Heredity **55** (1985) 105–110

Received 22 January 1985

A search for gametic disequilibrium in the plaice, *Pleuronectes platessa*

R. D. Ward* and B. J. McAndrew†

Department of Genetics, University College of Swansea, Swansea, SA2 8PP

Large samples of a natural population of the plaice were examined for evidence of gametic disequilibrium (*D*) at five and seven polymorphic enzyme loci (younger and older fish respectively). A single pairwise locus comprison gave a statistically significant value for *D*: *αGpdh*-1 and *Pgi*-2 in young fish. The mean *D* values of the 10 pairwise comparisons of the five loci in young and old fish were low, 0.00175 and 0.00420 respectively: corresponding values of *R* (the correlation of gene frequencies) were 0.01473 and 0.03002. The increase in these parameters in older fish might be due either to population admixture, natural selection, a smaller sample size, or to a combination of these factors. The mean *D* and *R* values for the 21 pairwise comparisons of the seven loci typed in older fish were 0·00394 and 0·02496 respectively. Two loci catalysing adjacent steps in the glycolytic pathway, *Pgm*-1 and *Pgi*-2, showed no evidence of epistatic interactions generating disequilibrium.

INTRODUCTION

Since it became apparent in the late sixties that natural populations of most plants and animals contain appreciable levels of genetically determined enzyme variation (as assessed by gel electrophoresis), a number of studies have been published which have looked for linkage disequilibrium between pairs of enzyme loci. Certain models of selection predict the existence of linkage disequilibrium, but the existence of linkage disequilibrium does not, in itself, predicate selection; genetic drift, migration and mutation can all, in principle, generate such an effect. Loci showing such disequilibria need not, in fact, be linked, and thus in this paper we have preferred to use the term "gametic disequilibrium" (Hedrick *et al.*, 1978).

Most of the searches for gametic disequilibrium have been carried out on Drosophila species (see, for example, Langley, 1977; Charlesworth *et al.*, 1979; Mukai *et al.*, 1974). These studies have revealed little evidence for gametic disequilibrium between pairs of enzyme loci, even between closely linked loci, but frequently significant disequilibrium is found between enzyme loci and closely linked polymorphic inversions. Few studies of gametic disequilibrium in vertebrates have been undertaken. In man, one large multilocus study of a Michigan population found gametic disequilibrium between two pairs of unlinked blood group loci, which the authors felt may have arisen, at least in part, from epistatic interactions (Sinnock and Sing, 1972), while a second study, of Yanomama Indians from South America, found no disequlibria which could not be explained by available information on population structure (Smouse and Neel, 1977). A survey of 12 polymorphic enzyme loci in the killifish found a single significant case of disequilibrium, between two esterase loci (Mitton and Koehn, 1975).

We report here the results of a search for gametic disequilibrium at seven polymorphic loci in large samples of plaice taken from a single panmictic population. Little evidence for gametic disequilibrium was found.

MATERIALS AND METHODS

The Bristol Channel population of plaice was studied. This is thought to comprise a panmictic and relatively isolated population, with rather little immigration from Irish Sea or English Channel stocks (Simpson, 1959; Macer, 1972). Older fish

Present addresses: * Department of Human Sciences, Environmental Biology Unit, Loughborough University, Loughborough, Leicestershire, LE11 3TU, U.K.
† Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, U.K.

(those more than one-year-old, hereafter referred to as adult fish) were collected from 1973 to 1977 by trawling, and in the present analysis are pooled into a single group. Fish less than one-year-old, the "O" group plaice, were collected by pushnetting in shallow water on the South Wales beaches of Swansea, Oxwich and Pendine in 1973, 1974 and 1975, and at Pendine only in 1977.

Five polymorphic loci (*Pgm*-1, *Ada*, *αGpdh*-1, *Mdh*-2 and *Pgi*-2) were assayed from muscle tissue of virtually all fish and a further two loci (*αGpdh*-2 and *Idh*-2) from liver tissue of most of the adult fish. Details of the electrophoretic procedures followed and information on allele frequencies are given in Ward and Beardmore (1977).

