DOMINANT MATERNAL-EFFECT MUTATIONS OF DROSOPHILA MELANOGASTER CAUSING THE PRODUCTION OF DOUBLE-ABDOMEN EMBRYOS

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ABSTRACT

Dominant mutations at two loci, BicaudalC (BicC) and BicaudalD (BicD), cause heterozygous females to produce double-abdomen embryos. These mutations cause the production of embryos with a range of defects extending from the anterior end of the differentiated embryo. The same array of defective embryos is caused by mutations at either locus and is similar to that produced by the original mutation at bicaudal (bic). The array of defective embryos suggests that these mutations cause the loss of positional values from the anterior end of the embryo, associated with a duplication of the posterior end if too few positional values remain. BicaudalD mutations appear to be antimorphic, gain-of-function mutations, whereas BicaudalC mutations are likely to be hypomorphic or amorphic mutations. Mutations at all these loci (bic, BicC and BicD) act as mutual enhancers of each other, and a number of other maternal-effect mutations also act to either enhance or suppress the expression of these dominant bicaudal mutations.

ONE of the most intriguing questions of developmental biology is how the pattern of the embryo is established. A number of zygotic mutations of Drosophila melanogaster have recently been isolated which disrupt the embryonic cuticle pattern (e.g., NÜSSLIN-VOLHARD, WIESCHAUSS and KLUDING 1984). However, none of these mutations affect the organization along the entire length of the anterior-posterior axis of the developing embryo. Instead, the effects of these mutations are limited to portions of the embryo or consist of small regional repeated defects along the length of the animal. It seems likely that the organization of the primary axes of the embryo is controlled by maternally expressed genes.

Of the recessive maternal-effect mutations of D. melanogaster that affect the organization of the entire embryo, the most dramatic of these act on the two perpendicular axes of the Drosophila embryo: dorsal (dl) and other dorsal-like mutations affect the dorsal-ventral axis of the embryo by expanding dorsal pattern elements at the expense of ventral pattern elements (ANDERSON and

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NUSSLEIN-VOLHARD 1984), bicaudal (bic) affects the anterior-posterior axis of the embryo (Bull 1966). The bic mutation causes a variety of defects among the progeny of homozygous females: in addition to normally developed embryos, some embryos develop without structures derived from the anterior end of the embryo; other embryos develop as double-abdomen embryos, with two posterior ends arranged in mirror-image symmetry. This mutation maps on the second chromosome to map position 67 near the vg locus and is believed to represent a hypomorphic allele of a gene within deficiencies Df(2R)vgB and Df(2R)vgP because of the enhancing effect of those deficiencies on the expression of the original bic mutation (NUSSLEIN-VOLHARD 1977a).

The development of screening methods whereby mutations can be detected by their effect on embryonic structure, rather than adult phenotype (NUSSLEIN-VOLHARD 1977b), made possible the ability to readily isolate incompletely penetrant maternal-effect mutations such as bic (in addition to the zygotic mutations described above). In this way, a number of dominant bicaudal mutations were isolated that cause females to produce variable numbers of double-abdomen embryos. This report characterizes a number of mutations at two loci, BicaudalC (BicC) and BicaudalD (BicD), in addition to a polygenic line YC67. We compare the effects of these mutations to those of the original recessive bic mutation and analyze the interaction of these mutations with each other and with other maternal-effect mutations.

MATERIALS AND METHODS

Genetics strains used: The dominant bicaudal mutations used in this study were obtained from a number of sources following mutagenesis with EMS (ethyl methanesulfonate, LEWIS and BACHER 1968). BicC796 was isolated in a screen designed to recover new alleles of the original bic mutation (C. NUSSLEIN-VOLHARD, personal communication). Mutations Bic7T54, BicFIF43, BicC793, BicC77, YC67 and E(2)Bic were recovered during screens for embryonic zygotic lethal mutations (NUSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984; WIESCHAUS, NUSSLEIN-VOLHARD and JURGENS 1984). Mutations BicC7F65 and BicC7R55 were isolated by their recessive female sterile phenotype by T. SCHUPBACH (personal communication).

Chromosomal aberrations used in association with the BicC locus were characterized by ASHBURNER et al. (1983) and by SIMPSON (1983). The cytology of the deficiencies and duplications are as follows: Df(2L)75c = Df(2L)35A1-2;35D4-7, Df(2L)H20 = Df(2L)36A1-2;36E1-2, Df(2L)osp29 = Df(2L)35B3;35E6, Df(2L)A446 = Df(2L)35B1;35F1-2 and Dp(2;3)osp3 = Dp(2;3)35B3-4;36C11. Deficiency Df(2L)TW119, used in association with studies on the BicD locus, is cytologically not detectable; however, Df(2L)TW119 fails to complement the dl (dorsal) locus that is associated with rearrangement breakpoints in 36C9-11 (STEWARD, MCNALLY and SCHEDL 1984). Deficiencies and mutations of the vg region were obtained from P. LASKO; the cytology of these deficiencies is unknown.

Female sterile mutations used in identifying dominant modifiers of bicaudal expression were obtained from T. SCHUPBACH. All were second chromosomal mutations induced by EMS on cn bw sp chromosomes and maintained heterozygous with a CyO balancer carrying a dominant temperature-sensitive mutation (DTS513). All other genetic strains are described by LINDSLEY and GRELL (1968).

Culture conditions: Flies were raised on a standard cornmeal-agar medium at either 18, 25 or 29°, depending on the experiment. For determination of the distribution of the frequency of different types of embryos produced by different dominant bicaudal
genotypes, females were raised in half-pint milk bottles at 25° and were allowed to lay eggs on 35 mm yeasted apple-juice agar plates. Between 400 and 1000 embryos were allowed to develop for 24-48 hr, were dechorionated with 5% w/v sodium hypochlorite and were mounted in Hoyer's medium (NÜSSEIN-VOLHARD, WIESCHAU and KLUDING 1984).

