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High level of residual heterozygosity in gynogenetic rainbow trout, *Salmo gairdneri*, Richardson

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Summary. Nine gynogenetic lines of rainbow trout and their full-sib controls were used to study genetic variation at 14 protein loci and at one body color locus. The maternal genotypes could be clearly determined from the analysis of the control full-sibs. Gynogenetic off springs only possessed the alleles of the mother. In these gynogenetic individuals, obtained by retention of the second polar body, the residual heterozygosity (r) was calculated at 8 loci. The following results were obtained:
(1) The values of r varied greatly from one locus to another (from 0.11 to 1.00);
(2) On the other hand, for a given locus, variations of r between females were not significant;
(3) Four loci reached residual heterozygosity close to 1.
The following hypotheses are put forward to explain these results: total interference, atypical meiosis and partial tetraploidization. The high residual heterozygosity mean observed in rainbow trout (0.71 ± 0.11) is discussed with respect to inbred line production and management and a breeding scheme alternating gynogenesis and full-sib mating is suggested.

Key words: Gynogenesis – Salmonids – Gene recombination – Electrophoretic variations

Introduction

Gynogenesis is a mode of reproduction in which the egg is activated by fertilization with genetically inert spermatozoa. It has been observed in several natural populations of lower vertebrates (Uzzel, reviewed by Chourrout 1982), but it has also been initiated artificially, mainly with the aim of obtaining inbred lines, for instance in fish species of economic interest (Cherfas 1965; Purdom 1969; Stanley 1976; Nagy et al. 1978). Experimental gynogenesis involves 1) induction of a haploid development by insemination with genetically inactivated semen and 2) diploidization of the female chromosome complement in order to produce viable diploid gynogenetic offspring.

Various cytological mechanisms may restore diploidy (Asher 1970); thus, the increase in homozygosity and hence the efficiency of artificial gynogenesis for production of inbred lines highly varies according to the mechanism involved. Up till now, attempts made to initiate gynogenesis by suppression of the first cleavage have not been successful (very low survival rates) Jaylet 1972; Steisinger et al. 1981; Chourrout, in press). Accordingly, endomitosis, which would lead to a total homozygosity after one generation, cannot be used now to produce inbred lines. However, production of diploid gynogenetic lines by retention of the second polar body is possible in a certain number of fishes and amphibians (reviewed by Chourrout 1982) and specially in rainbow trout (Chourrout 1980).

When diploidy results from the suppression of the second meiotic metaphase, the gynogenetic progeny of heterozygous females will be homozygous except in the case where a recombinaison occurs between the centromere and the locus involved. Thus, the reduction of heterozygosity, as well as the inbreeding obtained by gynogenesis, will depend on the frequency of gene recombinations which are in turn related to the distance between the loci and their centromere. The efficiency of gynogenesis as well as the gene-centromere distances can only be measured through direct observations of residual heterozygosity at specified loci. Studies concerning well characterized loci coding for mendelian traits such as body color, pigmentation pattern and enzyme systems have already been made in several species of amphibians (Volpe 1970; Nace et al. 1970; Ferrier et al. 1980; Ferrier 1981) and fishes (Purdom et al. 1976; Cherfas 1977; Cherfas and Truweller 1978; Nagy et al. 1978, 1979). In a recent study on recombination rate in diploid gynogenetic rainbow trout, Thorgaard et al. (1983) showed that high residual heterozygosity occurs in this species.

The present paper supplies new data on residual heterozygosity at 8 loci in gynogenetic lines of rainbow trout. The use of these data to estimate gene-centromere distances, as well as the analysis of linkages, is dis-

cussed. Suggestion pertaining to inbred line production and management are given.

Materials and methods

Animals

Rainbow trout females and males used in this study were supplied by experimental hatchery of Gournay. Fifteen gynogenetic lines were produced. Nine lines were chosen according to their survival rate and maternal electrophoretic phenotype.

