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Simulation of Quantitative Characters by Genes with Biochemically Definable Action

IV. The Analysis of Heritable Variation by the Diallel Technique[1,2])

S. JANA and W. SEYFFERT

Institut für Biologie der Universität Tübingen, Lehrstuhl für Genetik, Tübingen (BRD)

Summary. Six groups of genetic materials were developed in a cruciferous garden plant, *Matthiola incana* R. Br., to produce simplified genetic systems of a pair of loci in each group. There were only two alleles at each locus, which were directly involved in the modification of anthocyanins in the plant tissue. The parental lines and their F_1's in each group constituted an ideal 4 × 4 diallel cross and satisfied all but one condition necessary for a valid diallel analysis. Nonallelic interaction was the only possible offending postulate.

The diallel analysis of the data on anthocyanin content in the flower tissues and a comparison of the results with that of a relatively straightforward method of analysis indicated that in the presence of epistasis, the dominance ratio (H_1/D) ceases to be a reliable measure of the average degree of dominance. In such situations additional genetic information obtained from the diallel analysis are not in agreement with the expectations on the basis of the already available genetic information on the materials. The estimator $H_2/4\ H_1$, a measure of average value of the product of alleles with positive and negative effects, seemed to remain unaffected by epistasis. The Wr/Vr regression analysis does not always permit the detection of nonallelic gene interactions. The results suggest that duplicate interaction may escape detection by the regression analysis.

Introduction

In a previous paper of the series we presented the results of the matrix analysis of quantitative inheritance of anthocyanins in flower tissues of *Matthiola incana* R. Br. The materials consisted of six groups of isogenic lines. Each group contained four homozygous parents obtained from a pair of loci with two alleles each. The loci are involved in the modification of the anthocyanin molecule. Controlled crosses among the parents in each group produced a complete 4 × 4 diallel set, which was grown in the University experimental garden in Tübingen in 1965 and 1966. The concentration of anthocyanins in the flower tissues of the 16 progenies in a set were assessed separately. Because of the isogenic background each of the 4 × 4 diallel sets represents a simplified genetic system of two unlinked loci. The phenotypic values associated with the nine genotypes in a group were described in terms of nine biometrical quantities giving a set of nine equations in nine unknowns. The direct estimates of the nine parameters were obtained. Eight of the nine parameters were ascribable to the action of a pair of loci constituting the genetic system. Dominance constituted the most important part of the total genetic effects in these simple two-locus genetic systems. While additive effect was found to be of least importance, there was considerable nonallelic gene interaction.

The genetic material represented an ideal diallel system in each group of crosses giving an opportunity to compare the available genetic information about the materials with the results of diallel analysis. When the following assumptions are valid the diallel technique developed by Jinks and Hayman (1953 and later) provides reliable information regarding the genetic properties of the parents:

(1) homozygous parents,
(2) two alleles at each locus,
(3) random distribution of genes in the parents,
(4) diploid segregation,
(5) absence of reciprocal differences between crosses, and
(6) independent actions of nonallelic genes.

In the absence of epistasis the diallel analysis is expected to provide accurate genetic information about the parental lines in each of the six sets of diallel crosses repeated in two years. The presence of epistasis, however, should not offer any serious difficulty, because the diallel method provides a highly powerful regression analysis for detecting nonallelic gene interaction. In this report the results of diallel analyses of the data on anthocyanin content are presented, and are compared with the information available in Jana and Seyffert (1971). It is expected that useful information regarding reliability of the

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diallel cross technique would be available from these comparisons.

Materials and Methods

The experimental material has been described by Seyffert (1971) in a previous paper of this series. The crossing plan is given in Jana and Seyffert (1971). Table 1 contains a brief description of the six genetic systems. It should be noted that the parents within each group have identical genetic background. Within a group the parents and their F_1's differ genetically only with respect to the allelic combinations at the two loci under investigation.

Table 1. *The description of the homozygous parents and the genetic backgrounds of the six two-locus genetic systems in Matthiola incana R. Br.*

Group No.	Genetic background	Loci under investigation	Parents included in the diallel cross	
			Identification number	Genotype
I	$l^+l^+uud^+d^+$	b^+/b, v^+/v	22	$b^+b^+v^+v^+$
			20	b^+b^+vv
			02	bbv^+v^+
			00	$bbvv$
II	$l^+l^+u^+u^+dd$	b^+/b, v^+/v	22	$b^+b^+v^+v^+$
			20	b^+b^+vv
			02	bbv^+v^+
			00	$bbvv$
III	$b^+b^+lld^+d^+$	u^+/u, v^+/v	22	$u^+u^+v^+v^+$
			20	u^+u^+vv
			02	uuv^+v^+
			00	$uuvv$
IV	bbu^+u^+dd	l^+/l, v^+/v	22	$l^+l^+v^+v^+$
			20	l^+l^+vv
			02	llv^+v^+
			00	$llvv$
V	$b^+b^+uud^+d^+$	l^+/l, v^+/v	22	$l^+l^+v^+v^+$
			20	l^+l^+vv
			02	llv^+v^+
			00	$llvv$
VI	$uuvvd^+d^+$	b^+/b, l^+/l	22	$b^+b^+l^+l^+$
			20	b^+b^+ll
			02	bbl^+l^+
			00	$bbll$

The six sets of diallel crosses were grown in 1965 and 1966 without replication. The optical density peak of the 1% HCl-methanolic extracts of the petals sampled from each cross in a diallel set was recorded on a reading spectrophotometer at wave-lengths specific for anthocyanins (400—600 mμ). There were five observations in 1965 and sixteen in 1966, taken on different days extended over the flowering period of *M. incana* between June and August. The average extinction values were considered as the quantitative measures of the anthocyanin concentration in the flower tissues.

