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COMPETITION IN *DROSOPHILA*

III. A POSSIBLE MATERNAL EFFECT

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SUMMARY

The Wellington and Samarkand inbred lines differ in competitive ability when tested in competition with the 6CL stock. This difference was analysed using the eight true-breeding lines which comprise all the possible combinations of chromosomes I, II and III, treated as units, from Wellington and Samarkand.

Competitive ability was measured by ϕ, reflecting the proportion of wild-type as opposed to 6CL flies emerging from competitive cultures, the proportion being transformed to ϕ by the angular transformation.

The eight true-breeding lines were used as parents in a set of diallel crosses made in triplicate. The variance of the means of female arrays was greater than that of male arrays, suggesting some form of maternal effect.

The general consequences of maternal effects in diallel experiments are described, and the analysis of diallels in which the genetical constitution of the parents is defined, is developed.

Analysis of the results from the diallel experiment gives evidence of additive and dominance effects of the genes affecting competitive ability. These effects are constant over the three replicates and there is some indication of duplicate-type interaction among the genes. The analysis is also strongly suggestive of a maternal effect, associated chiefly with the distribution of the X chromosome and variable over the replicates.

The results of a non-diallel type of experiment are less informative but in general agreement. An apparent maternal effect of the kind found, when associated with the X chromosome, could in principle arise from the direct action of sex-linked genes, but reasons are advanced for not favouring this interpretation. Rather, the maternal effect is attributed to differences among the properties of females from the eight lines, probably in their behaviour in respect of egg-laying.

1. INTRODUCTION

IN their analysis of the genetical determination of competitive ability in *Drosophila melanogaster*, Mather and Cooke (1962) were able to show that chromosome III differed between the Oregon and Samarkand inbred lines in respect of its mediation of competitive ability. Further genetic differences between the lines were traced to the other two major chromosomes though by the nature of the experiments it was not possible to ascertain whether these differences were located in chromosome I, chromosome II or partly in each. This ambiguity sprang ultimately from Mather and Cooke's method of assessing relative competitive ability by competing the various genotypes, derived from Oregon and Samarkand, against one another in all possible combinations: this is a demanding technique which sets a sharp limit to the number of genotypes that can be tested and so did not allow them to effect a

full analysis. The use of a common tester strain against which all the derived genotypes can be competed and their competitive abilities compared is a more economical, even if in some respects a less informative, technique which allows more genotypes to be tested and compared within a given expenditure of experimental resources. Its use has allowed us to carry the analysis of the determination of competitive ability further and to separate the effects of all three major chromosomes.

2. The experiments

Mather and Cooke used the Oregon and Samarkand inbred lines, but the present experiments are concerned with the differences between the Wellington (W) and Samarkand (S) inbreds. Six homozygous lines were constructed, by the familiar technique using a stock with marked chromosomes, which together with the two parental lines comprised all eight combinations of the three major chromosomes taken as units. The composition of these lines will be denoted by WWW (Wellington), WWS, WSW, WSS, SWW, SWS, SSW and SSS (Samarkand), the three letters referring to the sources of the three major chromosomes in the order I, II, III. All the possible 27 genotypes, including all the 19 various heterozygotes, can be readily constructed by appropriate crosses among these eight homozygotes.

Flies of each of the 27 genotypes were competed against the 6CL stock, using Mather and Cooke's technique (see McGill and Mather, 1971, and Mather and McGill, 1972). Two females and two males of the genotype under test were placed on one side of the partition dividing a $3'' \times 1''$ tube into two chambers and two females and two males of the 6CL stock on the other. These parents were removed and the partition taken out after 3 days. The numbers of wild-type and 6CL flies emerging in each tube were recorded. The competitive ability ascribed to the genotype of the parents was measured by ϕ, derived by the angular transformation from the proportion of the emerging flies that were wild-type. As measured in this way competition will depend on the fecundity and laying behaviour of the parents and the hatchability of the eggs as well as the direct survival of the larvae. One aspect of the behaviour of the eight homozygous lines in competition with 6CL has been described by Mather and McGill (1972).

The experiments fell into two sets. In the first set all 27 genotypes were made up, using for the purpose whatever crosses were convenient, and tested in equal numbers of tubes against 6CL. In the second set, a diallel crossing programme was used among the eight homozygous lines. This gives $8 \times 8 = 64$ cells in the diallel table and contains the 27 genotypes in their F_2 proportions. It also takes into account the various ways of making up a heterozygous genotype. Thus, for example, the triple heterozygote is made up in all the eight possible ways (WWW × SSS, SSS × WWW, WWS × SSW, etc.) all of which appear once in the diallel set. This approach has the great advantage of allowing maternal effects to be detected and measured, and the results of the diallel experiments will hence be described first.

