SHORT COMMUNICATION

Microsatellite–Centromere Mapping in the Zebrafish (*Danio rerio*)

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Ten $(CA)_n$ microsatellite simple sequence repeat (SSR) markers, 1, 2, 12, 14, 16, 18, 20, 22, 26, and 29, were used to show high chiasma interference and to determine centromere–marker map distances in the zebrafish (*Danio rerio*). Of these, SSR 12 exhibited no recombinant tetratypes among 175 half-tetrad embryos, placing this marker within 1 cM of the centromere of Linkage Group XVII. Fractions of heterozygous half-tetrads for the remaining nine markers ranged from 0.64 to 0.89. Of these, six recombinant fractions were more than 0.67 ($P < 0.05$), indicating strong chiasma interference during female meiosis in the zebrafish. Consistent with previous mapping data, SSRs 2 and 20 of Linkage Group VI were tightly linked. Half-tetrad analysis will allow the mapping of the remaining centromeres and may be useful in the mapping of new genes and mutations in the zebrafish.

Factors that affect the uniformity of genetic crossovers influence the correlation between genetic and physical maps. Centromeres increase the regional density of genetic markers because they suppress recombination in their vicinity (7, 10, 22). To position centromeres on the zebrafish genetic map (18), we have used microsatellite analysis of half-tetrads (2).

Centromere mapping in the zebrafish uses meiotic half-tetrad embryos produced by activation of oocyte cell division with UV-irradiated sperm, followed by inhibition of the second meiotic disjunction using hydraulic pressure (early pressure, or "EP"). The central idea behind half-tetrad centromere mapping is that recombinant heterozygous maternal loci are heterozygous in half-tetrad gynogenetic progeny, while nonrecombinant loci are homozygous (17). (Fig. 1). A marker unlinked to its centromere has a half-tetrad recombinant fraction y of 2/3 (8). Preference for an odd number of crossovers (probably single) results in $y > 2/3$; this is called "chiasma interference." Values of $y > 2/3$ shown in fungi (17), carp (3), and rainbow trout (23) support the existence of chiasma interference. A y value of 0.89 inferred for one of four pigment loci studied by Streisinger *et al.* (21) suggested the existence of chiasma interference in the zebrafish. In the present report, we use 10 maternally heterozygous, codominant genetic markers (simple sequence repeat polymorphisms; SSRs) (6) to assess the frequency and degree of chiasma interference in zebrafish.

To generate gynogenetic diploid embryos (Fig. 1), we used early pressure (20) to cause retention of the second polar body during meiosis II. Since the originally reported pressure of 8000 psi (20, 24) resulted in egg lysis in our hands, modified pressures were used to create these embryos. Details will be presented elsewhere. Successful gynogenesis was demonstrated by the absence of paternal alleles in the progeny and evidence of diploid, maternally derived embryos (data not shown). Furthermore, some markers were heterozygous while others in the same embryo were homozygous due to varying positions of markers relative to crossovers during meiosis I (data not shown).

Among the EP-treated eggs from six experiments, the fraction y of heterozygous half-tetrads ranged from 0 to 0.89. The y values were then used to calculate SSR–centromere map distances in three ways (Table 1). In the first calculation, where $x = y/2$, complete interference is assumed (i.e., one recombinational exchange completely inhibits additional crossovers) (13). The second method for calculating map distance (15) is based upon the Kosambi equation (11), which assumes 50% interference; the third value is the Kosambi value calibrated to the published map (Table 1) (18).

The linkage of markers to recessive lethal alleles that prevent embryos from reaching the age at which DNA extraction is performed (several days) will cause one allele to predominate over the other type among homozygotes, leading in turn to an underestimation of the percentage of homozygous embryos. Of the 10 SSRs tested, only SSR 29 showed significant deviation from random segregation of alleles among homozygotes (χ^2

