Sex-Specific Recombination Rates in Zebrafish (*Danio rerio*)

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ABSTRACT

In many organisms, the rate of genetic recombination is not uniform along the length of chromosomes or between sexes. To compare the relative recombination rates during meiosis in male and female zebrafish, we constructed a genetic map based on male meiosis. We developed a meiotic mapping panel of 94 androgenetic haploid embryos that were scored for genetic polymorphisms. The resulting male map was compared to female and sex-average maps. We found that the recombination rate in male meiosis is dramatically suppressed relative to that of female meiosis, especially near the centromere. These findings have practical applications for experimental design. The use of exclusively female meiosis in a positional cloning project maximizes the ratio of genetic map distance to physical distance. Alternatively, the use of exclusively male meiosis to localize a mutation initially to a linkage group or to maintain relationships of linked alleles minimizes recombination, thereby facilitating some types of analysis.

In the 1920s, Haldane (1922) and Huxley (1928) noted that when meiotic recombination rates vary between the two sexes, it is usually the heterogametic sex that has suppressed recombination. In Drosophila, for example, this reduction is so dramatic that during male gametogenesis there are no chiasmata and hence no recombination (Morgan 1914). In mammals, many species show reduced recombination frequency in males, which are the heterogametic sex. Averaged over the entire genome, human female-to-male recombination rates are 1.6:1.0 (Dib *et al.* 1996). Ratios >1 are also found in dog (1.4:1.0), pig (1.4:1.0), and mouse (1.25:1.0) (Dietrich *et al.* 1996; Marklund *et al.* 1996; Neff *et al.* 1999).

Although the heterogametic sex usually has reduced recombination, there are exceptions to the rule. For example, in sheep, rams have 1.3 times as much recombination as ewes (Crawford *et al.* 1995), and in cattle there is no significant sex-specific difference (Kappes *et al.* 1997). Likewise, in birds, where the female is the heterogametic sex, hens and roosters have about the same rate of recombination (Groenen *et al.* 2000), and pigeon males and females have equal numbers of recombination nodules in sperm and eggs (Pigozzi and Solari 1999). Many species of fish do not have heteromorphic sex chromosomes, so the rule does not apply. Despite the absence of heteromorphic sex chromosomes in medaka, recombination is suppressed in both genotypic (XY) and phenotypic (XX) males on the chromosome bearing the sex-determination factor (Matsuda *et al.* 1998, 1999). Rainbow trout do have sex chromosomes and Sakamoto *et al.* (2000) found that male recombination is dramatically suppressed relative to females; in trout the female-to-male recombination ratio is 3.25:1.0.

While genetic maps are available for several fish species, comparisons between male and female recombination rates are not. The diploid map for Xiphophorus (Morizot *et al.* 1991) is sex averaged, the map for tilapia is gynogenetic and represents only female meiotic recombination events (Kocher *et al.* 1998), and the medaka map is based on meiosis from a single male (Naruse *et al.* 2000).

Knowing the relative rates of recombination in males and females has substantial practical significance for experimental design. For some types of experiments, a high rate of recombination is useful because it helps to distinguish closely linked markers. High rates of recombination thus facilitate the fine-structure genetic mapping necessary for positional cloning of mutations. In contrast, the initial approximate mapping of a mutation to a general region of the genome is easier in a system with a low rate of recombination. Furthermore, a system with a low rate of recombination tends to preserve alleles linked *in cis*, which is useful for the maintenance of linked double mutants for epistasis analysis. Thus, it is important to know the relative rates of recombination for both sexes of any species used in genomic analysis.

The zebrafish, *Danio rerio*, has recently emerged as a widely used model for large-scale analysis of genome function (Talbot and Hopkins 2000). This is based primarily on the ease with which gene functions can be inferred from the analysis of mutant animals and the similarity of developmental mechanisms controlling embryogenesis in zebrafish and mammals (Kimmel 1989).

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For efficient genetic analysis of mutations, it would be useful to manipulate the rate of recombination. Because zebrafish do not have heteromorphic sex chromosomes (Endo and Ingalls 1968), despite the application of a variety of banding techniques (Daga *et al.* 1996; Gornung *et al.* 1997; Amores and Postlethwait 1999), it is difficult to predict *a priori* the relative rates of recombination in the two sexes.