Results and Discussion

Table 2 (*a*—*l*) presents the mean extinction values of six diallel crosses in two years, averaged over the reciprocals and multiplied by 10^3. The differences between F_1's and parents are positive in all cases suggesting that the overall direction of dominance is positive in each cross.

When the assumptions underlying the diallel analysis are valid, the variance of each array (Vr) and the covariance between the non-recurrent parent and its offspring in each array (Wr) are independent of array. Consequently, the Wr—Vr values of all arrays should be homogeneous. The tests for heterogeneity are available for both replicated and unreplicated experiments (Hayman, 1954). In the absence of replication, a t-test with n — 2 degrees of freedom can be performed for determining the validity of the hypotheses by using the following formula:

$$t^2 = \left(\frac{n-2}{4}\right)\left(\frac{(\text{Var } Vr - \text{Var } Wr)^2}{\text{Var } Vr \cdot \text{Var } Wr - \text{Cov}^2(Vr, Wr)}\right)$$

where n is the number of parents included in a diallel cross. The estimated t^2 was significant at 5% level in group I cross in 1966 indicating failure of one or more of the hypotheses (Table 3). In the remaining eleven cases the estimates of t^2's were not significant.

When the postulates of diallel analysis hold, several second degree statistics are calculated from the diallel table and from them the least squares estimates of the parameters D, F, H_1, H_2 and h^2 are obtained by using the following equalities:

(1) Variance of the parents, $V_{oLo} = D$,

(2) Covariance between non-recurrent parents and the mean of their offspring,

$$W_{oLo} = \frac{1}{2}D - \frac{1}{4}F,$$

(3) Variance of the means of the arrays around the overall progeny mean,

$$V_{oL1} = \frac{1}{4}D - \frac{1}{4}F + \frac{1}{4}H_1 - \frac{1}{4}H_2,$$

(4) Mean array variance, $V_{1L1} = \frac{1}{4}D - \frac{1}{4}F + \frac{1}{4}H_1$, and

(5) (Mean of the entire n^2 progeny — mean of the parents)^2,

$$(m_{L1} - m_{L0})^2 = \frac{1}{4}h^2.$$

The estimates of the parameters, D, F, H_1 and H_2, and several useful ratios obtained from them are presented in Table 4. Due to the absence of replication an analysis of variation of the diallel table was not possible. The non-heritable component E could not be estimated. Thus the estimates of the parameters are exact solutions of the equations given in section 2:4 in Hayman (1954).

The estimator H_1/D is a measure of the average degree of dominance over all loci even when the test of heterogeneity indicates that Wr—Vr is not constant, implying the presence of linkage and/or multiple allelism. However, nonallelic gene interactions invalidate H_1/D as an estimator of the weighted overall degree of dominance (Hayman, 1957). The ratio

Table 2. *Mean extinction values of six sets of diallel crosses averaged over reciprocal crosses. The mean value of a cross was the average of five observations in 1965 and of sixteen in 1966. All original extinction values were multiplied by 10^3*

Parents	22	20	02	00
a. Group I; Year: 1965				
22	452.4	580.5	541.0	491.0
20		377.0	491.3	472.4
02			250.8	283.7
00				238.2
c. Group II; Year 1965				
22	864.8	883.4	1171.5	1044.4
20		1021.0	1128.8	785.5
02			813.4	781.0
00				745.4
e. Group III; Year 1965				
22	467.4	593.3	604.8	519.5
20		509.4	560.8	388.5
02			511.4	420.2
00				385.6
g. Group IV; Year 1965				
22	813.4	781.0	1049.5	1173.8
20		745.4	787.2	580.4
02			718.2	637.5
00				525.0
i. Group V; Year 1965				
22	452.4	580.5	588.3	513.7
20		377.0	493.4	379.6
02			511.4	420.2
00				385.6
k. Group VI; Year 1965				
22	377.0	379.6	472.4	457.5
20		385.6	500.6	373.7
02			238.2	227.1
00				164.6

Parents	22	20	02	00
b. Group I; Year 1966				
22	537.5	532.6	485.7	553.9
20		436.6	470.8	489.7
02			302.7	296.5
00				327.5
d. Group II; Year 1966				
22	1139.9	1199.6	1131.9	1060.2
20		1094.8	1212.6	1154.1
02			1032.9	1054.4
00				929.4
f. Group III; Year 1966				
22	536.9	595.6	581.3	495.1
20		488.3	624.3	448.8
02			525.7	475.9
00				333.8
h. Group IV; Year 1966				
22	1032.9	1054.4	1042.4	1081.2
20		929.4	1103.7	1105.4
02			708.2	724.5
00				540.0
j. Group V; Year 1966				
22	537.5	532.6	564.6	484.8
20		436.6	503.3	417.6
02			525.7	475.9
00				333.8
l. Group VI; Year 1966				
22	436.6	417.7	489.7	482.5
20		333.8	436.2	432.6
02			327.5	294.5
00				232.1

