GENE DOSAGE RELATIONS FOR HAPLOIDS AND DIPLOIDS OF HABROBRACON

A. M. CLARK AND C. J. MITCHELL

Department of Biological Sciences, University of Delaware

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GENE action has often been studied by investigating gene dosage relations. This has been done by the addition of chromosome fragments or of entire chromosomes to different genetic backgrounds, exemplified by the work of SCHULTZ (1935) for the gene shaven in Drosophila and of BLAKESLEE (1930) in Datura. STERN and HEIDENTHAL (1944) have shown by their work on position alleles in Drosophila that the effect of gene dosage may also be modified by changing the spatial relationships of genic material. In the above studies, interpretations are based to a certain extent upon changed genic balance conditions. To a limited degree the addition of entire chromosome sets has been used to study gene dosage. Here, the conditions of genic balance appear to remain constant.

In Drosophila, comparison of differences in gene quantity can be made normally only for factors that are sex-linked, since the male is simplex for these genes while the female is duplex. It has been shown, in general, that the gene in single quantity in the male produces an effect equal to that of the doubled gene condition found in the female. STERN (1929) and MULLER (1932, 1948) postulate a dosage compensation due principally to naturally selected intra-chromosomal modifiers. MULLER (1948) has presented evidence which indicates that the mechanism of dosage compensation holds for genes located in the X-chromosome of Drosophila melanogaster, but not for genes located in the autosomes since here males and females normally have the same dosage.

It appears that Habrobracon may offer favorable experimental conditions for the study of gene dosage relations since haploids and diploids normally occur within this organism. It would be interesting to determine if under these conditions of ploidy a system of compensating genes has been built up and also whether most of the mutant genes are hypomorphic such as those found in Drosophila. Three sex types exist in Habrobracon; haploid males arising from unfertilized eggs, diploid males and diploid females arising from fertilized eggs. Thus, comparison of mutant genes can be made between individuals of the same sex differing in the number of chromosome sets (haploid males and diploid males), as well as between individuals of different sexes which have the same number of chromosome sets (diploid males and diploid females).

Most of the work on the effects of extra chromosome sets on the morphology and physiology of organisms has been done on plants, where polyploidy

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can be readily induced. Little of this work has been done on animals since it is generally held that viable polyploids are not so readily obtained. For the amphibians, methods for the induction of polyploids have been developed (Fankhauser 1945), but these organisms have not been studied along genetic lines. Since comparison of phenotypic differences between diploids and polyploids are usually made with wild-type individuals, it is not possible to separate the effects due to increased gene dosage from those due to an increase in chromatin mass. In Habrobracon, individuals differing in the number of chromosome sets occur normally. Also, many mutant types have been isolated and bred (Whiting 1932, 1934). These mutant types affect eye color, eye size and shape, wing length, wing venation, antennal flagellar length and width. The antennae are particularly versatile, showing many types of inherited modification. It seems, then, that Habrobracon should be useful not only for genetic studies but also for the study of physiological and cytochemical differences between individuals differing in ploidy.

Organisms in which the number of sets of chromosomes has been changed usually show differences in cell and nuclear size. Dobzhansky (1929) has shown for Drosophila, that a single microchaeta corresponds to an epithelial cell making up the wings. This has also been observed for Habrobracon (Speicher 1935). It is possible, therefore, to obtain measurements of cell size by means of microchaetal counts. Comparison of wing microchaetae in Habrobracon shows that diploid male wing cells are larger than diploid female wing cells, which in turn are larger than the wing cells of the haploid males (Speicher 1935; Risman 1941; Grosch 1945). Diploid males have larger fat cells than haploid males and these are larger than the fat cells of the diploid females (Grosch 1948; Clark and Grosch 1948). Determination of cell and nuclear areas for the gut epithelium of adults (Grosch and Clark 1949) shows that the cell and nuclear size is identical for the diploid males and females but is smaller for the haploid males and that the nuclear-cytoplasmic ratio is the same for all three types. Thus, differences in size relationships hold for different tissues. Diploid males do not occur as often among offspring as might be expected on the basis of a random fertilization and are thought, therefore, to be less viable than the other two classes.

