Meiotic Exchange and Segregation in Female Mice Heterozygous for Paracentric Inversions

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ABSTRACT

Inversion heterozygosity has long been noted for its ability to suppress the transmission of recombinant chromosomes, as well as for altering the frequency and location of recombination events. In our search for meiotic situations with enrichment for nonexchange and/or single distal-exchange chromosome pairs, exchange configurations that are at higher risk for nondisjunction in humans and other organisms, we examined both exchange and segregation patterns in 2728 oocytes from mice heterozygous for paracentric inversions, as well as controls. We found dramatic alterations in exchange position in the heterozygotes, including an increased frequency of distal exchanges for two of the inversions studied. However, nondisjunction was not significantly increased in oocytes heterozygous for any inversion. When data from all inversion heterozygotes were pooled, meiotic nondisjunction was slightly but significantly higher in inversion heterozygotes (1.2%) than in controls (0%), although the frequency was still too low to justify the use of inversion heterozygotes as a model of human nondisjunction.

NUMERICAL chromosome abnormalities are present in 10–25% of all human conceptions (HASSOLD and HUNT 2001). This phenomenally high rate of nondisjunction in humans exceeds spontaneous aneuploidy rates in most model organisms by at least an order of magnitude (MERRIAM and FROST 1964; HODGKIN *et al.* 1979; BOND and CHANDLEY 1983; KOEHLER *et al.* 1996; ROSS *et al.* 1996). Since meiotic exchange is the primary mechanism responsible for ensuring segregation of homologous chromosomes, it is perhaps not surprising that the first identified molecular correlate of human nondisjunction was altered placement of recombination events, especially absent or single distal exchanges (LAMB *et al.* 1996, 1997). These types of chiasmate configurations are also known to be susceptible to nondisjunction in the fruit fly, as well as in yeast (KOEHLER *et al.* 1996; ROSS *et al.* 1996). However, no investigation has been made into whether homologous chromosome pairs or bivalents with altered patterns of recombination events may also be at increased risk for nondisjunction in mammals other than humans.

One situation that has long been noted to alter the frequency and/or position of meiotic recombination events is heterozygosity for a chromosomal inversion. Genetic maps compiled from progeny analysis of inversion heterozygotes are dramatically altered, often including reductions in the length of the map intervals

near or spanning the inverted region (STURTEVANT and BEADLE 1936; NOVITSKI and BRAVER 1954; EVANS and PHILLIPS 1975; MOSES *et al.* 1982; DRESSER *et al.* 1994; STEPHENSON *et al.* 1994; GORLOV and BORODIN 1995; ZHENG *et al.* 1999). However, these alterations are generally understood to arise primarily from a failure to recover many recombinant chromosomes. When meiotic exchange in an inversion heterozygote occurs within the inverted region, the result is frequently a chromosome aberration. Dicentric chromosomes and acentric fragments are formed if the inversion is *paracentric* (the centromere lies outside the inversion; Figure 1, A and B); duplication and deletion products result if the inversion is *pericentric* (the centromere lies inside the inversion). These aberrant chromosomes are frequently nontransmissible and/or lethal, although the details of transmission failure vary among organisms and among inversions (STURTEVANT and BEADLE 1936; NOVITSKI 1955; RHOADES 1955; KOEHLER *et al.* 2002b). However, there are exceptions; dicentric and duplication chromosomes can occasionally be inherited by progeny, albeit with potentially severe health consequences (WINSOR *et al.* 1978; JAAROLA *et al.* 1998; WHITEFORD *et al.* 2000; KOEHLER *et al.* 2002b).

Importantly, inversion heterozygosity generates structurally aberrant chromosomes only if exchange occurs within the inverted region; thus, a single inversion cannot prevent the recovery of recombination events in chromosomal regions outside of the inversion (Figure 1, C and D). Whole-chromosome “balancers,” composed of a series of inversions that collectively span the entire chromosome, are part of the standard genetic

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arsenal in *Drosophila melanogaster* (ASHBURNER 1989; LINDSLEY and ZIMM 1992). Balancers have been employed in the fly for nearly a century as a tool to maintain the integrity of a normal sequence chromosome through generations of transmission, *i.e.*, to keep several linked alleles *in cis* or to avoid losing a lethal mutation, by ensuring that only nonrecombinant chromosomes are transmitted (MULLER 1918; ASHBURNER 1989).

A sufficient number of strategically placed inversions on a single chromosome can give rise to a second type of exchange suppression: interfering with homolog pairing and synapsis so severely that exchange is unable to be initiated or established during meiotic prophase. For example, the *Drosophila* X chromosome balancer *FM7* is used to generate nonexchange chromosome pairs ~100% of the time (HAWLEY *et al.* 1992; DERNBURG *et al.* 1996).

However, such multiply inverted balancer chromosomes do not yet exist in mice or other mammals. Since the first engineered mouse chromosome bearing a single inversion was generated for balancing purposes (ZHENG *et al.* 1999), several other preexisting single inversions, often carrying a phenotypic marker and/or recessive lethal, have been successfully employed in isolating mutations from genetic screens and subsequently maintaining those mutations in stable breeding arrangements (see RINCHIK 2000 for review; KILE *et al.* 2003; NISHIJIMA *et al.* 2003). These partial-chromosome balancers are efficient at eliminating the transmission of recombinants (within the inverted region) to progeny (Figure 1, A and B), while exchange events occurring outside the inversion can ensure accurate segregation for the homologs (Figure 1, C and D). (One important caveat is that for a large single inversion, two-strand double crossover events occurring within the inverted region result in rescue from the formation of structural abnormalities and yield normal chromosomes bearing recombination events within the inversion.) Large inversions may also participate in an "antiparallel" pairing configuration, where an inversion loop is not formed; instead, chromosomes synapse homologously only *within* the inverted region, leaving the uninverted segments of the chromosome unpaired and the centromeres on opposite ends of the synaptonemal complex (SC; Figure 1, E and F).

Data on meiotic pairing in inversion heterozygotes exist for only a small handful of murine inversions; chiasma count data are sparse and segregation data for these genotypes are very limited, especially in females. However, it is clear from analyses of pairing in meiotic prophase that the timing and/or location of pairing or synaptic initiation events are altered in mice heterozygous for an inversion (FORD *et al.* 1976; BORODIN *et al.* 1990, 1992; GORLOV *et al.* 1991; ASHLEY *et al.* 1993; GORLOV and BORODIN 1995; RUMPLER *et al.* 1995).