The diallel experiment was carried out three times, one competition tube being set up from each of the 64 crosses in the table. The overall yields of flies (*i.e.* wild-type + 6CL) and the values of ϕ are shown in table 1, averaged over the three replicates. In 3 of the 64 cells of the table one of the three replicates failed, and it was accommodated in the analysis by using the missing

plot technique to provide a value for ϕ, the calculations being carried out within the relevant replicate of the experiment. Thus in these cases, the average given in table 1 is the mean of three values, two actually observed and one obtained by the missing plot technique. Although the results of the

TABLE 1

Results of diallel experiments—total yield and ϕ values averaged over the three replicates

		Male parents							
		WWW	WWS	WSW	WSS	SWW	SWS	SSW	SSS
Female parents	WWW	64·7	84·7	76·7	67·7	55·3	70·0	62·0	69·7
		75·3	64·0	70·3	87·0	92·0	75·0	56·3	91·3
	WWS	65·7	50·0	67·7	62·7	60·0	53·0	66·0	54·7
		68·3	55·0	49·3	63·3	90·0	54·7	68·7	90·7
	WSW	59·7	73·7	52·3	57·3	76·3	69·3	33·0	56·0
		70·7	70·3	77·3	57·3	112·0	70·0	45·0	74·7
	WSS	68·7	58·7	49·3	61·0	70·0	73·0	55·0	65·7
		67·3	74·0	60·3	71·7	95·7	47·7	58·7	64·3
	SWW	68·3	74·3	66·7	79·3	56·0	73·3	66·7	73·0
		90·3	106·0	89·3	79·3	46·3	77·3	51·7	66·0
	SWS	69·0	61·0	74·0	63·0	67·0	62·3	70·3	59·3
		68·0	77·7	82·3	61·7	71·3	54·0	53·0	58·0
	SSW	50·0	71·0	55·7	70·0	66·3	61·0	54·7	57·3
		50·0	75·7	50·3	46·0	77·0	39·0	44·3	52·0
	SSS	69·0	65·7	58·7	60·7	70·3	59·3	73·7	73·0
		51·7	61·7	49·3	39·7	67·7	64·0	79·3	52·0

In each cell of the table, the upper figure is ϕ and the lower figure is yield of flies averaged over the replicates.

three replicate diallels are not recorded individually in table 1, the analysis of the results was based on them and variation among them appears in the various analyses of variance as " block " interactions, each replicate being denoted as a " block " for this purpose.

3. MATERNAL EFFECTS IN DIALLELS

The first noteworthy feature of the diallel results is brought out by the simple analysis of variance of ϕ set out in table 2, where the variance is partitioned into that among male parents (*i.e.* between the arrays each of which has a common male parent), that among female parents, the interaction between male and female parents, and the variation between the blocks, or replicates of the diallel. The block interactions are homogenous and so have been pooled to give an error variance based on 126 degrees of freedom. The male × female interaction term is significant against this error, showing that more than additive variation is involved; but the most striking result is the great disparity between the components of variation traceable to male and female parents respectively. Even though the eight male parental

genotypes are exactly the same as the eight female parental genotypes, the variance between female arrays is over three times as large as that between males. The variance between female arrays is highly significant while that between male arrays is little larger than the error variance. Indeed the female variance is almost significantly larger than the male by a direct test (V.R. = 3·42, V.R. = 3·8 at P = 0·05).

Evidently females contribute something to their progeny, as tested against 6CL, which males do not. This could arise from either of two things. It could be due to a maternal effect, the females of the parental homozygous

TABLE 2

Simple analysis of variance of diallel results

Item	d.f.	M.S.	V.R.	P
Blocks	2	262·2	2·67	0·05-0·01
Female parents	7	383·4	3·91	0·001
Male parents	7	112·0	1·14	N.S.
M × F	49	222·6	2·27	0·001
F × blocks	14	142·4	—	—
M × blocks	14	67·6	—	—
M × F × blocks	98	96·1	—	—
Pooled error	126	98·09	—	—

First-order block interactions are non-significant and are therefore joined with the second-order block interaction, to give the pooled error.

line contributing to the properties of their offspring in these tests in a way which goes beyond the direct transmission of genes themselves, though of course such maternal effects may result from the action of genes in the mother. It could equally be due in the present case to sex-linkage of genes determining behaviour of the progeny when tested against 6CL. Normally sex linkage would be readily distinguishable from maternal effects in such a diallel as the male progeny would show differences relatable to their mother, which the female progeny would not. In the present case, however, this is not possible as the female and male progeny are used together in the tests against 6CL, so precluding any assessment of them separately. Taking the two sexes together, sex-linkage completely mimics a maternal effect where this is determined by the genotype of the mother. It will be assumed therefore, in the following, that reciprocal differences arise from maternal effects stemming from the genotypes of the mothers. This assumption is discussed further in Section 5.

(i) *Undefined diallels*

The general case of a single-gene difference with direct effects of d and h on the phenotype and a maternal effect of m is set out in table 3. It will be seen that, as would be expected, the variance of female array means reflects the maternal effect while that of male array means depends only on d and h. Equally, however, the variances within female arrays ($V_{r♀}$) receives no contribution from m while those for male arrays ($V_{r♂}$) depend on m as well as d and h. The covariances on the non-recurrent parents include items

depending on whether they are from the female arrays ($W_{r♀}$) or the male ($W_{r♂}$). In the former case, however, m is introduced only by the parents whereas in the male arrays it comes in from the progeny as well. In neither case is the difference between W_r from the two arrays (ΔW_r) the same as that between V_r from the two arrays (ΔV_r). The regression of W_r on V_r is

TABLE 3

The general case of a maternal effect in a diallel

Mean of parents $(d+m)(u-v)$		Male parents: AA $d+m$, u	Male parents: aa $-d-m$, v	Mean of array
Female parents	AA $d+m$, u	AA $d+m$, u^2	Aa $h+m$, uv	$ud+vh+m$
	aa $-d-m$, v	Aa $h-m$, uv	aa $-d-m$, v^2	$uh-vd-m$
Mean of array		$ud+vh$ $+m(u-v)$	$uh-vd$ $+m(u-v)$	$(u-v)(d+m)+2uwh$

(Below each genotype are shown the associated phenotype and the frequency of occurrence.)