Studies of haploid-diploid relations in Habrobracon have been made primarily to study the important problems of sex determination and evolution (Whiting 1943a, 1945). Whiting (1943b), in his studies on androgenesis noted that homozygous fused diploid males have shorter antennae than their fused haploid brothers. He regards this as being due to the doubled chromosome number. Clark and Mitchell (1948) noted that homozygous stubby diploid males have shorter antennae than the stubby haploid males. These observations indicated that a more detailed investigation of gene dosage relations among haploids and diploids might be fruitful.

MATERIALS AND METHODS

Sex in Habrobracon has been found to be determined by a series of multiple sex alleles, $xa$, $xb$, $xc$, etc. (Whiting 1943a). Haploid males arise from
unfertilized eggs and have any one of these alleles \((xa, xb, xc, \text{ etc.})\). Diploid males and diploid females arise from fertilized eggs. The female is heterozygous \((xa/xb, xa/xc, xb/xc, \text{ etc.})\) while the diploid male is homozygous \((xa/xa, xb/xb, xc/xc, \text{ etc.})\). Accordingly, diploid males are obtained from 2-allele crosses such as \(xa/xb\) by \(xb\), but not from 3-allele crosses such as \(xa/xb\) by \(xc\). Thus, in order to obtain diploid males it was necessary to synthesize 2-allele stocks. Number 25 stock was used as a base stock because a higher incidence of diploid males occurs in it than in the other available inbred stocks (GROSCH 1945).

Since, in the wild-type, haploid males cannot readily be distinguished from their diploid brothers, a recessive marker gene was used. Thus female homozygous for the recessive marker gene cantaloup (eye color) were mated to black-eyed haploid males. From this cross the unfertilized eggs result in cantaloup-eyed haploid males while the fertilized eggs give rise to black-eyed diploid offspring (male and female) which are heterozygous for eye color.

The breeding procedure was as follows: A haploid male carrying the mutant to be tested was crossed to a cantaloup-eyed female from stock 25. An \(F_1\) virgin female (heterozygous for the introduced mutant and the eye color) was selected and allowed to lay eggs. From these eggs, \(F_2\) haploid males were obtained. A cantaloup-eyed mutant male was crossed to his mother. From this cross a 2-allele stock was established which contained the mutant to be studied as well as the marker gene. All subsequent offspring were derived from this cross.

The breeding procedure used in the derivation of stocks does not insure that these stocks are homozygous for the residual genotype. However, all of the mutants were bred into the same base stock (No. 25c) and all of the progeny used in each of the final crosses were derived from a single mother to son mating. The nature of such crosses, in which diploid and haploid individuals are obtained, insure that the different classes of animals are genetically equivalent in modifiers.

In some of the mutants studied, comparisons were made on heterozygous as well as homozygous individuals. For other mutants, only homozygotes were studied.

For comparison of heterozygotes, six classes of offspring were obtained. For example: stubby \((sb)\)

\[
\begin{align*}
\text{haploids from} & \quad \text{unfertilized eggs} \\
\text{diploids from} & \quad \text{fertilized eggs}
\end{align*}
\]
For comparison of homozygotes, three classes of offspring were obtained. For example: cut (ct)

\[
\begin{array}{c|c|c}
\text{c} & \text{ct} & \chi \\
\hline
\text{c} & \text{ct} & \chi \\
\end{array}
\]

Females were placed in shell vials with Ephesia larvae which they parasitize and upon which they lay eggs. Transfer of females to vials with fresh Ephesia was made every four days. All wasps were reared at 30°C.

Individuals of similar body size were preserved in an alcohol-glycerine mixture. The structures to be studied were subsequently removed, washed in xylol and mounted on slides with balsam. Measurements were made directly from slides by means of an ocular micrometer or from camera lucida drawings.

**THE WILD-TYPE PHENOTYPE OF HAPLOIDS AND DIPLOIDS**

Haploids and diploids of the wild phenotype were compared in order to determine similarities and differences among them and the magnitude of these differences. This information was then used to evaluate the extent of the differences between haploid and diploid mutant types, and thereby determine whether the diploid mutants show a difference in phenotype.

Stock No. 25c females were crossed to No. 25+ males. Three classes of offspring were obtained: haploid males with cantaloup-eye color, diploid males with black eye-color, and diploid females with black eye-color. Fifty offspring of similar body lengths (2.65–2.75 mm) were selected from each class. The right primary wings, right secondary wings, and right antennae were removed and mounted.

