HOMEOSTASIS ASSOCIATED WITH HETEROZYGOSITY IN THE 
GENETICS OF TIME OF VAGINAL OPENING IN THE 
HOUSE MOUSE

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VARIATION in quantitative characters observed in long inbred strains of any 
organism may be interpreted as being due to environmental effects. On an ade-
quate scale, the magnitude of variation in such inbred strains should be equal, within 
the limit of sampling error, irrespective of the means of the different strains. In 
crosses between true breeding strains, the variation of the F1 generation should be 
equal to the variation in the parental strains.

When the variation in quantitative characters is approximately uniform in the 
parental strains and the F1 on some scale, it may be possible to perform a genetic 
analysis using the methods of Mather (1949). When no such scale is found or when 
the variation in F1 is markedly less than the variation in the parental strains, new 
assumptions must be made. Such a case was found in a genetic investigation of the 
time of vaginal opening in the house mouse, Mus musculus. The non-heritable varia-
tion of the F1 generation was much smaller than that of the parental strains. The 
non-heritable variation of the F2 generation and of the first backcross generations 
to males of either parental strains was also much smaller than that of parental 
strains, and did not differ in its magnitude from that of the F1 generation. The non-
heritable variation of the second backcross generations to males of either parental 
strains, however, showed some increase in its magnitude, as compared to the varia-
tion observed in the F1 and the first backcross generations. Even in these second 
backcross generations, the magnitude of the non-heritable variation was still much 
smaller than that of the parental strains.

Similar results have previously been reported by Livesey (1930) in rats, Mather 
(1949, 1950) in Petunia and Primula species, Rasmusson (1949, 1952) in sugar 
beets and Drosophila, Robertson and Reeve (1952) in Drosophila, and Dob-
zhansky and Wallace (1953) in Drosophila. With the exception of Livesey, these 
authors have linked their observations with the effect of different degrees of hetero-
yzogosity. It was similarly concluded, after a statistical analysis of the present data, 
that the magnitude of the non-heritable variation of a generation is associated with 
its degree of heterozygosity. The smaller variation in the F1, F2, and the first and 
second backcross generations was ascribed to their greater heterozygosity as com-
pared to that of the parental strains. To this effect of heterozygosity Dobzhansky 
and Wallace (1953) applied the term homeostasis.

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STRAINS OF MICE AND DESIGN OF EXPERIMENT

Long inbred strains of mice, NB, BALB/c, and C57BL/10, which were obtained from The Ohio State University genetics laboratory, were found to differ with respect to the mean time of vaginal opening. The times were about 22 days in BALB/c, about 24 days in NB, and about 36 days in C57BL/10. All mice were kept under as similar conditions as possible. Mated females were checked for pregnancy once each week, and after they were found pregnant they were checked once each day for litters. When a litter was born, the number of animals in the litter and the date of birth were recorded. As soon as a litter reached the age of ten days, the mice were classified for sex and the vagina of each female was examined daily thereafter. The time of vaginal opening was computed from the day of birth to its day of opening.

Crosses were made between NB and C57BL/10 (cross 1), and between BALB/c and C57BL/10 (cross 2). In cross 1 the NB strain was designated as P₁ and the C57BL/10 as P₂. In cross 2 the BALB/c strain was designated as P₁ and C57BL/10 as P₂. The mating system in cross 1 and cross 2 is similar. Males and females of P₁ were mated to males and females of P₂ to produce F₁’s. To produce F₂’s, F₁’s were mated to F₁’s. The first backcross generation, designated as B₁ or B₂, was produced by crossing F₁ females to either P₁ or P₂ males, B₁ being from P₁, and B₂ being from P₂. The second backcross generation, designated as B₁₁ or B₂₂, was produced by crossing B₁ or B₂ females to either P₁ or P₂ males, B₁₁ being from P₁, and B₂₂ being from P₂.

EXPERIMENTAL RESULTS

The experiment was begun in May, 1952 and was concluded in June, 1953, covering a little more than a year’s cycle. During this period a total of 1679 female mice were examined. Of these, 132 females were produced by a mating system not mentioned above. (This part of the data will be discussed separately.) The distribution and the mean time of vaginal opening in various generations of the remaining 1547 females are shown in table 1.

