EFFECTS OF MARKER CHROMOSOMES ON RELATIVE VIABILITY

C. CLARK COCKERHAM AND TERUMI MUKAI

Departments of Statistics and Genetics, North Carolina State University, Raleigh, North Carolina 27607

Manuscript received April 13, 1978
Revised copy received July 5, 1978

ABSTRACT

Viability relative to Cy/Pm as a standard was studied in Drosophila melanogaster. One experiment, E1, consisted of progeny from eleven distinct 7 x 7 factorial mating designs with reciprocals for second chromosomes extracted from a natural population. The other experiment, E2, consisted of two distinct sets of heterozygotes with reciprocals and corresponding homozygotes. It was established from E1 that there are little to no synergistic effects among different genotypes in a vial and that Cy and Pm heterozygotes vary almost as much as would be expected if one chromosome were held constant for wild-type heterozygotes. In wild-type heterozygotes, variances were estimated to be 0.0099 for average chromosomal effects, 0.0054 for interactions of chromosomes, 0.0021 for maternal effects, 0.0079 for paternal effects, and —0.0010 for the remaining interaction effects, all being significantly different from zero except the last. The variances of Cy and Pm heterozygotes, covariance of Cy and Pm heterozygotes, and covariances of Cy and Pm heterozygotes with wild-type heterozygotes, as well as the comparable statistics available in E2, all showed a large paternal component of variance and a smaller maternal component of variance, both unexpected results.—From E2 the variance of homozygotes, excluding error variance, was estimated to be 0.0149, and the covariances of homozygotes with wild-type heterozygotes to be 0.0056 for maternally derived chromosomes common and 0.0126 for paternally derived chromosomes common, again showing the larger paternal than maternal influence. The average genetic regression of heterozygotes on homozygotes of 0.61 was reduced only slightly to 0.56 by correcting for maternal and paternal variances. These genetic regressions, generally utilized as estimators of the average degree of dominance, are larger than any previously reported.—Differential meiotic drive in Cy and Pm parents was shown to be compatible with the large paternal and maternal variances, but other causes cannot be ruled out.—Approximations were developed for translating various variances, covariances, and regressions between single- and double-marker experiments, assuming that marker chromosomes behave as typical wild-type chromosomes in one case and assuming a (partially) recessive model with the population in mutation selection balance in another case. Various features, particularly the estimation of dominance, were compared and discussed between the two cases.

1 Paper No. 5012 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, North Carolina. This investigation was supported in part by Public Health Service Research Grant No. GM 11546.

2 Present address: Department of Biology, Kyushu University, Hakozaki, Fukuoka-shi, Japan.

FOR estimation of relative viability in Drosophila, marked inversion techniques have been traditionally employed. Historically, the Cy L technique (cf., WALLACE 1956) was employed at first. This corresponds to our Cy method (MUKAI 1964). We have used the Cy [In(2LR)SM1] chromosome instead of the Cy L chromosome of WALLACE (1956). In this method, accuracy of results depends on the Cy chromosome in heterozygous condition suppressing the effects of genes located in the homologous chromosomes, since the viability of wild-type flies is estimated relative to that of Cy heterozygotes. In order to remove this difficulty, WALLACE (1956) devised the Cy L/Pm, or double-marker, technique in which the viability of wild-type genotypes can be estimated relative to the constant double-marker genotype Cy L/Pm. Although the Cy L/Pm method has this advantage over the Cy method, it is a tedious and time-consuming experiment involving four-class segregation instead of two-class segregation for the Cy method. Also, the double-marker genotype may be too weak for a reliable standard as in the case of Cy/bwD in the experiments of GREENBERG and CROW (1960).

Experiments such as those of DOBZHANSKY, KRIMBAS and KRIMBAS (1960) and WALLACE and DOBZHANSKY (1962) in analyzing wild-type chromosomes with double-marker techniques have found little to no relationship between the relative viabilities of wild-type homozygotes and marker heterozygotes with the same wild-type chromosome, except for those chromosomes whose homozygous relative viabilities are above normal (i.e., near or above the mean relative survival for heterozygotes). These results have been interpreted variously. One interpretation is that marker chromosomes suppress the effects of wild-type chromosomes in heterozygotes. Another is that there is little correspondence between homozygotes and heterozygotes with a common chromosome. Actually, DOBZHANSKY, KRIMBAS and KRIMBAS (1960) found little to no correlation between the relative frequencies of wild-type homozygotes and heterozygotes with a common chromosome.

Two types of experiments should be distinguished. In one type, the primary interest is in making mean comparisons of relative viabilities, such as in studying the effects of radiation (WALLACE 1956) or of other treatments. The same conclusion may well be reached by single-marker as by double-marker techniques, the primary difference being in efficiencies of the procedures. In the other type of experiment, primary interest is in quadratic estimation (variances, covariances and regressions, as in MUKAI (1964) and on to MUKAI et al. (1974), where variation in single markers may be more critical.

Experiments were set up to determine the variances of relative viability of marker heterozygotes and wild-type heterozygotes in Drosophila melanogaster and the effects of using single-marker heterozygotes on the estimation of genetic variances and dominance.

EXPERIMENTAL PROCEDURES

The chromosomes used in the experiments were descended from second chromosomes extracted from a population of Drosophila melanogaster in Raleigh, North Carolina. The chromosomes
were isolated in a manner illustrated in Figure 1 of Mukai et al. (1974) and maintained in a mixed culture of +/+ and Cy(SM1)/+. To make crosses, Pm (plum) was paired with the wild-type chromosomes and all crosses were of the type 5(Cy/+/j) females from the ith line with 5(Pm/+/i) males from the jth line (the Cy L/Pm technique of Wallace 1956). In all cases the female parents were heterozygous for the Cy chromosome to avoid recombination. The following offspring, Cy/Pm, Cy/+/j, Pm/+/i, +i/+j, are expected to segregate in equal proportions.

Four days after the crosses were made, the flies were transferred to a second vial, and after four more days all parents were discarded. Offspring counts were continued until day 18 after the matings or transfers were made, and counts from the original and transfer vials were combined.

Chromosomes in the first experiment, E1, came from extractions made in 1970 and the experiments were done in 1973. Eleven separate 7 x 7 factorial mating designs (Design II of Comstock and Robinson 1952), each involving distinct sets of 14 wild-type chromosomes, were set up with reciprocals and with two replicates or duplicates of each cross. Four of the sub-experiments were complete, three had only missing duplicates and the other four had one or more missing crosses due to allelic lethals. These provided the following comparison of actual and intended.

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Number of Reciprocals</th>
<th>Duplicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intended</td>
<td>539</td>
<td>1078</td>
</tr>
<tr>
<td>Actual</td>
<td>532</td>
<td>1059</td>
</tr>
<tr>
<td>Missing</td>
<td>7</td>
<td>19</td>
</tr>
</tbody>
</table>

Chromosomes in the second experiment, E2, came from extractions in 1974 and the experiments were done in 1974. Two distinct sets of 67 chromosome lines were utilized. In each set homozygous progeny were produced by crossing Cy/+/i to Pm/+/i with four duplicates of each, and heterozygous progeny were produced in a chain block fashion by crossing Cy/+/i to Pm/+/i+i with reciprocals and two duplicates of each. All observations for a chromosome were deleted if the ratio of the number of homozygotes (+i/+i) to the number of Cy/+/i was less than 0.55, so that the data would be comparable to those analyzed by Mukai in the past. This restriction and some missing values reduced the number of distinct homozygotes in 59 in one set and to 63 in the other set with corresponding numbers of heterozygotes of 51 and 58.

DATA AND ANALYSES

The relative viabilities to be considered are given the following shorthand notations:

<table>
<thead>
<tr>
<th>Relative viability</th>
<th>Number of</th>
<th>Number of</th>
<th>Notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cy/+/j to Cy/Pm</td>
<td>x_j</td>
<td></td>
<td>x_j</td>
</tr>
<tr>
<td>Pm/+/i to Cy/Pm</td>
<td>y_i</td>
<td></td>
<td>y_i</td>
</tr>
<tr>
<td>+i/+j to Cy/Pm</td>
<td>z_ij</td>
<td></td>
<td>z_ij</td>
</tr>
<tr>
<td>+i/+j to Pm/+/i</td>
<td>u_ij</td>
<td></td>
<td>u_ij</td>
</tr>
<tr>
<td>+i/+j to Pm/+/i</td>
<td>v_ij</td>
<td></td>
<td>v_ij</td>
</tr>
</tbody>
</table>

When in progenies with homozygotes, they will be primed, x'_j, y'_i, z'_ij, u'_ij, and v'_ij.