For the determination of the frequency of double-abdomen embryos from double heterozygotes, females were raised at 18, 25 or 29° in 25 × 100 mm shell vials and were allowed to lay eggs in blocks on yeasted apple-juice agar plates (NÜSSEIN-VOLHARD 1977b). Between 100 and 300 embryos were allowed to develop to term, and the unhatched embryos were dechorionated with 5% sodium hypochlorite and were mounted in Hoyer's medium. The frequency of double-abdomen embryos was computed as the ratio of the number of embryos showing a reversal in polarity in the anterior-posterior axis to the total number of developed embryos. (In general, more double-abdomen embryos are produced by females raised in vial culture than by those raised in bottle culture; compare the compiled data from the figures with the analogous data from the tables.)

Revertant mutagenesis: Males heterozygous for a pr+ BicD mutation were either irradiated with 4000R from a 147Cs gamma-radiation source or were fed 25 mM EMS for 24 hr according to the procedure of LEWIS and BACHER (1968). Mutagenized males were mated to b pr cn sea females. F1 females heterozygous for the mutagenized BicD chromosome were mated to DTS91 pr cn sea/CyO males and were set up individually in blocks on apple-juice agar plates. Females which produced no double-abdomen embryos were retrieved from the block and were placed in individual vials at 25°. The BicD region of the mutagenized chromosome was recovered in the following generation using the closely linked pr+ marker and, subsequently, was retested heterozygous with Df(2L)TW119 to confirm the revertant phenotype. Approximately 5000 irradiated BicD71\(^{M4}\) chromosomes were analyzed in Heidelberg by one of us in association with C. NÜSSEIN-VOLHARD; revertants R26 and H68 resulted from this mutagenesis. Another 5000 irradiated BicD71\(^{M4}\) chromosomes, 5000 irradiated BicD\(^{HE48}\) and 3000 EMS-treated BicD\(^{HE48}\) chromosomes were analyzed in Princeton; two revertants of BicD\(^{HE48}\), R44 and M18 resulted from this mutagenesis.

RESULTS

Classes of bicaudal embryos: All mutations of both the BicaudalC (BicC) and BicaudalD (BicD) loci are incompletely penetrant, dominant maternal-effect mutations. Progeny embryos from mothers heterozygous for a BicC or BicD mutation or heterozygous for the YC67 polygenic line exhibit a wide range of embryonic defects, ranging from wild-type embryos to embryos which develop symmetrically. As shown in Figure 1, these defective embryos can be divided into a number of classes similar to those described by NÜSSEIN-VOLHARD (1977a) for the original bicaudal mutation (bic).

The unhatched normal class of defective embryos, of which a representative is shown in Figure 1d, appears to have a normal internal head skeleton (Figure 1a), at the end of embryogenesis, as well as an essentially wild-type external cuticular pattern with no polarity reversals. Some of the embryos have gaps or other pattern defects in cuticle structure (although the defects rarely extend more than a single segment), and many embryos appear perfectly normal, but simply fail to hatch.

The mouthparts-reduced class, shown in Figure 1e, have an essentially wild-type external cuticular pattern, but are lacking much of the elaborate head skeleton (Figure 1b). In these animals the external scleretonized structures of
FIGURE 1.—Phenotypic classes of embryos produced by females mutant for a bicaudal mutation (Bic"/CyO). a–b, Mouthparts and head skeleton of an unhatched normal embryo (a) and of a mouthparts-reduced embryo (b) (X150); c, detail of anterior region of the mixed embryo in (g) (X400); d–i, whole mount cuticle preparations of unhatched normal (d), mouthparts reduced (e), headless (f), mixed (g), asymmetric (h) and symmetric S2.5 (i) embryos (X90).
the mouthparts are present (such as the mouthhooks), whereas the internal structures (such as the labrum and cephalo-pharyngeal structure) are reduced or absent, and structures are missing progressively from the posterior-most portions of the head skeleton. These structures are derived from the most anterior region of the blastoderm and are subsequently brought into the interior of the embryo during head involution.

The headless class, shown in Figure 1f, completely lacks scleretonized head structures and often is missing more anterior thoracic structures. These embryos retain normal anterior-posterior polarity throughout the thoracic and abdominal regions of the larval cuticle. Occasionally, embryos from this class have rudimentary posterior spiracles and filzkorper duplicated at the anterior end.

The mixed class, an example of which is shown in Figure 1g, in general has normal polarity posterior to about the fourth abdominal segment (A4). Anterior to A4 the anterior-posterior polarity is disarranged, with some longitudinal strips retaining normal polarity and other longitudinal strips having reversed or ambiguous polarity. Thus, these embryos appear to have four or five normal abdominal denticle bands in their posterior half, but possess a disorganized array of denticles in their anterior half that cannot be readily distinguished as particular denticle bands or segments.

The symmetric class, shown in Figure 1i, is symmetric along the anterior-posterior axis of the differentiated embryo. Each half develops the structures posterior to the plane of mirror-image symmetry. The plane of symmetry is tangential to the segment boundary, such that approximately one more segment is formed on the ventral side of each half of the embryo than is formed on the dorsal side. The mirror-image pattern in these embryos may have anywhere from two to five segments along the ventral midline and one to four segments along the dorsal midline (designated here as S2 to S5). Along the ventral midline the plane of mirror-image symmetry can occur anywhere between or within the third and sixth abdominal segments (A3 through A6).

The asymmetric class of defective embryos (Figure 1h) also develops posterior structures at both the anterior and posterior ends of the embryo. In these embryos the number of segments formed in the anterior portion is less than that formed in the posterior portion of the embryo, and this smaller anterior portion has a reversed polarity. The most frequent type of asymmetric embryo has five segments with normal polarity (A4 to A8), with one or two segments anterior to A4 with reversed polarity. However, all possible types of asymmetric embryos are found, although no embryos with greater than nine or fewer than five total segments were observed.

