**Horka**, A Dominant Mutation of Drosophila, Induces Nondisjunction and, Through Paternal Effect, Chromosome Loss and Genetic Mosaics

János Szabad, *Endre Máthé* and Jaakko Puro

*Department of Biology, Albert Szent-Györgyi Medical University, H-6720 Szeged, Hungary, Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, H-6726 Szeged, Hungary, and Institute of Biology, University of Turku, SF-20500 Turku 50, Finland*

Manuscript received August 29, 1994
Accepted for publication November 17, 1994

**ABSTRACT**

*Fs(3) Horka* (*Horka*) was described as a dominant female-sterile mutation of *Drosophila melanogaster*. Genetic and cytological data show that *Horka* induces mostly equational nondisjunction during spermatogenesis but not chromosome loss and possesses a dominant paternal effect: the X, second, third and the fourth chromosomes, but not the Y, are rendered unstable while undergoing spermatogenesis and may be lost in the descending zygotes. The frequency of *Horka*-induced chromosome loss is usually 2–4% but varies with the genetic background and can be over 20%. The X chromosome loss occurs during the gomeric and the initial cleavage divisions. Loss of the X and fourth chromosomes shows no correlation. We propose, based on similarities in the mutant phenotypes with the chromosome destabilizing mutations nonclaret disjunctonal and paternal loss, that the normal *Horka* product is required for function of the centromeres and/or nearby regions. *Horka* is a convenient tool for the generation of gynandromorphs, autosomal mosaics and for the study of gene expression in mosaics.

**REPLICATION** and transmission of the genetic material to daughter cells are achieved with remarkable fidelity in all organisms. Although cell divisions have long been studied, rather little is known about the necessary proteins and how they control cell divisions (Baker and Hall 1976). Genetic dissection proved to be an efficient approach in understanding the mechanism of cell division (Endow 1992, 1993; Hawley and Theurkauf 1993). The cell division mutants of Drosophila have been useful in the elaboration of the cell cycle control mechanism (reviewed by Glover 1991). Of particular interest are those Drosophila mutants that destabilize chromosomes. Chromosomes exposed to the mutant environment become unstable and may be lost in the descending embryos. The best known examples of the chromosome-destabilizing mutants are nonclaret disjunctonal, originally known as claret nondisjunctonal (*ca* 

This paper provides genetic, developmental and cytological characterization of the dominant paternal effect of *Horka*.

Genetic mosaics generated by chromosome elimination have been useful tools in cell lineage studies and in analysis of gene expression during ontogenesis. Mutations that destabilize chromosomes (*ca*, *pal* and *mit*) and the unstable ring-X chromosomes, in particular *In(1) w*, are of special importance in the generation of genetic mosaics (for reviews see Hall et al. 1976; Janning 1978; Ashburner 1989). *Horka*, through its chromosome destabilizing effect, proved to be a useful tool for the generation of gynandromorphs (Szabad and Nöthiger 1992) and autosomal mosaics.

**MATERIALS AND METHODS**

*Fs(3) Horka* (*Horka*) is a dominant female-sterile mutation of *D. melanogaster* (Érdelyi and Szabad 1989). *Horka* is a gain-of-function mutation because it can be reverted to loss-of-function alleles (Érdelyi and Szabad 1989). The *Horka* mu-
tant phenotypes originate from one mutation, because they simultaneously disappear in the revertants of Horka. The Horka locus resides in the cytological region 87B (J. Szabad et al., unpublished data). Horka is not allelic with the other chromosome destabilizing mutations. The Horka-carrying third chromosome is labeled with the recessive marker mutations muh and v. Horka is kept in a self-propagating stock in which T(1;3) OR60/TM3, Sb Ser females are mated with Horka/TM3, Sb Ser males. The T(1;3) OR60 rearrangement induces dominant male lethality. For an explanation of the genetic symbols, see Lindsley and Zimm (1992).

Generation and detection of mosaics: Horka/TM3, Sb Ser males taken from the above stock (or following outcrosses) were mated with females that carried X-linked recessive marker mutations. Mosaics were detected in the Horka-derived offspring by identification of gynandromorphs (female/male), as well as haplo-4 mosaics (Janning 1978; Ashburner 1989). To detect the loss of major autosomes, Horka males were constructed that carried a G00 or a TM24 chromosome with a transgene. The transgene ensured an “all over” expression of β-galactosidase (Bellen et al. 1989). The transgene in the G00 chromosome is already expressed at the cellular blastoderm stage, whereas the TM24-linked transgene is expressed from the stage of germ band elongation. The diplo-/haplo-4 autosomal mosaics were detected after fixing and staining for β-galactosidase the embryos descending from the above males and y v mal females.

For the detection of Y chromosome loss, Horka males carrying the y'/Y chromosome were mated with X'X/+ or X't/+/+ females homozygous for the X-linked recessive marker mutations y or w, respectively. [The y' and the w' Y chromosomes carry the y' and the w' segment of the X chromosome (Lindsley and Zimm 1992).] Loss of the y'Y or the w'Y chromosome should lead to the formation of X'Y'/X'O or X'w'/X'O,Y', male mosaics.

Loss of the XY chromosome was studied in a cross between X'/X' females and males that carried the XY chromosome and Horka. The XY chromosome is XILY, y' su(w') w'. It is an intact Y chromosome with all the X chromosome euchromatin (Lindsley and Zimm 1992). Loss of the XY chromosome should lead to the formation of X''XY'/X'O gynandromorphs, many of which are expected to possess w'//w mosaicism.

Detection of nondisjunction: To test whether Horka can induce chromosome loss and/or nondisjunction during spermatogenesis, we made use of the compound second chromosome C(2)EN, bw sp (Gonzalez et al. 1989). In this system, cn bw/ed dp cl; Horka/TM3 (or +) males were mated with C(2)EN, bw sp females. Oocytes of the C(2)EN, bw sp females carry either two or none of the second chromosomes. Viable offspring do not result when such gametes are fertilized by regular sperm. However, viable offspring develop when the diplo-2 and nullo-2 oocytes fuse with aneuploid nullo-2 or diplo-2 sperm, respectively. The phenotype of the descending progeny allows conclusions to be made about the mechanism generating the aneuploid sperm.

Cytology: The chromosomes, the spindles and the sperm tail were made visible in the testes and in the newly fertilized eggs by double staining with Feulgen and Giemsa (Puro and Szabad 1989). The open male abdomens were immersed into a hypotonic (0.075 M) KCl solution for 10–13 min and fixed in a mixture of ethanol:chloroform:acetic acid 6:3:1 for 5 min. The abdomens were subsequently transferred to 99% ethanol and carried through reduced concentrations of alcohol to distilled water. A 30-min soaking in 1 N HCl followed first at room temperature and then at 60°C for 8 min to achieve hydrolysis. Feulgen staining was carried out by treatment with diluted Schiff’s reagent for 2–4 min. The abdomens were then transferred to a drop of distilled water, the testes were dissected and transferred to a slide. The water was replaced by 45% acetic acid. The testes were covered with a coverslip and gently squashed. The coverslip was removed on dry ice. The preparation was first dehydrated in 99% ethanol and then immersed in 100% methanol for 5 min. The preparations were dried and stained in 2% Giemsa in phosphate buffer (pH 6.8) for 15–30 min. Permanent preparations were made after a rinse in distilled water, drying and mounting in Entellan.