Variance of array means

Female arrays	$uv[d+2m+(v-u)h]^2$
Male arrays	$uv[d+(v-u)h]^2$

Variance within arrays (V_r)		Δ
Female arrays	$uv(d\mp h)^2$	$4uvdh$
Male arrays	$uv(d+2m\mp h)^2$	$4uvh(d+2m)$
Mean	$uv(d^2\mp 2dh+h^2+2m^2+2dm\mp 2hm)$	$4uvh(d+m)$

Covariance within arrays (W_r)		
Female arrays	$2uv(d+m)(d\mp h)$	$4uvh(d+m)$
Male arrays	$2uv(d+m)(d+2m\mp h)$	$4uvh(d+m)$
Mean	$2uv(d^2\mp dh+2dm\mp hm+m^2)$	$4uvh(d+m)$

thus a straight line of unit slope for neither female nor male arrays. Nevertheless, if we average $W_{r♀}$ and $W_{r♂}$ and also $V_{r♀}$ and $V_{r♂}$, we find that the familiar straight regression line of unit slope appears once again for the regression of W on V, on the assumption of independent distribution and independent action of the various genes where, as in the general case, a number of differences are expressing their effects simultaneously in the diallel.

The standard test for reciprocal differences (including maternal effects) in diallels is the analysis of variance derived by Hayman (1954, and see also Mather and Jinks, 1971). Application of this analysis to the present data is set out in table 4, the " blocks " being, of course, the replicates in the

experiment. The block interactions did not differ significantly among themselves and so have been pooled to give a joint estimate of error variation. Since this is based on 126 degrees of freedom it can be divided into the sums of squares for the main items to give χ^2 testing their significance. It is clear from the analysis that additive variation (*a*) and dominance (*b*) are significant. The near significant value of (*c*) points towards differences in the

TABLE 4

The Hayman analysis of variance of the diallel experiment

Item	d.f.	M.S.	χ^2	P
(*a*) Additive variation	7	295·45	19·46	0·01-0·001
(*b*) Dominance effects	28	281·56	74·17	< 0·001
(*c*) Overall maternal effects	7	199·95	13·17	0·10-0·05
(*d*) Other reciprocal differences	21	144·06	28·46	0·20-0·10
Blocks	2	262·19	4·93	0·10-0·05
Pooled error	126	106·30		

overall maternal effects among the eight parental lines in the determination of competitive ability, while item (*d*) shows no evidence of reciprocal differences beyond those ascribable to the overall effects. This analysis therefore bears out the test, referred to above, of the difference between the variances of means of female and male arrays.

Where the genotypes of the parents and therefore of the offspring in the diallel are not known individually, the analysis can be taken no further. In the present case, however, these genotypes are defined in terms of the three major chromosomes and further information can be obtained, as we shall now see.

(ii) *A defined diallel*

The eight parental lines comprise all the eight possible combinations of the three major chromosomes from W and S. Thus $u = v = \frac{1}{2}$ for all the three " genes ", and all three " genes " are independent in their distributions among the parents. Table 3 therefore immediately simplifies, for each of the " genes ", to table 5.

TABLE 5

The defined diallel where $u = v = \frac{1}{2}$

Parental mean = 0		Male parents: AA $d+m$ $\frac{1}{2}$	Male parents: aa $-d-m$ $\frac{1}{2}$	Mean of array
Female parents	AA $d+m$ $\frac{1}{2}$	AA $d+m$ $\frac{1}{4}$ (i)	Aa $h+m$ $\frac{1}{4}$ (ii)	$\frac{1}{2}(d+h)+m$
	aa $-d-m$ $\frac{1}{2}$	Aa $h-m$ $\frac{1}{4}$ (iii)	aa $-d-m$ $\frac{1}{4}$ (iv)	$\frac{1}{2}(h-d)-m$
Mean of array		$\frac{1}{2}(d+h)$	$\frac{1}{2}(h-d)$	$\frac{1}{2}h$

The expressions for the variances and covariances of array means, V_r and W_r, can easily be derived in the same way from those in table 3, and the types of information to be derived are of course essentially the same as with the general formulae. We can, however, go further with a defined diallel such as we are discussing, since, knowing the individual parental genotypes, we can identify the genotype of each set of progeny: in other words, in the case of the single difference we are discussing, we know which cell of the diallel table is AA, which Aa, which aA, and which aa. Denoting the phenotypes observed for AA, Aa, aA, and aa by (i)-(iv) respectively (see table 5) we can then describe the situation completely estimating three independent parameters corresponding to the three degrees of freedom among the four observations (i)-(iv). The most obvious parameters to estimate, using the marginal totals of table 5 are:

$$\begin{aligned} \tfrac{1}{2}d &= \tfrac{1}{4}[(\mathrm{i}) - (\mathrm{ii}) + (\mathrm{iii}) - (\mathrm{iv})] \\ \tfrac{1}{2}(d+2m) &= \tfrac{1}{4}[(\mathrm{i}) + (\mathrm{ii}) - (\mathrm{iii}) - (\mathrm{iv})] \\ \tfrac{1}{2}h &= \tfrac{1}{4}[-(\mathrm{i}) + (\mathrm{ii}) + (\mathrm{iii}) - (\mathrm{iv})] \end{aligned}$$

and the significance of each parameter can be tested by a sum of squares found as

$$\mathrm{SS}_{(\frac{1}{2}d)} = \tfrac{1}{4}[(\mathrm{i}) - (\mathrm{ii}) + (\mathrm{iii}) - (\mathrm{iv})]^2 \text{ etc.}$$

