Heredity (1976), **37** (2), 193-206

GENETIC ANALYSIS OF ENZYME POLYMORPHISMS IN PLAICE (*PLEURONECTES PLATESSA*)

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Received 17.ii.76

Summary

Genetic analysis was performed on five enzyme systems (G3PDH; GPI-A; GPI-B; PGM; MDH-A) in plaice (*Pleuronectes platessa*) collected in spawning condition from the North Sea. Conventional crosses, induced gynogenesis and induced triploidy were performed. The data conclusively demonstrated the inheritance of isozymes by co-dominant alleles at individual loci for each system. No linkage was observed but tests did not include MDH nor the possibility of linkage between G3PDH and GPI-A. Some anomalous segregation ratios were observed, particularly a deficiency of heterozygotes for GPI-A, but the data were largely in conformity with Mendelian expectations. At the PGM locus, five independent anomalous individuals were scored and interpreted as mutations with a mutation rate of $1{\cdot}1 \times 10^{-3}$ per gamete. Recombination with the centromere was assessed and induced triploidy and cross-over values of 41 per cent for PGM, 19 per cent for MDH-A and 9 per cent for GPI-B were derived on the assumption of complete interference. Amongst the parent fish, genotypic and phenotypic frequencies were largely consistent with the expectations of the Hardy-Weinberg Law, and allelic frequencies were not significantly different between year of collection or location of collection ground.

1. Introduction

Electrophoretic analysis of protein variation between individuals of a species is a powerful tool in the genetic study of natural populations. In marine fish, such studies have been widely concerned with the identification of stocks in species of commercial importance. The extensive literature in the field has been reviewed by de Ligny (1969).

More recent work has associated different allelic forms of enzymes with varying environmental conditions (Koehn, 1970; Johnson, 1971; Merritt, 1972; Powers and Powers, 1975).

Little attempt has been made to study the formal genetics of marine fish by breeding trials, and genetic interpretation has been by homology with similar systems in mammals and *Drosophila* and by compliance between observation and hypothesis on the basis of the Hardy-Weinberg Law.

Plaice (*Pleuronectes platessa* L.) is one of the few marine species of commercial importance which can be bred in captivity. A variety of polymorphic enzymes exist in this species although, as yet, there is no indication that their distributions vary between presumed stocks. This paper reports

studies on the inheritance of some of these enzyme variants, using conventional Mendelian methods, gynogenesis (Purdom, 1969) and induced triploidy (Purdom, 1972).

The haploid chromosome complement of plaice comprises 24 telecentric chromosomes (Barker, 1972) with an even-size distribution from the smallest to the largest, which is about twice the size of the smallest. The probability of finding linked loci is therefore small, but gynogenesis and induced triploidy supply a way of measuring recombination between single loci and the centromere and hence a method for producing chromosome maps.

2. Materials and methods

Sexually mature plaice were taken by trawl from several grounds in the North Sea. Individual fish were marked by tags or by cold branding. Skeletal muscle samples were taken by needle biopsy and analysed by starch gel electrophoresis for the following enzymes:

	International enzyme number
Glycerol-3-phosphate dehydrogenase (G3PDH)	1.1.1.8
Glucosephosphate isomerase-A (GPI-A)	5.3.1.9
Glucosephosphate isomerase-B (GPI-B)	5.3.1.9
Phosphoglucomutase (PGM)	2.7.5.1.
NAD-linked supernatant malate dehydrogenase-A (MDH-A)	1.1.1.37
NAD-linked supernatant malate dehydrogenase-B (MDH-B)	1.1.1.37

Selected parents were crossed by artificial fertilisation. Eggs were incubated at 7°C and larvae were either tested immediately upon hatching or were reared in batches of 200 for each cross in 301 tanks with static water at 11°C. The larvae were fed on a diet of *Artemia salina* nauplii and killed at or near metamorphosis 8-10 weeks after hatching. In the earlier work, offspring were deep frozen for storage prior to analysis, but better results were obtained latterly by performing electrophoresis on fresh tissues or on fresh whole larvae soon after hatching.

Vertical starch gel electrophoresis as described by Dando (1970) was used for enzyme analysis. For later work on G3PDH and MDH both horizontal and vertical gel electrophoresis was carried out using a pH 6·95 N-(3-aminopropyl)-diethanolamine-citric acid buffer (Clayton and Tretiak, 1972) instead of trisborate pH 8·7 buffer. This change in pH gave greater G3PDH and MDH stability and improved the resolution of the technique. Muscle biopsy samples and larvae were applied directly to slots in the gels.