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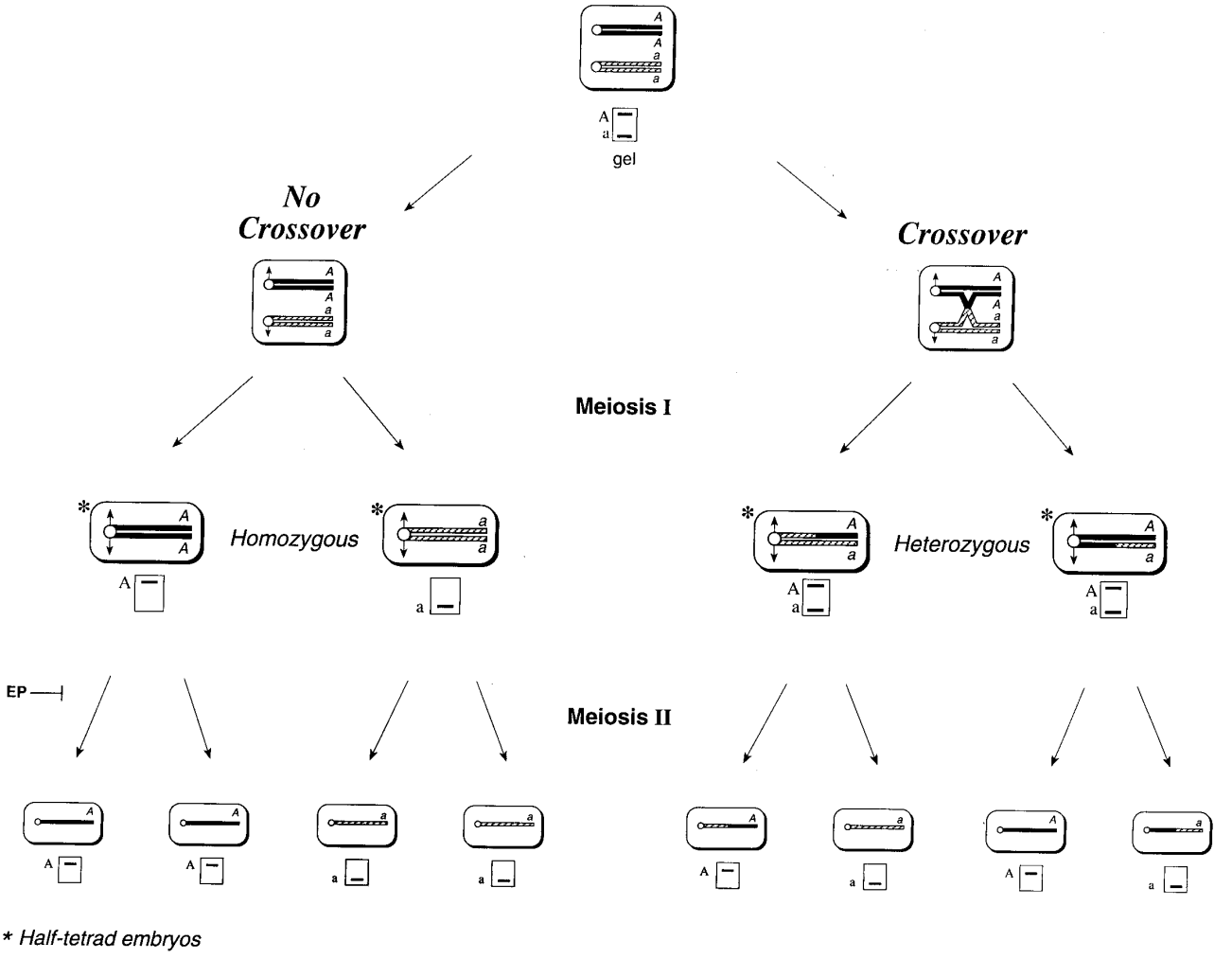

FIG. 1. Crossovers during meiosis yield heterozygous half-tetrads. In each intermediate, the chromosome configuration at hypothetical locus *A* is drawn. Two alleles, A and a, represent microsatellite alleles containing more (*A*), or fewer (*a*) repeat units. PCR amplification of this locus followed by electrophoretic fractionation of the products and autoradiography yields the corresponding schematicized gel genotypes. No crossovers or an even number of crossovers between a marker and its centromere yields homozygous half-tetrads (asterisks under "No crossovers"). In contrast, an odd number of crossovers between a marker and its centromere (indicated by the X-shaped structure between the inner nonsister chromatids under "Crossover") yields heterozygous half-tetrads. The two alleles would then segregate after meiosis II is completed. Early pressure parthenogenesis inhibits meiosis II segregation of sister chromatids, as indicated on the bottom left.

analysis, $P < 0.05$). Among 98 EP embryos, 7 were homozygous for SSR 29; all 7 had one of the two parental alleles. This unexpected segregation of alleles ($0.005 < P < 0.01$) suggests that the second allele is linked to a recessive lethal mutation. Since 7 additional embryos of the opposite homozygous genotype would be expected, the data for SSR 29 were corrected accordingly (Table 1).

