}^2) + \frac{3}{16}i_{ab}^2 + \frac{1}{2}d_{ab}d_{ab} + \frac{1}{2}d_{ba}d_{ba} + \frac{1}{4}h_{ab}^2 + \frac{1}{4}h_{ba}^2$		$(1) + \frac{1}{4}i_{ab}^2 + \frac{1}{4}(j_{ab}^2 + j_{ba}^2) + \frac{3}{16}i_{ab}^2 + \frac{1}{2}d_{ab}d_{ab} + \frac{1}{2}d_{ba}d_{ba} + \frac{1}{4}h_{ab}^2 + \frac{1}{4}h_{ba}^2$	
4. Linkage disequilibrium and epistasis present	$(2) + \frac{1}{4}i_{ab}^2 + p(1 - p)(j_{ab}^2 + j_{ba}^2) + (\frac{1}{4} - p^2)(1 - p^2)i_{ab}^2 + 2p(1 - p)(d_{ab}i_{ab} + d_{ba}i_{ba}) + \frac{1}{2}(1 - 2p)^2(l_{ab}(h_a + h_b)) + \frac{1}{2}(1 - 2p)(i_{ab}(h_a + h_b)) + \frac{1}{2}(1 - 2p)(1 - 2p)(1 - 2p)^2i_{ab}i_{ab}$		$(2) + \frac{1}{4}i_{ab}^2 + p(1 - p)(j_{ab}^2 + j_{ba}^2) + (\frac{1}{4} - p^2)(1 - p^2)i_{ab}^2 + 2p(1 - p)(d_{ab}i_{ab} + d_{ba}i_{ba}) + \frac{1}{2}(1 - 2p)^2(l_{ab}(h_a + h_b)) - \frac{1}{2}(1 - 2p)(i_{ab}(h_a + h_b)) - \frac{1}{2}(1 - 2p)(1 - 2p)(1 - 2p)^2i_{ab}i_{ab}$	
1. Linkage equilibrium and epistasis absent	$\frac{1}{2}d_a^2 + \frac{1}{2}d_b^2 + \frac{1}{4}h_a^2 + \frac{1}{4}h_b^2$	(b) B_1 variance + B_2 variance ($V_{1B1} + V_{1B2}$)	$\frac{1}{2}d_a^2 + \frac{1}{2}d_b^2 + \frac{1}{4}h_a^2 + \frac{1}{4}h_b^2$	
2. Linkage disequilibrium and epistasis absent	$(1) - (1 - 2p)d_ad_b + (1 - 2p)h_ah_b$		$(1) + (1 - 2p)d_ad_b + (1 - 2p)h_ah_b$	
3. Linkage equilibrium and epistasis present	$(1) + \frac{3}{8}i_{ab}^2 + \frac{1}{8}(j_{ab}^2 + j_{ba}^2) + \frac{3}{8}i_{ab}^2 + \frac{1}{2}(d_{ab}i_{ab} + d_{ba}i_{ba}) + \frac{1}{4}(d_{ab}i_{ba} + d_{ba}i_{ab}) + \frac{1}{2}i_{ab}(h_a + h_b) + \frac{1}{2}i_{ba}(h_a + h_b) + \frac{1}{4}i_{ab}i_{ab} - \frac{1}{4}i_{ab}i_{ba}$		$(1) + \frac{3}{8}i_{ab}^2 + \frac{1}{8}(j_{ab}^2 + j_{ba}^2) + \frac{3}{8}i_{ab}^2 + \frac{1}{2}(d_{ab}i_{ab} + d_{ba}i_{ba}) - \frac{1}{4}(d_{ab}i_{ba} + d_{ba}i_{ab}) - \frac{3}{2}i_{ab}(h_a + h_b) + \frac{1}{2}i_{ba}(h_a + h_b) - \frac{1}{4}i_{ab}i_{ab} + \frac{1}{4}i_{ab}i_{ba}$	
4. Linkage disequilibrium and epistasis present	$(2) + \frac{1}{2}(1 - p^2)i_{ab}^2 + \frac{1}{2}p^2(j_{ab}^2 + j_{ba}^2) + \frac{1}{2}(1 - p^2)i_{ab}^2 + p(d_{ab}i_{ab} + d_{ba}i_{ba}) + p(d_{ab}i_{ba} + d_{ba}i_{ab}) + (1 - p)(i_{ab}(h_a + h_b)) + (1 - p)(i_{ba}(h_a + h_b)) + (1 - p)i_{ab}i_{ab} - p i_{ab}i_{ba}$		$(2) + \frac{1}{2}(1 - p^2)i_{ab}^2 + \frac{1}{2}p^2(j_{ab}^2 + j_{ba}^2) + \frac{1}{2}(1 - p^2)i_{ab}^2 + p(d_{ab}i_{ab} + d_{ba}i_{ba}) - p(d_{ab}i_{ba} + d_{ba}i_{ab}) - (1 - p)(i_{ab}(h_a + h_b)) + (1 - p)(i_{ba}(h_a + h_b)) - (1 - p)i_{ab}i_{ab} + p i_{ab}i_{ba}$	