Preparations of fertilized eggs were made using a modification of the above procedure. Newly deposited eggs and those squeezed out from the uterus were put immediately into fresh fixative for 15–30 min. Ethanol (95% or 99%) was added to double the volume. Fixation was allowed to proceed for another 30 min. The rest of the procedure was as described above. Eggs were cut into an anterior and a posterior piece in distilled water and the chorion was removed. After dehydration in ethanol, the slides were immersed into glacial acetic acid for 20 sec. Staining was carried out in 4% Giemsa for 30 min.

Analysis of gynandromorphs: The adult gynandromorphs were recovered under a dissecting microscope based on the morphology of the sexually dimorphic structures and/or uncovering the X-linked recessive marker mutations in the XO male tissues. A random sample of 80 Horka-derived gynandromorphs were mounted and analyzed under the compound microscope (Szabad 1978). For comparison, 80 “traditional” gynandromorphs were generated by the unstable ring-X chromosome In(1)w'. They were also mounted and analyzed. The haplo-4 mosaics were detected on the basis of the thin short bristles and the rough eye phenotype (Ashburner 1989). Several Horka-derived gynandromorphs were also identified, using standard immunological methods, at the blastoderm stage by the Sex-lethal antibody that identifies female nuclei (Bopp et al. 1991). The blastoderm gynandromorphs were mounted and analyzed to determine the relative areas covered by the female and male cells.

RESULTS

The dominant paternal-effect of Horka: loss of the paternally derived X chromosome leads to formation of gynandromorphs: A cross between y v f mal females and Horka males yielded 20.5% gynandromorphs and 2.5% haplo-4 mosaics among the XX zygotes and initiated analysis of the Horka-related mosaicism. The following observations show that gynandromorph formation is indeed the consequence of the Horka mutation. Gynandromorphs did not develop among the progeny of y v f mal females and three different types of males: (1) muh e/TM3, (2) Fs(3)/TM3 or (3) revertants of Horka (Table 1). [Horka, like the Fs(3) mutations laborc17v, Kartal27v, Tolt18e and Toma116v, was induced on an muh e labeled chromosome (Erdeley and Szabad 1989).]

There were 1035 gynandromorphs recovered during the course of the present study, that originated from X'X zygotes. (X' and X stand for the y-labeled maternally and the y' allele-carrying paternally derived X chromosomes, respectively). The male parts were invariably X'O, indicating that the X chromosome insta-
any X chromosome  induced gynandromorph formation

<table>
<thead>
<tr>
<th>Features of Horka-induced gynandromorph formation</th>
<th>Zygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parents</strong></td>
<td><strong>Males</strong></td>
</tr>
<tr>
<td>Any X chromosome</td>
<td>Horka/any third chromosome</td>
</tr>
<tr>
<td>Any X chromosome</td>
<td>Horka/TM3, Sb Ser*</td>
</tr>
<tr>
<td>y v f mal</td>
<td>Horka/any third chromosome</td>
</tr>
<tr>
<td>y v f mal</td>
<td>Horka/TM3, Sb Ser*</td>
</tr>
<tr>
<td>y v f mal</td>
<td>Horka&quot; or TM3, Sb Ser*</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>y v f mal</td>
<td>msh e/TM3, Sb Ser*</td>
</tr>
<tr>
<td>y v f mal</td>
<td>Fs (3)/TM3, Sb Ser**</td>
</tr>
<tr>
<td>y v f mal</td>
<td>Horka**/TM3, Sb Ser</td>
</tr>
</tbody>
</table>

Nine different X chromosomes were used besides the one labeled with y v f mal (y w sn, y w w, w, fs(1)K10 w, M-5, y w c v f car, fs(1)K10 w f mal, wild type and one with a lacZ transgene). Thirty different third chromosomes were used, including 11 nonlabeled ones, four balancer chromosomes (TM3, TM2, TM6B and Csd from different strains) and three with lacZ transgenes. The TM3, Sb Ser* chromosome derives from the Fs (3)/T1(1; 2)Or60/TM3, Sb Ser stock. Horka* includes one EMS and eight P- element-induced revertants. Pooled data.

*Values are means ± SD.

*Pooled data from the dominant female-sterile (Fs) mutations Labor17c, Kartal21c, Tola20a and Tomat16d that were induced on msh e-labeled chromosomes (ERDÉLYI and SZABÓ 1989).

Bility is an inherent nature of the Horka-derived chromosomes.

In Drosophila eggs, the first mitotic (gonomeric) division occurs in parallel in both haploid genomes after fertilization. Chromosomes of maternal and paternal origin mix at the spindle poles and form two zygotic nuclei (LIN and WOLFLER 1991). The zygotic nuclei undergo a series of 13 rounds of rapid synchronous cleavage divisions without cytokinesis, before the formation of the cellular blastoderm (GLOVER 1991). The existence of the y male parts clearly shows that the Horka-derived X chromosome was either lost or did not segregate to the nuclei populating the male cells of the gynandromorphs.

Gynandromorphs may originate through either of two mechanisms: loss of an X chromosome during the gonomeric or the cleavage divisions, which leads to the formation of X*X//X'O type of gynandromorphs, and mitotic nondisjunction may result in the formation of XXX//X'O gynandromorphs. The following paragraphs present evidence that the Horka-induced gynandromorphs originate through loss and not nondisjunction of the paternal derived X chromosome.

It is known, from analysis of XXX//XX mosaics, that the first and second legs as well as the wings are very seldom composed of XXX cells due to the XXX-associated lethality mapping to the ventral thoracic region (SCHÜPBACH et al. 1978). Viability of the thoracic structures is not reduced in the Horka-induced gynandromorphs showing that they are X*X//X'O and not X*X//X'O type and hence originated after the loss of the Horka-derived X chromosome (Table 2).

The X*X//X'O zygotes are expected to live at least to the end of the larval life, because the XXX diploid metafemales die in the late larval and pupal stages (LINDSLEY and ZIMM 1992). There were 12 late third instar gynandromorph larvae isolated, showing y*/y mosaicism in the head skeleton, from the cross between y v f mal females and Horka/TM3, Sb Ser males. They were likely to have had mosaic central nervous systems and/or imaginal discs (cf. JANSS 1978). Cytological analysis clearly showed that their brains and/or imaginal discs were composed of only X and/or XO and not of XXX cells. Hence, the Horka-induced gynandromorphs are indeed X*X//X'O types and originate through elimination of the Horka-derived X chromosome.

To visualize cytological loss of the chromosomes, eggs of the y v f mal females that had been mated with Horka males were fixed within a few minutes after fertilization. The eggs were double stained with Feulgen and Giemsa and analyzed for the detection of lost chromosomes. As shown in Figure 1, chromosomes were identified in the zygotic nuclei (from different strains) and three with lacZ transgenes. Three with lacZ transgenes. The TM3, Sb Ser* chromosome derives from the Fs (3)/T1(1; 2)Or60/TM3, Sb Ser stock. Horka* includes one EMS and eight P-element-induced revertants. Pooled data.

