ESTIMATING THE COMPONENTS OF CONTINUOUS VARIATION

I. STATISTICAL

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Received 1.viii.61

1. THE PROBLEM

THE genetical description and analysis of continuous variation depends, not on the isolation and measurement of the effects of single gene differences in the classical Mendelian fashion, but on the biometrical interpretation of the various statistics derivable from observation of related individuals in terms of parameters measuring the non-heritable and the different heritable components of variation. Two processes are therefore involved in such an analysis; first the description of the available statistics in terms of the genetical quantities, components of variation as they have been termed, of which use is to be made; and secondly the extraction of these quantities from the values observed for the statistics.

The genetical descriptions of the statistics obtained by observations of individuals standing in various degrees of relationship rests on the assumption that the heritable determinants of continuous variation are nuclear genes transmitted from parent to offspring on the chromosomes, segregating and recombining just as the genes of major effect. This assumption has been fully validated by experiment and descriptions have been formulated in terms of components representing the effects of additive gene action, dominance, interaction of non-allelic genes and genotype-environment interactions (Mather, 1949; Mather and Vines, 1952; Lerner, 1950; Hayman and Mather, 1955; Mather and Jones, 1958; Kempthorne, 1957; Falconer, 1960). The only remaining divergence is in respect of the notation for representing these components of variation and the composition of the various statistics in terms of them. The components are composite, each representing the summed effects of all the genes which can contribute to it, and are derived by theoretical consideration. In no case yet has the check been made of comparing the values of the components derived by biometrical analysis in the now familiar way with their values as expected from the measurement and summation of the effects of the individual genetic differences. Neither the desirability nor the difficulty of making such a check requires stressing.

The estimation of the various components from constellations of observed statistics has been approached in several ways, of which the most generally applicable and useful is by a form of least squares analysis yielding estimates of the several components of variation. In its crudest form, as developed by Mather (1949), this consists of fitting constants for the various genetical components by minimising the sum of squares of the residuals of the several statistics, given equal weights. This process is simple but is open to several objections, especially that it takes regard, neither of the differences in precision with which the various statistics are observed experimentally, nor of the correlations that must exist between the values observed for them. Furthermore the standard errors which are obtained for the estimated values of the parameters are not fully reliable (Nelder, 1953). Recently Nelder (1960) and Hayman (1960) have devised a method of estimation which does pay regard to both the differences in precision and the correlation of the statistics forming the raw materials of the analysis, and which leads to more reliable values for the standard errors as well as a more informative test of goodness of fit. Involving as it does the use of weights which depend on the values of the parameters themselves, this method necessarily requires iterative calculations which can make prohibitively heavy demands where only a desk calculator is available. The task is, however, well suited to the use of an electronic computer and it was decided to carry out an analysis in this way both for its intrinsic content and for the test it would afford of the reliability of the estimates arrived at by the earlier and simpler method of estimation. The statistical aspects of this estimation will be considered in this first paper and the genetical aspects in a second one.

2. THE EXPERIMENT

The experiment was based on two inbred lines of *Drosophila melanogaster*, Samarkand (S) and the Birmingham line of Oregon (B), the character used being the number of sternopleural chaetæ. In the experiment with which we are concerned, the lines were crossed reciprocally and F_2's were raised from the resulting F_1's. From these F_2's, pair matings were made to the number of 346 from the cross $B \times S$ and 364 from its reciprocal, $S \times B$. The resulting families are of the types which Mather (1949) has called BIPS.

An experiment of this magnitude must obviously be spread over time and the BIPS were raised in groups of up to 30 or 40 at a time. On each occasion that a group of BIPS was being raised, up to 3 F_2's (in the case of $B \times S$) or 4 (in the case of $S \times B$) were also raised together with a single culture of each parental line and of each of the reciprocal F_1's. Parents, F_1's and F_2's were raised also at times when no BIPS were produced. The total numbers of cultures of P's, F_1's and F_2's appear in table 1. The chaetæ were counted on 10 females and 10 males from each culture in these generations and also in the

BIPS. Conditions were held as constant as possible over the period of the experiment and an analysis of variance of the F_2 results showed that the mean number of chaetæ did not vary significantly more between cultures raised on different occasions, than between cultures

TABLE 1

Total numbers of cultures scored, and numbers on each occasion

Generation	Parents		F_1's		F_2's		BIPS	
	B	S	B×S	S×B	B×S	S×B	B×S	S×B
Total No. .	29	29	29	29	44	49	346	364
Per occasion .	1	1	1	1	1·3	1·4	1·30	1·37

raised at the same time. A similar test was not possible with the results from the parents and F_1's, but no trend of the means could be observed graphically with time in these. There is thus no evidence that the spread of the experiment over time introduced any additional

TABLE 2

Mean and variance of parents, F_1's and F_2's

	No. of cultures	Mean	Mean variance within cultures	d.f.	Variance of mean of 10 over occasions	d.f.
Parents						
B ♂	29	22·4724	3·1376	261	0·6940	28
♀		22·9310	2·9628	261	0·5182	28
S ♂	29	18·2034	1·8333	261	0·4196	28
♀		18·5207	1·9374	261	0·2385	28
F_1						
B×S ♂	29	19·5828	2·2331	261	0·3272	28
♀		20·4586	2·0184	261	0·3354	28
S×B ♂	29	20·1172	2·6927	261	0·2419	28
♀		20·3138	1·8372	261	0·4618	28
F_2						
BS ♂	33	19·7091	4·8660	297	0·5033	32
♀		20·1205	3·1475	297	0·4139	32
SB ♂	37	19·8224	4·4303	333	0·5223	36
♀		20·4592	4·1654	333	0·5725	36

source of variation. The distinction between occasions has therefore been omitted from account in the analysis of the results.