G3PDH, MDH, PGM and GPI were stained by coupling their reactions to the reduction of the tetrazolium dye 3-(4:5-dimethyl-thiazolyl-2)-2:5-diphenyl-monotetrazolium bromide (thiazolyl blue). None of the activity bands developed in the absence of the specific enzyme substrate. Isoenzymes were numbered consecutively, on the basis of electrophoretic mobility, with the lowest number given to the form with the highest mobility towards the anode.

Gynogenesis was performed by the fertilisation of eggs with irradiated plaice spermatozoa, or with halibut (*Hippoglossus hippoglossus*) spermatozoa. Fertilisation was performed at approximately 7°C and after 15 minutes the eggs were subjected to a cold shock by instant immersion in sea water at less than 0°C. Eggs were held at a temperature of 0°C for 2-3 hours, after which a gradual return to the normal incubation temperature was permitted. Triploidy was induced in the same way following fertilisation with plaice spermatozoa.

3. Results

Table 1 lists the phenotypes of fish which comprised the parental pool during the 1972-75 seasons. Application of the Hardy-Weinberg Law gave no evidence of disparity between allelic and genotypic frequencies

Table 1

Distribution of phenotypes in fish collected from the North Sea

			Locus		
Phenotype	G3PDH	GPI-A	GPI-B	PGM	MDH-A
1-1	2	1	—	—	—
2-2	162	182	—	1	81
3-3	1	—	—	25	2
4-4	1	—	1	67	—
1-2	10	13	—	—	—
1-3	1	—	—	2	—
1-4	—	—	—	4	—
2-3	9	5	—	1	25
2-4	1	—	—	2	—
2-6	—	—	3	—	—
3-4	—	—	—	97	—
3-5	—	—	—	1	—
4-5	—	—	—	2	—
4-6	—	—	18	—	—
6-6	—	—	171	—	—
6-7	—	—	9	—	—
Untyped	18	4	3	3	—

except for an excess of homozygotes at the G3PDH locus ($\chi^2 = 3{\cdot}97$: $P = 0{\cdot}05$).* However, 9 per cent of the fish were untyped for this enzyme for technical reasons. Table 2 lists the major allele frequencies for each enzyme system against collection ground and year. There were no significant differences between samples with respect to individual loci.

(i) *Conventional crosses*

In over 70 crosses performed during the 1972-75 spawning seasons, the enzyme variants were predominantly transmitted in accordance with the simple hypothesis that they were determined by co-dominant alleles at individual loci for each system. In all crosses between a homozygote and a heterozygote the offspring segregated into only two classes. Single phenotypes are therefore assumed to be homozygotes for the alleles in question.

* This excess could be due to the presence of null alleles in the population rather than to the Wahlund effect or differential mortality.

TABLE 2

Allele distribution by year and fishing ground

Locus		G3PDH		GPI-A		GPI-B		PGM		MDH-A	
Allele ground	Year	2	Others	2	Others	6	Others	4	Others	2	Others
Silver Pits	1974	48	2	47	3	50	—	24	26	44	6
Tea Kettle	1973	97	13	120	4	117	9	71	55	—	—
	1974	44	4	47	1	42	6	30	18	43	5
West Mud Holes	1975	85	3	84	4	79	9	58	30	72	16
Other Grounds	1972	35	3	41	5	43	3	30	16	—	—
	1973	10	2	16	—	16	—	11	5	—	—
	1974	25	3	27	3	25	5	15	15	28	2
Totals		344	30	382	20	372	32	239	165	187	29
% others			8·0		5·0		7·9		40·8		13·4

Anomalies fell into two categories:

(*a*) Unexpected segregation which could be explained on the basis of mistyping of one parent or misreading of a parental tag. The results in these cases were consistent with a simple Mendelian model of inheritance providing assumptions were made about the parental genotypes. They will not be further discussed.

(*b*) Anomalies of segregation ratios or the appearance of individual genotypes which cannot be explained by mis-identification of parents.

