GENE-CENTROMERE MAPPING IN RAINBOW TROUT: HIGH INTERFERENCE OVER LONG MAP DISTANCES

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ABSTRACT

Ten enzyme loci were mapped in relation to their centromeres in gynogenetic diploid rainbow trout. Gene-centromere map distances, calculated under the assumption of complete interference, range from 1.1 cM for *Ldh4* to 50 cM for *Sod1*. The *Idh2* and *Est1* loci are linked on the same chromosome arm.——The observation of close to 100% heterozygous gynogenetic diploids for the *Sod1* and *Mdh3,4* loci suggests that near-complete interference occurs on the chromosome arms carrying these loci. The high interference observed in this study and in several other species of fish may be related to the small size of fish chromosome arms.——Comparisons of map locations for the *Ldh3* and *Ldh4* and the *Mdh3* and *Mdh4* loci, which were duplicated by a tetraploid event in the evolution of salmonid fish, reveal that they are located at similar distances from their centromeres. Comparative mapping of loci duplicated longer ago shows more variation in map location.——The high proportion of heterozygotes for some loci after gynogenesis involving second polar body retention demonstrates that this is not a practical method for producing homozygous inbred lines in rainbow trout; treatments suppressing the first cell division are more promising for this purpose.

GENE-centromere mapping is possible if two of the four strands (half-tetrads) from a single meiosis can be recovered. This mapping method has classically been applied in Drosophila studies using attached-X chromsomes (ANDERSON 1925; BEADLE and EMERSON 1935) or compound autosomes (BALDWIN and CHOVNICK 1967), and in Neurospora, which produce ordered tetrads (BARRATT *et al.* 1954; PERKINS 1962). Plants producing unreduced gametes (RHOADES and DEMPSEY 1966; MENDIBURU and PELOQUIN 1979) and mammalian ovarian teratomas resulting from fusion of the second polar body with the egg (OTT *et al.* 1976; EICHER 1978; EPPIG 1982) have also made gene-centromere mapping possible. Treatments inducing retention of the second polar body to produce triploids or gynogenetic diploids have been used for gene mapping in amphibians (LINDSLEY, FANKHAUSER and HUMPHREY 1956; VOLPE 1970; NACE, RICHARDS and ASHER 1970; DU PASQUIER and KOBEL 1979) and fish (PURDOM, THOMPSON and DANDO 1976; CHERFAS 1977; CHERFAS and TRUWELLER 1978; NAGY *et al.* 1978, 1979; THOMPSON, PURDOM and JONES 1981; STREISINGER *et al.* 1981).

Centromere mapping can rapidly provide considerable genetic information. In a species with many chromosomes, linkages between genes may be difficult to detect, but all genes can be mapped in relation to their centromeres. The degree of conservation of gene arrangement among species, and the levels of recombination and interference within species can be studied. The data can also be used to estimate the rate of inbreeding obtained with gynogenesis after retention of the second polar body (PURDOM 1969; NACE, RICHARDS and ASHER 1970; NAGY *et al.* 1978; THOMPSON, PURDOM and JONES 1981; NAGY and CSÁNYI 1982).

In this report, we describe the mapping of ten enzyme loci in relation to their centromeres, and the identification of a tight linkage between two of the loci (*Est1* and *Idh2*) through studies with gynogenetic diploid rainbow trout (*Salmo gairdneri*). Our results suggest that double crossovers may be rare on individual chromosome arms in rainbow trout, and possibly in other fish species.

MATERIALS AND METHODS

Rainbow trout eggs were obtained from the Spokane Trout Hatchery of the Washington Department of Game in December, 1981 and January, 1982. Eggs from sea-run rainbow trout (steelhead) were obtained from the Dworshak National Fish Hatchery, Ahsahka, Idaho in April and May, 1982. Sperm from brook trout (*Salvelinus fontinalis*) and westslope cutthroat trout (*Salmo clarki lewisi*) were obtained from the Ford Trout Hatchery of the Washington Department of Game; brook trout sperm was used in experiments with the Spokane rainbow trout eggs, and westslope cutthroat trout sperm was used in experiments with Dworshak steelhead eggs. Egg and sperm samples were transported on ice to Washington State University for the gynogenesis experiments.