*Values are means ± SD.

*Pooled data from the dominant female-sterile (Fs) mutations Labor17c, Kartal21c, Tola20a and Tomat16d that were induced on msh e-labeled chromosomes (ERDÉLYI and SZABÓ 1989).
Table 2

Frequencies (%) of adult structures in XXX//XX, In(1)w^Cc and Horka-induced mosaics

<table>
<thead>
<tr>
<th>Type of mosaic</th>
<th>Genetic constitution</th>
<th>First leg</th>
<th>Second leg</th>
<th>Wings (T2–T6)</th>
<th>Tergites (S1–S5)</th>
<th>Sternites (S1–S5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XXX//XX</td>
<td>XXX</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>XXX//XX</td>
<td>XXX</td>
<td>70</td>
<td>68</td>
<td>67</td>
<td>47</td>
<td>55</td>
</tr>
<tr>
<td>In(1)w^Cc-induced gynandromorphs</td>
<td>XX</td>
<td>45</td>
<td>44</td>
<td>46</td>
<td>51</td>
<td>59</td>
</tr>
<tr>
<td>Horka-induced gynandromorphs</td>
<td>XX</td>
<td>30</td>
<td>31</td>
<td>34</td>
<td>34</td>
<td>23</td>
</tr>
<tr>
<td>Horka-induced gynandromorphs</td>
<td>XX/XX</td>
<td>25</td>
<td>25</td>
<td>20</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>XX/XX</td>
<td>43</td>
<td>47</td>
<td>42</td>
<td>50</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>XX/XX</td>
<td>35</td>
<td>37</td>
<td>45</td>
<td>40</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>XX/XX</td>
<td>22</td>
<td>16</td>
<td>13</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

Data related to XXX//XX mosaicism is from Schüpbach et al. 1978.

These males originated from outcrosses with different types of females and Horka males. The frequency of gynandromorphs exceeded 20% when y v f mal females were mated with X/Y; Horka/TM3, Sb Ser males, taken from the Horka/T(1;3)OR60/TM3, Sb Ser stock. The above experiment was repeated 11 times over a span of 5 years. This last type of cross was also carried out at 18 and 29°C and yielded 10.5 (11/107) and 6.7% (14/209) gynandromorphs, respectively. These frequencies are significantly different from the 20.7% frequency (335/1614; Table 1; P < 0.05 and P < 0.01, respectively; chi-square test) determined at 25°C and indicates the temperature-sensitive nature of the Horka-induced X-chromosome loss.

The frequency of gynandromorphs was ~10% in those 17 experiments where the y v f mal females were mated with Horka males that originated from outcrosses between Horka-carrying males taken from the Horka/T(1;3)OR60/TM3, Sb Ser stock and five different types of females or carried, besides Horka, the TM3, Sb Ser chromosome that derived from the T(1;3)OR60 stock after three to four rounds of outcrosses. Results summarized in Table 1 clearly show that Horka brings about X chromosome instability that leads to the formation of X X/X0 mosaics and refer to at least two factors necessary for high frequency of chromosome loss, one being present in the Horka/T(1;3)OR60/TM3, Sb Ser and the other in the y v f mal stock.

The frequency of gynandromorphs dropped to 11.5% (59/514) in crosses where the females were y v f mal; Cy/Pm; CxD/TM3 and the Horka males derived from the Horka/T(1;3)OR60/TM3, Sb Ser stock. Similarly the frequency of gynandromorphs dropped to ~10% when the y v f mal females were mated with X/Y; Cy/Pm; Horka/TM3, Sb Ser males. When y w cu v f car or y w f mal (each sharing three marker mutations with the y v f mal chromosome) females were mated with Horka males from the Horka/T(1;3)OR60/TM3,

morpht formation, an indication of X chromosome loss, is nicely shown by the following observation. Among the progeny of y v f mal females and Horka/TM3, Sb Ser males, the frequencies of Horka/+ and TM3/+ gynandromorphs were not significantly different (46/173 vs. 29/150; P > 0.05) (Kastenbaum and Bowman 1970).

Unlike the case of In(1)w^Cc, there is no dominant lethality associated with the Horka mutation. Males and females developed with equal frequencies among the progeny of y v f mal females and Horka/TM3, Sb Ser males. The male:female ratio was 0.97 ± 0.10 (mean ± SD) in those 10 crosses where the number of males was recorded. When, however, the y v f mal females are mated with In(1)w^Cc/y^+Y males, an excess of males develop (5816 males and 2493 females) (Szabad et al. 1979) due to the dominant lethality associated with the In(1)w^Cc chromosome (for a review see Ashburner 1989).

**Parameters of the X chromosome loss:** We analyzed 55 crosses during the course of this study (Table 1). When males carrying Horka and any of the eight different third chromosomes were mated with females that carried eight different types of X chromosomes, 1.7% of the zygotes were gynandromorphs. The frequency of gynandromorphs was 3.2% when the X/Y; Horka/TM3, Sb Ser males were taken from the Horka/T(1;3)OR60/TM3, Sb Ser stock and the females carried one of four different types of X chromosomes. The frequency of gynandromorphs was 3.8% in those 12 crosses in which the females were y v f mal homozygous and the males carried Horka and one of nine different chromosomes, including TM2, TM6b and TM3, Sb Ser.

1 The X chromosome did or did not carry recessive marker mutations in the aforementioned experiments. The variation in the frequency of gynandromorphs can be accounted for to some extent by the fact that when the X chromosome is not labeled with recessive marker mutations only 93% of the gynandromorphs can be identified: those that show mosaicism in the sexually dimorphic structures.
Paternal Effect and Chromosome Loss

FIGURE 1.—Cytology of early embryogenesis in eggs of γ v f mal females that had been fertilized by Horka sperm. Gonomeric division, at prometa (A) and anaphase (D), showing side by side the maternally and the paternally derived chromosome sets. Please notice the monopolar nature of the spindle apparatus during the gonomeric division. The A1 inset is an enlargement of the gonomeric spindle apparatus. (B) Second embryonic division in anaphase. The B1 inset shows an enlargement of the right-hand spindle. (C) Gonomeric division at prometaphase. (E–G) Telophases in the cleavage divisions. The arrowheads point to the centrosomes. Note that one of the centrosomes is attached to the sperm tail (st) in A and B (cf. KARR 1991). Arrows point to displaced or lagging chromosomes prone to loss. Scale bar: 20 μm on A and B and 10 μm on A1, B1 and C–G.

Sb Ser stock, 15.5% (32/207) and 20.3% (35/172) of the XX zygotes were gynandromorphs. Attempts failed to identify the factor(s) of the γ v f mal chromosome responsible for the high frequency of chromosome loss. Recombinant chromosomes between a wild-type and the γ v f mal chromosome did not bring about high frequency of chromosome loss. A similar experience was reported following mapping factors that modify the frequency of pal-induced chromosome loss (BAKER 1975). The above data demonstrate that unknown genetic factors contributed by both parents can alter the frequency of X chromosome loss in the zygote.

The Drosophila females store sperm for ~10–12 days after copulation. When progeny derived from the stored sperm of γ v f mal females and Horka males, the frequency of gynandromorphs did not change significantly during a 10-day sperm storage period (P < 0.05; Table 3). This result shows the long-lasting action of the mutant Horka gene product.