of the basic generations the means of the families of a triple test cross (Jinks and Perkins, 1969; Jinks, 1978). Analyses of means are, therefore, no substitute for an analysis of the variances for detecting non-allelic interaction and linkage contributions to variation. Nevertheless we shall apply the relevant tests to the family means, some of which have already been published, to guide us in the interpretation of the tests on the variances.

3. EXPERIMENTAL DATA

The data come from two sets of association and dispersion crosses. The first set includes the P_1 , P_2 , F_1 , F_2 , B_1 and B_2 families of the cross between varieties V1 and V5 of *Nicotiana rustica* and between two of the B inbred lines B2 and B35. The allelic differences for final plant height and flowering time are largely dispersed between V1 and V5 while B2 and B35 are the tallest and shortest among a random sample of 82 highly inbred lines derived by single seed descent from the F_2 of the V1 $\times$ V5 cross; while they are not the latest and earliest flowering of these 82 lines they are more extreme than V1 and V5 (Jinks and Perkins, 1972). B2 and B35 should, therefore, differ for the same alleles at the same loci as V1 and V5 but these alleles should now be strongly associated for final height and relatively more associated for flowering time. The degrees of association-dispersion (r_d) for these pairs of parents for the two characters as estimated from their average phenotypes over 15 environments by the method of Jayasekara and Jinks (1976) are as follows:

	V1 $\times$ V5	B2 $\times$ B35
Final height	0.26	1.00
Flowering time	0.13	0.54

One thousand individually randomised plants were raised for each of the two crosses in 1973 as part of an experiment which also included a triple test cross of the 82 B inbred lines using V1, V5 and their F_1 as the testers. The design used was that of Jinks and Perkins (1969) for equalising the amounts of information about the family means. Further details are given by Jayasekara and Jinks (1976) and Pooni *et al* (1978). The success of this design in equalising the standard errors of the family means can be seen in table 2 where they are listed. The corresponding within family variances are given in table 3.

The second set of families of the basic generations comes from the cross between pure breeding varieties V2 and V12 and between the D inbreds D10 and D17. The cross of V2 and V12 displays a high level of non-allelic interaction and produces the most heterotic F_1 (Jinks, 1954; Jinks and Jones, 1958). The inbred lines D10 and D17 are again the extreme phenotypes among a random sample of 60 derived from the F_2 of the V2 $\times$ V12 cross by single seed descent (Jinks, Jayasekara and Boughey, 1977). The r_d values for final height and flowering time between these two pairs of parents based on their average phenotypes over 14 environments are as follows:

	V2 $\times$ V12	D10 $\times$ D17
Final height	0.35	1.00
Flowering time	0.52	0.77

TABLE 2

Family means and standard errors of the basic generations derived from the two sets of dispersion and association crosses V1×V5, B2×B35; V2×V12 and D10×D17 for final height and flowering time