(a) *Glycerol-3-phosphate dehydrogenase*

Teleosts have three loci coding for different subunits of this enzyme (Numachi, 1971; Engel, Schmidtke and Wolf, 1971; Clayton, Franzin and Tretiak, 1973). The nomenclature of the different G3PDH enzymes is confused and, in the absence of comparative kinetic or immunological data, the identity of the enzyme under discussion with any of the G3PDH enzymes found in these other teleosts cannot be defined. Two loci are known in plaice (R. Ward, pers. comm.) but only one is found in white skeletal muscle. This gives a simple zymogram pattern of a single band in homozygotes and three bands in heterozygotes. This pattern has been described for another flatfish, *Lepidorhombus whiff-iagonis* (Walbaum), by Dando (1970).

In this study G3PDH proved to be the most difficult enzyme to identify. Weak reactions were observed in much of the frozen material, particularly in 1973, but the use of the pH 6·95 buffer in 1974 gave stronger staining and better resolution.

Table 3 lists the results of crosses involving heterozygotes. Segregation was normal in all cases except the second and third crosses where significant deficiencies of heterozygotes were indicated. ($\chi^2 = 5{\cdot}4$, $P = 0{\cdot}02$ and $\chi^2 = 6{\cdot}8$; $P = 0{\cdot}01$ respectively). This could be attributed to the greater difficulty of scoring the weaker staining heterozygotes. Similar crosses in 1974 gave expected results. In 1972 and 1974 419 offspring from 2-2 × 2-2 crosses were also tested. All offspring were homozygous type 2-2. No anomalous phenotypes were observed in the total of 747 offspring examined.

(b) *Glucosephosphate isomerase*

Polymorphism for this enzyme in plaice has been described by Dando (1974). The enzyme is controlled by two loci, both forms being detectable in white muscle, and easily separated by electrophoretic mobility; GPI-A having the greatest mobility.

GPI-A: Seven crosses between a homozygote 2-2 and heterozygote 1-2 were performed in 1972 (table 4) and the offspring totalled 171 2-2, 118 1-2

TABLE 3

Segregation of G3PDH alleles

Cross*	Year	1-1	2-2	1-2	2-3	Not typed
1-2 × 2-2	1972	—	20	17	—	—
2-3 × 2-2	1972	—	35	—	18	5
1-2 × 1-2	1973	16	17	15	—	67
2-2 × 2-3	1973	—	8	—	11	1
1-2 × 1-2	1974	32	35	66	—	83
1-3 × 2-2	1974	—	—	9	7	4
2-2 × 1-2	1974	—	12	10	—	—

* Female first in all crosses.

TABLE 4

Offspring segregation in crosses between fish homozygous for allele 2 and heterozygous for alleles 1-2 (in brackets) for GPI-A, performed during 1972

Parents		Offspring	
		2-2	1-2
(7076)*	7064	25	29
7075	(7070)	22	12
(7073)	7074	21	5
(7068)	7064	21	16
(7076)	7069	35	23
(7076)	7080	44	31
(7076)	7084	3	2
Total		171	118

* Lowestoft identification numbers.

with a highly significant deficiency in heterozygotes ($\chi^2 = 9{\cdot}7$; $P = 0{\cdot}001$). Two similar crosses performed in 1974, however, produced totals of 100 2-2 and 101 1-2 individuals. The deficiency of the 1-2 genotype tended to occur in crosses with low survival. The data are therefore consistent with an hypothesis that the 1-2 genotype is less fit than the 2-2 genotype under adverse conditions although there is no corroborative evidence to support this. A 1975 cross between 2-2 and 2-3 produced a normal segregation of 32 2-2:28 2-3; and in a cross involving two heterozygous parents a normal segregation of 6 1-1:5 2-2 and 12 1-2 was observed. A total of 1883 offspring from 2-2 × 2-2 crosses were also tested. All the offspring were of homozygous type 2-2 except for one type 2-1, found in 1974. This allele has not been previously described and migrates faster than allele 1. As allele 1 was not present in the 1974 parent gene pool this abnormality cannot be the result of sample contamination.

GPI-B: Four alleles were examined. Table 5 lists the segregation ratios in 15 crosses between 6-6 homozygotes and the three heterozygotes. No significant departures from an expected 1:1 ratio were observed either in individual crosses or pooled totals for crosses of particular genotypes. In two crosses with 2-6 × 6-7 parents the total progeny were 18 6-6; 26 2-6; 20 6-7; 19 2-7 and in one cross 4-6 × 6-7 there were 6 6-6 and two each of 4-6, 6-7 and 4-7 with no significant departure from expectation. In one cross 4-6 × 4-6 the offspring numbered 4 4-4; 10 6-6; 7 4-6; the deviation from an expected 1:2:1 segregation was borderline ($\chi^2 = 5{\cdot}76$; $P = 0{\cdot}05$), but in view of the number of crosses tested, was not considered significant. Offspring from 6-6 × 6-6 crosses numbered 876 and all were homozygous.