Several meiotic maps for zebrafish have been constructed solely on the basis of meiosis in females using gynogenetic methodologies (Postlethwait *et al.* 1994, 1998, 2000; Johnson *et al.* 1996; Gates *et al.* 1999; Barbazuk *et al.* 2000; Kelly *et al.* 2000; Woods *et al.* 2000) and a sex-average map is available from natural matings (Knapik *et al.* 1996, 1998; Shimoda *et al.* 1999), but no estimate of recombination rates in males relative to females is yet available. While maps using radiation hybrids are useful for fine-structure mapping (Geisler *et al.* 1999; Hukriede *et al.* 1999; Kwok *et al.* 1999), they do not address the question of genetic recombination rates in meiosis. To address this gap in our knowledge, we constructed a map of the zebrafish genome based solely on recombination frequency during male meiosis. To accomplish this goal, we constructed an androgenetic haploid mapping panel from an individual male who was heterozygous for two highly polymorphic genomes. Constructing a meiotic map based on male meiosis from this mapping panel, and comparing the male map to the female map (Postlethwait *et al.* 1994, 1998), showed that recombination is drastically suppressed in male zebrafish relative to female meiosis, particularly around the centromeres.

MATERIALS AND METHODS

Animals: All fish and embryos were maintained at 28.5° on a 14-hr-light/10-hr-dark cycle. The *golden* strain (Streisinger *et al.* 1989) used for control experiments and the *AB zebrafish used to form the mapping panel were from University of Oregon lines (see http://www.zfin.org for information on fish strains).

Androgenetic mapping panel: Androgenetic haploid embryos were generated as described by Corley-Smith *et al.* (1996). Eggs from 10 females from the *AB line were pooled and exposed to 10 kR of gamma radiation from a cesium source. Eggs were irradiated with a dose found empirically to result in haploid progeny, but no diploid progeny. To determine the extent of maternal nuclear genes functional in the androgenetic progeny, we gamma-irradiated eggs of wild-type females and fertilized them with sperm from males homozygous for the *golden* (*gol*) body color mutation. Although a few of the offspring had an occasional black melanocyte, indicating the function of the wild-type allele of the *gol* gene (derived from the female) in these few cells, polymerase chain reactions (PCRs) always amplified single alleles for microsatellite markers in the embryos used for the mapping experiments, thus demonstrating that the few contaminating maternal alleles did not show up in our mapping reactions. Irradiated eggs were fertilized with sperm collected by gentle squeezing from a single, anesthetized male (Ransom and Zon 1999), the hybrid F_1 son of a *AB female mated to an SJD male. Because the most robust meiotic maps are made from highly inbred lines and because these are not usually available in fish, meiotic maps in fish are usually constructed by analyzing recombination in a single parent (Postlethwait *et al.* 1994, 1998; Kocher *et al.* 1998; Gates *et al.* 1999; Naruse *et al.* 2000; Sakamoto *et al.* 2000) or single pair of parents (Knapik *et al.* 1996, 1998; Shimoda *et al.* 1999). At 3 days after fertilization, DNA was collected from androgenetic embryos as described (Postlethwait *et al.* 1994). The mapping panel consisted of 94 individuals shown to be haploid by the presence of a single allele for each locus scored.

Genotyping: Primers for microsatellite markers were developed by Knapik *et al.* (1996, 1998) and Shimoda *et al.* (1999; Research Genetics, Huntsville, AL). Sequences for the primers used can be found at http://zebrafish.mgh.harvard.edu/mapping/ssr_map_index.html and http://zfin.org/ZFIN/. Loci were amplified using PCR protocols described by Shimoda *et al.* (1999). Polymorphic loci were scored for *AB or SJD alleles following gel electrophoresis in 3–4% agarose gels. Each locus was scored twice. To assure authenticity of double crossovers, each incident of apparent double crossover was rescored. If the double crossover could not be attributed to an error in scoring, the PCR was rerun on the individuals in question.

Map construction: Genetic maps were constructed using Map Manager at 99.9% limit (K. Manley and R. Cudmore, http://mcbio.med.buffalo.edu/mapmgr.html). Linkage was acceptable if the LOD score was 3 or higher. When applicable, each linkage group (LG) was subjected to the "rearrange" analysis seven times.