<table>
<thead>
<tr>
<th>Generation</th>
<th>Number of mice</th>
<th>X̄</th>
<th>sX</th>
<th>Number of mice</th>
<th>X̄</th>
<th>sX</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₁</td>
<td>86</td>
<td>24.640</td>
<td>0.872</td>
<td>145</td>
<td>22.193</td>
<td>0.659</td>
</tr>
<tr>
<td>P₂</td>
<td>111</td>
<td>36.712</td>
<td>0.754</td>
<td>(111)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₁</td>
<td>47</td>
<td>29.298</td>
<td>0.652</td>
<td>192</td>
<td>34.797</td>
<td>0.326</td>
</tr>
<tr>
<td>F₂</td>
<td>183</td>
<td>29.907</td>
<td>0.399</td>
<td>163</td>
<td>30.736</td>
<td>0.467</td>
</tr>
<tr>
<td>B₁</td>
<td>108</td>
<td>29.500</td>
<td>0.625</td>
<td>107</td>
<td>26.738</td>
<td>0.680</td>
</tr>
<tr>
<td>B₂</td>
<td>118</td>
<td>31.619</td>
<td>0.423</td>
<td>74</td>
<td>33.811</td>
<td>0.487</td>
</tr>
<tr>
<td>B₁₁</td>
<td>49</td>
<td>32.490</td>
<td>1.049</td>
<td>50</td>
<td>21.340</td>
<td>0.807</td>
</tr>
<tr>
<td>B₂₂</td>
<td>88</td>
<td>34.375</td>
<td>0.718</td>
<td>26</td>
<td>36.346</td>
<td>0.940</td>
</tr>
<tr>
<td>Total</td>
<td>790</td>
<td></td>
<td></td>
<td>757</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 1

Number of mice, mean times of vaginal opening, and standard error in various generations in cross 1 and cross 2. In cross 1, P₁ = NB, P₂ = C57BL/10; in cross 2, P₁ = BALB/c, P₂ = C57BL/10.
Effect of season

The effect of season on the time of vaginal opening is considerable. This may be made clear by figure 1, which shows the distribution of all the individuals in the C57BL/10 strain, arranged according to dates of birth. As seen in the figure, there is a definite tendency for the vagina to open early in summer and late in winter. This may be observed not only in three parental strains but also in every type of generation such as F_1, F_2, B_1, B_2, B_{11}, and B_{22}, except in a few cases where the collection of data covered only a part of a season.

In order to remove this effect from the data, it was assumed that a seasonal variation exists, and that this repeats itself once every year, reaching its minimum point sometime during the warmer season, and maximum point sometime during the cooler season. On this assumption the data were fitted with a sine curve of the form.

\[ \tilde{Y} = r \sin (X + U) + h, \]

where \( r \) is the amplitude, \( U \) the origin, and \( h \) the constant or mean level of the curve. This may be written in the form of a sine-cosine curve as:

\[ \tilde{Y} = f \sin X + g \cos X + h, \quad (1) \]

where \( r = \sqrt{f^2 + g^2} \), and \( \tan U = f/g \). The quantities \( f \) and \( g \) together determine the amplitude and the origin of the curve. Since this latter form is more amenable to the problem of estimating parameters by the least squares method, it was used for the curve fitting.

The quantity \( X \) in the sine-cosine function is an angle measured in degrees such that each day of a year represents a degree. For convenience a 30 day month was used and a month was divided into six parts. Arbitrarily 0 degree was assigned to the first part of June, the period from June 1st to 5th. After this the corresponding number of degrees for any part of any month can easily be found.

Figure 1. The time of vaginal opening arranged according to month of birth, dividing a month into two parts, a and b, in the C57BL/10 strain. The dotted line indicates expected means for various periods of time computed from sine-cosine curve (origin: July 16–20 or \( X = 9 \)). The solid line indicates the constant, \( h \). \(*_1 = \) point of maximum; \(*_3 = \) point of minimum; \(*_2\) and \(*_4 = \) points of inflexion.
From equation (1), the following can be written:

\[ \hat{Y}_j = f \sin X_j + g \cos X_j + h, \]  

(2)

where \( \hat{Y}_j \) denotes the expected mean for the period \( X_j \). Then let:

\[ Q = \sum (\hat{Y}_j - Y_{ij})^2 = \sum (f \sin X_j + g \cos X_j + h - Y_{ij})^2, \]

(3)

such that \( Q \) is the quantity to be minimized, \( Y_{ij} \) being the observed value for each individual at the time of \( X_j \).

By partial differentiation of the right side of the equation (3) with respect to \( f, g, \) and \( h, \) and by equating the derivative to zero, three normal equations result. Solving these three normal equations simultaneously, values of \( f, g, \) and \( h, \) can be obtained. Substituting these values of \( f, g, \) and \( h, \) and the values of \( X_j \) in (2), the expected means, \( \hat{Y}_j/3, \) for various periods of time can be found.