The overall mean numbers of chaetæ and variances, both of individuals within cultures and of means between cultures, are given for P's F_1's and F_2's in table 2, together with the numbers of degrees

of freedom on which the variances are based. The variances of parents and F_1's must obviously be non-heritable and provide the direct estimates of the non-heritable components of variation E_1 (of individuals within cultures) and E_2 (of means between cultures) used in the analysis. It will be seen that males always showed lower means and generally showed higher variances than did their sisters. The B parent also showed higher variances than S and the F_1's which, however, did not differ markedly or consistently among themselves. This difference in variances was accommodated by estimating E_1 and E_2 as the sum of one quarter of the variance of each parental line and one half of the variance of the corresponding F_1.

Strictly the BIPS should be raised by random pair matings among F_2 flies and this course was followed at first. For some reason, however, a correlation appeared between the chaeta counts of the male and

TABLE 3

Frequency distribution of F_2

Cross	15	16	17	18	19	20	21	22	23	24	25	26	Total
BS ♂	1	5	9	12	19	21	14	8	7	2	1	1	100
♀	...	1	7	14	16	21	18	15	6	2	...	...	100
SB ♂	1	4	8	13	21	15	13	14	7	3	...	1	100
♀	2	1	1	7	16	20	22	11	12	4	4	...	100

female parents taken. It was therefore decided deliberately to make up matings with frequencies corresponding approximately to those expected by combinations of the frequencies with which males and females were observed to fall in the various chaeta classes of the F_2's. The frequency distributions of chaeta number in 100 flies of each of the F_2's are shown in table 3 and the distribution of the matings used to raise BIP families in table 4.

The chaeta frequency distributions used in determining the mating frequencies were taken from 10 of the early F_2 families. The counts from these cultures are excluded from the calculations of the variances of individuals within F_2 cultures (V_{1F2}) used in both the analyses to be described and also from the variances between F_2 cultures used only in the second analysis. In addition one F_2 culture from $B \times S$ and two from $S \times B$ were omitted as the incubator temperature fluctuated unduly during the time they were being raised.

The BIPS yield, of course, values for overall mean number of chaetæ and also variances within (V_{2S3}) and between (V_{1S3}) cultures, as well as the covariance of BIP means with the parental averages (W_{1S23}). The notation is that of Mather and Vines (1952). Scaling tests using parental F_1, F_2 and BIP means were carried out on males and females separately by the method of Cavalli (1952). These

indicated the presence of interaction in females ($\chi^2_{[2]} = 25{\cdot}27$) and even more strongly in the males ($\chi^2_{[2]} = 48{\cdot}57$). As will be seen in the later paper these interactions were neither very large as compared

TABLE 4

Chaeta number in the F_2 parents of BIP families

Females \ Males	15	16	17	18	19	20	21	22	23	24	25	26	Total
15	...	...	...	...	...	...	...	...	...	...	...	...	0
	...	...	1	1	1	1	1	1	2	...	...	...	8
16	...	...	1	...	...	...	...	...	...	...	...	...	1
	...	...	...	...	1	2	...	1	...	...	...	...	4
17	...	1	2	3	4	5	3	2	2	1	1	...	24
	...	...	1	...	...	1	1	...	...	...	...	...	3
18	2	3	4	5	10	13	6	5	5	...	2	...	55
	...	1	2	3	5	4	3	3	2	1	...	1	25
19	1	3	5	7	11	12	8	6	5	2	...	1	61
	2	2	5	7	12	8	7	8	4	2	1	1	59
20	2	2	8	8	14	15	10	5	4	1	3	...	72
	1	3	5	9	16	10	8	10	4	3	1	...	70
21	1	3	6	7	11	13	9	4	4	1	...	2	61
	1	3	10	10	16	13	10	11	6	2	1	...	83
22	1	1	5	6	10	10	6	3	2	2	...	1	47
	...	2	3	5	8	6	6	4	3	1	...	...	38
23	...	1	2	2	4	4	3	2	...	...	...	...	18
	...	2	3	6	9	6	6	6	3	1	...	...	42
24	...	...	1	1	1	1	1	2	...	...	...	...	7
	...	1	2	2	3	2	2	1	1	...	...	...	14
25	...	...	...	...	...	...	...	...	...	...	...	...	0
	...	1	1	3	5	3	2	2	1	...	...	...	18
Total	7	14	34	39	65	73	46	29	22	7	6	4	346
	4	15	33	46	76	56	46	47	26	10	3	2	364

The figures in the body of the table are the numbers of matings with male parent having the chaeta number as at the head of the column and female parent the chaeta number as in the left margin. The upper figure in each cell is the number of matings from the F_2 of B×S, and the lower figure that from the F_2 of S×B.

with the main components of variation nor of a kind that could easily be scaled out, and so no rescaling was in fact attempted.

The statistics available for estimating the components of variation are collected together in table 5. They are given for sexes and reciprocal crosses separately, and for the whole experiment.