Table 3. *The estimates of t^2 for testing the validity of the hypotheses by Hayman's (1954) test (ii) in nonreplicated diallel crosses*

Background genotype	Loci under investigation	Year of investigation	Identification number of the cross	t^2 for detecting variation in $Wr - Vr$	Probability
$l^+l^+uud^+d^+$	$b^+/b, v^+/v$	1965	*a*	0.450	>0.5
$l^+l^+uud^+d^+$	$b^+/b, v^+/v$	1966	*b*	6.730*	0.02−0.05
$l^+l^+u^+u^+dd$	$b^+/b, v^+/v$	1965	*c*	0.100	>0.5
$l^+l^+u^+u^+dd$	$b^+/b, v^+/v$	1966	*d*	0.001	>0.5
$b^+b^+lld^+d^+$	$u^+/u, v^+/v$	1965	*e*	0.660	>0.5
$b^+b^+lld^+d^+$	$u^+/u, v^+/v$	1966	*f*	0.125	>0.5
bbu^+u^+dd	$l^+/l, v^+/v$	1965	*g*	0.717	>0.5
bbu^+u^+dd	$l^+/l, v^+/v$	1966	*h*	0.028	>0.5
$b^+b^+uud^+d^+$	$l^+/l, v^+/v$	1965	*i*	2.464	0.1−0.2
$b^+b^+uud^+d^+$	$l^+/l, v^+/v$	1966	*j*	0.131	>0.5
$uuvvd^+d^+$	$b^+/b, l^+/l$	1965	*k*	0.681	>0.5
$uuvvd^+d^+$	$b^+/b, l^+/l$	1966	*l*	0.439	>0.5

* Significant at 5% level.

of the absolute values of dominance (D) and additive (A) effects obtained by the solution of simultaneous equations was presented in Jana and Seyffert (1971) as a reliable measure of the average dominance even in the presence of epistasis. It appears worthwhile to compare the two estimators of dominance after converting them into the same scale. The $(H_1/D)^{1/2}$ values are presented in Table 5 along with the D/A and I/A ratios. The ratio of the absolute values of epistatic and additive effects (I/A), obtained from the matrix solution, is a measure of the average interactions among loci. Considerable nonallelic inter-

Table 4. *The estimates of second degree statistics, dominance ratios, relative frequency and distribution of alleles and the number of effective factors from twelve 4×4 diallel tables presented in Table 2*

Cross number	D	H_1	H_2	F	H_1/D	$\frac{V_{1L1}}{W_{0L01}}$	$2\left(\frac{V_{1L1}}{W_{0L01}}-\frac{1}{2}\right)$	$\frac{(4DH_1)^{1/2}+F}{(4DH_1)^{1/2}-F}$	$\frac{H_2}{4H_1}$	K*
a	10630.0	30163.6	28594.0	−7211.6	2.84	1.69	2.37	0.66	0.24	1.70
b	11654.9	13436.9	12912.4	−3567.8	1.16	1.07	1.13	0.75	0.24	0.87
c	13747.8	101787.0	85996.4	10687.6	7.40	6.24	11.48	1.33	0.21	0.29
d	8307.9	13748.6	11701.0	782.3	1.65	1.34	1.69	1.08	0.21	1.43
e	3462.3	15998.0	11833.3	−3015.8	4.62	2.26	3.52	0.66	0.18	0.40
f	8819.3	6847.6	6445.9	−2883.0	0.78	0.90	0.81	0.69	0.24	1.50
g	15292.0	166954.8	120671.7	−4610.6	10.22	4.74	8.49	1.10	0.19	0.34
h	48995.5	90327.7	86963.1	12342.1	1.84	1.48	1.96	1.20	0.24	1.21
i	3965.9	17293.4	13007.5	−1055.9	4.36	2.48	3.97	0.88	0.19	0.78
j	8928.8	1758.5	1697.6	391.7	0.20	0.59	0.19	1.10	0.24	1.92
k	11703.2	44969.2	44540.8	898.8	3.84	2.47	3.96	1.04	0.25	0.62
l	6981.2	19273.2	18948.6	709.4	2.76	1.93	2.85	1.06	0.25	1.03

* $K = 4$ (Mean of progeny − mean of parents)$^2/H^2 = h^2/H_2$ = number of effective factors.

actions are evident from the I/A ratios in all the 12 cases, but compared with the rest of the crosses, *b, f, h, j,* and *i* show a relatively small role for epistasis. In four of the latter crosses, there is good agreement between the $(H_1/D)^{1/2}$ and D/A values. Although there is an appreciable difference, both ratios indicate partial dominance in the cross *j*. Crosses with larger contributions of epistasis show greater difference between the two dominance ratios. The cross *g* is particularly disturbing, because not only $(H_1/D)^{1/2}$ is far from D/A, but also it indicates overdominance while the latter ratio reveals partial dominance. Thus in epistatic genetic systems, $(H_1/D)^{1/2}$ tends to be inflated or depressed, with no systematic trend in the spuriousness introduced into the estimator.

