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GENETIC ANALYSIS OF SPONTANEOUS GYNOGENETIC DIPLOIDS IN THE PLAICE *PLEURONECTES PLATESSA*

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1. Introduction

THE genetic analysis of enzyme polymorphisms in induced diploid gynogenesis in the plaice, *Pleuronectes platessa*, demonstrates that the duplication of chromosome sets is caused by the suppression of the formation of the second polar body at egg activation (Purdom *et al.*, 1976). Recombination between loci and centromeres in the first meiotic phase is shown by the appearance of heterozygotes in broods of diploid gynogenetic offspring; the frequency of these heterozygotes indicates the extent of such recombination and therefore the efficiency of induced diploid gynogenesis as a method for the rapid increase of inbreeding coefficients.

Diploidy can be induced by a variety of physical means but it may also occur spontaneously at very low levels following gynogenetic egg development. In some species, *e.g.*, the rainbow trout, *Salmo gairdneri*, such spontaneous diploids are all that can be obtained at present as satisfactory methods of induction have not yet been developed. It is therefore of value to determine whether or not the genetical consequences of spontaneous diploidy are similar to those of induced diploidy. In other words, do spontaneous diploids arise by suppression of meiosis II and have retention of the genetic material of the second polar body? This paper reports genetic analysis of spontaneous gynogenetic diploids in plaice and compares them with previous genetical data on induced gynogenesis in this species.

2. Materials and methods

The spermatozoa from 12 sexually mature male plaice were irradiated with Co^{60} gamma radiation. A dose of 100,000 rad had previously been found to be necessary to ensure total inactivation of the chromosomes (Purdom, 1969). If kept at 0°C such irradiated spermatozoa retain activity for up to 5 days.

The success of any gynogenetic experiment depends upon high egg quality and previous experience has shown that eggs obtained from freshly caught, sexually mature females are superior to those obtained from females kept in a hatchery. Consequently all fertilisations were carried out at sea aboard ship. To allow the maximum amount of time for these fertilisations to be carried out, the spermatozoa were not collected and irradiated until immediately before the research vessel sailed.

Within four days of sailing 12 fertilisations were made, each using eggs from a different female and each kept in 20 l of sea water in a plastic bin

lined with a polythene bag. The necks of the bags were sealed to prevent spillage and contamination. Every 24 h the water in the bins was changed and any dead eggs removed. Upon completion of the voyage the surviving eggs were incubated in the laboratory at 7°C, as described by Riley and Thacker (1969).

A sample of white skeletal muscle was removed from each female parent and deep frozen. The muscle samples were analysed by vertical starch gel electrophoresis for polymorphisms at the phosphoglucomutase (E.C. number 2.7.5.1) and glucose phosphate isomerase (E.C. number 5.3.1.9) loci using the technique of Dando (1970). Staining methods and isozyme numbering were as described by Purdom *et al.* (1976). Within two or three days of hatching fresh whole larvae were similarly analysed electrophoretically.

3. Results

In the skeletal muscle of plaice, phosphoglucomutase (PGM) is controlled by a single locus (Purdom *et al.*, 1976) whilst two forms of glucose phosphate isomerase (GPI-A and GPI-B) are found, each controlled by an independent locus (Dando, 1974). The analysis of the tissue samples from the female plaice showed that of the 12 fish, five were heterozygous at the PGM locus, two at the GPI-A locus and four at the GPI-B locus. Two fish were heterozygous at more than one locus and three fish were homozygous at all three loci. However, irrespective of parental genotype, all 12 batches of eggs were incubated. The number of eggs developing successfully was very low and the majority of the eggs died within three or four days of fertilisation, at the time of gastrulation. Of the remainder, many produced short, misshapen embryos which failed to hatch, a typical fate of haploid eggs. In three batches all the eggs developed in this way and none survived to hatch into normal larvae. The numbers in seven of the remaining batches were very low, eight or less, but two batches produced 21 and 52 larvae respectively. All the larvae hatched successfully were tested for all three enzymes. Details of the results from each heterozygous female are given in table 1. Also included in this table are results showing that homozygous females breed true (in a total progeny of 130).

If diploidy is restored in spontaneous gynogenetic diploids by the same method as in induced gynogenetic diploids, *i.e.*, the retention of the chromosomes destined for the polar body from the second meiotic division and their inclusion in the egg pronucleus, the expected segregation in broods of offspring from heterozygous females will consist of each type of homozygote, and heterozygotes of the parental type. The proportion of heterozygotes will depend on the extent of recombination between the locus and the centromere during meiosis I. These values are included in table 1. The two types of homozygote should be equally frequent but this is not always the case, although the numbers of offspring are low in individual broods. In only one brood, from female number 12, do the numbers of homozygotes differ significantly from the expected 1:1 ratio ($\chi^2 = 4{\cdot}00$, df = 1, $0{\cdot}05 > P > 0{\cdot}02$). This may be by chance or because of differences in viability *per se* or because of close linkage with a deleterious allele at another locus. Where differences occur between homozygote frequencies

TABLE 1

Distribution of genotypes at the PGM, GPI-A and GPI-B loci in gynogenetic diploid offspring from homozygous and heterozygous females

Parent genotype		Fish No.	Offspring genotype											Untyped	% recombination	
			1^a-1^a	2-2	3-3	4-4	5-5	6-6	7-7	1^a-2	3-4	5-6	6-7		On both homozygotes	On the most frequent homozygote
PGM 3-4		6			—	1					5			3	83·3	—
		9			4	3					13			1	65·0	61·9
		11			—	1					2				66·7	—
		12			4	12					36			2	69·2	60·0
	Totals				8	17					56				69·1	62·2
PGM 4-4		∑ 5 fish				34										
GPI-A 1^a-2		4	1	3						3					42·9	33·3
GPI-A 2-2		∑ 8 fish		114												
GPI-B 5-6		4					—	2				5			71·4	—
		5					4	4				—			NIL	NIL
		9					5	10				6			28·6	23·1
	Totals						9	16				11			30·6	25·6
GPI-B 6-7		2						4	—				1		20·0	—
Overall GPI-B	Totals						9	20				11	1		29·3	23·1
GPI-B 6-6		∑ 5 fish						82								

it may be more realistic to assess recombination levels on the basis of the more frequent homozygote; table 1 includes these values.