The figures are as consistent as could be expected except for the females of B×S. These give a very high value for W_{1S23} accompanied by low values for V_{1F2} and V_{2S3}. Furthermore, the values for the

B × S females are not merely inconsistent with those for the other three parts of the experiment, they are inconsistent with one another. Thus W_{1S23}, whose expectation is $\frac{1}{4}D$, falls short of that of V_{1S3}, whose expectation is $\frac{1}{4}D+\frac{1}{16}H+E_2$, by only 0·1377, a value which is less than one third the direct estimate of E_2. Again W_{1S23} is only 1·8289 less than V_{2S3} whose expectation includes E_1, of which the direct estimate is 2·4906. This set of results must therefore be regarded as

TABLE 5

Statistics used in estimation of the components of variation and, in brackets, the degrees of freedom on which they are based. The expectation in terms of the components of variation is given for each statistic

Statistics	BS ♂	BS ♀	SB ♂	SB ♀	Overall Average
V_{1F_2} . . . $\frac{1}{2}D+\frac{1}{4}H+E_1$	4·8660 (297)	3·1475 (297)	4·4303 (333)	4·1654 (333)	4·1606
V_{1S_3} . . . $\frac{1}{4}D+\frac{1}{16}H+E_2$	1·0673 (345)	1·1921 (345)	1·2002 (363)	1·1960 (363)	1·1648
$W_{1S_{23}}$ . . . $\frac{1}{4}D$	0·5797 (345)	1·0544 (345)	0·5376 (363)	0·7318 (363)	0·7236
V_{2S_3} . . . $\frac{1}{4}D+\frac{3}{16}H+E_1$	3·2767 (3114)	2·8733 (3114)	3·3076 (3276)	3·1315 (3276)	3·1491
E_1 . . .	2·6854 (522)	2·4906 (522)	2·2630 (522)	1·8873 (522)	2·3316
E_2 . . .	0·4881 (56)	0·4268 (56)	0·3308 (56)	0·3502 (56)	0·3990

suspect on genetical grounds. The other three sets of results are not open to any such suspicion. The consequences of these inconsistencies, for which no reason can be advanced, will be seen in the results of the analysis.

3. THE UNWEIGHTED ANALYSIS

The analysis by the crude unweighted procedure follows the same pattern as the example described by Mather (1949, pages 66-68 and 95-96) and his matrices were used in the calculations. It should be observed that this analysis is strictly valid only where all the genetic differences follow an autosomal pattern of inheritance, whereas in fact, the sex chromosomes must be expected to contribute to the differences between B and S. If we neglect any possible effect of the Y chromosome, the males cannot be heterozygous for any differences, so that in segregating generations they will contribute less to H and more to D than the assumption of autosomal inheritance allows. The females of a family receive one common X from their fathers and so will always give backcross ratios for any segregating gene. They will

therefore, on the average contribute less to D and more to H than the autosomal formula allows. There is thus some tendency towards compensation between the sexes and in any case, as will be seen from the later paper, the contribution of the X chromosome to the parental difference, taken as a unit, was not large. The use of autosomal formulæ should not therefore lead to any major disturbances in the estimates of the components of variation and indeed the difficulty it introduces must be considerably less than that springing from the unexplained inconsistencies of the statistics noted above.

Since the available statistics include V_{2S3}, a test for the effects of recoverable linkage on the variation is possible as this statistic is of the second rank in relation to recombination, whereas V_{1F2}, V_{1S3}

TABLE 6

Analyses of variance for linkage. The entries are mean squares

Data	Linkage 1 df	Remainder 1 df	VR	P (per cent.)
BS ♂ . .	0·3861	0·0275	14·0400	...
BS ♀ . .	0·0949	0·0023	41·2609	10·5
SB ♂ . .	0·0510	0·0015	34·0000	...
SB ♀ . .	0·0057	0·0034	1·6765	...
Overall . .	0·0204	0·0012	17·0000	...

and W_{1S23} are of the first rank (Mather, 1949; Mather and Vines, 1952). If, therefore, recombination is materially affecting the components of variation the D and H of V_{2S3} will not be the same as the D and H of the other statistics. Two analyses are therefore conducted, the inclusive which brings in V_{2S3} and the exclusive which omits it, so that in effect it becomes its own expectation and thus accommodates any change in the values of D and H. The comparison of the expectations, found from inclusive and exclusive estimates of the components, with the values observed for the statistics, allows an analysis of variance to be carried out in which a mean square for linkage is compared with a mean square for residual variation.

Since six statistics are available and four components of variation are estimated in the inclusive analysis, two degrees of freedom remain for assessing the variation arising from differences between observation and inclusive expectation. The exclusive analysis in effect estimates five parameters, leaving one degree of freedom for residual variation. The difference between these two mean squares springs from linkage effects (or from interactions indistinguishable from them—see Opsahl (1956)), so that the linkage item in the analysis of variance takes one degree of freedom, the residual variation in the analysis being the residual variation of the exclusive calculations. The analyses of variance are set out in table 6, for the males and

females of the two reciprocals separately and for the combined data. There is a suggestion of linkage effects in the B×S females, whose results however we have judged to be suspect on other grounds. The overall results give no significant indication of linkage effects. However, if we pool over the individual observations we can obtain a variance ratio of 15·50 for four and four degrees of freedom which has a P≏0·01. This would seem to imply some degree of heterogeneity in respect of linkage between the four parts of the experiment. There is therefore no good evidence of consistent disturbances due to linkage and we have taken the inclusive estimates of the components, obtained ignoring linkage effects, for further consideration.

