THE NATURE OF QUANTITATIVE GENETIC VARIATION IN DROSOPHILA. III. MECHANISM OF DOSAGE COMPENSATION FOR SEX-LINKED ABDOMINAL BRISTLE POLYGENES

R. FRANKHAM

School of Biological Sciences, Macquarie University, North Ryde, N.S.W. 2113, Australia

Manuscript received November 3, 1975
Revised copy received May 18, 1976

ABSTRACT

Seventeen lines, each homozygous for a different X chromosome but all with a common autosomal genetic background, were constructed and assayed for abdominal bristle number to determine whether dosage compensation operates for sex-linked genes affecting this character. The regression coefficient of male mean on female mean using a logarithmic scale was 0.90 ± 0.13 and the genetic regression coefficient 0.92, neither differing significantly from unity. The genetic components of variance in males and females were also very similar (0.000234 or 0.000228, respectively). These results indicate that dosage compensation is complete (or nearly so) for sex-linked genes affecting this character. The bristle scores of females did not differ in reciprocal crosses between these lines, thus dosage compensation does not operate by paternal X inactivation. The question of an adequate scale for abdominal bristle number had to be examined during the study. A logarithmic scale appeared to be adequate for both genotypic and environmental differences.

THERE has been considerable confusion in the literature concerning the covariances between relatives and of effectiveness of selection for sex-linked genes (see James 1973). This confusion has arisen from two sources. Firstly, several authors have failed to consider the question of dosage compensation, and secondly, there has been confusion interpreting Bohidar's (1964) paper on the relations between relatives for sex-linked genes. Much of this confusion was clarified by James (1973). The question of response to selection for sex-linked genes was considered in the first paper of this series (Frankham 1976) where it was shown that the response differed markedly depending on the presence or absence of dosage compensation.

The available evidence (see Lucchesi 1973 for review) from visual mutants and structural genes for enzymes indicates that there is complete dosage compensation for a number of sex-linked genes in Drosophila and possibly for all of them. However, Muller and Kaplan (1966) suggested that dosage compensation for sex-linked genes in Drosophila is achieved by the evolution of separate modifiers for different genes. If this were the case it is possible that different sex-linked genes may show different amounts and types of dosage compensation.

1 Present address: Institute of Animal Genetics, Edinburgh EH9 3JN, Scotland.
Conversely, Lucchesi (1973) has suggested the existence of a composite mechanism producing complete dosage compensation for all sex-linked genes in Drosophila. This controversy is not satisfactorily resolved so it is not safe to assume that information on one set of sex-linked genes will apply to all others. The functioning of loci such as bobbed which are found on both the X and Y chromosomes must also be considered as they may affect a quantitative character and influence the response to selection for such characters.

The purpose of this study was to determine whether dosage compensation exists for sex-linked abdominal bristle polygenes and, if so, what form it takes. This is necessary so that appropriate prediction equations can be chosen for work on this character. The approach taken was to construct lines with different homozygous X chromosomes in a common genetic background. On the appropriate scale, the regression of male mean on female mean in these lines is expected to be unity with complete dosage compensation and 0.5 with no dosage compensation. Also, the genetic component of variance among lines in males will equal that in females with complete dosage compensation but will be only half the female value when there is no dosage compensation. An additional experiment was carried out to determine whether the paternal X inactivation form of dosage compensation, as found in kangaroos (Cooper et al. 1975) occurs for sex-linked abdominal bristle polygenes.

There is known to be a scale difference between sexes for abdominal bristle number (Frankham 1968) so it was necessary to determine an adequate scale for this character. This was done by investigating the relation between male and female means in a set of homozygous lines differing widely in abdominal bristle number. The relation between means and within culture standard deviations was also investigated in these lines.

**MATERIALS AND METHODS**

The character studied was the sum of bristle numbers on the fifth and sixth abdominal sternites in females and on the fourth and fifth sternites in males. Culture conditions were similar to those described by Frankham, Jones and Barker (1968) and all lines used were originally derived from the Canberra outbred population described by Latter (1964).

For the scale experiment six of the selection lines of Jones, Frankham and Barker (1968), one line of Hollingdale and Barker (1971) and the Canberra base population were made homozygous by the usual procedure using the following balancer stock (LT):

- In (1) sc81F sc81R+8, sc81 sc8 w* B;
- In (2LR) SMI, al2 Cy cn2 sp2/
- In (2LR) bwV1, ds310 dp87* bwV1;
- In (3LR) UbxL0, UbxL0 p0/

Each resulting line has its cytoplasm and Y chromosome from the LT stock and is homozygous within the limits of the ability of the balancers to prevent the recovery of recombinants (see MacIntyre and Wright 1966) except that the small fourth chromosome was not controlled.

Abdominal bristle number was scored for these lines for another purpose on one, two or three occasions with a maximum of 25 pairs of progeny being scored from each of four bottles on each occasion. Ten pairs of parents were used in each bottle.