For clarification, part of the data are illustrated (Figure 1) for the progeny of one of the 11 distinct factorial mating designs in E1. The viabilities relative to Cy/Pm for cells involving the matings of chromosomes 1 and 2 with 8 and 9, with reciprocals, but not duplicates, are given. From these the pattern for the other cells can be quickly ascertained. Note that each y_i and x_i and y_j and x_j.
Figure 1.—Relative viabilities of progenies from factorial mating design in E1.*

*Reciprocals below dashed line.

occurs in seven cells. Also illustrated are various types of relatives for the ‘s; $z_{18}$ and $z_{81}$ are reciprocal full-sibs, $z_{18}$ and $z_{19}$ are maternal half-sibs, $z_{18}$ and $z_{28}$ are paternal half-sibs, and $z_{18}$ and $z_{91}$ are reciprocal half-sibs.

Estimates of means and variances were obtained for each of the 11 distinct experiments in E1, pooled or averaged, and standard errors of all pooled estimates were estimated directly from the variation among the 11 independent estimates. Results from the two subexperiments in E2 were also pooled, but standard errors were computed using normal distribution theory as well as approximate theory for ratios (Moore and Robinson 1959). A comparison of both methods for six estimates of variances in E1 resulted in the directly estimated errors being larger in each case than those calculated from normal distribution theory, averaging 1.33 times larger.

**Variance and covariances of marker chromosomes, x and y**

As illustrated in Figure 1, each $x_{i}$ or $j$ and each $y_{i}$ or $j$ occurred in seven cells. This permitted a breakdown for $x$ and for $y$ of the total variance within each factorial experiment into components $V_d$ for duplicates, $V_c$ for cells, and $V_e$ for chromosomes. The averages of the 11 estimates of each component are summarized in Table 1, and the standard errors are the standard deviations for the average.

Neither of the cell components of variance, but all of the other components of variance, are significantly different from zero. Between $x$ and $y$, only the means and chromosome components of variance are significantly different. The differ-
Mean and variances of relative viability for marker heterozygotes in El

<table>
<thead>
<tr>
<th>Component</th>
<th>%</th>
<th>Component</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomes $-V_c$</td>
<td>0.0104 ± 0.0021</td>
<td>0.0064 ± 0.0011</td>
<td>13</td>
</tr>
<tr>
<td>Cells $-V_c$</td>
<td>0.0012 ± 0.0017</td>
<td>0.0014 ± 0.0018</td>
<td>3</td>
</tr>
<tr>
<td>Duplicates $-V_d$</td>
<td>0.0443 ± 0.0034</td>
<td>0.0412 ± 0.0027</td>
<td>84</td>
</tr>
<tr>
<td>Total</td>
<td>0.0559</td>
<td>0.0490</td>
<td>100</td>
</tr>
<tr>
<td>Mean relative viability</td>
<td>1.009 ± 0.012</td>
<td>1.110 ± 0.013</td>
<td>100</td>
</tr>
</tbody>
</table>

ence $V_{cx} - V_{cy}$ = 0.0040 ± 0.0024 could be due to differences between the marker chromosomes, but could be due to the $+$ chromosomes in $x$ being paternally derived and the $+$ chromosomes in $y$ being maternally derived.

The covariance, $C_{cxy}$, of $x_i$ with $y_i$ involves the same wild-type chromosome. This covariance is actually the component of variance for wild-type chromosomes appropriate for the combined analysis of $x$ and $y$. In this context, there is a chromosomal component of variance, which is actually $C_{cxy}$, and an interaction component of variance, $V_{cxy}$, due to the interaction of wild-type chromosomes with $Cy$ vs. $Pm$. The relationship between this partitioning of the variance and the separate components, $V_{cx}$ and $V_{cy}$, is

$$ C_{cxy} + V_{cxy} = (V_{cx} + V_{cy})/2 $$

Consequently,

$$ V_{cxy} = \frac{V_{cx} + V_{cy}}{2} - C_{cxy} = \frac{(\sqrt{V_{cx}} - \sqrt{V_{cy}})^2}{2} + \sqrt{V_{cx}V_{cy}} (1 - r_{cxy}) $$

where the correlation of chromosomal effects is

$$ r_{cxy} = C_{cxy}/\sqrt{V_{cx}V_{cy}}. $$

These pooled estimates are

$$ \hat{C}_{cxy} = 0.0034 ± 0.0013 \quad \hat{V}_{cxy} = 0.0051 ± 0.0014 $$

$$ \hat{r}_{cxy} = 0.646 ± 0.448 \text{ average over subexperiments} $$

$$ = 0.417 \text{ from averages of components.} $$

A more useful breakdown reflecting paternal, $p$, and maternal, $m$, effects may be to consider the variances additional to the covariance, i.e.,

$$ \hat{V}_{cp} = \hat{V}_{cx} - \hat{C}_{cxy} = 0.0070 ± 0.0022 $$

$$ \hat{V}_{cm} = \hat{V}_{cy} - \hat{C}_{cxy} = 0.0030 ± 0.0015 $$

By paternal and maternal effects is meant those effects due to the origins of the wild chromosomes. Recall that the wild-type chromosome is always paternally derived in $x$ and maternally derived in $y$. 
The other experiment, E2, provides less reliable estimates, which are collected into Table 2. Since the cell component, $V_e$, was concluded to be zero in E1, this source of variation was pooled with duplicates in the analysis of $x'$ and $y'$, i.e., with homozygotes in the progeny. All possible covariances of marker heterozygotes are given in Table 2. In no case where the marker heterozygotes with the same wild-type chromosome are in the same duplicate as for $x_i'$ and $y_i'$ were these included in the covariance.

Since $x$ and $x'$ involve the same chromosomes, as do $y$ and $y'$, the individual chromosomal components are pooled in Table 2 to provide overall estimates. While the chromosomal components are different from those in E1, that for $x$ being larger and that for $y$ being less than in E1, the differences are probably due to sampling errors. The results do substantiate the finding that $V_{cx}$ is greater than $V_{cy}$.

**Variances of relative viabilities of wild-type heterozygotes**

These heterozygotes with reciprocals were analyzed according to the model

$$z_{ijl} = \mu + n_i + n_j + t_{ij} + m_i + p_j + k_{ij} + d_{ijl}$$

where $z_{ijl}$ is the observation for the $l$th duplicate of the cross of the $i$th chromosome from the maternal parent with the $j$th chromosome from the paternal parent. The mean is $\mu$ and the effects are $n$ for nuclear gametic effects, $t$ for interaction of the two nuclear gametic effects ($t_{ij} = t_{ji}$), $m$ for extranuclear maternal effects, $p$ for extranuclear paternal effects, $k$ for several interaction effects ($k_{ij} = (nm)_{ij} + (np)_{ij} + (nm)_{ji} + (np)_{ji} + (tm)_{ij} + (tp)_{ij}$) and $d$ for the remainder. All distinct effects are assumed to be uncorrelated and to have variances, $V_n$, $V_t$, $V_m$, $V_p$, $V_k$ and $V_d$. A more detailed description of the model

<table>
<thead>
<tr>
<th>Components of variance</th>
<th>$x'$</th>
<th>$y'$</th>
<th>$x$</th>
<th>$y$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_c$</td>
<td>0.0168 ± 0.0029</td>
<td>0.0020 ± 0.0012</td>
<td>0.0176 ± 0.0032</td>
<td>0.0047 ± 0.0016</td>
</tr>
<tr>
<td>$V_d$</td>
<td>0.0227 ± 0.0017</td>
<td>0.0261 ± 0.0019</td>
<td>0.0249 ± 0.0020</td>
<td>0.0257 ± 0.0020</td>
</tr>
<tr>
<td>Mean relative viability</td>
<td>0.935 ± 0.014</td>
<td>1.048 ± 0.008</td>
<td>0.932 ± 0.014</td>
<td>1.043 ± 0.010</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Components of covariance</th>
<th>$x'y'$</th>
<th>$xy'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_{cx}$</td>
<td>0.0162</td>
<td>0.0031</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pooled components*</th>
<th>$x$</th>
<th>$y$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_c$ or $L_{cx}$</td>
<td>0.0167</td>
<td>0.0032</td>
</tr>
</tbody>
</table>

* ($L_{cab} + L_{ca'b} + L_{ca'v} + L_{cab'})/4$, $L_{cab} = V_{ca}$, $a = x'y$; $b = x'y$. 

\[ \rho_{cy} = 0.479 \]
and analyses is given by COCKERHAM and WEIR (1977). The pooled results for the eleven experiments in E1 are displayed in Table 3.