Table 1. Survival rates in full-sib and gynogenetic lines

Females	Gynogenetic lines		Full-sub families	
	Egg no.	Survival rates	Males	Survival rates
5	1106	53%[a]	1	93%[a]
6	1450	9%[a]	1	80%[a]
7	1537	12%[a]	2	70%[a]
8	1485	28%[a]	2	87%[a]
9	1198	69%[b]	3	95%[b]

[a] Survival rate at 60 days; [b] Survival rate at 120 days

The gynogenetic lines were obtained according to Chourrout's method (Chourrout 1980; Chourrout and Quillet 1982). Heat shocks of 26 °C were applied to the eggs for 20 min after a fertilization period of 10 min and an incubation period of 25 min at 10 °C. Females were individually fertilized with irradiated sperm. A small number of eggs from each female were not subjected to any heat shock so as to obtain a haploid control. Another small sample of eggs were collected from each female and fertilized with normal sperm in order to produce full-sib controls. Controls and gynogenetic lines were reared at 12±1 °C.

Progeny survival rates were recorded on day 60 and 120.

Electrophoresis

Nine biochemical systems, usually polymorphic in rainbow trout, were analysed: aspartate aminotransferase (AAT), creatine phosphokinase (CPK), glycerol-3-phosphate dehydrogenase (G3P), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), phosphoglucomutase (PGM), superoxyde dismutase (SOD), transferrin (TFN), para-albumine (P-ALB). A body color marker, golden color, was also studied. Golden color proves to be coded by one locus and the golden allele is dominant on the wild type (Chevassus, unpublished results). Starch gel electrophoretic methods applied to AAT, CPK, G3P, IDH, MDH, PGM, SOD and TFN have been described by Guyomard (1981). For P-ALB, a buffer described by Khanna et al. (1975) was used. Gel slices were scanned densitometrically for MDH. The locus nomenclature chosen was that described by May (1980).

Table 2. Genetic inheritance and parental genotype of systems examined in this study

Tissue system	AAT Muscle	P-ALB Serum	G3P Muscle	IDH-m Muscle	IDH-S Liver		MDH Muscle		PGM Muscle	SOD Liver	
Genetic control	*Aat-1* and *2*	*P-alb 1* and *2*	?	*Idhm-1* and *2*	*Idhs-3* and *4*		*Mdh-3* and *4*		*Pgm-2*	*Sod-1*	
,Polymorphic Loci in this study	*Aat-1*	*P-alb-1*	*G3p-1*	*Idhm-2*	*Idhs-3*	*Idhs-4*	*Mdh-3*	*Mdh-4*	*Pgm-2*	*Sod-1*	Gold
Female 1	aa	ab	aa	ab	ab[a] and aa[a] or ac and ab		ab and cc or ac and bc		ab	aa	nn
– 2	ab	n.s.	aa	ab	n.s.	n.s.	aa	aa	ab	aa	nn
– 3	aa	n.s.	aa	aa	n.s.	n.s.	aa	aa	aa	ab	nn
– 4	ab	n.s.	aa	ab	n.s.	n.s.	ab	aa	ab	aa	nn
– 5	aa	n.s.	aa	ab	n.s.	n.s.	ab	aa	aa	bb	nn
– 6	ab	n.s.	aa	aa	n.s.	n.s.	bc and aa or ab and ac		aa	n.s.	G
– 7	aa	n.s.	aa	ab	n.s.	n.s.	ac and bb or ab and bc		aa	n.s.	G
– 8	ab	n.s.	ab	aa	n.s.	n.s.	bc[a] and aa[a] or ac and ab		ab	aa	nn —
– 9	ab	n.s.	aa	ab	n.s.	n.s.	ab	aa	aa	aa	nn
Male 1	ab	n.s.	aa	ab	n.s.	n.s.	aa	aa	ab	ab	nn
– 2	aa	n.s.	aa	ab	n.s.	n.s.	aa and bb or ab[a] and ab[a]		aa	aa	nn
– 3	ab	n.s.	aa	aa	n.s.	n.s.	aa	aa	ab	ab	nn

G = Golden phenotype; n.s. = not studied

[a] Genotypes determined from full-sib families or gynogenetic progeny analysis

Table 3. Observed and expected segregations in full sib families for five protein systems