The analysis of synapsis and exchange in mammalian inversion heterozygotes is complicated by the pachytene

FIGURE 1.—The meiotic consequences of heterozygosity for a paracentric inversion. (A and B) When an exchange occurs within the inverted region (which commonly forms an inversion loop; A), the two recombinant chromatids form a dicentric bridge and an acentric fragment (B). (C and D) When exchange occurs outside, but not inside, the inverted region (C), there are no adverse meiotic consequences for the cell (D). (E and F) In some cases, an inversion loop may not form and the inverted region may undergo antiparallel synapsis, pairing homologously only within the inverted region (E). An exchange within the inversion then results in a dicentric bridge and an acentric fragment (F). (G and H) A "straight" or linear SC may arise through synaptic adjustment in an inversion heterozygote of either an inversion loop (A, adjusting to G) or an antiparallel configuration (E, adjusting to H).

phenomenon of synaptic adjustment. Through this process, the inversion loop (or other configuration) that is formed by homologous pairing and synapsis is gradually "adjusted out" via local desynapsis and nonhomologous resynapsis; by the end of pachytene, the loop is no longer present, resulting in a "straight" or linear SC (Figure 1, G and H; POORMAN *et al.* 1981; MOSES *et al.* 1982). Recombination events that have occurred within the loop do not block synaptic adjustment (MOSES *et al.* 1982).

Some inversion heterozygotes (as well as translocation heterozygotes) engage in direct heterologous synapsis instead of homologous synapsis and synaptic adjustment (ASHLEY and RUSSELL 1986; ASHLEY 1988). This process differs from synaptic adjustment in that its timing is much earlier, concomitant with homologous synapsis, and is therefore not preceded by the formation of a loop. On the basis of the available experimental data, ASHLEY (1988, 1990) proposed that the complexities of meiotic inversion behavior are determined by the type of chromatin that contains the inversion breakpoint(s);

that is, if at least one breakpoint lies within a G-band, then direct heterologous pairing will occur and exchange within the inverted region will be suppressed. If both inversion breakpoints lie in R-bands, normal homologous pairing will take place, including the formation of an inversion loop, followed by synaptic adjustment, and the potential for recombination is present.

To expand existing knowledge of the relationship between inversion heterozygosity and exchange patterns established in meiotic prophase and to determine whether altered recombination affects the fidelity of meiotic chromosome segregation in female mice, we studied meiotic exchange and segregation in 2728 mouse oocytes from heterozygotes for five different paracentric inversions and controls: In(X)1H, In(2)2H, In(2)5Rk, In(2)40Rk, and In(19)37Rk.

We find that while recombination is rarely absent in female mice heterozygous for paracentric inversions, its patterns are dramatically altered. While no single inversion elevates nondisjunction when heterozygous, inversion heterozygotes do have slightly but significantly increased nondisjunction in comparison to controls when treated as a group. Thus, our studies supply a detailed set of important observations about the meiotic behavior of inversions in female mice.

MATERIALS AND METHODS

Production of inversion homozygotes, heterozygotes, and normal sequence controls: Breeding stock of control C57BL/6J inbred mice and of mice carrying the inversions In(X)1H, In(2)2H, In(2)40Rk, and In(19)37Rk was obtained from the Jackson Laboratory (TJL) and maintained as inbred stocks via brother $\times$ sister matings. Stock carrying the homozygous lethal inversion In(2)5Rk was also obtained from TJL and was maintained by crossing normal sequence females to males heterozygous for the inversion. Inversion heterozygotes were generated by crossing C57BL/6J females to a male hemi- or homozygous for the inversion, except for In(2)5Rk, for which normal sequence littermates were used. Mice carrying In(2)2H *in trans* to In(2)40Rk were generated by crossing females homozygous for In(2)2H to males homozygous for In(2)40Rk. Mice simultaneously heterozygous for In(X)1H, In(2)2H, and In(19)37Rk were created in two generations:

1. Females homozygous for In(X)1H were crossed to males homozygous for In(2)2H.
2. Females homozygous for In(19)37Rk were crossed to the sons of the first mating, who were of the genotype In(X)1H/Y In(2)2H/+.
3. From the second mating, daughters of the genotype In(X)1H/+ In(2)2H/+ In(19)37Rk/+ were selected [and daughters lacking In(2)2H were rejected] by their coat color, *suppressor of agouti* (*A^s*), a dominant mutation conferred by the distal In(2)2H breakpoint (EVANS and PHILLIPS 1978).

Inversion breakpoint determination: We selected a series of nonchimeric yeast artificial chromosomes (YACs) from the WI/MIT-820 Mouse YAC library (Research Genetics, Birmingham, AL; see also HALDI *et al.* 1996) for each inversion-bearing chromosome to define approximate centimorgan positions for the breakpoints for the inversions (Figure 2). YACs

were labeled using the Bionick labeling system (GIBCO BRL, Gaithersburg, MD) and assigned centimorgan positions along the chromosome using the Mouse Genome Database (MGD) map. To do this, each YAC's position on the MIT map was converted to a position on the MGD map by comparing the positions of simple sequence length polymorphism markers on the YAC for both maps. Pairwise, 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). Our findings for In(X)1H and In(19)37Rk have been reported previously (KOEHLER *et al.* 2002b).

Oocyte collection and fixation: For pachytene preparations, oocytes were collected from mice on the day of birth, when the majority of the synchronously developing oocyte population is expected to be in late pachytene (SPEED 1982). Surface-spread synaptonemal complex preparations were prepared as described in PETERS *et al.* (1997).

For MII-arrested preparations, all oocytes were collected either from $\sim$ 4-week-old females or, when specified, from females of "advanced" maternal age (8–12 months). Oocytes 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).

Immunostaining: The immunostaining protocol for pachytene oocytes is a modification of that used by ANDERSON *et al.* (1999), as described in KOEHLER *et al.* (2002a). Antibodies to the synaptonemal complex protein SCP3 were used to identify the lateral elements of the SC (SCHALK *et al.* 1998) and antibodies to MLH1, a meiotic mismatch repair protein, bound to the putative late recombination nodules or exchange events (BAKER *et al.* 1996).

Fluorescence microscopy and digital imaging: Slides were examined on a Zeiss Axiophot epifluorescence microscope and imaged with a CCD camera and computer using Vysis Quips PathVysion SmartCapture VP 1.4 software (Digital Scientific).