Gynogenetic diploid rainbow trout were produced by fertilizing rainbow trout eggs with brook or cutthroat trout sperm that had been genetically inactivated with ultraviolet (UV) light, and shocking with heat 10 min after fertilization for 10 min at 28–29° to induce retention of the second polar body (CHOURROUT 1980). For sperm inactivation, 0.5 ml of brook trout or westslope cutthroat trout sperm was diluted into 4.5 ml of a chilled extender solution of 0.076 M Tris (hydroxymethyl) aminomethane, 0.005 M monohydrate citric acid, 0.064 M glycine, 0.04 M KCl and 10% (w/v) polyvinyl pyrrolidinone (ZELL 1978). The optical density at 265 nm of a 1/100 dilution of a typical sperm-extender mixture was about 0.45. The sperm was spread into a thin layer in a glass baking dish on ice and irradiated at a distance of 30 cm from a Westinghouse G25T8 Sterilamp bulb for 2 min. This provided a UV dose of approximately 3600 ergs/mm^2 as measured with an Ultra-Violet Products, Inc. (San Gabriel, CA) model J-225 short wave meter.

Eggs and fry were reared in a recirculating water system at 10 ± 1°. Haploid embryos are morphologically distinguishable and die about the time of hatching (30 days postfertilization under our conditions). The surviving gynogenetic diploids were sampled for electrophoresis as advanced yolk sac fry or 2- to 5-month-old fish.

Tissue extracts were prepared and horizontal starch gel electrophoresis was accomplished as described by ALLENDORF *et al.* (1977). The nomenclature of loci and allelic variants follows the system proposed by ALLENDORF and UTTER (1979). Allozymic variants were found for the following seven enzymes: esterase, EST, EC 3.1.1.1; glycyl-L-leucine peptidase, GLP, EC 3.4.-.-; isocitrate dehydrogenase, IDH, EC 1.1.1.42; lactate dehydrogenase, LDH, EC 1.1.1.27; malate dehydrogenase, MDH, EC 1.1.1.37; phosphoglucomutase, PGM, EC 2.7.5.1; and superoxide dismutase, SOD, EC 1.15.1.1.

RESULTS

Gynogenesis: Brook and westslope cutthroat trout sperm were used to allow identification of any paternal contribution to the gynogenetic diploids. Rainbow × brook trout hybrids survive to hatching but typically die before yolk absorp-

tion (BUSS and WRIGHT 1956; G. H. THORGAARD, unpublished results). In addition, there are at least 20 fixed differences at enzyme loci between rainbow trout and brook trout that can be detected with electrophoresis. We found no evidence of male contribution among 10 families of gynogenetic diploid rainbow trout produced from eggs from the Spokane Trout Hatchery fertilized with UV-inactivated brook trout sperm.

Rainbow × westslope cutthroat trout hybrids are viable, but can be identified because different alleles are present in the two species at the *Gpi3* locus (REINITZ 1977a), and allelic frequencies are different at the *Glp1* and *Ldh4* loci. Among 16 families of gynogenetic diploid rainbow trout produced from eggs from the Dworshak hatchery fertilized with UV-inactivated westslope cutthroat trout sperm, we detected apparent hybrids in two families. In one family (I86D) four of eight fish tested appeared to be hybrids on the basis of their *Gpi3*, *Glp1* and *Ldh4* phenotypes, whereas one of eight fish tested in a second family (I87D) appeared to be a hybrid. These families were not used for the mapping study. Some sperm in these two cases apparently escaped UV inactivation, probably because of screening of UV light resulting from incomplete mixing of the sperm with the extender or too thick a film on the dish during irradiation. TROTTIER and ARMSTRONG (1976) also found evidence of male contribution in some cases in gynogenesis studies in axolotls (*Ambystoma mexicanum*). These results illustrate the importance of diluting and dispersing the sperm before irradiation and of using genetically distinct sperm donors in gynogenesis studies.

