Sex-Specific Differences in Meiotic Chromosome Segregation Revealed by Dicentric Bridge Resolution in Mice

Kara E. Koehler,*[,1] Elise A. Millie,* Jonathan P. Cherry,* Paul S. Burgoyne,[†] Edward P. Evans,[‡] Patricia A. Hunt* and Terry J. Hassold*

**Department of Genetics and the Center for Human Genetics, Case Western Reserve University and the University Hospitals of Cleveland, Cleveland, Ohio 44106-4955, [†]Division of Developmental Genetics, MRC National Institute for Medical Research, London NW7 1AA, United Kingdom and [‡]Department of Zoology, University of Oxford, Oxford OX1 3PS, United Kingdom*

Manuscript received April 18, 2002
Accepted for publication August 13, 2002

ABSTRACT

The meiotic properties of paracentric inversion heterozygotes have been well studied in insects and plants, but not in mammalian species. In essence, a single meiotic recombination event within the inverted region results in the formation of a dicentric chromatid, which usually breaks or is stretched between the two daughter nuclei during the first meiotic anaphase. Here, we provide evidence that this is not the predominant mode of exchange resolution in female mice. In sharp contrast to previous observations in other organisms, we find that attempts to segregate the dicentric chromatid frequently result not in breakage, stretching, or loss, but instead in precocious separation of the sister centromeres of at least one homolog. This often further results in intact segregation of the dicentric into one of the meiotic products, where it can persist into the first few embryonic divisions. These novel observations point to an unusual mechanism for the processing of dicentric chromosomes in mammalian oogenesis. Furthermore, this mechanism is rare or nonexistent in mammalian spermatogenesis. Thus, our results provide additional evidence of sexual dimorphism in mammalian meiotic chromosome behavior; in "stressful" situations, meiotic sister chromatid cohesion is apparently handled differently in males than in females.

MEIOSIS is the process by which the genetic material is divided in half in preparation for the next generation. This reduction occurs at the first meiotic division and is achieved by the pairing and disjunction of homologous chromosomes. Resolution of meiotic crossing over at anaphase I allows homologs to move freely to opposite poles. However, sister chromatid cohesion is maintained at the centromere of each homolog until anaphase II, when sister chromatids segregate from each other. These two processes—chiasma resolution at anaphase I and release of sister centromere cohesion at anaphase II—are crucial for accurate partitioning of the genetic material to daughter cells. Indeed, interference with these processes can result in adverse consequences for the cell. For example, in certain situations, crossover resolution may not always remove all physical hindrance to segregation. The paracentric inversion heterozygote, in which crossing over can result in the formation of a dicentric anaphase bridge, is a classic example (Figure 1).

The fundamental properties of meiosis in inversion heterozygotes were first elucidated early in the last century, via cytological observations in *Zea mays* by McClintock (1931, 1933) and genetic studies in *Drosophila melanogaster* by Sturtevant and Beadle (1936). Crossing over within the inversion loop results in the formation of a dicentric bridge and an acentric fragment, as well as two structurally normal chromatids (Figure 1B). These early studies suggested that, in maize, the dicentric bridge was broken during anaphase I. In flies, by contrast, the dicentric was selectively eliminated from inclusion in the gamete; *i.e.*, it was stranded in the plane of the first meiotic division and thus unable to participate in the second. However, studies in the subsequent decades demonstrated that, in fact, paracentric inversions in both maize and flies can exhibit a variety of behaviors, including breakage at anaphase I or mechanical elimination in both organisms, depending on the specific inversion (Novitski 1955; Rhoades 1955). In contrast to the many analyses in flies and maize, inversions in mice have been identified and studied only within the past several decades and are far less well understood. In fact, the original approach to identify inversions in mice was based on observations in maize and flies: male progeny of mutagenized mice were tested for inversion carrier status by screening for anaphase I bridges (Roderick 1971). However, a high rate of bridge formation is not a universal characteristic of paracentric inversions, and may in fact appear to be so only in mice because most extant paracentric inversions were selected on the basis of this property (Roderick and Hawes 1974; Beechey and Evans 1996). For example, In(2)2H, an

[1]*Corresponding author:* Department of Genetics and the Center for Human Genetics, Case Western Reserve University and the University Hospitals of Cleveland, 10900 Euclid Ave., Cleveland, OH 44106-4955. E-mail: kek4@po.cwru.edu

inversion picked up due to its association with the *suppressor of agouti* mutation, forms no detectable bridges in heterozygous males (Evans and Phillips 1978).

Since their identification, studies of murine paracentric inversions have largely focused upon meiotic prophase at the expense of later stages, analyzing synaptonemal complex formation and behavior in a handful of inversion heterozygotes and double heterozygotes (mice heterozygous *in trans* for two different inversions of the same chromosome; Ford *et al.* 1976; Chandley 1982; Moses *et al.* 1982; Tease and Fisher 1986; Borodin *et al.* 1990, 1992; Gorlov and Borodin 1995; Rumpler *et al.* 1995). Furthermore, most of these studies have focused on males, so little is known about inversion behavior in female mice, and virtually nothing is known about the segregation of inversion products in either sex.

In this report, we describe studies of the products of meiosis I in female mice heterozygous for different inversions. Our analyses indicate that, unexpectedly, the most frequent result of dicentric bridge formation is precocious loss of sister chromatid cohesion at one and often both homologous centromeres. Furthermore, the dicentric chromatid often is retained within a meiotic product and continues to persist, via replication and segregation, through the first few mitotic divisions. This is an unequivocal departure from the expected meiotic progression: homologous centromeres usually segregate intact from each other at anaphase I, and the centromeres of sister chromatids normally maintain cohesion until anaphase II. We propose that the forces exerted on the dicentric chromatid by the attempted disjunction of physically linked homologous centromeres are responsible for the precocious centromere separation event(s).

Strikingly, although this unusual behavior is typical for female mice, it is not observed in the male germline. The disparity between the meiotic outcomes in females *vs.* males is dramatic and provides additional evidence of sex-specific differences in the control of mammalian meiotic chromosome segregation.

MATERIALS AND METHODS

Production of inversion homozygotes and heterozygotes: For meiotic studies, breeding stock of control C57BL/6J inbred mice and of mice carrying the In(X)1H and In(19)37Rk inversions was obtained from Jackson ImmunoResearch Laboratories (West Grove, PA) and maintained as inbred stocks via brother × sister matings. Inversion heterozygotes were generated by crossing C57BL/6J females to a male hemi- or homozygous for the inversion. All oocytes were collected from ~4-week-old mice. For studies of preimplantation embryos, In(X)1H animals were obtained from Harwell.

Meiocyte and embryo culture conditions and fixation: Oocytes for the analysis of both metaphase II and anaphase I were collected and cultured as previously described (Hunt *et al.* 1995). For cytogenetic analysis, air-dried preparations of MII-arrested oocytes were made according to the method of Tarkowski (1966). For anaphase I analysis, GV-stage oocytes were cultured for 12 hr, embedded in fibrin clots, and fixed as described previously (Hunt *et al.* 1995). Air-dried spermatocyte preparations were made as described in Evans *et al.* (1964). The culture, fixation, and analysis of preimplantation-stage embryos were performed as described previously (Burgoyne 1993).

Fluorescence *in situ* hybridization analysis of oocytes and spermatocytes at metaphase II: Chromosome paint probes for mouse chromosomes X and 19 were obtained from Vysis. Unless otherwise noted, all incubations were in a 37° humid chamber and all solutions were pH 7.0.

Slides were soaked in 2× SSC for 30 min at 37°, dehydrated in a cold ethanol series (70, 80, 90, and 100%; 2 min each), and air dried. Slides were denatured in 70% formamide/2× SSC for 2 min, dehydrated in a cold ethanol series, and air dried. Each slide received 10 μl of chromosome paint probe that had been denatured at 72° for 10 min and preannealed at 37° for 2 hr. Slides were incubated overnight, washed in 2× SSC at 72° for 5 min, and washed in PN buffer, pH 8.0 (0.1 M $NaH_2PO_4 \cdot H_2O$, 0.1 M NaH_2PO_4, 0.05% Nonidet P-40). Slides were incubated with 200 μl of blocking solution (3% BSA/4× SSC) for 5 min, incubated with 30 μl of fluorescein- or rhodamine-labeled antidigoxigenin (Boehringer Mannheim, Indianapolis; diluted 1:200 in blocking solution) for 20 min, washed in three changes of PN buffer for 2 min each, and stained with 4′,6-diamidino-2-phenylindole.