The variance of estimate of \( f \) can be computed as:

\[ s_f^2 = \frac{\sum (Y_{ij} - \hat{Y}_j)^2}{df}, \]

(4)

the standard deviation being \( s_f = \sqrt{s_f^2}, \) and the degree of freedom being \( N - 3, \) since three degrees of freedom are sacrificed because of the estimate of \( f, g, \) and \( h. \)

The variances of quantities \( f, g, \) and \( h \) may be computed by replacing the right sides of the three normal equations just mentioned, successively by 1, 0, 0; 0, 1, 0; and 0, 0, 1 to give three sets of three equations. Solving these three sets of three equations gives a matrix of multipliers. From this and the variance of \( V \), the variances of \( f, g, \) and \( h \) can be obtained.

Tests of whether the seasonal variation is statistically significant were made by using the test of curvilinearity (Snedecor 1950). Tests were performed for three parental strains; \( F_1, F_2, B_1, B_2, B_{11}, \) and \( B_{22} \) in cross 1; and \( F_1, F_2, B_1, \) and \( B_2 \) in cross 2. From a total of 13 tests, 8 indicated a seasonal effect with significance at the 1% level, and 3 at the 5% level, with only 2 indicating non-significance. It may be concluded that the effect of season on the time of vaginal opening is unmistakable.

The curve with dotted line in figure 1 was constructed by connecting the points of expected means computed by the method described above for various periods of time for C57BL/10 strain. The straight line drawn horizontally in the center of the figure represents the constant, \( h, \) around which the curve repeats its cycle. This value of \( h \) may now be taken as the mean of the strain.

Fitting the data with the curve becomes especially important when means of two or more different generations, the data of which have been collected at different seasons of a year, are compared. For example, if a mean from data collected during a season of early vaginal opening is compared, without this adjustment, with another mean from data collected during a season of late vaginal opening, the result would lead to biased conclusion. In order to avoid this possible bias, the statistic, \( h, \) was used for the comparison of generations.

However, for the generations other than parental strains, fitting the data with the sine-cosine curve (1) was not feasible, since the data of these generations do not
cover a full year. Instead, a sine curve of the following form was used for the purpose,

\[ \hat{Y} = f \sin X + h. \] (5)

The principal properties of (5) are the same as (1), except that here the quantity \( f \) determines the amplitude of the curve, and the origin of the curve is assigned \textit{a priori}.

The origin of the curves for generations other than parental strains was estimated from the origins of the curves of parental strains. Thus the period between June 26th and 30th, which is the midpoint of origins of NB and C57BL/10, was taken as the origin for all the generations coming from the cross between NB and C57BL/10. For various generations coming from the cross between BALB/c and C57BL/10, the origin of C57BL/10, which is between July 16th and 20th, was chosen as their origin. The reason for not taking the midpoint of the origins of parental strains is that, since the origins of both NB and C57BL/10 are earlier than that of BALB/c, if the midpoint is taken, there may be a risk of fixing the origin in this cross much later than it actually is. Since no adjustment was made of the origin for C57BL/10 in this cross, no adjustment was made for BALB/c either, leaving the origin of the BALB/c strain between July 26th and 31st.

After this decision about the origin of the curves was made, the data for all the generations in cross 1 and cross 2, including those of parental strains, were fitted with the sine curve of (5). The values of \( h \) for these generations are shown in Table 2.

**TABLE 2**

Values of \( h \), variances free from seasonal effect, and variances due to seasonal effect, for \( P_1 \), \( P_2 \), and subsequent generations in cross 1 and cross 2*