The haplo-4 mosaicism: There were 269 haplo-4 mosaics and 781 gynandromorphs recovered in those 39

| TABLE 3 |
|------------------|------------------|
| **The relationship between sperm storage and the frequency of gynandromorphs** | |
| Days after | Zygotes | |
| copulation | Total XX | XX//XO (%) |
| 0–2 | 820 | 20.5 ± 2.1 |
| 2–4 | 561 | 24.6 ± 1.9 |
| 4–6 | 397 | 26.4 ± 4.2 |
| 6–8 | 189 | 23.3 ± 3.2 |
| 8–10 | 67 | 22.4 ± 3.5 |
| Total | 2034 | 25.1 ± 1.6 |

γ v f mal females were mated with Horka/TM3, Sb Ser males that derived from the Horka/T(1;3)OR60/TM3, Sb Ser stock. Three parallel samples were studied. The males were discarded after 2 days, and eggs were collected subsequently from the females in 2-day intervals.

Average of three samples ± SD.
TABLE 4
Features of Horka-induced haplo-4 mosaic formation

<table>
<thead>
<tr>
<th>Parents</th>
<th>Females</th>
<th>Males</th>
<th>Total</th>
<th>%</th>
<th>No. of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>y v f mal</td>
<td>Control*</td>
<td>5740</td>
<td>0</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>y v f mal</td>
<td>Horka/TM3, Sb Ser*</td>
<td>3735</td>
<td>16</td>
<td>2.1 ± 0.4</td>
<td>4</td>
</tr>
<tr>
<td>Any X chromosome</td>
<td>Horka/TM3, Sb Ser*</td>
<td>132</td>
<td>25</td>
<td>1.4 ± 0.9</td>
<td>7</td>
</tr>
<tr>
<td>y v f mal</td>
<td>Horka/any third chromosome A</td>
<td>3678</td>
<td>61</td>
<td>1.6 ± 0.7</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1690</td>
<td>62</td>
<td>3.8 ± 0.7</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1120</td>
<td>78</td>
<td>6.9 ± 0.7</td>
<td>7</td>
</tr>
<tr>
<td>Any X chromosome</td>
<td>Horka/any third chromosome</td>
<td>4741</td>
<td>27</td>
<td>0.7 ± 0.5</td>
<td>6</td>
</tr>
</tbody>
</table>

The TM3, Sb Ser* chromosome derives from the Es(3)/T(1; 3)OR60/TM3, Sb Ser stock. A, at least third generation outcross; B, second generation outcross; C, first generation outcross.

* Pooled data from mwh e/TM3, Sb Ser, the dominant female-sterile mutations Labor 27c, Karta 216, Toll 50a and Tomaj 33d as well as the nine Horka revertants.

experiments where haplo-4 mosaics were scored. (Only the XX; haplo-4 mosaics were recorded. The XY; haplo-4 mosaics develop with similar frequencies as the XX; haplo-4 ones.) Formation of haplo-4 mosaics is a consequence of the Horka mutation: haplo-4 mosaics did not develop among the progeny of (1) males carrying the mwh labeled chromosome, (2) either of the four F(3) mutations listed in Table 1 and (3) any of the Horka revertants. The frequency of the haplo-4 mosaicism varied between 0.7% and 2.1% among the Horka-derived XX zygotes in 26 of the 39 crosses (Table 4) and did not seem to depend on the male and female genotype. However, when the Horka males carried the “sensitive” fourth chromosomes the frequency of haplo-4 mosaics increased significantly (P < 0.01; Table 4). The sensitive fourth chromosomes derived from eight different stocks. The frequency of haplo-4 mosaics was 6.9% and 3% when the Horka males were derived from the second or first generation outcrosses, respectively (Table 4, B and C).

The relationship between elimination of the X and the fourth chromosomes is linear and is shown on Figure 2. In six crosses the frequency of haplo-4 mosaics exceeded those of the gynandromorphs. The Horka males were derived from first generation outcrossings in six of the eight crosses and mothers of the Horka males provided the sensitive fourth chromosome. (The gynandromorph/haplo-4 mosaic ratio was 2.6 ± 0.6; r = 0.92, P < 0.01; Figure 2, line B). In 14 crosses many more gynandromorphs developed than haplo-4 mosaics. In 7 of the 14 crosses the Horka males were directly taken from the

Figure 2.—The linear relationship between the frequency of gynandromorphs and the frequency of haplo-4 mosaics. The gynandromorph/haplo-4 mosaic ratio was 0.6 ± 0.2 in 6 (●, line A), 2.6 ± 0.6 in 17 (□, line B) and 7.7 ± 2.5 in 14 of the crosses (▲, line C).

Although detection of gynandromorphs is based on coexistence of female and male structures over the entire body, the haplo-4 mosaics were identified based on short head and notum bristles and/or the rough eye phenotype (ASHBURNER 1989). Haplo-4 mosaicism restricted to the abdomen went undetected. Of the 80 Horka-induced gynandromorphs analyzed in a compound microscope, 55 (71%) carried male clones on the head and/or the notum, leaving the abdomen composed of entirely male or female structures. This observation suggests that ~29% of the haplo-4 mosaics went undetected.
Horka/ T(1;3) OR60/ TM3, Sb Ser stock and mated with y v f mal females. In the remaining seven crosses the males were derived from outcrossings with different females that did not carry the sensitive fourth chromosome. (The gynandromorph h-4 ratio was 7.7 ± 2.5; r = 0.88, P ≈ 0.01; Figure 2, line C). The slope of line B is the average of lines A and C (Figure 2).

The above results show the presence of factor(s) in the different genotypes that render the fourth chromosomes highly or only moderately sensitive to Horka action. The linear relationship between the Horka-related X and fourth chromosome instability points to a common source for loss of the X and the fourth chromosomes.

Independent loss of the X and the fourth chromosomes: Unlike in ca ad and pal, where loss of the X and fourth chromosomes mostly takes place simultaneously and, as a rule, the male parts of the gynandromorphs are also haplo-4 mosaics, the Horka-induced X and fourth chromosome losses are independent. In one experiment a total of 1695 zygotes were XX at fertilization. Of the 1695 zygotes, 130 (7.7%) were gynandromorphs. Loss of the fourth chromosome took place in 55 (3.2%) of the zygotes. It was expected, based on frequencies of X and fourth chromosome losses, that four of the zygotes would bear XO and haplo-4 tissues simultaneously. XO and haplo-4 tissues were simultaneously present on 3 of the 1695 zygotes. Borders of the XO and haplo-4 clones did not coincide in two of the above three mosaics. In one case, part of the XO clone was also haplo-4, indicating that loss of the fourth chromosome took place in a nucleus that had already lost the X chromosome.