Habrobracon females normally have shorter antennae and fewer antennal segments than do males (fig. 1). This represents a secondary sex character in the wild-type state. Although haploid males and diploid males are similar in appearance, statistical differences do exist. GROSCH (1945), in a study of structural differences among haploids and diploids in wild-type stocks, showed that the antennal flagella length is the same for haploid and diploid males, but that the haploids had more flagellar segments and, therefore, shorter segments. He also showed that the haploid males have longer flagellar segments than females. The data reported here (table 1) are in accord with these observations: The measurements for segment length were obtained from the seventh segment of the males and the fifth segment of the females. These were selected because they were middle segments of the flagellum,
TABLE 1

Mean measurements* of wild-type offspring from 25c ♀ × 25 + ♂.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>2♂ ± ♂</th>
<th>2♀ ++ ♀</th>
<th>2♂ ++ ♀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary wing length (mm)</td>
<td>2.262 ± 0.012</td>
<td>2.298 ± 0.012</td>
<td>2.395 ± 0.013</td>
</tr>
<tr>
<td>Secondary wing length (mm)</td>
<td>1.958 ± 0.012</td>
<td>1.960 ± 0.013</td>
<td>2.051 ± 0.007</td>
</tr>
<tr>
<td>Secondary wing area (mm²)</td>
<td>0.772 ± 0.008</td>
<td>0.765 ± 0.008</td>
<td>0.825 ± 0.010</td>
</tr>
<tr>
<td>Number of microchaetae per 0.01 mm² of wing surface in radial cell</td>
<td>15.36 ± 0.16</td>
<td>10.41 ± 0.12</td>
<td>13.73 ± 0.14</td>
</tr>
<tr>
<td>Antennal flagellar length (mm)</td>
<td>1.8634 ± 0.0149</td>
<td>1.8359 ± 0.0120</td>
<td>1.1080 ± 0.0081</td>
</tr>
<tr>
<td>Segment lengtha (mm)</td>
<td>0.0988 ± 0.0009</td>
<td>0.1026 ± 0.0008</td>
<td>0.0902 ± 0.0007</td>
</tr>
<tr>
<td>Segment widtha (mm)</td>
<td>0.0722 ± 0.0004</td>
<td>0.0641 ± 0.0008</td>
<td>0.0765 ± 0.0004</td>
</tr>
</tbody>
</table>

*Measurements are based upon 50 individuals per group.

aMeasurements made of the 7th segment for the males and of the 5th segment for the females.

not in the tapered part of the normal antennae. Comparison of segment width among these three types shows that the females (0.0765±0.0004 mm) are wider than the haploid males (0.0722±0.0004 mm), and the haploid males are wider than the diploid males (0.0641±0.0008 mm). Correlation of segment width with segment length shows an inverse relationship. The diploid males have longer and thinner segments while the females have shorter and thicker segments. On this basis it is assumed that each segment has the same volume and that an increase in length is compensated for by a decrease in width.

Comparison of primary wing lengths (table 1) for the diploid males (2.298 ± 0.012 mm) and the haploid males (2.262 ± 0.012 mm) shows that the difference is on the borderline of statistical significance. The primary wing length for the females (2.395 ± 0.013 mm) is significantly greater than the other two groups. Differences in secondary wing length between haploid males (1.958 ± 0.013 mm) and diploid males (1.960 ± 0.013 mm) are non-significant, but both of these groups have wings that are significantly shorter than the wings of the females (2.051 ± 0.007 mm). The females, then, have longer primary and secondary wings than the males. Measurements of wing areas for the secondary wings show that the females (0.825 ± 0.010 mm²) are significantly greater than the diploid males (0.765 ± 0.008 mm²) and the haploid males (0.772 ± 0.008 mm²), with the latter two groups being statistically similar. The method used in estimating statistical significance was the standard error of the difference.

Microchaetal counts in the radial cell of the primary wing show that the average wing cell size for these groups decreases in the order of diploid male, diploid female, and haploid males (table 1). These observations are in accord with the findings of Speicher (1934) and Grosch (1945).

The foregoing shows that there is an overlapping of phenotype between the wild-type haploid and the diploid males with no extreme structural differ-
ences. Therefore, the doubled chromosomal complement found in the diploid male produced no marked qualitative or quantitative differences in gross structure.