All components except $V_k$ are significantly different from zero. The paternal component is much larger than the maternal component. The difference, $V_p - V_m = 0.0058$, is reasonably close to that, $V_{cz} - V_{cy} = 0.0040$, in Table 1, and may well account for this latter difference.

Only a partial breakdown of the total variance is available from E2: among entries, $2V_n + V_t = 0.0100 \pm 0.0033$, and between reciprocals, $V_m + V_p + V_k = 0.0115 \pm 0.0033$. These are in reasonable agreement with comparable estimates from Table 3, $2V_n + V_t = 0.0153$, and $V_m + V_p + V_k = 0.0090$.

The same analysis as in Table 3 was made of the viability of wild-type heterozygotes relative to single-marker heterozygotes, i.e., of $u$ and $v$, for comparison of single-marker techniques with the double-marker technique, and the results appear in Table 4.

There are differences between $u$ and $v$ for the components, and the overall picture for $u,v$ is different from that for $z$, although the magnitude of such differences is difficult to specify because of the standard errors. Standardizing to the same mean would affect the magnitudes of the components, $v$ in comparison to

### Table 3

Mean and variances of relative viability of heterozygotes, $z$ in E1

<table>
<thead>
<tr>
<th>Description</th>
<th>Component</th>
<th>Estimate</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>$2V_n$</td>
<td>$0.0099 \pm 0.0039$</td>
<td>14</td>
</tr>
<tr>
<td>Specific</td>
<td>$V_t$</td>
<td>$0.0054 \pm 0.0022$</td>
<td>7</td>
</tr>
<tr>
<td>Maternal</td>
<td>$V_m$</td>
<td>$0.0021 \pm 0.0009$</td>
<td>3</td>
</tr>
<tr>
<td>Paternal</td>
<td>$V_p$</td>
<td>$0.0079 \pm 0.0023$</td>
<td>11</td>
</tr>
<tr>
<td>Reciprocal</td>
<td>$V_k$</td>
<td>$-0.0010 \pm 0.0021$</td>
<td>—</td>
</tr>
<tr>
<td>Duplicate</td>
<td>$V_d$</td>
<td>$0.0481 \pm 0.0039$</td>
<td>66</td>
</tr>
<tr>
<td>Total</td>
<td>$V_z$</td>
<td>$0.0724$</td>
<td>100</td>
</tr>
</tbody>
</table>

Mean relative viability $0.979 \pm 0.020$

### Table 4

Means and variances of the survival of heterozygotes relative to marker heterozygotes, $u$ and $v$ in E1

<table>
<thead>
<tr>
<th>Component</th>
<th>Estimate</th>
<th>$u$</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2V_n$</td>
<td>$0.0039 \pm 0.0016$</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>$V_t$</td>
<td>$0.0033 \pm 0.0022$</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>$V_m$</td>
<td>$0.0024 \pm 0.0012$</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>$V_p$</td>
<td>$0.0009 \pm 0.0015$</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>$V_k$</td>
<td>$0.0016 \pm 0.0019$</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>$V_d$</td>
<td>$0.0367 \pm 0.0029$</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>$0.0488$</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Mean relative viability $0.976 \pm 0.008$

### Table 4 (continued)

<table>
<thead>
<tr>
<th>Component</th>
<th>Estimate</th>
<th>$v$</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2V_n$</td>
<td>$0.0011 \pm 0.0017$</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>$V_t$</td>
<td>$0.0066 \pm 0.0039$</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>$V_m$</td>
<td>$0.0008 \pm 0.0011$</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>$V_p$</td>
<td>$0.0079 \pm 0.0012$</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>$V_k$</td>
<td>$0.0028 \pm 0.0019$</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>$V_d$</td>
<td>$0.0324 \pm 0.0032$</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>$0.0516$</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Mean relative viability $0.886 \pm 0.016$
$u$ and $z$ for example, but would not change the percentages. Many of the differences can be accounted for by the covariances of $x$ and $y$ with $z$, since $u = z/x$ and $v = z/y$. These covariances are considered next.

**Covariances of marker heterozygotes with wild-type heterozygotes and homozygotes**

Because of the reciprocals in $E_1$, pairs of marker heterozygotes and wild-type heterozygotes with one chromosome in common fall into two classes: in the same factorial and in reciprocal factorials. (See Figure 1.) When in reciprocal factorials, the common wild-type chromosome is maternally derived for one variable and paternally derived for the other, e.g., for $x_i$ and $z_{ij}$

\[
\begin{array}{c|c|c}
\text{factorial} & x_i & z_{ij} \\
\hline
\text{maternal} & \frac{x_i}{y} & \frac{z_{ij}}{y} \\
\text{paternal} & \frac{x_i}{y} & \frac{z_{ij}}{y} \\
\end{array}
\]

We call this covariance $C_m$. When in the same factorial, the common chromosome is always maternally derived if $y$ and paternally derived if $x$. We designate these two covariances $C_m$ and $C_p$, respectively. In addition, there is a duplicate covariance, $C_d$, for observations in the same duplicate. These, along with comparable types of estimates in $E_2$ between marker heterozygotes, $x'$ and $y'$ reared with homozygotes, and wild-type heterozygotes, are given in Table 5. The two sets of estimates agree very well and further substantiate the large component contributed by the paternal chromosome.

In homozygotes the same chromosome is both maternally and paternally derived, of course. Because of paternal effects, the chromosomal covariances, $C_o$, of homozygotes with marker heterozygotes should be larger for $x$ and $x'$ than for $y$ and $y'$, which turns out to be the case (Table 6).

**Table 5**

*Covariances between relative viabilities of marker heterozygotes and wild-type heterozygotes*

<table>
<thead>
<tr>
<th>Covariance</th>
<th>$E_1$ estimates</th>
<th>$E_2$ estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_n$</td>
<td>$0.0033 \pm 0.0014$</td>
<td>$0.0034$</td>
</tr>
<tr>
<td>$C_p$</td>
<td>$0.0099 \pm 0.0020$</td>
<td>$0.0051$</td>
</tr>
<tr>
<td>$C_m$</td>
<td>$0.0274 \pm 0.0035$</td>
<td>$0.0146$</td>
</tr>
<tr>
<td>$C_d$</td>
<td>$0.0239 \pm 0.0022$</td>
<td>$0.0033$</td>
</tr>
</tbody>
</table>

Explanation of covariances given in text.
TABLE 6

Covariances of marker heterozygotes with homozygotes in E2

<table>
<thead>
<tr>
<th>Covariance</th>
<th>Estimate $x'z'$</th>
<th>Estimate $y'z'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomal $-C_c$</td>
<td>0.0127</td>
<td>0.0038</td>
</tr>
<tr>
<td>Duplicate $-C_d$</td>
<td>0.0101</td>
<td>0.0094</td>
</tr>
<tr>
<td>Chromosomal $-C_c$</td>
<td>0.0138</td>
<td>0.0026</td>
</tr>
</tbody>
</table>

Variance of homozygotes and covariances and regressions of heterozygotes on homozygotes (E2)

Only two components of variance, that for chromosomes, $V_c$, and that for duplicates, $V_d$, are available for homozygotes. The results for $u'$ and $v'$ are given in Table 7 for comparison with those of $z'$. As expected there are differences dependent upon the marker used as a standard.

The covariances of heterozygotes with homozygotes and the genetic regressions of heterozygotes on homozygotes were computed separately for the common chromosome maternally and paternally derived in the heterozygotes. For genetic regressions, the chromosomal component of variance, $V_c$, is used as the variance of the dependent variable (see Mukai et al. 1972). The same computations were made for $u$ and $v$, and the results are given in Table 8. The maternal and paternal effects are large, particularly when using single markers as the standard. Since the denominators are the same for maternal and paternal regressions, the averages are the same as the regressions from pooling the components. The differences due to the use of different markers are striking.

These genetic regressions are utilized to estimate the degree of dominance in Cy experiments (Mukai et al. 1972; Mukai and Yamaguchi 1974). While the regressions for $u, u'$ differ considerably between the maternally and paternally derived chromosomes, the average, 0.18, is not significantly different from that, 0.21, found by Mukai et al. (1972) and, 0.29, found by Mukai and Yamaguchi (1974) using Cy heterozygotes as the standard. In view of the maternal and paternal effects, the analyses involving $u$ and $u'$ are not really comparable to a Cy experiment, however.

