AN ANALYSIS OF RECOMBINATION DATA IN GYNOGENETIC DIPLOID RAINBOW TROUT

D. THOMPSON AND A. P. SCOTT

*Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Research,
Fisheries Laboratory, Lowestoft, Suffolk NR33 0HT*

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SUMMARY

Electrophoretic analyses of genetically controlled enzyme polymorphisms were carried out on broods of diploid gynogenetic rainbow trout alevins, produced by heat-shocking eggs which had been fertilised with u.v.-irradiated sperm. The results showed that diploidisation was caused by the retention of the second polar body and also demonstrated complex cross-over phenomena.

1. INTRODUCTION

The use of induced diploid gynogenesis as a means of rapidly developing inbred lines in fish and the potential value of these lines in the genetical improvement of commercial fish cultivation was proposed by Purdom (1969). Stanley and Sneed (1974) listed 17 species of fish in which gynogenesis had been achieved by successful manipulation of the embryo whilst Kirpichnikov (1981) discussed the occurrence and mechanism of both natural and induced gynogenesis in fish. Reviews have been presented of gynogenesis in all vertebrates by Chourrout (1982*a*) and, more recently, of induced gynogenesis in fish by Purdom (1983).

The application of electrophoretic techniques specifically identifying genetically-controlled polymorphic enzymes demonstrated that in broods of induced diploid gynogenetic offspring of the plaice, *Pleuronectes platessa*, diploidy was created by interruption of the second meiotic division of the egg; chromosomes destined to form the second polar body were retained in the egg pronucleus (Purdom *et al.*, 1976). Recombination data obtained from similar studies using genetically-controlled markers were used by Nagy *et al.* (1979) to estimate that in the carp, *Cyprinus carpio*, the inbreeding effect of one generation of induced diploid gynogenesis was equivalent to between 10 and 12 generations of conventional sib mating. However, similar data for the plaice indicated a lower effect (Thompson *et al.*, 1981) and a more comprehensive analysis of recombination data suggested that for both species a generation of induced diploid gynogenesis may be equivalent to between seven and eight generations of sib mating (Thompson, 1983).

Recent developments in inducing diploid gynogenesis in the rainbow trout, *Salmo gairdneri*, involving heat treating the eggs (Chourrout, 1980) and fertilising them with sperm rendered genetically inactive by ultraviolet irradiation (Chourrout and Quillet, 1982; Chourrout, 1982*b*) have allowed the original ideas of Purdom (1969) to be applied to a genetical improvement programme in the United Kingdom. In this paper, genetical variation of four enzyme loci is used to demonstrate the absence of any paternal contribution to the gynogenetic offspring genome and to consider the effect

of recombination upon the inbreeding efficiency of induced diploid gynogenesis. These data will be compared to those recently presented by Thorgaard *et al.* (1983) in their study of recombination data and its use in gene-centromere mapping in rainbow trout.

2. MATERIALS AND METHODS

Adult rainbow trout were individually tagged and small amounts of skeletal muscle were taken from the dorsal side, between the adipose and caudal fins. These tissue samples were tested for polymorphism in three enzymes using horizontal starch gel electrophoresis as described by Smithies (1955) followed by histochemical staining.

The enzymes and the electrophoretic buffer systems used, were as follows: malate dehydrogenase (MDH), E.C. number 1.1.1.37, using a continuous amine-citrate buffer system, pH 6.1, described by Clayton and Tretiak (1972); superoxide dismutase (SOD, also known as tetrazolium oxidase), E.C. number 1.15.1.1, using a continuous Tris-EDTA-borate buffer system, pH 8.7, described by Markert and Faulhaber (1965); phosphoglucomutase (PGM), E.C. number 2.7.5.1, using a discontinuous tris-citrate gel buffer, pH 8.5 and a lithium hydroxide-boric acid bridge buffer, pH 8.1, as described by Ridgway, *et al.* (1970). Stains were as described by Shaw and Prasad (1970).