Loss of the major autosomes: Loss of the second chromosome was studied in embryos descending from y v f mal females and males that carried a nonmarked X chromosome and a CyO d chromosome with a β-galactosidase transgene ensuring “all over” expression of the enzyme at the cellular blastoderm stage. In this cross, 50% of the embryos should stain blue. However, if Horka induces loss of the paternally derived CyO d chromosome, a few of the zygotes will be diplo-2//haplo-2 mosaics and show blue//white mosaicism. Seven (3.4%) of the 216 embryos with the transgene showed blue//white mosaicism (Table 5 and Figure 5). The existence of blue//white mosaics clearly shows that in addition to the X and the fourth chromosomes, Horka also renders the second chromosomes unstable. (The blue//white mosaic pattern did not develop in the 603 blue-staining embryos that descended from the control males who carried CyO d but not Horka.) A proportion of the sibling embryos developed to adulthood. The frequencies of X and fourth chromosome loss was 3.4% and 7.8%, respectively (Table 5).

The Horka males carried the TM2 d chromosome with the β-galactosidase transgene in experiments where loss of the third chromosome was assayed. Two (0.8%) of the 251 TM2 d-carrying embryos possessed blue//white mosaicism, indicating that Horka also renders the third chromosome unstable (Table 5). (None of the 408 TM2 d-carrying control embryos showed blue//white mosaicism.)

Detection of the diplo-3//haplo-3 mosaicism was limited. Expression of the β-galactosidase transgene commences only during germ band elongation, at a stage when viability of the haplo-2 or haplo-3 cells is already reduced. A comparison between the frequencies of X, fourth, second and third chromosome loss is complicated by the low viability of the haplo-2 or haplo-3 cells. Monosomy for either chromosome 2 or 3 leads to embryonic lethality (Wright 1970). The stage of death of the diplo-2//haplo-2 and diplo-3//haplo-3 mosaics may also depend on the size and/or position of the haplo clones.

Horka-derived Y chromosomes are not lost: Six experiments were conducted to evaluate whether Horka induces loss of a y’ Y chromosome (Table 6). First, X’/ X’ females were mated with X/ y’ Y Horka males. The body of the regular X’/y’ Y male offspring is wild type. Loss of the y’ Y chromosome in the X’/y’ Y zygotes should lead to the formation of X’y’//X’O wild-type//yellow male mosaics. None of the 3267 X’/y’ Y males were wild-type//yellow mosaic (Table 6).

The Horka-derived X chromosome was nonmarked in the above experiments. Its loss could be detected in the sibling X’X zygotes. Of the 5557 X’X zygotes 237 (4.2%) were gynandromorphs and 99 (1.8%) haplo-4 mosaics. Assuming similar frequencies for the elimination of the X, fourth and the y’ Y chromosomes, formation of ~139 or 38 wild-type//yellow mosaic males was expected. As stated above, none was detected. There were 68 yellow males identified in the above cross besides the 3267 wild-type looking ones. They all were sterile, most likely X’O males. In a second experiment, loss of a w’ Y chromosome was studied: X’w’/ X’w’ females were mated with X/ w’ Y; Horka males (Table 6). Of the 858 X’w’/ w’ Y males, none was X’w’//X’O mosaic showing red//white eye mosaicism. There were 1014 X’X sibling zygotes recovered. Forty-five (4.4%) and 12 (1.1%) were gynandromorphs and haplo-4 mosaics, respectively. Based on these frequencies, formation of 38 or 9 red//white male mosaics was expected. Again, none developed. The above results show that Horka-induced loss of the Y chromosome is at least an order of magnitude less than that of the X or any autosome and may in fact never occur.

Loss of the XY chromosome: Lack of Y chromosome loss could be the consequence of absence of factors that render the Y chromosomes susceptible to Horka action or Y-linked factors that may inhibit the Horka destabilizing action. These alternatives were investigated by studying...
the action of *Horka* on an *XY* chromosome. In this experiment, *X''/X''* females were mated with *XY; Horka* males. Loss of the *XY* chromosome should lead to the formation of *X''XY//X''O* gynandromorphs with white (male) clones in the red (female) eye. Among the 989 *X''XY* zygotes, 21 (2.1%) were gynandromorphs, as identified based on the sexually dimorphic structures. Nine of the 21 *X''XY//X''O* gynandromorphs showed red//white eye mosaicism. Formation of the *X''XY//X''O* mosaics clearly indicates that loss of the *Y* chromosome takes place when it is attached to the *X* chromosome. We thus conclude that the *X* chromosomes and autosomes, but not the *Y* chromosome, carry factor(s) that render them susceptible to *Horka* action.

The origin of the *XO* males: The *Y* chromosomes carried the *y*+ or the *w*+ segment of the *X* chromosome in 11 crosses. The partner females were homozygous for the marker mutations *y* or *w*. There were *y* or *w* exceptional males in addition to the regular *X'/y+Y* or *X'/w+Y* ones. All the exceptional males were sterile, thus likely *XO*. As shown in Figure 3, the frequency of *XO* males is directly proportional to the frequency of gynandromorphs (*r* = 0.96, *P* < 0.01). This result indicates that the formation of *XO* males and gynandromorphs has a *Horka*-related common source. *XO* males also develop among the progeny of males carrying the unstable ring-*X* chromosome *In(1)w''*. In a cross where *y v f mal* females were mated with *In(1)w''/y+Y* males, 712 (10.9%) of the 6528 males were *XO* (SZABAD et al. 1979). Of the sibling 2493 *X'/In(1)w''* zygotes, 854 (34.3%) were gynandromorphs. The *In(1)w''*-related *XO* male and gynandromorph frequency data fit the *Horka*-related ones (Figure 3) and suggest a common route for the formation of *XO* males in the two systems.

Because it is very unlikely that the *XO* males originated through the simultaneous loss of the unstable *X* chromosomes from both zygotic nuclei during the gonomeric division, it may be suggested that the *XO* males developed due to fertilization of the *X*-bearing eggs by nullo-*X* sperm.

*Horka* induces mostly equational nondisjunction during spermatogenesis: The *Horka*-derived *XO* males apparently originated through the fertilization of the *X*-bearing eggs by nullo-*X* sperm. The nullo-*X* sperm may originate due to chromosome loss and/or nondisjunction during spermatogenesis. To decide between the above possibilities, *X/Y; cn bw/ed dp cl; Horka/TM3*,

| TABLE 5 |
| Features of *Horka*-induced major autosome loss |

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Male type</th>
<th>Diplo autosome zygotes</th>
<th>Diplo haplo mosaiscs</th>
<th>XX zygrotes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>%</td>
<td>Total</td>
</tr>
<tr>
<td>Second</td>
<td>Control</td>
<td>603</td>
<td>0</td>
<td>204</td>
</tr>
<tr>
<td></td>
<td><em>Horka</em></td>
<td>216</td>
<td>7</td>
<td>Not determined</td>
</tr>
<tr>
<td>Third</td>
<td>Control</td>
<td>408</td>
<td>0</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td><em>Horka</em></td>
<td>251</td>
<td>2</td>
<td>Not determined</td>
</tr>
</tbody>
</table>

*Only the transgene-carrying zygotes were included.*

| TABLE 6 |
| The lack of *Horka*-induced *Y* chromosome loss |

<table>
<thead>
<tr>
<th>Females</th>
<th>Males</th>
<th>Regular</th>
<th>Obs.</th>
<th>Expb</th>
<th>XO Females</th>
<th>Total</th>
<th>%a</th>
<th>Total</th>
<th>%a</th>
</tr>
</thead>
<tbody>
<tr>
<td>*X'/X'</td>
<td><em>X'/Y; Horka</em></td>
<td>3267</td>
<td>0</td>
<td>199; 58</td>
<td>68</td>
<td>5221</td>
<td>237</td>
<td>4.2</td>
<td>99</td>
</tr>
<tr>
<td>*X''/X''</td>
<td><em>X'/w+Y; Horka</em></td>
<td>858</td>
<td>0</td>
<td>38; 9</td>
<td>20</td>
<td>957</td>
<td>45</td>
<td>4.4</td>
<td>12</td>
</tr>
</tbody>
</table>

Six crosses, with females and males of different genotypes, were studied to assay possible loss of the *y*+*Y* chromosome. The maternally derived chromosomes were labeled with *y*, a recessive marker mutation. Possible loss of the *w''Y* chromosome was analyzed in two crosses. The maternal chromosomes were *w* labeled.

a% of XX zygotes.