The numbers of half-tetrad offspring genotyped yielded significant lod scores for SSRs 1, 2, 12, 20, 22, 26, and 29 (Table 1) despite distances of >30 cM. Similar numbers of offspring from traditional crosses did not produce significant lod scores over comparable intervals (data not shown).

The gene–centromere recombinant frequencies were significantly greater than 0.67 for 6 of 10 informative SSRs: 1, 2, 20, 22, 26, and 29 (Table 1), indicative of significant chiasma interference in zebrafish. Of these 6, 5 were between 0.84 and 0.89, but none were greater than 0.89. Determining more exactly the proportion of the genome susceptible to chiasma interference and the degree of that interference (i.e., how closely y values approach unity) will require additional markers.

It is also of interest that SSR 12 was tightly linked to the centromere on Linkage Group XVII. Of 175 embryos tested, none was heterozygous at this locus. This places SSR 12 less than 1 cM from its centromere (χ^2, $P < 0.05$). These data also indicate that meiosis I nondisjunction, which would artifactually contribute nonrecombinant heterozygous half-tetrads, is not significant, at least for this linkage group.

SSR 12 on Linkage Group XVII of Postlethwait *et al.* (18) is 8.5 cM from one end and 78 cM from the other end of Linkage Group XVII, i.e., very close to one end of this medium-sized linkage group. Since the only two acrocentric zebrafish chromosomes are very small (5,

TABLE 1

Fraction of Heterozygous Half-Tetrads (y) and Centromere–SSR Map Distances for 10 Loci

SSR	Linkage group	Heterozygous/total[a]	y	Map distance			SSR–centromere lod
				$x = y/2$	Kosambi	Adjusted Kosambi[b]	
1	XXV	155/190	0.81	40.5	56.4	45	4.24
2	VI	153/171	0.89	44.5	71.1	57	10.54
12	XVII	0/175	<0.006	<0.3	<0.29	<0.23	83.5
14	III	21/32	0.66	33	39.6	32	0.003
16	XXIX	181/257	0.70	35	43.4	35	0.27
18	XV	55/86	0.64	32	37.9	30	0.06
20	VI	80/89	0.89	44.5	71.1	57	5.72
22	V[c]	100/114	0.88	44	68.8	55	5.85
26	VI	82/98	0.84	42	61.1	49	3.03
29	IV	91/105[d]	0.87	48	96	52	4.8

[a] Results from six sets of EP embryos are combined (the majority of the data comes from the last two sets of EP embryos).

[b] Kosambi map distance 0.795, which is the ratio between the published map distance between SSRs 20 and 26 and the sum of centromere–marker distances for the same markers.

[c] SSR 22 has recently been placed on Linkage Group V by Postlethwait *et al.* (Eugene, OR, pers. comm., July 1994).

[d] Corrected for recessive lethal mutation linked to one of two alleles (see text). Uncorrected values for this data set are 91/98, 0.93, 47, 82, 66, and 8.41, respectively.

19), and this is a medium-sized linkage group, one of the small supernumary linkage groups of the current map is expected to be attached distally.

The large centromere–marker map distances between SSRs 2/20 and SSR 26 and previous knowledge of synteny with SSR 26 together place the centromere between these markers. The centromere is predicted to lie near the 16.2-cM interval between 5N.800(D) and 6AB.450(A) on the published map. SSR 16 is 2.7 cM from the end of Linkage Group "XXIX," whose entire current length is 17.6 cM. Since the centromere–SSR 16 map distance is 35 cM, this linkage group must also be attached to one of the others of Postlethwait *et al.* (18). The remaining centromere assignments are approximate due to the large map distances involved. By conventional linkage analysis, SSR 1 is 14.1 cM from the upper end of Linkage Group XXV, whereas the gene–centromere distance for this marker is 50 cM. This suggests that the centromere is near 7N.350. Placing the centromere 50 cM in the other direction from SSR 1 seems unlikely, since such a placement would require an additional 30 cM missing in this linkage group; the four remaining supernumerary linkage groups containing only 21.5, 17.6, 16.4, and 10 cM remain. Similarly, SSR 18 and 29 half-tetrad genotypes place the centromeres for Linkage Groups XV and IV near RAPD loci 6G.1300 and 17P.875, respectively. SSR 14, on Linkage Group III, is roughly 32 cM from its centromere. The metacentric configuration of the largest zebrafish chromosomes in the karyotype (19) favors a centromere assignment in the 26-cM interval between 14J.1090(D) and 20Y.670(A) over the alternative acrocentric centromere placement on Linkage Group III.