The estimates are set out in table 7. Two points require comment. The standard errors shown in the body of the table are derived from

TABLE 7

Estimates of the components of crosses from the unweighted inclusive analysis

	BS ♂	BS ♀	SB ♂	SB ♀	Overall average	Pooled s.e.
D	2·8813±1·6010	3·8534±0·7761	2·5123±0·5704	2·9550±0·2387	3·0480±0·3659	±0·9420
H	1·8795±3·9571	−4·5432±1·9184	3·3469±1·4111	3·0118±0·5902	0·9550±0·9043	±2·3284
E_1	2·4616±0·4417	2·5363±0·2142	2·2175±0·1575	1·8834±0·0656	2·3125±0·1010	±0·2600
E_2	0·3588±0·3455	0·4698±0·1676	0·3469±0·1233	0·3096±0·0520	0·3711±0·0787	±0·2032

The standard errors derived from the error variances in the unweighted analyses are shown by each statistic, and those for the pooled estimates of error are given in the right-most column (see in text).

the differences between the values observed for the six statistics and the values expected using the inclusive estimates of the four components of variation. The residual variation, and by derivation the standard errors of the components, are assessed from two degrees of freedom in each of the four parts of the experiment as noted above. The standard errors should therefore be used with corresponding caution. Since, however, the four parts of the experiment may be regarded as affording independent values of the six statistics and independent estimates of the four components their sum of squares for residual variation may be pooled to yield a combined estimate of residual variation based of course on $2 \times 4 = 8$ degrees of freedom. Common standard errors, applying to the estimated components from all four parts of the experiment have been calculated from this combined residual mean square and are shown at the end of the table. The overall estimates of each component is virtually the mean of the four estimates for the four parts of the experiment and so will take therefore a standard error half that of the common standard error applying to each of the four individual estimates.

The second point requiring comment is the difference between the components as estimated from the B×S females as compared with

their brothers and the two sexes from S×B. As would be expected from the high covariance and the low variances B×S females yielded as compared with the rest of the experiment, they give a high value for D while H appears negative and this negative value approaches significance ($t_{[2]} = 2{\cdot}3683$ and $P = 0{\cdot}2\text{-}0{\cdot}1$ using the individual estimate of its standard error, and $t_{[8]} = 1{\cdot}9512$ and $P = 0{\cdot}1\text{-}0{\cdot}05$ using the common standard error based on the pooled residual variation). The estimates from the S×B females and the two groups of males are both reasonable and consistent among themselves. Even the difference of H from the B×S males (1·88) on the one hand and the average H of the sexes from S×B (3·18) is not significant. Our view that the B×S females are aberrant in the data they yielded is thus further strenthened.

4. WEIGHTED ANALYSIS

The unweighted analysis is simple to use in that the matrices it involves may be inverted once and for all (Mather, 1949), but it takes no account either of the differences in precision with which the various statistics are found experimentally or of the correlations which must exist between the values observed for them.

It may be expected to extract the greater part of the information from the data, but in so far as there is any loss of efficiency, the final test of goodness of fit between observation and expectation must be, to that extent, suspect. Furthermore, the standard errors it yields for the estimates of the components of variation will be unreliable, partly because of this loss of efficiency and partly because any positive correlations among the initial statistics will tend to cause them to be underestimated. Unbiased estimates of the standard errors applicable to estimates obtained by unweighted analysis could be found empirically where the experiment is replicated, by obtaining separate estimates of D, H and E from each section of the experiment and deriving the standard errors of the components from the variation between these replicated estimates (Nelder, 1953). The numbers of degrees of freedom available for finding these empirical standard errors must, however, be small unless the sub-division of the experiment is extreme.

An analysis in which the statistics are weighted to take account of their precisions and their correlations, will overcome these difficulties and Nelder (1960) and Hayman (1960) have independently shown how the weights may be derived and the analysis carried out. Full accounts of the method will be found in these papers.

In the unweighted analysis certain genetic sampling terms which depend on the size of family, plot or culture, as the case may be, and enter into the expectations of statistics such as the variance of BIP means, are neglected. In the weighted analysis no further complication arises from the inclusion of these terms and also of the variance of F_2 culture means as an additional statistic. The comparison

between weighted and unweighted analysis is thus slightly complicated, but in the experiments reported here much the greater part of the difference in precision is due to the weighting itself rather than to these other refinements.

For most purposes it is sufficient to calculate the weights and correlations from a model which assumes that the character under consideration is normally distributed in the various families, and that the correlations are normal. For example, if $v_0 = V_{1F2}$, $v_1 = V_{1S3}$, $w = W_{1S23}$, based on N degrees of freedom and $v_2 = V_{2S3}$ based on N′ degrees of freedom and V_0, V_1, W and V_2 denote the corresponding expectations, this gives for the sampling variance matrix of the observed variances and covariances (v_0, v_1, w and v_2)

$$\begin{bmatrix} 2V_0^2/N & 2W^2/N & 2V_0W/N & 0 \\ 2W^2/N & 2V_1^2/N & 2V_1W/N & 0 \\ 2V_0W/N & 2V_1W/N & (W^2+V_0V_1)/N & 0 \\ 0 & 0 & 0 & V_2^2/N' \end{bmatrix}$$

Nelder (1960) has considered, in certain cases, how far this approximate variance matrix represents the true sampling variances and covariances of the observed statistics and has found it satisfactory, at least when the number of genes is not too small. However, V_0, V_1, W and V_2 are not known in advance, but have to be estimated from the analysis. An iterative process is therefore necessary starting with the empirical values v_0, v_1, w and v_2. Such a process has been carried out by Hayman (1960) but is clearly a very heavy operation unless an electronic computer is available.