Duplicated loci: Many duplicated loci in salmonids show no evidence of structural divergence. For example, BAILEY *et al.* (1970) have shown that the skeletal muscle form of MDH (MDH-B) in rainbow trout is encoded by two loci (*Mdh3,4*) that have the same most-frequent allele. Three such duplicated loci were segregating in the gynogenetic diploid families we examined.

Two loci (*Mdh1,2*) in rainbow trout encode the form of MDH that predominates in liver and neural tissues, MDH-A (BAILEY *et al.* 1970). These loci share the same most-frequent allele. One gynogenetic family from Dworshak fish was segregating for one of these loci (Table 1). We were thus able to estimate the proportion of heterozygous gynogenetic diploid progeny at only one of these two loci. We have arbitrarily designated this locus *Mdh1*.

Two loci (*Idh3,4*) sharing alleles encode the cytosolic form of IDH in rainbow trout (ALLENDORF and UTTER 1973; REINITZ 1977b). We examined six families segregating for *Idh3,4*; however, only one of the two loci was segregating in each of these families. Therefore, we cannot be sure if these families were segregating for the same locus or if different families were segregating for different loci. We have made the conservative assumption that all families were segregating for the same locus, *Idh3*.

Six of the Spokane females producing gynogenetic diploid families were apparently heterozygous for at least one of the two MDH-B loci (Table 2). Two of the families (I64S and I67S) consisted only of individuals homozygous for the common allele (100 or B) at one locus and heterozygous (74/100 or Bb) at the other locus. Three families (I61S, I66S, and I69S) consisted of three different progeny phenotypes: BBBb, BBbb, Bbbb. These progeny phenotypes are ex-

pected only if the female was heterozygous at both loci. Thus, we were able to estimate the proportion of heterozygous progeny for both MDH-B loci (*Mdh3,4*).

Gene-centromere recombination: The proportion of homozygous and heterozygous gynogenetic diploid progeny at eight enzyme loci is shown in Table 1. The frequency of heterozygotes is expected to vary according to the frequency of second division segregation (y). No heterozygotes are expected if a crossover never takes place, 100% heterozygotes are expected if a single crossover always takes place, and about 2/3 heterozygotes are expected if the gene is assorting independently in relation to the centromere. The proportion of heterozygotes can be used to estimate the gene-centromere map distance, but several types of tests should be made before such estimates are made.

If one of the homozygous progeny classes had significantly reduced viability (because of homozygosity for the locus itself or a linked gene), y (frequency of second division segregation) might be better estimated by (number of heterozygotes)/(number of heterozygotes plus twice the number in the most common class of homozygotes). We tested for significant differences in numbers in the two homozygous classes in the various families but found a significant deviation in only one case: the *Idh3,4* locus in family I62S ($\chi^2 = 7.12$; d.f. = 1; P = 0.008). A deviation of this magnitude is not significant when the number of tests being made simultaneously ($n = 19$) is taken into consideration (COOPER 1968). Consequently, the y value in individual families could appropriately be estimated by (proportion of heterozygotes)/(total progeny).

Before pooling results from the different families, we tested for heterogeneity among families at the six loci for which both homozygotes and heterozygotes were present in more than one family. The only significant deviation was among families for the *Glp1* locus ($\chi^2 = 9.77$; d.f. = 3; P = 0.02). A deviation of this magnitude, again, is not significant when the number of tests made simultaneously ($n = 6$) is taken into account (COOPER 1968). Consequently, we are justified in pooling results from different families to estimate y.