As indicated in Figures 2 and 4, the same classes of embryos are produced by mutations in the BicaudalC and BicaudalD loci, as well as by the YC67 line, but various mutations and genetic constitutions differ in the exact frequencies of the various classes. These classes of defective embryos can be divided into two distinct types: (1) head-defective embryos, which are missing structures from the anterior end but retain normal polarity throughout the body length (headless and reduced mouthparts embryos) and (2) double-abdomen embryos,
which have a reversal of polarity along the anterior-posterior axis somewhere in the animal (symmetric, asymmetric and mixed classes).

The BicaudalC locus: The proportion of double-abdomen embryos produced by heterozygous BicC females depends on the temperature and genetic background of the female. As shown in Table 1, females heterozygous for a BicC mutation and the CyO balancer produce a substantial frequency of double-abdomen embryos. Females heterozygous for the same BicC mutation and a different second chromosome, cl b, rarely produce any double-abdomen embryos, but some weaker phenotypes associated with the bicaudal mutation (headless and reduced mouthpart embryos) are consistently observed. BicC heterozygotes with these two second-chromosomes represent extremes in the frequency of double-abdomen embryo production by BicC; other second-chromosomes (including SM1, In(2L+2R)Cy, cn bw, b pr cn sca, b Tft and Oregon-R) fall in-between (J. Mohler, unpublished results). Females heterozygous for any BicC mutation and the CyO balancer produce more double-abdomen em-
TABLE 1
The frequency (expressed as percent) of double-abdomen embryo production by females heterozygous for a BicC mutation and either CyO or cl b

<table>
<thead>
<tr>
<th>BicC allele</th>
<th>18° cl b</th>
<th>25° cl b</th>
<th>29° cl b</th>
<th>18° CyO</th>
<th>25° CyO</th>
<th>29° CyO</th>
</tr>
</thead>
<tbody>
<tr>
<td>YC33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>39</td>
<td>6</td>
</tr>
<tr>
<td>C96</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>HF34</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WC45</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RU35</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Double-abdomen embryos are those in the mixed, symmetric and asymmetric classes of Figure 1. Females were raised and embryos collected at either 18, 25, or 29°. All tests were done at the same time in parallel to minimize uncontrolled environmental factors. In each case, 200–600 differentiated embryos were counted from two to five females.

Females homozygous for any allele of the BicaudalC locus are sterile. In these homozygous BicC females, germ-cell differentiation is blocked during oogenesis at stage 8 or 9 (at the beginning of vitellinogenesis; for a review of oogenesis, see King 1970). This can clearly be seen in Figure 3, where most mature egg chambers in each ovariole are usually at stage 8. However, differentiation of follicle cells continues past this point; follicle cells often migrate off the nurse cells onto the developing oocyte, but this migration usually leaves bryos at 25° than at 18°; these females produce very few double-abdomen embryos at 29° (Table 1). The strongest allele, BicC^{HC35}, produces about 30% double-abdomen embryos at the temperature most permissive for expressivity (25°) in combination with the CyO balancer (Figure 2a and Table 1).

![Figure 3](image-url)
the last tier of nurse cells covered with a thick follicle cell epithelium. Follicle cells never completely surround the oocyte or separate it from the nurse cells. When rarely a chorion is secreted, it is cup shaped and open at the anterior end, often with nondegenerated nurse cell material. Ovaries from these females frequently contain large numbers of degenerating egg chambers.

The alleles of the BicC locus all fail to complement inter se for their female sterility phenotype. The female sterility phenotype of these mutations was mapped between b and pr to 2-52.0±0.8(2 se). The homozygous female sterility phenotype of these mutations is complemented by deficiencies Df(2L)75c and Df(2L)H20 (T. Schupbach, personal communication), but not by deletions Df(2L)A446 and Df(2L)osp 29. The female sterility of homozygous BicC females is also complemented by Dp(2;3)osp 3. The BicC locus, therefore, appears to map to between 35D4,7 and 35E6 on the polytene chromosome map, between the distal breakpoints of Df(2L)75c and Df(2L)osp 29.

Two alleles of the BicC locus were isolated by their homozygous female sterile phenotype by T. Schupbach, the other four by their dominant bicaudal phenotype. Because of the ease of isolation of BicC mutations and because alleles isolated for the recessive female sterility phenotype also produce the dominant bicaudal phenotype, it is likely that the dominant bicaudal phenotype is due to a haplo-insufficiency of the BicC locus. This is confirmed by the fact that females heterozygous for Df(2L)osp 29 and CyO produce 5% double-abdomen embryos at 25°.

The BicaudalD locus and the YC67 polygenic line: The BicaudalD locus has two alleles: BicD 71.34 and BicD III 48, both of which cause heterozygous females to lay double-abdomen embryos. (Both mutations have similar map positions on the second chromosome BicD 71.34,53.3, BicD III 48-52.4). In mutagenesis screens to revert the bicaudal production of BicD 71.34 and BicD III 48 (see below), a revertant of each was found to be a deficiency of the region around 36C including the maternal-effect dorsal (dl) locus, which has been found to lie very close to the BicD locus; no recombinants between BicD 71.34 and dl were found out of 981 progeny. Therefore, both of these mutations appear to map in the 36C region of the polytene chromosome map close to the dorsal locus.