Contingency tables of dilocus genotypes were drawn up and analysed by the chi-square test developed by Nass (1959), which is robust to the small expectations caused by the presence of rare alleles.

More than two alleles were present at each of the seven loci, and for the purposes of calculating the coefficient of gametic disequilibrium, D (Kimura, 1956), the rarer alleles at each of the loci were pooled. Values of D were estimated for all possible dilocus comparisons using the maximum likelihood procedure for estimating gametic frequencies given by Bennett (1965) and Hill (1974), and their significances assessed by the chi-square test. Values of R (the correlation of gene frequencies, Hill and Robertson, 1968) are also presented.

RESULTS

A total of 41 dilocus genotype contingency tables were drawn up, made up of 10 tables for each of the two large "O" group samples (1975, samples from the three sites pooled, and 1977), sampled for five loci, and 21 tables for the adult fish, sampled for seven loci. In these tables no alleles or genotypes were pooled. Only two of these tables gave significant departures from expectations after employing the χ^2 test of Nass (1959): *Pgm*-1 and *Mdh*-2 in "O" group fish ($\chi^2 = 6{\cdot}44$, $v = 1{\cdot}90$, $0{\cdot}05 > P > 0{\cdot}025$), and *αGpdh*-2 and *Ada* in adults ($\chi^2 = 14{\cdot}12$, $v = 2{\cdot}56$, $P = 0{\cdot}001$). The *Pgm*-1/*Mdh*-2 association may well be a sampling artefact, particularly since this pair of loci show no such association in the 1975 "O" group fish nor in the adult fish. The *αGpdh*-2/*Ada* association is caused by the presence of a single fish having a very rare genotype at each of the two loci; if this fish is left out of the analysis, no significant association remains ($\chi^2 = 1{\cdot}24$, $v = 2{\cdot}89$, $P > 0{\cdot}5$). Thus these analyses gave no evidence for non-random association of genotypes generated by genetic interactions.

Following pooling of alleles at each locus into two classes (one comprising only the most frequent allele, the other all remaining alleles), a total of 17 single locus genotype distributions, made up of five loci each for the 1975 and 1977 "O" group fish, and seven loci for the adult fish, were tested for goodness-of-fit to Hardy–Weinberg expectations. Two distributions differed significantly from expected: *Pgm*-1 in 1977 "O" group fish ($\chi^2 = 8{\cdot}84$, $P = 0{\cdot}003$) and *αGpdh*-2 in adults ($\chi^2 = 4{\cdot}79$, $P = 0{\cdot}029$). At the *Pgm*-1 locus there was a heterozygote deficiency, but no significant deviations were observed at this locus in 1975 "O" group fish nor in adult fish. The *αGpdh*-2 deviation, also caused by a heterozygote deficiency, is less statistically significant; this liver-specific locus was not typed in the small, "O" group fish. It is possible that both of these deviations were sampling artefacts.

Table 1 gives gametic frequencies (following pooling of alleles into two classes) and values of D and R for pairwise comparisons of the five loci scored in all three groups of fish. A single value of D out of the 30 calculated is significant, that between *Pgi*-2 and *αGpdh*-1 in 1975 "O" group fish ($P = 0{\cdot}035$). Interestingly, this comparison is nearly significant in the 1977 "O" group fish ($P = 0{\cdot}067$) although non-significant (and of a different sign) in adult fish. These two significant, or nearly significant, values of D are also associated with the two largest values of R recorded for the "O" group fish. It should be noted that Hill's (1974) method for estimating dilocus gametic frequencies assumes that both loci are in Hardy–Weinberg equilibrium. This condition is clearly fulfilled for most of the samples, and the two loci which do show small, but, given the large sample sizes, significant departures from Hardy–Weinberg equilibrium, are not involved in the generation of the significant D values.

Table 2 summarises data from, firstly, pooled collections of "O" group fish, adding the comparatively small samples collected in 1973 and 1974 to the 1975 and 1977 samples, and, secondly, pooled collections of all fish ("O" group and adult fish combined). Again, the only significant values of D are for *Pgi*-2 and *αGpdh*-1.