Meiocyte fluorescence *in situ* hybridization analysis: Chromosome paint probes for mouse chromosomes 2, 19, and X were obtained from Vysis. Fluorescence *in situ* hybridization (FISH) on MII-arrested oocytes was performed as described in KOEHLER *et al.* (2002b). FISH on pachytene oocytes was executed subsequent to antibody staining, imaging, and recording of cell coordinates; a FISH protocol identical to that for MII-arrested oocytes was followed, except that the formamide washes were omitted. FISH images of pachytene oocytes were aligned and superimposed on antibody-stained oocyte images in Adobe Photoshop 7.0 using SCP3 staining patterns common to both images.

Scoring of pachytene oocytes: Two independent observers scored blind-coded digital images, at $\sim$ $\times$ 2600 magnification, of each pachytene oocyte for the number of MLH1 foci on every SC; if the observers did not agree on the number of foci present, the cell was discarded.

Measurement of exchange positions: The software package MicroMeasure (REEVES and TEAR 2000) was utilized to precisely localize the MLH1 foci or exchange events along the length of the FISH-identified SC in all pachytene oocytes.

Scoring of MII-arrested oocytes: Blind-coded slides of air-dried MII-arrested oocytes were scored for hyperploidy, the presence of an extra chromosome (accounting for half of all nondisjunction events), on a Zeiss Axiophot epifluorescence microscope by two independent observers. If the observers did not agree on the number of chromosomes present in a cell, it was discarded. Guidelines for interpreting the recombinant/

aberrant products of inversion heterozygotes have been described previously (RHOADES 1955; KOEHLER *et al.* 2002b).

Calculating nondisjunction: In situations where only one meiotic product can be scored, it is standard practice in many organisms to calculate nondisjunction conservatively as twice the observed hyperploidy, to avoid artificially inflating the figure with chromosome loss due to methodological issues. Likewise, in situations where all products of a single meiosis can be analyzed ($\sim 10\%$ of all oocytes studied here), no factor of two is necessary. Thus, we have calculated nondisjunction as the number of hyperploidy oocytes where both products of meiosis I could be scored (only one) plus twice the number of hyperploidy oocytes where only one product could be scored, divided by the total number of oocytes observed.

RESULTS

Inversion breakpoint mapping: Cytological breakpoints have been reported previously for In(X)1H (as A1-F4) and for In(2)2H [as D-H1 (EVANS and PHILLIPS 1978) or E1-H1 (RUMPLER *et al.* 1995)]. No breakpoints have been reported for In(2)40Rk or In(19)37Rk, although genetic data have suggested approximate inversion sizes/shapes (BEECHEY and EVANS 1996). The breakpoints of In(2)5Rk have been reported variously as D-F (DAVISON and RODERICK 1973), D-H1 (POORMAN *et al.* 1981), and A3-F3 (RUMPLER *et al.* 1995).

We used nonchimeric YACs from the WI/MIT-820 Mouse YAC library to define approximate genetic intervals of ~ 3 –12 cM each that contained the inversion breakpoints (Figure 2). For In(X)1H, In(2)2H, In(2)40Rk, and In(19)37Rk, the inversion breakpoints determined by our YAC analysis were consistent with the previously published breakpoints determined through either cytogenetic banding or genetic mapping studies. For In(2)5Rk, we found that our results were consistent with the cytogenetic breakpoints D-H1 (POORMAN *et al.* 1981; BEECHEY and EVANS 1996). These are the same bands in which breakpoints have been reported for In(2)2H (EVANS and PHILLIPS 1978), but our analysis shows that In(2)5Rk is in fact slightly larger than In(2)2H (Figure 2B). The distal breakpoint of In(2)2H was known to be extremely close to the *nonagouti* locus at 89 cM but has now been defined even more precisely; it is located within an ~ 820 -kb YAC (345E6) from the WI/MIT-820 Mouse YAC library (Figure 2B; HALDI *et al.* 1996).

Thus, In(19)37Rk and In(X)1H are large inversions that span $>85\%$ of the chromosome length and each have their proximal breakpoint quite near the centromere (Figure 2A). Indeed, although the most proximal YAC in our analysis of In(X)1H was ~ 3.6 cM from the centromere (KOEHLER *et al.* 2002b), previous studies by other investigators have shown that this inversion's proximal breakpoint lies between the centromere and *DXWas70* at 0.2 cM (FISHER and TEASE 1992). In(19)37Rk's proximal breakpoint is within 5 cM of the centromere (KOEHLER *et al.* 2002b). In(2)2H, In(2)5Rk, and In(2)40Rk are inversions comprising ~ 40 , ~ 45 , and $\sim 75\%$, respectively, of chromosome length. In(2)40Rk's proximal breakpoint

lies 2–11 cM away from the centromere, leaving a small but detectable interstitial region; In(2)2H and In(2)5Rk have substantial interstitial regions of ~ 40 –50 cM (Figure 2B).

Timing of meiotic recombination analysis: Although precise substaging of individual pachytene cells is more difficult in female mice than in males due to the absence of a reliable stage-specific marker, it is likely that most oocytes studied here were in late pachytene. Oocytes were collected at birth, a timepoint at which a large fraction of oocytes are normally in or near late pachytene (SPEED 1982) and thus are finished with synaptic adjustment (MOSES 1980). Two pieces of evidence suggest that this was indeed the case: (1) only a single (small) inversion loop [for In(2)2H] was observed in 1 of the 129 heterozygous oocytes examined in this study, and (2) thickened “knobs” characteristic of late pachytene cells were frequently observed at the ends of SCs (ASHLEY and PLUG 1998).

Inversion heterozygosity can alter exchange frequency: We selected three paracentric inversions for an analysis of meiotic exchange patterns: one inversion on chromosome 2 [In(2)2H], one inversion on the X chromosome [In(X)1H], and one inversion on chromosome 19 [In(19)37Rk; Figure 2]. Recombination events in inversion heterozygotes and homozygotes were detected at pachytene by scoring for MLH1 foci (Figure 3). Immunostaining with fluorescently labeled antibodies to SCP3 to illuminate the lateral elements of synaptonemal complexes and MLH1 foci to mark the sites of exchange has recently been demonstrated to be an accurate surrogate for chiasma analysis at diakinesis in both mice and humans (BARLOW and HULTEN 1998; ANDERSON *et al.* 1999; FROENICKE *et al.* 2002; KOEHLER *et al.* 2002a; LYNN *et al.* 2002; TEASE *et al.* 2002). We subsequently used FISH to identify the inversion-bearing chromosome in 215 oocytes from inversion heterozygotes and controls (Table 1). SCs were classified as being “normally” synapsed if they were indistinguishable from the SCs of other noninverted chromosomes in the cell: *i.e.*, if the SC was completely linear, without any obvious bubbles, forks, loops, or irregularities, and if the 4',6-diamidino-2-phenylindole (DAPI)-bright pericentromeric heterochromatin was visible at one end of the SC (since all mouse chromosomes are acrocentric).