Like H_1/D, the ratio V_{1L1}/W_{0L01} is a measure of the average degree of dominance. But these two ratios measure dominance on different scales. If the frequencies of dominant alleles, *u*, and of recessive alleles, *v*, are the same $\left(\text{i.e., } u = v = \frac{1}{2}\right)$ they can be converted to the same scale as (Jinks, 1954):

$$H_1/D = 2\left(\frac{V_{1L1}}{W_{0L01}} - \frac{1}{2}\right).$$

A comparison of the ratios presented in 6th and 8th columns in Table 4 would indicate that with the exception of cross *c* there is a good agreement between the two ratios, confirming the expectation on the basis of the qualitatively determined effect of the alleles in the parents.

The parameter F provides information on the distribution of dominant and recessive alleles in the parents. A value of F close to zero implies equality of *u* and *v*, i.e., 1/2, or that no gene in the parents has dominant effect. A positive value of F indicates excesses of dominant alleles and a negative value implies an excess of recessive alleles in the parents. If the dominance effects of different genes are unequal, the values of F will be weighted in favour of the genes with larger dominance. The ratio $\{(4\,D\,H_1)^{1/2} + F\}/\{(4\,D\,H_1)^{1/2} - F\}$ is then a measure of the proportion of dominant and recessive alleles in the parents. In the present analyses, the ratio should be equal to unity since the dominant and recessive alleles appear in equal frequencies in the parents. The observed values seem to have varying degrees of closeness to unity. In view of the large estimates of F, both positive and negative, such a discrepancy is not unexpected.

The equality of the parameters H_1 and H_2 indicates equality of the number of dominant and recessive homozygotes among the parents. If the frequency of positive and negative alleles in the parents are *u* and *v*, respectively, the ratio $H_2/4\,H_1$ estimates the average value of the product, *u v*, to a maximum value of 1/4 when $u = v = 1/2$. The ratio measures

Table 5. *The estimates of average degree of dominance and epistasis of a pair of loci controlling the biosynthesis of anthocyanins in M. incana. The dominance (D/A) and epistasis (I/A) ratios are reproduced from Table 3 of Jana and Seyffert (1971), $(H_1/D)^{1/2}$ is obtained from Table 4*

Cross no.	Dominance ratio (Diallel model) $(H_1/D)^{1/2}$	Dominance ratio (Matrix model) D/A	Epistasis ratio (Matrix model) I/A
a	1.68	2.61	1.96
b	1.08	0.95	0.91
c	2.73	1.41	4.09
d	1.29	1.63	1.44
e	2.15	1.63	4.91
f	0.88	1.07	0.79
g	3.20	0.85	2.18
h	1.36	1.44	0.82
i	2.09	1.80	3.31
j	0.45	0.77	0.65
k	1.96	1.35	1.05
l	1.66	1.49	0.63

the relative distribution of only those alleles which exhibit dominance and is biased upwards. Since both loci involved in each diallel cross exhibited dominance, and since the frequency of dominant and recessive alleles are known to be equal in each cross, an estimate of $H_2/4\ H_1$ is expected to be close to the highest value of 1/4. The calculated ratio, free from the effect of epistasis, is reasonably close to 1/4 in each cross (Table 4, Column 10).

When the dominance effects (h) at different loci are equal and in the same direction, the ratio h^2/H_2 is a minimum estimate of the number of the genes with some degree of dominance controlling the character. The results given in the last column in Table 4 confirm the general belief that h^2/H_2 is an inefficient measure of the number of effective factors (k's).

The parental order of dominance determined by Wr + Vr, and the coefficient of correlation between the parental measurements and (Wr + Vr) values are presented in Table 6. The coefficients of correlation (r's) are negative in most cases indicating dominance of the genes for higher anthocyanin content. The only exception is cross *e* where negative effects of the dominant genes are indicated. A small value of r in cross *c* suggests that the dominant genes are positive and negative in equal proportion. The estimates of correlation coefficients are significant in crosses *a*, *g*, *h* and *j* and only in these crosses the squares of the coefficients (r^2's) are close to unity. Hence, the regression of the parental values on (Wr + Vr) exists in these crosses, and predictions of the measurements of completely dominant and completely recessive parents are possible.

Table 6. *The estimates of coefficients of correlation (r's) between the parental values and their dominance order, squares of the coefficients of correlation and the parental orders of dominance (Wr + Vr)*

Cross no.	Coefficient of correlation (r)	(r^2)	Order of dominance of the patents ($Wr + Vr$)			
a	−0.972	0.945	22	20	00	02
b	−0.888	0.788	22	20	02	00
c	−0.064	0.004	22	00	20	02
d	−0.835	0.698	20	22	02	00
e	0.886	0.785	00	22	02	20
f	−0.579	0.336	22	02	00	20
g	−0.980	0.960	20	22	02	00
h	−0.980	0.961	20	22	02	00
i	−0.469	0.220	22	00	02	20
j	−0.999	0.999	22	02	20	00
k	−0.918	0.843	22	20	00	02
l	−0.865	0.749	22	20	02	00

Earlier in this section a comparison of the dominance ratio $(H_1/D)^{1/2}$ with the D/A ratio of a previous analysis of the same genetic system showed that in the epistatic diallels $(H_1/D)^{1/2}$ is a spurious measure of the average degree of dominance. However, a test of epistasis is obtained from the analysis of the relationship between array variance (Vr) and array covariance (Wr) calculated from the diallel table. If epistasis is absent, the line of regression of Wr on Vr has a unit slope. The geometric representation of the (Vr, Wr) graphs are produced in Figure 1 (a–l). No regression coefficient is significantly different from unity (Table 7). Despite the fact that they are nearer to unity, the estimates of regression coefficients in crosses *c*, *d*, *e*, *f*, *g* and *i* are not significantly different from zero, which may be due to the following causes:

(a) no genetic variance,
(b) no dominance,
(c) epistasis.