The mean D value of the "O" group fish, calculated from table 2 as the absolute sum of D divided by the number of dilocus comparisons (10), is 0·00175; the corresponding figure for the adult fish is 0·00420. $\bar{R}$ increases in a similar

Table 1 Gametic frequencies, values of disequilibrium (D) and correlations of gene frequencies (R) for *Pgm*-1, *Ada*, *Mdh*-2, *Pgi*-2 and *αGpdh*-1

Locus pair	Fish group	Gametic frequencies 11	12	21	22	N	χ^2_1	D	R
Pgm-1	1975 "O"	0·445	0·170	0·281	0·103	1629	0·109	−0·0018	−0·0082
Ada	1977 "O"	0·444	0·155	0·294	0·107	1914	0·166	0·0020	0·0093
	adults	0·456	0·148	0·285	0·111	870	1·282	0·0082	0·0384
Pgm-1	1975 "O"	0·534	0·082	0·339	0·045	1631	0·736	−0·0034	−0·0212
Mdh-2	1977 "O"	0·532	0·067	0·348	0·053	1914	1·857	0·0049	0·0311
	adults	0·523	0·079	0·346	0·052	924	0·004	−0·0003	−0·0021
Pgm-1	1975 "O"	0·576	0·040	0·363	0·021	1631	0·762	−0·0025	−0·0216
Pgi-2	1977 "O"	0·557	0·042	0·377	0·024	1914	0·691	−0·0023	−0·0190
	adults	0·566	0·036	0·382	0·016	918	1·810	−0·0048	−0·0444
Pgm-1	1975 "O"	0·539	0·076	0·338	0·046	1631	0·042	−0·0008	−0·0050
αGpdh-1	1977 "O'	0·515	0·084	0·347	0·054	1914	0·161	−0·0016	−0·0092
	adults	0·512	0·090	0·352	0·046	924	2·117	−0·0080	−0·0479
Ada	1975 "O"	0·632	0·094	0·241	0·033	1629	0·328	−0·0021	−0·0142
Mdh-2	1977 "O"	0·654	0·083	0·226	0·036	1914	2·384	0·0050	0·0353
	adults	0·639	0·102	0·228	0·031	870	0·537	−0·0037	−0·0249
Ada	1975 "O"	0·684	0·042	0·255	0·019	1629	0·638	0·0021	0·0198
Pgi-2	1977 "O"	0·688	0·049	0·245	0·018	1914	<0·001	<0·0001	0·0003
	adults	0·699	0·041	0·248	0·011	870	0·688	−0·0027	−0·0281
Ada	1975 "O"	0·640	0·087	0·238	0·035	1629	0·283	0·0019	0·0132
αGpdh-1	1977 "O"	0·633	0·105	0·229	0·033	1914	0·806	−0·0031	−0·0205
	adults	0·629	0·111	0·231	0·028	870	2·609	−0·0083	−0·0548
Mdh-2	1975 "O"	0·821	0·052	0·118	0·009	1631	0·276	0·0010	0·0130
Pgi-2	1977 "O"	0·823	0·057	0·110	0·009	1914	0·533	0·0013	0·0167
	adults	0·826	0·044	0·123	0·008	918	0·251	0·0012	0·0165
Mdh-2	1975 "O"	0·767	0·106	0·111	0·016	1631	0·077	0·0007	0·0069
αGpdh-1	1977 "O"	0·759	0·122	0·103	0·016	1914	0·036	−0·0005	−0·0043
	adults	0·756	0·114	0·108	0·022	924	1·390	0·0045	0·0388
Pgi-2	1975 "O"	0·828	0·111	0·049	0·012	1631	4·462*	0·0041	0·0523
αGpdh-1	1977 "O"	0·808	0·125	0·054	0·013	1914	3·360	0·0036	0·0419
	adults	0·819	0·129	0·045	0·007	918	0·017	−0·0003	−0·0043

* $P = 0·035$.

Loci: *Pgm*-1, phosphoglucomutase-1; *Ada*, adenosine deaminase; *Mdh*-2, malate dehydrogenase-2; *Pgi*-2, phosphoglucose isomerase-2; *αGpdh*-1, α-glycerophosphate dehydrogenase-1.