When the fish reached sexual maturity they were stripped of gametes which, depending upon the parental genotype, were used to produce broods of either diploid gynogenetic alevins or normal diploid controls. Induced gynogenetic offspring were produced by fertilising eggs from individual females with genetically-inert spermatozoa and restoring diploidy by heat-treating the developing eggs to interrupt the second meiotic division.

Usually the eggs from females showing heterozygosity for at least one of the loci tested were chosen for the induced diploid gynogenesis experiments, although some experiments using eggs from homozygous females and milt from males of a different genotype were carried out to demonstrate the absence of paternal alleles in the offspring.

Normal diploid control matings were made between fish of various genotypes to demonstrate the Mendelian control of the enzyme polymorphisms.

The alevins were reared until absorption of the yolk sac when they were killed and stored in liquid nitrogen until electrophoretically tested, using the same methods as for the adult fish.

Genetically-inert spermatozoa were produced by irradiation of fresh milt with ultraviolet (u.v.) light. The milt was diluted with an equal volume of ice-cold modified Cortland's diluent (Scott and Baynes, 1980) and a 10 ml aliquot, with a final packed cell volume value of between 6 and 10 per cent, was transferred to a 100 ml disposable polystyrene weighing dish on a bed of crushed ice 15 cm beneath four 36 W u.v. germicidal tubes, wavelength 254 μm , fixed 7 cm apart. A magnetic stirrer was embedded in the ice beneath the weighing dish so that throughout irradiation the milt could be continuously agitated by a 20 mm stirrer. The whole apparatus was mounted in a sealed box to prevent leakage of the u.v. light. The intensity of irradiation at the surface of the milt was between 4.7 and 5.5 mW cm^{-2} as measured by a digital radiometer. Irradiation was carried

out for 25 min. Two ml of 0.9 per cent saline were added to batches of between 1000 and 2000 eggs which were then fertilised by irradiated milt and placed in water at 10°C. After 40 min they were transferred to a water bath held at 28°C for 10 min and then returned to water at 10°C for incubation.

The dosage of u.v. light required to inactivate the sperm DNA totally was determined by removing aliquots of sperm at intervals during the irradiation procedure and using these to fertilise batches of 200–300 eggs. After incubation at 10°C for 30 days (*i.e.*, to within about three days of hatching) the eggs were killed and cleared in a solution of equal volumes of glacial acetic acid, methanol and water. They were then examined to determine whether the developing embryos had haploid or diploid morphological characteristics. Egg development following control fertilisations using normal active sperm was also carried out and monitored.

3. RESULTS

(i) *Induction of gynogenesis*

The results of four experiments to determine the dose of u.v. light required to inactivate sperm DNA totally showed that the numbers of haploid embryos per batch of eggs increased proportionally to the u.v. irradiation of the sperm used to fertilise them. Sperm exposed to 1 min of irradiation at $5 \text{ mW} \cdot \text{cm}^{-2}$ produced only a few haploid-like embryos whilst sperm exposed to 15 min of irradiation produced 98–100 per cent haploids. When the dose rate exceeded 30 min there was a reduction in the number of motile spermatozoa and an associated decrease in the fertilisation rate.

Control fertilisations produced normal diploid embryos.

Haploid and diploid embryos were identified by certain morphological features which have been well described (Purdom, 1969; Chourrout *et al.*, 1980; Reftsie, 1983) and related to the ploidy by cytological investigations (Chourrout *et al.*, 1980). Haploids have small eyes, short thin bodies occupying only a part of the egg and are surrounded by a light yellow yolk, while diploids have large eyes, long bodies curving round the egg and are surrounded by a dark orange yolk. Only a few deformed embryos, which had characteristics intermediate between haploids and diploids, were noted in the experimental batches. The proportion of embryos dying during the early stages of development was much higher in irradiated batches than in the controls; 7–24 per cent in the former and 1–2.5 per cent in the latter.