**THE MUTANT PHENOTYPES OF HAPLOIDS AND DIPLOIDS**

*Stubby (sb), Fused (fu), Coalescent (co)*

The mutants, stubby, fused and coalescent, affect antennal flagellar length. In all of these mutant types the flagella are shorter than the wild type. This shortening is accompanied by varying degrees of fusion of the segments, apparently due to the inability of the antennae to differentiate properly into flagellar segments. Since antennal flagellar length is a secondary sex character, the mutant type females have shorter flagella than their corresponding mutant type males (Plate 1).

*Fused*—Fusion of flagellar segments is so complete that the flagellum appears unsegmented. There is also a fusion of the segments of the tarsus into a single segment, and the maxillary palpi and the labial palpi are each fused into single segments.

*Stubby*—Varying degrees of fusion and aberrant articulation of flagellar segments occur. These are never as extreme as in fused. There is no effect upon the palpi and the tarsi.

*Coalescent*—Varying degrees of fusion and aberrant articulation of flagellar segments occur. There is an overlapping with the stubby phenotype. Segments making up the palpi show aberrant articulation. The normally occurring 5-jointed tarsus is not found for many coalescent individuals; tarsal segments may be split transversally into two, thereby giving the appearance of additional segments.

Comparison of antennal flagellar lengths between the haploid and diploid males for each mutant type (stubby, fused and coalescent) shows that the diploid male flagellum is shorter (Plate 1). However, there is some overlapping since a few of the diploid male antennae are as long as the mutant haploid male antennae, while others are as short as the shortest female antennae.

For the mutant type stubby, sufficient diploid males were obtained for statistical treatment (table 2). The haploid stubby males have a mean flagellar length of 0.8562 ± 0.0164 mm as compared with 0.5399 ± 0.0174 mm for

<table>
<thead>
<tr>
<th>TABLE 2</th>
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</thead>
<tbody>
<tr>
<td><strong>Mean antennal flagellar length (mm) of offspring from</strong></td>
</tr>
<tr>
<td>$\frac{c+}{c sb}$ $\varphi \times \frac{+}{+ sb}$ $\delta$.</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>Number of flagella measured</strong></td>
</tr>
<tr>
<td><strong>Antennal flagellar length</strong></td>
</tr>
</tbody>
</table>
the homozygous diploid stubby males. The difference between these means is significant. The shorter antennal flagella found for the homozygous diploid, when compared with the haploid males, indicates greater gene dosage effects for the diploid males.

Comparison of the homozygous diploid stubby male flagella (0.5399 ± 0.0174 mm) with those of the homozygous diploid stubby females (0.4394 ± 0.0071 mm) shows that while the males approach the females in antennal flagellar length, the difference between the means is significant. However, since the females normally have shorter antennae than the males, direct comparisons of flagellar length for males and females may not suffice for an accurate determination of quantitative genic differences.

There is a difference in expressivity between diploid males and females heterozygous for stubby. Examination of the segments making up the flagellum of the antenna shows a certain amount of aberrant articulation for some of these heterozygous individuals. For the heterozygous diploid males, 24 out of 72 showed aberrant articulation while for the heterozygous females only 3 out of 70 show this trait. For the females this aberrant articulation was restricted to the distal segments while in the males it was found also for middle segments. The expressivity was also greater for the diploid males than the females. Essentially the same condition is found when comparing males and females heterozygous for the mutant type fused. Thus, examination of heterozygotes shows that stubby and fused are semi-dominant in the diploid male state, but are masked by the wild-type allele in the diploid female state.

In the preceding section the data indicate that the mutants stubby, fused and coalescent show marked differences in phenotype between the haploid and diploid males. For other mutants reported below, obvious differences were not found. In the study of some of these mutants it was discovered that certain of the characters do not lend themselves to quantitative analysis or are influenced by sex or male diploidy.

**Thin (th)**

This is an antennal mutant in which the flagellar segments are thinner than wild-type. Comparison between wild-type and thin phenotypes of haploid males, diploid males, and diploid females show that thin individuals have shorter flagella (table 3). The mean segment length is also shorter. The effect of male diploidy upon segment length is similar here as in wild-type, in that the diploid males have longer segments than the haploid males or females. There is no effect upon segment number. Comparison of segment widths between $c^{th}$ males and $c^{th}+th$ males shows that the homozygous diploid males have thinner antennae. However, this difference in structural dimension between these groups, although statistically significant, is not sufficiently great to be explained in terms of enhanced gene dosage. It seems better to attribute this difference to the effects of male diploidy, since differences of a similar magnitude are found in the wild-type.
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TABLE 3
Antennal flagellar measurements of offspring from $^{c + \varphi}_{c + lb}$ $\times ^{l + I}_{tb} \delta$.