Gamete frequency notation: gamete 11 has allele 1 at both loci, gamete 12 has allele 1 at the first locus, allele 2 at the second locus, and so on.

N is the number of fish screened for that locus pair.

fashion, from 0·01473 in "O" group fish to 0·03002 in adult fish.

Adult fish were screened for two more loci than the "O" group fish, and table 3 presents the results of the 11 additional dilocus comparisons. None of these D values is significant. Values of $\bar{D}$ and $\bar{R}$ over the 21 dilocus comparisons in adult fish are 0·00394 and 0·02496 respectively.

DISCUSSION

Gametic or linkage disequilibrium can be generated by natural selection, genetic drift, migration or (at least in principle) mutation. It may also arise from small founding populations or from bottlenecks generating disequilibria which, for closely linked loci, decay slowly. Furthermore, Thompson (1977) has demonstrated that significant disequilibrium can be generated between two unselected or neutral loci through the evolution of a linked selected locus, an example of genetic "hitchhiking". In attempting to detect disequilibria which can be ascribed to selective forces, it is necessary to sample large panmictic populations which have not gone through recent bottlenecks and into which there is, ideally, zero immigration. Even then, disequilibrium between very closely linked loci may

Table 2 Values of D (above diagonal) and R (below diagonal) in pooled groups* of fish

		Pgm-1	*Ada*	*Mdh*-2	*Pgi*-2	*αGpdh*-1
Pgm-1	O group		−0·0004	0·0027	−0·0029	−0·0020
	All fish		0·0010	0·0021	−0·0033	−0·0030
Ada	O group	−0·0020		0·9926	0·0010	−0·0009
	All fish	0·0047		0·0014	0·0004	0·0023
Mdh-2	O group	0·0168	0·0178		0·0007	−0·0008
	All fish	0·0132	0·0099		0·0008	0·0002
Pgi-2	O group	−0·0246	0·0092	0·0093		0·0035†
	All fish	−0·0278	0·0040	0·0101		0·0027‡
αGpdh-1	O group	−0·0122	−0·0061	−0·0070	0·0432	
	All fish	−0·0180	−0·0154	0·0015	0·0334	

* O group: pooled collections from 1973, 1974, 1975, 1977, $\bar{N} = 4201{\cdot}8$, range 4198–4205. All fish: all O group and adult fish: $\bar{N} = 5102{\cdot}4$, range 5068–5127.
† $P = 0{\cdot}005$, $\chi^2_1 = 7{\cdot}84$.
‡ $P = 0{\cdot}017$, $\chi^2_1 = 5{\cdot}70$.

Table 3 Gametic frequencies, values of disequilibrium (D) and correlations of gene frequencies (R) for comparisons involving two further loci studied only in adult fish

	Gametic frequencies							
Locus pair	11	12	21	22	N	χ^2_1	D	R
αGpdh-2 : *Idh*-1	0·320	0·292	0·210	0·179	671	0·161	−0·0038	−0·0155
Pgm-1 : *Idh*-1	0·311	0·287	0·226	0·176	770	1·276	−0·0100	−0·0407
Ada : *Idh*-1	0·389	0·355	0·142	0·114	716	0·576	−0·0062	−0·0284
Mdh-2 : *Idh*-1	0·464	0·407	0·073	0·056	770	0·335	−0·0035	−0·0209
Pgi-2 : *Idh*-1	0·507	0·442	0·030	0·021	764	0·400	−0·0025	−0·0229
αGpdh-1 : *Idh*-1	0·471	0·398	0·066	0·065	770	0·612	0·0047	0·0282
Pgm-1 : *αGpdh*-2	0·367	0·232	0·244	0·158	671	0·023	0·0014	0·0058
Ada : *αGpdh*-2	0·457	0·291	0·154	0·098	671	<0·001	<0·0001	0·0002
Mdh-2 : *αGpdh*-2	0·536	0·335	0·075	0·054	671	0·398	0·0040	0·0243
Pgi-2 : *αGpdh*-2	0·582	0·366	0·029	0·023	671	0·436	0·0028	0·0255
αGpdh-1 : *αGpdh*-2	0·529	0·340	0·083	0·049	671	0·090	−0·0019	−0·0116

The two additional loci are *αGpdh*-2, α-glycerophosphate dehydrogenase-2, and *Idh*-1, isocitrate dehydrogenase-1.

simply reflect ancient reductions in population size or linkage with a locus under selection. The plaice population of the Bristol Channel is large and ancient, and thus seems a suitable candidate for the examination of gametic disequilibrium, although it should be noted that there is limited immigration from other Irish Sea populations (Macer, 1972).