Families		T_5 (♀5×♂1)		T_8 (♀8×♂2)						T_9 (♀9×♂3)	
		Observed	Expected	Observed	Expected under model ( )					Observed	Expected
					(1)	(2)	(3)	(4)	(5)		
MDH	Parents F	aaab	ab aa	aabc	T	bc/aa	ab/ac	bc/aa	ab/ac	aaab	ab aa
	M	aaaa	aa aa	aabb	T	aa/bb	aa/bb	ab/ab	ab/ab	aaaa	aa aa
	Progeny aaa	7	7.5	0	1.3	0	0	0	2.95	14	15.5
	aaab	8	7.5	6	7.8	0	11.75	5.9	8.8	17	15.5
	aaac			8	2.6	0	0	5.9	2.95		
	aabb			14	11.8	23.5	11.75	11.7	8.8		
	aabc			10	11.8	23.5	11.75	11.7	8.8		
	abbb			3	2.6	0	0	5.9	2.95		
	abbc			6	7.8	0	11.75	5.9	8.8		
	bbbc			0	1.3	0	0	0	2.95		
	Chi-square		N.S.		15.8*	–	–	N.S.	18.4**		N.S.
AAT	Parents F	aaab	ab aa	aaab			ab aa			aaab	ab aa
	M	aaaa	aa aa	aaaa			aa aa			aaab	ab aa
	Progeny aaaa	5	7.5	19			23			4	7.75
	aaab	10	7.5	27			23			19	15.5
	aabb									8	7.75
	Chi-square		N.S.				N.S.				N.S.
G3P	Parents F	aa		ab						aa	
	M	aa		aa						aa	
	Progeny aa	15	15	23			19			31	31
	ab	0	0	15			19			0	0
	Chi-square						N.S.				
IDHm	Parents F	ab		aa						ab	
	M	aa		aa						aa	
	Progeny aa	n.s.		47			47			18	15.5
	ab			0			0			13	15.5
	Chi-square										N.S.
PGM	Parents F	aa		aa						aa	
	M	aa		aa						ab	
	Progeny aa	15	15				47			13	12
	ab	0	0	0			0			11	12
	Chi-square										N.S.

Genotypes of systems coded for by two loci are symbolized by fractions (eg. ab/ac) where the numerator is the first locus genotype and the denominator the second loci genotype
N.S. = not significant; n.s. = not studied; F = female; M = male
* $P = 0.05$;
** $P = 0.01$
In family T_8, five genetic models were tested (colums (1) to (5)); T = tetrasomic heredity

Results

Survival rates

Survival rates were calculated in the gynogenetic lines and full-sib controls 5, 6, 7, 8 and 9 on day 60 and day 120 just before the electrophoretic analysis (Table 1). No hatching was observed in haploid controls.

Interpretation of maternal electrophoregrams

Genetic control of the systems studied has been well established (May 1980; May et al. 1982) and is shown in Table 2. However, some questions still remain for G3P which might be coded by one or two loci in muscle (May 1980). Some systems, i.e. AAT, CPK, IDH-m and MDH in muscle, IDH-s in liver and P-ALB in serum are

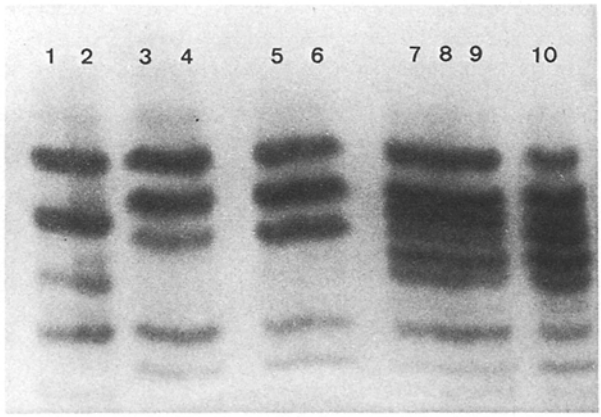

Fig. 1. MDH electrophoregrams in rainbow trout muscle: *1, 2* aaab; *3, 4* aaac; *5, 6* aacc; *7, 8, 9* aabc; *10* abcc; Rf: a = 1.00; b = 0.70; c = 0.79

coded for by two loci. It is now generally admitted that this duplication results from a tetraploidization (Allendorf 1975). Thus, a total of 14–15 loci were analysed. Four loci (*Cpk-1, Cpk-2, Idh_m-1, Tfn*) were monomorphic in all females examined. System SOD was heterozygous in female 3, but this enzyme was not typed in the gynogenetic offsprings.