Some general trends were evident. First, while the SC of the inversion pair was always synapsed normally in control genotypes, all inversion heterozygotes sometimes displayed synaptic irregularities, including antiparallel synapsis (pericentromeric heterochromatin visible at both ends of the SC; Figure 3D), synaptic failure [*i.e.*, appearing as unpaired SCs or axial elements (AEs); Figure 3E], or other unusual configurations. Only one inversion loop was observed [in an In(2)2H heterozygote; Figure 3B]. It was likely in the process of synaptic adjustment and destined to become a linear SC; how-

FIGURE 2.—Cytogenetic breakpoint locations for paracentric inversions. YACs from the WI/MIT-820 Mouse Library, indicated here with their approximate positions in centimorgans (cM) on the Mouse Genome Database map, were used to cytogenetically identify the inversion breakpoints for paracentric inversions used in this study. (A) Schematic of breakpoint locations for all five paracentric inversions used in this study. (B) Arrows and brackets indicate the map intervals in which each breakpoint occurs for the three inversions on chromosome 2. Detailed breakpoint information on In(X)1H and In(19)37Rk is given elsewhere (KOEHLER *et al.* 2002b). (C) YAC FISH on a partial fetal liver metaphase from an In(2)2H heterozygote, using YACs from the map in B (YAC 345E6 in red, 88C11 in green). The normal sequence chromosome is indicated with a white arrow and the inverted chromosome with an open arrowhead. In this case, the distal breakpoint bisects YAC 345E6, so that two red signals result on the inverted chromosome.

ever, since there was only one such SC in our data set, it was not scored as normal.

Second, among the normally synapsed inversion chromosomes, exchange frequencies for the inversion chromosome were not significantly altered from control levels in heterozygotes for In(X)1H or In(19)37Rk; similar results were obtained when SCs with synaptic irregularities were included in the analysis (Table 1). However, In(2)2H heterozygotes (with or without irregular SCs) displayed an increased recombination frequency over controls (Table 1; $P = 0.046$ when irregular SCs are excluded). [Controls for In(2)2H consist of both inversion homozygotes and normal sequence C57BL/6; since no statistical differences were identified between these two control groups, they were pooled for the remainder of the analyses. These findings are consistent with previous failures to detect differences in recombination in either mice or *Drosophila* in inversion homozygotes *vs.*

normal sequence animals (ASHBURNER 1989; GORLOV *et al.* 1991).] This elevation was largely due to an increase in the frequency of SCs with two MLH1 foci at the expense of those with one focus, although as many as four MLH1 foci were observed on SCs in In(2)2H heterozygotes but not controls (Table 1).

In(19)37Rk was the only inversion for which nonexchange chromosome pairs were observed; 3/37 heterozygous oocytes analyzed (8%) contained axial elements devoid of MLH1 foci (Figure 3E). Both AEs were colocalized to the same small area of the cell in all three cases. MLH1 foci were never absent on normally synapsed SCs (Table 1).

We were also interested in determining whether there were differences in SC length between inversion heterozygotes and controls. SC length has recently been demonstrated to have a positive linear relationship with genetic length at the cellular level (LYNN *et al.* 2002) as

FIGURE 3.—Synaptic configurations in pachytene oocytes from inversion heterozygotes. Cells were immunostained with antibodies to MLH1 (yellow) and antibodies to SCP3 (red); chromosome-specific paint probes were used to identify individual chromosomes (green; shown only in A). Bright DAPI-stained swirls at one end of each SC correspond to pericentromeric heterochromatin. (A) Oocyte from an In(2)2H heterozygote with normal synapsis for all SCs. Chromosome 2 has a single MLH1 focus (arrow). (B) Oocyte from an In(2)2H heterozygote, showing a small inversion loop (nearing the end of synaptic adjustment) and three MLH1 foci on chromosome 2 (arrow). (C) Oocyte from an In(2)2H heterozygote, with an unusual, abnormal SC configuration (arrow) and four MLH1 foci. (D) Oocyte from an In(X)1H heterozygote, exhibiting antiparallel synapsis (arrows denote visible heterochromatin at both ends of SC). (E) Oocyte from In(19)37Rk heterozygote, where the two chromosome 19 homologs have localized to the same region of the cell (arrows) but have not synapsed, remaining as AEs.

TABLE 1
Frequencies of exchange events (MLH1 foci) in inversion heterozygotes and controls

Inversion	Genotype	N (SCs)	No. of SCs with synaptic irregularities	No. (%) of normally synapsed SCs with					Foci/SC for normal synapsis	Foci/SC for all synaptic configurations
				0 foci	1 focus	2 foci	3 foci	4 foci		
In(X)1H	Homozygotes	24	0	0	19 (79.2)	5 (20.8)	0	0	1.21	1.21
	Heterozygotes	24	10 ^a	0	13 (92.9)	1 (7.1)	0	0	1.07	1.13
In(2)2H ^b	Homozygotes	25	0	0	11 (44.0)	13 (52.0)	1 (4.0)	0	1.60	1.60
	C57BL/6	21	0	0	8 (38.0)	12 (57.1)	1 (4.8)	0	1.67	1.67
In(19)37Rk	Heterozygotes	68	3 ^c	0	16 (24.6)	43 (66.2)	5 (7.7)	1 (1.5)	1.86	1.93
	Homozygotes	15	0	0	14 (93.3)	1 (6.7)	0	0	1.07	1.07
	Heterozygotes	37	9 ^d	0	27 (96.4)	1 (3.6)	0	0	1.04	0.95

^a Antiparallel synapsis (8 SCs with 1 MLH1 focus; 2 with 2 foci).

^b In addition to inversion homozygotes, chromosome 2 FISH was carried out in cells from C57BL/6 females as a second control genotype.

^c One inversion loop; two synaptic “bubble” abnormalities.