Analysed in this way, however, the value of m cannot be estimated directly, but only by a subtraction involving the estimation of d. A different set of parameters can be chosen which gives m directly, thus:

$$\begin{aligned} d+m &= \tfrac{1}{2}[(\mathrm{i}) - (\mathrm{iv})] \\ m &= \tfrac{1}{2}[(\mathrm{ii}) - (\mathrm{iii})] \\ \tfrac{1}{2}h &= \tfrac{1}{4}[-(\mathrm{i}) + (\mathrm{ii}) + (\mathrm{iii}) - (\mathrm{iv})] \end{aligned}$$

with sums of squares found, again, as:

$$\mathrm{SS}_{(d+m)} = \tfrac{1}{2}[(\mathrm{i}) - (\mathrm{iv})]^2 \text{ etc.}$$

This approach can be readily extended to the two gene case (table 6). The situation shown in the table is that of four parental lines comprising all homozygous combinations of two gene pairs, A-a and B-b. Thus, $u = v = \frac{1}{2}$ for both gene differences and they are independent of one another in their distribution. The diallel contains 16 cells each of whose genotype is unique when maternal effects are taken into account. So 15 parameters are required to describe the data completely. These could be derived from the single gene pattern of d, $d+2m$, h; but as the maternal effect is of special interest the m, $d+m$, h will be used. Of the 15 parameters, six are taken up by m, $d+m$ and h for each of the two genes. The remaining nine are the interaction between the three parameters measuring the effects of one gene difference with the three measuring the effects of the other. These 15 parameters are listed down the left margin of table 6 which also shows the coefficients with which the phenotypes of the 16 crosses must be combined to give estimates of them together with the divisors to be applied in arriving at the estimates. Thus the parameter measuring interactions between m_a and m_b is estimated as

$$m_a \times m_b = \tfrac{1}{4}([\mathrm{AB/ab}] - [\mathrm{Ab/aB}] - [\mathrm{aB/Ab}] + [\mathrm{ab/AB}]),$$

where [AB/ab] is the phenotype of the class of progeny derived from the

TABLE 6

A defined diallel with two gene differences and $u = v = \frac{1}{2}$

Female parent	AB				Ab			
Male parent	AB	Ab	aB	ab	AB	Ab	aB	ab
Phenotype of offspring	d_a+d_b m_a+m_b	d_a+h_b m_a+m_b	h_a+d_b m_a+m_b	h_a+h_b m_a+m_b	d_a+h_b m_a-m_b	d_a-d_b m_a-m_b	h_a+h_b m_a-m_b	h_a-d_b m_a-m_b
Group	(i)	(ii)	(iii)	(iv)	(ii)	(i)	(iv)	(iii)
m_a	—	—	1	1	—	—	1	1
m_b	—	1	—	1	−1	—	−1	—
$m_a \times m_b$	—	—	—	1	—	—	−1	—
d_a+m_a	1	1	—	—	1	1	—	—
d_b+m_b	1	—	1	—	—	−1	—	−1
$(d_a+m_a)(d_b+m_b)$	1	—	—	—	—	−1	—	—
$\frac{1}{2}h_a$	−1	−1	1	1	−1	−1	1	1
$\frac{1}{2}h_b$	−1	1	−1	1	1	−1	1	−1
$\frac{1}{2}h_a \times \frac{1}{2}h_b$	1	−1	−1	1	−1	1	1	−1
$m_a(d_b+m_b)$	—	—	1	—	—	—	—	−1
$m_b(d_a+m_a)$	—	1	—	—	−1	—	—	—
$m_a \times \frac{1}{2}h_b$	—	—	−1	1	—	—	1	−1
$m_b \times \frac{1}{2}h_a$	—	−1	—	1	1	—	−1	—
$(d_a+m_a) \times \frac{1}{2}h_b$	−1	1	—	—	1	−1	—	—
$(d_b+m_b) \times \frac{1}{2}h_a$	−1	—	1	—	—	1	—	−1

cross AA BB × aa bb, etc. The sum of squares for which this parameter accounts in the analysis of variance is found as

$$\mathrm{SS}(m_a \,.\, m_b) = \tfrac{1}{4}([\mathrm{AB/ab}] - [\mathrm{Ab/aB}] - [\mathrm{aB/Ab}] + [\mathrm{ab/AB}])^2.$$

It will be observed that the 16 classes of progeny fall into four groups each of four, distinguished by the numerals (i) to (iv) in table 6. One group includes the four homozygous classes (i), and another the four double heterozygotic (iv), with another group (ii) including all four combinations of $\pm(d_a+m_a)$ and $(h_b \pm m_b)$, and the last group (iii) including all combinations of $(h_a \pm m_a)$ and $\pm(d_b+m_b)$. Each group is orthogonal to both female and male parents, and each contributes information about eight of the 15 parameters, while each parameter may draw on information from one, two or all four of the groups, as shown in the right margin of the table.