The expected figures were calculated based on the frequency of gynandromorphs and haplo-4 mosaics, respectively.
Sb Ser (or +) males were mated with C(2)EN, bw sp females. Progeny derive from the above cross only if the males produce nullo-2 and/or diplo-2 sperm. bw sp flies originate after fertilization of diplo-2 oocytes by nullo-2 sperm. Wild-type, cn bw and ed dp cl flies originate after fertilization of nullo-2 oocytes by diplo-2 sperm. The wild-type class is an indicator of reductional nondisjunction. The cn bw and the ed dp cl classes originate from equational nondisjunction and should theoretically be present in equal numbers.

A single offspring developed in the control (Table 7). The 53 Horka-carrying males yielded altogether 1085 offspring, showing that Horka males do indeed produce sperm with unusual numbers of chromosomes. Only 35 wild-type flies developed among the 1085 offspring (Table 7). There were also 382 cn bw and 122 ed dp cl offspring flies recovered, indicating that Horka induces mostly equational nondisjunction. (The difference between the frequencies of the cn bw and the ed dp cl flies presumably stems from the difference in their viabilities.)

The ratio of nullo-2 and diplo-2 sperm is 1:1 after nondisjunction during spermatogenesis. If the nullo-2 sperm originate only from nondisjunction, the number of the bw sp progeny flies, indicators of the nullo-2 sperm, should be similar to the sum of the wild-type, the cn bw and the ed dp cl classes. If, however, Horka induces loss of the second chromosomes during spermatogenesis, an excess of the bw sp progeny flies should develop. Because the number of bw sp flies (546) was similar to the sum of the others (539) and no excess in the bw sp class emerged (Table 7), we propose that Horka does not induce detectable frequency of chromosome loss during spermatogenesis.

The nullo-2 sperm are likely to be the result of nondisjunction during spermatogenesis. This assumption is supported by the following observation. Both products (the nullo-2 and the diplo-2 sperm) of the equational nondisjunction were recovered from every one of the 53 tested males (Table 7): all 53 yielded bw sp offspring and in addition either cn bw (14 males) or ed dp cl (2 males) or both cn bw and ed dp cl flies (37 males). In the case of loss of the second chromosome, some of the males should have given rise to only bw sp offspring.

Cytological analyses confirmed the presence of aneuploid sperm in the testes of Horka males. The “onion” stage spermatids were analyzed first on squashes of the Horka testes (GONZÁLEZ et al. 1989). In the Horka testes, the diameter of ~10% of the onion stage spermatid nuclei was either larger or smaller than those of the normal spermatid nuclei. These spermatids were aneuploid and carried more or fewer chromosomes than the normal ones (Figure 4). It should be mentioned that

![Figure 3](image-url)  
**Figure 3.**—The linear relationship between the frequency of gynandromorphs and XO males ($r = 0.96$, $P < 0.01$). Notice that the value related to the unstable ring-X chromosome $In(1)w^{tc}$ (in the top right corner) fit nicely the line based on the Horka data (●).

### TABLE 7

<table>
<thead>
<tr>
<th>Males</th>
<th>Males tested</th>
<th>Test period (days)</th>
<th>Total</th>
<th>Wild type</th>
<th>bw sp</th>
<th>cn bw</th>
<th>ed dp cl</th>
<th>Yield of progeny (progeny/male/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mwh e</td>
<td>10</td>
<td>170</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>$5.9 \times 10^{-4}$</td>
</tr>
<tr>
<td>Horka</td>
<td>53</td>
<td>636</td>
<td>1085</td>
<td>35</td>
<td>546</td>
<td>382</td>
<td>122</td>
<td>$3.3 \times 10^{-2}$</td>
</tr>
<tr>
<td>Horka&quot;m&quot;</td>
<td>30</td>
<td>222</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>$4.5 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

The second chromosome constitution of the males was cn bw/ed dp cl. The mwh e, Horka and Horka"m" males also carried the TM3, Sb Ser chromosome. Single males were mated with C(2)EN, bw sp females. Horka"m" stands for the EMS-induced revertant of Horka. Wild-type flies indicate reductional nondisjunction. The bw sp class results from nullo-2 sperm. The cn bw and ed dp cl classes are indicators of equational nondisjunction. The “test period” is the sum of days through which the lots were tested.
all the spermatids with reduced diameter were associated with a nucleus of increased size, and vice versa, supporting the genetic data: *Horka* induces nondisjunction during spermatogenesis and not loss of chromosomes.

Cytological analysis of chromosome content of primary and secondary spermatocytes confirmed the genetic data. The first meiotic divisions appeared normal in testes of the *Horka* males. Abnormalities were de-
ected primarily in the second meiotic division. The abnormalities include (1) abnormal condensation and disarrangement of chromosomes at metaphase, (2) lagging of chromosome arms that appear to reach the telophase nuclei late and (3) uneven distribution of chromatin at telophase, which is an indication of aneuploid segregation (Figure 4). It is an important observation that satellite nuclei, which represent lost chromosomes (Puro 1991), were not detected. Thus, it is very likely that the aneuploid sperm originate through nondisjunction rather than chromosome loss.

The frequency of nullo-2 and diplo-2 sperm was determined as follows. Larvae hatched from 94 (2.2%) of the 4362 eggs collected from C(2)EN, bw sp females mated with cn bw/ed dp cl; Horka/TM3 (or +) males. Because nullo-, mono- and trisomy of the second chromosome results in embryonic lethality, the eclosing larvae represent the viable diploid combinations. Viable offspring originate from 50% of the aneuploid sperm, that is, when they randomly combine with C(2)EN-derived ova. Thus, the frequency of aneuploid sperm is $2 \times 2.2\% = 4.4\%$. It should be mentioned that 4.8% of the descending females were gynandromorphs in the C(2)EN experiment.

Aneuploid sperm may, in principle, originate through nondisjunction during mitotic proliferation of the spermatogonial cells (Szabad 1986). Wild-type and cn bw plus ed dp cl flies should develop with equal frequencies after mitotic nondisjunction. As the results presented in Table 7 contradict the expectations, it is safe to conclude that Horka does not induce nondisjunction during the mitotic proliferation period of the spermatogonial cells.