Contingency tests of homozygote and heterozygote distribution between offspring from each female showed homogeneity at the PGM locus ($\chi^2 = 0{\cdot}73$, df = 3, $0{\cdot}90 > P > 0{\cdot}80$) but heterogeneity at the GPI-B locus ($\chi^2 = 9{\cdot}52$, df = 3, $0{\cdot}05 > P > 0{\cdot}02$). The latter result was due to a significant excess of heterozygotes in the offspring of female number 4 ($\chi^2 = 6{\cdot}00$, df = 1, $0{\cdot}02 > P > 0{\cdot}01$). Although the numbers under consideration are small, possible explanations for such a discrepancy may be similar to those suggested previously for anomalous homozygote ratios.

4. Discussion

Genetic studies of plaice have shown that polymorphic forms of the enzymes PGM, GPI-A and GPI-B are under the control of codominant alleles at individual, independent loci (Purdom *et al.*, 1976). These authors also determined recombination frequencies for the PGM and GPI-B loci in induced diploid gynogenetic plaice; additional data for the GPI-A locus have since been obtained. These data are summarised in table 2, together with the results of the experiments described in this paper.

There are three possible methods of restoring diploidy in gynogenesis, namely: the retention of a product of the first polar body and its fusion with the egg pronucleus, the retention of the chromosomes of the second polar body and their fusion with egg pronucleus or the suppression of the telophase following the first mitotic division of the zygote. Only the second method can generate the range of frequencies observed for the PGM, GPI-A and GPI-B loci in gynogenetic plaice. If diploidy arose by involvement of the first polar body a 1 : 2 : 1 segregation would occur in eggs from heterozygous parents giving 50 per cent heterozygosity amongst the offspring for any locus. If diploidy arose through fusion of the products of first mitosis the offspring would all be homozygotes, segregating in a 1 : 1 ratio.

Contingency tests on the distribution of homozygotes and heterozygotes in induced and spontaneous gynogenetic diploid offspring show heterogeneity at the PGM locus ($\chi^2 = 6{\cdot}68$, df = 1, $0{\cdot}01 > P > 0{\cdot}001$) but homogeneity at both GPI loci (GPI-A: $\chi^2 = 0{\cdot}01$, df = 1, $0{\cdot}95 > P > 0{\cdot}90$; GPI-B: $\chi^2 = 2{\cdot}73$, df = 1, $0{\cdot}10 > P > 0{\cdot}05$). When the results are expressed as proportions, the recombination values for induced and spontaneous gynogenomes at all three loci are not significantly different (table 2). However the interpretations of both of these statistical comparisons may be restricted by the low numbers of individuals involved. The results of the present experiments are sufficiently similar to those found following induced diploid gynogenesis to indicate that diploidy is due to the retention of the chromosomes of the second polar body in both induced and spontaneous events.

Accepting that this is the method of diploidisation the efficiency of spontaneous gynogenetic diploidy as an inbreeding mechanism depends upon the frequency of crossing over in the first meiotic division. The recombination frequencies observed in gynogenesis represent twice the values obtained from conventional recombination analysis. In gynogenesis each heterozygote represents one recombined chromosome pair whereas

TABLE 2

Summary of observed recombination frequencies at three loci in gynogenetic offspring and an estimate of map length between the centromere

	On both homozygotes			On one homozygote				
Locus	*n*	Observed recombin. (%)	95% conf. level	*n*	Observed recombin. (%)	95% conf. level	Mean map length	Source of data
PGM								
Induced	396	81·8	±3·9	204	79·4	±5·7	40·3	Purdom *et al.* (1976)
Spontaneous	81	69·1	±10·3	45	62·2	±14·5	32·8	This paper
GPI-A								
Induced	60	45·0	±12·9	33·5	40·3	±17·0	21·3	Thompson (in prep.)
Spontaneous	7	42·9	±37·4	4·5	33·3	±44·4	19·1	This paper
GPI-B								
Induced	352	18·5	±4·1	206·5	15·7	±5·1	8·6	Purdom *et al.* (1976)
Spontaneous	41	29·3	±14·2	26	23·1	±16·5	13·1	This paper

in conventional recombination between pairs of linked loci a crossover event produces only a single recombined chromosome in the offspring. Therefore estimates of the crossover frequency or map length between the centromere and each locus may be obtained by halving the observed heterozygote frequencies (table 2).

The coefficient of inbreeding after one generation of gynogenesis is: $F = \frac{1}{2}(2-x)$, where x is the mean probability of crossing over (Purdom, 1969). Taking the mean of the crossover frequencies obtained for the 3 loci, (table 2), $x = 0{\cdot}22$, suggesting that for first generation spontaneous gynogenetic diploid plaice the coefficient of inbreeding is $\frac{1}{2}(2-0{\cdot}22)$ or 0·890. A similar value is obtained for the induced gynogenetic diploid plaice, where $x = 0{\cdot}023$ and the coefficient of inbreeding is 0·885. These values are equivalent to the effect of between three and four generations of conventional sib-mating. Thus a single generation of diploid gynogenesis, either induced or spontaneous, may aid the production of inbred lines and eliminate generations of sib-mating and several years of fish husbandry.

5. References

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