Values of y range from 0.021 to 1.000 for the eight loci in Table 1. Only *Idh3* showed variation in both Spokane and Dworshak fish; similar estimates of y were obtained in both strains.

Gynogenetic diploid progeny in families showing variation at the duplicate *Mdh3,4* loci demonstrated a high y value for these loci (Table 2). In families I64S and I67S, the females were apparently heterozygous at a single locus; the observation of 100% BBBb progeny (heterozygous at a single locus) indicated a y value of 1.000. In families I61S, I66S and I69S, the females were apparently heterozygous at both loci; either a double crossover or the absence of a single crossover between the gene and the centromere for one of the two loci generated the occasional BBBb and Bbbb progeny. These results also indicated a high y value for the *Mdh3,4* loci. The 100% BBbb progeny in family I63S could have resulted from the female being homozygous BB at one locus and homozygous bb at the other locus, or from a female heterozygous (Bb) at both loci in which a single crossover occurred between each of the *Mdh* loci and the centromere in all cases ($y = 1.000$).

The high y values (near 1.000) observed for the *Sod1* and *Mdh3,4* loci suggest that a single crossover occurs between these genes and their centromeres in

TABLE 1

Progeny genotypes at eight loci in gynogenetic diploid rainbow trout

Locus	Family[a]	Maternal genotype	Progeny genotypes[b] 11	12	22	Proportion heterozygotes (y)
Est1	I61S	100/95	10	114	14	0.826
	I65S	100/95	1	34	4	0.872
			11	148	18	0.836
Glp1	I71D	100/70	9	19	9	0.404
	I72D	100/85	12	17	19	0.354
	I78D	100/85	12	22	14	0.458
	I79D	100/70	8	32	8	0.667
			41	90	50	0.497
Idh2	I61S	100/140	27	94	17	0.681
	I62S	100/140	6	34	6	0.739
			33	128	23	0.696
Idh3	I61S	100/71	27	74	37	0.536
	I62S	100/40	3	23	14	0.575
	I63S	100/40	4	27	9	0.675
	I65S	71/40	9	25	6	0.625
	I69S	100/40	9	23	8	0.575
	I79D	100/71	11	29	8	0.604
			63	201	82	0.581
Ldh4	I72D	100/76	17	1	30	0.021
	I82D	100/76	22	1	25	0.021
			39	2	55	0.021
Mdh1	I85D	100/140	11	5	5	0.238
Pgm2	I64S	100/90	21	9	26	0.161
	I65S	100/90	25	5	18	0.106
			46	14	44	0.135
Sod1	I63S	100/152	0	94	0	1.000
	I66S	100/152	0	35	0	1.000
			0	129	0	1.000

[a] Family abbreviations ending with the letter S represent families of Spokane hatchery origin and those ending with D represent families of Dworshak hatchery origin.

[b] 11 and 22 are homozygotes for the first and second maternal allele, respectively, and 12 are heterozygotes.

virtually all cases. The results also indicate that multiple crossovers rarely, if ever, occur; there appears to be a high level of chiasma interference on these chromosomes.

Linkage of Est1 *and* Idh2: We were able to test for joint segregation of the following loci in families of gynogenetic diploid rainbow trout: *Idh3* with *Idh2* (in two families); *Idh3* with *Est1* (in two families); *Idh3* with *Pgm2*; *Idh3* with *Glp1*; *Est1* with *Pgm2*; and *Est1* with *Idh2*. The tests were made by comparing

TABLE 2

Progeny genotypes at the duplicate Mdh3,4 *loci in gynogenetic diploid rainbow trout*

Family	Maternal genotype[a]	Progeny phenotypes[b]					y
		BBBB	BBBb	BBbb	Bbbb	bbbb	
I61S	Bb; Bb	0	3	140	3	0	0.979
I63S	BB; bb or Bb; Bb	0	0	48	0	0	—
I64S	BB; Bb	0	55	0	0	0	1.000
I66S	Bb; Bb	0	0	34	1	0	0.986
I67S	BB; Bb	0	65	0	0	0	1.000
I69S	Bb; Bb	0	3	44	1	0	0.958
							0.981

[a] The semicolon separates the genotypes at the two loci, e.g., a Bb; Bb individual is heterozygous at both loci.