^d Axial elements (without MLH1 foci) present in 3 cells; antiparallel synapsis in 6 cells (each with 1 MLH1 focus).

well as that of the individual chromosome (FROENICKE *et al.* 2002; ANDERSON *et al.* 2003). We measured all normally synapsed SCs, as well as those in the antiparallel synaptic configuration. The overall mean SC length was 13.8 μm for chromosome 2, 11.5 μm for chromosome X, and 6.0 μm for chromosome 19; the mean SC lengths for various inversion genotypes and synaptic configurations are presented in Table 2. The only significant difference in mean SC length was between In(19)37Rk controls and heterozygotes ($P = 0.012$); yet there were no differences in recombination frequency between these groups (Table 1). On average, control SC 19's were 6.9 μm , ~ 1.2 μm longer than those of inversion heterozygotes with either normal synapsis (5.7 μm) or antiparallel synapsis (5.6 μm ; Table 2).

Inversion heterozygosity alters exchange position: Although inversion heterozygosity significantly altered exchange frequency for only one of the three inversions studied, we found that the positioning of meiotic recombination events was dramatically different between heterozygotes and controls in all inversion genotypes (Figure 4). We divided each SC into 20 equal intervals of relative physical length for both controls and normally synapsed inversion heterozygotes. For In(X)1H and In(19)37Rk, we observed a striking enrichment for single exchanges in the 3 most distal intervals (distal 15%) of the SC in heterozygotes (Figure 4, A and B). The distal 15% of the SC contained 59.3 and 69.2% of MLH1 foci in In(19)37Rk and In(X)1H heterozygotes, respectively, as compared to 0 and 5.3% in controls.

For the same two inversions, we also examined the locations of MLH1 foci to assess exchange patterns for SCs from inversion heterozygotes that were in the antiparallel configuration (Figure 5). Our observations at late pachytene revealed linear SCs with pericentromeric heterochromatin at or near both ends (Figure 3D), suggesting that the unpaired ends had gone through synaptic adjustment. Consistent with this idea, all MLH1 foci

observed on SCs in the antiparallel configuration fell in the central 66% (homologously synapsed) of the SC, leaving the terminal 17% (nonhomologously synapsed via synaptic adjustment) on each end free of meiotic recombination events (Figure 5).

For In(2)2H, the locations of both single and double exchange events were shifted in heterozygotes (Figure 4C). Single MLH1 foci, most of which were located in the distal half of the SC (which contains much of the inversion) in control genotypes, were generally shifted toward the proximal half of the SC in heterozygotes. For SCs with two MLH1 foci, foci in controls appeared in all intervals except the most distal and most proximal; in heterozygotes, foci rarely (2.3% of total) appeared in six medial intervals comprising 30% of the chromosome length, but did appear in the most distal interval (Figure 4C).

The large changes in exchange distribution observed for double exchanges in In(2)2H heterozygotes raised the question of whether the distance between the two crossovers might also be substantially altered. We calculated the interfocal distance in micrometers between MLH1 foci for every linear SC with two foci in our study (Table 3); many genotypes had only one or two SCs with two MLH1 foci. For In(2)2H, the only inversion for which a substantial number of double crossovers were observed, no significant difference in interfocal distance between controls (7.1 μm) and heterozygotes (8.0 μm) was detected. Furthermore, the relative interfocal distance (expressed as a fraction of total SC length) ranged from 22.2 to 83.5% in controls and 27.7 to 84.1% in heterozygotes. Therefore, although inversion heterozygosity affected exchange positioning, it did not significantly alter the distance between two exchanges on the same chromosome.

Inversion heterozygosity does not substantially elevate nondisjunction: The accuracy of meiotic chromosome segregation was initially analyzed in a total of 2044

TABLE 2
SC lengths in inversion heterozygotes and controls

Inversion	Genotype	N (SCs)	Mean SC length, in μm (SD)	Shortest SC, in μm (no. of MLH1 foci)	Longest SC, in μm (no. of MLH1 foci)
In(X)1H	Homozygotes	24	12.5 (3.5)	8.4 (1)	25.7 ^a (2)
	Heterozygotes, normal synapsis	14	11.0 (2.6)	6.3 (1)	15.7 (1)
	Heterozygotes, antiparallel synapsis	10	11.2 (1.6)	9.7 (1)	14.2 (2)
In(2)2H	Controls ^b	46	13.8 (2.1)	10.0 (1)	18.4 (3)
	Heterozygotes ^c	65	13.8 (1.9)	8.4 (1)	19.2 (2)
In(19)37Rk	Homozygotes	15	6.9 (1.8)	5.5 (1)	12.9 (2)
	Heterozygotes, normal synapsis	28	5.7 (0.7)	4.4 (1)	7.3 (1)
	Heterozygotes, antiparallel synapsis	6	5.6 (1.1)	4.5 (1)	7.1 (1)

^a From a pachytene cell with unusually long SCs for all chromosome pairs; next longest X chromosome SC in this set was 16.8 μm with 1 MLH1 focus.

^b Control data on chromosome 2 from In(2)2H homozygotes and C57BL/6, combined.

^c The single SC with a loop and 2 aberrant "bubble" SCs were omitted from heterozygotes.

MII-arrested oocytes from females heterozygous and/or homozygous for five different paracentric inversions: In(X)1H, In(2)2H, In(2)5Rk, In(2)40Rk, and In(19)37Rk (Table 4). The genotypes studied included female mice heterozygous for both In(2)2H and In(2)40Rk *in trans*, as well as mice simultaneously heterozygous for In(X)1H, In(2)2H, and In(19)37Rk. Chromosome-specific paint probes were used in each case to monitor whether segregation of the inversion chromosome had resulted in nondisjunction (Figure 6).

As noted above, exchange within the inverted region in a paracentric inversion heterozygote produces structurally aberrant meiotic products, including dicentric chromosomes and acentric fragments (Figure 1, A and B). We sometimes observed such meiotic products for all inversion heterozygote genotypes, indicating that recombination within the inversion is not prohibited. These dicentric chromosomes exhibited novel segregational properties and their behavior is described in detail elsewhere (KOEHLER *et al.* 2002b). Since this study is concerned with the missegregation of whole chromosomes rather than segmental imbalances or chromosome aberrations, oocytes containing dicentric chromosomes or other structural abnormalities were not considered aneuploid.