Seven polymorphic loci were screened, and of the 21 possible dilocus combinations, only the *Pgi*-2 and *αGpdh*-1 loci showed gametic disequilibrium. This disequilibrium is evident in "O" group fish (values of D and R being consistent between collections made in 1975 and 1977) but disappears in adult fish. The consistency found between "O" group fish of different years suggests it is a real phenomenon and not a sampling artefact, but it is then perhaps surprising that it is not present in older fish. The D value found in pooled collections of "O" group plaice, 0·0035, is too large to be explained simply by a mixing of parental stocks, each in equilibrium but having different gene frequencies at the two loci. Neither of these two loci is highly polymorphic, frequencies of the two loci. Neither of these two loci is highly polymorphic, frequencies of the most common alleles at *Pgi*-2 and *αGpdh*-1 in the Bristol Channel population being around 0·943 and 0·872 respectively, with corresponding values of 0·927 and 0·867 in a north-east Irish Sea population (Ward and Beardmore, 1977). Using the formula of Cavalli-Sforza and Bodmer (1971), this small differentiation in gene frequencies would, by itself, generate a maximum D value (when the two stocks contribute equally to the gametic pool) of only 0·00002. If the disequilibrium in young fish was

established by selection, one must suppose that selective forces in older fish are different and happen to restore equilibrium. The two enzymes are not functionally very closely related to each other, although the substrate of *αGPDH*, dihydroxyacetone phosphate, is formed in the glycolytic pathway two steps away from the reaction catalysed by *PGI*. No information is available on the linkage relationships of these two loci. Thus this observation of disequilibrium remains tantalising and it is difficult to assess its real significance.

Linkage data are only available for three of the locus pairs screened here; viz. *Pgm*-1/*Mdh*-2, *Pgm*-1/*αGpdh*-1, and *Pgm*-1/*Pgi*-2, and none of these pairs shows evidence of linkage (Purdom *et al.*, 1976; Ward and Beardmore, 1977). Note, however, that epistatic interactions can, in principle, generate gametic disequilibrium between unlinked loci, although such interactions have to be markedly stronger than for closely linked loci.

It is interesting that values of $\bar{D}$ and $\bar{R}$ increase two to three fold in the adult fish. Disequibria formed as a result of epistatic interactions are expected to be more pronounced in older fish, which have survived increased selective culling (although of course the *Pgi*-2/*αGpdh*-1 association in young fish conflicts with this view), and thus the observation of increased $\bar{D}$ in older fish might reflect the action of selection. However, it should be pointed out that sample sizes for the adult fish are substantially smaller than for the "O" group fish, and thus part of this increase might be attributable to sampling error. A small part of this increase might also be attributable to population admixture of adult fish (which are far more active and mobile than young fish), although, as indicated above, genetic differentiation of Irish Sea stocks appears to be very limited.

The general scarcity of examples of gametic disequilibrium is sometimes viewed as a finding contrary to the expectations of selective theories for the maintenance of enzyme polymorphisms, although it may simply be a consequence of looking at insufficiently closely linked loci. Furthermore, Brown (1975) showed that the number of samples required to reject the null hypothesis $D = 0$ is quite large, expecially when allele frequencies are far from equality. This is not a problem with our data set where sample sizes are large. Zouros and Johnson (1976) suggest, no doubt correctly, that disequilibria are most likely to be found between loci which are functionally related. Two such loci in our survey are *Pgi*-2 and *Pgm*-1 which catalyse neighbouring steps in the glycolytic pathway; they show no evidence of epistatic interactions. However, since *Pgi*-2 is not a highly polymorphic locus in the plaice, such interactions may not be detectable even in the large samples employed here.

