THE TRUE NATURE OF THE NON-ALLELIC INTERACTIONS IN *NICOTIANA RUSTICA* REVEALED BY ASSOCIATION CROSSES

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1. INTRODUCTION

JINKS and JONES (1958) showed that the additive genetic, $[d]$ and the additive × additive, $[i]$ and additive × dominance, $[j]$ interaction components of means estimated from the means of two pure-breeding lines (P_1 and P_2) and of their F_1, F_2, B_1 and B_2 families are functions of the degree of association (r) of alleles of like effect in the parents. Their real magnitudes and signs are therefore revealed only when the alleles of like effect are completely associated ($r = 1$) in the parents. In order to obtain a true picture of the gene action and interaction in *Nicotiana rustica* we have therefore investigated two crosses between pure breeding lines in which there is a high degree of association for the genes controlling most of the characters of interest.

2. MATERIAL

The experiment consists of the P_1, P_2, F_1, F_2, B_1 and B_2 families of the cross between varieties 2 and 12 and between two of the D inbred lines, D_{10} and D_{17}. Varieties 2 and 12 are two of the most extreme in the Birmingham collection. They display a high level of non-allelic interaction and produce the most heterotic F_1 (Jinks, 1954). D_{10} and D_{17} are the extreme selections for final height from a random sample of 60D inbreds derived by single seed descent from the F_2 of the $V_2 \times V_{12}$ cross (see Jinks *et al.*, 1977). The coefficients of gene association/dispersion in these two pairs of parents estimated as described by Jayasekara and Jinks (1976) as the ratio of the difference between the parental means and the difference between the extremes of the random sample of D inbred lines for each character are as follows;

Character	"r" for $V_2 \times V_{12}$	"r" for $D_{10} \times D_{17}$
Height-1, H_1	0·45	0·82
Height-2, H_2	0·82	0·74
Height-3, H_3	0·95	0·35
Flowering time, *FT*	0·59	0·85
Height at Flowering, *HFT*	0·21	0·82
Leaf length, *LL*	0·13	0·84
Leaf width, *LW*	0·25	0·96
Final height, *FH*	0·35	0·94

One thousand individually randomised plants were raised for each of the two crosses as part of a much larger experiment during the summer of

1975. The design used was that of Jinks and Perkins (1969) for equalising the amount of information about the P_1, P_2, F_1, F_2, B_1 and B_2 family means. The plants were scored in cm for all the height and leaf measurements while flowering time was scored from date of sowing. The data for each cross were processed separately through the University of Birmingham 1906A computer.

3. RESULTS

We shall analyse only those combinations of cross and character where the degree of association is greater than $r = 0.8$. These are summarised in table 1 where the family means and their standard errors are listed. The standard weighted least squares model fitting procedures were used to find the best fitting model for each combination. A simple additive, dominance model was first tried and was inadequate in every case ($\chi^2_{(3)}$ testing goodness-of-fit significant).

The perfect fit, six parameter model for digenic interactions was then fitted (Jinks and Jones, 1958). No simpler model would fit seven of the eight combinations. For H_1, however, a model containing the four parameters, m, $[d]$, $[h]$ and $[l]$ was adequate ($\chi^2_{(3)} = 3.93$). The adequacy of the six parameter model for the remaining seven characters can only be tested by including more generations. On adding the means of the L_1, L_2 and L_3 generations of an F_∞ triple test cross to the analysis, the digenic interaction model proved to be adequate for H_2, H_3 and FH ($\chi^2_{(3)}$ not significant). For FT, HFT, LW and LL, however, it was inadequate ($\chi^2_{(3)}$ significant) suggesting that more complex effects were also present.

The results of the model fitting (table 2) show that the $[l]$ component always takes the opposite sign to $[h]$. For the seven characters for which they are significant $[i]$ and $[j]$ are both positive having the same sign as $[h]$ and the opposite sign to $[l]$. For these seven characters, therefore, $[l]$ opposes all of the other kinds of gene action and interaction.

Jayasekara and Jinks (1976, table 4) have reported similar results for flowering time and height at flowering for the cross between inbred lines B_2 and B_{35} of *N. rustica* where the degree of association/dispersion was also relatively high ($r \geq 0.7$). The reciprocal situation where $[h]$, $[i]$ and $[j]$ are negative and $[l]$ is positive has only been found in a cross between varieties V_2 and V_{42} for flowering time where there was also a high level of association (Pooni, 1972).