<table>
<thead>
<tr>
<th>Generation</th>
<th>( h )</th>
<th>( t_h )</th>
<th>Variances free from seasonal effect</th>
<th>Variances due to seasonal effect</th>
<th>( h )</th>
<th>( t_h )</th>
<th>Variances free from seasonal effect</th>
<th>Variances due to seasonal effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Seasonal variance</td>
<td>Average</td>
<td></td>
<td></td>
<td>Seasonal variance</td>
<td>Average</td>
</tr>
<tr>
<td>( P_1 )</td>
<td>24.803</td>
<td>0.856</td>
<td>62.627 16.888 9.8365</td>
<td></td>
<td>21.054</td>
<td>0.651</td>
<td>53.231 9.707 14.0425</td>
<td></td>
</tr>
<tr>
<td>( P_2 )</td>
<td>34.939</td>
<td>0.700</td>
<td>46.194 2.785</td>
<td></td>
<td>35.110</td>
<td>0.695</td>
<td>44.704 18.378</td>
<td></td>
</tr>
<tr>
<td>( F_1 )</td>
<td>30.154</td>
<td>0.620</td>
<td>15.683 4.270 2.7175</td>
<td></td>
<td>32.340</td>
<td>0.673</td>
<td>19.085 1.297 0.9080</td>
<td></td>
</tr>
<tr>
<td>( F_2 )</td>
<td>29.279</td>
<td>0.440</td>
<td>27.917 1.165</td>
<td></td>
<td>28.741</td>
<td>1.058</td>
<td>34.938 0.519</td>
<td></td>
</tr>
<tr>
<td>( B_1 )</td>
<td>27.731</td>
<td>0.800</td>
<td>39.257 3.198 3.0595</td>
<td></td>
<td>25.895</td>
<td>1.554</td>
<td>50.221 0.759 0.2830</td>
<td></td>
</tr>
<tr>
<td>( B_2 )</td>
<td>30.822</td>
<td>0.422</td>
<td>17.878 2.921</td>
<td></td>
<td>31.346</td>
<td>0.988</td>
<td>16.250 1.325</td>
<td></td>
</tr>
<tr>
<td>( B_{11} )</td>
<td>29.396</td>
<td>1.413</td>
<td>48.761 4.977 10.0690</td>
<td></td>
<td>32.551</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( B_{22} )</td>
<td>31.353</td>
<td>0.739</td>
<td>30.341 15.161</td>
<td></td>
<td>22.960</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Adjusted origins of curves were used for the computation.
† Unadjusted values: Therefore contain the variances due to seasonal effect.
Effect of age of parents, suckling litter, and size of litters

It was found that the age of parents does not affect the time of vaginal opening of their young. The effect of size of litter was found to be statistically significant. However, no constant correlation was found between the size of litter and the mean time of vaginal opening of three parental strains. So this effect was disregarded in the analysis. The effect of suckling litter was experimentally avoided. Females suckling litters were separated from males, and were not mated until the litters were weaned.

Test of difference between parental strains

The difference between three parental strains was tested using the values of $h$, computed from unadjusted origins. The difference between NB and C57BL/10, between BALB/c and C57BL/10, and BALB/c and NB were all found to be significant at the 1% level. It was, therefore, concluded that these three strains are genetically different with respect to the time of vaginal opening. No reason was found to suspect that these three parental strains may not be homozygous in loci affecting the time of vaginal opening.

No significant difference was observed between reciprocal F1's. A significant difference was observed between a backcross generation to males of a parental strain and that to females of a parental strain. This difference will be discussed later.

Test of adequacy of scale with means of various generations

The adequacy of a scale may be tested by comparing the means of various generations. Such a test reveals, in addition, the presence or absence of dominance or balance of dominance. It will also indicate any non-allelic interaction involved in the inheritance of the character tested. Therefore, taking the values of $h$ in table 2 as the means of various generations, adequacy of the time scale was tested, according to the methods described by Mather (1949).

The results of such tests showed that none of the deviations were significant at the 1% level, but the deviations of B2 and B11 in cross 1, and of B2 in cross 2 were significant at the 5% level. From these facts it may be concluded that the scale used is in general adequate for both cross 1 and cross 2, and that the gene effects are additive on the scale as a whole, but that there seem to be certain complications which tend to lower the means of backcross generations to P2, since in both crosses the first backcross means are lower than expected.

Partitioning the variance

Association between the non-heritable variation and the degree of heterozygosity in various generations

Mather (1949) divided components of variation in quantitative characters into non-heritable and heritable. The non-heritable variation results from the action of environment and is designated as $E$. The heritable variation results from the action of genes controlling these quantitative characters, and is divided into fixable and un-fixable.

Fisher, Immer, and Tedin (1932) developed a method of determining the con-
tribution of each gene to the fixable and unfixable components. Using the method of these authors, the following relations were found:

\[ V_{F_2} = \frac{1}{2}D + \frac{1}{4}H + E \]  

\[ V_{B_1} + V_{B_2} = \frac{1}{2}D + \frac{1}{4}H + 2E \]  

\[ V_{B_{11}} + V_{B_{22}} = \frac{2}{3}D + \frac{2}{3}H + 2E \]  

\[ W_{B_{11}B_{11}} + W_{B_{22}B_{22}} = \frac{1}{2}D + \frac{1}{4}H \]  

where \( D \) denotes the sum of fixable components of variation contributed by all the genes involved, \( H \), the sum of unfixable components of variation also contributed by all the genes involved, and \( W_{B_{11}B_{11}}, \) the covariance between \( B_1 \) and \( B_{11} \). In equations (7) and (8) the non-heritable variation, \( E \), is multiplied by 2, because each of the two generations in these equations contributes \( E \) to the total variation.