The results of this study support the general conclusion of other workers that gametic disequilibrium between enzyme loci is a rare phenomenon. With the possible exception of *αGpdh*-1 and *Pgi*-2 in "O" group fish, we find no evidence for such disequilibria in the plaice.

Acknowledgment. This work was supported by NERC grant GR3/1558 and by the award of a NERC studentship to B. J. McAndrew. We thank Professor J. A. Beardmore for his advice and encouragement.

REFERENCES

BENNETT, J. H. 1965. Estimation of the frequencies of linked gene pairs in random mating populations. *Amer. J. Hum. Genet.*, *17*, 51–53.

BROWN, A. H. D. 1975. Sample sizes required to detect linkage disequilibrium between two or three loci. *Theor. Pop. Biol.*, *8*, 184–201.

CAVALLI-SFORZA, L. L., AND BODMER, W. F. 1971. *The Genetics of Human Populations.* Freeman, San Francisco.

CHARLESWORTH, B., CHARLESWORTH, D., AND LOUKAS, M. 1979. A study of linkage disequilibrium in British populations of *Drosophila subobscura*. *Genetics*, *92*, 983–994.

HEDRICK, P., JAIN, S., AND HOLDEN, L. 1978. Multilocus systems in evolution. *Evol. Biol.*, *11*, 101–184.

HILL, W. G. 1974. Estimation of linkage disequilibrium in randomly mating populations. *Heredity*, *33*, 229–239.

HILL, W. G. AND ROBERTSON, A. 1968. Linkage disequilibrium in finite populations. *Theor. Appl. Genet.*, *38*, 226–231.

KIMURA, M. 1956. A model of a genetic system which leads to closer linkage by natural selection. *Evolution*, *10*, 278–287.

LANGLEY, C. H. 1977. Nonrandom associations between allozymes in natural populations of *Drosophila melanogaster*. In Christiansen, F. B. and Fenchel, T. M. (eds.) *Measuring Selection in Natural Populations.* Springer-Verlag, Berlin, pp. 265–273.

MACER, C. T. 1972. The movements of tagged adult plaice in the Irish Sea. *Fish. Investig., London, Ser.* 2, 27(6), 41pp.

MITTON, J. B. AND KOEHN, R. K. 1975. Genetic organization and adaptive response of allozymes to ecological variables in *Fundulus heteroclitus*. *Genetics*, *79*, 97–111.

MUKAI, T., WATANABE, T. K. AND YAMAGUCHI, O. 1974. The genetic structure of natural populations of *Drosophila melanogaster*. XII. Linkage disequilibrium in a large local population. *Genetics*, *77*, 771–793.

NASS, C. A. G. 1959. The χ^2 test for small expectations in contingency tables, with special references to accidents and absenteeism. *Biometrika*, *46*, 365–385.

PURDOM, C. E., THOMPSON, D. AND DANDO, P. R. 1976. Genetic analysis of enzyme polymorphisms in the plaice (*Pleuronectes platessa*). *Heredity*, *37*, 193–206.

SIMPSON, A. C. 1959. The spawning of the plaice (*Pleuronectes platessa*) in the Irish Sea. *Fish. Investig., London, Ser.* 2, 22 (8), 30pp.

SINNOCK, P. AND SING, C. F. 1972. Analysis of multilocus genetic systems in Tecumseh, Michigan. II. Consideration of the correlation between nonalleles in gametes. *Amer. J. Hum. Genet.*, *24*, 393–415.

SMOUSE, P. E. AND NEEL, J. V. Multivariate analysis of gametic disequilibrium in the Yanomama. *Genetics*, *85*, 733–752.

THOMPSON, G. 1977. The effect of a selected locus on linked neutral loci. *Genetics*, *85*, 753–788.

WARD, R. D. AND BEARDMORE, J. A. 1977. Protein variation in the plaice, *Pleuronectes platessa* L. *Genet. Res.*, *30*, 45–62.

ZOUROS, E. AND JOHNSON, W. 1976. Linkage disequilibrium between functionally related enzyme loci of *Drosophila mojavensis*. *Can. J. Genet. Cytol. 18*, 245–254.