4. DISCUSSION

For a single pair of loci all of the known classical non-allelic interactions can be directly equated with a unique combination of relative signs and magnitudes of d, h, i, j and l (Mather and Jinks, 1971). In the general case of many loci with association at some pairs and dispersion at others only two types of non allelic interactions can be recognised; those in which $[h]$ and $[l]$ have the same sign (complementary) and those in which they have opposite signs (duplicates) (Jinks and Jones, 1958). On this basis all of the examples we have analysed show duplicate interactions. But we are now in the unique position of being able to take the magnitudes and signs of our estimates of $[d]$, $[i]$ and $[j]$ at their face values since they are not

TABLE 1

Family means and their standard errors for the basic generations derived from $D_{10} \times D_{17}$ cross for the six characters for which parents D_{10} and D_{17} display a high degree of gene association ($r \geqq 0.8$) and the corresponding statistics for the basic generations of $V_2 \times V_{12}$ cross for H_2 and H_3 for which V_2 and V_{12} take an r value of 0.82 and 0.95 respectively

				$D_{10} \times D_{17}$ cross					$V_2 \times V_{12}$ cross	
Generations	n	Height-1	Flowering time	Height at flowering	Leaf length	Leaf width	Final height	n	Height-2	Height-3
P_1^*	100	3·58±0·11	97·12±0·43	122·13±1·07	23·07±0·19	22·34±0·25	160·33±1·30	20	24·53±1·36	82·05±2·65
P_2	20	10·20±0·49	73·90±0·30	54·40±1·09	10·28±0·47	6·22±0·30	75·90±2·05	20	5·53±0·31	17·61±0·98
F_1	80	7·90±0·19	79·59±0·44	79·08±1·47	18·37±0·33	14·91±0·35	138·38±1·84	60	15·88±0·70	62·69±1·60
F_2	400	6·76±0·11	81·31±0·31	77·08±0·88	18·02±0·17	14·68±0·19	126·53±1·07	360	13·02±0·32	47·94±1·02
B_1	240	4·86±0·09	92·41±0·38	116·09±1·24	23·22±0·17	21·58±0·19	160·19±0·93	160	20·46±0·46	73·15±1·07
B_2	160	8·02±0·21	77·35±0·42	64·30±0·79	15·11±0·24	11·37±0·30	105·91±1·33	380	9·86±0·24	36·71±0·85

* P_1 refers to D_{17} and V_{12} for the respective set of generations.

TABLE 2

Components of generation means for various characters as obtained by weighted least squares analysis together with χ^2 values for testing the goodness of fit of a simple additive/dominance model

Parameter	$D_{10} \times D_{17}$						$V_2 \times V_{12}$ cross	
	Height-1	Flowering time	Height at flowering	Leaf length	Leaf width	Final height	Height-2	Height-3
m	6·93±0·18	71·11±1·70	35·81±4·67	12·10±0·94	7·10±1·06	98·73±5·72	7·69±1·43	29·51±3·74
$[d]$	3·36±0·16	11·61±0·26	33·87±0·76	6·40±0·25	8·06±0·20	42·22±1·21	9·50±0·70	32·22±1·41
$[h]$	-2·30±0·54	32·32±4·30	121·83±11·64	17·43±2·39	22·51±2·72	70·44±14·59	14·70±3·81	48·44±9·55
$[i]$	—	14·40±1·68	52·46±4·60	4·58±0·91	7·18±1·05	19·38±5·59	7·34±1·24	20·32±3·47
$[j]$	—	7·02±1·24	35·85±3·32	3·43±0·78	4·30±0·81	21·13±4·49	3·30±1·63	9·53±3·54
$[l]$	3·27±0·53	-23·84±2·77	-78·55±7·62	-11·15±1·60	-14·70±1·81	-30·79±9·66	-6·51±2·74	-15·26±6·62
$\chi^2_{(3)}$†	42·00*	117·26*	199·57*	107·87*	131·73*	83·49*	34·40*	49·70*

* $P < 0.001$.
† $\chi^2_{(3)}$, testing goodness of fit of m, $[d]$, $[h]$ model.

distorted by low levels of association. For seven of the eight characters in table 2 (omitting Height-1) $[d]$, $[h]$, $[i]$ and $[j]$ are positive and $[l]$ alone is negative. This is clearly incompatible with duplicate genes which require that $[i]$, $[j]$ and $[l]$ all have the opposite sign to $[h]$ when the distortions due to low levels of association are removed.