(ii) *Enzyme polymorphism*

The different alleles at the four enzyme loci studied were labelled according to their rates of electrophoretic mobility either observed in these experiments or attributed to them by Allendorf (1973); the most common allele was given a distance of 1.00 and all others identified by their relative migration.

Two polymorphic MDH loci with equal migration rates were observed. Five alleles were identified and the electrophoretic patterns of heterozygotes were typical of polymorphism at a dimeric enzyme locus. As usual with equally migrating duplicate loci, single heterozygotes were identified by

differential band-staining on the gels and polymorphism could not be attributed to a particular locus. Similarly, some double heterozygotes and unlike double homozygotes could not be distinguished. A slower migrating monomorphic MDH locus was also observed.

A single locus for SOD was found with three alleles controlling production of dimeric molecules.

A single polymorphic locus was also found for PGM; here single- and double-banded phenotypes were interpreted as the monomeric products of a two allele system.

Details of the alleles observed at each locus and their frequencies are given in table 1.

TABLE 1

Allele frequencies found at the four loci examined in the adult fish. The values for the MDH-3 and MDH-4 loci are identical

MDH-3,4		SOD		PGM	
Allele	Frequency	Allele	Frequency	Allele	Frequency
1.33	0.0101	1.52	0.1881	1.00	0.7230
1.00	0.8164	1.00	0.8096	0.85	0.2770
0.80	0.0056	0.48	0.0023		
0.67	0.1655				
0.54	0.0023				
N = 222		N = 218		N = 222	

(iii) Control crosses

In the inheritance experiments the results of the control crosses showed that polymorphism at each of the four loci is controlled by codominant alleles segregating in a Mendelian manner.

The results for the MDH-3,4 loci are given in table 2. The offspring of matings between parents of similar genotype have been pooled as the segregation in individual matings did not significantly deviate from the expected.

TABLE 2

Segregation of offspring in conventional crosses made at the MDH-3,4 loci. Numbers in parentheses are the expected segregations according to Mendelian ratios

Parental genotypes (female first)	No. of matings	Offspring genotypes			χ^2	Probability
		100 ⁴	1.00 ³ , 0.67	1.00 ² , 0.67 ²		
1.00 ⁴ × 1.00 ³ , 0.67	4	98 (103)	108 (103)		0.49	0.50 > 0.30
1.00 ³ , 0.67 × 1.00 ⁴	4	126 (138.5)	151 (138.5)		2.26	0.20 > 0.10
1.00 ³ , 0.67 × 1.00 ³ , 0.67	2	18 (22)	46 (44)	24 (22)	1.00	0.70 > 0.50

The results for the SOD locus are given in table 3; again there were no deviations from the expected segregation ratios either in individual broods or the pooled data.

Details of the offspring segregation ratios at the PGM locus are given in table 4. All of the matings except one complied with the expected; in

TABLE 3

Segregation of offspring in conventional crosses made at the SOD locus. Numbers in parentheses are the expected segregations according to Mendelian ratios

Parental genotypes (female first)	No. of matings	Offspring genotypes			χ^2	Probability
		1.52, 1.52	1.00, 1.00	1.52, 1.00		
1.00, 1.00 $\times$ 1.52, 1.00	2		126 (134.5)	143 (134.5)	1.07	0.30 > 0.20
1.52, 1.00 $\times$ 1.00, 1.00	9		341 (342)	343 (342)	0.01	0.95 > 0.90
1.52, 1.00 $\times$ 1.52, 1.00	5	61 (59)	60 (59)	115 (118)	0.16	0.95 > 0.90

TABLE 4

Segregation of offspring in conventional crosses made at the PGM locus. Numbers in parentheses are the expected segregations according to Mendelian ratios