The non-random choice of matings among the F_2's in the *Drosophila* experiment must presumably have reduced the variance of W_{1S23} and also the effective number of degrees of freedom in V_{1S3}, but it was not practicable to correct for this in the weighted analysis. The fact that, as we shall see, the total residual χ^2 was 7·449 with 8 d.f. suggests that the weights were not seriously wrong.

The iterative weighted least squares procedure has now been programmed for the Elliott 401 computer at Rothamsted. The programme is sufficiently general to deal with most, if not all, cases which are likely to arise in practice. For example, the statistics may fall into groups such that any pair belonging to the same group are correlated, but statistics in different groups are uncorrelated. This is the most frequent situation, a group of statistics consisting of the variances of a set of variables and all possible correlations between pairs of these variables. The details of the procedure are as set out in Nelder (1960) except that the rules for forming the variance matrix of the statistics have been generalised, but the correction for kurtosis has not been included.

The computer is able to handle a number of observed variances and covariances considered as a $n \times 1$ column vector $\mathbf{x}$, and a number

of estimated parameters considered as a $m \times 1$ column vector $\boldsymbol{\theta}$ where $n \leqq 16$, $m \leqq 10$ and $nm \leqq 128$. The greater part of the calculation consists of standard operations on matrices. The only special feature of the programme is the method used in providing all the information required for the calculation of the variance matrix $\mathbf{V}$ of $\mathbf{x}$.

Suppose there are s associated sets of variances and covariances, the tth set having v_t variances, c_t covariances and N_t degrees of freedom. Form a symmetric matrix Z by placing all the variances in the diagonal positions and each covariance in the same row and column as its associated variances. Let x_i, the ith element of the observation vector $\mathbf{x}$, be in the r_ith row and the c_ith column above the diagonal of the Z matrix.

Then

$$\operatorname{cov}(x_i, x_j) = (X_{r_i r_j} X_{c_i c_j} + X_{r_i c_j} X_{r_j c_i})/N_i$$

where X_k is the expected value of x_k as estimated from the previous cycle of the iteration. In the first cycle $X_k = x_k$.

In order to economise in storage space, the Z matrix is stored in the computer as the $n \times 1$ column vector $\mathbf{x}$ and a $n \times 1$ position vector $\mathbf{p}$. The ith element of $\mathbf{p}$ contains in packed form, the position of x_i in the Z matrix and also additional information which enables the covariance of x_i and x_j to be either calculated using the above formula or to be set to zero. (This is the usual case when x_i and x_j belong to different sets.) An approximation to the special case in which a covariance is present but one of the associated variances is missing may be made by giving the missing variance an arbitrary value with a small non-zero fractional value for the degrees of freedom.

The iteration is continued until $M_\theta = \max \dfrac{(\theta_r - \theta_{r-1})^2}{\operatorname{var} \theta_r}$ is less than a prescribed quantity a^2, the suffices r, $r-1$ referring to the cycle of iteration and the output then comprises M_θ, the total (weighted) sum of squares, the fitted sum of squares, the estimated expected values, X, of the variances and covariances, x, the estimated components, θ, and the variance matrix of $\boldsymbol{\theta}$. The difference between the total and fitted sum of squares gives a χ^2 for goodness of fit.

The principle of the test for the effects of linkage is the same as in the unweighted analysis, but the actual application is a little different because in the construction of Mather's (1949) matrices no allowance was made, in formulating the expectation of V_{1S3}, for the small items representing the genetic sampling variation which must be shown by mean values of finite families. This term reflects the genetic variation within families and is therefore related to V_{2S3}. Neglecting it, as Mather did in the interests of simplicity, allows the effect of linkage to be accommodated by omitting V_{2S3} from the analysis, but its inclusion brings the second rank components D_2 and H_2 into the expectation for V_{1S3} which otherwise depends solely

on the first rank components D_1 and H_1, together of course with E_2. This term for genetic sampling variation was included in the weighted analysis, so that the linkage effects cannot be accommodated by the simple exclusion of V_{2S3}. Rather V_{2S3} must be retained and a quantity $G = \frac{1}{4}D_2 + \frac{3}{16}H_2$ must be estimated alongside D_1, H_1, E_1 and E_2, this quantity appearing in V_{2S3} and, by virtue of the sampling term, also in V_{1S3}. D_2 and H_2 appear in the same combinations in both statistics so that this inclusion requires and indeed permits the estimation of only the single additional component G as defined above. The difference between the two residual χ^2, one from the analysis when D and H are assumed to be homogeneous (*i.e.* when no allowance is made for linkage, comparable with the inclusive unweighted analysis) and the other from the analysis where G is introduced (*i.e.* where allowance is made for linkage effects comparable with the exclusive analysis) provides a χ^2 testing for linkage effects in essentially the same way as the linkage mean square does in the unweighted analysis. The only difference is that it provides a test of significance in its own right without any comparison with the residual variation unless of course the χ^2 reflecting this residual variation is itself significant. In fact from table 8 (a) we obtain by summing the residual χ^2 from the four exclusive analyses a value of 7·449 with 8 d.f., which agrees very well with expectation.