[b] B = *Mdh3,4* (100) and b = *Mdh3,4* (74).

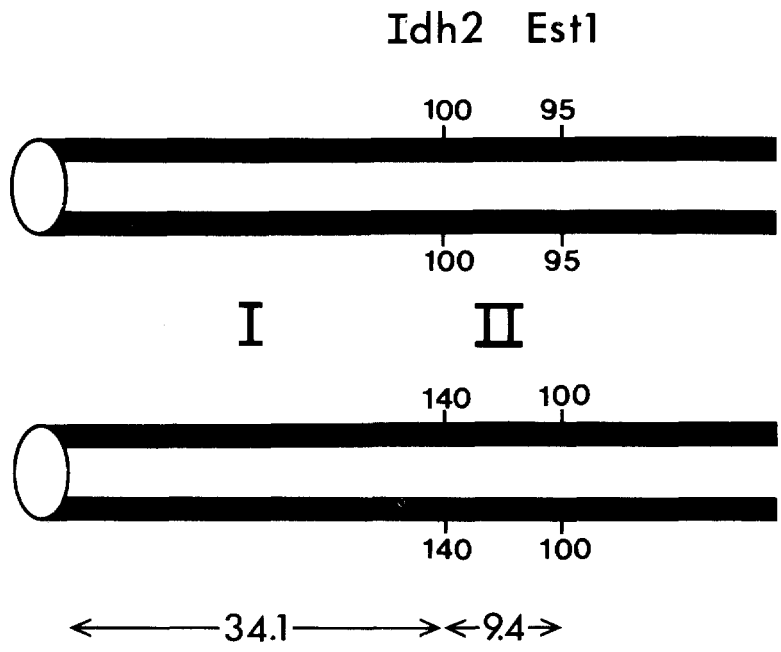

FIGURE 1.—Genetic map of centromere—*Idh2*—*Est1* linkage group showing assumed meiotic tetrad in female producing family I61S. The numbers below the tetrad represent the map distances (cM) estimated from this family.

the observed numbers of individuals with the numbers expected if the loci were inherited independently using a 3 x 3 contingency table. All tests were nonsignificant except the comparison for *Est1* and *Idh2* in family I61S (Table 3; χ^2 = 122.1; d.f. = 4; $P < 0.005$).

The results are consistent with the gene order centromere—*Idh2*—*Est1* (Figure 1). The probable origins of the various classes of gynogenetic diploids are shown in Table 3. In the interval between the centromere and *Idh2* (region I), there

TABLE 3

Joint segregation of Idh2 *and* Est1 *in gynogenetic diploid rainbow trout*

Alleles at *Idh2*	Alleles at *Est1* 100/100	95/100	95/95
100/100	0 (1.9)[a] [4st DCO, II][b]	13 (18.2) [SCO, II]	14 (1.9) [NCO]
100/140	2 (8.2) [2st DCO, I, II or 4st DCO, I, II]	92 (77.6) [SCO I or 3st DCO I, II]	0 (8.2) [2st DCO, I, II or 4st DCO, I, II]
140/140	8 (1.9) [NCO]	9 (18.2) [SCO, II]	0 (1.9) [4st DCO, II]

[a] The numbers in parentheses are the expected numbers if *Idh2* and *Est1* are on different chromosomes.