<table>
<thead>
<tr>
<th></th>
<th>$c + \varphi$</th>
<th>$c + lb$</th>
<th>$c + \varphi + lb$</th>
<th>$c + lb + I$</th>
<th>$c + \varphi + lb + I$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number measured</td>
<td>62</td>
<td>71</td>
<td>52</td>
<td>38</td>
<td>49</td>
</tr>
<tr>
<td>Flagellar length</td>
<td>1.824 ± .012</td>
<td>1.621 ± .014</td>
<td>1.789 ± .024</td>
<td>1.552 ± .023</td>
<td>1.040 ± .010</td>
</tr>
<tr>
<td>(mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number flagellar</td>
<td>19.45 ± .09</td>
<td>19.35 ± .09</td>
<td>17.54 ± .14</td>
<td>17.39 ± .18</td>
<td>12.33 ± .07</td>
</tr>
<tr>
<td>segments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Segment length</td>
<td>.0877 ± .0009</td>
<td>.0777 ± .0012</td>
<td>.0992 ± .0007</td>
<td>.0899 ± .0012</td>
<td>.0865 ± .0006</td>
</tr>
<tr>
<td>(mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Segment width</td>
<td>.0736 ± .0004</td>
<td>.0495 ± .0005</td>
<td>.0664 ± .0005</td>
<td>.0445 ± .0008</td>
<td>.0763 ± .0005</td>
</tr>
<tr>
<td>(mm)</td>
<td></td>
<td></td>
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</table>

**Long (l)**

This is an antennal mutant in which each flagellar segment is longer than the wild-type, thereby resulting in longer flagella. Comparison between wild-type and long phenotypes of haploid males, of diploid males and of diploid females shows that the long mutant types have longer antennae than the corresponding wild type (table 4). In each case the mean segment length is also longer. There is no apparent effect upon segment number. Comparison of mean segment length shows that the homozygous long diploid males exceed dimensions for the long haploid males, and the heterozygous long diploid males exceed dimensions for the wild-type haploid males. These differences are not sufficiently large to attribute them to enhanced gene dosage. The increased segment length, therefore, seems to be due to the effects of male diploidy.

For the antennal mutants thin and long, statistically significant differences between diploid males and haploid males were obtained. However, these differences were of the same order of magnitude as those found between the wild-type haploid and diploid males. These differences, therefore, seem better

**TABLE 4**
Antennal flagellar measurements of offspring from $^{c + \varphi}_{c + l}$ $\times ^{l + I}_{l}$ $\delta$.

<table>
<thead>
<tr>
<th></th>
<th>$c + \varphi$</th>
<th>$c + l$</th>
<th>$c + \varphi + l$</th>
<th>$c + l + I$</th>
<th>$c + \varphi + l + I$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number measured</td>
<td>26</td>
<td>24</td>
<td>24</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Flagellar length</td>
<td>1.9699 ± .0431</td>
<td>2.3759 ± .0114</td>
<td>1.9112 ± .0164</td>
<td>2.1053 ± .0164</td>
<td>1.2681 ± .0300</td>
</tr>
<tr>
<td>(mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number flagellar</td>
<td>19.69 ± .18</td>
<td>19.79 ± .18</td>
<td>17.07 ± .16</td>
<td>16.91 ± .20</td>
<td>12.60 ± .16</td>
</tr>
<tr>
<td>segments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Segment length</td>
<td>.1000 ± .0011</td>
<td>.1202 ± .0009</td>
<td>.1113 ± .0016</td>
<td>.1244 ± .0015</td>
<td>.1006 ± .0009</td>
</tr>
<tr>
<td>(mm)</td>
<td></td>
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explained as due to the effects of male diploidy rather than to enhanced dosage of these genes in the diploid state.

**Kidney-Heidenthal (K^h)**

This phenotype is distinguished from wild-type by the presence of a smaller head. The compound eyes are also smaller and sometimes do not appear. Examination of K^h haploids and diploids failed to reveal any noticeable difference in phenotype expression.