If there is no interaction of the non-heritable components and if the non-heritable variation is independent of genotypes, the quantity \( E \) should be the same in all generations within the limit of sampling error. \( E \) can be estimated from the magnitude of variation of parental strains and the \( F_1 \) generations.

The variance of various generations may be computed by (4) with results shown in table 2 for both cross 1 and cross 2. These variances are, of course, free from variances due to seasonal effect. The variances of the three parental strains do not differ from each other beyond the limit of sampling error. However, the variances of parental strains evidently differ significantly from those of \( F_1 \) generations, contrary to expectation. The variances of parental strains are much greater than those of their \( F_1 \) generations in both crosses. These differences are both significant at the \( 1\% \) level. Not only are the variances of the \( F_1 \) generations much smaller than expected, but other subsequent generations also show smaller variances than expected.

To clarify these points, a closer examination of the variances of cross 1 will be made. As shown in equation (6), the variance of \( F_2 \) is \( \frac{1}{2}D + \frac{1}{4}H + E \). It should, therefore, contain the variance due to the non-heritable factors plus variance due to genetic segregation. If the variance of \( P_1 \) or \( P_2 \) or the average variance of \( P_1 \) and \( P_2 \) is taken as an estimate of the non-heritable portion of the \( F_2 \) variance, the variance of \( F_2 \) is evidently too small, since it is smaller than the variance of parental strains. The variances of \( B_1 \) and \( B_2 \), when examined by the use of the equation (7), show that they are smaller than expected if the non-heritable portion of the variances is estimated from \( P_1 \) or \( P_2 \) or both. This is true with the variances of \( B_{11} \) and \( B_{22} \). The examination of variances in cross 2 yields a similar conclusion. This difference in magnitude of the non-heritable variance can be explained, if it is assumed that the increase of heterozygosity of a generation tends to decrease the magnitude of its non-heritable variance.

However, there are two explanations possible. The first is that the degree of heterozygosity of the generation in question is associated with the magnitude of its non-heritable variance. The second is that the degree of heterozygosity of the parent population of a generation in question is associated with its magnitude. There is evidence that both of these factors are responsible, but for the sake of simplicity, it
will be assumed for this moment that the degree of heterozygosity of the generation in question alone is responsible.

If the heterozygosity of \( F_1 \) is taken as unity, the relative heterozygosity of the other generations may be computed as by Wright (1921) as

\[ q_{F_1} = 1, \quad q_{F_2} = \frac{3}{4}, \quad q_{B_1} = \frac{1}{2}, \quad q_{B_2} = \frac{1}{2}, \quad q_{B_{11}} = \frac{1}{2}, \quad q_{B_{22}} = \frac{1}{4} \]  

where \( q_{F_2} \) denotes the heterozygosity of the \( F_2 \) generation, etc.

The heterozygosity in \( F_2 \) is, on the average, \( \frac{1}{2} \) of that of \( F_1 \). A problem arises as to whether this much of a decrease in heterozygosity increases the non-heritable variance of \( F_2 \), as compared to the non-heritable variance of \( F_1 \). Since the variance of \( F_2 \) contains \( D \) and \( H \), it is not clear whether the portion of the non-heritable variance in \( F_2 \) is greater than that of \( F_1 \). A study of variance due to the seasonal effect, however, has thrown some light on this question. As shown in table 2, the magnitude of the seasonal variance is not the same in all types of generations. There is a clear indication that variance due to seasonal effect is also associated with the degree of heterozygosity of a population. But the variance due to seasonal effect in \( F_2 \) in both cross 1 and cross 2 is not greater than that of \( F_1 \). Therefore, it was assumed that the loss of \( \frac{1}{2} \) of the \( F_1 \) heterozygosity does not raise the non-heritable variance in \( F_2 \) in both crosses. Since the amount of heterozygosity in \( B_1 \) and \( B_2 \) is the same, on the average, as in \( F_2 \), the value still being \( \frac{1}{2} \), it was also assumed that the non-heritable variance does not increase in \( B_1 \) and \( B_2 \) either, as compared to that of \( F_1 \).