Generation	V1×V5	B2×B35	V2×V12	D10×D17
<i>Final height</i>				
P ₁	127.34±2.09	143.84±1.88	118.43±2.12	160.33±1.30
P ₂	103.17±1.27	92.27±2.12	86.56±2.13	75.90±2.05
F ₁	130.24±1.81	126.29±1.74	140.27±1.93	138.38±1.84
F ₂	126.50±1.50	124.89±1.52	124.45±1.25	126.53±1.07
B ₁	132.30±1.63	138.61±1.88	127.07±1.35	160.19±0.93
B ₂	118.19±1.70	111.05±1.57	120.30±2.30	105.91±1.88
<i>Flowering time</i>				
P ₁	77.26±0.65	83.65±0.70	91.33±1.42	97.12±0.43
P ₂	72.18±0.61	69.84±0.76	75.05±0.66	73.90±0.30
F ₁	73.68±0.57	73.83±0.57	78.78±0.42	79.59±0.44
F ₂	76.13±0.58	76.60±0.53	82.53±0.43	81.31±0.31
B ₁	77.25±0.59	80.16±0.70	85.75±0.30	92.47±0.38
B ₂	73.60±0.60	73.41±0.49	76.55±0.81	77.35±0.42

TABLE 3

Within family variances and their degrees of freedom for the basic generations derived from the crosses V1×V5, B2×B35; V2×V12 and D10×D17 for final height and flowering time

Generation	V1×V5		B2×B35		V2×V12		D10×D17	
	Variance	df	Variance	df	Variance	df	Variance	df
<i>Final height</i>								
P ₁	302.82	137	347.09	194	90.32	19	164.95	96
P ₂	96.03	117	134.98	58	85.56	18	83.81	19
F ₁	228.30	136	209.64	136	224.43	58	270.69	78
F ₂	267.04	229	275.19	230	564.04	355	452.36	394
B ₁	266.02	190	278.08	150	291.31	153	207.44	235
B ₂	229.78	152	246.71	191	471.91	373	279.72	153
<i>Flowering time</i>								
P ₁	29.81	138	48.19	194	38.15	18	17.86	96
P ₂	22.04	118	17.36	58	8.68	19	1.83	19
F ₁	22.93	136	23.03	136	10.54	58	15.31	78
F ₂	40.19	232	33.28	231	65.33	355	38.79	394
B ₁	34.41	192	38.38	149	63.31	373	33.62	235
B ₂	28.71	152	24.03	191	14.47	153	27.90	153

Again one thousand individually randomised plants of the basic generations were raised for each cross in 1975. The experiment also included two triple test crosses of 60 D inbred lines using V2, V12 and their F₁ and D10, D17 and their F₁ as the two sets of testers. Further details are given by Pooni and Jinks (1981). The family means and standard errors of the basic generations are given in table 2 and the corresponding within family variances in table 3.

4. RESULTS

(i) Family means

We shall begin by analysing the family means to identify the likely level of complexity of the genetical control. Models have been fitted to the

combined family means of the basic generations of the $V1 \times V5$ and $B2 \times B35$ pair of crosses and of the $V2 \times V12$ and $D10 \times D17$ pair of crosses (table 2) using the procedures of Jayasekara and Jinks (1976) to take account of the differing levels of association and dispersion in the parents of the pair of crosses in each set. This is achieved by allowing $[d]$, $[i]$ and $[j]$ to differ between the pair of crosses in each set while keeping m , $[h]$ and $[l]$ the same. The full digenic non-allelic interaction model, therefore, contains nine parameters for fitting to 12 family means (tables 2 and 4). Hence, weighted least squares procedures can be used to estimate the parameters and to test the goodness of fit of the model. By elimination of non-significant parameters and the inclusion of additional parameters, if the model fails the test of goodness of fit, we obtain the simplest adequate models listed in table 4. These are the genetically simplest models that give a non-significant χ^2 for the test of goodness of fit and in which all the estimates of the parameters are significant.