As with mutations at the BicC locus, the degree of expression of the BicD mutations is dependent on environmental conditions and genetic background. Females heterozygous for BicD alleles in any given genetic background produce highest frequencies of double-abdomen embryos at 18° and lowest frequencies at 29° (Table 2). The actual frequency of double-abdomen embryos for a given strain of flies bearing a single BicD mutation is variable. For example, females heterozygous for BicD 71.34 and CyO grown at 25° will produce between 3 and 30% double-abdomen embryos, depending on uncontrolled environmental factors. To avoid problems associated with this variability, no conclusions about the effect of a particular genetic construction were considered valid unless the same or similar effects were observed with all combinations of alleles, thereby observing the phenomenon in a number of genetic backgrounds.

The bicaudal phenotype of BicD mutations is not due to a haplo-insufficiency for this locus, because Df(2L)TW119 heterozygotes (with a single wild-type
The frequency (expressed as percent) of double-abdomen embryos produced by females heterozygous for a BicD mutation or YC67 and either a wild-type allele of BicD (Bic/CyO; Bic/h pr cn bw wxt; Bic/+; TM3/+), a BicD deficiency (Bic/Df(2R)TW119) or two wild-type copies of BicD (Bic/+; Dp(2,3)osp³/+).

<table>
<thead>
<tr>
<th>BicD</th>
<th>Dose of Bic⁺</th>
<th>71.34</th>
<th>IIIE48</th>
<th>YC67</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bic/+; Dp(2,3)osp³/+</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Bic/+; TM3/+</td>
<td>1</td>
<td>46</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Bic/CyO</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Bic/b pr cn bw wxt</td>
<td>1</td>
<td>26</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Bic/Df(2L)TW119</td>
<td>0</td>
<td>72</td>
<td>58</td>
<td>25</td>
</tr>
</tbody>
</table>

Experiments with Dp(2,3)osp³, TM3 and Df(2L)TW119 were done at the same time in parallel to control for unknown environmental effects. Experiments with CyO and b pr cn bw wxt were done on separate occasions. These data represent the counts of 200–500 differentiated embryos from two to five females.

allele of the BicD locus) do not produce double-abdomen embryos and because BicD/+; Dp females still produce rare double-abdomen embryos despite having two wild-type copies of the BicD locus (Table 2). Instead, these mutations (BicD71.34 and BicDIIIE48) appear to be antimorphic mutations, in which the mutated gene product is antagonistic to the wild-type gene product. Females heterozygous for a BicD allele and Df(2L)TW119, which is deficient for the region around the dl locus (Steward, McNally and Schell 1984), also produce higher levels of double-abdomen embryos (about 60%) than do females heterozygous for a BicD mutation and a wild-type allele. In contrast, females heterozygous for a BicD mutation and a wild-type second chromosome, which in addition carried a duplication for the region including the BicD locus (Bp(2, 3)osp³), produced a greatly reduced proportion of double-abdomen embryos (<1%). Thus, the expression of the BicD mutations is affected by the number of wild-type copies of the BicD locus, the expression dropping with increasing amounts of the wild-type gene (BicD/Df > BicD/+ > BicD/+/+).

Unlike the mutations at the BicC locus, females homozygous for either of the BicD mutations are not sterile. Although such homozygous females yield the same range of defective embryos as heterozygous females, the proportion of double-abdomen embryos then is much larger. Whereas females heterozygous for a BicD mutation and a wild-type allele produce about 10% double-abdomen embryos (Table 2), females homozygous and trans-heterozygous for BicD mutant alleles produce between 50 and 95% double-abdomen embryos (Table 3). In no case were the females ever completely sterile; in all combinations at least 1% of the embryos manage to hatch. At 18°C, homozygous females also show a slight change in egg shape and chorion morphology. In 90% of such eggs, the dorsal appendages are fused to form a single medial structure. More rarely, the appendages are reduced in length or are totally absent.
The frequency of double-abdomen embryos (expressed as percent) produced by females doubly heterozygous for a dominant \textit{bicaudal} mutation (\textit{BicC}, \textit{BicD} and \textit{YC67}) and either another dominant \textit{bicaudal} mutation (\textit{BicD} or \textit{YC67}) or a dominant \textit{bicaudal} enhancer (\textit{Df(2R)vgD} or \textit{E(2)Bic})

<table>
<thead>
<tr>
<th>Bicaudal</th>
<th>\textit{BicD} \textit{YC67}</th>
<th>Enhancers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textit{18°}</td>
<td>\textit{25°}</td>
</tr>
</tbody>
</table>

\textit{BicD} \textit{18°} | 94 | 91 | 76 | 78 | 79 | 73 | 35 | 95 | 66 | 98 |
\textit{BicD} \textit{25°} | 41 | 30 | 85 | 80 | 67 | 50 | ND | ND | 79 | 93 |
\textit{YC67} \textit{18°} | 80 | 60 | 41 | 54 | ND | ND | 32 | 92 | |
\textit{YC67} \textit{25°} | 68 | 77 | 71 | 87 | 78 | 83 | 0 | 9 | 32 | 92 |
\textit{BicC} \textit{18°} | 80 | 71 | 88 | 48 | 77 | 48 | 0 | 2 | 0 | 18 |
\textit{BicC} \textit{25°} | 98 | 45 | 72 | 76 | 50 | 68 | ND | ND | ND | ND |
\textit{WC45} \textit{18°} | 83 | 100 | 91 | 56 | 93 | 93 | 0 | 1 | ND | ND |
\textit{WC45} \textit{25°} | 74 | 68 | 80 | 57 | 48 | 70 | 2 | 1 | ND | ND |

\textbf{ND = not determined.}

Experiments using two dominant \textit{bicaudal} mutations were done in parallel to control for unknown environmental factors. Experiments measuring the effects of the enhancers on dominant \textit{bicaudal} mutations were done at separate occasions. In all cases, 100–300 differentiated embryos were counted from two to five females.