[b] The symbols in square brackets indicate the probable origins of the indicated class of progeny. NCO = noncrossover, SCO = single crossover, DCO = double crossover; I indicates exchange in region between the centromere and *Idh2*, II indicates exchange in region between *Idh2* and *Est1*; 2st, 3st and 4st indicate number of strands (chromatids) involved in the double crossover.

were 94 exchanges (92 + 2) among the 138 progeny, for a recombination fraction of 0.681. Three-strand double crossovers in regions I and II are indistinguishable from single crossovers in region I; they are expected to be as frequent as two-strand double crossovers plus four-strand double crossovers in these regions (PERKINS 1962). Thus, we can estimate that there were 26 exchanges (9 + 13 + 2 + 2) between *Idh2* and *Est1* (region II). The 26 exchanges correspond to a recombination fraction of 0.188 in region II. Assuming there were no double crossovers within this region, this indicates a map distance of 9.4 cM between *Idh2* and *Est1*. We have since confirmed the linkage of *Est1* and *Idh2* in both females and males through family studies (F. W. ALLENDORF and K. L. KNUDSEN, unpublished results).

Using an estimate of four double crossovers in regions I and II (two observed two-strand or four-strand double crossovers plus two estimated three-strand double crossovers) and 17.8 for the expected number [(0.68)(0.19)(138)], the coefficient of coincidence is 4/17.8 = 0.22. This corresponds to an interference value of 0.78.

DISCUSSION

High interference: The most surprising aspect of our results is the high level of chiasma interference that they demonstrate. This conclusion is based on second division segregation (y) values near 1.000 for *Mdh3,4* and *Sod1*, suggesting that exactly one crossover takes place between the gene and the centromere on the chromosomes carrying these loci. The interference value found in the family showing joint segregation for *Idh2* and *Est1* (0.78) is also higher than interference values observed over even shorter map intervals in other organisms

(PERKINS 1962). *Mdh3*, *Mdh4*, *Sod1* and *Idh2* are all known to be unlinked to each other (MAY, WRIGHT and JOHNSON 1982), suggesting that high interference over long map distances is common on at least four pairs of chromosome arms.

High interference is the only good explanation for our observations. Simultaneous lethality of both classes of homozygotes in the two families with *Sod1* variation is unlikely; the homozygotes are known to exist in nature and to have good viability. Similarly, all the phenotypes at the *Mdh3,4* loci are found in nature and most were found in this study. Most of the families showing the "surprising" results for *Sod1* and *Mdh3,4* also showed variation at other loci. In these cases, they had homozygous and heterozygous progeny for the other loci in proportions typical of other families.

Second division segregation frequencies greater than two-thirds, reflecting high interference, have previously been observed in other organisms, particularly fungi (PERKINS 1955). Such results are noticeably absent, however, among studies of animals and plants (see references in the introduction). Two other cases of high interference have been observed in fish; y values of 0.97 for the *N* gene in carp, *Cyprinus carpio* (GOLOVINSKAYA and ROMASHOV 1966; CHERFAS 1977), and 0.89 for the *gol-1* gene in zebra fish, *Brachydanio rerio* (G. STREISINGER, personal communication) suggest high interference on the chromosomes carrying these loci. Linkage studies in the fish genus Xiphophorus have provided mixed evidence about interference but indicated high interference over some intervals (MORIZOT, WRIGHT and SICILIANO 1977; MORIZOT and SICILIANO 1979).

These results suggest that high interference is more common in fish than in other animals and in plants. This difference could be related to the relatively small size of fish chromosomes. Below a minimum size, chromosomes may frequently have only a single obligate chiasma (MATHER 1937). The average chromosome arm in rainbow trout has about the same DNA content as chromosome 21 in humans (OHNO, WOLF and ATKIN 1968; MENDELSOHN *et al.* 1973), which normally has a single chiasma in males (HULTÉN 1974). If interference acts at similar levels among vertebrates, rainbow trout and other fish species might thus be expected to have an obligate single chiasma on many of their chromosome arms and to show high second division segregation (y) values for distal loci on those chromosome arms.