TABLE 7

Means and variances of the survival of homozygotes relative to double and single markers (E2)

<table>
<thead>
<tr>
<th>Component</th>
<th>Estimate Variances $u$</th>
<th>Estimate Variances $v'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomes $-V_c$</td>
<td>0.0149 ± 0.0025</td>
<td>0.0057 ± 0.0014</td>
</tr>
<tr>
<td>Duplicates $-V_d$</td>
<td>0.0189 ± 0.0014</td>
<td>0.0206 ± 0.0015</td>
</tr>
<tr>
<td>Total</td>
<td>0.0338</td>
<td>0.0263</td>
</tr>
</tbody>
</table>

Means

|                | 0.729 ± 0.013 | 0.790 ± 0.009 | 0.702 ± 0.011 |
TABLE 8

Covariances and genetic regressions of heterozygotes on homozygotes with the different markers as standards (E2)

<table>
<thead>
<tr>
<th>Chromosome common</th>
<th>Covariances $zz'$</th>
<th>Covariances $uu'$</th>
<th>Covariances $vu'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal $- C_M$</td>
<td>0.0056 $\pm$ 0.0023</td>
<td>0.0024 $\pm$ 0.0011</td>
<td>0.0025 $\pm$ 0.0021</td>
</tr>
<tr>
<td>Paternal $- C_P$</td>
<td>0.0126 $\pm$ 0.0026</td>
<td>$-$0.0005 $\pm$ 0.0010</td>
<td>0.0074 $\pm$ 0.0022</td>
</tr>
<tr>
<td>Average</td>
<td>0.0090 $\pm$ 0.0023</td>
<td>0.0010 $\pm$ 0.0009</td>
<td>0.0050 $\pm$ 0.0019</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genetic regressions $uu'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paternal $- b C_P$</td>
</tr>
<tr>
<td>Maternal $- b C_M$</td>
</tr>
<tr>
<td>Average</td>
</tr>
</tbody>
</table>

We may attempt to remove the maternal and paternal effects in the covariances and the regressions, although no direct estimates are available. We let

$$
C_{zz'} = C_{nzz'} + C_{mzz'}
$$

where $C_{nzz'}$ is the covariance of nuclear effects and $C_{mzz'}$ is the covariance of maternal effects. Correspondingly, for the paternal covariance,

$$
C_{ppzz'} = C_{nzz'} + C_{pzz'}.
$$

For the chromosomal component of variance for homozygotes we let

$$
V_{zz'} = V_{nzz'} + V_{mzz'} + V_{pzz'},
$$

where $V_{nzz'}$, $V_{mzz'}$, and $V_{pzz'}$ are the respective nuclear, maternal, and paternal components of variance in homozygotes. It seems reasonable to assume that the maternal and paternal effects in homozygotes are less than in heterozygotes because of the reduced viability of homozygotes. Logically, the effects in homozygotes should be approximately $\bar{z}' = 0.729$ times the effects in heterozygotes. With this assumption

$$
C_{nzz'} \equiv \bar{z}' V_{nzz}, \quad C_{pzz'} \equiv \bar{z}' V_{pzz},
$$

and the corrected values are: $V_{nzz'} = 0.0096$.

<table>
<thead>
<tr>
<th>Covariances $nzz'$</th>
<th>Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td>0.0041</td>
</tr>
<tr>
<td>Paternal</td>
<td>0.0068</td>
</tr>
<tr>
<td>Average</td>
<td>0.0054</td>
</tr>
</tbody>
</table>

There are still considerable differences between maternal and paternal covariances and regressions.
There is little doubt about the variation among single-marker heterozygotes. A question that arises is how the variation involving marker chromosomes compares with that of wild-type chromosomes.

**Do the marker chromosomes act as typical wild-type chromosomes?**

If one were to hold a random, maternally derived wild-type chromosome constant, one would expect a chromosomal component of variance of \( z \) of

\[
V_{cz} = V_{nz} + V_{tz} + V_{pz} + V_{hz}
\]

where the components may be identified in Table 3. For a random, paternally derived chromosome held constant, the expected chromosomal component of variance is

\[
V_{cmz} = V_{nz} + V_{tz} + V_{mz} + V_{hz}.
\]

If the marker chromosomes act as random chromosomes on the average (i.e., \( Cy \) and \( Pm \) each play the role of a typical + chromosome), then \( V_{cz} = V_{cps} \) and \( V_{cy} = V_{cmz} \), and \( C_{cuy} = V_{nz} \). These comparisons are, where estimates are in parentheses,

<table>
<thead>
<tr>
<th>Component</th>
<th>Table 1</th>
<th>Table 2</th>
<th>Table 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{cz} )</td>
<td>(0.0104)</td>
<td>(0.0167)</td>
<td>(0.0172)</td>
</tr>
<tr>
<td>( V_{cy} )</td>
<td>(0.0064)</td>
<td>(0.0032)</td>
<td>(0.0115)</td>
</tr>
<tr>
<td>( C_{cuy} )</td>
<td>(0.0034)*</td>
<td>(0.0035)</td>
<td>(0.0049)</td>
</tr>
</tbody>
</table>

* In text.

It would appear that the marker chromosomes, particularly \( Pm \), suppress the variation to some extent in comparison to the average for random chromosomes.

On the same assumptions, equalities between components of Tables 5 and 3, and estimates of the components, are

<table>
<thead>
<tr>
<th>Component</th>
<th>Table 5</th>
<th>Table 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{nzz} )</td>
<td>(0.0033)</td>
<td>(0.0049)</td>
</tr>
<tr>
<td>( C_{nzy} )</td>
<td>(0.0038)</td>
<td>(0.0051)</td>
</tr>
<tr>
<td>( C_{pzz} )</td>
<td>(0.0099)</td>
<td>(0.0146)</td>
</tr>
<tr>
<td>( C_{mzy} )</td>
<td>(0.0059)</td>
<td>(0.0033)</td>
</tr>
</tbody>
</table>

Agreement is better in this case, although there is still the suggestion of some suppression of variation by the marker chromosomes.
Important to the question being asked is the nature of the paternal variation, $V_p$. One possible explanation is differential meiotic drive of the wild-type chromosomes in male parents, which always contained the $Pm$ marker. For this model, the foregoing comparisons and conclusions still hold because female parents always carried the $Cy$ chromosome and the male parents always carried the $Pm$ chromosome. Comparisons among $Cy$, $Pm$, and wild-type chromosomes in combination with sex are not available.

Meiotic drive

The large component of variance for paternally derived chromosomes, $\hat{V}_p = 0.0079 \pm 0.0023$, in Table 3 was unexpected. Even the lesser maternal component $\hat{V}_m = 0.0021 \pm 0.0009$ was not expected to be so large. By the method of procedure, the cytoplasm of all experimental material was that of the $Cy/Pm$ stock, and any variation in fecundity and fertility of the $Cy$ heterozygous female parents should cancel out in the ratio nature of the relative viabilities that were analyzed.

In a model considering the effects of differential meiotic drive alone, we let the gametic frequencies be changed from $1/2$ to those shown:

<table>
<thead>
<tr>
<th>Parents</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gametes</td>
<td>$Cy$</td>
<td>$+_i$</td>
</tr>
<tr>
<td>Frequencies</td>
<td>$1 + a_i$</td>
<td>$1 - a_i$</td>
</tr>
</tbody>
</table>

where $a_i$ is the effect of the $i$th chromosome with $Cy$ in females on meiotic drive and $b_j$ is the effect of the $j$th chromosome with $Pm$ in males on meiotic drive. The effects may be plus or minus. In the absence of selection, the expected frequencies in the offspring are

<table>
<thead>
<tr>
<th>Pm</th>
<th>$+_j$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Cy$</td>
<td>$(1/4) (1+a_i) (1+b_j)$</td>
</tr>
<tr>
<td>$+_i$</td>
<td>$(1/4) (1-a_i) (1+b_j)$</td>
</tr>
</tbody>
</table>

with contributions to the relative viabilities of

\[
x_{ij} \text{ of } [(1-b_j)/(1+b_j)] - 1 \approx -2b_j
\]

\[
y_{ij} \text{ of } [(1-a_i)/(1+a_i)] - 1 \approx -2a_i
\]

\[
z_{ij} \text{ of } [(1-a_i)/(1+b_j) - 1] \approx -2a_i - 2b_j
\]

\[
u_{ij} \text{ of } [(1-a_i)/(1+a_i)] - 1 \approx -2a_i
\]

\[
v_{ij} \text{ of } [(1-b_j)/(1+b_j)] - 1 \approx -2b_j
\]

The contribution of meiotic drive to the variance due to paternally derived chromosomes is $4\sigma_{a_i}^2$, while that to the variance due to maternally derived chromosomes is $4\sigma_{b_j}^2$. For some of the components of variance, there could be a contribution due to the covariance, $C_{ab}i$, of $a_i$ with $b_i$. There is evidence that the
covariance is zero, however, from comparing various $V_m$’s and $V_p$’s from Tables 3 and 4.