TABLE 5

Segregation of GPI-B alleles

Cross	No. of matings	6-6	2-6	4-6	6-7	χ^2	P
6-6 × 2-6	2	288	256	—	—	1·88	0·15
6-6 × 4-6	8	174	—	149	—	1·93	0·15
6-6 × 6-7	5	97	—	—	120	2·44	0·10
Totals	15	559		525		1·07	0·25

(c) *Phosphoglucomutase*

Plaice has two separate zones in PGM activity on zymograms of tissue extracts. The most anodal zone is found only in erythrocyte extracts. The second zone is present in all other tissues, including skeletal muscle, and consists of two or four bands, in presumed homozygotes and heterozygotes respectively.

The results of crosses involving five alleles at the PGM locus are listed in table 6. Where more than one cross of particular genotypes occurred, and there was no evidence of heterogeneity, the results are pooled. In the majority of cases, segregation ratios were consistent with expectation on the basis of a simple Mendelian mode of inheritance and there was no evidence of selective advantage for particular genotypes. There were several discrepancies.

A noticeable departure from expectation occurred in the first of the 3-4 × 1-4 crosses, with a highly significant deficiency of allele 1. The full cross was 2-2, 2-2, 6-7, 3-4 × 2-2, 2-2, 2-6, 1-4 in the order G3PDH, GPI-A, GPI-B and PGM. No departure from expectation was observed at the GPI-B locus which segregated 15 6-6; 19 2-6; 16 2-7; 12 6-7; ($\chi^2 = 1{\cdot}6$; $P = 0{\cdot}65$) and therefore there was no evidence of sample contamination from another cross which would have to involve a parent genotype GPI-B 1-3 with either PGM 3-4 or PGM 4-4—no such genotypes were present in the pool of parental fish. The PGM allele 1 itself, however, showed no abnormal segregation in four other crosses, one of which employed the same genotypes but not the same fish as in the abnormal cross.

A further anomaly in the above cross was the appearance of a PGM 3 phenotype which could represent a mutation to allele 3 or to a null allele.

Possible mutations also occurred in three other crosses, a 4 phenotype was found in a 3-4 × 2-3 cross, a 3 phenotype in a 3-4 × 4-4 cross and a 2-3 phenotype in a 3-4 × 3-4 cross. The first cross was also significantly

deficient of allele 2, but segregation for this cross at the GPI-B locus (parents 4-6 × 6-6) was as expected, 6-6 23; 4-6 18 ($\chi^2 = 0{\cdot}61$; P = 0·42). The second cross showed no other abnormalities at the PGM locus, nor at the

TABLE 6

Segregation of PGM alleles

Cross	No. of matings	Offspring phenotypes 3-3	1-3	2-3	3-4	3-5	4-4	1-4	2-4	χ^2	P
3-3 × 3-4	4	165	—	—	172	—	—	—	—	0·15	0·70
3-3 × 4-5	1	—	—	—	30	28	—	—	—	0·1	0·75
3-4 × 1-3	1	2	7	—	7	—	—	5	—	3·19	0·40
	1	28	34	—	16	—	—	20	—	7·96	0·05
	1	20	22	—	16	—	—	20	—	0·97	0·85
3-4 × 1-4	1	(1)	2	—	25	—	24	10	—	24·00	0·0001
	1	—	18	—	18	—	31	28	—	5·80	0·12
3-4 × 2-3	1	58	—	50	43	—	—	—	40	4·04	0·25
	1	14	—	3	14	—	(1)	—	7	11·01	0·01
3-4 × 2-4	1	—	—	42	37	—	42	—	32	1·8	0·60
3-4 × 3-3	2	63	—	—	57	—	—	—	—	0·30	0·60
3-4 × 3-4	6	42	—	—	101	—	36	—	—	3·36	0·20
	1	18	—	—	12	—	4	—	—	14·47	0·0007
	1	19	—	—	31	—	5	—	—	8·02	0·017
	1	69	—	(1)	48	—	8	—	—	66·26	0·0001
	1	15	—	—	11	—	5	—	—	9·06	0·01
3-4 × 4-4	8	—	—	—	150	—	151	—	—	0·003	0·99
	1	—	—	—	37	—	21	—	—	4·41	0·03
	1	—	—	—	69	—	44	—	—	5·53	0·02
	1	(1)	—	—	29	—	22	—	—	1·23	0·25
4-4 × 3-4	3	—	—	—	193	—	207	—	—	0·29	0·58