Available information indicates presence of genetic variation among the parents. Presence of dominance is revealed in all the six cases by the dominance ratios given in Table 5. The absence of regression can therefore be concluded to be due to epistasis. The interaction ratios (I/A) presented in the last column in Table 5 indicate that there is substantial epistasis in these crosses. The information from the regression analyses appears to be quite consistent with the already available genetic information.

Table 7. *Estimates of regression coefficients of Wr on Vr, their respective standard errors (S. E.) and the t values, (b − 1)/S. E.*

Cross no.	Regression of *Wr* on *Vr*			
	b	S. E.	*t*	*P*
a	0.931	0.087	−0.785	0.5
b	0.842	0.055	−2.877	0.1
c	0.914*	0.486	−0.177	0.4
d	0.848*	0.385	−0.394	0.5
e	0.703*	0.241	−1.237	0.3
f	0.800*	0.283	−0.706	0.5
g	0.236*	0.384	−1.991	0.1
h	0.944	0.163	−0.341	0.5
i	0.553*	0.197	−2.275	0.1
j	0.988	0.029	−0.393	0.5
k	1.067	0.094	0.707	0.5
l	0.873	0.147	−0.864	0.4

* Regression coefficient is not significantly different from zero at $P = .05$ by the t test.

In six cases, *a*, *b*, *h*, *j*, *k*, and *l*, the slope of the regression line was significantly different from zero, but not from unity, implying no epistasis. The diallel analysis of these crosses are normally expected to provide reliable genetic information regarding these simple two-locus systems. At least in five out of the above six cases, the overall degree of epistasis measured as the I/A ratio is relatively small (see Table 5). In the cross *a*, however, inspite of substantial epistasis, the (Vr, Wr) graph fails to detect nonallelic interaction. A careful examination of the estimates of the

components of genetic effects provides a clue to the probable cause of the failure. The estimates of the genetic parameters indicate preponderance of dominance type of gene action. The d's are large and positive; the dd_{bv} value is also large, but in the opposite direction ($-dd_{bv}$), resulting in an overall negative epistasis. These observations are suggestive of a duplicate type of gene interaction operating in the genetic system. Duplicate gene interaction also causes clustering of the (Vr, Wr) points on both ends of the regression line, and an examination of the graph suggests that this may be the case here (see Figure 1 a). That duplicate interaction escapes Wr/Vr regression analysis is more clearly evident in the cross *h*. The estimates of genetic parameters for this cross are reproduced below from Table 2 of Jana and Seyffert (1971):

$a_l = 178.5$	$aa_{lv} = -16.2$
$a_v = 67.9$	$ad_{lv} = -13.6$
$d_l = 268.2$	$da_{lv} = -102.5$
$d_v = 86.8$	$dd_{lv} = -68.9$

Here dominance and additive components constitute the major part of the genetic effect, the magnitude of epistasis is relatively small, but while the a's and d's have positive values, all the interaction components are negative, a characteristic of the classical duplicate gene relationship (Jana, 1971). The shape of the graph and clustering of the (Vr, Wr) points in two distinct groups on both ends of the regression line clearly indicate a duplicate relationship.

The 22 parent has dominant alleles at both the loci and the double recessive homozygote is 00. In the (Vr, Wr) graphs of the respective diallels, therefore, one would expect the parents 22 and 00 to occupy the lowest and the highest positions, respectively, along the regression line of unit slope while the 20 and 02 parents would have intermediate positions between the two extremes. However, this expectation is based on the supposition that the quantitative behaviour of the genes controlling anthocyanin concentration at the biochemical level is analogous to their qualitative behaviour. Results corroborating this expectation are obtained in crosses *b*, *j*, *k* and *l*. It is interesting to note that the estimates of epistasis in these crosses are relatively small. The crosses with large estimates of epistasis show little or no correspondence with the expected relative position of the parents along the regression line. On the basis of their dominance order determined from the Wr-intercept of the regression line, the crosses can be classified into the following groups:

(1) partial dominance : *b*, *f*, and *j*;

(2) overdominance : *a*, *c*, *d*, *e*, *g*, *h*, *i*, *k*, and *l*.

In general the estimates of the average degree of dominance by the D/A ratio agree with the above grouping. As in the (Vr, Wr) graph, the dominance ratios also indicate overdominance in all crosses in 1965 and in four crosses in the following year. In cross *f*, however, the D/A ratio indicates slight overdominance, whereas, both $(H_1/D)^{1/2}$ and the Wr-intercept indicate partial dominance. The overall picture which emerges from both types of genetic analyses is that the gene action in 1965 was one of dominance type leading to overdominance. Substantial epistasis was also present in that year. In the next year the role of epistasis was highly reduced. Dominance was preponderant in both the years.