Yeast artificial chromosome fluorescence *in situ* hybridization to fetal liver metaphases: The fluorescence *in situ* hybridization (FISH) procedure was as described above for oocytes, except that after the overnight incubation, slides were washed in 50% formamide/2× SSC at 43° for 15 min, in 2× SSC at 37° for 8 min, and in PN buffer, pH 8.0. Additionally, 50 μl of fluorescein- or rhodamine-labeled antidigoxigenin or avidin was used for detection.

FISH analysis of intact oocytes captured at anaphase I: The methodology used has been previously described in Hunt *et al.* (1995). Chromatin was counterstained with propidium iodide.

Scoring: Coded slides of air-dried oocytes and spermatocytes were scored on a Zeiss epifluorescence microscope by two independent observers using guidelines for interpreting the recombinant/aberrant products of inversion heterozygotes as described previously (Figure 1; Rhoades 1955).

Inversion breakpoint determination: Cytological breakpoints have been reported previously for In(X)1H (as A1-F4), but not for In(19)37Rk (Beechey and Evans 1996). To define approximate centimorgan positions for the breakpoints for both inversions, we selected a series of nonchimeric yeast artificial chromosomes (YACs) for each inversion-bearing chromosome (Figure 2). Individual YACs from the WI/MIT-820 Mouse YAC library were obtained from Research Genetics (Huntsville, AL) and labeled using the Bionick labeling system (GIBCO BRL, Gaithersburg, MD). YACs were assigned centimorgan positions along the chromosome using the Mouse Genome Database (MGD) map. To do this, we converted each YAC's position on the MIT map to a position on the MGD map by comparing the positions of simple sequence length polymorphism markers on the YAC for both maps. Two at a time, in all possible combinations, the YACs were hybridized to fetal liver metaphases from inversion heterozygotes, prepared as described by Bean *et al.* (2001). By comparing the relative position and order of each pair of YACs in a heterozygous animal, the two YACs spanning each breakpoint location were identified (Figure 2).

Although chromosomes X and 19 are quite different in size, both inversions cover the majority of the chromosome, with

a proximal breakpoint that leaves an interstitial region of negligible size between the centromere and the breakpoint (Figure 2).

RESULTS

Interpreting the products of meiosis I in female inversion heterozygotes: To examine the products of meiosis I (MI), we analyzed a total of 310 MII-arrested oocytes from heterozygotes and 316 oocytes from homozygotes for inversions In(X)1H and In(19)37Rk (for breakpoints, see Figure 2). FISH paint probes for chromosomes X or 19 were used to identify the inversion-bearing chromosome. Approximately 80% of the time, it was possible to accurately analyze only the oocyte, as the polar body chromatin was degraded. Examples of observed segregation products are illustrated in Figure 3.

The data in Table 1 summarize our direct cytological observations of the products of MI. For simplicity in interpretation, the observed products have been grouped into three categories, as follows:

1. *Normal segregation*: This classification involves meioses in which there were no apparent exchanges within the inversion loop (but see Figure 1, A and C). Such situations are effectively normal and result in homologous products that resolve into physically distinct and freely segregable entities at anaphase I (Figure 3A). Overall, 154/310 (49.7%) MII-arrested oocytes from inversion heterozygotes and all 316/316 (100%) oocytes from inversion homozygotes fell into this category.
2. *Precocious sister chromatid separation and intact dicentric segregation*: This category includes cells with an intact dicentric chromosome in one of the two meiotic products (oocyte or first polar body). In these cases, exchange within the inversion loop produced a dicentric chromatid at metaphase I, but premature loss of sister centromere cohesion resulted in intact segregation of the dicentric to one pole, with or without an accompanying single sister chromatid and/or acentric fragment (Figure 3, B and C). Overall, 106/310 (34.2%) MII-arrested oocytes from inversion heterozygotes fell into this category.

There were also cells in which the presence of an intact dicentric was not directly observed but could be inferred; *i.e.*, only one meiotic product was analyzable and it contained a single sister chromatid from the inversion chromosome, with or without an accompanying acentric fragment. Because single chromatids and acentric fragments were never observed in oocytes from inversion homozygotes, we concluded that, in these cases, the reciprocal (unscorable) product contained the intact dicentric chromatid. The inclusion of the 33 such cells identified in heterozygotes brings the total of MII-arrested oocytes

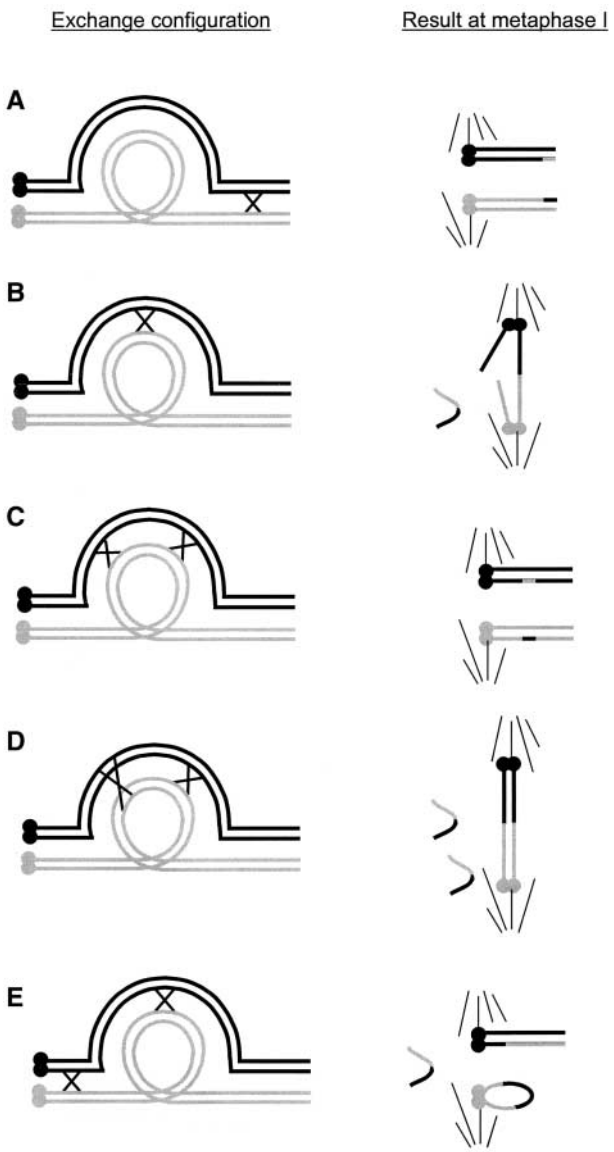

Figure 1.—Meiotic exchanges and their consequences in a paracentric inversion heterozygote. (A) An absence of exchange within the inversion loop results in unhindered segregation of the two homologs from each other at the first meiotic division, just as they would in a situation of homozygosity for either the inverted or the normal sequence chromosome. (B) A single crossover within the loop results in the formation of a dicentric chromatid and an acentric fragment. The two nonrecombinant chromatids remain intact. Most multiple exchange configurations will also yield these products at anaphase I. (C) A two-strand double crossover within the loop results in rescue from dicentric chromatid formation. Two chromatids are recombinant. (D) A four-strand double crossover within the inversion loop results in the formation of two dicentric chromosomes (double bridge) and two acentric fragments. (E) A three-strand double crossover—with one exchange event within the inversion loop and the second in the interstitial region—results in the formation of one normal homolog, one ring chromosome, and one acentric fragment. The dicentric ring will form a bridge during anaphase II, when sister centromeres normally lose cohesion and segregate from each other.