The weighted analysis also differed from the unweighted in the present case in one final respect. In the unweighted analysis, no account was taken of the variance between the mean of F_2 cultures, the variance of F_2 being found solely from differences among individuals within cultures. In the weighted analysis V_{1F2}, found as the variance within F_2 cultures and having the expectation $\frac{1}{2}D + \frac{1}{4}H + E_1$, was used exactly as in the unweighted, but an additional statistic was introduced, found as the variance between the means of F_2 cultures, these means being based on 10 flies and having the expectation $\frac{1}{20}D + \frac{1}{40}H + E_2$. The number of components estimated in the weighted analysis was thus the same as in the unweighted, but the number of statistics used in providing the estimates was raised from six to seven, so that there is one extra degree of freedom for residual variation in the weighted as compared with the unweighted treatment. The inclusive weighted analysis provides therefore a χ^2 for 3 degrees of freedom for residual variation and in the equivalent of the exclusive analysis, one for 2 degrees of freedom, their difference being a χ^2 testing linkage and having one degree of freedom just as the linkage mean square had one degree of freedom in the unweighted treatment.

The results of that test for the effects of linkage can be seen from the bottom lines of table 8 (a) where are set out the final values of χ^2 from the inclusive and exclusive analyses of the four parts of the experiment. The linkage $\chi^2_{[1]}$ is significant (P = just over 0·02) in the B×S males, but not significant in any of the other three, the χ^2 being indeed rather small for the S×B females. If, however, we

add all four linkage χ^2 together to find $\chi^2_{[4]} = 9{\cdot}595$ the joint evidence again appears to be strongly suggestive of linkage effects since P is only just over 0·02. As with the unweighted analysis, however, the evidence is not as good as might seem at first sight since if we take

TABLE 8

The effect of iteration on :

(a) *The values of* χ^2

	BS ♂			SB ♂		
	Inclusive (3 df)	Exclusive (2 df)	Linkage (1 df)	Inclusive (3 df)	Exclusive (2 df)	Linkage (1 df)
After 1 cycle .	6·502	3·585	2·917	1·282	0·101	1·181
After 2 cycles .	9·448	4·306	5·142	1·458	0·102	1·356
Final value .	9·276	4·105	5·171	1·454	0·102	1·352
	BS ♀			SB ♀		
After 1 cycle .	4·413	0·750	3·663	2·738	2·632	0·106
After 2 cycles .	3·685	0·592	3·093	2·733	2·725	0·008
Final value .	3·638	0·582	3·056	2·676	2·660	0·016

(b) *The estimates, as measured by* $\sqrt{M_\theta}$

	BS ♂		SB ♂	
	Inclusive	Exclusive	Inclusive	Exclusive
After 1 cycle .	0·122	0·447	0·025	0·008
After 2 cycles .	0·008	0·097	0·000	0·000
	BS ♀		SB ♀	
After 1 cycle .	0·138	0·099	0·235	0·149
After 2 cycles .	0·006	0·003	0·032	0·015

This table shows the maximum change in an estimate in the next cycle, relative to its standard error. Thus the entry 0·122 implies that the difference between the 1st and 2nd cycle estimates is at most 0·122 of its standard error.

the $\chi^2_{[1]}$ for linkage from the pooled data, comparable with the overall linkage test in the unweighted analysis (table 6), we find it to be only 0·7189. This implies that the individual groups of data must be heterogeneous in the evidence they provide for linkage. It should, however, be realised that in any case the evidence for linkage in biometrical genetics is not to be judged in the same way as that from the linkage experiments of classical genetics, because very loose or very tight linkage will have little effect in changing the statistics by which variation is measured in biometrical analyses. In other words, the failure to detect an effect of linkage biometrically does not imply

that linkage and recombination in the classical sense are effectively inoperative in the system: it merely implies that the change recombination produces from generation to generation in the components of variation is not large, even where its ultimate consequences for, for example, progress under selection may be far from negligible.

The final values yielded by the weighted analysis for the components of variation are set out in table 9. The figures shown are those from the inclusive analysis and they may therefore be regarded as invalidated in the strict sense by the evidence for linkage effects. They are nevertheless taken so as to facilitate comparisons with the results of the unweighted analysis where the inclusive results are used. In any case, the differences between the estimates of D and H from the inclusive weighted analysis are hardly likely to differ materially from the values yielded for D_1 and H_1 by the exclusive operation.

TABLE 9

Estimates of the components of variation from the weighted inclusive analysis

	BS ♂	BS ♀	SB ♂	SB ♀	Averaged overall
D	2·4882±0·4429	3·9784±0·4683	2·3083±0·4423	2·7499±0·4573	2·8970±0·2269
H	0·0944±1·1216	−3·8713±1·0596	2·8432±1·0489	2·5477±0·9983	0·3783±0·5300
E_1	2·6954±0·1442	2·5636±0·1338	2·2314±0·1237	1·9525±0·1104	2·3617±0·0648
E_2	0·4277±0·0616	0·3916±0·0557	0·3359±0·0520	0·3166±0·0493	0·3695±0·0283

Comparing the weighted estimates of the components with the unweighted, the most striking feature is the close similarity of the two sets of estimates. The weighted estimates of D and H are, however, on the whole smaller than the unweighted, the difference being more noticeable for H than for D. Even so, no comparable figures differ by amounts even approaching significance and the very same unexpectedly high value for D and negative value for H are obtained for B×S females, with this difference, that the value of H is now significantly negative. Since by definition, H is a quadratic quantity, a negative value is nonsensical and once again the aberrant nature of the results from this part of the experiment is emphasised, but this time even more strongly because of the greater precision of the weighted analysis. This finding also serves further to emphasise the obvious point, if further emphasis be needed, that no refinement of statistical procedure can bring sense out of data which are suspect by genetical criteria.