The second chromosomal double-abdomen-producing line, \textit{YC67}, behaves similarly to the \textit{BicD} mutations in most respects, except that it appears polygenetic. Females homozygous for the \textit{YC67} chromosome produce higher frequencies of double-abdomen embryos than do females heterozygous for the \textit{YC67} chromosome. Like \textit{BicD} mutations, females produce higher frequencies of double-abdomen embryos at 18° than at 25°, producing none at 29°. In addition, the expression of the \textit{YC67} chromosome is dependent on the number of wild-type copies of the \textit{BicD} locus. Females heterozygous for \textit{YC67}, a wild-type \textit{BicD} allele, plus a wild-type \textit{BicD} gene duplication (\textit{YC67}/\textit{+}/\textit{Dp(2,3)osp}^3, Table 2) produce fewer double-abdomen embryos than do females heterozygous for \textit{YC67} and one wild-type \textit{BicD} gene (\textit{YC67}/\textit{CyO}). In addition, females heterozygous for \textit{YC67} and a deletion of \textit{BicD} (\textit{YC67}/\textit{Df(2L)TW119}) produce more double-abdomen embryos than \textit{YC67}/\textit{BicD}^+. However, there are at least two lesions on the \textit{YC67} chromosome that are necessary for the production of double-abdomen embryos, as indicated by the fact that most recombinants with a multiply marked non-\textit{bicaudal} second chromosome (\textit{al b pr cn} or \textit{b dl}) fail to produce double-abdomen embryos. It seems likely that one of these lesions is at the \textit{BicD} locus, due to the similarities of the genetic characteristics of the \textit{YC67} mutation and \textit{BicD} mutations.

Figure 4 shows the distribution of defective embryos from homozygous or \textit{trans}-heterozygous \textit{BicD}^71.34/\textit{BicD}^{HE48}, the same classes of defective embryos were observed as in the heterozygous \textit{bicaudal} case, but the frequency of all classes of symmetric double-abdomen embryos is much higher than from heterozygous females. However, in the case of \textit{BicD}^71.34/\textit{Df(2L)TW119} females,
the classes of defective embryos are qualitatively different, in that the symmetric double-abdomen embryos have between 3½ and 6 segments in mirror-image symmetry instead of between 2½ and 5 segments in the case of homozygous BicD and YC67 and heterozygous BicC, BicD and YC67 individuals.

Revertants of BicaudalD: Because of the antimorphic nature of the BicD mutations, a second amorphic mutation in the mutant BicD gene should relieve the dominant expression of the BicD mutations. In order to produce such amorphic mutations, approximately 10,000 BicD$^{71,34}$/BicD$^{IHE}$ and 5000 BicD$^{71,34}$/Df(2R)TW119 chro-

**FIGURE 4.**—Frequency distribution of embryonic phenotypic classes from females homozygous, trans-heterozygous and hemizygous for dominant bicaudal mutations and raised at 25°. Left axis indicates the frequency of each class of embryo among all fertilized embryos; the right axis indicates the frequency among unhatched fertilized embryos. Vertical lines indicate the standard error of the frequency of each class among the total unhatched fertilized embryos. Class designations are the same as in Figure 2. a, YC67/YC67; b, BicD$^{71,34}$/BicD$^{IHE}$; c, BicD$^{71,34}$/Df(2R)TW119.
mosomes were irradiated with 4000R, and F₁ females heterozygous for these irradiated chromosomes were screened for the ability to produce double-abdomen embryos. In addition to a number of lines which showed reduced double-abdomen embryo production, we isolated two revertants of BicD<sup>71</sup>,<sup>34</sup> (R26 and H68). Also, one revertant of BicD<sup>HIE</sup><sup>48</sup>, M18, was isolated out of approximately 3000 BicD<sup>HIE</sup><sup>48</sup> chromosomes mutagenized with EMS. Revertants H68 (BicD<sup>71</sup>,<sup>34</sup>) and M18 (BicD<sup>HIE</sup><sup>48</sup>) proved to be deficiencies. Df(2L)H68 is deficient for 36B1.2;37A1.B1 (C. Nüsslein-Volhard, personal communication), and Df(2L)M18 is deficient for 36B3.8;D1.E1; both deficiencies are lethal when heterozygous with Df(2L)TW119. These deficiencies, therefore, confirm that both BicD<sup>HIE</sup><sup>48</sup> and BicD<sup>71</sup>,<sup>34</sup> map to this region.

The other revertant, R26 (BicD<sup>71</sup>,<sup>34</sup>), is not a cytologically visible deficiency. R26 when homozygous or heterozygous with Df(2L)TW119 is viable, but is a nonegg-laying female sterile. Ovaries from females homozygous for R26 possess egg chambers with 16 nurse cells and no oocyte surrounded by follicle cells, instead of the normal arrangement of 15 nurse cells and an oocyte. Examination of ovaries from BicD<sup>71</sup>,<sup>34</sup> homozygotes demonstrates that these ovaries also occasionally contain such egg chambers in addition to many morphologically normal ones. Thus, the R26 mutation appears to shift the frequency at which these 16 nurse egg chambers are produced and, at the same time, to eliminate the production of double-abdomen embryos. The defects during oogenesis observed in the R26 line are not due to the induction of modifier mutations elsewhere in the genome, since the R26 sterility maps less than 0.01 map units to the right of dl (R. Stewart, personal communication).

Seven other chromosomes, six derived from BicD<sup>71</sup>,<sup>34</sup> and one from BicD<sup>HIE</sup><sup>48</sup>, were retained which initially behaved as if the dominant bicaudal mutation had been reverted. The six putative revertants of BicD<sup>71</sup>,<sup>34</sup> proved to yield lower, nonzero frequencies of double-abdomen embryos. It is not clear whether these represent weak mutations at BicD or second-site modifiers of BicaudalD expression. The one putative revertant derived from BicD<sup>HIE</sup><sup>48</sup> following gamma irradiation, R44, failed to produce double-abdomen embryos on any subsequent retest. R44 is viable, fertile and apparently wild type when heterozygous to Df(2L)TW119. Although this may represent another type of BicD revertant different from R26, R44 may also be the product of gene conversion or recombination from the wild-type chromosome following irradiation.