The genetic interpretation of the electrophoregrams for systems MDH and AAT of the muscle as well as for IDH-s and P-ALB is more complicated because of gene duplication. The duplicated loci coding for these systems may share common alleles. For instance, *P-Alb-1* and *2* are both variable for two alleles, a and b (Gall and Bentley 1981); three combinations involving these two alleles (aaab, aabb, abbb) are possible and exhibit the same qualitative electrophoretic pattern; these combinations are usually differentiated by relative banding intensities. In addition, it is not always possible to assign the alleles to their respective loci and to determine allele pairs. In this study, two different genotypes may account for the MDH electrophoregrams of female 1 and 8 as well as for the IDH-s electropherogram of female 1. Presumed parental genotypes are given in Table 2. Examples of MDH electropherograms observed in rainbow trout muscle are shown in Fig. 1.

Table 4. Observed and expected segregation of gold in full sib families

Full-sib family		T_6 (♀6×♂2)			T_7 (♀7×♂3)		
		obs.	exp.		obs.	exp.	
Parents	F	{G}	Gn	GG	{G}	Gn	GG
	M	nn	–	–	nn	–	–
Progeny	{G}[a]	116	121,5	243	36	36	72
	nn	127	121,5	0	36	36	0
Chi-square			N.S.			N.S.	

{G}[a] = Golden phenotype; G = golden allele; n = wild allele; F = female; M = male

Table 5. Joint segregations in full sib families

Family	Parents	Genotype Progeny genotype						Chi-square
T_8	*Mdh-3*	*Aat-1*	a_2b_1[a] (1)		b_1b_2(*) (2)	a_2c_1(*) (3)	c_1b_2(*) (4)	$\frac{\{(1)+(4)-(2)-(3)\}^2}{(1)+(4)+(2)+(3)}$
F	b_1c_1	a_2b_2						
M	a_1b_1	a_2a_2	11		11	8	16	1.4
T_9	Idh_m-2	*Mdh-3*	a_1a_2(*) (1)		a_1b_2(*) (2)	a_2b_1(*) (3)	b_1b_2(*) (4)	
F	a_1b_1	a_2b_2						
M	a_1a_1	a_2a_2						
T_9	*Aat-1*	Idh_m-2	$\frac{a_1a_1}{a_2a_2}$ (1)	$\frac{a_1b_1}{a_2a_2}$ (2)	$\frac{b_1b_1}{a_2b_2}$ (3)	$\frac{a_1b_1}{a_2b_2}$ (4)	$\frac{b_1b_1}{a_2a_2}$ (5)	$\frac{a_1a_1}{a_2b_2}$ (6)	$\frac{\{(1)+(3)-(5)-(6)\}^2}{(1)+(3)+(5)+(6)}$
F	a_1b_1	a_2b_2							
M	a_1b_1	a_2a_2	3	1	8	11	3	5	1.2
T_9	*Aat-1*	Mdh-3							
F	a_1b_1	a_2b_2							
M	a_1b_1	a_2a_2	2	2	9	10	7	1	3
T_8	*Mdh-3*	Mdh-4	$\frac{aa}{ab}$ (1)	$\frac{aa}{ac}$ (2)	$\frac{aa}{bb}+\frac{ab}{ab}$ (3)	$\frac{aa}{bc}+\frac{ab}{ac}$ (4)	$\frac{ab}{bb}$ (5)	$\frac{ab}{bc}$ (6)	$\frac{\{(1)+(2)+(5)+(6)-(3)-(4)\}^2}{(1)+(2)+(3)+(4)+(5)+(6)}$
F	aa	bc							
M	ab	ab	6	8	14	10	3	6	0.2

[a] Only maternal alleles are considered; F = female; M = male
For statistical methods, see May et al. (1979)

Table 6. Progeny genotypes at ten loci in diploid gynogenetic rainbow trout and residual heterozygosity (r) at 8 loci