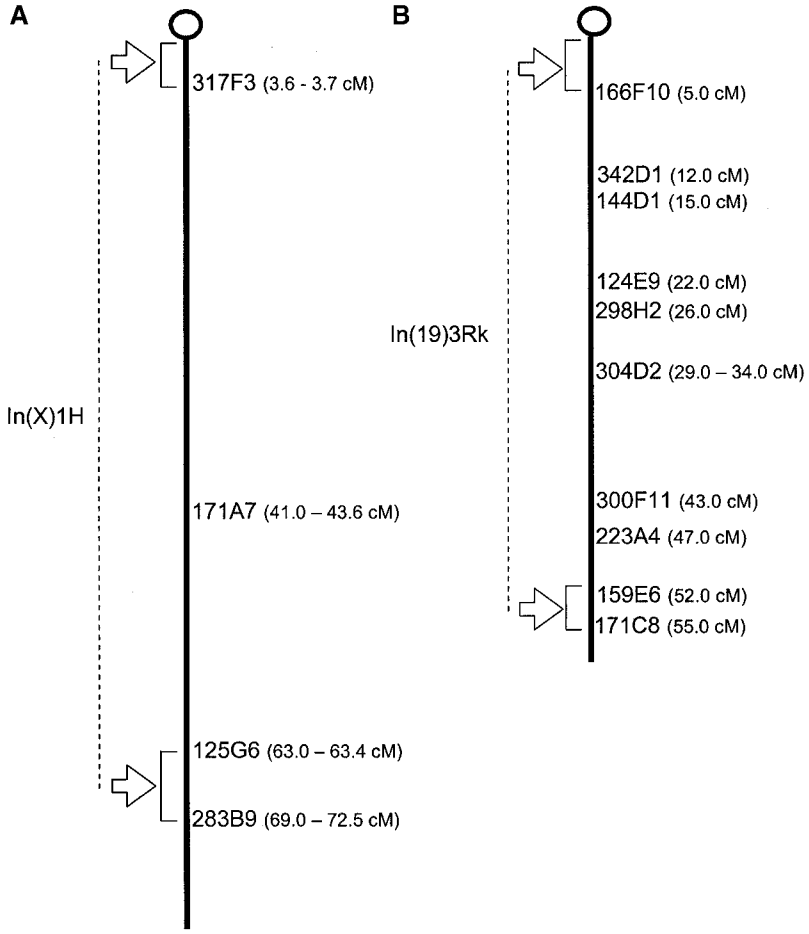

FIGURE 2.—Cytogenetic breakpoint locations for paracentric inversions. (A) Chromosome X (81 cM). (B) Chromosome 19 (57 cM). YACs from the WI/MIT-820 Mouse Library, indicated here with their approximate positions in centimorgans on the Mouse Genome Database map, were used to cytogenetically identify the inversion breakpoints for paracentric inversions used in this study. Arrows and brackets indicate the map intervals in which each breakpoint occurs for (A) In(X)1H and (B) In(19)37Rk.

containing an intact dicentric chromatid with at least one precociously separated sister centromere to 139/310 (44.8%).

3. *Other meiotic products*: This category applies to 17/310 (5.5%) of MII-arrested oocytes from inversion heterozygotes and includes less commonly observed cells in which a dicentric chromatid formed but broke and/or stretched instead of segregating (Figure 3E). Broken chromatids, usually accompanied by an acentric fragment, were observed in 9/310 (2.9%) MII-arrested oocytes. Stretched chromatin bridges were observed rarely and were not included in the total cell count because it was generally difficult to obtain accurate chromosome counts for the oocyte and polar body involved (*e.g.*, Figure 3D). Products of this type were extremely rare in both genotypes studied.

We have also included in this category 8/310 (2.6%) oocytes in which a double dicentric bridge formed (resulting from a four-stranded double crossover within the inversion loop; see Figure 1D) and segregated intact to one pole. While such intact segregation is as much a deviation from the normal meiotic process as the dicentrics described in category 2, in this situation there was no precocious loss of centromere cohesion between sister chromatids.

Analysis of dicentric chromosome behavior in intact oocytes: Because the fixation technique for conventional cytogenetic preparations destroys the three-dimensional architecture of the cell, we analyzed intact oocytes from In(19)37Rk heterozygotes to verify that premature separation of the dicentric chromosome from its sister chromatids was not an artifact. Intact oocytes fixed at anaphase I were immunostained to visualize the spindle and centromeres and counterstained with a chromatin stain. The dicentric chromosome was clearly visible in many oocytes, lagging at the metaphase plate while the two groups of homologous chromosomes moved toward opposite poles (Figure 4). Strikingly, in most anaphase I preparations the dicentric appeared to have already lost cohesion with both structurally normal sister chromatids.

Analysis of dicentric chromosome behavior in preimplantation embryos: To determine whether the dicentric chromatid was able to segregate intact not only during the meiotic divisions but also during the early cleavage divisions, we karyotyped 214 two- to eight-cell embryos derived from female In(X)1H heterozygotes. Thirty-five

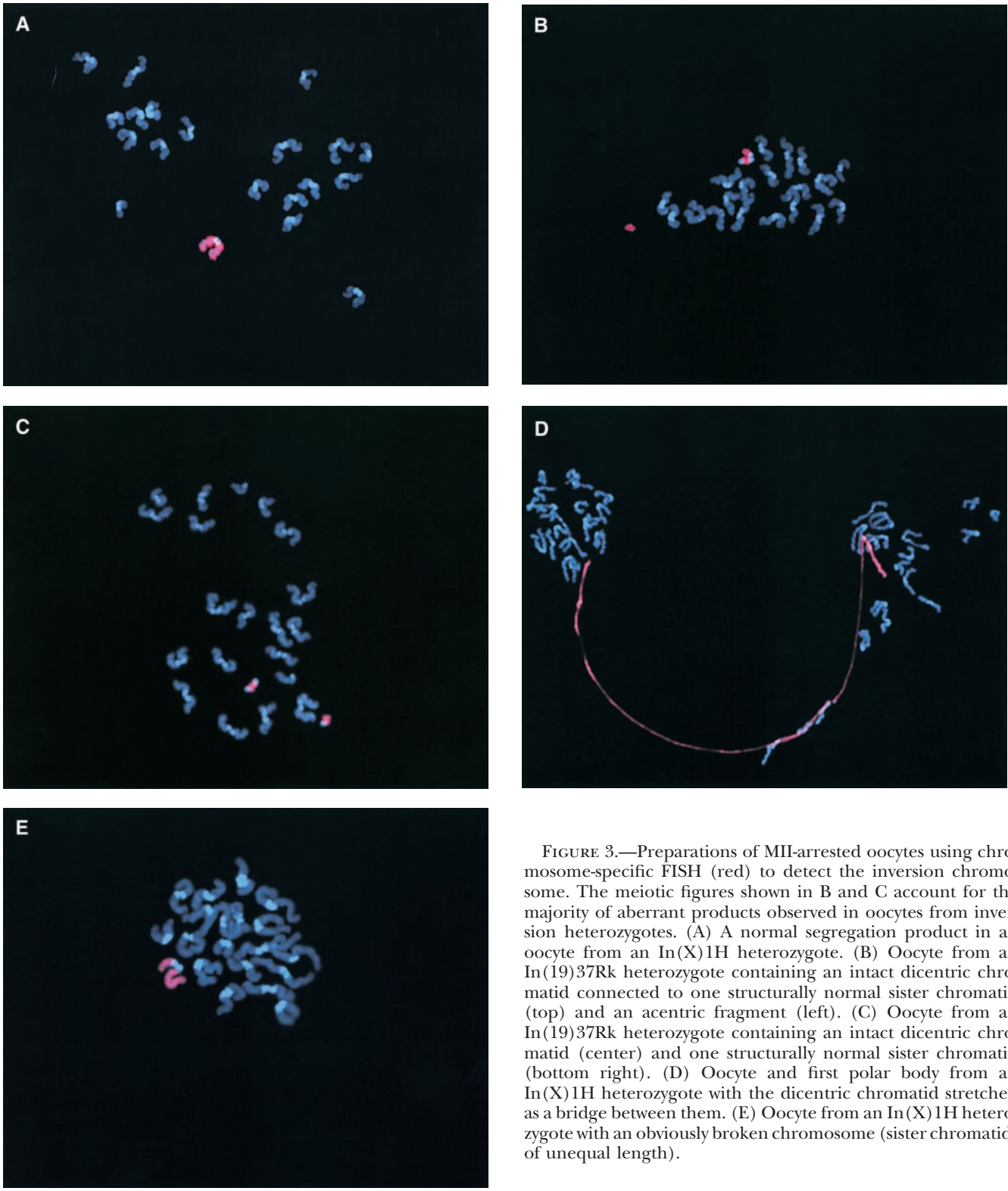

Figure 3.—Preparations of MII-arrested oocytes using chromosome-specific FISH (red) to detect the inversion chromosome. The meiotic figures shown in B and C account for the majority of aberrant products observed in oocytes from inversion heterozygotes. (A) A normal segregation product in an oocyte from an In(X)1H heterozygote. (B) Oocyte from an In(19)37Rk heterozygote containing an intact dicentric chromatid connected to one structurally normal sister chromatid (top) and an acentric fragment (left). (C) Oocyte from an In(19)37Rk heterozygote containing an intact dicentric chromatid (center) and one structurally normal sister chromatid (bottom right). (D) Oocyte and first polar body from an In(X)1H heterozygote with the dicentric chromatid stretched as a bridge between them. (E) Oocyte from an In(X)1H heterozygote with an obviously broken chromosome (sister chromatids of unequal length).