TABLE 4

Estimates of the parameters in the simplest adequate model ($\chi^2_{(n-p)}$ non significant) in which every parameter is significant, for the twelve family means, six derived from the dispersion cross between pure breeding varieties and six from the corresponding association cross between extreme inbreds

Parameter	V1 $\times$ V5 and B2 $\times$ B35 crosses		V2 $\times$ V12 and D10 $\times$ D17 crosses	
	Final height	Flowering time	Final height	Flowering time
m	117.68 $\pm$ 0.81***	74.67 $\pm$ 0.44***	102.02 $\pm$ 1.48***	83.25 $\pm$ 0.34***
$[d]_1$	13.37 $\pm$ 1.03***	2.77 $\pm$ 0.39***	15.93 $\pm$ 1.50***	8.70 $\pm$ 0.70***
$[d]_2$	26.30 $\pm$ 1.22***	6.81 $\pm$ 0.44***	42.08 $\pm$ 1.21***	11.44 $\pm$ 0.66***
$[h]$	12.70 $\pm$ 1.51***	6.41 $\pm$ 1.56***	59.27 $\pm$ 5.72***	—
$[i]_1$	—	—	—	—
$[i]_2$	—	2.15 $\pm$ 0.64***	16.41 $\pm$ 1.88***	2.52 $\pm$ 0.77***
$[j]_1$	—	—	-20.62 $\pm$ 9.38**	—
$[j]_2$	—	—	26.71 $\pm$ 7.54***	7.72 $\pm$ 2.15***
$[l]$	—	-7.33 $\pm$ 1.45***	-22.01 $\pm$ 5.32***	-3.97 $\pm$ 0.67***
$[pi]$	—	—	—	3.17 $\pm$ 0.70***
$\chi^2_{(n-p)}$	14.43 (8) ^{NS}	9.20 (6) ^{NS}	5.09 (4) ^{NS}	10.29 (5) ^{NS}

NS $P > 0.05$; * $0.05 \geq P > 0.01$; ** $0.01 \geq P > 0.001$; *** $P \leq 0.001$.

The estimates of $[d]$, $[i]$ and $[j]$ show the expected effects of the different levels of association and dispersion between the pair of crosses of each set in that $[d]_1 < [d]_2$, $[i]_1 < [i]_2$ and $[j]_1 < [j]_2$. Furthermore, the adequate models all contain a single m , $[h]$ and $[l]$, that is, $m_1 = m_2$, $[h]_1 = [h]_2$ and $[l]_1 = [l]_2$ thus confirming that the parents of the association and of the dispersion cross of each set differ only in the way the same allelic differences at the same loci are distributed between them. Otherwise, the outcome of the model fitting confirms that a simple additive dominance model is sufficient for final height in $V1 \times V5$ and $B2 \times B35$ whereas there are non-allelic interactions for flowering time in the same crosses and for final height and flowering time in the $V2 \times V12$ and $D10 \times D17$ crosses (Jayasekara and Jinks, 1976; Pooni and Jinks, 1981). The only evidence for linkage comes from the significant $[pi]$ parameter for flowering time in the $V2 \times V12$ and $D10 \times D17$ crosses (Jinks and Perkins, 1969).

The more specific tests for a linkage disequilibrium involving interacting genes which are based upon comparisons of B_1 , B_2 and F_2 family means

with the corresponding L_1 , L_2 and L_3 family means of the triple test cross (Jinks, 1978) are summarised in table 5. For the first set these comparisons are confined to the $V1 \times V5$ cross but for the second set they are available for both the $V2 \times V12$ and $D10 \times D17$ crosses (see section 3). For the $\bar{F}_2 = \bar{L}_3$ comparison the $V2 \times V12$ and $D10 \times D17$ crosses have been combined into a single one way analysis of variance as all family means are expected to be equal in the absence of a linkage disequilibrium of interacting genes. Similarly the F_2 families of the $V1 \times V5$ and $B2 \times B35$ crosses and the L_3 family of the $V1 \times V5$ triple test cross have been combined into a single test (table 5).