For comparison to the androgenetic haploid map, we used markers scored on a gynogenetic haploid map (Postlethwait *et al.* 1998) constructed from the same genetic strains. The use of the same genetic strains for the male and female maps controls for any hypothetical strain-specific recombination rates. The total number of markers used to draw the female map was 242 simple sequence length polymorphisms (SSLPs), 22 genes, and 10 randomly amplified polymorphic DNA markers for a total of 274 loci.

The diploid genetic map was adapted from Shimoda *et al.* (1999). Only SSLPs that were mapped on the male and female maps were used on the adapted sex-average map. The distances between markers were calculated by adding the intervals between the markers of interest as shown in Shimoda *et al.* (1999) and the sex-average map website (http://zebrafish.mgh.harvard.edu/mapping/ssr_map_index.html). Several SSLPs (z1313, z3309, z5503, z4161, z1396, and z5500) that were mapped on the male map do not currently appear on the sex-average map website. The positions of these loci were intercalated on the sex-average map based on their positions with respect to closely linked flanking markers located on both the sex-average map and either the LN54 radiation hybrid (RH) map (Hukriede *et al.* 1999; http://eclipse.nichd.nih.gov/nichd/lmg/lmgdevb.htm) or the gynogenetic doubled haploid (heat-shock) panel (Woods *et al.* 2000; http://zebrafish.stanford.edu/).

Some linkage groups in the male map have markers that are not located on either gynogenetic map. For example, for LG13, z1774, the top marker on the male map, is not on the female map, and z4252, the bottom marker on the female map, is not on the male map. To deal with such situations, we calculated for comparative purposes the "greatest distance in common." The greatest distance in common on LG13 is between z1531 and z6657, which is 28.2 cM on the male map, 130.6 cM on the female map, and 52.5 cM for the sex-average map.

Statistical analyses: To analyze the data for LG5, LG7, and LG14 each interval distance was standardized as a percentage of total LG length [standardized interval distance = 100 *

(interval distance/total LG length)]. We plotted male standardized intervals against female standardized intervals for each LG. Visual examination of the resultant graph reveals three areas of distinct slope along the length of each LG. Because the slope of the lines may represent biologically real differences in recombination rate between the sexes we analyzed the slopes of the lines by simple linear regression of two continuous variables (male and female) in groups defined as left, middle, and right thirds. The left-third data points included the first three intervals of LG5 and LG14 and the first four intervals of LG7. The middle-third data points included the next four intervals for LG5 and LG14 and the middle five intervals of LG7. The right third included the last three intervals for all three LGs.

RESULTS

Compression of the male map: To understand the relative rates of recombination in male and female zebrafish, we constructed a genetic map based on male meiosis and compared it to female and sex-average maps for this species. The male mapping panel consisted of 94 haploid embryos that were the androgenetic progeny (Corley-Smith *et al.* 1996) of a single male heterozygous for *AB and SJD chromosomes. In general, genetic markers for the male map were chosen that were localized on both a gynogenetic haploid map (Postlethwait *et al.* 1998) and a sex-average map (Knapik *et al.* 1996, 1998; Shimoda *et al.* 1999) and that were approximately uniformly distributed across the full length of each chromosome in both maps. Of 325 markers tested, 185 were polymorphic and therefore usable on the androgenetic haploid panel. There remains a discrepancy as to the location of the marker z1322. This marker maps to the same location on LG7, between z9323 and z4507, on the androgenetic haploid map, the gynogenetic haploid map (Postlethwait *et al.* 1998), and the gynogenetic double haploid (heat-shock) map (Woods *et al.* 2000), but maps to LG9 on the sex-average map (Shimoda *et al.* 1999).

Figure 1 compares the androgenetic haploid map to the female and sex-average maps for each of the 25 zebrafish chromosomes. A total of 141 SSLPs make up the male map, and the map has no gaps between mapped markers. The average interval between markers is 7.2 cM, and the largest interval is 30.4 cM. The total length of the mapped intervals is 1009.9 cM.

For nearly all linkage groups, the male map is much shorter than the female map, and the sex-average map is intermediate in length between the maps from meiosis in individual sexes. Summing the lengths of the greatest common interval for each linkage group on both the female and male maps gave lengths of 2582.7 and 942.5 cM, respectively. This is a female-to-male ratio of 2.74:1.0. We conclude that recombination is dramatically suppressed in male meiosis compared to female meiosis, substantially more than in humans (Dib *et al.* 1996) and other mammals (Dietrich *et al.* 1996; Marklund *et al.* 1996; Neff *et al.* 1999).