No significant differences in nondisjunction frequency were detected between inversion heterozygotes and controls for any specific inversion (Table 4). However, when the sum of the hyperploid oocytes from all inversion heterozygote genotypes (7/1108, 0.6% hyperploidy or 1.2% nondisjunction) was compared to the observed nondisjunction in the controls (0/936, 0%), a slight but statistically significant increase in the level of nondisjunction in inversion heterozygotes was detected ($P = 0.003$). Much of this effect appears to be

contributed by females heterozygous for In(2)5Rk (4/7 hyperploid oocytes; Table 4), although it is not significant when this strain is considered alone.

We therefore studied an additional 469 oocytes from heterozygous and control females of advanced maternal age for the inversions In(2)5Rk and In(2)2H. However, no age-related increase in nondisjunction was seen in either case (Table 5).

DISCUSSION

These experiments were initiated as part of a search for a mammalian model that mimicked the recombination frequency and exchange patterns observed in human aneuploidy. Thus, we were especially interested in situations that might abolish exchange between homologs altogether or those that would generate an increase in single, distal exchanges—that is, situations enriched for the types of chiasmate configurations that are known to be susceptible to nondisjunction in humans, *Drosophila*, and yeast (KOEHLER *et al.* 1996; LAMB *et al.* 1996; Ross *et al.* 1996).

Most inversion studies in the mouse have analyzed synapsis and earlier stages of meiosis at the expense of segregation studies; many of them have also focused on the male (FORD *et al.* 1976; POORMAN *et al.* 1981; CHANDLEY 1982; MOSES *et al.* 1982; TEASE and FISHER 1986; GORLOV *et al.* 1991; BORODIN *et al.* 1992; ASHLEY *et al.* 1993; GORLOV and BORODIN 1995; RUMPLER *et al.* 1995). Furthermore, these studies of early meiosis have often focused on synapsis rather than on recombination; synapsis creates the intimate homologous pairing context required for exchange but does not ensure that it occurs. Here we provide a detailed analysis of meiotic

FIGURE 4.—Positioning of exchange events (MLH1 foci) along normally synapsed SCs in inversion heterozygotes and controls. The SC is divided into 20 equal intervals of relative physical length, centromere (left) to telomere (right). Arrows indicate approximate location of inversion. Bars represent fraction of total SCs with an MLH1 focus in the given interval. Graphs depict distributions of (A) single exchange positions for chromosome 19 bivalents in oocytes from In(19)37Rk heterozygotes (solid) and controls (open), (B) single exchange positions for X chromosome bivalents in oocytes from In(X)1H heterozygotes (solid) and controls (open), and (C) single and double exchange positions for chromosome 2 bivalents in oocytes from In(2)2H heterozygotes (solid) and controls (open).

recombination and segregation in five different female paracentric inversion heterozygotes, as well as two other genotypes bearing multiple inversions.

Inversion heterozygosity can alter exchange frequency: In control genotypes, the X chromosome was 60.5 cM in length, chromosome 2 was 81.5 cM, and

FIGURE 5.—Positioning of exchange events (MLH1 foci) along SCs with antiparallel synapsis. Each circle represents one MLH1 focus from In(19)37Rk heterozygotes (single exchanges, open circles) or In(X)1H heterozygotes (single exchanges, solid circles; double exchanges, shaded circles). Since SCs with antiparallel synapsis have ends that are indistinguishable from each other (a centromere on either side), relative positions of foci were measured from the center of the SC. Arrows denote approximate distal breakpoint locations, separating homologous from nonhomologous synapsis.

chromosome 19 was 53.5 cM (mean number of MLH1 foci/SC from Table 1, multiplied by 50 cM/focus). These genetic lengths are all considerably shorter than the current values from established linkage maps, which are based on the analysis of molecular markers inherited by progeny (*e.g.*, DIETRICH *et al.* 1996). The Mouse Genome Database consensus map (<http://www.informatics.jax.org>) gives the genetic length of the X chromosome as 96.7 cM, chromosome 2 as 114 cM, and chromosome 19 as 57 cM. Since recombination rates in mammalian females are generally higher than those in mammalian males (POLANI 1972; SPEED 1977; BROMAN *et al.* 1998; LYNN *et al.* 2000), the fact that these linkage maps are sex averaged cannot be responsible for this discrepancy. However, studies using cytogenetic methods to assess exchange levels directly in mice, humans, and maize have all consistently yielded maps significantly shorter than those produced through linkage analysis (LAURIE and HULTEN 1985; LAWRIE *et al.* 1995; FROENICKE *et al.* 2002; KOEHLER *et al.* 2002a; LYNN *et al.* 2002; TEASE *et al.* 2002; ANDERSON *et al.* 2003). The reasons for these differences remain unclear (but see FROENICKE *et al.*

2002 and ANDERSON *et al.* 2003 for further discussion). Nevertheless, our control estimates of genetic length are consistent with previous studies in the female mouse using chiasma analysis at diakinesis (HULTEN *et al.* 1995; LAWRIE *et al.* 1995).

In inversion heterozygotes (including all SC configurations), the X chromosome was 56.5 cM, chromosome 2 was 96.5 cM, and chromosome 19 was 47.5 cM (mean number of MLH1 foci/SC from Table 1, multiplied by 50 cM/focus). In(2)2H was the only inversion to trigger a significant difference in genetic length, an increase in recombination frequency; however, the overall linear SC length for chromosome 2 in In(2)2H heterozygotes remained the same (Table 2). In contrast, In(19)37Rk heterozygotes, which had a recombination rate similar to that of controls (Table 1), exhibited a significantly shorter mean SC length (Table 2). Thus, this study does not support previous observations that increasing genetic length is correlated with increasing SC length (LYNN *et al.* 2002).

We observed the absence of MLH1 foci on the SC of an inversion heterozygote only when synapsis had failed

TABLE 3

Interfocus distances between MLH1 foci on the same SC in inversion heterozygotes and controls

Inversion	Genotype	No. of SCs with two MLH1 foci	Mean interfocus distance, in μm (SD)
In(X)1H	Homozygotes	5	7.8 (3.3)
	Heterozygotes, normal synapsis	1	5.3 (0)
	Heterozygotes, antiparallel synapsis	2	3.7 (1.5)
In(2)2H	Controls ^a	25	7.1 (2.2)
	Heterozygotes ^b	43	8.0 (2.0)
In(19)37Rk	Homozygotes	1	7.0 (0)
	Heterozygotes, normal synapsis	1	3.9 (0)
	Heterozygotes, antiparallel synapsis	0	NA

NA, not applicable.