Gene mapping: Gene-centromere map distances can be estimated from the fraction of heterozygous half-tetrads (y). The relationship between the two numbers will vary depending on the level of interference (PERKINS 1962; NACE, RICHARDS and ASHER 1970). When interference is complete (no double crossovers), the map distance in centimorgans will be equal to $(100)(y/2)$. Because of the extremely high level of interference demonstrated in this study, we have used this relationship for calculating the gene-centromere map distances for the ten loci we studied (Table 4). The gene-centromere map distances range from 1.1 cM for *Ldh4* to nearly 50 cM for *Sod1*, *Mdh3* and *Mdh4*, and include a full range of intermediate values (Table 4).

The tight linkage of *Est1* and *Idh2* identified in this study represents the third pair of loci showing classical linkage to each other in rainbow trout. MAY, WRIGHT and JOHNSON (1982) showed that *Idh3-Me2* and *Ada1-G3p3* were tightly

TABLE 4

Gene-centromere map distances and 95% confidence intervals for ten loci in rainbow trout

Locus	No. of families	No. of individuals	y	Gene-centromere map distance (cM)[a]
Est1	2	177	0.836 ± 0.055	41.8 ± 2.8
Glp1	4	181	0.497 ± 0.073	24.9 ± 3.7
Idh2	2	184	0.696 ± 0.066	34.8 ± 3.3
Idh3	6	346	0.581 ± 0.052	29.0 ± 2.6
Ldh4	2	96	0.021 ± 0.029	1.1 ± 1.5
Mdh1	1	21	0.238 ± 0.182	11.9 ± 9.1
Mdh3,4[b]	5	289	0.981 ± 0.016	49.1 ± 0.8
Pgm2	2	104	0.135 ± 0.066	6.8 ± 3.3
Sod1	2	129	1.000	50
Mean			0.554	27.7

[a] Map distances were calculated under the assumption of complete interference.

[b] Our results indicate that both the *Mdh3* and *Mdh4* loci are close to 50 cM from their centromeres, but do not identify distances for each locus. The *Mdh3,4* results were only counted once in calculating the average y value among the loci.

linked in males. All of these three pairs of loci are unlinked to each other (MAY, WRIGHT and JOHNSON 1982), indicating that we may now have identified linked loci on three distinct chromosomes in rainbow trout.

Comparative mapping: Gene-centromere mapping has considerable potential for comparative mapping studies. Because of the tetraploid ancestry of salmonid fish (OHNO, WOLF and ATKIN 1968), comparisons of the map locations of pairs of loci duplicated by the tetraploid event are of special interest, in addition to comparisons of map locations between species.

There is evidence that two pairs of loci duplicated by the salmonid tetraploid event are located at similar distances from their centromeres. *Mdh3* and *Mdh4* are both far from their centromeres (Table 4). *Ldh4* is very near the centromere (Table 4), and *Ldh3* is also apparently very near to the centromere, on the basis of studies with triploid rainbow trout (WRIGHT *et al.* 1983). As previously discussed, we cannot be certain that the variation at *Idh3,4* was not all at a single locus, and thus cannot compare the map locations of *Idh3* and *Idh4*.

Some of the loci in salmonids that were duplicated by the tetraploid event show no obvious structural or regulatory divergence from each other and are termed isoloci (e.g., *Mdh3,4* and *Idh3,4*). Others show marked structural and regulatory divergence, and are termed paralogous (e.g., *Ldh3* and *Ldh4*). Loci that are isoloci in one species also tend to be isoloci in other species. Loci might be prevented from diverging by residual homeologous pairing between chromosomes, which sometimes takes place in male salmonids and results in unusual inheritance patterns that have been termed "pseudolinkage" or "residual tetrasomic inheritance" (WRIGHT *et al.* 1980; WRIGHT *et al.* 1983).