Many cysts of the secondary spermatocytes appeared to be normal in the Horka males. However, most if not all the nuclei appeared abnormal in the affected cysts showing an all or none reaction (Figure 4). This observation is related to the fact that spermatids develop side by side in the cytoplasm of a common syncytium (Lindsley and Tokuyasu 1980). A mutant cytoplasmic Horka factor thus may exert its effect on every nucleus within the cyst.

Arrangement of the spermatid heads was abnormal in many sperm bundles in the males showing abnormal cysts (Figure 4). Whether sperm of the abnormal bundles participate in fertilization is not clear. Lischutz and Meyer (1977) and Casllillon et al. (1993) described recessive male-sterile mutants in which sperm arrangement within the bundles was similar to that seen in abnormal bundles of the Horka males.

Features of the Horka-induced gynandromorphs: To determine the average frequency of maleness (the probability that a given structure is male), we analyzed 80 Horka-derived adult gynandromorphs with 42 landmarks each. The average frequency of maleness was 47 ± 27%, and this is in agreement with the hypothesis that the Horka-derived X chromosome can be lost as early as the gonomeric division. The proportion of the male structures varied between 6% and 94% in the different gynandromorphs, indicating that the Horka-derived X chromosome can be lost during the gonomeric plus the subsequent cleavage divisions and first during the cleavage divisions. We mention, for comparison, that the average frequency of maleness in gynandromorphs generated by the unstable ring-X chromosome, In(1)w^{ec}, is ~46–48% (Table 8) (Hall et al. 1976; Janning 1978; Szabad et al. 1979).

The conclusion that the Horka-derived X chromosomes may indeed be lost during different stages after fertilization is confirmed by analysis of 47 blastoderm stage Horka-derived gynandromorphs. Two- to 4-hr-old embryos were collected and fixed from a cross between y f mal females and Horka/TM3, Sb Ser males. The female cells were identified with the Sex-lethal antibody using immunohistochemical techniques (Bopp et al. 1991). Presence of female (staining) cells was apparent in 756 of the 1821 embryos. Seventy-eight (10.3%) of the 756 embryos were gynandromorphs because they were composed of both female and male cells. (Mosaic embryos did not develop among the >2000 embryos that descended from y f mal females and mus e/TM3, Sb Ser males.) The mosaic pattern of 47 gynandromorphs was drawn onto standard grids. The relative size of the female and male patches was measured by planimetry. (The 31 gynandromorphs that developed beyond the blastoderm stage were omitted from the present study.) The average size of the female and male patches was 50 ± 28% and varied between 9% and 90% (Table 8 and Figure 5). Analysis of blastoderm gynandromorphs clearly showed that the Horka-derived X chromosome, like the unstable In(1)w^{ec} ring-X chromosome, can be lost (1) during the gonomeric division (in 13 embryos the ratio of the staining and nonstaining area was $\sim1:1$), (2) during the gonomeric and the subsequent cleavage divisions (in 18 embryos the nonstaining area included 60–90% of the blastoderm surface) and (3) during the cleavage divisions (16 embryos had small nonstaining clones; Figure 5).

For a comparison of gynandromorphs generated by Horka and the unstable In(1)w^{ec} ring-X chromosome, 80 abdomens of each type of adult gynandromorph were analyzed under the compound microscope. Distribution of the number of male bristles on mosaic hemitergites is shown in Figure 6. The frequency of mosaic hemitergites with more than one yellow bristle/mosaic hemitergite was not significantly different between the two types of gynandromorphs ($P > 0.05$, chi-square test; 88/800 vs. 117/800 for Horka and In(1)w^{ec} gynandromorphs, respectively; Figure 6). This result may suggest similar mechanisms for loss of the Horka exposed and the In(1)w^{ec} X chromosomes. However, in the In(1)w^{ec} gynandromorphs, 71 of the 800 hemitergites
carried XO clones composed of a single y bristle each (see Ripoll 1972). Only four such clones developed on the 800 hemitergites of the Horka-generated gynandromorphs (Figure 6). The majority of the small XO clones originated most likely from the loss of the In(1)wC chromosome during proliferation of the histoblasts, precursors of the tergites (Garcia-Bellido 1973; Guerra et al. 1973; Sanchez and Nöthiger 1983). It is also possible that some of the small XO y clones originate due to spontaneous mitotic recombination between the In(1)wC and the y v f mal chromosome (Merriam et al. 1972). The absence of small male clones in the Horka-generated gynandromorphs may indicate decay, dilution and/or replacement of the Horka-derived mutant gene product during the course of the cleavage divisions.

**DISCUSSION**

**Horka**, a new chromosome destabilizing mutation: Understanding the possible function of the normal Horka gene product may be facilitated by a comparison of the Horka mutant phenotypes with those characteristic of the other chromosome-destabilizing mutants. There have been three such mutations characterized in detail so far: nonclaret disjuncional (ncd), which was originally identified by the claret nondisjuncional (cnd) alleles; mitotic loss inducer (mit) and paternal

<table>
<thead>
<tr>
<th>Type of gynandromorph</th>
<th>Stage of examination</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blastoderm</td>
<td>Larval epidermis</td>
<td>Adult cuticle</td>
</tr>
<tr>
<td><em>Horka</em></td>
<td>50 ± 28 (47)</td>
<td>Not determined</td>
<td>47 ± 27 (80)</td>
</tr>
<tr>
<td>In(1)wC</td>
<td>34 ± 25° (18)</td>
<td>46 ± 24° (140)</td>
<td>49 ± 29 (80)</td>
</tr>
</tbody>
</table>

Values are means ± SD with number of cases in parentheses.

* From Table 2 of Zalokar et al. 1980.
* From Szabad et al. 1979.
loss (*pal*) (Baker and Hall 1976; Ashburner 1989; Lindsley and Zimm 1992). When exposed to mutant *ncd*, *pal* or *mit* gametogenesis, the chromosomes are rendered unstable and can be lost in the descending zygotes. The mutations *ncd* and *mit* act maternally, and *pal* acts paternally. In *ncd* mostly the maternally, in *mit* both the maternally and the paternally, and in *pal* only the paternally derived homologs of the zygote chromosomes are affected. *Horka* is a new chromosome destabilizing mutation. Although *ncd*, *mit* and *pal* are recessive, *Horka* is dominant. Chromosome losses take place in the zygotes descending from *Horka* males; thus *Horka* possesses a dominant paternal effect. Like in the case of *pal*, only the paternally derived chromosomes are lost in the *Horka*-derived zygotes. The chromosome destabilizing effect of *Horka* is reminiscent of chromosome imprinting inasmuch as the paternally derived chromosomes are eliminated (Moore and Haig 1991). (The dominant maternal effect of *Horka* and the loss-of-function phenotypes will be described elsewhere; J. Szabad et al., in preparation.)

Like in the case of *ncd*, *mit* and *pal*, the chromosome destabilizing effect of *Horka* depends on the genetic background. As in *ncd*, *mit* and *pal*, the frequency of *X* and fourth chromosome loss is usually in the range of 2–4%. However, factors, not readily identifiable, can increase the frequency of *X* chromosome loss to over 20%. Furthermore, the different chromosomes possess different *Horka* sensitivity. Although the *Horka*-exposed *X*, second, third, fourth and the *XY* chromosomes can be lost, *Y* and *w* *Y* chromosomes do not respond to *Horka* action, confirming that the *Y* chromosome is more stable than either the *X* or the fourth chromosomes (Ashburner 1991). Gelbart (1974) reported that neither a marked *Y* nor the *XY* chromosomes are frequently eliminated by *mit*.