The sex-average map was constructed from the F_2 of a standard diploid cross (Knapik *et al.* 1996, 1998; Shimoda *et al.* 1999). This sex-average map has a length of 1635.6 cM for the sum of the chromosome lengths held in common with the male map. For comparison, the calculated average of the lengths of the male and female maps [(m + f)/2] is 1762.6 cM. Thus, the sex-average map approximates a linear average of the male and female maps, as expected.

Pericentromeric depression of recombination in male zebrafish: The comparison of linkage group lengths showed that the male map is much shorter than the female map. However, the difference is not equally distributed over the entire chromosome; it is concentrated in special regions of the chromosome. Examination of Figure 1 shows that, in general, most linkage groups on the male map are compressed toward the centromeres relative to the corresponding intervals on the female map. The locations of the centromeres for each linkage group (Kane *et al.* 1999; Mohideen *et al.* 2000) are shown in Figure 1, and, in general, the regions of map compaction correspond to the chromosome regions surrounding the centromeres. Regions around the centromere that occupy ≥30 cM on the female map can encompass only 1 or 2 cM on the male map. For example, on LG5, z647 to z3804, a 60.8-cM region on the female map occupies only 1.3 cM on the male map. Toward the telomeres, however, the male map is expanded.

To provide a more detailed comparison of relative interval sizes along the length of chromosomes in male and female meioses, we mapped a larger number of markers distributed approximately uniformly along the length of LG5, LG7, and LG14. Figure 2 compares standardized interval sizes on the male and female maps along each of these three chromosomes from the upper to the lower end of the chromosome. This figure confirms that recombination in the region around the centromeres is preferentially lower in male meiosis than in female meiosis. When broken into thirds, regression analyses verify our conclusions. The average slope of the three lines in the middle third of the graph (the region encompassing the centromeres) is 0.26 (±0.14) and is significantly different from 1 ($P < 0.05$), the slope expected if there were no sex-specific difference in recombination rates. We conclude that, in general, most of the decrease of male recombination relative to female recombination in zebrafish takes place around the centromere. In contrast, toward the telomeres, the slopes for the left and right thirds were 1.71 (±0.24) and 1.83 (±0.36), significantly >1 ($P < 0.05$ for each). We conclude that near the telomeres the trend is for the male map to be expanded relative to the female map.

DISCUSSION

The results presented here show that zebrafish males have an overall reduction in recombination rates rela-