The treatment can easily be extended to the corresponding case of three gene differences. There will now be eight homozygous parents and 64 classes of progeny, each of which is unique when maternal effects are taken into account. There will thus be 63 parameters, of which nine, *i.e.* three for each of the three differences, will measure the various m, $d+m$, and h effects, 27 the first-order interactions among the nine main parameters, and 27 the second-order interactions. The 64 genotypes fall into eight groups of eight, orthogonal to female and male parents. One of them comprises the eight classes covering in their phenotypes all possible combinations of $\pm(d_a+m_a)$, $\pm(d_b+m_b)$ and $\pm(d_c+m_c)$, and a second the eight classes covering in their phenotypes all possible combinations of $(h_a \pm m_a)$, $(h_b \pm m_b)$ and $(h_c \pm m_c)$. The remaining six groups will be three of a type covering all the combinations of, for example, $\pm(d_a+m_a)$, $\pm(d_b+m_b)$ and $(h_c \pm m_c)$, and three of a type covering all the combinations of, for example, $\pm(d_a+m_a)$, $(h_b \pm m_b)$ and $(h_c \pm m_c)$.

Functions for the estimators of the 63 parameters can be written down in a way parallel to those for the two gene case in table 6, and again each group contributes to the information about certain of the parameters and not others, every class in a group being used where the group contributes. It would, however, be tedious to reproduce in detail the 63 functions, and indeed only 36 of them have in fact been used, viz. those for the nine main parameters

aB				ab					
AB	Ab	aB	ab	AB	Ab	aB	ab		
h_a+d_b $-m_a+m_b$ (iii)	h_a+h_b $-m_a+m_b$ (iv)	$-d_a+d_b$ $-m_a+m_b$ (i)	$-d_a+h_b$ $-m_a+m_b$ (ii)	h_a+h_b $-m_a-m_b$ (iv)	h_a-d_b $-m_a-m_b$ (iii)	$-d_a+h_b$ $-m_a-m_b$ (ii)	$-d_a-d_b$ $-m_a-m_b$ (i)	Divisor	Groups
−1	−1	—	—	−1	−1	—	—	8	(iii)+(iv)
—	1	—	1	−1	—	−1	—	8	(ii)+(iv)
—	−1	—	—	1	—	—	—	4	(iv)
—	—	−1	−1	—	—	−1	−1	8	(i)+(ii)
1	—	1	—	—	−1	—	−1	8	(i)+(iii)
—	—	−1	—	—	—	—	1	4	(i)
1	1	−1	−1	1	1	−1	−1	16	all
−1	1	−1	1	1	−1	1	−1	16	all
−1	1	1	−1	1	−1	−1	1	16	all
−1	—	—	—	—	1	—	—	4	(iii)
—	—	—	−1	—	—	1	—	4	(ii)
1	−1	—	—	−1	1	—	—	8	(iii)+(iv)
—	1	—	−1	−1	—	1	—	8	(ii)+(iv)
—	—	1	−1	—	—	−1	1	8	(i)+(ii)
1	—	−1	—	—	−1	—	1	8	(i)+(iii)

and the 27 first-order interactions, in the analysis of the present data to which we now turn.

4. The results

(i) *The diallel experiment*

The diallel whose results are summarised in table 1 was carried out in triplicate. The full experiment therefore includes $64 \times 3 = 192$ observations on competitive ability, measured by ϕ, and so yields 191 degrees of freedom. Of these 63 are assignable to the 63 parameters discussed above, two to overall differences between the three replicates or blocks, and $63 \times 2 = 126$ to the variation of the parameters over blocks. These 126 are divisible into $9 \times 2 = 18$ for variation of main parameters over blocks, $27 \times 2 = 54$ for variation of first-order interactions and $27 \times 2 = 54$ for variation of the second-order interactions.

The full analysis of variance of ϕ, measuring competitive ability, is overlong for complete presentation and a summary setting out the chief results is given in table 7. The error variance was based on the variation of the second-order interactions over blocks. This gave a sum of squares of 4322·261 for 54 degrees of freedom and hence a mean square of 80·042. The overall second-order interactions were tested against this and with a S.S. of 2868·997 for d.f. 27 are not significant. The test used is a χ^2_{27} obtained as the ratio of the S.S. to the error M.S. (80·042) which, being based on 54 d.f. can be used as a theoretical variance. χ_{27} obtained in this way is 35·84 with a probability of 0·20-0·10. The overall second-order interactions were consequently pooled with their variation over blocks to give a new error variance of 88·781 for 81 d.f. The variation of first-order interactions over blocks (S.S. = 5645·291 for 54 d.f.) was found not to be significant whether tested against the original error variance ($\chi_{54} = 70{\cdot}259$, P = 0·10-0·05) or against the new ($\chi^2_{54} = 63{\cdot}587$, P = 0·20-0·10) and it too was therefore rolled into the estimate of error which then becomes 95·085 for $81 + 54 = 135$ d.f. This is the error mean square used in all the later tests of significance.