**Bulge (Bu)**

The mutant type bulge is recognizable by the marked transverse protrusion of the eyes. Examination of bulge haploid males, homozygous bulge diploid males, and homozygous bulge diploid females did not reveal noticeable differences among these classes.

**Small-wings (sw)**

This phenotype is characterized grossly by the presence of shorter primary and secondary wings. Lengths of primary and secondary wings were determined for \( c^+ \) males, \( c \, sw \) males, \( c^+ \, sw \) males, \( c \, sw^+ \) males, and \( c^+ \, sw^+ \) females, and \( c^+ \, sw^+ \) females obtained from the cross \( C^c \, sw^c \, sw^+ \) females \( \times + \, sw \) males. Differences in wing length between the haploid and diploid mutants were not sufficiently great to be interpreted as clear cut dosage differences. Homozygous diploid male wings were longer than were the small wing haploid males, but this condition also exists in the wild-type.

**Reduced (r)**

The reduced mutant type shows shorter primary and secondary wings than the wild-type and also the absence of the medio-cubital crossvein and a certain amount of vein disorganization. Comparison of haploid males and diploid males showed no apparent difference in phenotype, either in wing length or vein disorganization.

**Cut (ct)**

The mutant cut affects primary and secondary wings. For the primary wing the margin from the stigma to the apex is straightened instead of being rounded as is normally found for the wild-type. The secondary wings show varying degrees of scalloping recognizable by a decrease in wing area size and the absence of bristles at the margin of scalloped regions. Cut is variable in expression in both the secondary and primary wings. There is, however, a certain degree of correlation between the amount of scalloping in the secondary wing and wing area.

Comparison of mean areas for the secondary wings shows that the diploid male wings are significantly smaller than those of the haploid males or diploid females (table 5). Since differences in secondary wing areas for the wild-type
haploid and diploid males are non-significant, it appears that in cut diploid males the doubled gene content enhances the dosage effect upon the secondary wing. Cut diploid females do not differ from cut haploid males with respect to secondary wing areas. However, wild-type females have a larger secondary wing area than do wild-type haploid males. Thus, the phenotype cut is more strongly expressed in the female than in the haploid male. These data do not indicate whether or not a difference in gene dosage between diploid males and diploid females exists.

**DISCUSSION**

Studies of gene dosage relations in organisms differing in the number of chromosome sets seem to offer opportunity for the analysis of gene action. Such studies differ from work in which entire chromosomes or chromosome fragments are added to the genetic complex in that the addition of an entire genome would not be expected to alter the conditions of genic balance. Comparison of diploids and polyploids has shown that quantitative differences exist, however, in some cases, qualitative differences have also been shown. In these studies differences in phenotype are usually attributed to the larger cell and nuclear size in polyploids. It is difficult to determine how much of the developmental process can be credited to the changed physical relationships due to the increase in the mass of chromatin material, and how much to the changed chemical relationships due to a differential action of individual genes in the polyploid state.

In the present study, an attempt is made partially to separate the effects of individual genes from an increase in chromatin mass by comparing haploids and diploids in the wild-type state and by the substitution of mutants into these systems. It is never possible to effect completely such a separation since the mutant genes in the diploid are operating in a different genetic system than those in the haploid.

A comparison of wild-type haploid and diploid males shows that there are no distinctive structural differences which can be used to separate them. Differences do exist, but these are of a statistical nature. As a group, diploid males have larger wing cells and fewer, longer, and thinner antennal flagellar segments than haploid males. Thus, an increase from one to two sets of chromosomes results in no striking phenotypic differences. It would appear that haploid and diploid mutant types would contain the same mass of chro-
matin as their comparable wild-types and would differ only in a single gene locus. Therefore, marked phenotypic differences above those shown in the wild-type could be attributed to a change at a single gene locus.