The 17 lines used for the dosage compensation experiment were constructed from lines used by Frankham (1976). These consisted of the lines with selection line X chromosomes (with the omission of lines 10(20%)a and 10(20%)c) plus 3 of the lines with X chromosomes from the base population (ICan 35, ICan 76 and ICan 105) and an additional one (ICan 9) from the
homozygous base population line mentioned above. Each of these lines had a common autosomal genetic background (that of ICan 9). The X chromosomes in each of these lines were made homozygous using the procedure described in Figure 1. The FM7 chromosome used in the procedure is a highly efficient balancer (Merriam 1969). Its constitution is:

\[
\text{In}(1) \, \text{sc}^8 + 15D-E; 20 \text{A.E} + dl-49, \, \gamma' \text{sc}^8 \text{uOf}^P \text{t}^B
\]

Nondisjunction appears to be relatively common in FM7 heterozygotes but the procedure used allowed nondisjunctional products to be detected and discarded. No crossover progeny were detected in the construction of any of the stocks. Y chromosomes in these stocks all come from the LT stock and all lines had the same cytoplasm. The fourth chromosome constitution of the stocks was not completely controlled.

For each line, four bottles were set up with 20 pairs of parents, the parents emptied after 3 days and 25 pairs of progeny scored per bottle ten days after setting up.

The experiment to check for paternal X inactivation was done by testing for differences between reciprocal crosses in females. Reciprocal crosses were carried out between ICan 9 and all other lines and between ICan 35 and 13 of the other lines. Two bottles of each reciprocal cross were set up, as above, and 25 females scored per bottle.

**EXPERIMENT I SCALE**

From previous studies (Frankham 1968) it was known that for a single abdominal segment male score was approximately 0.8 female score. Consequently, a logarithmic transformation of the data was the most obvious scale to consider. If this provides an adequate scale for genetic effects (sex) it should result in a regression of male mean on female mean not significantly different from unity. Further, if this scale is adequate for environmental effects it should result in similar standard deviations in the two sexes and a regression of standard deviation on mean for the homozygous lines which does not deviate significantly from zero.

Means and pooled-within-bottle standard deviations for males and females from all lines on the logarithmic scale (base 10) are presented in Table 1. The
Means and within-culture standard deviations (SD) on the logarithmic scale for each sex in the homozygous lines

<table>
<thead>
<tr>
<th>Line</th>
<th>Females n*</th>
<th>Mean</th>
<th>SD</th>
<th>Males n</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICan 9</td>
<td>138</td>
<td>1.603</td>
<td>0.0302</td>
<td>104</td>
<td>1.546</td>
<td>0.0257</td>
</tr>
<tr>
<td>I10(20%)a</td>
<td>100</td>
<td>1.859</td>
<td>0.0233</td>
<td>100</td>
<td>1.774</td>
<td>0.0239</td>
</tr>
<tr>
<td>I10(20%)d</td>
<td>100</td>
<td>1.899</td>
<td>0.0225</td>
<td>100</td>
<td>1.717</td>
<td>0.0284</td>
</tr>
<tr>
<td>I10(40%)b</td>
<td>200</td>
<td>1.749</td>
<td>0.0282</td>
<td>194</td>
<td>1.675</td>
<td>0.0268</td>
</tr>
<tr>
<td>I20(10%)b</td>
<td>300</td>
<td>1.811</td>
<td>0.0291</td>
<td>288</td>
<td>1.750</td>
<td>0.0264</td>
</tr>
<tr>
<td>I20(20%)a</td>
<td>153</td>
<td>1.787</td>
<td>0.0234</td>
<td>148</td>
<td>1.727</td>
<td>0.0241</td>
</tr>
<tr>
<td>I20(40%)b</td>
<td>300</td>
<td>1.774</td>
<td>0.0230</td>
<td>300</td>
<td>1.719</td>
<td>0.0248</td>
</tr>
<tr>
<td>ISO+3</td>
<td>185</td>
<td>1.780</td>
<td>0.0226</td>
<td>178</td>
<td>1.724</td>
<td>0.0247</td>
</tr>
</tbody>
</table>

* Number of observations.

regression coefficient of male mean on female mean of $0.91 \pm 0.07$ does not differ significantly from unity. The averages of within-culture standard deviations for males and females from Table 1 are very similar, being 0.0256 and 0.0253 respectively. The regression coefficients of standard deviation on mean for females, males and both sexes combined were $-0.027 \pm 0.014$, $-0.005 \pm 0.009$ and $-0.014 \pm 0.008$, respectively. None of these three regression coefficients differs significantly from zero. All the above four regression coefficients have a slight downward bias as there is an error variance about the values of the independent variable (SNEDECOR and COCHRAN 1967). No attempt was made to adjust for this due to the unbalanced nature of the data. The adjustment would only improve the agreement with the expectations for adequacy of the logarithmic scale.

Thus, the logarithmic scale meets the above criterion for an adequate scale for both genotypic and environmental effects. The logarithmic scale is used in what follows.