GPI-B locus (parents 6-7 × 6-7; offspring 6-6 11, 7-7 12, 6-7 29; $\chi^2 = 1{\cdot}35$; P = 0·60). The third cross, 3-4 × 3-4, was significantly deficient of allele (see table 7), but again segregation at the GPI-B locus was as expected (parents 6-6 × 4-6 offspring 6-6 61; 4-6 65; $\chi^2 = 0{\cdot}03$, P = 0·95).

TABLE 7

Segregation of MDH-A alleles

Cross	No. of matings	Offspring phenotypes 2-2	3-3	2-3	χ^2	P
2-2 × 2-3	1	58	—	55	0·08	0·80
2-3 × 2-2	6	182	—	175	0·14	0·70
2-3 × 2-3	4	29	37	69	1·01	0·62

Further segregation ratio anomalies occurred in three other 3-4 × 3-4 crosses, all showing a deficiency of 4-4 genotypes. Six similar crosses, however, gave expected results. An excess of 3-4 genotypes was observed in two of the 11 3-4 × 4-4 crosses tested. One of these crosses was also heterozygous as the MDH-A locus where segregation was as expected (parents 2-2 × 2-3; offspring 2-2 58, 2-3 55, $\chi^2 = 0{\cdot}08$; P = 0·85).

Offspring from one 3-3 × 3-3 cross and four 4-4 × 4-4 crosses comprising 216 individuals were all expected homozygotes.

(d) *Malate dehydrogenase*

Only the supernatant form of the enzyme has appreciable activity in plaice white muscle. The zymogram pattern is similar to that described for supernatant MDH in several other teleosts (Clayton *et al.*, 1971; Wheat *et al.*, 1971; Wheat *et al.*, 1972). In these species two loci code for supernatant MDH subunits in muscle and zymograms show three bands for the enzyme in homozygous individuals, two homodimers and a heterodimer. Variation only occurred in the most anodal form of supernatant MDH in plaice. The two loci have been designated B and C by Clayton *et al.* (1971) and A and B by Wheat *et al.* (1971, 1972). This paper will refer to them as A and B.

Technical problems with this system were not solved until the 1975 season. Four crosses of the type 2-3 × 2-3, six of the type 2-3 × 2-2 and one of the type 2-2 × 2-3 were performed. The results, in table 7, are in accordance with expectations.

(e) *Linkage*

Many of the crosses which were performed involved heterozygotes at more than one locus. There was no evidence of linkage between any of the pairs of loci listed in table 8. This table shows the total offspring in

TABLE 8

Complementary segregation class frequencies from crosses between male double heterozygotes at one or both loci

Loci		No. of offspring in complementary segregation classes	
PGM	GPI-B	343	342
PGM	G3PDH	53	45
PGM	GPI-A	77	89
GPI-B	G3PDH	41	47
GPI-A	GPI-B	85	81

the two complementary segregation classes which arise when one parent is heterozygous at more than one locus and produces four types of gamete, e.g. parent type 2-3, 2-3 produces 2·2 + 3·3 gametes and 2·3 + 3·2 gametes, respectively. There were no significant deviations from an expected 1:1 ratio. No tests have yet been made of possible linkage between the MDH locus and the others, nor between GPI-A and G3PDH.

(f) *Individual anomalous FI phenotypes*

Mention has already been made of the appearance of unexpected phenotypes in five independent instances, four at the PGM locus, one at the GPI-A locus. Another instance occurred at the PGM locus, where a 2-4 phenotype was found in a 3-4 × 4-4 triploid cross.

The single nature of these anomalies in broods of 216, 39, 126, 62, 52 and 47 offspring respectively is unlikely to arise by contamination and

indeed can be eliminated in the first case due to the absence of GPI-A allele 4 in the 1974 stock of potential parents. The simplest interpretation is that they represent mutations.