(16.4%) embryos either were unanalyzable or had no dividing blastomeres. Of the remaining 179 embryos, 129/179 (72.1%) had normal karyotypes, 31/179 (17.3%) contained no maternal X chromosome contribution (*i.e.*, were XO or OY), 11/179 (6.1%) contained a dicentric chromosome, and 5/179 (2.8%) contained one or more acentric fragments (Table 2; Figure 5). Among the dicentric chromosomes, a few "mirror image" dicentrics of varying sizes were also observed (*e.g.*, Figure 5C). The chromosome constitution of each cell scored from embryos with these chromosome abnormalities is presented in Table 3.

TABLE 1

Products of MI in female inversion heterozygotes and homozygotes

Genotype of female	*N*	Normal segregation[a] (%)	PSSC/intact dicentric[b] (%)			Broken (%)	Double dicentric (%)	Stretched bridges
In(X)1H/In(X)1H	133	133 (100)	0	0	0	0	0	0
In(X)1H/+	121	56 (46.3)	39	2 (42.1)	10	6 (5.0)	8 (6.6)	3 —
In(19)37Rk/In(19)37Rk	183	183 (100)	0	0	0	0	0	0
In(19)37Rk/+	189	98 (51.8)	60	5 (46.6)	23	3 (1.6)	0 —	0 —

[a] Despite the absence of exchange within the inverted region, these chromosome pairs are not nonexchange. In some cases, exchange occurs outside the inversion (K. E. Koehler and T. J. Hassold, unpublished observations; see Figure 1A). In a few cases, a two-strand double exchange within the inversion loop may have occurred (Figure 1C).

[b] Considering only situations where a single dicentric chromatid was formed, precocious separation of the sister centromeres (PSSC) occurred in 89.5% (51/57) and breakage in 10.5% (6/57) of cases for In(X)1H heterozygotes. For In(19)37Rk heterozygotes, PSSC occurred in 96.7% (88/91) of cases and breakage in 3.3% (3/91).

Since acentric and dicentric products were present at the second and third cleavage divisions, we made preparations from embryos at 3.5 days *post coitum*, when embryos are expected to be at late morula or blastocyst stages. Out of 180 embryos processed, 109 had at least one analyzable metaphase. Of these, 6/109 (5.5%) had dicentrics and 1/109 (0.9%) had acentrics. All those with dicentrics were retarded or grossly abnormal, some with remnants of dead blastomeres; furthermore, one had a tetraploid metaphase and two others had a very large nondividing nucleus.

Analysis of dicentric chromosome behavior during male meiosis: To determine whether the unusual premature loss of sister chromatid cohesion between the dicentric and its structurally normal sister chromatids is unique to female meiosis, we analyzed meiosis II chromosomes from male mice heterozygous and homozygous for In(19)37Rk. We analyzed 51 haploid products of meiosis I, 34 from heterozygotes and 17 from homozygotes, and classified them as described above for our oocyte studies. We found that 11/34 (32.4%) spermatocytes from heterozygotes and all 17/17 (100%) spermatocytes from homozygotes exhibited normal segregation of the inversion chromosome (Figure 6A; Table 4). Intact single dicentric chromosomes were not observed in heterozygous males, but 11/34 (32.4%) cells had obviously broken chromosomes, suggesting that the most frequent consequence of dicentric bridge formation in males is chromosome breakage (Figure 6B). However, seven cells with a single sister chromatid from the inversion chromosome were observed, as well as one cell containing only an acentric fragment. Thus, the possibility of rare precocious sister chromatid separation and intact dicentric segregation in heterozygous males cannot be ruled out. As in females, an occasional double dicentric bridge (formed via a four-stranded double crossover in the inversion loop; see Figure 1D) was observed.

DISCUSSION

Dicentric chromatid behavior violates meiotic and mitotic expectations in female mice: In this study, we examined the behavior of dicentric chromatids generated in paracentric inversion heterozygotes during meiosis. Our results suggest that new paradigms are required for understanding the meiotic behavior of chromosome aberrations in mammals. We found that in mice, the majority of oocytes in which a dicentric chromosome was generated experienced premature loss of sister chromatid cohesion at one or both centromeres. Although the dicentric chromatid was sometimes stretched or broken, the most frequent means of resolving the dicentric bridge at anaphase I was through the precocious release of sister centromere cohesion. This violates the normal meiotic prohibition against disrupting cohesion at sister centromeres prior to anaphase II, since that cohesion is required for accurate segregation. Furthermore, the dicentric chromatid persisted at a surprisingly high frequency, apparently both replicating and sometimes segregating through the first few embryonic divisions. This behavior represents a second violation of normal chromosome behavior, namely that the presence of one and only one centromere is required to ensure segregation during cell division. These are novel cytogenetic observations and, in fact, represent one of the first investigations of meiotic and early mitotic chromosome segregation in mammalian inversion heterozygotes.