^a Control data on chromosome 2 from In(2)2H homozygotes and C57BL/6, combined.

^b The single SC with a loop and 2 aberrant SCs were omitted from heterozygotes.

TABLE 4
Chromosome segregation in MII-arrested oocytes from inversion heterozygotes and controls

Category	Genotype	N	No. of cells hyperploid for inversion chromosome	% hyperploidy	% nondisjunction ^a
Controls	+ / + (sibs of In(2)5Rk) ^b	145	0	0	0
	In(2)2H/In(2)2H	402	0	0	0
	In(2)40Rk/In(2)40Rk	73	0	0	0
	In(X)1H/In(X)1H	133	0	0	0
	In(19)37Rk/In(19)37Rk	183	0	0	0
Heterozygotes	In(2)5Rk/+	237	4	1.7	3.0
	In(2)2H/+	243	1	0.4	0.8
	In(2)40Rk/+	137	0	0	0
	In(X)1H/+	121	1	0.8	1.7
	In(19)37Rk/+	189	0	0	0
	In(X)1H/+, In(2)2H/+, In(19)37Rk/+	139	1 ^c	0.7	1.4
	In(2)2H/In(2)40Rk	42	0	0	0

^a See MATERIALS AND METHODS.

^b In(2)5Rk is homozygous lethal; see MATERIALS AND METHODS.

^c Hyperploid for X chromosome.

altogether. In 3/37 oocytes (8%) from In(19)37Rk heterozygotes, the SCs remained as axial elements, although the chromosome 19's were colocalized, suggesting that some initial pairing steps had taken place (Table 1; Figure 3E). This figure, if indicative of the nonexchange chromosome pair frequency at the first meiotic metaphase, suggests that 8% of all chromosome 19 pairs lack a crossover in oocytes from inversion heterozygotes. This contrasts sharply with our finding that the rate of achiasmate or nonexchange chromosome pairs at pachytene (as judged by MLH1 foci on pachytene-stage SCs for all chromosomes) in normal mice from a variety of inbred strains is quite low, ~0.1% in males (autosomes only; KOEHLER *et al.* 2002a) and ~0.2–0.3% in females (K. KOEHLER and T. HASSOLD, unpublished observations).

However, it is not clear that the frequency of nonexchange or unpaired homologs at pachytene is an accu-

rate reflection of their frequency at metaphase I. A well-documented checkpoint exists at pachytene in many organisms, including yeast and male mice (ODORISIO *et al.* 1998; ROEDER and BAILIS 2000), that effectively arrests cells with asynapsed or unpaired chromosomes and prevents them from exiting pachytene. Although some quality control mechanisms and other meiotic checkpoints have been shown to be less restrictive or even nonfunctioning in female mammals (HUNT and HASSOLD 2002), it is at present unknown how effective the pachytene checkpoint is in the female mouse, although many cells are lost around the pachytene stage (McCLELLAN *et al.* 2003). However, it is possible to conclude from the data presented here that, in general, nonexchange chromosomes are not a major consequence of heterozygosity for a single inversion in the female mouse.

Inversion heterozygosity alters exchange position: For

TABLE 5
Chromosome segregation in MII-arrested oocytes from inversion heterozygotes and controls of advanced maternal age

Category	Genotype of female	N	No. of cells hyperploid for inversion chromosome	% hyperploidy	% nondisjunction ^a
Controls	In(2)2H/In(2)2H	91	0	0	0
	+ / + (sibs) ^b	88	0	0	0
Heterozygotes	In(2)2H/+	136	0	0	0
	In(2)5Rk/+	154	1	0.6	1.3

^a See MATERIALS AND METHODS.

^b In(2)5Rk is homozygous lethal; see MATERIALS AND METHODS.

FIGURE 6.—Preparations of MII-arrested oocytes using chromosome-specific FISH (green) to detect the inversion chromosome. (A) A normal oocyte from an In(2)5Rk heterozygote, with a euploid chromosome complement of 20 and a single copy of chromosome 2. The arrow denotes the degrading chromatin of the first polar body. (B) An aneuploid oocyte from an In(2)5Rk heterozygote, with 21 chromosomes, including two copies of chromosome 2.

SCs with normal synapsis as well as those with antiparallel synapsis, inversion heterozygosity causes exchange events to be placed in patterns quite different from their

normal distributions (Figure 4). Two of the inversions, In(X)1H and In(19)37Rk, exhibited intriguing similarities in meiotic behavior when heterozygous in oocytes. Although mouse chromosomes X and 19 are substantially different in size, both inversions have similar “shapes” (*i.e.*, breakpoint locations and proportion of total chromosome length covered by the inversion; see Figure 2). Both exhibited some degree of antiparallel synapsis when heterozygous, as might be expected in a situation where the longest stretch of homology is the inverted region. However, the most remarkable similarity between these two inversions was their exchange placement profiles when synapsed normally, notably the large increase in exchange in the distal 15% of the chromosome, which roughly corresponds to the uninverted region (Figure 4, A and B). The high level of recombination in this chromosomal segment may be related to the tendency for chromosomes to begin pairing at their ends (ASHLEY *et al.* 1993; GORLOV and BORODIN 1995; SCHERTHAN *et al.* 1996; ZICKLER and KLECKNER 1998). It would be interesting to examine other heterozygotes with similar breakpoint positioning to determine whether such behavior is a general property of inversion shape.

For In(2)2H, the difference in exchange positioning is accompanied by an increase in exchange frequency. This is consistent with previous suggestions that the sequence discontinuities created by inversion heterozygosity (or other structural abnormalities) can result in several chromosomal regions becoming available for pairing or recombination independent of other intervals on the chromosome (*e.g.*, GORLOV and BORODIN 1995). Indeed, crossover interference (and hence exchange positioning) appears to be mediated by the SC (SYM and ROEDER 1994; ZICKLER and KLECKNER 1999; YUAN *et al.* 2002). The breakpoints of In(2)2H divide the chromosome into three intervals; double crossovers in inversion heterozygotes appeared to preferentially span the inversion rather than fall within it (Figure 4C); yet, perhaps surprisingly, the distance between the two exchanges is not altered (Table 3). This exchange pattern suggests that In(2)2H might be good balancer chromosome material; furthermore, it already has a dominant genetic marker (*suppressor of agouti*; *A^s*) at its distal breakpoint (EVANS and PHILLIPS 1978).