Lines	*Aat-1*	*P-alb-1*	*G3p*	*Idh$_m$-2*	*Idh$_s$-3*	*Mdh-3*	*Mdh-4*	*Pgm*	*Sod-1*	*Gold*
1*	49 aa	23 ab 2 bb	49 aa	7 aa 32 ab 10 bb	6 bb 15 bc 5 cc	46 ab 2 ac 1 ab	cc cc ac	25 aa 2 ab 22 bb	49 aa	
r		0.92		0.65	0.58	0.94		0.04		
1**						46 ac 2 ac 1 aa	bc cc bc			
r						0.97	0.97			
2	29 ab 2 bb	n.s.	31 aa	9 aa 17 ab 5 bb	n.s.	31 aaaa		16 aa 2 ab 13 bb	31 aa	
r	0.94			0.55				0.06		
3	31 aa	n.s.	31 aa	31 aa	n.s.	31 aaa		31 aa	n.s.	
4	43 ab 1 aa	n.s.	41 aa	7 aa 29 ab 8 bb	n.s.	44 ab aa		16 aa 10 ab 18 bb	44 aa	
r	0.98			0.66		1.00		0.23		
5	31 aa	n.s.	31 aa	n.s.	n.s.	31 ab aa		31 aa	31 bb	
r						1.00				
6	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.	n.s.	83 GG + Gn 49 nn
r										0.26
7	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.	n.s.	170 Gn + GG 14 nn
r										0.85
8	27 ab 2 aa 2 bb	n.s.	31 ab	31 aa	n.s.	30 bc aa 1 bb aa		31 aa	31 aa	
r	0.87		1.00			0.97				
9	29 ab 1 aa 2 bb	n.s.	aa	5 aa 17 ab 10 bb	n.s.	32 ab aa		32 aa	32 aa	
r	0.91			0.53		1.00				

1* = *Mdh-4* monomorphic; 1** = Both *Mdh-3* and *Mdh-4* polymorphic
n.s. = not studied; G = golden allele; n = wild allele

Analysis of full-sib controls

Full-sib controls were used in order to prevent any genetic misinterpretation in the case of loci *Mdh-3* and *4*, *Aat-1* and *2*, *Idh-3* and *4*, *P-Alb-1* and *2*. Only full-sib controls corresponding to females 5 through 9 were analysed and the results reported in Table 3. These genetic interpretations were tested. Five different models were considered for the MDH phenotype of female 8. Only model 3, in which both loci were variable in the male and only one in the female, was in keeping with our observations. All the genotypes suggested in the other cases were confirmed by the analysis of controls. Golden females 6 and 7 proved to be heterozygous at the golden locus (Table 4). The results of six pairwise examinations of joint segregation involving loci *Aat-1*, *Idh$_m$-2*, *Mdh-3* and *Mdh-4* are shown in Table 5. No linkage disequilibrium was detected.

Gynogenetic lines

Genotypes of gynogenetic offsprings of each line are indicated in Table 6. MDH scanner analysis was in good agreement with the genetic interpretation. For example, all the densitometric profiles of the gynogenetic offsprings of line 9 were identical to those of their mother. For IDH-s, it may be deduced from the absence of aaac, acbb and abcc phenotypes and from the large pro-

Table 7. Joint segregation in gynogenetic lines

Line	Female		PD[a]	NPD[b]	Chi-square
	Idh$_s$-3	*Pgm*	$b_1b_1a_2a_2 + c_1c_1a_2a_2$	$b_1b_1b_2b_2 + c_1c_1a_2a_2$	
1	b_1c_1	a_2b_2	6	4	0.4
	Idh$_m$-2	*Pgm*	$a_1a_1a_2a_2 + b_1b_1b_2b_2$	$a_1a_1b_2b_2 + b_1b_1a_2a_2$	
1	a_1b_1	a_2b_2	6	9	0.6
5	–	–	7	7	0
8	–	–	6	6	0

[a] Parental ditypes; [b] Non-parental ditypes

portion of aacc and aabb individuals that female 1 was only heterozygous at one of the two loci. In the same line, the two supposed MDH maternal genotypes still remained possible. If only one locus was heterozygous, the ccac and acab genotypes could result from allele exchanges between *Mdh-3* and *Mdh-4*. Such exchanges between homoeologous chromatides have already been demonstrated in rainbow trout (May et al. 1982).

The gynogenetic progeny possessed only the alleles of their mother. At heterozygous loci, no significant difference between the two homozygote frequencies was detected in the gynogenetic lines.

The residual heterozygosity values are reported in Table 6. Lines 1 and 4 showed a significant difference in the residual heterozygosity at locus *Pgm-2* (corrected chi-aquare = 5.6). The average heterozygosity level was 0.71 ± 0.11 after one generation of gynogenesis.

When joint segregations are examinated in gynogenetic lines, the most satisfactory criterion for detection of random segregation is the difference between parental (PD) and non parental (NPD) ditypes (Perkins 1953; Volpe 1970) as in tetrad analysis. There is no evidence of linkage between any of the locus pairs tested in this study (Table 7).