<table>
<thead>
<tr>
<th>From Table</th>
<th>Component</th>
<th>Contribution</th>
<th>Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>$V_{mx}$</td>
<td>$4\sigma^2_a - 4C_{ab}$</td>
<td>0.0021</td>
</tr>
<tr>
<td>3</td>
<td>$V_{px}$</td>
<td>$4\sigma^2_b - 4C_{ab}$</td>
<td>0.0079</td>
</tr>
<tr>
<td>4</td>
<td>$V_{mu}$</td>
<td>$4\sigma^2_a$</td>
<td>0.0024</td>
</tr>
<tr>
<td>4</td>
<td>$V_{pu}$</td>
<td>zero</td>
<td>0.0009</td>
</tr>
<tr>
<td>4</td>
<td>$V_{mv}$</td>
<td>zero</td>
<td>0.0008</td>
</tr>
<tr>
<td>4</td>
<td>$V_{pv}$</td>
<td>$4\sigma^2_h$</td>
<td>0.0079</td>
</tr>
</tbody>
</table>

Under the assumption that $C_{ab} = 0$, $V_{mx} = V_{mu}$ and $V_{px} = V_{pu}$, and the agreement among the estimates is unusually good. Note also under this model $V_{pu} = 0$ and $V_{mv} = 0$, and the estimates are small and not significantly different from zero.

While the results are consistent with a model of meiotic drive, caution must be exercised in this interpretation since any other types of effects, either multiplicative or additive, which are associated with maternally and paternally derived chromosomes, and not correlated, will give the same results.

**Cy vs. Cy/Pm methods**

No direct comparisons of the methods are available. The results for $u$ or $u'$ involving $Cy$ heterozygotes as a standard are not really comparable to those for the $Cy$ method, particularly in terms of maternal and paternal effects. For the $Cy$ method, in experiments like E1,

$$f_{ij} = \frac{2(+/+j)}{(Cy/+i) + (Cy/+j)}$$

where the number 2 approximates the standardizing factor for a mean $f$ of one. The expression is equivalent to

$$f_{ij} = \frac{2z_{ij}}{x_j + y_i}$$

except that $y$ now is for $Cy$ heterozygotes with maternally derived wild-type chromosomes, and of course not distinguishable from $z$.

The components of variance for $f$ corresponding to the model at (1), and the analysis of $z$ (Table 3) can be derived approximately in terms of the variances and covariances involving $z$, $x$ and $y$ if one assumes that marker chromosomes act as typical wild-type chromosomes. This is done in Appendix A with the following results:

$$V_{nf} \approx \frac{V_{nx}}{4} + \frac{V_{tz}}{4}$$

$$V_{mf} \approx \frac{V_{mx}}{4} + \frac{V_{kz}}{4}$$

$$V_{tf} \approx V_{tx}$$

$$V_{pf} \approx \frac{V_{pz}}{4} + \frac{V_{kz}}{4}$$

$$V_{kf} \approx V_{kz}.$$
The total variance for \( f \), excluding error, is related to the components of \( z \) as

\[
2V_{nf} + V_{tf} + V_{mf} + V_{pf} + V_{bf} \equiv \frac{V_{nz}}{2} + \frac{3}{2} V_{tz} + \frac{V_{ms}}{4} + \frac{3}{2} V_{bz} .
\]

For maternal and paternal effects we may alternatively look at the contributions of these effects to \( f_{ij} \) in the manner that they were treated in the previous section on meiotic drive. These are

\[
f_{ij} : \frac{(1-a_i)(1-b_j)}{1-a_ib_j} - 1 \equiv -a_i - b_j .
\]

Thus, the contributions to the variances are approximately one-fourth those by the \( C_y/P_m \) method.

The foregoing translations of variances depend on marker chromosomes acting as typical wild-type chromosomes, and, if different marker chromosomes are involved, additionally on the different marker heterozygotes providing the same paternal-maternal picture. Paternal effects for \( C_y \) heterozygotes and \( P_m \) heterozygotes used as male parents may well be different.

It was the assumption that \( C_y \) acts as a typical wild-type chromosome that allowed Mukai et al. (1974) to estimate additive and dominance variances. While their arguments were based on additive and dominance effects, these effects operationally are synonymous with average and interaction effects, respectively, of chromosomes. Also, they assumed no maternal or paternal effects, since a test of reciprocal effects was not significant. They used row, \( VR \), column, \( VC \), and interaction, \( VRO \), components of variance for the factorial designs. With row being maternal and column paternal, the translation of these components into those used herein is

\[
V_R = V_{nf} + V_{mf}, \quad V_C = V_{nf} + V_{pf}, \quad V_{RO} = V_{tf} + V_{bf} .
\]

With the assumption of no epistasis, the additive \( V_A \) and dominance \( V_D \) genetic variances are related to the design components of variance as \( V_A = 8(V_{nf} - V_{tf}) = 2V_{nz} \) and \( V_D = V_{tf} = V_{tz} \). As would be appropriate under the assumption of no reciprocal effects, Mukai et al. (1974) used the following estimators

\[
\hat{V}_A = 4\hat{V}_R + 4\hat{V}_C - 2\hat{V}_{RO} = 0.0096
\]

\[
\hat{V}_D = \hat{V}_{RC} = 0.0010
\]

Those from the current experiment, excluding reciprocal effects, are

\[
\hat{V}_A = 2\hat{V}_{nz} = 0.0099 , \quad \hat{V}_D = \hat{V}_{tz} = 0.0054 .
\]

The discrepancy involves \( V_D \). It was found by Carrellino and Mukai (1975) that \( V_D \) increased with the number of generations that the wild-type chromosomes were maintained in balanced stocks before initiation of the experiments. They laid the blame on mutator factors in some of the isochromosomal lines that
led to male recombination among other things, and concluded that increased linkage disequilibrium led to the increased variance for \( V_{tf} \). Whatever the cause, there was a large increase in \( V_{tf} = V_D \) and some in \( V_A \).

Many of the chromosome lines in the current study were the same as those of Mukai et al. (1974), but utilized in generation zero for their experiment 1 and about generation ten for their experiment 2 but 20 generations later for ours.

It was possible that some maternal and paternal variances contributed to the estimate of \( V_A \) of Mukai et al. (1974). They found the overall pooled test of reciprocal effects to be nonsignificant. A comparable test is also nonsignificant in our case assuming \((1/4)(V_{mx} + V_{px})\), as would be appropriate for the Cy method. It is necessary to break out the mean square for \( V_m + V_p \) separately from \( V_k \) to show significance. On reanalysis of their data, these separate tests were also nonsignificant.

We are on much less sound ground in comparing covariances and regressions between Cy and Cy/Pm methods because homozygotes are functions of genetic, maternal, and paternal effects involving the same chromosomes and the components are not experimentally separable. Approximate formulations relating the two are given in Appendix B as a matter of record. Also, the regression is developed following the approach of Mukai et al. (1972) and compared with the one based on marker chromosomes being typical.

**General comments**

One potential problem, the interaction or competitive effects of different genotypes in a vial on relative survival, did not materialize. The factorial experiments in E1 provide an excellent test of these effects in that each marker heterozygote survived in mixtures involving seven distinct marker heterozygote and wild-type heterozygote combinations. The components of variance for these effects were (Table 1) \( 0.0012 \pm 0.0017 \) for Cy heterozygotes and \( 0.0014 \pm 0.0018 \) for Pm heterozygotes, neither of which is significantly different from zero. In any case, the components amount to only 2% and 3% of the total variance, respectively, and these effects should not materially influence other results.

Also, there was no problem about the suitability of Cy/Pm heterozygotes as a standard; the average viabilities of the four heterozygous classes were very close to each other (within 3%).