Parental genotypes (female first)	No. of matings	Offspring genotypes			χ^2	Probability
		1.00, 1.00	0.85, 0.85	1.00, 0.85		
1.00, 1.00 $\times$ 1.00, 0.85	4	141 (146.5)		152 (146.5)	0.41	0.70 > 0.50
0.85, 0.85 $\times$ 1.00, 0.85	2		34 (42.5)	51 (42.5)	3.40	0.10 > 0.05
1.00, 0.85 $\times$ 1.00, 1.00	9	537 (559.5)		582 (559.5)	1.81	0.20 > 0.10
1.00, 0.85 $\times$ 1.00, 0.85	2	71 (69.25)	51 (69.25)	155 (138.5)	6.82	0.05 > 0.02

this cross a deficiency of offspring of the genotype 0.85, 0.85 was found (offspring distribution: 1.00, 1.00, observed 33, expected 25; 0.85, 0.85, observed 14, expected 25; 1.00, 0.85, observed 53, expected 50; $\chi^2 = 7.58$; $0.05 > P > 0.02$) which caused a similar deficit in the pooled data. When these offspring were tested at the SOD locus the segregation was as expected.

(iv) *Induced diploid gynogenomes*

The segregations of offspring at the MDH-3,4, SOD and PGM loci in broods of induced diploid gynogenomes are given in tables 5, 6 and 7 respectively. The presence of two homozygote classes and heterozygotes of the parental type confirm that the meiotic division of the egg, not the first mitotic division of the zygote, was interfered with to produce diploidy. The frequencies of heterozygotes below 50 per cent observed in some broods confirm that meiosis II was suppressed.

In broods of induced diploid gynogenomes the two types of homozygote should be equally frequent and in the majority of the experiments this was the case. However, significant deviations from the 1:1 ratio were found in two broods for each of the three enzymes (see tables 5, 6 and 7). It was not possible to test statistically the distribution in all the individual broods at the SOD locus or the MDH-3,4 loci; however, for both of these sets of data the overall distribution of homozygotes was as expected, although this was not so at the PGM locus.

For the MDH-3,4 and SOD loci it seems unlikely that these deviations in two individual broods indicate the superiority of a particular genotype; the overall deviation at the PGM locus may suggest this, but in the two individual broods, where a significant deviation from expected was

TABLE 5

Distribution of genotypes at the MDH-3,4 loci in broods of gynogenetic diploid offspring. All the female parents were of genotype 1.00³, 0.67

Parents*		Offspring genotypes			% recombination		Strain of origin of female parent
Female	Irrad. Male	1.00 ⁴	1.00 ² , 0.67 ²	1.00 ³ , 0.67	On both homozygotes	On most frequent homozygote	
32	6	1	1	26	92.9	92.9	Caribou‡
67	33	8	4	51	81.0	76.1	Whitebrook
73	16	3	2	36	87.8	85.7	Whitebrook
79	16	9	9	29	61.7	61.7	Whitebrook
322	323	2	9†	89	89.0	83.2	Christiansen
326	323	33	28	77	55.8	53.9	Christiansen
329	465	24	28	44	44.9	44.0	Christiansen
331	465	—	1	15	93.7	—	Christiansen
336	324	—	1	30	96.8	—	Christiansen
343	461	10	12	105	82.7	81.4	Christiansen
345	346	—	—	52	100.0	—	Christiansen
451	346	—	5†	2	28.6	—	Christiansen
Totals		90	100	556	74.5	73.5	

* Numbers of the tags used to identify each fish.

† Significant deviation from 1:1 ratio in homozygote distribution.

‡ Names indicate origin of strains kept at Lowestoft laboratory.

TABLE 6

Distribution of genotypes at the SOD locus in broods of gynogenetic diploid offspring. All the female parents were of genotype 1.00, 1.52

Parents		Offspring genotypes			% recombination		Strain of origin of female parent
Female	Irrad. Male	1.00, 1.00	1.52, 1.52	1.00, 1.52	On both homozygotes	On most frequent homozygote	
24	16	3	—	105	97.2	—	Whitebrook
67	33	4	—*	69	94.5	—	Whitebrook
68	16	10	16	116	81.7	78.4	Whitebrook
70	16	5	—*	66	93.0	—	Whitebrook
322	323	4	2	94	94.0	92.2	Christiansen
325	55	—	—	96	100.0	—	Christiansen
336	324	—	—	31	100.0	—	Christiansen
336	475	—	—	89	100.0	—	Christiansen
342	465	2	4	118	95.2	93.7	Christiansen
345	346	—	—	52	100.0	—	Christiansen
453	324	—	—	13	100.0	—	Christiansen
459	324	—	—	48	100.0	—	Christiansen
472	475	2	—	64	97.0	—	Christiansen
Totals		30	22	961	94.9	94.1	

* Significant deviation from 1:1 ratio in homozygote distribution.