Genetic factors modulating the frequency of double-abdomen embryo production: Females heterozygous for two dominant bicaudal mutations, as shown in Table 3, produce high proportions of double-abdomen embryos. This level is higher than the 1–30% found in heterozygotes of any dominant bicaudal mutation alone, and is considerably more than additive. The double-abdomen embryo production of heterozygous BicD or YC67 females can also be enhanced by the deficiency Df(2R)vg<sup>0</sup>, which is deficient for bic (Nüsslein-Volhard 1977a). Females heterozygous for a BicD allele or YC67 and Df(2R)vg<sup>0</sup> produce between 40 and 95% bicaudal embryos (Table 3). In contrast, females heterozygous for both a BicC allele and Df(2R)vg<sup>0</sup> do not appear to produce fre-
Figure 5.—Enhancing effects of vg-region deficiencies on the frequency of double-abdomen embryos produced by females heterozygous for a dominant bicaudal mutation. Solid bars indicate which visible or lethal complementation groups are deleted by a given vg-region deficiency (data courtesy of P. Lasko, personal communication). The SM5 balancer is used to balance these vg-region mutations and is wild type for this region. The frequencies of double-abdomen embryos produced at 25°C by females heterozygous for a given vg-region deficiency or vg-region lethal and either 71.34 or Yc67 are indicated in the table. The SM5/Bic females that were tested are sibs of the Df/Bic females.

Using a series of deficiencies of the vg locus, it was possible to map the locus within Df(2R)vgD responsible for enhancing BicD bicaudal production. These deficiencies were created and genetically characterized by P. Lasko (unpublished data), and the relevant complementation data is diagramed in Figure 5. As is shown in Figure 5, only vg region deficiencies that deleted the l(2)vr22 locus, a postembryonic lethal, were capable of enhancing BicD71.34 or Yc67 bicaudal production. In addition the sole allele of l(2)vr22 (P3) also enhances bicaudal production of heterozygous BicD71.34 and Yc67 females. In contrast, these deficiencies did not enhance the double-abdomen production of BicC alleles (Yc33 and C96) above that of BicC heterozygotes with Cy0 (data not shown). Therefore, lesions of the l(2)vr22 locus appear to be specific dominant enhancers of the production of double-abdomen embryos by BicD and Yc67, but not by BicC.

Another dominant enhancer of double-abdomen embryo production by BicC, BicD and Yc67 is E(2)Bic [2-64.0 + 1.2 (2 se)]. Females heterozygous for E(2)Bic and BicD71.34 produce 97% double-abdomen embryos at 25°C; other mutations are similarly enhanced (Table 3). This mutation was isolated as a recombinant from a polygenic line that infrequently produced double-abdomen embryos on its own; however, the E(2)Bic mutation itself is not sufficient for the production of double-abdomen embryos. The E(2)Bic mutation alone is an incompletely penetrant, dominant female-sterile mutation: females heterozygous for E(2)Bic and wild-type chromosome produce approximately 60% apparently unfertilized eggs.
The experiments presented in this table were done in parallel to control for unknown environmental factors. In each case 100–300 differentiated embryos were counted from two to five females.

Because the penetrance of the dominant bicaudal mutations could be influenced readily, it seemed appropriate to look for other dominant modifiers of BicD expression. Special candidates were other known female-sterile mutations in anticipation that mutations affecting the same system affected by bicaudal mutations would be identified. The rationale is that changing the dose of a gene used in the same pathway affected by the bicaudal mutation would alter the extent of perturbation caused by the bicaudal mutation and, hence, the penetrance of the bicaudal mutation. Thirty-one second-chromosomal female-sterile mutations (mostly maternal-effect mutations) were obtained from T. SCHUPBACH and were tested for their dominant effect on expression of BicD(BicD71.34) and YC67 (these maternal-effect mutations will be described in a forthcoming manuscript by T. SCHUPBACH and E. WIESCHAUS). Of these mutations, only five were found to have a major effect on the expression of BicD and YC67. The two alleles of egalitarian, RC12 and WU50, both suppress as dominants (i.e., in heterozygous females) the expression of BicD and YC67 (Table 4). Females homozygous for egalitarian produce no oocytes; the ovaries of these females contain egg chambers with 16 nurse cells and no oocyte, instead of the usual 15+1 arrangement of wild-type egg chambers (T. SCHUPBACH, personal communication). This phenotype is identical to the R26 revertant of BicD(BicD71.34), however, these mutations map to 2-105, far from the BicD locus.

The other three mutations that are dominant modifiers of BicD and YC67 expression, tudor, vasa and staufen, have a similar homozygous maternal effect phenotype to each other. Females homozygous for any of these mutations produce embryos lacking some anterior abdominal segments [in a way reminiscent of the zygotic mutation, knirps ([JURGENS et al. 1984]) and germ cells; embryos from females homozygous for staufen are also missing the most an-
terior head structures (T. Schupbach and E. Wieschaus, unpublished results). Females heterozygous for tudor or vasa and BicD$^{71,34}$ or YC67 produce very few double-abdomen embryos, around 1% at 25°. Females heterozygous for staufen and BicD$^{71,34}$ or YC67 produce high frequencies (above 50%) of double-abdomen embryos. Thus, females heterozygous for this particular type of recessive maternal-effect mutation are either more or less sensitive to the antimorphic action of the BicD or YC67 mutations. In contrast, heterozygosity for any of the many other recessive maternal-effect mutations tested does not greatly affect the frequency of double-abdomen embryo production by BicD or YC67 (data not shown).