One further matter requires discussion before we leave the weighted analysis. The process of estimation is iterative and while the number of iterations necessary is relatively unimportant when an electronic computer is available, it becomes of serious moment should, for any reason, a weighted analysis be carried out with the aid of no more

than a desk calculating machine. The χ^2 values after the first and second cycles of calculation are shown for comparison with the final values in table 8(a) and the values of $\sqrt{M_\theta}$ which give an upper bound for the computational error of the estimates as a fraction of their standard errors, are set out correspondingly in the second part of the same table. The values of both χ^2 change very much more between the results of the first and second cycles than between the latter and final values and the value of $\sqrt{M_\theta}$ is quite small after the second cycle. The conclusion, already reached by Hayman (1960) from a different set of data, would seem clear; that while two cycles of calculation lead to a correct interpretation, a single cycle is unreliable. It is of interest that the χ^2 for B×S males actually rises from the first to the second cycle, no doubt because the weights used initially failed to give full emphasis to some differences between the seven statistics from which the estimates are obtained.

The weights used in the first cycle were empirical in that they were derived from the values actually observed for the statistics. The weights in the subsequent cycles were derived from the values expected for the statistics on the basis of the last set of estimates of the components. If therefore a set of weights approximating better to the true ones could be found with which to start the first cycle, a single cycle of the weighted least squares procedure might be sufficient. Possibly weights derived from the results of the simple unweighted analysis might serve for this purpose and if that were so, the unweighted analysis, followed by a single cycle of the weighted, might well be regarded as a not intolerably heavy task, even where no electronic computer were available. This possibility has not, however, been tested.

5. THE EFFICIENCY OF UNWEIGHTED ANALYSIS

The unweighted analysis yielded values for the components of variation which differ from the weighted estimates to only a minor extent. There is thus no reason to suspect that the failure to use weights introduces any material bias into the estimates but one is led to enquire into the relative efficiency of the unweighted method. This may be determined by dividing the sampling variance of the estimates given by the computer by that of the estimate from the unweighted analysis. The variance matrix of the latter set of estimates, $\boldsymbol{\theta}$, is (Nelder, 1960)

$$\text{var } \theta = (\mathbf{a}'\mathbf{a})^{-1}\mathbf{a}'\mathbf{v}\mathbf{a}(\mathbf{a}'\mathbf{a})^{-1} = \mathbf{g}'\mathbf{v}\mathbf{g}$$

where the matrix $\mathbf{g} = \mathbf{a}(\mathbf{a}'\mathbf{a})^{-1} = \mathbf{a}\mathbf{c}$ depends only on the generations included in the experiment and is easy to calculate once the inverse c-matrix is known.

This procedure gives, of course, the sampling variances expected for the estimates from the unweighted analysis. The standard errors shown in table 7 are, on the other hand, observed errors in that they

are derived from the differences between the values observed and expected for the statistics in the particular experiment. Measured in this way, these standard errors will themselves be subject to sampling variation which will be large in cases such as the present where errors are derived from a very small number of degrees of freedom. The standard errors of the unweighted estimates actually found in an experiment will thus fluctuate round the values expected from the calculations outlined above.

Reference to tables 7 and 10 for the *Drosophila* results, and to table 12 for others from an experiment with *Nicotiana*, shows this to be the case in the sense that the unweighted standard errors do not bear to the weighted the relation that the comparative efficiencies

TABLE 10

Comparative efficiencies of the unweighted estimates of the components from the four parts of the unweighted inclusive analysis

	BS ♂	BS ♀	SB ♂	SB ♀
D	93·8	98·1	94·0	96·3
H	76·0	71·5	76·6	74·6
E_1	81·3	77·5	85·9	88·5
E_2	71·4	71·4	59·5	57·1

would suggest; sometimes they are larger and sometimes smaller than would be expected on the basis of the measures of relative efficiency which, as we have observed, were calculated from the sampling variation expected for the unweighted estimates obtained from an experiment of this kind. Such expected sampling variances must obviously be used in assessing the relative efficiencies for the purpose of planning future experiments. They are general properties of the type of experiment and therefore applicable to all experiments of the kind in question, whereas the relative efficiencies actually observed in any given experiment necessarily reflect its own special circumstances and are therefore applicable to it alone.

Applying this method to the four parts of the experiment gives the percentage efficiencies set out in table 10 for the components of variation in the unweighted analysis. For the two genetical parameters D and H and for E_1, the efficiency is never less than 75 per cent. and indeed for D the unweighted analysis sacrifices only about one part in fourteen of the information that the weighted treatment can extract. Even for E_2, which is of lesser interest than the genetical parameters, well under half the information is lost.

The relative efficiencies will depend on the structure of the experiment, that is on the generations included in it, on the statistics it yields, and on the relative number of individuals and families from which the various statistics are calculated. It is therefore of interest

to look at a further experiment of the same general kind as that with *Drosophila* but which is smaller and shows a somewhat different balance of structure. Data are available for flowering time and plant height in *Nicotiana rustica*.