An unexpected problem that arose was the large component of variance for paternally derived chromosomes and a smaller component for maternally derived chromosomes. The results from all experiments substantiate these effects. While they could be estimated and accounted for in the factorial experiments, the contribution of paternal and maternal effects could not be accounted for in homozygotes directly, but only indirectly by making assumptions about the relationship between maternal and paternal variances of homozygotes and heterozygotes.

The cause(s) of these maternal and paternal variances is not known, although meiotic drive is a logical candidate. Also, whether such variances are peculiar to the marker chromosomes, Cy and Pm, utilized in the parents is not known. To sort this out would require similar experiments with other markers and with
each marker utilized in both male and female parents. It is indeed unfortunate that marker chromosomes other than \( Cy \) with sufficient inversions to suppress crossing over in females are not available.

Another problem that arose, not known in advance of the experiment but discovered by Cardellino and Mukai (1975), is that wild-type chromosomes kept balanced in the laboratory under certain conditions for some time show a different variance picture from those freshly introduced. In their case, wild-type chromosomes were kept balanced by \((5) Cy/Pm \) female \( \times (1) Pm/+; \) male matings, and the experiments were conducted by the \( Cy \) technique. The variance due to interaction of chromosomes increased almost 100-fold and that due to average effects of chromosomes doubled between generation 0 and 30. They attributed this increase to male recombination caused by mutators. On the other hand, there was a ten-generation difference between the material in experiments 1 and 2 in Mukai et al. (1974), which gave essentially the same variance picture. In this case the wild-type chromosomes were maintained by \( Cy/+; X Cy/+; \) matings, and the experiments were conducted by the \( Cy \) technique. The chromosomes were maintained prior to the current experiments by this latter method, but it took four to five generations, on the average, to balance \( Pm \) with the wild-type chromosomes and set up all the matings for the \( Cy/Pm \) experiments. Consequently, there was opportunity for recombination in \( Pm/+; \) males prior to that in the parents of the experiments.

Considerable research has been conducted on mutator phenomena in Drosophila, most of which is reviewed by Kidwell, Kidwell and Sved (1977). They termed this phenomenon hybrid dysgenesis and listed seven traits affected. The important traits in our experiments would appear to be male recombination and transmission distortion (which we have called meiotic drive). We have already shown how meiotic drive in parents can affect the maternal and paternal components of variance. On the other hand, the effects of male recombination are not clear. Recall that five \( Pm/+; \) males were used in each cross. If recombination had occurred previously, then they could vary in their second chromosome constitution, and families would represent an average for the five male parents. Male recombination in the gametes of the parents would lead to additional segregation within families. Recall also in the factorial experiments that there were reciprocals and duplicates with other samples of parents. The estimation of the average chromosomal variance, \( V_{nz} \), and the interaction variance, \( V_{ts} \), involves the average products of the relative viabilities of reciprocal full sibs, \( \hat{P}_{rs} \), of reciprocal half sibs, \( \hat{P}_{rs} \), and of unrelated families, \( \hat{P}_o \), as

\[
\hat{V}_{nz} = \hat{P}_{rs} - \hat{P}_o
\]

\[
\hat{V}_{ts} = \hat{P}_{rs} - 2\hat{P}_{rs}.
\]

Any general recombination with the \( Pm \) chromosome would tend to increase \( \hat{P}_o \), i.e., unrelated families would tend to share some \( Pm \) genes and be more alike than otherwise. On the other hand, reciprocal half-sib families would segregate for
Pm and + genes and would tend to be less alike than if they had exactly the same wild-type second chromosome. The same would hold for $P_m$ between reciprocal full-sib families. Thus, we would expect $V_{ns}$ to be less than if there were no recombination. The situation is not as clear for $V_{ts}$ but it should not be increased appreciably.

Recombination means also that $Pm$ in the $Cy/Pm$ standard is variable. This should operate further to reduce $V_{ns}$ and not affect $V_{ts}$ appreciably in the manner illustrated theoretically for the $Cy$ technique.

These conclusions do not agree with the results of Cardellino and Mukai (1975). In their experiments, a single wild-type chromosome for each chromosomal line was utilized by the $Cy$ technique. Assuming no recombination in males balanced with $Cy$, there would be no segregation within families. Also, reciprocal crosses were pooled. Nevertheless, prior recombination in the lines in $Pm/+\$ males would work to introduce $Pm$ genes in some or all of the families, with effects of the same nature as described in our experiments. It would take large interactions of $Pm$ genes (introduced) with the wild-type genes to generate the large increase in variances. The accumulation of unique mutations, probably enhanced by mutator factors, would work toward increasing the variances. Chromosomal aberrations, another hybrid dysgenic trait listed by Kidwell, Kidwell and Sved (1977), could also increase the variances. A combination of these traits may have been responsible.

The genetic regressions estimated in this study, even when corrected for maternal and paternal effects, were in the vicinity of 0.5. When used as an estimate of the average degree of dominance, $h$, it implies that genes are additive in their effects, on the average. However, such a conclusion is incompatible with the large inbreeding depression found universally for relative viability and demonstrated by the low relative viability of homozygotes in these experiments. Previous estimates by Mukai et al. (1972) and by Mukai and Yamaguchi (1974) using single-marker experiments were in the vicinity of 0.25, which is compatible with a large inbreeding depression.

Katz and Cardellino (1978), utilizing a circulant mating design with the $Cy/Pm$ technique, found variation among marker heterozygotes. However, their design was not sufficient to allow the depth of analysis that we have accomplished. Also, they introduced the confusing factor of $Pm$ heterozygotes as maternal parents in which recombination may occur.

We also found, and have established with little doubt, that there is variation among marker heterozygotes with the same marker chromosome. This result is intuitively reasonable. The result is also compatible with the lack of any substantial relationship between the average relative viabilities of wild-type homozygotes and marker heterozygotes with the same wild-type chromosome (Dobzansky, Krimbas and Krimbas 1960; Wallace and Dobzhansky 1962), although this was not the case in our experiments. Variation in marker heterozygotes used as standards for relative viabilities will affect estimates of variances.
and can affect estimates of regressions, although there may be cancelling contributions to both numerator and denominator.

Approximations were developed assuming marker chromosomes to behave as typical random chromosomes for translating variances between double-marker experiments and single-marker experiments and which allow estimation of the desired components of variance from single marker experiments. More information involving other marker chromosomes is needed to learn the extent to which results are specific to each marker chromosome, before much reliability can be placed in the approximations.

LITERATURE CITED


Corresponding editor: W. J. EWENS
MARKER CHROMOSOME EFFECTS

APPENDIX A

We can derive approximately (using methods outlined by Mode and Robinson 1959) the components for \( f \) in terms of variances and covariances involving \( z, x \) and \( y \). Using \( \mathcal{C} \) for covariance as before,

\[
V_{nf} = \mathcal{C}_{f_i f_j k} = \frac{4V_{nz} + V_{tz}}{A^2} - \frac{8\bar{z}}{A^2} \left( \mathcal{C}_{nzz} + \mathcal{C}_{nyz} \right) + \frac{8\bar{z}^2}{A^4} \mathcal{C}_{cyy},
\]

where \( A = \bar{x} + \bar{z} \) and bars indicate mean values. For standardized means \( \bar{z} = \bar{x} = \bar{z} = 1 \), and with the assumption that markers act as typical random chromosomes,

\[
\mathcal{C}_{nzz} = \mathcal{C}_{nyz} = V_{nz}, \quad \mathcal{C}_{cyy} = V_{nz} + V_{tz}, \text{ so that } V_{nf} \approx \frac{V_{nz}}{4} + \frac{V_{tz}}{4}.
\]

By the same procedure and assumptions:

\[
2V_{nf} + V_{tf} = \mathcal{C}_{f_i f_j k} = \frac{4(2V_{nz} + V_{tz})}{A^2} - \frac{8\bar{z}}{A^2} \left( \mathcal{C}_{nzz} + \mathcal{C}_{nyz} \right) + \frac{8\bar{z}^2}{A^4} \mathcal{C}_{cyy},
\]

\[
\vdots \quad V_{tf} \approx V_{tz},
\]

\[
V_{nf} + V_{mf} = \mathcal{C}_{f_i f_j k} = \frac{4(V_{nz} + V_{nz})}{A^2} - \frac{8\bar{z}}{A^2} \left( \mathcal{C}_{nzz} + \frac{\bar{z}^2}{A^4} \mathcal{C}_{cyy} + V_{cy}
\]

\[
\vdots \quad V_{mf} \approx \frac{V_{nz}}{4} + \frac{V_{tz}}{4}
\]

\[
V_{pf} \approx \frac{V_{nz}}{4} + \frac{V_{tz}}{4} \quad \text{(by symmetry)}
\]

\[
2V_{nf} + V_{tf} + V_{mf} + V_{pf} + V_{kf} = \mathcal{C}_{f_i f_j k} = \frac{4}{A^2} \left( 2V_{nz} + V_{tz} + V_{nz} + V_{pz} + V_{hz} \right)
\]

\[
- \frac{8\bar{z}}{A^2} \left( \mathcal{C}_{nzz} + \mathcal{C}_{cyy} \right) + \frac{4\bar{z}^2}{A^4} \left( V_{cz} + V_{cy} + 2 \mathcal{C}_{cyy} \right)
\]

\[
\vdots \quad V_{kf} \approx V_{hz}
\]