TABLE 7

Distribution of genotypes at the PGM locus in broods of gynogenetic diploid offspring. All the female parents were of genotype 1.00, 0.85

Parents		Offspring genotypes			% recombination		Strain of origin of female parent
Female	Irrad. Male	1.00, 1.00	0.85, 0.85	1.00, 0.85	On both homozygotes	On most frequent homozygote	
15	6	29	15*	4	8.3	6.5	Caribou
24	16	45	47	51	35.7	35.2	Whitebrook
68	16	44	76*	22	15.5	12.6	Whitebrook
73	16	11	13	30	55.6	53.4	Whitebrook
79	16	18	31	7	12.5	10.1	Whitebrook
322	323	47	46	10	9.7	9.6	Christiansen
326	323	47	49	5	5.0	4.9	Christiansen
331	465	4	11	1	6.3	4.3	Christiansen
342	465	65	71	28	17.1	16.5	Christiansen
343	461	52	74	1	0.8	0.7	Christiansen
451	346	3	1	3	42.9	33.3	Christiansen
453	324	5	9	1	6.7	5.3	Christiansen
459	324	20	25	3	6.3	5.7	Christiansen
475	340	20	16	1	2.7	2.4	Christiansen
Totals		410	484*	167	15.7	14.7	

* Significant deviation from 1:1 ratio in homozygote distribution.

observed, the first was due to an excess of one homozygote whilst the second was due to an excess of the other homozygote. In the absence of further data and, considering the compliance with Mendelian inheritance observed in the majority of conventional crosses involving these genotypes, it does not seem reasonable to attach particular significance to these anomalies.

Contingency tests of homozygote and heterozygote distribution between offspring from each brood showed heterogeneity for all three enzymes (MDH-3,4, $\chi^2 = 130.98$, D.F. = 11, $0.001 > P$; SOD, $\chi^2 = 70.98$, D.F. = 12, $0.001 > P$; PGM, $\chi^2 = 157.01$, D.F. = 13, $0.001 > P$).

This significant heterogeneity was retained when the broods were separated on the basis of the strain of origin of the female parent and contingency tests were made on the resulting homozygote and heterozygote distributions, see table 8.

TABLE 8

The results of contingency tests made for the distribution of homozygotes and heterozygotes between broods from female parents of like origin

Enzyme	Whitebrook			Christiansen		
	χ^2	D.F.	Probability	χ^2	D.F.	Probability
MDH-3,4	9.42	2	$0.01 > 0.001$	114.37	7	$0.001 > P$
SOD	20.08	3	$0.001 > P$	17.86	8	$0.05 > 0.02$
PGM	42.32	3	$0.001 > P$	39.44	8	$0.001 > P$

Eight control gynogenetic experiments were made between homozygous females and inactivated males of a different genotype in order to confirm that there was no male parental contribution to the offspring genome. Ninety-two offspring from three broods were tested at the MDH-3,4 loci, 84 offspring from two broods at the SOD locus and 234 offspring from five broods at the PGM locus; all had the same homozygous genotype as the female parent.

4. DISCUSSION

The use of u.v. irradiation to inactivate fish spermatozoa is becoming widespread (Ijiri and Egami, 1980; Chourrout, 1982*b*; Thorgaard *et al.*, 1983) and is replacing the γ -irradiation used by earlier studies (Purdom, 1969; Purdom *et al.*, 1976; Chourrout, 1980). Apart from the potential danger of γ -irradiation and its limited availability, there is good evidence that it is less efficient than u.v. irradiation. Ijiri (1980) showed that even after exposing spermatozoa to relatively large doses of γ -rays (100 and 125 kRad) the male genome of *Oryzias latipes* was not totally inactivated, whereas after exposure to a wide range of moderate u.v. doses there was no evidence at all of male genes being incorporated into gynogenetic embryonic genomes.