The total numbers of gametes in which mutation could have been scored for individual alleles at the PGM and GPI-A loci are shown in table 9.

TABLE 9

Estimated numbers of gametes under test for possible mutations at the PGM and GPI-A loci and numbers of presumed mutations to each allele

	Allele				
	1	2	3	4	5
PGM					
Estimated No. of gametes under test	6712	6656	1283	1019·5	7096
No. of presumed mutations	—	2	2	1	—
GPI-A					
Estimated No. of gametes under test	4683	—	5374	5464	—
No. of presumed mutations	—	—	—	1	—

The mutation rates expressed as the number of mutations divided by the mean number of tested gametes were $1{\cdot}1 \times 10^{-3}$ mutations per gamete for PGM and $2{\cdot}6 \times 10^{-4}$ mutations per gamete for GPI-A.

(ii) *Gynogenesis*

The genotypes of gynogenetic offspring from females heterozygous at three of the enzyme loci are shown in table 10 together with the percentage recombination expressed as the frequency of heterozygous offspring. In

TABLE 10

The genotypes of gynogenetic offspring from individual Po females heterozygous at the loci indicated

	Po genotype	Offspring genotypes					% recombination	
	PGM MDH-A	2-2	3-3	4-4	2-3	3-4	on both	on one
Locus	GPI-B	4-4	6-6	7-7	4-6	6-7	homozygotes	homozygote
PGM								
	3-4	—	6	—	—	82	93·2	—
		—	—	1	—	7	87·5	—
		—	—	—	—	8	100·0	—
		—	5	12	—	45	72·6	65·2
		—	6	10	—	43	72·9	68·3
		—	13	19	—	139	81·3	78·5
Totals		—	30	42	—	324	81·8	79·4
GPI-B								
	6-7	—	41	42	—	5	5·7	5·6
	4-6	3	4	—	1	—	12·5	—
		68	129	—	59	—	23·0	18·6
Totals		71	174	42	65		18·5	15·7
MDH-A								
	2-3	19	29	—	32	—	40·0	35·6
		51	95	—	92	—	38·7	32·6
Totals		70	124	—	124	—	39·0	33·3

conventional recombination analysis, with pairs of linked loci, one cross-over event produces only a single recombined chromosome whereas after diploid gynogenesis, one cross-over between the centromere and a locus produces one recombined chromosome pair in the zygote. In conventional terms, therefore, the observed recombination frequency in gynogenesis is an estimate of twice the map length between the locus and the centromere. The data in table 10 therefore suggest cross-over values of about 41 per cent for PGM, 19 per cent for MDH-A and 9 per cent for GPI-B, all in relation to the centromere which is terminal or sub-terminal in plaice chromosomes.

The recombination values from different females were consistent at the MDH locus but ranged from 72·6 per cent to 100 per cent at the PGM locus and from 5·7 per cent to 23·0 per cent at the GPI-B locus.

The genotypic arrays in the four larger broods for PGM are significantly different ($\chi^2 = 14{\cdot}04$; $P = 0{\cdot}007$) and the difference is not only in the frequencies of the heterozygous class but also in the relative frequencies of the two homozygotes. Similar genetic imbalance is shown by the two large broods for GPI-B ($\chi^2 = 13{\cdot}04$; $P = 0{\cdot}001$) again with a significant difference between the frequencies of the two homozygotes in the larger of the two broods. Although the recombination values at the MDH-A locus were consistent, the second brood tested here also showed a significant difference between the frequencies of the two homozygotes. Normal expectation after gynogenesis would be equal frequencies of the two homozygotes, but this is clearly not the case in these instances. This phenomenon could arise through linkage of lethal or deleterious genes with one or other allele in the heterozygous P_0 female. It might therefore be better to estimate recombination values on the basis of the most common homozygote where there is evidence of disparity between the frequencies of the two homozygotes. Such estimates are included in the final column of table 10. This, however, does not entirely remove the anomaly for GPI-B where the frequencies of the heterozygotes relative to the most common homozygote still show significant differences between the two large groups of offspring ($\chi^2 = 13{\cdot}07$; $P = 0{\cdot}001$). A more comprehensive explanation would require the postulation of differing viabilities of the homozygotes within broods and between broods but this is difficult to quantify without backcross or F_2 data. The observed recombination in gynogenesis can therefore only give rough estimates of map size. These are all fairly large for the loci studied and multiple crossing-over must also be considered. This will be dealt with in the discussion.