Conclusions

An important source of error in the present studies could be sampling, particularly in 1965, since there were only five observations available for each cross in a diallel set. Secondly, the experiments were not replicated. Therefore, an analysis of variation was not possible. The genetic parameters, D, H_1, H_2, F and h^2 were estimated without any correction for the non-heritable component, E. Such an estimation would lead to serious error when non-heritable effect is not negligible. The H_1/D ratio ceases to be a reliable measure of the average degree of dominance because what is estimated as H_1/D is really, $(H_1 + 2E)/(D + E)$ when $n = 4$. It is then likely that the inflated dominance ratios (H_1/D) presented in Table 4 are partly due to the error in ignoring E. Since the standard errors of the statistics could not be estimated, there is no information about their accuracy. Furthermore, the relatively small estimate of additive genetic effects suggests that heritability for the trait may be low. The efficiency of the diallel analysis with a character with low heritability is doubtful (Allard, 1956 a and b). These insuperable limitations should be kept in mind while interpreting the results of the present analyses.

The parental lines included in each 4×4 diallel cross were isogenic except for a pair of genes defined in each case. Therefore, each diallel set represents a genetic system consisting of two distinct loci. These loci are directly involved in the modification of anthocyanins in the flower tissues. We are then concerned with the quantitative analysis of the biochemical product of the enzyme systems controlled by these loci. The diallel analysis indicated a major contribution of dominance in the production of anthocyanins in these simplified genetic systems. Epistasis was found to be a common feature of the genetic systems in 1965, but in most cases had a considerably reduced role in 1966. There was year-to-year variation in the nature of the gene action, which is attributable to the environmental difference between the years.

Within the limitations of the experiments it can be observed that in certain situations nonallelic interaction may remain undetected by diallel analysis. In such cases the dominance ratio (H_1/D) may be a spurious measure of dominance. The random trend in spuriousness introduced into the estimator confirms Hayman's (1957) observation that epistasis is unrelat-

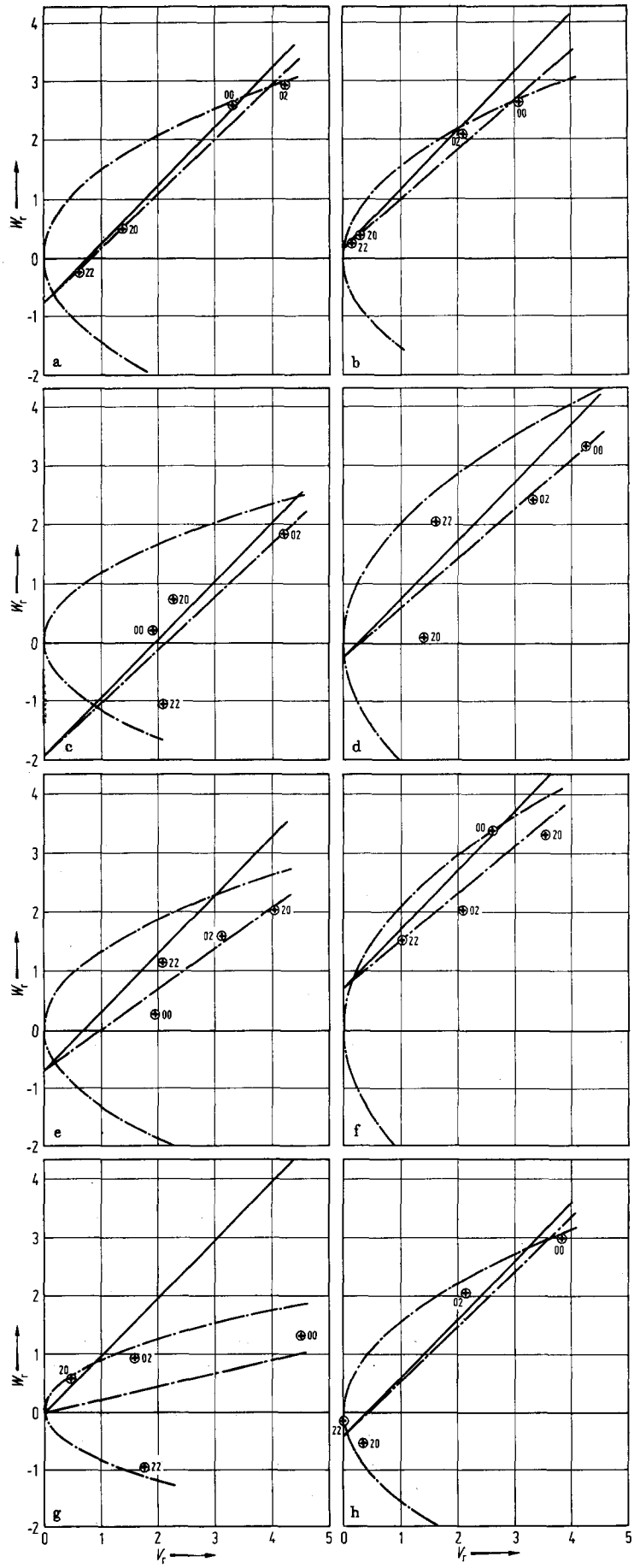

Fig. 1a. Loci investigated: b^+/b, v^+/v
Background: $l^+l^+uud^+d^+$, Year: 1965
One axis unit = 5000
V_{OLO} = 10630.00
b = 0.9315