The enzyme polymorphisms observed in the adult fish complied with previous descriptions of genetic variation at the loci studied. At the MDH-3,4 loci the results were similar to those originally described by Bailey *et al.* (1970) and to the more detailed observations of Clayton *et al.* (1975). Polymorphism at the SOD locus was as reported by Utter (1971) and Utter and Hodgins (1972) whilst the two alleles observed at the PGM locus were similar to those reported by Roberts *et al.* (1969).

The results of the conventional crosses made at the MDH-3,4 loci confirm the Mendelian control of polymorphism by codominant alleles at two disomic loci as described by Utter *et al.* (1975).

Similarly the crosses made at the SOD locus all complied with the concept of Mendelian inheritance again as first reported by Utter *et al.* (1975). At the PGM locus the offspring of all except one cross segregated into expected ratios; the simplest interpretation is that polymorphism at this locus is also under Mendelian control and that such a deviation may occur by chance when several crosses at any single locus are studied.

In the broods of induced diploid gynogenetic alevins the frequencies of the maternal type heterozygotes observed confirm that the restoration of diploidy is due to interference with meiosis II and the retention of chromatids destined to form the second polar body, as described by Thompson *et al.* (1981). Similar conclusions have been made from inheritance studies in gynogenetic diploids of the plaice (Purdom *et al.*, 1976; Thompson *et al.* 1981), the carp (Golovinskaia, 1968) and more recently the rainbow trout (Thorgaard *et al.*, 1983), although the data for the carp are complicated by lethal combinations and dominance effects. Thompson (1983) presented a review of all the previously published data on plaice and carp species.

As each heterozygous genotype represents a recombined chromosome pair in the zygote caused by a crossover event between the centromere and

the locus in question, the heterozygote frequencies observed in broods of induced diploid gynogenomes of plaice (Thompson *et al.*, 1981, Thompson, 1983) and carp (Nagy *et al.*, 1979) have been used to estimate the inbreeding effect in these species of one generation of induced diploid gynogenesis. In the plaice, although the recombination frequencies were different for all the five loci examined, at four of them there were no significant differences in heterozygote frequencies between broods from different females. Such consistency between broods allowed the use of the recombination data to calculate the coefficient of inbreeding of one generation of gynogenesis, as suggested by Purdom (1969).

The data presented here for rainbow trout appear to be more complex in that variations in the frequencies of heterozygotes observed occurred between broods for each of the enzymes studied.

The data observed for the MDH-3,4 loci may represent a mixture of recombination frequencies for two loci each with a different map length. The female parents used in the experiments, although only single heterozygotes, may have been heterozygous at either the MDH-3 or MDH-4 locus. Allendorf *et al.* (1975) demonstrated by inheritance experiments that there is no linkage between these two loci and that they are on separate chromosomes; during evolutionary changes to the rainbow trout genome the distances between these loci and their respective centromeres may have changed.

However, Thorgaard *et al.* (1983) found recombination frequencies of between 96 per cent and 100 per cent for MDH-3,4 in five broods of gynogenetic diploid offspring, three of these being from females heterozygous for one of these loci and two from females heterozygous for both loci and the homogeneity of these data suggested that both the MDH-3,4 loci have similar map lengths from the centromere on their respective chromosomes.