APPENDIX B

Comparisons of covariances and regressions between Cy and Cy/Pm methods

We may expand the expressions into approximations involving the variances and covariances of the different types of flies as before.

\[
\mathcal{C}_{Mff} = \mathcal{C}_{f_i f_j k} = \frac{4\mathcal{C}}{AB} \mathcal{C}_{zz'} - \frac{4\bar{z}'}{AB^2} \left( \mathcal{C}_{nz'z} + \mathcal{C}_{M'yz} \right) - \frac{4\bar{z}^2}{A^2 B} \mathcal{C}_{cyy'}
\]

\[
\vdots \quad + \frac{4\bar{z}^2}{A^2 B^2} \left( \mathcal{C}_{cz'y' + \mathcal{C}_{cyy'}} \right)
\]

where \( A = \bar{z} + \bar{z}' \) and \( B = \bar{z}' + \bar{z}' \). There should be no difference between \( x \) and \( x' \) between \( y \) and \( y' \); the distinctions are for bookkeeping. For standardized means, we let all means be one except \( z' \) for homozygotes. Then, assuming marker chromosomes to be typical random wild-type chromosomes and making use of previously developed relationships,

\[
\mathcal{C}_{Mzz} = \mathcal{C}_{cyy} = \mathcal{C}_{nzz} + \bar{z}V_{nz} + \bar{z}V_{mx}, \quad \mathcal{C}_{nx'z} = \mathcal{C}_{M'y'z} = 2V_{nz} + V_{mx}
\]

\[
\vdots \quad + \mathcal{C}_{cyy} = 2V_{nz} + V_{mx} + 2V_{nz} + V_{tz} + V_{hz}
\]
the expression reduces to

\[ C_{Mff} = \frac{C_{nzz}}{2} - \frac{\bar{z}'}{4} \left( 2V_{nz} - V_{mz} - 2V_{tz} - V_{kz} \right) \]

\[ \approx \frac{C_{nzz}}{2} - \bar{f} \left( 2V_{nf} - V_{mf} - V_{tf} \right) . \]

The latter equation involves components that are estimable from a Cy experiment, and could be utilized in estimating \( C_{nzz} \). The corresponding expression for the paternal covariances is

\[ C_{Dff} = \frac{C_{nzz}}{2} - \frac{\bar{z}'}{4} \left( 2V_{nz} - V_{pz} - 2V_{tz} - V_{kz} \right) \]

\[ \approx \frac{C_{nzz}}{2} - \bar{f} \left( 2V_{nf} - V_{pf} - V_{tf} \right) . \]

For the chromosomal variance among homozygotes the expansion into variances and covariances of the components in the ratio is

\[ V_{cf'} = \frac{4V_{er} - 8\bar{z}}{B^2} \left( C_{xe'r'} + C_{ec'r'} \right) + \frac{4(\bar{z})^2}{B^4} \left( V_{er} + V_{cv} + 2C_{ec'r'} \right) . \]

Using the following equivalences,

\[ V_{er} = V_{nz} + (\bar{z})^2 (V_{pz} + V_{mz}), C_{xe'r'} + C_{ec'r'} = 2C_{nzz} + \bar{z}^2 (V_{mz} + V_{pz}), \]

\[ V_{er} + V_{cv} + 2C_{xe'r'} = 4V_{nz} + V_{mz} + V_{pz} + 4V_{tz} + 2V_{kz}; B = 2 \]

then

\[ V_{cf'} \approx V_{nz} - 2\bar{z} C_{nzz} + \frac{(\bar{z})^2}{4} (4V_{nz} + V_{mz} + V_{pz} + 4V_{tz} + 2V_{kz}) \]

\[ \approx V_{nz} - 2\bar{f} C_{nzz} + (\bar{f})^2 (4V_{nf} + V_{mf} + V_{pf}) . \]

No clear picture emerges as to the biases, particularly in \( V_{cf'} \), and the regressions. The difficulties do not involve just maternal and paternal variances, but nuclear variances and covariances as well. We may project, but with little reliability, the components for a Cy experiment by substitution of estimates from our experiments in the right-hand sides of the equations. In these projections, it is assumed also that \( P_m \) in male parents provides the same variance picture as \( Cy \), which may be in considerable error.

\[ \hat{C}_{Mff} = 0.0025 \quad \hat{V}_{cf'} = 0.0082 \quad \hat{b}_{Mff} = 0.30 \]

\[ \hat{C}_{Dff} = 0.0048 \quad \hat{b}_{Dff} = 0.59 \]

Average 0.0036 0.44

The average of the two \( \hat{C}_{nzz} \)'s was used in projecting \( \hat{V}_{cf'} \). The average regression is higher than any given by Mukai et al. (1972) and Mukai and Yamaguchi (1974).

Alternatively, from Cy experiments of the E1 and E2 type, one can reverse the procedure and estimate \( b_{nzz} = C_{nzz}/V_{nz} \) by making the appropriate substitutions in the formulations

\[ b_{nzz} = \frac{C_{Mff} + C_{Dff} + \bar{f} (4V_{nf} - 2V_{pf} - V_{mf})}{V_{cf'} + 2\bar{f} (C_{Mff} + C_{Dff}) + (\bar{f})^2 (4V_{nf} - 4V_{pf} - 3V_{mf} - 3V_{pf})} \]
Even without maternal and paternal effects, i.e., \( V_{mf} = V_{pf} = 0 \) and \( C_{Mf} = C_{Df} \), there are adjustments to the regression stemming from variation among marker heterozygotes.

In the estimation of dominance by Mukai et al. (1972), it was assumed that \( Cy \) carried the best gene at each locus and that \( Cy \) heterozygotes were constant in relative viability. In the foregoing, statistical methods were developed to overcome these assumptions, but these methods required other assumptions about the behavior of marker heterozygotes.

We now modify the approach of Mukai et al. (1972) to allow for gene effects at a locus to be the same for \( Cy \) heterozygotes and wild-type heterozygotes, and for \( Cy \) to carry some poor genes as well as good genes. First, consider the situation where \( Cy \) contains the good gene, \( A^* \) (the asterisk denotes that the gene is in \( Cy \)). The frequencies and relative viabilities of the heterozygotes and sums of homozygotes are as follows, where viabilities are depicted on a 1, \( 1 - hs \), \( 1 - s \) scale for \( AA, Aa, aa \) respectively.

<table>
<thead>
<tr>
<th>Frequencies</th>
<th>( p^2 )</th>
<th>( 2pq )</th>
<th>( q^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygotes</td>
<td>( 2AA )</td>
<td>( 2Aa )</td>
<td>( 2aa )</td>
</tr>
<tr>
<td>( A^*A + A^*a )</td>
<td>( A^*A + A^*a )</td>
<td>( A^*a + A^*a )</td>
<td></td>
</tr>
<tr>
<td>Relative viability of heterozygotes — ( Y )</td>
<td>1</td>
<td>( \frac{2(1-hs)}{2-hs} )</td>
<td>( \frac{1-s}{1-hs} )</td>
</tr>
<tr>
<td>Sum of homozygotes</td>
<td>( 2(2AA) )</td>
<td>( 2AA )</td>
<td>( 2aa )</td>
</tr>
<tr>
<td>( A^*A + A^*A )</td>
<td>( A^*A + A^*A )</td>
<td>( A^*a + A^*a )</td>
<td>( A^*a + A^*a )</td>
</tr>
<tr>
<td>Relative viability of sum of homozygotes — ( X )</td>
<td>2</td>
<td>( 1 + \frac{1-s}{1-hs} )</td>
<td>( \frac{2(1-s)}{1-hs} )</td>
</tr>
</tbody>
</table>