We observed aberrant chromosomal products of recombination within the loop in oocytes after the first meiotic division for all inversions studied (KOEHLER *et al.* 2002b). This suggests that the inversions studied here all participated in homologous synapsis (including loop formation) followed by synaptic adjustment, rather than direct heterologous synapsis. However, our analysis of pachytene oocytes did not allow us to precisely localize the inversion boundaries along SCs, and it is difficult to accurately determine the level of recombination (or recombination suppression) within the inversion without this information. Currently, the detailed integration

of mouse cytogenetic and linkage maps required to refine these inversion breakpoints further is not yet available, although such anchoring efforts have begun (KORNERBERG *et al.* 1999; FROENICKE *et al.* 2002). However, recent studies of inversions in humans (GABRIEL-ROBEZ and RUMPLER 1994; JAAROLA *et al.* 1998) have provided support for the Ashley model, in which the type of chromatin present at the breakpoints influences meiotic pairing behavior (ASHLEY 1990).

Inversion heterozygosity causes low but significant levels of nondisjunction: The finding that paracentric inversions do not cause high rates of aneuploidy was somewhat unanticipated. Meiotic exchange frequency and positioning is under tight genetic control and exchange is a crucial prerequisite for proper meiotic chromosome segregation (see HAWLEY 1988 for review). Despite dramatic alterations in patterns of meiotic exchanges and even the possible formation of a few nonexchange chromosome pairs, we did not observe any increase in nondisjunction in inversion heterozygotes. The original studies on In(X)1H suggested that heterozygous females produced not only aneuploid oocytes, including those with extra chromosomes, but also a high frequency of X_pO daughters (PHILLIPS *et al.* 1973; PHILLIPS and KAUFMAN 1974; EVANS and PHILLIPS 1975). Although our study utilized chromosome-specific FISH to unambiguously identify the X chromosome, we did not observe a significant increase in aneuploidy in oocytes from females heterozygous for In(X)1H (Table 4). An alternative mechanism for the chromatid loss that results in the production of X_pO daughters has been proposed on the basis of cytological observations of the aberrant chromosomal products during the meiotic and early mitotic divisions (KOEHLER *et al.* 2002b).

The unsynapsed axial elements observed in In(19)37Rk heterozygotes (Figure 3E) may, at least in theory, mature into nonexchange chromosome pairs. On the basis of our data, this would suggest that 8% (3/37) of all cells have an achiasmate chromosome 19 pair. Assuming random segregation of nonexchange chromosomes, ~4% nondisjunction of chromosome 19 would be expected; none was observed (Table 4). As discussed earlier, one possible explanation for this discrepancy is that cells with unsynapsed chromosomes arrest and fail to exit pachytene (see above). Another possibility is that mammals have some form of “backup” segregational mechanism reminiscent of that in *D. melanogaster* females (for review, see KOEHLER and HASSOLD 1998). Such a system could be as simple as the presence of an activity similar to that of NOD, a kinesin-like *Drosophila* protein that acts as a “chiasma substitute” and helps ensure the correct segregation of nonexchange and even some distal-exchange chromosome pairs (CARPENTER 1973; ZHANG *et al.* 1990; RASOOLY *et al.* 1991; AFSHAR *et al.* 1995a,b).

The inversion with the greatest number of nondis-

junction events was In(2)5Rk (Table 4). In(2)5Rk has been reported to have a maternal age effect on the frequency of fetal loss in heterozygous females (FORD *et al.* 1976), which we initially suspected could potentially be due to an increase in aneuploidy with age. Although we found that 4/237 oocytes were hyperploid for chromosome 2 in oocytes from 4-week-old In(2)5Rk heterozygotes, only 1/136 oocytes had an extra chromosome 2 in aged females of the same genotype. We also examined In(2)2H for a possible effect of maternal age on chromosome segregation, but did not observe one (Table 5). Therefore, we conclude that maternal age does not increase chromosome nondisjunction in inversion heterozygotes and that the maternal age effect on fetal loss observed in In(2)5Rk heterozygotes is not the result of aneuploidy (FORD *et al.* 1976). However, we also failed to observe an increase in dicentric bridge formation with advancing maternal age (K. KOEHLER and T. HASSOLD, data not shown), which FORD *et al.* (1976) suggested might be responsible for the fetal wastage; thus, the source of the increased fetal loss in older female mice remains unclear.

Although no individual inversion generated an increase in aneuploidy when heterozygous, when all inversion heterozygote genotypes are considered as a single group, the collective level of nondisjunction is significantly higher than that in controls (1.2% *vs.* 0, $P = 0.003$). This aneuploidy rate is so low that inversion heterozygosity does not represent an efficient or practical model for human aneuploidy. Interestingly, though, data from *Drosophila* also indicate that females heterozygous for balancer chromosomes have a very small but significantly increased level of nondisjunction over chromosome pairs where both homologs are normal sequence or inverted (ZITRON and HAWLEY 1989; HAWLEY *et al.* 1992).

Multiple inversions and sexual dimorphism: Even the analysis of a single inversion heterozygote can be very complicated, since in a number of cases the same inversion can exhibit direct nonhomologous synapsis in some cells and inversion loop formation in others (CHANDLEY 1982; BORODIN *et al.* 1990, 1992; RUMPLER *et al.* 1995). In male mice carrying multiple inversions of the same chromosome *in trans*, serious synaptic abnormalities have been observed, concomitant with sterility (CHANDLEY 1982; RUMPLER *et al.* 1995). However, extrapolating meiotic chromosome behavior between the sexes is frequently unreliable (HUNT and HASSOLD 2002; KOEHLER *et al.* 2002b), so any comparisons between males and females should be made with caution. In oocytes from females of two genotypes with multiple paracentric inversions, neither meiotic progression nor segregation appeared to be compromised (Table 4) and structurally aberrant recombinant chromosomes were generated at rates typical for single paracentric inversion heterozygotes, suggesting that large numbers of oocytes were not ar-

resting at pachytene (KOEHLER *et al.* 2002b; K. KOEHLER and T. HASSOLD, unpublished observations). Females simultaneously heterozygous for In(X)1H, In(2)2H, and In(19)37Rk, as well as In(2)2H/In(2)40Rk heterozygotes, are fertile, but quantitative comparisons of fertility levels relative to controls were not made. Thus, heterozygosity for multiple inversions may not pose as serious a problem in female meiosis as in male meiosis.

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