The high frequencies of heterozygotes, 81 per cent and above, observed in some of the broods are higher than the theoretical maximum of 67 per cent postulated by Purdom *et al.* (1976) for an infinite number of crossovers between a locus and the centromere during the first meiotic division. This is not an unusual occurrence and has been observed in similar experiments at these loci in rainbow trout (Thorgaard *et al.*, 1983) and at other loci in induced gynogenetic diploids in carp (Golovinskaia, 1968; Cherfas, 1977) and plaice (Purdom *et al.*, 1976; Thompson *et al.*, 1981; Thompson 1983). It has consistently been interpreted as due to positive interference occurring after a single chiasma, resulting in total heterozygosity at loci distal to the centromere.

The results at the remaining two loci, SOD and PGM, also show significant heterogeneity in the distribution of homozygotes and heterozygotes between broods, although for SOD the range of recombination frequencies (81 per cent to 100 per cent) is not as great as that for PGM (1 per cent to 56 per cent). Thorgaard *et al.* (1983) reported recombination frequencies of 100 per cent at the SOD locus in two broods of gynogenetic diploids. Twelve of the broods examined in our study gave recombination frequencies greater than 93 per cent and in six of these 100 per cent recombination was observed, only one brood showing a lower (81 per cent) recombination frequency (see table 6). It can be concluded that these data support those of Thorgaard *et al.* (1983).

Similarly, at the PGM locus, Thorgaard *et al.* (1983) observed recombination frequencies of 10 per cent and 16 per cent in two broods which fall within the range of 1 per cent to 56 per cent recombination in 14 broods reported here.

The retention of variation in recombination frequencies when the data are divided according to the strain of origin of the female parent suggest that the possibilities of interstrain differences in chiasma frequency and position do not apply.

This range of recombination frequencies may be due to loci being on acrocentric chromosomes which become involved in the Robertsonian fusion of two such pairs of chromosomes to form a single pair of metacentrics. The occurrence of these fusions in some of the parent fish and the resulting effects upon crossover events between a particular locus and the centromere may explain the variations observed between broods.

Considerable chromosome polymorphism caused by Robertsonian centric fusion has been reported in rainbow trout (Ohno *et al.*, 1965; Thorgaard, 1976) when both inter and intra individual variations in diploid numbers were found, even although a constant chromosome arm number of 104 was maintained. The results of these authors varied slightly in that Ohno *et al.* (1965) reported chromosome numbers of 58–65 with considerable intra-individual variation and Thorgaard (1976) reported chromosome numbers of 58–60 with a low level of intra-individual variation. Thorgaard (1976) also found inter-strain differences in the frequencies of fish with different chromosome numbers which he suggested could indicate that inter-individual differences in chromosome number are inherited and not somatic.

Alternative genetical reasons for the ranges of recombination frequencies observed may be that either pericentric inversions within a chromosome or even translocation between chromosome arms may alter map lengths.

As yet it has not been possible to repeat the experiments using the same female parents to test the consistency of recombination in broods from one fish from year to year nor to test the inheritance of recombination frequencies by a second generation of gynogenesis. Such evidence must be obtained before further interpretations can be made.

Apart from demonstrating the restoration of diploidy by interruption of meiosis II, the data available do not allow any other firm conclusions to be made on the chromosome behaviour necessary to cause the observed recombination frequencies; because of this it does not seem reasonable to attempt to assess the inbreeding effects of a generation of induced diploid gynogenesis.

Thorgaard *et al.* (1983) suggest that, because high levels of interference cause high recombination values for distal parts of chromosomes, the production of gynogenetic diploids by retention of the second polar body cannot be an efficient technique for inducing inbreeding; the data presented here at the MDH-3,4 and SOD loci support this. The problem of producing inbred lines may be diminished by alternating a generation of gynogenesis with one or more generations of self-fertilisation by the use of hormone-induced hermaphroditism, as suggested by Purdom (1983).

The differences between our recombination data for rainbow trout and that previously published for carp and plaice are consistent with differences observed between salmonids and other fish in both biochemical and cytogenetical studies. Further inheritance data from induced diploid gynogenetic

offspring may contribute to the understanding of chromosome behaviour in salmonids; the results so far emphasise the uniqueness of this group with its retention of disomic inheritance, frequently at duplicate loci, following a tetraploid event in their evolution.

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