The heterozygosity in \( B_{11} \) and \( B_{22} \), however, decreases from \( \frac{1}{2} \) in \( F_2 \) to \( \frac{1}{4} \) in these generations. With this decrease of heterozygosity, the variance of \( B_{11} \) and \( B_{22} \) increases to a considerable degree in cross 1. This increase is also seen in the variance due to seasonal effect. Therefore, it was assumed that the critical point of heterozygosity lies between \( \frac{1}{2} \) and \( \frac{1}{4} \) of that of \( F_1 \) in cross 1, and that when the amount of heterozygosity of any generation passes this critical point, the generation begins to show an increase of the non-heritable variance, as compared to that of \( F_1 \). This, however, does not seem to hold true for all types of crosses. In cross 2 the variances of \( B_{11} \) and \( B_{22} \) do not seem to increase to any considerable degree, as compared to the variance of \( F_1 \). The data for these generations in cross 2 were not fitted with the sine curve, because the period in which the data were collected was too short. Therefore, the variances of \( B_{11} \) and \( B_{22} \) may be a little inflated. This may be expected, because, when any two inbred strains of mice are compared, the degree of heterozygosity may certainly not be the same.

From the above considerations, the non-heritable variance was divided into two portions; one which is residual variance, \( E_r \), obtainable in the generation of maximum heterozygosity, and therefore the minimum variance observed in a group of generations of a cross, and the other, \( E_n \), which is due to an absence of heterozygosity, the maximum value of which is obtained in the long inbred strains. After this, the assumptions made about the association between the degree of heterozygosity and the magnitude of the variance were tested as below.

An estimate of \( E_r \) may be obtained from the variance of \( F_1 \). This is 15.683 in cross 1. The estimate of \( E_r \) may be obtained from the average variance of \( P_1 \) and \( P_2 \) after
$E_r$ is subtracted. This value in cross 1 is

$$\frac{1}{2}(62.627 + 46.194) - 15.683 = 38.7275.$$  

Then the components of variances of various generations, and their observed and expected values in cross 1 becomes as shown in table 3. The expected values were found by the methods described by MATHER (1949).

The coefficient of $E_i$ in $V_{B11} + V_{B22}$ was determined as follows. The excess loss of heterozygosity in $B_{11}$ and $B_{22}$, as compared to $F_2$, $B_1$, and $B_2$ is $\frac{1}{2} - \frac{1}{4} = \frac{1}{4}$. If the critical point is assumed to lie at $\frac{1}{2}$ on the scale of heterozygosity, and the increase of $E_i$ is linearly proportional to the loss of heterozygosity after this critical point, $B_{11}$ and $B_{22}$ will each contribute $\frac{1}{2}E_i$ to the total variance, the summation of the two being $\frac{3}{2}E_i$. For a reason, which will be discussed later, this $\frac{1}{2}$ was multiplied by 2, and the resulting figure was taken as a coefficient of $E_i$.

From table 3 it can be seen that the deviations of $V_{D}$ from expectation is nearly zero. The deviations of the other variances and covariances, $E_r$, and $E_i$ are not very large. Therefore, it may be concluded that the assumptions tested were given a rather strong support.

The value of $H$, compared to that of $D$, is quite large, but, since the standard error of $H$ is large, this value of $H$ may not be dependable. However, since the variance of $B_2$ is much smaller than that of $B_1$, and the variance of $B_{zz}$ is also smaller than that of $B_{11}$, a high value of $H$ in $P_2$ seems to exist. The probable reason for the $F_1$ mean being so close to the midpoint, instead of being nearer the $P_2$ mean, will be discussed later.

**TABLE 3**

<table>
<thead>
<tr>
<th>Generation</th>
<th>Components of variance</th>
<th>Cross 1</th>
<th>Cross 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{D}$</td>
<td>$\frac{1}{2}D + \frac{1}{4}H + \frac{1}{2}E_r$</td>
<td>27.9170</td>
<td>27.91766</td>
</tr>
<tr>
<td>$V_{B1} + V_{B2}$</td>
<td>$\frac{1}{4}D + \frac{1}{4}H + \frac{1}{2}E_r$</td>
<td>57.1350</td>
<td>52.63776</td>
</tr>
<tr>
<td>$V_{B11} + V_{B22}$</td>
<td>$\frac{3}{4}D + \frac{3}{4}H + \frac{1}{2}E_r + E_i$</td>
<td>79.1020</td>
<td>82.91674</td>
</tr>
<tr>
<td>$W_{B1/B11} + W_{B2/B22}$</td>
<td>$\frac{1}{4}D + \frac{1}{4}H$</td>
<td>5.9920</td>
<td>10.86630</td>
</tr>
<tr>
<td></td>
<td>$E_r$</td>
<td>15.6830</td>
<td>17.05136</td>
</tr>
<tr>
<td></td>
<td>$E_i$</td>
<td>38.7275</td>
<td>34.91274</td>
</tr>
</tbody>
</table>