Importantly, not all maternal-effect mutations that affect pattern along the anterior-posterior axis act as dominant modifiers of BicD. In particular, mutations at the torso, trunk and exuperentia loci do not affect the expression of the BicD$^{71,34}$ and YC67 mutations. These mutations are recessive maternal-effect mutations which produce embryos lacking the most anterior and posterior structures (T. Schupbach and E. Wieschaus, unpublished results); the head defects of these embryos resemble the head defects of the reduced head skeleton and headless classes of bicaudal embryos. Thus, only the recessive maternal-effect mutations affecting the anterior-posterior pattern by deleting anterior abdominal segments (the knirps-like mutations) appear to have any dominant effect on the expression of the BicD and YC67 mutations.

DISCUSSION

Mutations at three loci, BicC, BicD and bic, in addition to a polygenic line YC67, cause females to produce double-abdomen embryos. Mutations at all three of these loci cause females to produce the same array of intermediate phenotypes. As described above, females mutant for BicC, BicD or YC67 produce embryos with reduced head-structures, headless embryos, embryos with mixed regions of polarity reversal and normal polarity, and asymmetric and symmetric double-abdomen embryos, although at different frequencies depending on the locus, the allele and the environmental conditions. Identical classes of embryos were observed for the bic locus (Nüsslein-Volhard 1977a) as were identified here for BicD, BicC and YC67. Due to the high degree of similarity of these phenotypes and the array of phenotypes observed, it seems likely that these mutations are interfering with the same developmental system. This conclusion seems especially warranted in light of the high degree of mutual enhancement of the frequency of double-abdomen embryos in double heterozygotes.

The conclusion that mutations at the bic locus enhance the expression of the BicD locus is based on the assumption that the enhancing activity of Df(2R)vg$^b$ (and l(2)ur22) on BicD mutations is due to an insufficiency of the bic gene product. The exact relationship between l(2)ur22 and bic is difficult to resolve. Although bic/l(2)ur22 trans-heterozygotes did not produce double-abdomen embryos (J. Mohler, unpublished results), in our hands no double-abdomen embryos were produced by bic/Df(2R)vg$^b$ or bic/Df(2R)vg$^b$ double heterozygotes either, in contrast to results of Nüsslein-Volhard (1977a). These results
could be due to an accumulation of modifiers in the original bic stock that suppress double-abdomen production in this line or due to our inability to recreate the appropriate environmental conditions for double-abdomen embryo production by this line. Our results are still consistent with the idea that the $l(2)vr22$ and bic loci are identical. At this time it seems most likely that the $l(2)vr22^{2+}$ mutation represents a null or strong hypomorphic mutation of the bic locus and that the original bic mutation represents a leaky, hypomorphic mutation (as suggested by Nüsseim-Volhard 1977a) at the same locus. However, it is also possible that the apparent noncomplementation of bic and Df(2R)vg$^0$ observed by Nüsseim-Volhard (1977a) may be due to a dominant enhancing effect of the deficiency upon the bic locus, which may be located near to but not within Df(2R)vg$^0$.

A number of parameters of the distribution of the intermediate phenotypes from mutant bicaudal females deserve emphasis. The array of different bicaudal embryo phenotypes can be arranged in a linear series depending on the most anterior structure still present in the abnormal pattern. That this series is, in fact, a series of progressively stronger phenotypes (the reduced head-skeleton embryos representing weak expression of a bicaudal mutation and symmetric double-abdomen embryos representing strong expression of bicaudal mutations) is suggested by the frequency distributions of embryos from females of different genotypes. From genotypes which produce a low proportion of unhatched embryos (e.g., YC67/CyO), a greater proportion of the unhatched embryos develop as headless or reduced head-skeleton embryos than do those from genotypes which produce higher proportions of unhatched embryos (e.g., BicO$^{77,34}$/BicD$^{III,48}$). In addition, in genotypes which produce mostly unhatched embryos (e.g., YC67/YC67), a large proportion of the embryos develop into the class of the smallest symmetric double-abdomen embryos (S2.5). Thus, the types of bicaudal embryos can be arranged from weak to strong as reduced head-skeleton embryos; headless embryos; mixed and asymmetric embryos; large-segment number, symmetric double-abdomen embryos; and low-segment number, symmetric double-abdomen embryos.

There appear to be defined limits in segment number in each of these classes. Embryos which have defects limited to head structures do not possess polarity reversals. The embryos which are missing the most structures but which retain normal polarity have all eight abdominal segments, most or all of the third thoracic segment and parts of the second thoracic segment. In most bicaudal strains, the symmetric double-abdomen embryo with the largest number of segments has a mirror-image plane in the denticle band of the fourth abdominal segment (A4) or in the naked cuticle of the third abdominal segment (A3) (see Figures 2 and 4; an exception is embryos from BicD$^{71,34}$/Df(2L)TW119 females, see below). In the majority of asymmetric double-abdomen embryos, the most anterior segment in normal polarity is the fourth abdominal segment (A4); anterior to it are often one or two reversed-polarity segments. In most mixed-class embryos, polarity reversals are restricted to strips running along the anterior-posterior axis of the embryo: strips with polarity reversals are usually asymmetric and have the polarity reversal in or
just anterior to the third abdominal segment; strips without polarity reversals are segmented normally into the thorax (a similar phenomenon is seen after ligation of Callosobruchus embryos (Van der Meer 1984)). Thus, it appears that there is a transitional point wherein defects from the head into the thorax retain normal polarity, and deeper defects result in mirror-image embryos with a symmetry point in A3 or A4. The mixed and asymmetric embryos appear to be relatively rare transitional types between headless embryos, where some embryos have normal polarity throughout, and symmetric double-abdomen embryos. In the case of the mixed class, this is apparent in the fact that some longitudinal strips are reversed, and others are not (Figures 1c and 1g). In the asymmetric class where the polarity is reversed around the entire circumference, the intersegmental distances have not been equalized along the length of the embryo, and segments in the posterior portion are frequently longer than those in the reversed polarity anterior portion. The limit of defects extending from the anterior end is in the embryos with a plane of mirror-image symmetry in the sixth abdominal segment; embryos with two or fewer mirror-image segments are extremely rare.