Discussion

High level of residual heterozygosity

Four of the polymorphic loci (*Mdh-3, Aat-1, P-alb-1, G-3-p*) exhibited a high residual heterozygosity (95–100%). An erroneous interpretation of the maternal genotypes is not possible since the latter were checked with full-sib mating. Likewise, there was no lasting non-genetic maternal effect since this phenomenon could not be observed in the full-sibs at day 60.

Our results were compared to theoretical values derived from retention of the second polar body assuming (1) a disomic inheritance, (2) a tetrasomic and (3) a pseudotetrasomic inheritance. Pseudotetrasomy or residual tetrasomy has been described in some salmonids and especially in rainbow trout (Morrisson 1970; Wright et al. 1975; Wright et al. 1980; May et al. 1982). At metaphase 1, pseudotetrasomy leads to the formation of tetravalents involving two chromosome pairs resulting from the duplication of an ancestral pair (homoeologous chromosomes). In rainbow trout, such homoeologous pairing have been suggested by cytological observations of tetravalents and evidenced by segregation analysis of duplicated loci (May 1980). Since high levels of residual heterozygosity were mainly observed at duplicated loci, we examined the effects of residual and true tetrasomy on post-reduction rates (Appendix 1). Our results are not consistent with the retention of the second polar body, assuming a random association of chromosomes as well as the absence of selection and interference. In such a model, the maximum recombination value is always lower than or equal to 67% (⅔) with disomic heridity as well as with tetrasomic and pseudotetrasomic inheritances (0.57 for true tetrasomy, Appendix 1). Values exceeding 67% have often been reported in other gynogenetic vertebrates (Lindsley et al. 1956; Nace et al. 1970; Volpe 1970; Ferrier et al. 1980; Ferrier 1981; Nagy and Csanyi 1982; Thompson 1983). However, these values always ranged around 0.67 and, as no survival rates are indicated, they may be due to selection against homozygotes. Thorgaard et al. (1983) also observed rates of residual heterozygosity close to 1 at the *Mdh 3–4* and *Sod-1* loci and considered selection against heterozygotes to be unlikely. In our study, this hypothesis can also be exclused because of the observed high survival rates. However, on account of the low survival rates in lines 6 and 7, a selective mortality of homozygous individuals may be at the origin of the two recombination values observed in the case of the gold locus. High interference seems to be the best explanation for these observations (Thorgaard et al. 1983). When only one crossing-over occurred systematically the distal loci would exhibit 100% recombination. This hypothesis is supported by cytological evidence: there are species in which some chromosomes always show 1 chiasma, never 0 or 2 (White 1973, page 180).

Apart from the recombination involving a total interference, other less likely phenomena could lead to high residual heterozygosity levels. For example, meiosis could be atypical for some chromosomes which