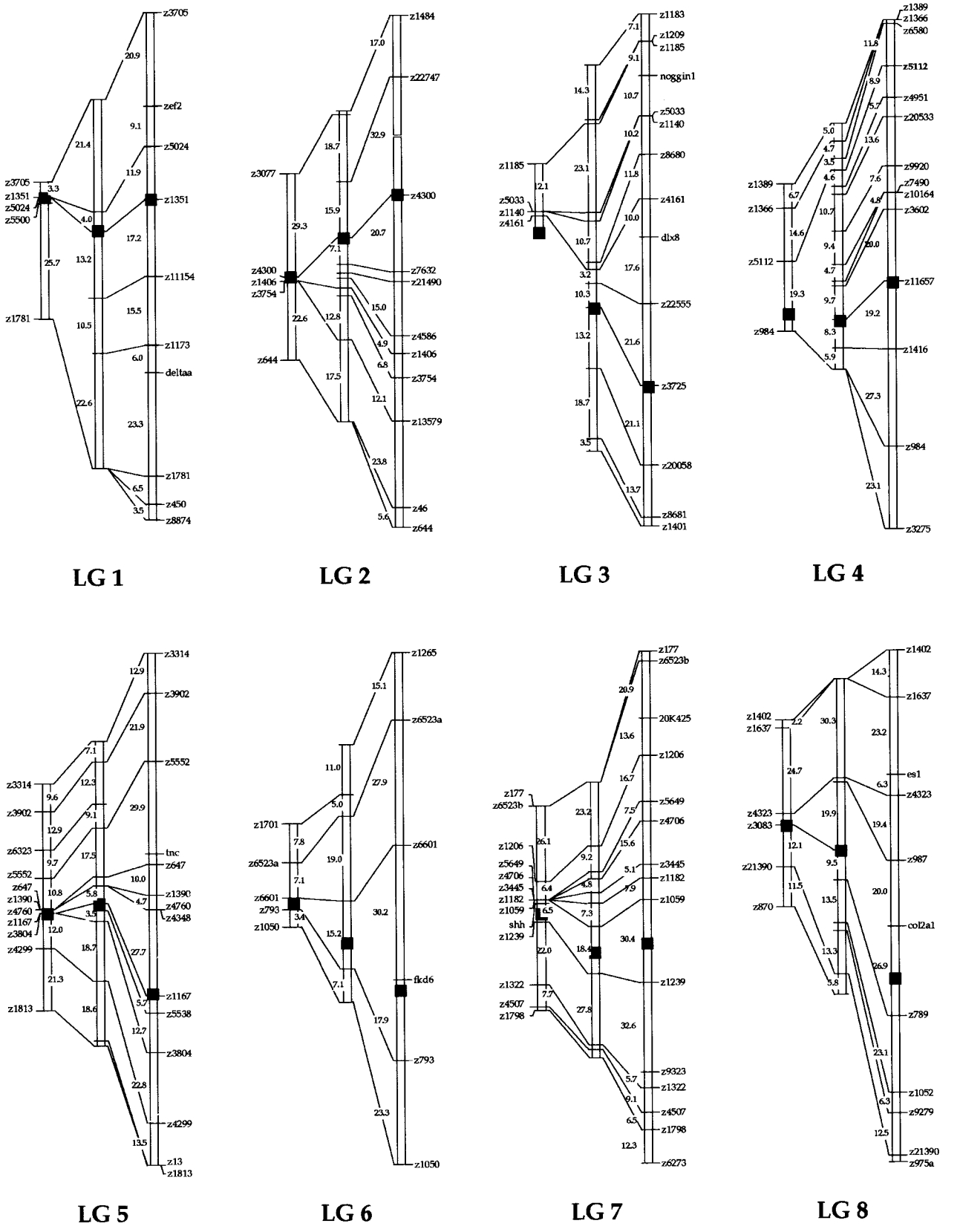


FIGURE 1.—Male, sex-average, and female genetic maps. The male genetic map is compressed compared to female and sex-average maps. For each chromosome, the figure shows the male map on the left, the sex-average map in the middle (KNAPIK *et al.* 1998; SHIMODA *et al.* 1999), and the female map on the right (POSTLETHWAIT *et al.* 1998). Centromeres are indicated by solid boxes (KANE *et al.* 1999; MOHIDEEN *et al.* 2000).