To produce these observed relationships we would require that $d_a = d_b = h_a = h_b = i_{ab} = j_{ab} = j_{ba} = -l_{ab}$ at the level of a single pair of interacting loci. This does not correspond with any of the classical interactions. It would, for example, lead to a $5:4:7$ segregation in an F_2. This is a modification of the $9:7$ segregation of classical complementary genes in which the double heterozygote is showing the equivalent of duplicate genes and the rest of the genotypes complementary interactions (fig. 1). The reciprocal situation, observed only in the $V_2 \times V_{42}$ cross for flowering time, is equivalent to $d_a = d_b = -h_a = -h_b = -i_{ab} = -j_{ab} = -j_{ba} = l_{ab}$ which is the classical dominant epistasis that produces an F_2 segregation of $12:3:1$ (fig. 1).

(a)

AABB (5)	AABb	AAbb
AaBB	AaBb (4)	Aabb
aaBB	aaBb (7)	aabb

(b)

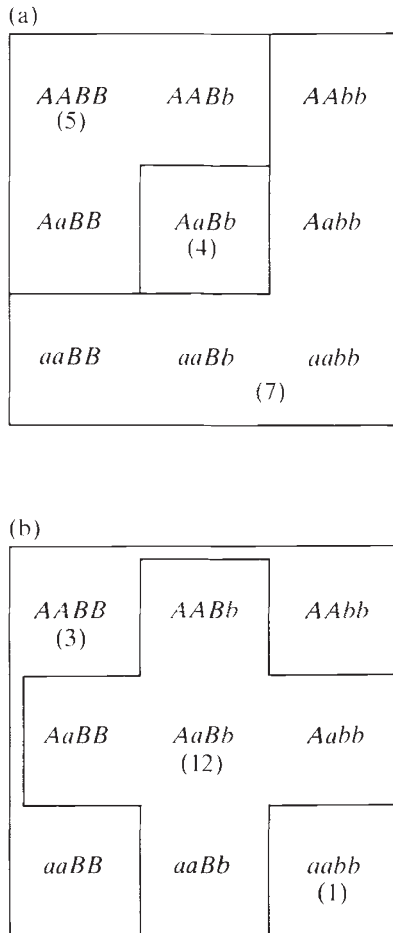|		
AABB (3)	AABb	AAbb
AaBB	AaBb (12)	Aabb
aaBB	aaBb	aabb (1)

FIG. 1.—Segregation ratios in F_2 where (a) represents $d_a = d_b = h_a = h_b = i_{ab} = j_{ab} = j_{ba} = -l_{ab}$ and (b) represents $d_a = d_b = -h_a = -h_b = -i_{ab} = -j_{ab} = -j_{ba} = l_{ab}$.

These conclusions have profound consequences for understanding the genetical basis of heterosis in *Nicotiana rustica* and the prospects for producing superior inbred lines. The deviation of the mean of the F_1 hybrid from m is the sum of $[h]$ and $[l]$ and these have opposite signs. The deviation of the best inbred line extractable from the hybrid is the sum of $[d]$ and $[i]$ providing that they are estimated from a cross between associated parents. These have the same sign in the examples in table 2. As a consequence $[d]+[i]>[h]+[l]$ for every character. While, therefore, $V_2 \times V_{12}$ is the most heterotic cross, we expect and observe that the best inbred lines extractable from it are superior to the F_1 for every character.

One last point, by analysing each of the character cross combinations with the lower degree of association we can examine its distorting effects on the significant positive estimates of $[i]$ and $[j]$ given in table 2. For the seven combinations the lower degree of association makes $[i]$ and $[j]$ negative in three cases and non-significant in four. It would, therefore, have led us to underestimate the importance of the additive × additive and additive × dominance components and possibly misinterpret the kind of interaction.

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