(iii) *Induced triploidy*

Many triploid crosses were set up but the majority failed for technical reasons possibly associated with the difficulty of inducing viable triploids in eggs of poor quality. Two successful crosses involving females heterozygous at the MDH locus were also invalid because of the earlier difficulty in scoring MDH in small fish.

Six progeny were scored from a triploid cross with parents of the G3PDH and PGM genotypes 2-3:4-4 × 2-2:3-4. For PGM, there were four 4 and two 3-4 phenotypes with low staining for the 3 isozyme in both 3-4 fish. This is consistent with the presumed genotypes 4-4-4 and 3-4-4, but differential staining was not always observed in other triploid material.

At the G3PDH locus the segregation was two 2, four 2-3 with presumed genotypes 2-2-2 and 2-2-3 or 2-3-3. A preponderance of 2-3 phenotypes would be consistent with a high cross-over frequency between the locus and the centromere, although the data are too few for quantitative assessment. At the PGM locus, a triploid cross of genotypes 3-4 × 1-4 produced the following segregation: 4 4-4; 4 1-3; 2 1-4; 7 3-4; 8 1-3-4.

The 1-3-4 phenotype was clearly of triploid origin, but differential staining in the other heterozygotes could not be scored. The two cross-over classes are 3-3-3 and 1-3-4 and the former could not be distinguished from 3-4. Amongst zygotes with allele 1, the proportion of recombinants was 57 per cent, which is lower than that observed in gynogenesis (80 per cent) but the data are too sparse amongst the triploids for meaningful comparison.

4. Discussion

Polymorphism was observed for five enzymes in sexually mature plaice (*Pleuronectes platessa*) collected from various grounds in the North Sea. The results of crosses between parents of known phenotypes demonstrated conclusively that the polymorphisms were determined by the inheritance of co-dominant alleles at independent loci for each enzyme system.

Amongst the parents, allele frequencies, excluding the commonest allele at each locus, ranged from 0·007 to 0·376 with all but two having low frequencies of 0·05 or less. All the alleles found have also been found in plaice from the Western English Channel and the Irish Sea. There was no evidence of heterogeneity in a comparison of different spawning grounds on the basis of the frequency of the commonest allele at each locus, and on this basis no evidence of genetic isolation of the individual spawning grounds. Samples were small, however, and came predominantly from grounds on the southern edge of the Dogger Bank. Only a few parents were obtained from East Coast or Southern Bight grounds which might conceivably represent populations isolated from the Dogger Bank populations (Simpson, 1959). This possibility is being examined by typing juveniles reared from eggs collected from the different grounds.

The great majority of crosses produced segregation ratios which were consistent with normal Mendelian inheritance in which the different genotypes were of equal fitness. One possible exception to this general rule occurred in the GPI-A system, where there was a significant deficiency of heterozygotes throughout one year and some evidence that this deficiency was related to poor survival conditions. Low survival of heterozygotes for transferrin alleles in tuna has been recorded by Fujino and Kang (1968) and for esterase polymorphism in *Zoarces* by Christiansen, Frydenberg and Simonsen (1973). Each of these instances occurred under natural conditions, over a longer period of the life-cycle than in the present case and may be alternatively explained by differential migration. There was no lack of GPI-A heterozygotes among parental plaice, but the frequency of allele 1 was low (0·04) in relation to the sample size (200) and comparison with results from the hatchery work was inconclusive.

Other anomalies of segregation ratios occurred at the PGM locus, where a significant deficiency of the allele 1 arose in a cross 3-4 × 1-4, and an excess of 3-4 and 3-3 genotypes respectively in four separate crosses 3-4 × 3-4. Such anomalies may occur by chance in a large number of crosses. The

possibility of linked deleterious genes remains but is untestable without recourse to further test generations or to further testing of parents. The latter was not practical since parents were not retained after spawning due to lack of holding facilities.