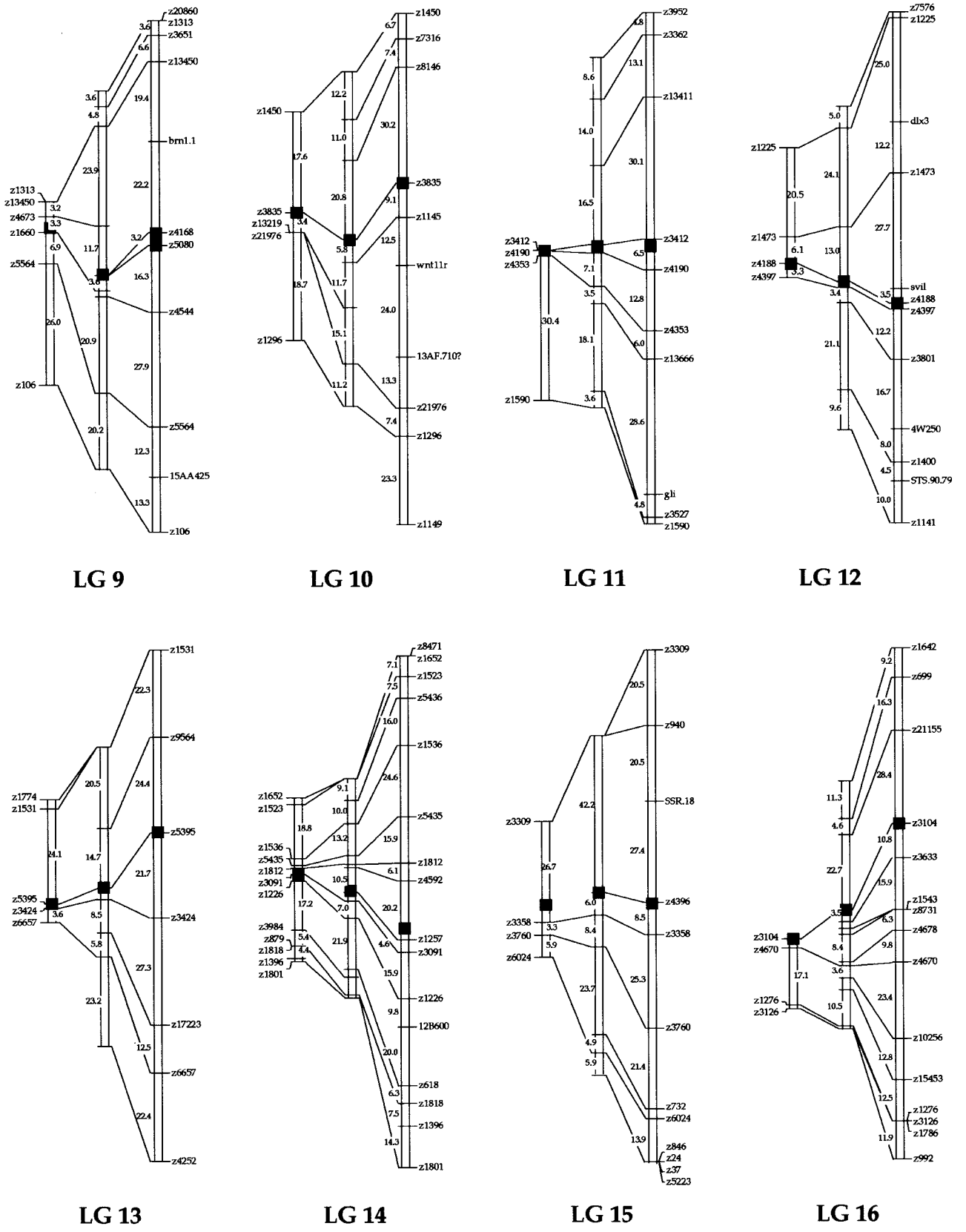
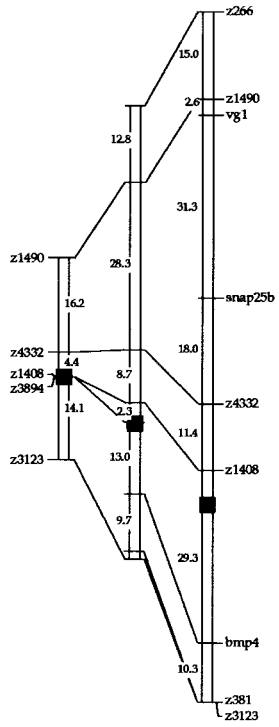
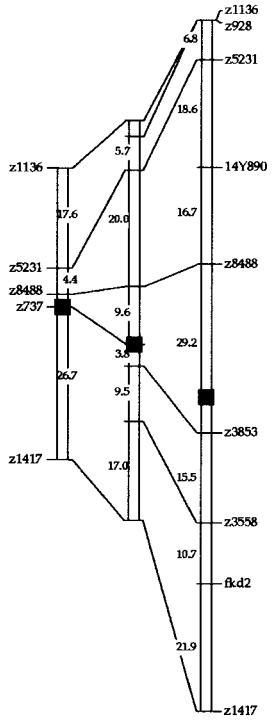