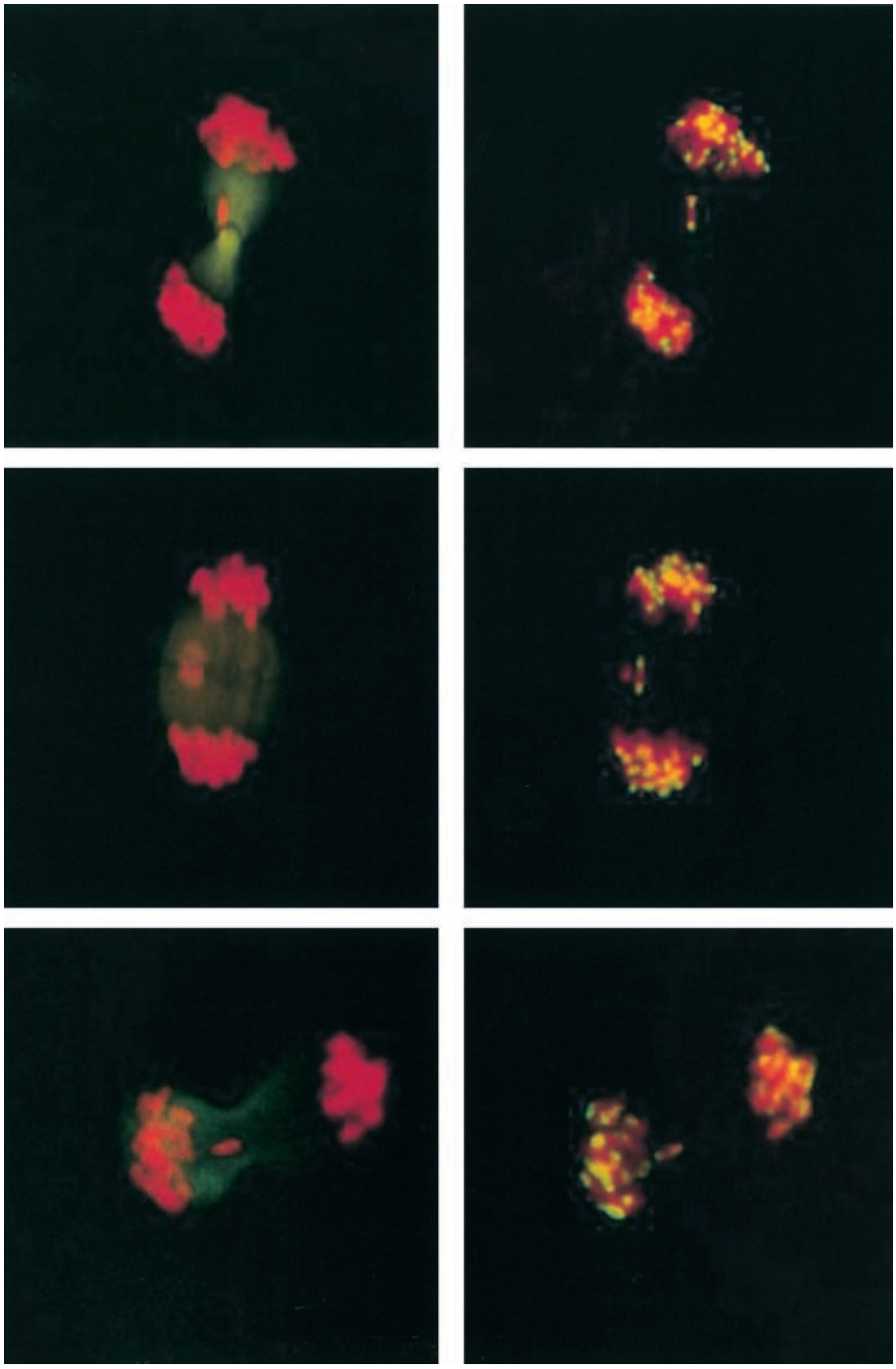

FIGURE 4.—Intact oocytes from In(19)37Rk heterozygotes captured at anaphase I. Confocal micrographs of three different oocytes show the dicentric chromosome lagging at or near the spindle equator. (Left) Spindle (green) and chromosomes (red). (Right) The same oocytes hybridized with a pan-centromere probe (yellow).

Both inversions used in this study have pericentromeric proximal breakpoints (Figure 2). While this raises the possibility that the proximity of the breakpoint to the centromere has an impact on how well the centromere is able to maintain its integrity under physical stress, we suggest that this is not the case. We have examined three additional inversions with substantial interstitial regions (up to 40% of the chromosome length) between the centromere and the proximal breakpoint and found high rates of precocious sister centromere separation and intact dicentric chromatid segregation: specifically, we observed values of 69.2, 76.9, and 100%, respectively, for In(2)5Rk, In(2)40Rk, and In(2)2H female heterozygotes among the cases in which a single dicentric chromatid is formed (K. E. KOEHLER and T. J. HASSOLD, unpublished observations), similar to the values of 89.5 and 96.7% we observed for In(X)1H and In(19)37Rk heterozygotes (Table 1). Thus, this unusual meiotic behavior appears to be a general property of paracentric inversions when heterozygous in female mice and may be common among other mammals as well.

The bulk of the information regarding paracentric inversion heterozygotes and dicentric chromatid behavior has come from studies in flies and maize and sharply

TABLE 2

Karyotypes of preimplantation embryos derived from females heterozygous for In(X)1H

Stage	*N*	XO	OY	XX[a]	XY[b]	+Ac[c]	+Dic[d]	Other
2–4 cell	128	13	8	43	53	2	7	2[e]
4–8 cell	51	8	2	17	16	3	4[f]	1[g]

[a] XX and In(X)X pooled.
[b] XY and In(X)Y pooled.
[c] One cell scored had two or more acentrics.
[d] One or more cells with dicentrics.
[e] One triploid; one 41,In(*X*)XX.
[f] One was tetraploid.
[g] One 39,XY.

contrasts with our findings. However, a few reports suggest that our observations in female mice have parallels in other organisms. Interestingly, genetic studies in yeast have demonstrated that dicentric chromosomes are capable of segregating intact through meiosis (Haber *et al.* 1984). Additionally, there are at least three reports of a human paracentric inversion carrier transmitting a dicentric chromosome to her offspring (Mules and Stamberg 1984; Worsham *et al.* 1989; Whiteford *et al.* 2000). These observations suggest that female mice may not be alone in their unusual response to the meiotic dilemma posed by the presence of a dicentric chromatid. However, data from these organisms provide little insight into the actual mechanism through which precocious centromere separation and intact dicentric chromatid segregation occurs.

Deducing the mechanism and sequence of events in the processing of the dicentrics: The mechanism behind this phenomenon may be complex, since there are several possible outcomes for the meiotic cell confronted with a dicentric bridge. However, the multiple timepoints assayed in our studies offer clues to the sequence

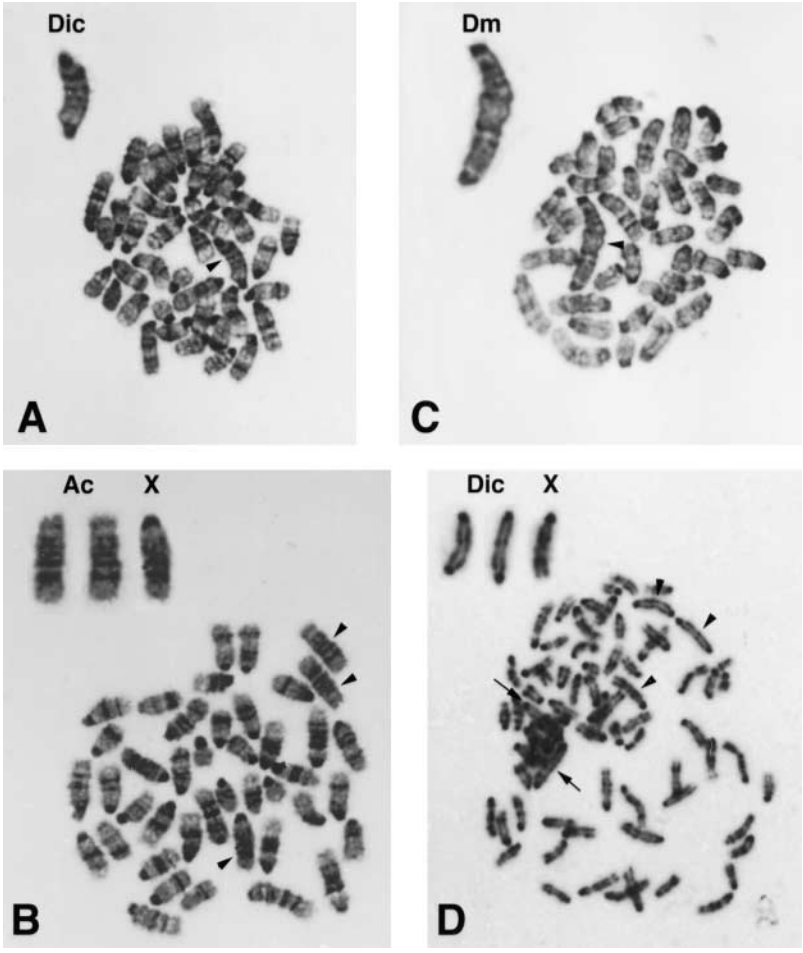

Figure 5.—Preimplantation embryos derived from female mice heterozygous for In(X)1H. Structurally aberrant recombinant inversion products are indicated by arrows, as is one normal X chromosome in some cases. Enlargements in each panel are of chromosomes marked by arrowheads. (A) 40,Y plus "regular" dicentric (generated via meiotic exchange); (B) 42,XX plus two acentric fragments; (C) 40,X plus "mirror image" dicentric (resulting from breakage and subsequent fusion of a regular dicentric); (D) tetraploid cell containing four dicentric X chromosomes.

TABLE 3

Chromosome constitution of individual cells from abnormal embryos

Embryo ID	*N* (cells)	Metaphases	Cell 1	Cell 2
23-7	2	2	41,XX + Dic[a]	?,XX + Dic
27-13	2	2	41,XX + Dic	?,XY + Dic
29-9	2	2	41,XY + Dm[b]	39,Y
30-8	2	2	40,X + Dm[c]	40,X + Dm
30-9	2	2	41,XX + Dic	40,XX?
34-3	2	2	40,Y + Dic	40,Y + Dic
34-8	2	2	41,XX + Dic	41,XX + Dic
35-6	2	2	40,XX	?,XX + 2 Ac
35-13	2	2	40,XX	41,XX + Ac
6-1	3	1	84,XX + 4 Dic[d]	
12-2	5	1	41,X + 2 Dic	
12-11	6	2	40,XX	?,XX + 4 Ac
12-12	5	2	40,XY	43,XY + 3 Ac
13-3	5	2	?,XX	42,XX + 2 Ac[e]
14-6	6	2	?,X? + Dm	?
14-8	6	2	40,Y + Dic[f]	40,Y + Dic

Abnormal embryos [from In(X)1H/+ mothers] are reported in Table 2.