Fig. 1b. Loci investigated: b^+/b, v^+/v
Background: $l^+l^+uud^+d^+$, Year: 1966
One axis unit = 5000
V_{OLO} = 11654.91
b = 0.8420

Fig. 1c. Loci investigated: b^+/b, v^+/v
Background: $l^+l^+u^+u^+dd$, Year: 1965
One axis unit = 10000
V_{OLO} = 13747.82
b = 0.9138

Fig. 1d. Loci investigated: b^+/b, v^+/v
Background: $l^+l^+u^+u^+dd$, Year: 1966
One axis unit = 2000
V_{OLO} = 8307.86
b = 0.8484

Fig. 1e. Loci investigated: u^+/u, J^+/v
Background: $b^+b^+lld^+d^+$, Year: 1965
One axis unit = 2000
V_{OLO} = 3462.28
b = 0.7025

Fig. 1f. Loci investigated: u^+/u, v^+/v
Background: $b^+b^+lld^+d^+$, Year: 1966
One axis unit = 2000
V_{OLO} = 8819.30
b = 0.8003

Fig. 1g. Loci investigated: l^+/l, v^+/v
Background: bbu^+u^+dd, Year: 1965
One axis unit = 20000
V_{OLO} = 15291.98
b = 0.2362

Fig. 1h. Loci investigated: l^+/l, v^+/v
Background: bbu^+u^+dd, Year: 1966
One axis unit = 20000
V_{OLO} = 48995.48
b = 0.9443

Fig. 1i. Loci investigated: l^+/l, v^+/v
Background: $bbuud^+d^+$, Year: 1965
One axis unit = 2500
$V_{OLO} = 3965.95$
$b = 0.5526$

Fig. 1j. Loci investigated: l^+/l, v^+/v
Background: $bbuud^+d^+$, Year: 1966
One axis unit = 1600
$V_{OLO} = 8928.83$
$b = 0.9884$

Fig. 1k. Loci investigated: b^+/b, l^+/l
Background: $uuvd^+d^+$, Year: 1965
One axis unit = 5000
$V_{OLO} = 11703.16$
$b = 1.0666$

Fig. 1l. Loci investigated: b^+/b, l^+/l
Background: $uuvd^+d^+$, Year: 1966
One axis unit = 3000
$V_{OLO} = 6981.22$
$b = 0.8729$

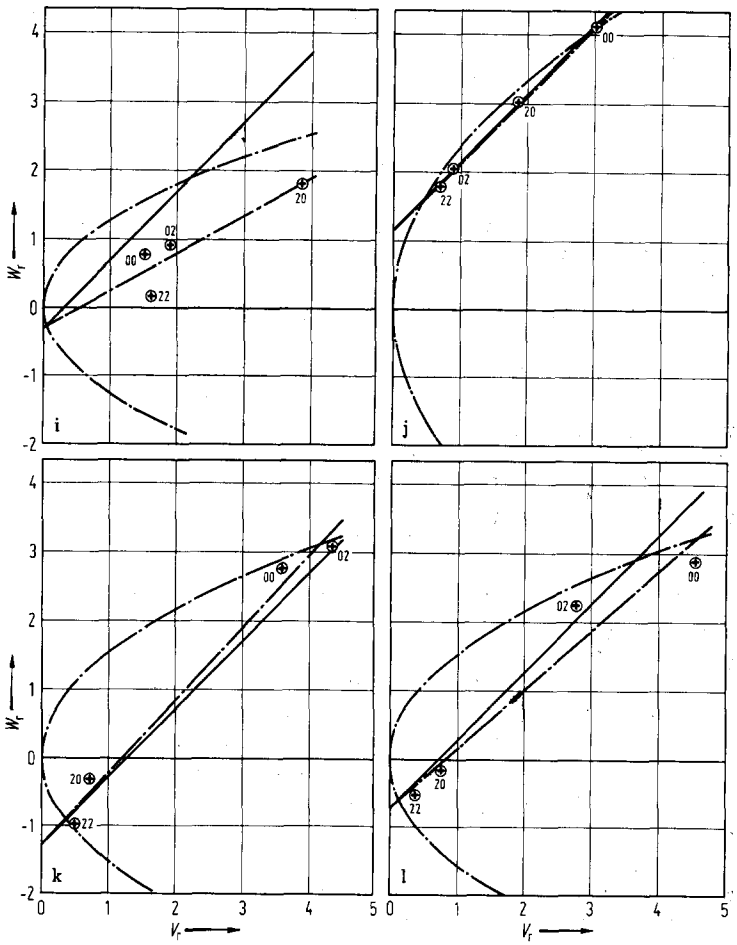

Figure 1. (V_r W_r) graphs of twelve 4×4 diallel crosses in *Matthiola incana*.
The description of the genotypes of the parents represented by the (V_r, W_r) points, 22, 20, 02, and 00, corresponds to the identification number given in table 1. The dotted line represents the calculated line of regression of W_r on V_r, the solid line is the theoretical regression of unit slope. The parabola is given by $W^2 = V_{OLO}\ V_r$