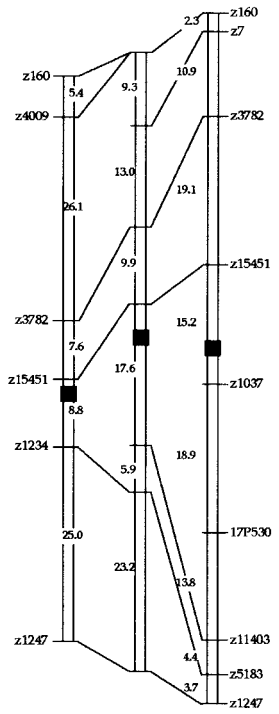
FIGURE 1.—*Continued.*



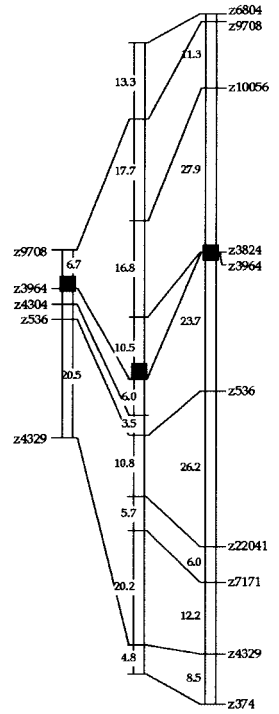
LG 17



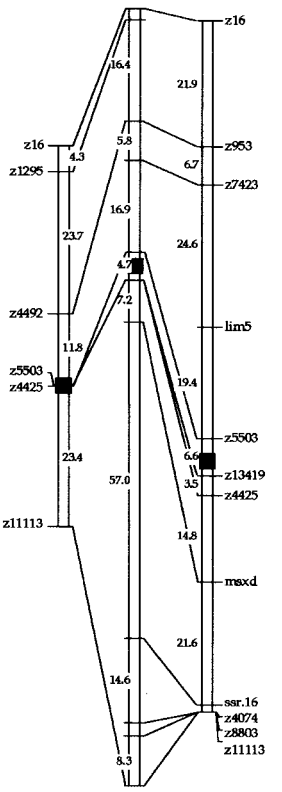
LG 18



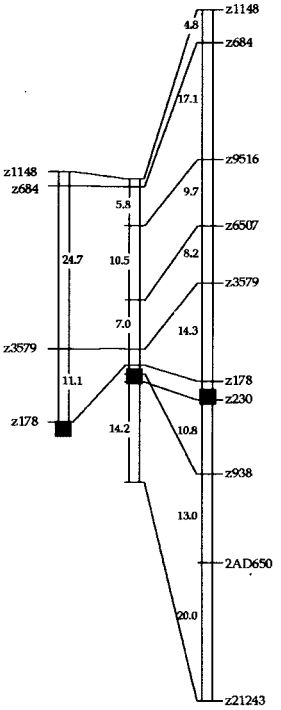
LG 19



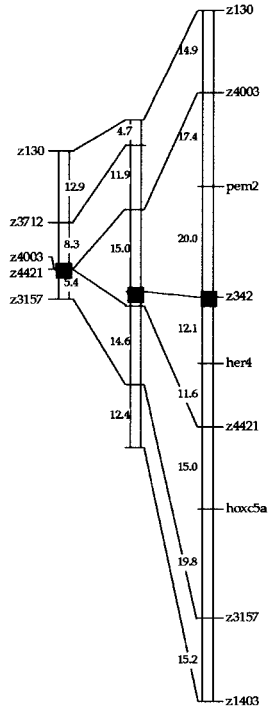
LG 20



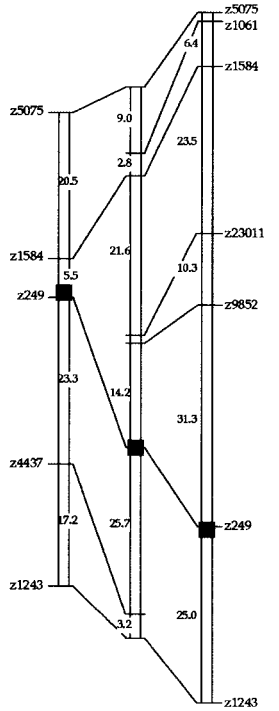
LG 21



LG 22

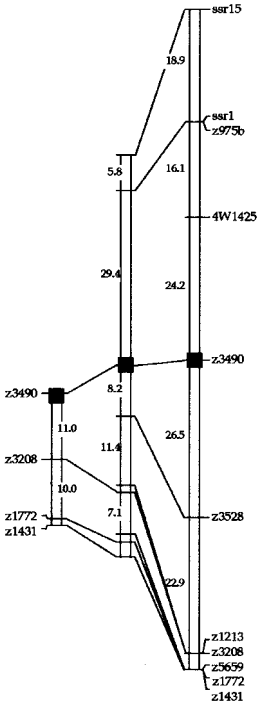


LG 23



LG 24

FIGURE 1.—*Continued.*



LG 25

FIGURE 1.—*Continued.*

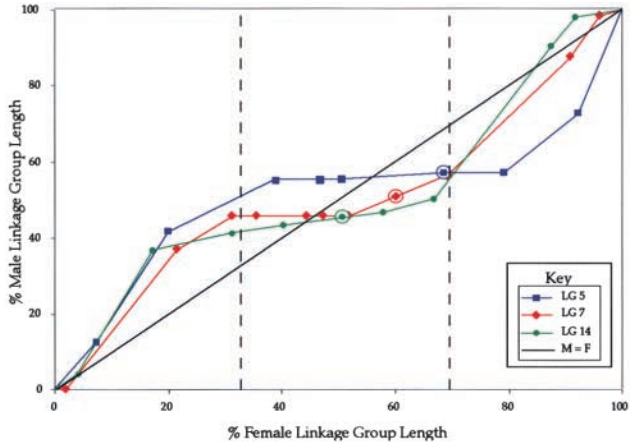


FIGURE 2.—Comparison of standardized male and female maps. Comparison of standardized intervals reveals variable recombination along the male chromosomes. Standardized interval distances along the chromosome are compared for the male and female maps of LG5, LG7, and LG14. Centromeric locations for each linkage group are circled (KANE *et al.* 1999; MOHIDEEN *et al.* 2000). Regression analyses were performed by dividing the graph into thirds as approximated by the vertical dashed lines. The male map is compressed near the centromeres and expanded near the telomeres. The slope of the lines near the centromeres (middle third of graph) is 0.26 and is significantly different from 1 ($P < 0.05$). The average male-to-female recombination ratio near the centromeres is 1.0:3.63, indicating a male "cold-spot." The slopes of the lines near the telomeres are 1.71 ($P < 0.05$) for the left third of the graph and 1.83 ($P < 0.05$) for the right third of the graph.

tive to females. This is shown by short androgenetic haploid maps for each chromosome compared to the gynogenetic haploid maps and the intermediate length of the sex-average diploid maps. Considering the greatest common interval distances, the female map is 2.74 times as long as the male map and the sex-average map is 1.87 times as long as the male map.

The expansion of the male genetic map near the telomeres may be due to a regional increase in the incidence of chiasmata. These results would predict that chiasmata would be found more frequently near the telomeres in meiosis in males compared to females. Likewise, the conclusions would predict that chiasmata in cells undergoing oogenesis will be found at regular intervals due to the more evenly distributed recombination frequencies observed in the female map.

The molecular mechanisms responsible for the differences in recombination rates between the two sexes are not currently well understood. In principle, the mechanism could be investigated by isolating mutations that alter recombination frequency. Males made homozygous for mutagenized chromosomes could be screened for their ability to produce progeny recombinant for two linked markers, such as z4003 and z4421, that are at the same position on the male map, but are 17.4 cM apart on the sex-average map and 61.1 cM distant on the female map. This strategy should identify mutations in genes necessary for recombination during vertebrate meiosis.