Because the thorax arises in the developing embryo from a region about 50–60% egg length from the posterior end in the cellular blastoderm of wild-type embryos (Lois-Schardin, Cremer and Nüsslein-Volhard 1979), it appears that all embryos retaining normal polarity retain over one-half of the positional values of the egg; the smallest headless embryo retains just over one-half of the positional values. Symmetric (and asymmetric) double-abdomen embryos are missing much more than one-half of the wild-type positional values, retaining only the posterior one-quarter to one-third of the positional values of the egg (A3 derives from about 35% egg length, and A6 derives from about 25% egg length, in wild-type embryos). Thus, the distribution of embryos from females bearing a bicaudal mutation behaves as if the primary effect of the bicaudal mutation on the development of embryos is a loss of positional values from the anterior end of the embryo. In embryos where more than one-half of the positional values are missing, the embryonic field is organized to produce a mirror-image duplication that results in a double-abdomen embryo.

The reorganization of a field containing less than one-half of the normal positional values into a duplicated pattern appears to be a generalized phenomenon. Duplication of the remaining positional values of a fragment of wing imaginal discs often occurs during regeneration if the fragment contains less than one-half of the entire disc (see review by Bryant 1975). Embryonic lethal mutations, such as runt and patch, which cause the deletion of pattern elements in apparent double- and single-segment fields, respectively, appear to cause mirror-image duplications of the remaining pattern elements only if the allele used is strong enough to eliminate over one-half of the pattern elements of the field (runt: J. P. Gergen and E. Wieschaus, unpublished results; patch: E. Wieschaus and C. Nüsslein-Volhard, unpublished results). In all of these cases, the mirror image provides an intermediate pattern that smooths out irregularities resulting from the juxtaposition of normally distant positional
values. These patterns can all be modeled as examples of shortest route intercalary regeneration as proposed by FRENCH, BRYANT and BRYANT (1976). In contrast to discs or segments, the field affected by the bicaudal mutations is linear, rather than circular or repeating. It has defined endpoints, and deletions at the anterior end would offer no opportunity for intercalary regeneration. Therefore, it seems likely that the formation of duplications after the removal of more than one-half of the positional values of a field is a more general phenomenon than can be explained by intercalary regeneration. Mosaic analysis of runt embryos also suggests that the pattern duplications of runt embryos are not due to intercalary regeneration (GERGEN and WIESCHAUS 1985).

The other two apparent transition points, A3 and A6, appear as distinct limits to the sizes of double-abdomen embryos. Although these transition points may reflect other significant aspects of the organization of the embryo along the anterior-posterior axis, at present the nature of these constraints on double-abdomen embryo size is not easily assessed. It should be emphasized that these transition points are somewhat plastic and can be altered by genetic background; for example, in embryos from BicD<sup>D71.34</sup>/Df(2L)TW119 females, the sizes of symmetric double-abdomen embryos are increased, such that the most anterior structure of these embryos may be anywhere between A2 and A5 (Figure 4c). This appears to be due to an effect of a haplo-insufficiency of the dorsal locus, which is also deleted by Df(2L)TW119, on the bicaudal pattern, rather than an effect of a deletion of a wild-type BicD allele. Compound heterozygotes of YC67, BicD<sup>D71.34</sup> or BicD<sup>D1ras</sup> with some <i>dl</i> alleles also produce six-segment embryos (J. MOHLER, unpublished results). The haplo-insufficiency of dorsal causes cells close to the ventral midline to give rise to cuticle structures, rather than internal structures (NÜSSLIN-VOLHARD et al. 1980). In the typical symmetric double-abdomen embryo, the plane of mirror-image symmetry is skewed relative to the dorsal-ventral axis, such that more segments are formed on the ventral than on the dorsal side of the embryo. In a dorsalized double-abdomen embryo, the difference between the dorsal and ventral cuticular patterns would be expected to be even greater, because the ventral most cuticular structures would arise from cells close to the ventral midline, rather than from the ventral-lateral region. Thus, the apparent increase in size of double-abdomen embryos associated with dorsal heterozygosity may be a result of our scoring primarily ventral cuticle structures, and it need not reflect an alteration in the pattern of anterior-posterior positional values or a change in the more typical A3 and A6 transition points.

The analysis presented in this paper is based on the cuticle phenotype secreted by epidermal cells at the end of embryogenesis. Given the maternal nature of the bicaudal mutations, it is attractive to extrapolate from these patterns back to some early alteration in the egg cytoplasm. Although the deletions and duplication might formally be described as resulting from cell death and subsequent regeneration, observation of early development in bicaudal embryos suggests that this is not the case. Gastrulation of double-abdomen embryos from bic females appears symmetric with an invagination correspond-
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According to the posterior midgut invagination duplicated at the anterior end of the gastrula (NÜSSLER-VOLHARD 1979); similar effects on gastrulation can be observed in embryos from females mutant for YC67 and BicD (MOHLER and WIECHHAUS 1985). Embryos from females mutant for BicD also show an altered spatial transcription pattern of fushi tarazu (futz), a gene involved in proper segmentation of the larva, during the blastoderm stage (MOHLER and WIECHHAUS 1985). These results indicate that the bicaudal mutations alter the spatial pattern of cell determinations at the blastoderm stage, primarily by excluding the most anterior positional values and secondarily by reorganizing into a duplicated field if fewer than one-half of the positional values remain.

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