In contrast to *ncd* and *pal*, where loss of the *X* and the fourth chromosomes is positively correlated, implying that the *XO* and the haplo-4 clones are almost always coincident, loss of the *Horka*-derived *X* and fourth chromosomes is not correlated; *XO* cells are not haplo-4 and vice versa.

As in the case of *ncd* and *pal*, loss of the *Horka*-derived *X* chromosome can take place during the gonomic division, as indicated by the production of gynandromorphs with 50% female and 56% male tissues (Figure 5) (Baker 1974; Portin 1978). However, multiple and late losses are also apparent as revealed by gynandromorphs with male clones comprising larger or smaller than 50% of the zygote’s tissues, respectively. *Horka*, like the other chromosome destabilizing mutations and the unstable ring-*X* chromosome *In(1)* *w*+, is a useful tool for the generation of gynandromorphs (Szabad and Nothiger 1992) as well as diplo//haplo mosaics.

Beside its chromosome destabilizing action, *Horka* induces nondisjunction, primarily equational, but not chromosome loss during spermatogenesis. (Note that the *Horka*-related mutant phenotypes stem from the same mutation as shown by the linear relationship between the frequencies of *X* chromosome loss and the aneuploid sperm and also the concomitant reversion of these effects.) Of the other chromosome destabilizing mutants, *ncd* induces nondisjunction and *pal* induces chromosome loss. (cf. Lindsley and Zimm 1992).

The possible function of the *Horka* protein: It is rather unlikely that the normal *Horka*–gene product specifies a component of the spindle apparatus, because only the paternally derived chromosomes are lost in the offspring. In the case of cytoplasmic factors, elimination of both maternally and paternally derived chromosomes would be expected as was described for *mit* (Gelbart 1974). We propose, based on the chromosome destabilizing mutant phenotypes, that the normal *Horka*–gene product is required—directly or indirectly—for appropriate chromosome segregation. Because the segregation behavior of the chromosomes resides in the centromere and/or in the neighboring region [as has been elegantly shown based on the segregation patterns of the yeast chromosomes with replaced centromeres (Simchen and Hugger 1993)], we also propose that the *Horka*–gene product is required for normal function of the centromere and/or nearby chromosome regions.

The *Horka* mutant phenotype is analogous to those described for *ncd*: (1) an elevated frequency of nondisjunction in the *ncd* homozygous females. (2) *ncd* is maternal in action and (3) mostly the maternally derived chromosomes are lost in the descending zygotes (Davis 1969; Sequiera et al. 1989). *Horka* induces nondisjunction chromosome loss in the zygotes and only chromosomes originating from the mutant...
parent are lost. Molecular analysis of the *ned* gene showed that the *ned* gene encodes a kinesin-like minus end-directed microtubule motor protein required for chromosome segregation through association with the kinetochore (YAMAMOTO et al. 1989; ENDOW et al. 1990; MCDONALD and GOLDSTEIN 1990; MCDONALD et al. 1990; ENDOW 1992, 1993; THEURKAUF and HAWLEY 1992). Thus, it appears likely that the normal *Horka* + product is required for centromere-associated function.

Baker (1975) proposed, based on the *pal*-related mutant phenotypes, that the normal *pal* gene "specifies a product that is component of, or interacts with, the centromeric region of chromosomes." The chromosomes may have defective centromeric regions in the *pal* mutant males and can be lost. The molecular nature of the *pal* product is not known. There are apparent similarities between *pal* and *Horka*: both act parentally and only the paternal chromosomes are lost in the zygotes. The similar mutant phenotypes further support the hypothesis that the normal *Horka* + product is likely to be required for a centromere-related function.

The gain-of-function nature of *Horka* and its relevance to the *Horka* + function: Whether the normal *Horka* + product does indeed function in the centromere (or in a nearby chromosome region) cannot be taken for granted. The "traditional" genetic dissection makes use of loss-of-function recessive mutations and deduces the normal gene function from the mutant phenotype, as in the case of, for example, *ned* and *pal*. *Horka* is a dominant gain-of-function mutation. It can be reverted and its encoded mutant gene product perdured in the nonmutant germ line cells (ERDÉLYI and SZÁBAD 1989). *Horka* is neomorphic: its dominant paternal effect does not depend on the number of wild-type copies present in the *Horka* males and the *Horka* / deficiency combination is lethal (J. SZÁBAD, unpublished data). Thus, in principle, the mutant *Horka* product may interfere with another process from that in which the normal *Horka* + product is involved.

However, results to be described in the forthcoming paragraph support our hypothesis concerning the possible function of the normal *Horka* + product. It is apparent that mutant products encoded by all the dominant female-sterile (Fs) mutations studied thus far disturb the same process in which the normal protein functions. [Recall that *Horka* is one of the Fs mutations; ERDÉLYI and SZÁBAD 1989.] The dominant as well as the recessive *Toll*, *easter* and *dorsal* Fs alleles interfere with establishment of the dorsoventral embryonic polarity. Although the dominant alleles induce the formation of ventralized and/or lateralized embryos, dorsalized embryos develop when function of the above genes is eliminated (GOVIND and STEW-ARD 1991; ST. JOHNSTON and NÜSSLIN-VOLHARD 1992). The Fs (2) *torso* + revertible allele leads to the formation of "all terminal" embryos (KLINGLER et al. 1988; SZÁBAD et al. 1989). In contrast, terminal structures do not develop when function of the *torso* gene product is absent (SCHÜPBACH and WIESCHAUS 1986). The *rolled* Fs allele disturbs function of the same signal transducing pathway in which the *rolled* + product functions (BRUNNER et al. 1994). The *Tomaj* Fs alleles, which identify the so-called maternal alpha-tubulin gene, interfere with microtubule formation (K. JOSVAY, E. MÁTÉ and J. SZÁBAD, unpublished data). Microtubules do not form in the absence of the maternal alpha-tubulin molecules (MATTHEWS et al. 1993). Although the data listed above support our hypothesis concerning the function of *Horka* + in the centromere and/or nearby regions, direct evidence will emerge after only molecular cloning of the gene.

We are grateful to Drs. D. BOPP for providing the Sex-lethal antibody; H. BELL for the lines with the "all over" expression of the β-galactosidase transgene and M. ASHBURNER, ROLI NÖTHEGER, TRUDI SCHÜPBACH and an anonymous reviewer for discussions and comments on the manuscript. The "Horka project" was supported by grant no. 922 from the Hungarian National Science Foundation to J. SZÁBAD and also by the Bástyi-Holczer Memorial Foundation.

Note added in proof: Complementation analyses with alleles of genes situated around the *Horka* locus showed that *Horka* is allelic to the mutagen-sensitive mutant mus309 (BOYD et al. 1981) and to *ck12* (GAUSZ et al. 1981).

LITERATURE CITED


Paternal Effect and Chromosome Loss