Dosage relations for ten mutant types are reported, involving antennal, head or wing structure. Study of these mutants indicates that no generalization can be made for doubled gene dosages in Habrobracon, since some show increased phenotypic effect while others do not. These data are in accord with the views of Goldschmidt (1938) who, in considering dosage effects due to the addition of entire chromosome sets, states "The threshold for the maximum effect of gene doses may be different for different genes; therefore, at higher dosage some may increase their effect proportionately, others not (when their threshold is reached)." Thus, the conditions of genic balance would not appear to hold for some of these genes. The homozygous stubby, fused and coalescent diploid males have markedly shorter antennae than their corresponding mutant haploid brothers. This indicates that for these mutants the doubled gene condition results in an enhanced effect upon phenotype. In both the haploid and diploid males the ratio of gene to background is identical, since a doubling of any particular gene is accompanied by a doubling of all the genes in the system. According to the theory of genic balance a resulting phenotype is effected by plus and minus modifiers present throughout all of the chromosomes. These conditions for genic balance do not appear to hold for the mutants stubby, fused and coalescent of Habrobracon.

For the ten mutant types reported here, the most marked differences were obtained for those mutants having a similar phenotype. The mutant types stubby, fused and coalescent have shorter antennae and show fusion of antennal segments. This expression is increased in the diploid male. This indicates that the doubled gene dosage has no meaning in itself but only in so far as these genes are able to act during particular stages of development. Certain developmental processes may be considered more susceptible to doubled gene quantity than others. Goldschmidt (1938), in attempting to explain phenotypic differences between males and females comparable in genetic constitution for the specified gene loci, has postulated that these genes are operating in different genetic systems. It seems that such an explanation could be used here.

In Habrobracon, haploid males, diploid males and diploid females may differ during certain periods of development and not during others. Genes operating during the developmental periods which differ among these three groups would act differentially, while genes acting during developmental periods which did not differ would show no such differential effect. Pertinent to this is the observation (unpublished) that irradiation of diploid male larvae in cocoons, at a period 72-84 hours after oviposition, results in a shortening of antennae while similar treatment of comparable haploid males and diploid females does not. There is apparently a difference in the sensitive period among these groups. There is also an indication of changed dosage relationships when comparing males and females heterozygous for stubby or fused.
Almost all of the heterozygous females are of wild-type phenotype, while about 30 percent of the heterozygous males show a certain amount of antennal fusion. Thus, the mutants stubby and fused are masked by their wild-type alleles in the female, but are semi-dominant in the male.

**Summary**

1. Gene dosage effects for haploid males, diploid males, and diploid females were investigated in the parasitic wasp Habrobracon. Determinations were made of phenotypic differences for the wild-type and for ten mutant types. An attempt is made to separate partially the effect of enhanced gene dosage from increased chromatin mass upon the phenotype.

2. Stubby, fused and coalescent, mutant types which are recognizable by antennal shortening and aberrant articulation of antennal segments, are more extreme in the diploid males than in the haploid males. Thus, the conditions of genic balance do not seem to hold for these mutants.

3. The heterozygous stubby and heterozygous fused diploid males show a certain degree of antennal fusion (about 30 percent penetrance). Few heterozygous stubby or fused females show this fusion. These genes, then, are masked by their wild-type alleles in the female state, but are semi-dominant in the diploid male state.

4. The mutant type cut shows greater effect upon scalloping of the secondary wing in the diploid male and diploid female than in the haploid male.

5. The mutant types long, thin, kidney-Heidenthal, bulge, small wing, and reduced show no marked phenotypic differences between haploid males, diploid males, and diploid females. These genes in doubled dose seem to have no greater effect upon phenotype than when present in single dose.

6. The data show that some genes in doubled quantity have an enhanced effect while others do not. Of the ten mutants studied, the three showing the most marked effects were those affecting the same structure in the same way.

**Plate 1**

Drawings of antennae for wild-type, stubby, fused, and coalescent in haploid males, diploid males, and diploid females.

- Figure 1.—Wild-type haploid male.
- Figure 2.—Wild-type diploid male.
- Figure 3.—Wild-type diploid female.
- Figures 4-5.—Stubby haploid male.
- Figures 6-8.—Homozygous stubby diploid male.
- Figures 9-10.—Homozygous stubby diploid female.
- Figure 11.—Heterozygous stubby diploid male.
- Figures 12-13.—Coalescent haploid male.
- Figures 14-15.—Homozygous coalescent diploid female.
- Figures 16-17.—Homozygous coalescent diploid male.
- Figures 18-20.—Fused haploid male.
- Figures 21-23.—Homozygous fused diploid male.
- Figures 24-25.—Homozygous fused diploid female.
DOSAGE RELATIONS IN HABROBRACON
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