[a] "Regular" dicentric generated through meiotic exchange in the inverted region.

[b] "Mirror image" dicentric generated through mitotic breakage and fusion of a "regular" dicentric.

[c] This cell is shown in Figure 5C.

[d] This cell is shown in Figure 5D.

[e] This cell is shown in Figure 5B.

[f] This cell is shown in Figure 5A.

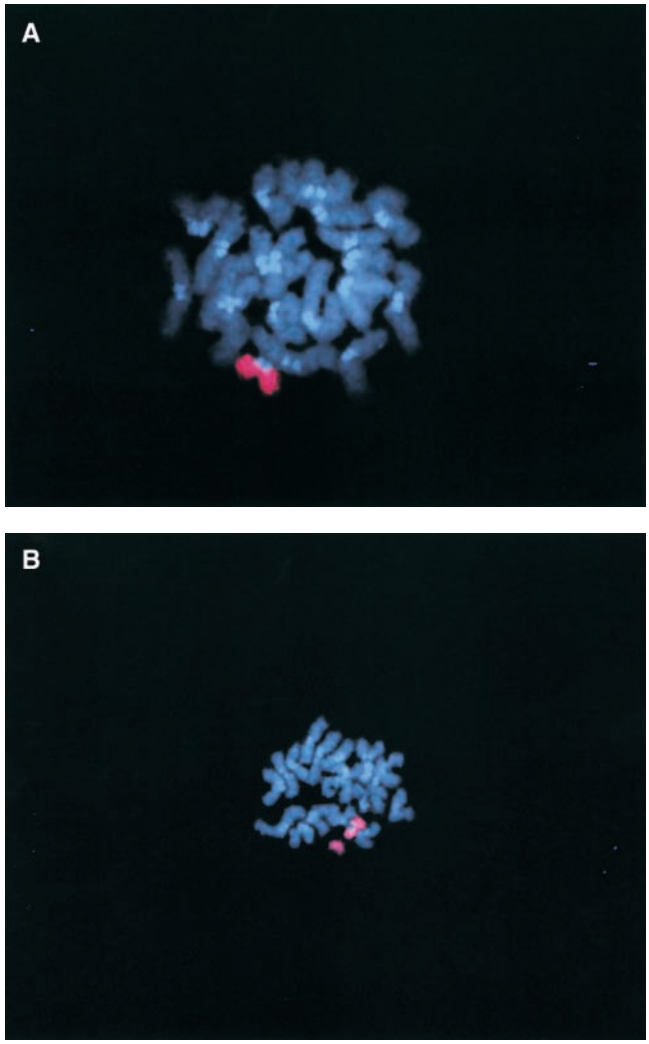

FIGURE 6.—Metaphase II cells from In(19)37Rk male heterozygotes. Chromosome-specific FISH (red) has been used to detect the segregation products of the inversion chromosome. (A) Normal segregation; (B) apparently "normal" chromosome plus acentric fragment, indicating breakage.

of events in this decidedly curious chromosome behavior.

First, confocal images of intact anaphase I-stage oocytes from female inversion heterozygotes revealed frequent lagging of the dicentric chromatid at the spindle equator, often with at least one of its structurally normal sister chromatids missing (Figure 4). We therefore suggest that the spindle-generated tension exerted upon the homologous centromeres connected by the dicentric chromatid is the primary cause of the loss of cohesion between the sister centromeres (Figure 7).

Second, the mechanism by which the lagging dicentric chromatid eventually moves intact into one of the two products of the first meiotic division is not clear, but may involve the "choice" of a pole through the inactivation of one centromere. It seems likely that the stress exerted on the dicentric's opposing centromeres by the spindle eventually results in the loss of cohesion at at least one and, often, both pairs of sister centromeres. This is followed by late migration of the dicentric toward one pole, regardless of whether centromere inactivation or some other mechanism facilitates detachment of the dicentric chromatid from one of the two opposing spindle poles.

Third, once the dicentric chromatid has been included in a meiotic product, it apparently not only can persist, but also can replicate and segregate at least into the first several mitotic divisions of embryogenesis, as our observations of two-, four-, and eight-cell preimplantation embryos demonstrate. Among two-cell embryos, if one cell contained a dicentric chromosome, it was almost always found in the other as well (Table 3).

However, segregation during meiosis II and/or mitosis is quite likely fraught with the typical problems encountered by a chromosome with two active centromeres, since mirror image dicentric chromosomes of varying sizes were also observed (Figure 5; Table 3). These are almost certainly products of breakage-fusion-bridge cycles typical of dicentric chromosomes (McClintock 1938). Therefore, if one of the dicentric's centromeres is inactivated during anaphase I, it is a fairly transient state from which the centromere can be reactivated within the next several cell divisions. Alternatively,

TABLE 4

Products of MI in male inversion heterozygotes and homozygotes

Genotype of male	*N*	Normal segregation (%)	Origin unknown[a] (%)		Broken (%)	Double dicentric (%)	Stretched bridge
In(19)37Rk/In(19)37Rk	17	17 (100)	0	0	0	0	0
In(19)37Rk/+	34	11 (32.4)	7 (20.3)	3 (8.8)	11 (32.4)	1 (2.9)	1 —

[a] These products may be the result of dicentric breakage, PSSC, or some other event.

it is also possible that some dicentrics escape centromere inactivation or that the segregation of the dicentric occurs through a completely different process.

Acentric fragments were also sometimes observed in embryos from mothers heterozygous for In(X)1H, although in this case they tended to accumulate in the same cell, suggesting that, not surprisingly, they replicate but cannot segregate.

Despite the fact that they produce oocytes and embryos of abnormal chromosome constitution at high frequency, female heterozygous carriers of both inversions examined in this study are fertile. The abnormal products were never observed in postimplantation embryos (P. S. Burgoyne and E. P. Evans, unpublished observations). Acentric fragments may be eventually lost, cells containing them may be diluted out, or the cells with several acentric fragments may die due to imbalances in gene expression. Embryonic cells containing dicentrics undoubtedly suffer segregation problems with associated polyploidy due to failure of cytokinesis (Table 3; Figure 5D). Our observations on later preimplantation embryos show that the embryos with dicentrics become retarded and abnormal, probably as a consequence of the increasing incidence of blastomeres that are tetraploid or have higher levels of ploidy. However, embryos with other abnormal karyotypes may survive.

In fact, females heterozygous for In(X)1H have been studied for decades because they produce XO female offspring at a high frequency. The investigators who made the original observation suggested that the nullo-X ova being produced were the result of nondisjunction (Phillips and Kaufman 1974), and this phenomenon continues to be so attributed (*e.g.*, Evans and Phillips 1975). Although no offspring with extra sex chromosomes were produced (Phillips *et al.* 1973), the original studies also included one experiment that examined chromosome number in a small number of oocytes and found a significant increase in hyperploidy in the gametes from female inversion heterozygotes as compared to controls (Phillips and Kaufman 1974). However, in our own studies of these females we did not observe elevated nondisjunction [1/121 oocytes and 1/179 embryos derived from In(X)1H heterozygotes were hyperploid for the X chromosome]. Instead, we suggest that the high level of chromosome loss that unquestionably does occur in the oocytes of In(X)1H/+ females (31/179 embryos were hypoploid for the X chromosome; Table 2) is the result of precocious sister chromatid separation. The subsequent intact segregation of one normal chromatid with the dicentric to one pole, while the other sister chromatid segregates to the other daughter cell, would often result in the formation of a hypoploid oocyte after completion of the second meiotic division (Figure 7B).

Sex influences dicentric chromatid behavior: A sexual dimorphism exists between male and female mice with respect to dicentric chromatid processing, since premature chromatid separation was not observed in inversion-carrying male mice. Two other previous studies have described the products of MI in male paracentric inversion heterozygotes (Gorlov and Borodin 1995; Burgoyne and Evans 2000). Both reported dicentric chromatids, often in incompletely separated or diploid restitution nuclei. Burgoyne and Evans (2000) also observed intact dicentric chromatids in haploid MII cells at levels approximating that of broken anaphase bridges in male mice, but since their studies involved an aberrant XY pair, detailed comparisons are not straightforward.