TABLE 5

Tests of linkage between the genes displaying non-allelic interactions as obtained by comparing the family means of B_1 , B_2 , and F_2 generations of various crosses with the respective L_1 , L_2 and L_3 means of the corresponding triple test crosses

Test of linkage	df	V1 $\times$ V5 and B2 $\times$ B35 crosses	
		Final height $\chi^2_{(df)}$	Flowering time $\chi^2_{(df)}$
1. $B_1(V1 \times V5)$ vs. $L_1(V1 \times V5)$	1	3.08 ^{NS}	8.75**
2. $B_2(V1 \times V5)$ vs. $L_2(V1 \times V5)$	1	0.00 ^{NS}	5.80*
3. $F_2(V1 \times V5)$ vs. $F_2(B2 \times B35)$ vs. $L_3(V1 \times V5)$	2	4.43 ^{NS}	3.62 ^{NS}
Test of linkage	df	V2 $\times$ V12 and D10 $\times$ D17 crosses	
		Final height $\chi^2_{(df)}$	Flowering time $\chi^2_{(df)}$
1. $B_1(V2 \times V12)$ vs. $L_1(V2 \times V12)$	1	2.36 ^{NS}	0.00 ^{NS}
2. $B_2(V2 \times V12)$ vs. $L_2(V2 \times V12)$	1	0.00 ^{NS}	1.99 ^{NS}
3. $B_1(D10 \times D17)$ vs. $L_1(D10 \times D17)$	1	4.97*	28.86***
4. $B_2(D10 \times D17)$ vs. $L_2(D10 \times D17)$	1	0.57 ^{NS}	13.44***
5. $F_2(V2 \times V12)$ vs. $F_2(D10 \times D17)$ vs. $L_3(V2 \times V12)$ vs. $L_3(D10 \times D17)$	3	1.71 ^{NS}	10.59*

See table 4 for probability levels.

As there was no evidence of non-allelic interaction for final height in the $V1 \times V5$ and $B2 \times B35$ pair of crosses there is, as expected, no evidence of linkage of interacting genes. On the other hand, flowering time in these crosses where there was clear evidence of non-allelic interactions shows a significant linkage disequilibrium among the interacting genes, which confirms the results of earlier experiments (Jinks and Perkins, 1969).

Although both characters in the $V2 \times V12$ and $D10 \times D17$ pair of crosses showed highly significant non-allelic interactions there is little evidence of a linkage disequilibrium among the interacting genes for final height but quite strong evidence for flowering time (table 5). The latter, of course, agrees with the model fitting to the basic generations (table 4).

(ii) Within family variances

V_{1F2} and $(V_{1B1} + V_{1B2})$ for corresponding association and dispersion pairs of crosses have been compared by iterative weighted least squares model fitting procedures which lead to a χ^2 test of goodness of fit (Mather and Jinks, 1971). The comparisons have been carried out independently

for the F_2 and backcross variances using a technique which we will illustrate for V_{1F2} in the $V1 \times V5$ and $B2 \times B35$ pair of crosses.

A single model was fitted to the two estimates of V_{1F2} and the estimates of the environmental component of variation E obtained as $\frac{1}{4}V_{P1} + \frac{1}{4}V_{P2} + \frac{1}{2}V_{F1}$ for the $V1 \times V5$ and $B2 \times B35$ crosses (Mather and Jinks, 1971). To test specifically the hypothesis that the genetical component (G) of the two estimates of V_{1F2} is the same, the following three parameter model was fitted to the four estimates.

$$V_{1F2}(V1 \times V5) = G + E'$$

$$V_{1F2}(B2 \times B35) = G + E''$$

$$\frac{1}{4}V_{P1} + \frac{1}{4}V_{P2} + \frac{1}{2}V_{F1}(V1 \times V5) = E'$$

$$\frac{1}{4}V_{P1} + \frac{1}{4}V_{P2} + \frac{1}{2}V_{F1}(B2 \times B35) = E''$$

The $\chi^2_{(1)}$ testing the goodness of fit of this model will be significant only when G differs significantly between the dispersion and association pairs of crosses.