The first-order interactions were isolated individually but most of them

TABLE 7

Analysis of variance of ϕ measuring competitive ability

	Item	Overall S.S.	Overall M.S.	Overall d.f.	Overall χ^2	Overall P	Block interactions S.S.	Block interactions M.S.	Block interactions d.f.	Block interactions χ^2	Block interactions P
Main parameters	m_1	425·0		1	4·47	0·05-0·02	685·3	342·6	2	7·21	0·05-0·02
	m_2	28·2		1	0·30	0·70-0·50	453·6	226·8	2	4·77	0·10-0·05
	m_3	247·0		1	2·60	0·20-0·10	809·1	404·6	2	8·51	0·02-0·01
	d_1+m_1	60·2		1	0·63	0·50-0·30	323·1	161·5	2	3·40	0·20-0·10
	d_2+m_2	1001·0		1	10·53	0·01-0·001	23·3	11·6	2	0·25	0·90-0·80
	d_3+m_3	32·7		1	0·34	0·70-0·50	196·3	98·2	2	2·06	0·50-0·30
	$\frac{1}{2}h_1$	20·0		1	0·21	0·70-0·50	608·5	304·3	2	6·40	0·05-0·02
	$\frac{1}{2}h_2$	1376·0		1	14·47	<0·001	67·6	33·8	2	0·71	0·70
	$\frac{1}{2}h_3$	2200·5		1	23·14	<0·001	86·8	43·4	2	0·91	0·70-0·50
First-order interaction	mm	819·1	273·0	3	8·61	0·05-0·02					
	$m(d+m)$	1063·0	177·2	6	11·18	0·10-0·05					
	$(d+m)(d+m)$	1812·1	604·0	3	19·06	0·001					
	hm	874·0	145·7	6	9·19	0·20-0·10					
	$h(d+m)$	1370·4	228·2	6	14·41	0·02-0·01					
	hh	178·7	59·6	3	1·88	0·70-0·50					
	Second-order	2869·0	106·3	27			4322·3	80·0	54		

	Overall χ^2	Overall P	Block interactions χ^2	Block interactions P
Main parameters	7·37	0·10-0·05	20·49	0·01-0·001
	11·50	0·01-0·001	5·71	0·50-0·30
	37·82	<0·001	8·02	0·30-0·20
First-order interaction	35·85	<0·001		
	23·60	0·05-0·02		

	Block interactions S.S.	Block interactions M.S.	Block interactions d.f.
First-order interaction	5645·3	104·5	54

Pooled error variance 95·085

were clearly not significant. As a group, however, they account for a S.S. of 6117·313 for 27 degrees of freedom, so proving highly significant with $\chi^2_{27} = 64{\cdot}335$ and P = 0·001. They have been divided into groups of like-type interactions for presentation in table 7; thus all three $m \times m$ type interactions have been grouped as have the six $m \times h$ type interaction and so on. The S.S. for the main parameters and for their variation over blocks are also presented individually in the table. In all cases the subscripts 1-3 denote that the parameter in question refer to the effects of the I-III chromosomes respectively. The χ^2's obtained by dividing the S.S. by the error variance, 95·085, are also shown in the table together with the corresponding probabilities.

It is clear from the table that there is dominance for competitive ability, notably in chromosomes II and III. It is also clear that the combination of additive and maternal variation as represented by $d+m$ is effective, at any rate in chromosome II, but this item does not, of itself, tell us whether it is additive genic variation or the maternal component that is important. The evidence about the maternal component, m, is less clear. The distribution of the X chromosome has a just significant maternal effect associated with it. Chromosome II has no evidence of such an association and the evidence for chromosome III is not good. The three chromosomes taken together show a just not formally significant effect with P = 0·10-0·05. If this were all, the evidence would not carry much weight. We should note, however, that while the $(d+m)$ and h components show no evidence of variation among the three replicates, there is clear evidence that the m effects, especially those associated with chromosomes I and III do have such variation. And if there were no m component, it could not vary over the replicates which are otherwise consistent with one another. Furthermore, when we turn to the first order interaction there is evidence that those of type $m \times m$ occur, though those of type $(d+m) \times (d+m)$, where interpretation must be ambiguous, are even stronger. The only group wholly and clearly free from m, the $h \times h$ interactions, are insignificant.

The indications are therefore that there is a maternal component in the determination of competitive ability, though the evidence is less clear than one might wish. This component is particularly related to the distributions of chromosomes I and III. Dominance is very clearly present, especially in relation to chromosomes II and III. There would seem also to be an additive, or d component, recognisable in relation to chromosome II, but further analysis of the data will help us to obtain a clearer picture of the d component and of the interactions of d and h.

We saw in Section 3 (ii) that in the case of a single gene difference comparisons can be chosen to yield estimates, orthogonal to one another, of $\frac{1}{2}d$, $\frac{1}{2}(d+2m)$ and $\frac{1}{2}h$. This breakdown can be readily extended to the two and three gene cases in the same way as the analysis which has already been used. The comparisons yielding estimates of the h's and the $h \times h$ interactions are exactly the same as those used above but we obtain estimates of d and $(d+2m)$ in place of m and $(d+m)$. The estimates and corresponding S.S.'s of the d's and the $d \times d$ interactions are obtained from the totals of the male arrays. The d's, but not the $d \times d$ interactions, are equally obtainable as the difference of the comparisons already used in finding $(d+m)$ and m. The $d \times h$ interactions can similarly be found by the differences of the $h \times (d+m)$ and $h \times m$ comparisons already used.