In contrast, we never observed an intact dicentric chromatid in male In(19)37Rk heterozygotes (Table 4). However, a handful of haploid cells contained a single chromatid from the inversion chromosome that could have separated precociously from a dicentric bridge, but might also have arisen through another mechanism. Examination of an additional inversion in males [In(2)5Rk; K. E. Koehler and T. J. Hassold, unpublished observations] also failed to reveal any intact dicentric chromatids present in isolated haploid MII cells.

However, in this and both previous reports, dicentric chromatid breakage at anaphase I was observed in the male mouse at frequencies far exceeding the rate of breakage we observed in females. Thus, it is clear that precocious sister centromere separation and subse-

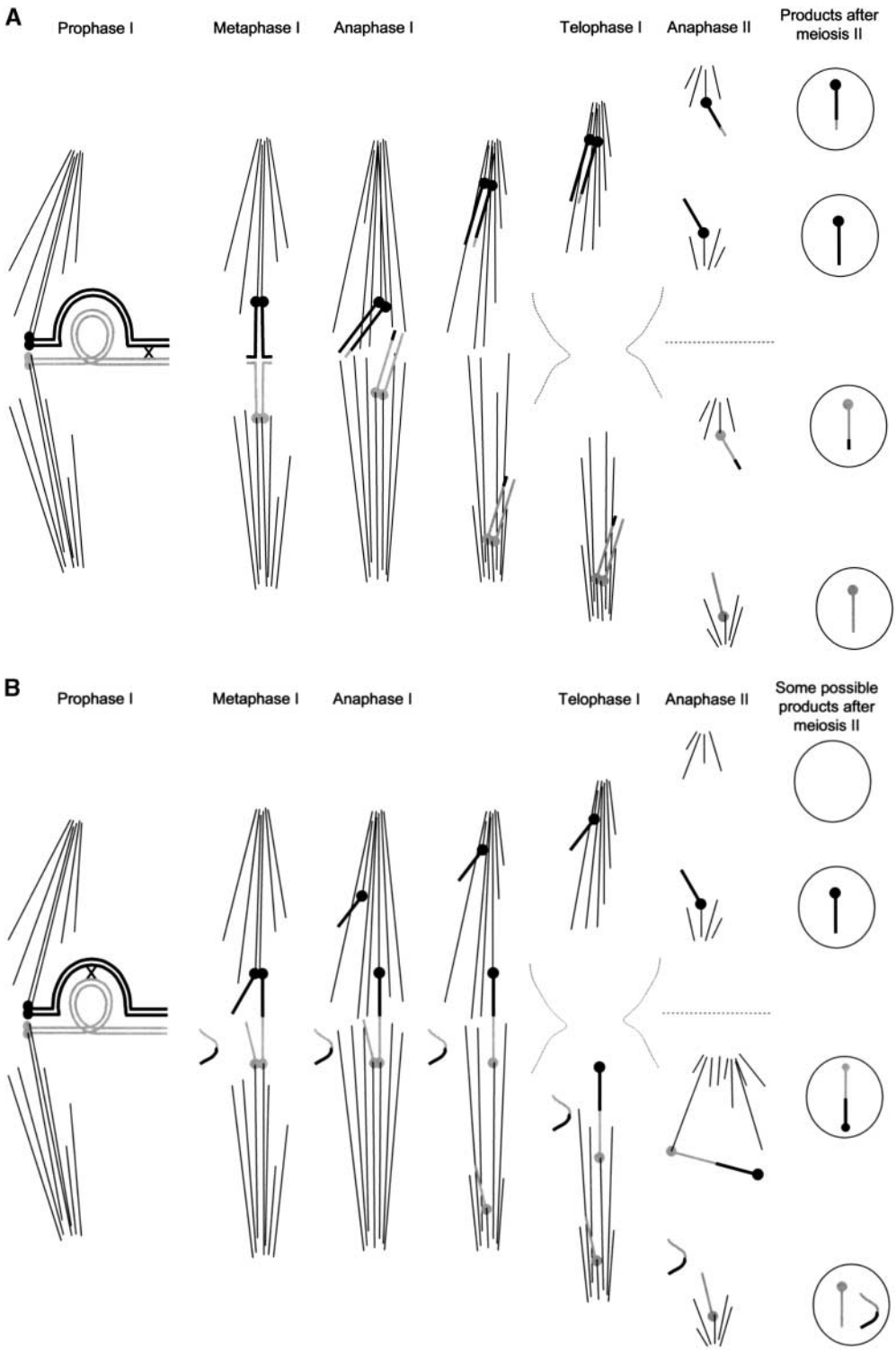

FIGURE 7.—Model for precocious sister chromatid separation followed by intact dicentric chromatid segregation during meiosis in mammalian females heterozygous for a paracentric inversion. (A) Segregation proceeds normally after a single exchange occurs outside the inverted region. Resolution of the chiasma at anaphase I leads to free segregation of homologous chromosomes to opposite poles. This is followed at anaphase II by normal loss of sister chromatid cohesion at the centromeres of each homolog. One of four possible meiotic products with respect to the inversion chromosome will be formed, each containing a single monocentric or "normal" chromosome. (B) Oocyte faced with a dilemma at anaphase I after a single exchange occurs within the inverted region. This generates a dicentric chromatid bridge that is physically linked to both poles and thus is hindered from segregating correctly at anaphase I. The physical strain exerted on the homologous centromeres of the dicentric chromatid by the poleward microtubules may result in the premature loss of sister chromatid cohesion at at least one centromere. The dicentric chromatid subsequently lags behind the other chromosomes and may eventually "choose" a pole through inactivation of one centromere or some other mechanism. A few of the many segregation products possible after anaphase II are shown. Significantly, this model also provides an explanation for the high frequency of XO daughters born to females heterozygous for In(X)1H, as a large number of ova hypoploid for the inversion chromosome are expected to arise through the process illustrated here. Such XO daughters are unlikely to be produced through nondisjunction, as increased hyperploidy was not detected in either oocytes or embryos in this study (see text).

quent intact dicentric segregation are not the major pathway of resolution in male paracentric inversion heterozygotes, as in female mice.

Indeed, sex-specific differences in inversion heterozygotes are evident earlier in meiotic prophase. Dramatic differences in the frequency of inversion loop and anaphase bridge formation have been documented for the few paracentric inversions that have been studied in

both sexes (for review, see Beechey and Evans 1996), although this may reflect, at least in part, differences in levels or patterns of recombination between the sexes. Nevertheless, our current data demonstrate that, once formed, dicentric chromosomes are processed in a sex-specific manner as well.

Recent studies have established other significant differences between male and female gametogenesis (reviewed in Hunt and Hassold 2002). Ova, with their vast cytoplasm and stockpiles of maternal gene products and energy supplies, are biologically more "expensive" to create than sperm, which contain tightly packaged chromatin and little else. Furthermore, female mammals are born with a finite, nonrenewable supply of ova, which may provide a biological incentive to salvage an imperfect ovum whenever possible. For example, mammalian female meiosis seems to have less stringent quality control than male meiosis (Hunt and Hassold 2002). Nonrandom segregation, a phenomenon in which perceived "extra" chromatin segregates preferentially to the oocyte pole over the first polar body, has been observed in female mammals by several groups (LeMaire-Adkins and Hunt 2000; Pardo-Manuel de Villena and Sapienza 2001). However, in this study, no evidence for the nonrandom segregation of the dicentric chromatid to either pole was observed (K. E. Koehler and T. J. Hassold, data not shown).

Time is another crucial factor in the disparity between male and female meiotic processes. Because female meiosis begins before birth, arrests in prophase, and does not resume until shortly before ovulation, the length of meiotic arrest in female mice is ~6 weeks to 18 months, and in the human female ranges from ~10 to 50 years. In contrast, spermatocytes do not undergo a comparable meiotic arrest and, once formed, generally have a fleeting existence on the order of a few days. Sister chromatid cohesion—which our observations suggest is much more fragile and susceptible to loss in females than in males—is established during DNA replication, which occurs prior to meiotic onset and arrest in females (Wang *et al.* 2000; Carson and Christman 2001). Thus, the weeks or years that elapse between the initial establishment of sister chromatid cohesion in female mammals and the eventual reliance on that cohesion for proper distribution of homologs and sister chromatids during the meiotic divisions may be responsible for the apparent fragility it displays in female meiosis, especially when compared to male meiosis. Indeed, sex-specific differences in at least two putative cohesion components' presence during various stages of meiosis have already been demonstrated (Hodges *et al.* 2001).