The χ^2 obtained on fitting such a model to the observed variances in table 3 are presented in table 6 along with the observed values of the environmental component E .

TABLE 6

Genetic components (V_{1F2}) and ($V_{1B1} + V_{1B2}$) and the estimate of environmental component E of the $V1 \times V5$, $B2 \times B35$, $V2 \times V12$ and $D10 \times D17$ crosses and the tests of goodness of fit of a model which assumes the same genetic component for the corresponding association and dispersion crosses

Component	$V1 \times V5$ cross	$B2 \times B35$ cross	$\chi^2_{(1)}$	$V2 \times V12$ cross	$D10 \times D17$ cross	$\chi^2_{(1)}$
<i>Final height</i>						
V_{1F2}	53.18	49.85	0.01 ^{NS}	407.86	254.83	5.92*
$V_{1B1} + V_{1B2}$	68.08	74.11	0.01 ^{NS}	450.86	92.09	7.41**
E	213.86	225.33		156.19	197.54	
<i>Flowering time</i>						
V_{1F2}	15.76	5.38	3.50†	48.35	26.21	4.98*
$V_{1B1} + V_{1B2}$	14.27	6.11	4.54*	43.83	36.37	0.49 ^{NS}
E	24.43	27.90		16.98	12.58	

For probability levels see table 4.

† $P = 0.06$.

The results can be interpreted by reference to the categorisation in section 2 ((i) to (iii)). For final height in the $V1 \times V5$ and $B2 \times B35$ pair of crosses the genetical components of V_{1F2} and ($V_{1B1} + V_{1B2}$) do not differ significantly so that there is no evidence for either non-allelic interaction or a linkage disequilibrium. For flowering time in the same crosses V_{1F2} shows a marginally significant difference and ($V_{1B1} + V_{1B2}$) a significant difference. Furthermore, the genetical components are larger for the dispersion than for the association cross and therefore the cause can only be a linkage disequilibrium of interacting genes. This same pattern also applies to flowering time in the $V2 \times V12$ and $D10 \times D17$ pair of crosses and hence there is a linkage disequilibrium of interacting genes. For final height in

the same crosses the genetical components of both V_{1F2} and $(V_{1B1} + V_{1B2})$ differ significantly and they are markedly larger for the dispersion than for the association cross. But again the only explanation compatible with this pattern is a linkage disequilibrium of interacting genes.

5. DISCUSSION

The expected variances in table 1 show that non-allelic interaction, linkage disequilibrium of non interacting genes and linkage disequilibrium of interacting genes can be individually identified by comparison of the within family variances of the basic generations of matched pairs of association and dispersion crosses. Comparison of these variances in two sets of matched association and dispersion crosses between pure breeding varieties of *Nicotiana rustica* and between highly inbred selections derived from them confirm these theoretical expectations. Indeed for final height the comparison of the within family variances of the basic generations was as informative as the most sensitive analysis of the means of the families of the basic generations and of a triple test cross. But whereas an analysis of means can detect only contributions to the variation that have a net directional effect, the comparison of variances is equally sensitive to ambidirectional effects.

For flowering time the comparison of variances is less conclusive than the analysis of means in one pair of association and dispersion crosses although there is no disagreement between them. This is almost certainly due to the small difference in the degree of association/dispersion (section 3) between the association and dispersion pair of crosses rather than to an inherent lower sensitivity of the variances to the presence of non-allelic interaction or a linkage disequilibrium.

Provided, therefore, that we have available the basic generations of a matched pair of crosses differing markedly in their degrees of association/dispersion for the characters of interest, a simple experimental design of modest dimensions will allow the detection of the most complex contributors to variation that have been encountered in biometrical genetical analyses (Mather and Jinks, 1982).

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