The S.S.'s and tests of significance of the comparisons yielding estimates of $\frac{1}{2}d$, etc., their interaction with one another and with the $\frac{1}{2}h$'s are set out in table 8. Since these are alternatives to the components isolated in the full analysis of variance of table 7, the same estimate of error variance may be used in testing their significance. The value of d_2 is verging on significance though neither of the other d's approaches at all closely to a significant level. That there are d effects is, however, strongly suggested by the near significant value of χ^2_3 obtained when all three d's are tested together. The three $d \times d$ interactions show sub-normal variation as a group. Only one of the $d \times h$ interactions is significant, viz. $d_3 \times h_2$, the remaining five being insignificant

TABLE 8

The alternative analysis of ϕ

Item	S.S.	d.f.	M.S.	χ^2	P	χ^2	d.f.	P
Main effects								
$\frac{1}{2}d_1$	82·7	1	82·7	0·87	0·05-0·30			
$\frac{1}{2}d_2$	346·7	1	346·7	3·65	0·05	6·93	3	0·10-0·05
$\frac{1}{2}d_3$	229·7	1	229·7	2·42	0·20-0·10			
First-order interactions								
$\frac{1}{2}d \times \frac{1}{2}d$ (3 items)	74·7	3	24·9	0·79	0·90-0·80			
$\frac{1}{2}d \times \frac{1}{2}h$ (5 items)	550·4	5	110·1	5·79	0·50-0·30	6·57	8	0·70-0·50
$\frac{1}{2}d_3 \times \frac{1}{2}h_2$	553·5	1	553·5	5·82	0·02-0·01			

when tested as a group. The significance of $d_3 \times h_2$ should not be over-stressed since it was selected as the single large value out of six interactions of this kind, but even so with a probability below 2 per cent. the evidence for this interaction is at least reasonable.

To summarise the outcome of this analysis, there are suggestive indications of maternal effects, which however vary over replicates. The largest of these effects appears to be associated with the distribution of the X chromosome. There is evidence of a d effect stemming from chromosome II and perhaps a hint of one from chromosome III. Dominance associated with chromosomes II and III is clear and there is evidence of a j-type interaction ($d \times h$) between chromosomes II and III. The d and m effects take sign according to whether enhancement of competitive ability is associated with the Wellington chromosome (+) or with the Samarkand homologue (−). Dominance takes sign according to whether it is in the direction of higher competitive ability (+) or lower (−). The j-type interaction $d_3 \times h_2$, is of the opposite sign to d_3 and is therefore of the kind that will tend to reduce variation in an F_2 where compound items of the form $(d+\frac{1}{2}j)$ appear (Mather and Jinks, 1971). It would thus indicate a duplicate type interaction (Mather, 1967).

(ii) *A non-diallel experiment*

Prior to the diallel experiment described above and the appreciation that reciprocal crosses did not behave alike, an earlier experiment had been undertaken. In this the eight homozygous lines were used in whatever combinations were convenient to produce the 27 possible genotypes in respect of the three chromosomes. These 27 genotypes were tested in equal numbers

against 6CL for competitive ability by the same technique as was later used in the diallel experiment. The experiment was carried out in duplicate. In the absence of complications the 27 values of ϕ, one for each genotype, afford the means of estimating the 26 independent parameters, $3d$'s, $3h$'s, 12 first-order interactions (being 3 $d \times d$, 6 $d \times h$, and 3 $h \times h$), and 8 second-order interactions (being 1 $d \times d \times d$, 3 $d \times d \times h$, 3 $d \times h \times h$, and 1 $h \times h \times h$) necessary to specify the genetical situation and of testing their significance.

Full reproduction of the analysis is not, however, justified as the estimates of only three of the parameters proved to be significant. Two of these are mean effects, d_3 and h_3, and these are set out in the last column of table 9,

TABLE 9

Estimates of the parameters from the analysis of ϕ

The first two columns are from the diallel experiment and the third from the non-diallel experiment

m_1	−2·10*	d_1	1·31	" d_1 "	1·1
m_2	0·54	d_2	2·69*	" d_2 "	1·8
m_3	1·60	d_3	−2·18	" d_3 "	3·7**
d_1+m_1	−0·79	h_1	−0·65	h_1	2·0
d_2+m_2	3·23**	h_2	5·35**	h_2	2·2
d_3+m_3	−0·58	h_3	6·77**	h_3	10·3**

* Significant at 5 per cent. level.
** Significant at 1 per cent. level.

where the remaining four main parameters are also given albeit they are not significant. The third significant value was obtained for $d_2 \times d_3$. The d values from this experiment will each contain a component depending on the corresponding m but this will vary according to the way in which the crosses were made to produce the various genotypes that are being compared. The estimates for this experiment are therefore shown as " d_1 ", etc., in the table to indicate this uncertainty. Comparison of their values with those from the diallel suggest that, in so far as comparisons among insignificant estimates are meaningful, " d_1" has been little affected by m, " d_2" has been affected a little more and " d_3" most of all. That d_3 and m_3 between them are exerting an effect can, however, now be hardly doubted though neither of them was established as significant in the earlier analysis. The values of the h's are likely to be less affected by the m's and the comparison between the h's from this experiment and from the diallel is remarkably good. Both experiments yield significant evidence of h_3; h_2 is significant in the diallel and, though not significant from the 27 genotypes, is moderately high, and only in the case of h_1 where neither estimate is significant, is there any appearance of disparity. The significant value of " d_2" × " d_3" obtained from the 27 genotypes is not surprising in view of the behaviour of the m's in the diallel experiment.