| Sum of deviations squared | 74.96101 | 6.85920 |

| Expected value of $D$ | 6.39514 | 8.55742 |
| $s_D$                | 11.838  | 4.728  |
| Expected value of $H$ | 30.67493 | 39.31680 |
| $s_H$                | 15.334  | 6.048  |
Similar tests were made for cross 2. Since in these tests the estimate of \(E_1\) could not be tested, only the estimate of \(E_2\) was tested. The observed values of variances and covariances of various generations are shown in table 3, together with their expected values. The deviations from expectation are again small, rendering further support to the assumptions tested. The value of \(H\) is also high and the variances of backcross generations to \(P_2\) are much smaller than those of backcross generations to \(P_1\). Thus, as in cross 1, the existence of a high value of \(H\) in \(P_2\) is strongly indicated.

Effect of degree of heterozygosity of female parents on the magnitude of the non-heritable variance

It was stated previously that 132 females were produced by a mating system not mentioned so far. They were produced in cross 2 by backcrossing males of \(F_1\), \(B_1\), or \(B_2\) to females of parental strains, in contrast to backcrossing hybrid females to parental males. In order to distinguish these two types of backcross generations, the following designations were used. When \(F_1\) females were backcrossed to parental males, the offspring generation was designated as \(B_1(1(F_1))\) or \(B_2(2(F_1))\), depending on which parental males were used. When \(F_1\) males were backcrossed to parental females, the offspring generation was designated as \(B_1((F_1)1)\) or \(B_2((F_1)2)\). Designations for the second backcross generations are based on the same principle. The data of the backcross generations so far presented are, of course, those of \(B_1(1(F_1))\), \(B_2(2(F_1))\), \(B_11(1(B_1))\), and \(B_22(2(B_2))\), which were simply referred as \(B_1\), \(B_2\), \(B_11\), and \(B_22\).

Since animals to be backcrossed to parental strains were chosen at random, two types of \(B_1\) or \(B_2\) should be identical as to their genetic components within the limit of sampling error. The same will be true with \(B_11\) and \(B_22\). The only difference between two contrasting types of generations in any pair is that female parents of one type are from parental strains while those of the other type are hybrids. Therefore, any difference found between two contrasting types of generations may be attributed

<table>
<thead>
<tr>
<th>Generation</th>
<th>Number of mice</th>
<th>Mean</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(B_1(1(F_1)))</td>
<td>107</td>
<td>26.738</td>
<td>49.462</td>
</tr>
<tr>
<td>(B_1(1(F_1)1))</td>
<td>27†</td>
<td>28.741</td>
<td>57.500</td>
</tr>
<tr>
<td>(B_2(2(F_1)))</td>
<td>74</td>
<td>33.811</td>
<td>17.575</td>
</tr>
<tr>
<td>(B_2(2(F_1)2))</td>
<td>55†</td>
<td>37.127</td>
<td>69.185</td>
</tr>
<tr>
<td>(B_11(1(B_1)))</td>
<td>50</td>
<td>21.340</td>
<td>32.551</td>
</tr>
<tr>
<td>(B_22(2(B_2)))</td>
<td>28†</td>
<td>30.750</td>
<td>120.926</td>
</tr>
<tr>
<td>(B_22(2(B_2)))</td>
<td>26</td>
<td>36.346</td>
<td>22.960</td>
</tr>
</tbody>
</table>

Since the data of the second backcross generations were collected during a short period of time, no curve fitting was made. It was decided, therefore, to use the unadjusted data for the first backcross generations also.

† These are 132 females produced by the mating system not mentioned in the first part of the discussion.
either to cytoplasmic factors or environmental factors which different genotypes of female parents provide for their young.

The means and variances of various backcross generations are shown in table 4. The difference of means between two contrasting types in $B_2$, and $B_{11}$ were found to be significant at the 1% level. The difference in $B_1$ was found to be significant at the 5% level, with only the difference in $B_{22}$ not being significant. The difference of variances between the two contrasting types is also clear in the same table. The difference in $B_2$, $B_{11}$, and $B_{22}$ were all found to be significant at the 1% level, with only the difference in $B_1$ not being significant.

Since the possibility of cytoplasmic factors was eliminated in the tests of reciprocal F₁'s, these differences were interpreted as being due to the fact that different degrees of heterozygosity of female parents provide different environmental conditions, which affect means and variances of the distributions of time of vaginal opening of their offspring. The greater the degree of homozygosity of female parents, the greater the increase of both the means and variances of their offspring.

The previous analysis of variance was made upon an assumption that the degree of heterozygosity of parents has no effect upon the variance of their offspring. This assumption is supportable except in the second backcross generations because of the following reasons.