ed to the degree of dominance. Hayman also suggested that the dominance ratio is more seriously affected by epistasis than by failure of the assumptions like no multiple allelism and no linkage. Jinks (1954 and 1955) carried out extensive investigation on the problem of epistasis in diallel analysis and illustrated the effectiveness of the analysis of (Vr, Wr) graph in determining the presence of epistasis. He also showed how by systematic elimination of parents from the diallel set the offending parents can be detected and removed so that an epistatic diallel is converted to a non-epistatic one. With lima bean seed size, a character with high heritability, Allard (1956 a and b) elaborated the technique and demonstrated the sensitivity of the analysis of (Vr, Wr) graph not only in detecting epistasis, but from the characteristic modification of the graph, also its type. He concluded that the inheritance pattern of seed-size in his materials was complementary type of gene interaction superimposed upon a system of nearly complete dominance. Even with a character like yield which presumably has high environmental influence and low heritability, Johnson (1963) provided an excellent example from barley diallel cross elucidating the usefulness of the analysis of Wr/Vr regression in detecting complementary type of gene interaction. More recently Mather (1967) examined the effect of complementary and duplicate interactions between a pair of loci on the (Vr, Wr) graph and showed that while complementary interaction causes the Wr/Vr line to be concaved upward, the concavity is downward with duplicate gene interaction, but the latter disturbance is much less than the former. Therefore, duplicate gene interactions might be more difficult to detect than complementary in diallel crosses. Present analyses provide experimental evidence in support of this view.

Nonallelic interaction was found to effect not only the slope of the regression line, but also the position and scatter of the (Vr, Wr) points along the line. In crosses with relatively low epistasis, the position of the (Vr, Wr) points on the graph, as well as the order of dominance of the parents measured as (Wr + Vr) generally conformed to the expectation on the basis

of the qualitative behaviour of the genes. The estimator $H_2/4\ H_1$ measuring the proportions of positive and negative alleles in the parents remained unaffected by epistasis. In most cases the effective factors (k) were underestimated. In view of the highly unlikely assumptions concerning the minimal estimates of effective factors, such a result is not unexpected.

A comparison of the direct estimates of genetic parameters obtained by the matrix solution method with the results of diallel analysis reveals that in some systems the latter analysis may fail to detect epistasis, and consequently lead to incorrect conclusion. However, in non-epistatic genetic systems the diallel cross technique is a useful means of understanding the genetic properties of a number of parental lines. Its chief advantage lies in the rapid and systematic evaluation of breeding materials and prediction of the performance of the progeny of selected parents.

Present studies involved simplified genetic systems. The diallel analysis of such systems is expected to provide relatively reliable information on the genetic nature of the materials than can be expected from the analysis of highly complex genetic systems usually encountered in populations for which the diallel technique is called for. With the increasing complexity of genetic situation, it is expected to be more and more difficult to interpret the results of diallel analysis. Its primary limitation appears to be the oversimplified genetic assumptions underlying the analysis. Reports of clear genetic information emanating from the diallel analysis exist throughout the literature. It should be interesting to explore the effects of joint failures of two or more hypotheses on the results of diallel analysis and to examine how many of the apparently clear results might be due to the balanced failures of more than one postulates.

Zusammenfassung

Anhand der Crucifere *Matthiola incana* wurde ein genetisches Material aufgebaut, das es erlaubt, in 6 verschiedenen Gruppen je 2 Loci in allen Kombinationen systematisch zu variieren. Je Locus liegen 2 Allele vor, die in die Modifikation der Anthocyane eingreifen.

Die Elterlinien und ihre F_1-Nachkommen bilden ein ideales 4×4-Diallel und erfüllen bis auf eine alle für eine gültige Diallelanalyse erforderlichen Bedingungen. Die einzige nicht erfüllte Voraussetzung ist die Abwesenheit nichtalleler Interaktionen.

Die diallele Analyse der Daten über den Anthocyangehalt der Petalen und ein Vergleich der Resultate mit den aufgrund einer sehr direkten und detaillierten Untersuchungsmethode erhaltenen zeigten, daß das Dominanzverhältnis (H_1/D) bei Vorliegen von Epistasie keine brauchbare Messung des durchschnittlichen Dominanzgrades mehr ist. In derartigen Situationen stimmen auch die zusätzlich aus der diallelen Analyse zu erhaltenden Informationen nicht mit den Erwartungen überein, die auf der bereits vorliegenden Kenntnis der Genetik des Materials beruhen.

Der Schätzwert $H_2/4\ H_1$ als Messung des Durchschnittswertes des Produktes der Allele mit positiven und negativen Effekten scheint dagegen durch Epistasie unbeeinflußt zu bleiben.

Die W_r/V_r Regressionsanalyse führt nicht in allen Fällen zum Nachweis nichtalleler Interaktionen. Die Ergebnisse zeigen, daß eine „duplicate gene action" dem Nachweis durch die Regressionsanalyse entgeht.

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Dr. S. Jana
Crop Science Dept.
University of Saskatchewan
Saskatoon (Canada)
Prof. Dr. W. Seyffert
Institut für Biologie der Universität Tübingen
Lehrstuhl für Genetik
Auf der Morgenstelle 1
D-74 Tübingen (Germany/BRD)