One general point should be noted about the d's and h's from the two experiments. The various genotypes occur with their F_2 frequencies in the diallel and the orthogonal comparisons employed in the analysis will yield d's and h's conforming to the definition used by Hayman and Mather (1955). These do not conform to the more general definitions used by Mather and Jinks (1971). Indeed, when transformed into Mather and Jinks notation,

Hayman and Mather's d contains elements of the j-type interactions and their h contains elements of l-type interactions. Thus, for example, d_1 and h_1 as found from the diallel experiment would be respectively $d_1+\frac{1}{2}j_{12}+\frac{1}{2}j_{13}$ and $h_1+\frac{1}{2}l_{12}+\frac{1}{2}l_{13}$ in the more general notation used by Mather and Jinks. The 27 genotypes occur all equally often, and not with their F_2 frequencies, in the second experiment described immediately above. The d's and h's therefore conform to neither Hayman and Mather's nor Mather and Jinks' definitions. They again contain elements of j- and l-type interactions but now are $d_1+\frac{1}{3}(j_{12}+j_{13})$, etc., and $h_1+\frac{1}{3}(l_{12}+l_{13})$, etc., in the more general notation. Thus, even apart from the effects of the m's, the d's and h's from the 27 genotypes do not correspond precisely with those from the diallel, though the difference in structure is so small as to be negligible by comparison with the error variance.

5. Conclusion

The results of the present experiments still leave questions open about the determination of the difference in competitive ability between the Wellington and Samarkand lines. There can be little doubt that chromosomes II and III are exerting direct genetic effects, for in the diallel experiment both h_2 and h_3 emerge clearly as significant and d_2 is near significant, while h_3 again emerges as significant from the 27 genotypes, where "d_3" is also significant though not capable of unambiguous interpretation. The X chromosome has yielded no evidence of a direct genic action on competitive ability.

Perhaps the most important finding is the indication of differences in competitive ability between the offspring of reciprocal crosses and the major component of this kind is associated with the distribution of the X chromosome. This might be taken as indicating that the differences between reciprocals are due to the direct effects of sex-linked genes, which as we have seen earlier cannot be distinguished analytically from maternal effects in the diallel experiment as carried out. There is, however, some evidence against this view. In the first place if the significant value of m were due to the genetic differences between the X's of the males from reciprocal crosses, one would expect to find a d_1 component ascribable to the females which will be alike in reciprocal crosses. No good evidence of such a component has emerged. This is, of course, no more than negative evidence which might be reversed by a larger experiment or a more precise test. A second point is, however, somewhat more positive. The m's proved to be significantly variable over replicates, though the h's and the d's were not. Similarly, wherever m's were involved there appeared to be a greater propensity for interactions to appear, there being in fact only one clear interaction not involving m, viz. that between d_2 and h_2 in table 8. There is thus an indication that the m's are intrinsically more subject to variation than the h's or the d's, and this is most readily interpreted as implying a difference in the basic nature of the parameters—in other words that m is not measuring a direct genetic effect though it must be measuring a transmissible property.

The component of variation measured by the m's is therefore provisionally taken as a maternal effect. Just how it is determined is, however, another matter. For the purpose of analysis it has been assumed to be determined by the mother's genotype, as in the classical case of coiling direction in the snail *Limnaea peragra*, and this assumption is not unreasonable

on other grounds. It is very unlikely that the differences of ϕ in reciprocal crosses merely reflect differences in fecundity of the wild-type mothers, as neither a simple analysis of variance nor a Hayman analysis reveals evidence of a maternal effect in respect of total yield of flies similar to the maternal effect in respect of ϕ. The reciprocal differences must, therefore, spring from differences in competitive ability. Now the genotypes of the larvae competing with 6CL will be the same in reciprocal matings (apart, of course, from the X chromosomes in males from certain crosses); but their fate may well be affected by the mating and, more importantly, laying behaviour of the mothers, whose genotypes will not be the same in the crosses. Now if, for example, one type of female is slower than the other in starting to lay, its offspring could be at a relative disadvantage, since Bakker (1961) has shown that even a difference in age of a few hours affects the prospects of larvae in a competitive situation. This would lead to a maternal effects comparable with that in *Limnaea*.

It might be, however, that the difference between reciprocals sprang from a determinant extra-chromosomal in nature and transmitted through the female parent. In this case the apparent association with the chromosomes would not be causal and would reflect no more than the lines of descent of the chromosomes in building up the eight homozygous lines used as parents in the diallel. It would nevertheless represent a maternal effect, albeit one that is commoner in plants than in animals. Only further experiments can distinguish among these various possibilities.

One final point remains to be made. The three major chromosomes were manipulated as units in this experiment. The effective factor is thus the whole chromosome and we must expect it to have properties which transcend those of the individual genes, borne by the chromosome, from which the various effects ultimately spring. Thus the association of a maternal effect as well as a *d* or *h* with a given chromosome does not mean that it is produced by the same gene or genes. Nor does the observation of a larger *h* than *d* mean that there is overdominance, since if more than one gene of the chromosome is involved such an effect could imply no more than that other genes are distributed between the W and S homologous in such a way that their *d*'s balance even though their *h*'s may be reinforcing.

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