**Experiment II Dosage Compensation**

Mean abdominal bristle number for males is plotted against that for females (both on a log scale) in Figure 2 for each of the lines with homozygous X chromosomes in a common autosomal genetic background. Hierarchical analyses of variance and covariance for these data and the variance and covariance components are presented in Table 2. The components of variance for individuals and bottles were similar in males and females providing a further indication of the adequacy of the logarithmic scale.

Two approaches to evaluating the form of dosage compensation are possible with these data, namely, a comparison of genetic components of variance in males and females and the regression of males on females. The genetic components of variance of 0.000234 and 0.000228 in males and females are close to equality as is expected for complete dosage compensation. The regression of male mean on
female mean was $0.89 \pm 0.12$ and after adjustment for the fact that the independent variable was subject to error (see SNEDECOR and COCHRAN 1967) the coefficient became $0.90 \pm 0.13$. This value does not differ significantly from the value of unity expected for complete dosage compensation but differs significantly from the value of 0.5 expected when there is no dosage compensation. The genetic regression coefficient of 0.92, obtained from the genetic components of variance and covariance also does not differ significantly from unity.

If paternal X inactivation operates one expects a difference between reciprocal crosses in the females with the F, females being similar to their maternal parent in crosses between homozygous lines. There was no significant difference in

| TABLE 2 |
| Analyses of variance and covariance and components of variance and covariance for the dosage compensation experiments |

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>$\eta$ Mean square</th>
<th>Mean cross product</th>
<th>$\delta$ Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lines (L)</td>
<td>16</td>
<td>0.024166</td>
<td>0.021503</td>
<td>0.024693</td>
</tr>
<tr>
<td>Bottles/L</td>
<td>51</td>
<td>0.001356</td>
<td>0.000465</td>
<td>0.001268</td>
</tr>
<tr>
<td>Individuals/B/L</td>
<td>1632</td>
<td>0.000809</td>
<td>0.000833</td>
<td>0.000833</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>$\eta$ Variance components</th>
<th>Covariance components</th>
<th>$\delta$ Variance components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lines</td>
<td>0.000228</td>
<td>0.000210</td>
<td>0.000234</td>
</tr>
<tr>
<td>Bottles</td>
<td>0.000022</td>
<td>0.000019</td>
<td>0.000017</td>
</tr>
<tr>
<td>Individuals</td>
<td>0.000809</td>
<td></td>
<td>0.000833</td>
</tr>
</tbody>
</table>
females between reciprocal crosses for either of the two series of crosses carried out, the means being 1.6245 and 1.6272 for the crosses with ICan 9 as male and as female parents ($t = 1.07$ for a paired $t$ test with 14 d.f.), respectively, and 1.6224 and 1.6237 for the corresponding crosses involving ICan 35 ($t = 0.95$ for a paired $t$ test with 12 d.f.). In addition, the $F_1$ female means for the ICan 9 crosses, which were set up contemporaneously with the parental lines, were intermediate between the female means for the lines used as parents in the crosses. These results conflict with the expectations for paternal X inactivation.

**DISCUSSION**

The results presented above indicate that most, or all, sex-linked abdominal bristle polygenes show complete, or nearly complete, dosage compensation so that the level of their gene products is similar in males and females. This is similar to the results obtained for sex-linked sternopleural bristle polygenes (A. Robertson in Cock 1964), sex-linked wing length polygenes (see Cock 1964) and for the glucose-6-phosphate dehydrogenase, tryptophane pyrrolase, fumarase and 6-phosphogluconate dehydrogenase structural gene loci (see Lucchesi 1973). It now seems likely that all sex-linked genes in Drosophila show complete dosage compensation (with the exception of the alleged sex-determinants which, according to Bridge's theory of sex determination, must be dosage dependent). However, genes found on both the $X$ and $Y$ chromosomes, probably show similar activity per gene dose in both sexes. The results do rule out the possibility that dosage compensation for sex-linked abdominal polygenes is achieved by paternal X inactivation.

The correct relationships between relatives and the equations for prediction of selection response for sex-linked genes in Drosophila and eutherian mammals can be obtained by using the expressions for the case of complete dosage compensation from James (1973) in conjunction with Bohidar (1964) and Griffing (1965). The expressions for birds and Lepidoptera await unequivocal evidence concerning the existence and mechanism of dosage compensation for them. The expressions for the case of paternal $X$ inactivation in kangaroos (and possibly all marsupials) remain to be determined.

This work was made possible by Macquarie University and A.R.G.C. research grants. The technical assistance of Mrs. Anne Hately and Mr. Eric van der Wal is gratefully acknowledged, as are the helpful comments from Dr. D. A. Briscoe, Associate Professors J. S. F. Barker and D. W. Cooper, Drs. I. R. Franklin and J. W. James, Professors B. D. H. Latter and G. B. Sharman and a reviewer.

**LITERATURE CITED**


DOSAGE COMPENSATION FOR POLYGENES


Corresponding editor: R. C. LEWONTIN