If divergence of homeologs takes place gradually or is an incomplete process (as the unusual inheritance patterns observed in one sex, but not the other, suggest), distal loci on chromosome arms might be expected to diverge less

rapidly than loci near the centromere (ALLENDORF and THORGAARD 1983). The gene mapping results provide mixed evidence concerning this model: *Ldh3* and *Ldh4* (diverged loci) are near the centromere, whereas the *Mdh3,4* loci are farther from the centromere. At least one of the undiverged *Idh3,4* loci is also relatively far from its centromere. On the other hand, at least one of the undiverged *Mdh1,2* loci is relatively near its centromere. More mapping results are needed to test the model.

Comparisons of map locations can also be made for loci that were duplicated by an earlier tetraploid event in the evolution of fish (FISHER *et al.* 1980). *Mdh1* is much nearer the centromere than *Mdh3* and *Mdh4* (11.9 cM *vs.* 49.1 cM). *Idh2* and *Idh3* are both at intermediate distances from the centromere (34.8 and 29.0 cM).

There are very little gene-centromere map distance data available in other fish species that can be compared to our results. PURDOM, THOMPSON and DANDO (1976), in studies with plaice (*Pleuronectes platessa*), estimated gene-centromere map distances of 41 cM for *Pgm* (compared to 6.8 cM for *Pgm-2* in rainbow trout) and 19 cM for *Mdh-A* (compared to 11.9 cM for *Mdh1*, the homologous locus in rainbow trout). These comparisons are obviously too limited for any conclusions about the extent of genome conservation among fishes, but do illustrate the potential for comparisons as more information accumulates. The similar 48 acrocentric chromosome diploid karyotype observed in many distantly related fish species (OHNO 1974) suggests that there might be considerable genome conservation among species.

Rate of inbreeding with gynogenesis: One of the potential applications of gynogenesis in vertebrates is the rapid production of inbred lines (NACE, RICHARDS and ASHER 1970; STANLEY and SNEED 1974; PURDOM 1976; NAGY *et al.* 1978; STREISINGER *et al.* 1981). Diploid gynogenesis may be achieved by retention of the second polar body of the egg, as in this study, or by suppression of the first cell division. Suppression of the first cell division results in 100% homozygosity, whereas retention of the second polar body produces varying levels of homozygosity depending on the amount of crossing-over in meiosis.

Among the loci mapped in this study, the average proportion of heterozygous progeny was 55% (Table 4). This corresponds to an inbreeding coefficient (F) of 0.45 in one generation. Studies in plaice (PURDOM, THOMPSON and DANDO 1976; THOMPSON, PURDOM and JONES 1981) have shown an average of 46% heterozygotes among four loci analyzed. An average of 35% heterozygotes was observed among nine loci analyzed in carp, *Cyprinus carpio* (CHERFAS 1977; CHERFAS and TRUWELLER 1978; NAGY *et al.* 1978, 1979; NAGY and CSÁNYI 1982).

A major problem with inbreeding by inducing retention of the second polar body is that not all loci become homozygous at the same rate (NAGY and CSÁNYI 1982). This is illustrated by comparing our results for *Ldh4* with results for *Mdh3*, *Mdh4* and *Sod1*. Virtually all individuals are homozygous for *Ldh4* after gynogenesis whereas very few are homozygous for *Mdh3*, *Mdh4* or *Sod1*. It would be impossible to produce homozygous lines in a reasonable length of time in rainbow trout by gynogenesis involving retention of the second polar body.

Production of inbred lines in rainbow trout by diploid gynogenesis involving suppression of the first cell division may, however, be feasible. The first cell division has been successfully suppressed in rainbow trout to induce tetraploidy (THORGAARD, JAZWIN and STIER 1981; CHOURROUT 1982). Using such treatments after gynogenesis should make it possible to produce homozygous diploid individuals that could then be used to produce homozygous lines in rainbow trout as has been done in zebra fish (STREISINGER *et al.* 1981).

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