With the following approximations:

\[
\frac{2(1-hs)}{2-hs} \approx 1 - \frac{hs}{2}, \quad \frac{1-s}{1-hs} \approx 1 - s(1-h),
\]

the means, variances and covariance are found to be

\[
\bar{X} = 1 - qgs(1-h), \quad \bar{Y} = \frac{\bar{X}}{2} + pqg(1-2h),
\]

\[
V_X = \frac{pq}{2} s^2h^2 + pq^2g^2(1-2h) + pqg^2(1-2h)^2,
\]

\[
\bar{C}_{XY} = pqg^2h(1-h) + 2pq^2g(1-2h)(1-2h)
\]

When \( Cy \) contains the poor gene, \( a^* \), the following situation obtains,

<table>
<thead>
<tr>
<th>Frequencies</th>
<th>( p^2 )</th>
<th>( 2pq )</th>
<th>( q^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygotes</td>
<td>( 2AA )</td>
<td>( 2Aa )</td>
<td>( 2aa )</td>
</tr>
<tr>
<td>( Aa^* + Aa^* )</td>
<td>( Aa^* + aa^* )</td>
<td>( aa^* + aa^* )</td>
<td></td>
</tr>
<tr>
<td>Relative viability — ( Y )</td>
<td>( \frac{1}{1-hs} )</td>
<td>( \frac{2(1-hs)}{2-s-hs} )</td>
<td>1</td>
</tr>
<tr>
<td>Sum of homozygotes</td>
<td>( 2(2AA) )</td>
<td>( 2AA )</td>
<td>( 2aa )</td>
</tr>
<tr>
<td>( Aa^* + Aa^* )</td>
<td>( Aa^* + Aa^* )</td>
<td>( aa^* + aa^* )</td>
<td>( aa^* + aa^* )</td>
</tr>
<tr>
<td>Relative viability — ( X )</td>
<td>( \frac{2}{1-hs} )</td>
<td>( \frac{1}{1-hs} + 1 )</td>
<td>2</td>
</tr>
</tbody>
</table>
With the approximations
\[
\frac{1}{1 - hs} \approx 1 + hs, \quad \frac{2(1 - hs)}{2 - s - hs} \approx 1 + \frac{s(1 - h)}{2}
\]
the means, variances and covariance in this case are
\[
\frac{\bar{X}}{2} = 1 + psh, \quad \frac{\bar{Y}}{2} = \frac{\bar{X}}{2} + pqs(1 - 2h)
\]
\[
V_y = \frac{pq}{2} s^2 (1 - h)^2 - 2pq^2qs^2h(1 - 2h) - p^2q^2s^2(1 - 2h)
\]
\[
V_x = 2pq^2h^2
\]
\[
C_{xy} = pq^2h(1 - h) - 2pq^2qs^2h(1 - 2h)
\]

For the estimation of \( \hat{h} \) as \( b_{yx} = C_{xy}/V_x \), it is assumed that the population is in mutation selection balance so that \( q = u/hs \), where \( u \) is the mutation rate, and terms involving \( q^2 \) or higher powers of \( q \) can be ignored. With this assumption and summing over loci indexed by \( i \) for \( A^* \)'s and \( j \) for \( a^* \)'s,
\[
b_{yx} = \frac{\sum_i u_i s_i (1 - h_i) - \sum_j u_j s_j (1 - 3h_j)}{\sum_i 2u_i s_i (1 - h_i)^2 + \sum_j 2u_j s_j h_j}
\]

If we assume \( u, s, \) and \( h \) to be uncorrelated and take mean values of the numerator and denominator, the expression reduces to
\[
b_{yx} = \frac{n_i(1 - \bar{h}_i) - n_j(1 - 3\bar{h}_j)}{2n_i \left( \frac{1}{h_i} - 2 + \bar{h}_i \right) + 2n_j \bar{h}_j}
\]
where \( \bar{h} \) is the arithmetic mean and \( \bar{h} \) is the harmonic mean. If we let the proportion of \( a^* \) genes be \( e = n_j/(n_i + n_j) \) and omit the \( i \) and \( j \) distinction on \( h \),
\[
b_{yx} = \frac{\bar{h}}{2(1 - \bar{h})} \left[ \frac{1 - \bar{h} - \bar{h} + \bar{h}\bar{h} - 2e(1 - 2\bar{h})}{1 - 2\bar{h} + \bar{h}\bar{h} - e(1 - 2\bar{h})} \right].
\]
The ratio \( \bar{h}/2(1 - \bar{h}) \) is a good approximation to the one for \( A^* \) genes alone, i.e., \( e = 0 \). The proportion of \( a^* \) genes is expected to be very small. With \( e \) negligible,
\[
b_{yx} \approx \frac{\bar{h}}{2(1 - \bar{h})} \quad \text{or} \quad \frac{2b_{yx}}{1 - 2b_{yx}}.
\]
The results now are in terms of the harmonic mean, which is always less than the arithmetic mean. Note that \( b_{yx} \) and \( \bar{h} \) are approximately the same for \( \bar{h} \), near 1/2. While one may wish to use this reformulation for estimating the average degree of dominance, one would still want to correct the variance and covariance for maternal and paternal effects, if any. This could be done by separate covariances on single homozygotes or by a single covariance on sums of homozygotes. For separate covariances
\[
b_{yx} = \frac{C_{Mff'} + C_{Gff'} - \bar{f}(V_{p} + V_{mf})}{2[V_{cf} - (f')^2(V_{p} + V_{mf})]}
\]
and

\[
\hat{h} = \frac{C M + C D - \bar{f} (V_{pf} + V_{mf})}{V_{cf} + C M + C D - \bar{f} (1+\bar{f}) (V_{pf} + V_{mf})}
\]  

(3)

There are some similarities between this formulation and that for \( b_{nzz} \), at (2), also an estimator of \( h \); however, they are quite different, having been derived on different premises.

For this model for an \( A^* \) gene, the contribution to

\[
V_{nf} \text{ is } p q \left[ (1/4) - 2 q^2 (p-q) \right] s^2 h^2 + p q^2 (1-4 q) s^2 h + p q^2 s^2
\]

and to

\[
V_{tf} \text{ is } p^2 q^2 s^2 (1-2 h)^2.
\]

If we view \( z \) as measured on the \( 1, 1 - hs, 1 - s \) scale, then \( V_{tf} = V_{tz} \) as in the general model, but \( V_{nf} \neq (V_{nz} + V_{tz})/4 \) unless we ignore terms involving \( q^2 \) and higher powers of \( q \). If we ignore these terms and let \( q = u/hs \) for an equilibrium population, then

\[
\begin{align*}
V_{nf} &= \Sigma u_i s_i h_i / 4, V_{tf} = 0 \\
C M - \bar{f} V_{mf} &= C D - \bar{f} V_{pf} = \Sigma u_i s_i (1-h_i) / 2 \\
V_{cf} - (\bar{f})^2 (V_{mf} + V_{pf}) &= \Sigma u_i s_i (1-h_i)^2 / h_i
\end{align*}
\]

(4)

There is still no real agreement between (3) and (2) unless gene effects are additive, i.e., \( h = 1/2 \). Then,

\[
4 V_{nf} = 2 (C M - \bar{f} V_{mf}) = 2 (C D - \bar{f} V_{pf})
\]

\(= V_{cf} - (\bar{f})^2 (V_{mf} + V_{pf}), \bar{f} = 1
\]

and both regressions are 1/2.

An obvious weighted average of \( h \) from the formulations at (4) is

\[
\begin{align*}
\hat{h} &= \frac{\Sigma u_i s_i h_i}{\Sigma u_i s_i} = \frac{4 V_{nf}}{4 V_{nf} + C M - \bar{f} V_{mf} + C D - \bar{f} V_{pf}} \\
&= \frac{1}{1 + 2 b_{nf} f}
\end{align*}
\]

(5)

where \( b_{nf} f \) is a concocted genetic regression of homozygotes on heterozygotes (it involves only the additive or average chromosomal variance of heterozygotes).

The estimator at (5) is still different from that for \( b_{nzz} = C_{nzz} / V_{nz} \) on the \( 1, 1 - hs, 1 - s \) scale. With the same assumptions involving an equilibrium population,

\[
b_{nzz} = \frac{\Sigma u_i s_i}{\Sigma u_i s_i \cdot h_i}
\]

is a weighted harmonic mean of \( h \), which is approximated by a single-marker experiment at (2).