The results reported here are significant for their utility in facilitating more efficient experimental design in zebrafish genomic research. Thousands of zebrafish mutations are now known, but only a few have been identified molecularly (TALBOT and HOPKINS 2000). Greater efficiency in molecular cloning of genes identified by mutations could accelerate our understanding of gene functions in zebrafish and, through the connectivity of vertebrate genomes, suggest functions for human genes known only by sequence and map location. One of the main ways to identify the molecular nature of genes disrupted by mutation is positional cloning.

Because positional cloning requires "chromosome walks" based on the recombination of polymorphic markers genetically linked to the mutation in meiotic mapping experiments, it is advantageous to have a high recombination rate in mutant mapping crosses. Thus, crosses used for positional cloning should take advantage of the relatively higher recombination rate between the mutant phenotype and DNA polymorphism markers in female meiosis. Because a single centimorgan can be >10 times longer in kilobase pairs in male recombination than in female recombination, a chromosome walk that uses exclusively male recombination will be slower and more expensive.

On the other hand, in some types of genomic analysis,

a decreased rate of recombination can be a benefit. For example, mapping a mutation to a chromosome arm can quickly rule out many possible candidate genes for the mutation. Likewise, such mapping data can focus attention on alternative candidates that can be checked by subsequent genetic analysis. For this approach, mapping should use male meiosis, significantly decreasing the number of individuals that need to be examined to detect linkage. Decreased rates of recombination are also useful when trying to maintain stocks with alleles linked *in cis* on individual chromosomes. For example, when making double-mutant combinations of linked genes for epistasis analysis, the two mutant alleles would be retained *in cis* more frequently when passed through a male parent than through a female parent.

With this new knowledge of the dramatic differences in recombination rates between male and female meiosis in zebrafish, a number of types of experiments can now be conducted with greater efficiency, and hence these results should accelerate our understanding of vertebrate genome function.

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