We thank R. Scott Hawley and an anonymous reviewer for helpful comments on the manuscript and Jodi Jackson for technical assistance. This work was funded by grants HD21341 (to T.J.H.) and HD37502 (to P.A.H.). K.E.K. is the recipient of a postdoctoral fellowship (96994) from the American Cancer Society.

LITERATURE CITED

Bean, C. J., P. A. Hunt, E. A. Millie and T. J. Hassold, 2001 Analysis of a malsegregating mouse Y chromosome: evidence that the earliest cleavage divisions of the mammalian embryo are nondisjunction-prone. Hum. Mol. Genet. **10:** 963–972.

Beechey, C. V., and E. P. Evans, 1996 Chromosomal variants, pp. 1452–1511 in *Genetic Variants and Strains of the Laboratory Mouse*, Ed. 3, edited by M. F. Lyon, S. Rastan and S. D. M. Brown. Oxford University Press, Oxford.

Borodin, P. M., I. P. Gorlov and T. Ladygina, 1990 Synapsis in single and double heterozygotes for partially overlapping inversions in the chromosome 1 of the house mouse. Chromosoma **99:** 365–370.

Borodin, P. M., I. P. Gorlov and T. Ladygina, 1992 Synaptic interrelationships between the segments of the heteromorphic bivalent in double heterozygotes for paracentric inversions in chromosome 1 of the house mouse. Chromosoma **101:** 374–379.

Burgoyne, P. S., 1993 A Y chromosomal effect on blastocyst cell number in mice. Development **117:** 341–345.

Burgoyne, P. S., and E. P. Evans, 2000 A high frequency of XO offspring from X(Paf)Y* male mice: evidence that the Paf mutation involves an inversion spanning the X PAR boundary. Cytogenet. Cell Genet. **91:** 57–61.

Carson, D. R., and M. F. Christman, 2001 Evidence that replication fork components catalyze establishment of cohesion between sister chromatids. Proc. Natl. Acad. Sci. USA **98:** 8270–8275.

Chandley, A. C., 1982 A pachytene analysis of two male-fertile paracentric inversions in chromosome 1 of the mouse and in the male-sterile double heterozygote. Chromosoma **85:** 127–135.

Evans, E. P., and R. J. Phillips, 1975 Inversion heterozygosity and the origin of XO daughters of Bpa/+ female mice. Nature **256:** 40–41.

Evans, E. P., and R. J. S. Phillips, 1978 A phenotypically marked inversion (In(2)2H). Mouse News Lett. **58:** 44–45.

Evans, E. P., G. Breckon and C. E. Ford, 1964 An air-drying method for meiotic preparations from mammalian testes. Cytogenet. Cell Genet. **3:** 289–294.

Ford, C. E., E. P. Evans and M. D. Burtenshaw, 1976 Meiosis and fertility in mice heterozygous for paracentric inversions, pp. 123–132 in *Current Chromosome Research, Kew Chromosome Conference*, edited by K. Jones and P. E. Brandham. Elsevier/North Holland Biomedical Press, Amsterdam.

Gorlov, I. P., and P. M. Borodin, 1995 Recombination in single and double heterozygotes for two partially overlapping inversions in chromosome 1 of the house mouse. Heredity **75:** 113–125.

Haber, J. E., P. C. Thorburn and D. Rogers, 1984 Meiotic and mitotic behavior of dicentric chromosomes in *Saccharomyces cerevisiae*. Genetics **106:** 185–205.

Hodges, C. A., R. LeMaire-Adkins and P. A. Hunt, 2001 Coordinating the segregation of sister chromatids during the first meiotic division: evidence for sexual dimorphism. J. Cell Sci. **114:** 2417–2426.

Hunt, P., and T. Hassold, 2002 Sex matters in meiosis. Science **296:** 2181–2183.

Hunt, P., R. LeMaire, P. Embury, L. Sheean and K. Mroz, 1995 Analysis of chromosome behavior in intact mammalian oocytes: monitoring the segregation of a univalent chromosome during female meiosis. Hum. Mol. Genet. **4:** 2007–2012.

LeMaire-Adkins, R., and P. Hunt, 2000 Nonrandom segregation of the mouse univalent X chromosome: evidence of spindle-mediated meiotic drive. Genetics **156:** 775–783.

McClintock, B., 1931 Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*. Mol. Agric. Exp. Stn. Res. Bull. **163:** 30.

McClintock, B., 1933 The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. Z. Wiss. **19:** 191–237.

McClintock, B., 1938 The fusion of broken ends of sister half-chromatids following chromatid breakage at meiotic anaphases. Missouri Agric. Exp. Stn. Bull. **290:** 1–48.

Moses, M. J., P. A. Poorman, T. H. Roderick and M. T. Davisson, 1982 Synaptonemal complex analysis of mouse chromosomal rearrangements. IV. Synapsis and synaptic adjustment in two paracentric inversions. Chromosoma **84:** 457–474.

Mules, E. H., and J. Stamberg, 1984 Reproductive outcomes of paracentric inversion carriers: a report of a live born dicentric recombinant and literature review. Hum. Genet. **67:** 126–131.

Novitski, E., 1955 Genetic measures of centromere activity in *Drosophila melanogaster*. Symp. Genet. Recomb. **45:** 151–169.

Pardo-Manuel de Villena, F., and C. Sapienza, 2001 Female meiosis drives karyotypic evolution in mammals. Genetics **159:** 1179–1189.

Phillips, R. J., and M. H. Kaufman, 1974 Bare-patches, a new sex-linked gene in the mouse, associated with a high production of XO females. II. Investigations into the nature and mechanism of the XO production. Genet. Res. **24:** 27–41.

Phillips, R. J., S. G. Hawker and H. J. Moseley, 1973 Bare-patches, a new sex-linked gene in the mouse, associated with a high production of XO females. I. A preliminary report of breeding experiments. Genet. Res. **22:** 91–99.

Rhoades, M., 1955 The cytogenetics of maize, pp. 123–219 in *Corn and Corn Improvement*, edited by G. F. Sprague. Academic Press, New York.

Roderick, T. H., 1971 Producing and detecting paracentric chromosomal inversions in mice. Mutat. Res. **11:** 59–69.

Roderick, T. H., and N. L. Hawes, 1974 Nineteen paracentric chromosomal inversions in mice. Genetics **76:** 109–117.

Rumpler, Y., O. Gabriel-Robez, V. Volobouev, W. Yu, P. Rasamimanana *et al.*, 1995 Male sterility and double heterozygosity for chromosomal inversion. Cytogenet. Cell Genet. **69:** 66–70.

Sturtevant, A. H., and G. W. Beadle, 1936 The relations of inversions in the X chromosome of Drosophila melanogaster to crossing over and disjunction. Genetics **21:** 554–604.

Tarkowski, A. K., 1966 An air-drying method for chromosome preparations from mouse eggs. Cytogenetics **5:** 394–400.

Tease, C., and G. Fisher, 1986 Further examination of the production-line hypothesis in mouse foetal oocytes. I. Inversion heterozygotes. Chromosoma **93:** 447–452.

Wang, Z., I. B. Castano, A. De Las Penas, C. Adams and M. F. Christman, 2000 Pol kappa: a DNA polymerase required for sister chromatid cohesion. Science **289:** 774–779.

Whiteford, M. L., C. Baird, S. Kinmond, B. Donaldson and H. R. Davidson, 2000 A child with bisatellited, dicentric chromosome 15 arising from a maternal paracentric inversion of chromosome 15q. J. Med. Genet. **37:** E11.

Worsham, M. J., D. A. Miller, J. M. Devries, A. R. Mitchell, V. R. Babu *et al.*, 1989 A dicentric recombinant 9 derived from a paracentric inversion: phenotype, cytogenetics and molecular analysis of centromeres. Am. J. Hum. Genet. **44:** 115–123.

Communicating editor: R. S. Hawley