In $B_1$ and $B_2$ the female parents were F₁ hybrids, since no data of $B_1$ or $B_2$ generation in which female parents were from parental strains was included in the discussion under the assumption. Therefore, no consideration for the absence of heterozygosity of female parents was necessary. However, the situation with the F₁ generation is different since the female parents of this generation are from inbred strains in which the heterozygosity is absent or nearly so. It was assumed, however, that in a generation of such a high degree of heterozygosity as the F₁ in cross 1 and cross 2, the absence of heterozygosity of its female parents does not affect the magnitude of its variance. This assumption was based on the observation of the small seasonal variance of the F₁ generation.

Thus only the second backcross generations, $B_{11}$ and $B_{22}$ present a problem. The female parents of these generations were $B_1$ and $B_2$ hybrids. (The second backcross generations whose female parents were from inbred strains were not included in the discussion under the assumption.) In the analysis of variance in cross 1, it was arbitrarily decided, in the absence of definite evidence, that the decrease of heterozygosity in $B_1$ and $B_2$ females affects the variance of their offspring in $B_{11}$ and $B_{22}$, the heterozygosity of which is, on the average, $\frac{1}{4}$. The magnitude of the effect of decrease of heterozygosity in female parents was assumed to be equal to the magnitude of the effect of decrease of heterozygosity in the $B_{11}$ and $B_{22}$ generations themselves. This is why the coefficient of $E_i$ in $V_{B_{11}} + V_{B_{22}}$ in the test of variance of cross 1 was multiplied by 2.

**DISCUSSION**

The lesser variance of heterozygotes, as compared to the greater variance of homozygotes, may be interpreted as an expression of the better ability of hetero-
zygotes to maintain their own constancy among varying environmental conditions, or of the superior developmental homeostatic functions of heterozygotes compared with those of homozygotes.

DOBZHANSKY and WALLACE (1953) quoted CANNON: "In an open system such as our bodies, compounded of unstable materials and subjected continually to disturbing conditions, homeostasis is in itself evidence that agencies are acting or ready to act, to maintain this constancy." The data of the present report indicate that heterozygosity is one of these agencies.

The homeostatic function of heterozygosity was pointed out by RASMUSSON (1949, 1952), ROBERTSON and REEVE (1952), and DOBZHANSKY and WALLACE (1953). RASMUSSON (1952) stated that heterozygosity increases the resistance of the fly to environmental changes and brings about the greater uniformity of heterozygotes, as compared to homozygotes. DOBZHANSKY and WALLACE (1953) stated that "heterozygotes are more uniformly successful in a variety of environments than are homozygotes; this suggests that the heterozygotes are better able than homozygotes to cope with those different environments and to maintain their internal milieu in functional order."

However, the present data indicate that not only animals themselves but also the developmental environment the female parents provide, which is in turn controlled by genes, play an important role in homeostasis. It appears that not only the lack of heterozygosity in animals themselves leads to a decreased resistance of the animals toward environmental changes but also the lack of heterozygosity in female parents leads to an unfavorable condition for the homeostatic function of their offspring. This unfavorable condition may be overcome to a varying extent if the heterozygosity in the offspring is high enough.

SUMMARY

1. Long inbred strains of house mouse, *Mus musculus*, NB, C57BL/10, and BALB/c were chosen for the study of the inheritance of the time of vaginal opening. During the course of the study it was found that the magnitude of the non-heritable variation of a generation is associated with its degree of heterozygosity.

2. The effect of season on the time of vaginal opening was found to be considerable. In order to remove this effect, a sine-cosine curve (and a sine curve) was fitted to the data.

3. The difference between three parental strains, NB, BALB/c, and C57BL/10 were found to be statistically significant. Therefore, it was concluded that these three strains are genetically different. No difference was found between reciprocal F1's.

4. Tests of additivity of gene effects showed the scale used is adequate as a whole but with some complications.

5. The following assumptions were made, tested and affirmed about the behavior of non-heritable variation. (a) The magnitude of the non-heritable variation of a generation is associated with its degree of heterozygosity and the heterozygosity of its female parents. (b) The magnitude of the non-heritable variation increases as the degree of homozygosity increases. (c) There is a certain quantitative relation as well
as critical points between the magnitude of the non-heritable variation and the degree of heterozygosity.

6. The lesser variation of heterozygotes, as compared to the greater variation of homozygotes, was interpreted as an expression of the better ability of heterozygotes to maintain their own constancy among varying environmental conditions. In other words, the developmental homeostatic functions in heterozygotes are superior, as compared to homozygotes.

LITERATURE CITED