The statistics available for both characters in this plant are the same as with *Drosophila*. Table 11 shows the number of degrees of

TABLE 11

Degrees of freedom for the statistics in the Nicotiana *experiment and the balances of the* Nicotiana *and* Drosophila *experiments*

Statistic	*Nicotiana*		*Drosophila*
	Degrees of freedom	Balance	Balance
V_{1F_2}	80	40	10
V_{1F_3}	24	12	11
$W_{1S_{23}}$	24	12	11
V_{2S_3}	200	100	100
E_1	200	100	16
E_2	42	21	2

freedom on which each statistic is based. An attempt has also been made in this table, to illustrate and compare the relative balances of the two experiments by showing the number of degrees of freedom for each statistic expressed relative to the number for V_{2S_3} taken as 100. Thus in *Nicotiana*, V_{2S_3} is based on 200 degrees of freedom and

TABLE 12

Components of variation and efficiency of unweighted estimates in Nicotiana

	Flowering time components		Relative efficiency	Plant height components		Relative efficiency
	Unweighted	Weighted		Unweighted	Weighted	
D	$6{\cdot}51 \pm 12{\cdot}63$	$3{\cdot}21 \pm 7{\cdot}30$	95	$19{\cdot}45 \pm 6{\cdot}95$	$20{\cdot}54 \pm 11{\cdot}71$	86
H	$30{\cdot}50 \pm 31{\cdot}22$	$43{\cdot}72 \pm 13{\cdot}56$	79	$10{\cdot}98 \pm 17{\cdot}18$	$9{\cdot}28 \pm 19{\cdot}36$	66
E_1	$4{\cdot}91 \pm 3{\cdot}49$	$4{\cdot}56 \pm 0{\cdot}45$	60	$7{\cdot}48 \pm 1{\cdot}91$	$7{\cdot}26 \pm 0{\cdot}72$	71
E_2	$2{\cdot}44 \pm 2{\cdot}73$	$1{\cdot}52 \pm 0{\cdot}32$	11	$5{\cdot}48 \pm 1{\cdot}50$	$6{\cdot}20 \pm 1{\cdot}26$	55

V_{1F_2} on 80 so that when V_{2S_3} takes 100 on the relative scale, V_{1F_2} takes 40. The chief differences between the experiments lie in the greater relative weight of V_{1F_2}, E_1 and E_2 in *Nicotiana*.

There was no evidence of linkage exerting an effect in respect of either character and the estimates given are therefore taken from the inclusive evaluation. The weighted and unweighted estimates of the components of variation are shown for both characters of *Nicotiana* in table 12 together with their standard errors and the relative

efficiencies of the unweighted estimates. As in *Drosophila*, the unweighted estimates are encouragingly similar to the weighted, the greatest relative discrepancy being in the D of flowering time. Neither this difference, nor any other between the weighted and unweighted estimates, is anywhere near significance. The standard errors of both sets of estimates are large as would be expected from an experiment much smaller in size than that with *Drosophila*, but even so the weighted analysis clearly establishes the presence of dominance in respect of flowering time which the unweighted analysis fails to do.

The efficiencies of the unweighted estimates relative to the weighted are very much the same for *Nicotiana* as for *Drosophila* apart from the case of E_2 in flowering time. Evidently the difference in balance of the two experiments has had little effect on the efficiency of the unweighted analysis. In an example discussed by Nelder (1960), however, the efficiency of the unweighted method was distinctly lower. This case contrasted with the present examples in that the ratio of the greatest to the smallest weights it involved was 100 : 1, whereas in our experiments this ratio always lay between 15 : 1 and 30 : 1. This presumably accounts for the difference in efficiency of the unweighted analyses. It would thus seem that despite its shortcomings, the simple unweighted treatment may be expected to yield reasonable estimates of the components of variation, though the values found for their standard errors cannot be regarded as fully reliable.

6. SUMMARY

Two inbred lines, Oregon (B) and Samarkand (S) of *Drosophila melanogaster* were crossed reciprocally. In addition to the F_1's and F_2's, biparental progenies of the third generation (BIPS) were raised from both crosses. The number of sternopleural chaetæ were counted in all these generations and the components of variation (D, H, E_1 and E_2) in respect of this character were estimated using the method devised by Mather (1949). Estimates were obtained in quadruplicate by using data from males and females separately in the two halves of the experiment stemming from the reciprocal crosses. The results from the B × S females proved to be aberrant for reasons which are not known. The analyses yielded no clear indication of the presence of linkage on the variation in respect of this character.

This unweighted method of analysis is easy to use but makes no allowance for the differences in the precision of the statistics for the various generations or for correlations among these statistics. A weighted method which accommodates these differences in precision and correlation has been described by Nelder (1960) and this was applied to the results, the iterative calculations being carried out on the Rothamsted electronic computer. Two cycles of iteration would appear generally to be adequate in using this method.

The simple unweighted analysis yields estimates of the components

of variation which do not differ significantly from those resulting from the weighted analysis. The relative efficiency of the unweighted analysis varies from 60 to over 90 per cent. for the various components of variation over the four parts of the experiment.

A further set of data for flowering time and plant height in *Nicotiana rustica* were similarly analysed by both methods. Though the broad structure of the experiment was similar to that of the one with *Drosophila* the relative precisions of the statistics it yielded were somewhat different. The unweighted estimates of the components stood in very much the same relation to the weighted estimates as in *Drosophila* and the efficiency of the weighted method was also much the same.

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