The tight linkage of SSRs 2 and 20 at one end of Linkage Group VI reported by Postlethwait *et al.* (18) is supported by their having identical centromere–marker map distances (57 cM, Table 1) and by allelic coupling (Table 2). In Table 2, large and small alleles of each SSR are represented by upper and lowercase letters respectively, and were assigned arbitrary letters, beginning with *A* and *a*, for SSR 1. SSR genotypes of the first 50 of 160 genotyped embryos from a single mother are shown to illustrate three observations (Table 2). First, of the 160 embryos examined, 17 were homozygous for both SSRs 2 and 20. From the 50 sample genotypes (Table 2), embryos 9, 11, 25, 48, and 50 were homozygous for both SSRs 2 and 20; in every case in which one was homozygous, the other was as well. Second, in each of these homozygous embryos, either large alleles *B* and *K* or small alleles *b* and *k* were coupled with each other. Third, SSR 2 and 20 alleles were not coupled with those of SSR 26, which lies 105 cM from SSRs 2 and 20 on Linkage Group VI (18). Despite the small number of embryos homozygous for SSRs 2 and 20, the gene centromere data offer significant support for linkage of SSRs 2 and 20 ($\hat{Z} = 13.7$, $\theta = 0.0$). For comparison, two-point haploid data (J. Postlethwait, Eugene, OR, pers. comm., Oct. 1994) yield a lod score of 20.5 for 1 cM. The lack of coincident homozygosity or coupling of the alleles of SSRs 1, 12, 16, 18, and 22 (Table 2) supports the assignment of those markers to different linkage groups.

Continued half-tetrad analysis using the available markers will lead to the mapping of the remaining zebrafish centromeres. Furthermore, as suggested by our coupling data, mapping of new codominant markers may be aided by half-tetrad analysis. Strong chiasma interference implies minimal numbers of multiple exchanges; minimal multiple exchanges in turn imply that homozygous half-tetrads, even those associated with large marker–centromere distances, lack a ge-

TABLE 2

Linkage of Alleles among One Set of Gynogenetic Embryos (D51)

	SSR							
Embryo	2	20	12	1	16	18	22	26
1			d			J	l	
2			D		h	J		
3			d		H			
4			D					
5			d					N
6			d			j		—
7			D		h			—
8			D			j		
9	B	K	D	A				N
10			d					N
11	B	K	d			J		—
12			d			J		n
13			D			j	l	
14			d					
15			D		h			
16			d		H			
17			d		H	j		
18			d			J		
19			D	a				
20			D					
21			D	a				
22			D			j		
23			d		H	J		
24			d					
25	B	K	d					
26			D					n
27			d	—	H	J		
28			D				—	n
29			d		h			
30			D	a	h	J		
31			D					
32			D				L	
33			d					
34			d			—		
35			D		h	J		
36			D		h	j		
37			D			J		
38			d		H	j		
39			D	A	h	j		
40			d					—
41			d		h		L	
42			D					—
43			d					
44			d					
45			d				—	
46			D					
48	B	K	D	a	H	J		n
49			d					
50	b	k	D					n

Note. Blank spaces indicate heterozygosity, and a dash indicates that the corresending embryo/SSR combination was not scored.

netic exchange between the centromere and the marker (rather than double or quadruple crossovers). Should this assumption be confirmed using intervening markers, homozygous markers on the same chromosome arm would show allelic coupling. This prediction and the availability of genetically divergent zebrafish strains (9; Kauffman and Cheng, unpublished observations) suggest that half-tetrad mapping may become a useful adjunct to the mapping of genes and mutations in the zebrafish.

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