Single anomalous individuals were observed in six independent crosses and five of these involved the PGM locus. In no case was there any evidence for contamination and the simplest interpretation was that the anomalies arose through mutation. On this basis the estimated mutation rates of $1{\cdot}1 \times 10^{-3}$ mutations per gamete at the PGM locus and $2{\cdot}6 \times 10^{-4}$ mutations per gamete at the GPI-A locus were extremely high compared with normal spontaneous rates of 10^{-5} or less. No mutations were observed at the other three loci studied and estimated values of less than $2{\cdot}9 \times 10^{-4}$, $9{\cdot}0 \times 10^{-4}$ and $1{\cdot}4 \times 10^{-3}$ mutations per gamete for the GPI-B, G3PDH and MDH-A loci respectively were obtained. High mutation rates have been described at the blood group B-system locus of cattle, 2×10^{-3} (Stormont, 1969), in the lactate dehydrogenase B subunit in brook trout, 2×10^{-2} (Wright and Atherton, 1968), and at the 6-phosphogluconate dehydrogenase locus in Japanese Quail, 1×10^{-2} (Ohno *et al.*, 1969). The latter observed mutations from known alleles to previously undescribed variants with slight differences in electrophoretic mobility as well as mutations from one known allele to another as described here. No new variants were observed in any of the experiments in this study.

At the PGM locus three mutations were from a heterozygous parent, one from a homozygote and one from a heterozygote × homozygote triploid cross in which either parent could have been responsible. The GPI-A mutant was from a homozygous cross. The possibility exists that high mutation rates may arise from intra-cistronic recombination. To date too few crosses at this locus have been tested to draw significant conclusions and further studies are intended.

Linkage was not observed between any two loci, but MDH was not included in the tests nor tests between GPI-A and G3PDH. With a haploid complement of 24 small telocentric chromosomes, the probability of linkage is low amongst four random loci. However, two of the loci were concerned with one enzyme (GPI) and had they arisen by tandem duplication—there is no evidence that pleuronectids are ancient tetraploids—they might have been expected to show close linkage.

The extent of linkage between loci and centromeres was estimated by the production of diploid gynogenetic offspring from heterozygous females. Since diploidy arises by the suppression of the second meiotic metaphase (Purdom, 1969) offspring will be homozygous except where recombination occurred between the centromere and the locus under consideration. One cross-over will produce a heterozygous pair of chromosomes in the egg. Two cross-overs involving only one pair of chromatids will not produce recombination, but where the involvement of chromatids is random, the situation is more complex. The addition of a cross-over to a situation otherwise leading to homozygosity will produce a heterozygote. Of the four possibilities arising when the extra cross-over is applied to a heterozygous situation, two lead to homozygosity and two to heterozygosity. The probability of heterozygosity with x cross-overs is therefore

$$P_{(x)} = 1 - \tfrac{1}{2}P_{(x-1)}$$

and this will tend towards 0·67. For two of the loci, MDH-A and GPI-B, the frequency of recombination is well below this limit, but for PGM it is significantly higher (0·82, s.e. 0·02).

A high level of observed recombination could arise through loss of homozygotes due to lethal or deleterious genes, but at the PGM locus there was no evidence of disparity between the frequencies of the two homozygous classes.

Cross-over interference could also explain the high level of recombination for PGM and several models can be envisaged.

To arrive at minimum estimates of chromosome map length, the simplest assumption is that interference is absolute and only one cross-over permitted per chromosome. On this basis, the three loci studied are approximately 41 units (PGM), 19 units (MDH-A) and 9 units (GPI-B) from the centromere on each of the chromosomes on which they are located. The first two represent high cross-over values for fish. Older studies with aquarium fish (Winge, 1927; Gordon, 1937) report cross-over values up to 10 units, but usually very much less. These data, however, refer to sex-linked loci controlling secondary sexual characters and may not be representative of autosomal situations. Morrison and Wright (1966) report linkage between two lactate dehydrogenase loci in *Salvelinus* hybrids with recombination of about 15 per cent. Similarly, in segregation analyses using hybrids of *Lepomis* in which the parents showed differing mobilities for homologous enzymes, Wheat *et al.* (1973) reported linkage between G3PDH and 6-phosphogluconate dehydrogenase with 15-22 per cent recombination. A high recombination frequency is also suggested between the centromere and the G3PDH locus from a triploid cross in the present series in which four of the six offspring were recombinants. Assuming that the PGM locus is terminal on one of the large chromosomes the total map length of the 24 telocentric chromosomes in plaice (Barker, 1972) in which the largest is about twice the size of the smallest, can be estimated to be about 720 units. Considering that the Pleuronectidae, like most teleosts, have a genome only 20 per cent the size of the mammalian genome (Ohno and Atkins, 1966), this represents a very high total map length and may indicate a very high level of euchromatin.

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