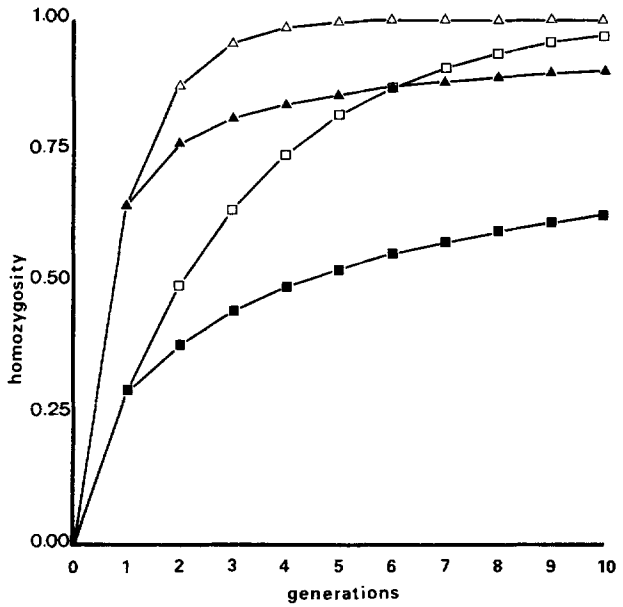

Fig. 2. Effect of the interlocus variation of r, residual heterozygosity, on the change in homozygosity. Assuming that r_i is the observed value of residual heterozygosity at locus i after one generation of gynogenesis and remains constant over generations, the expected value of H (homozygosity level at generation n is given by $H_n = 1 - \left\{ \frac{\sum_{i=1}^{L} r_i^n}{L} \right\}$ (L = number of loci analysed): -■-: H_n in trout; -▲-: H_n in carp (from Nagy and Csanyi 1982). If all the loci would have the same residual recombination value, $r = \sum_{i=1}^{L} r_i/L$ (no interlocus variation), the expected value of H at generation n would be $H'_n = 1 - \left\{ \frac{\sum_{i=1}^{L} r_i}{L} \right\}^n$. -□-: H'_n in trout; -△-: H'_n in carp

would first divide equationally and then reductionally. For these chromosomes, heterozygosity would be maintained when no crossing-over occurs. It may also be assumed that because of the gynogenesis treatment some chromosomes do not undergo either a reductional or an equational division. Thus, the high level of residual heterozygosity may result from a partial tetraploidization. However, it is difficult to imagine that such a frequent event (four loci out of eight studied) has not been detected through the karyological analysis of the diploid gynogenetic individuals (Chourrout 1980).

Gene mapping and linkage

On account of the different mechanisms likely to induce high levels of residual heterozygosity (interference, atypical meiosis, partial tetraploidization) it is not possible in our case to draw any conclusion about the locus–centromere distances. According to the hypothesis of total interference, *Mdh-3, P-Alb-1, Aat-1* and *G3p* would be located far from the centromere. In the case of an atypical meiosis, they would be close to the centromere and in the case of a partial tetraploidization they could be located anywhere on the chromosome.

Our results point out that gynogenesis is less efficient than full-sib crossing for the analysis of joint segregations. When highly recombinant loci are examined the number of gynogenetic individuals used to detect the linkages (PD + NPD) is small and the test efficiency low.

Evolution of inbreeding

In rainbow trout, average heterozygosity is maintained at a high level after one generation of gynogenesis ($r = 0.68 \pm 0.36$, results of Thorgaard et al. 1983 included). This value is quite high as compared to estimates in carp ($r = 0.35 \pm 0.33$, Nagy et al. 1982) and plaice (0.42 ± 0.24, Thompson 1983). However, The standard errors are still too large in the three species to allow accurate estimations.

In rainbow trout, the residual heterozygosity widely varies from one locus to another and ranges from 0.11 to 0.99. Thus, if these values remain constant over generations, some genes (*Pgm-2*) will be fixed after two generations while gynogenesis will be inefficient at other loci (*Aat-1, Mdh-3, P-Alb-1*). This interlocus heterogeneity of r tends to reduce the increase in homozy-

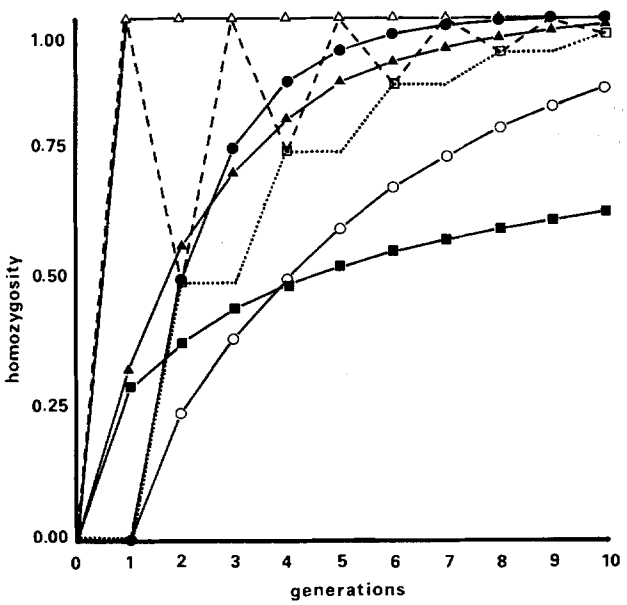

Fig. 3. Theoretical changes in homozygosity in different breeding plans: -○-: full-sib mating; -●-: selfing; -△-: gynogenesis with recombination rate = 0.66; -▲-: gynogenesis with recombination rate = 0; ···□···: alternate gynogenesis with recombination rate = 1; -□-: alternate gynogenesis with recombination rate = 0; -■-: see definition in Fig. 2

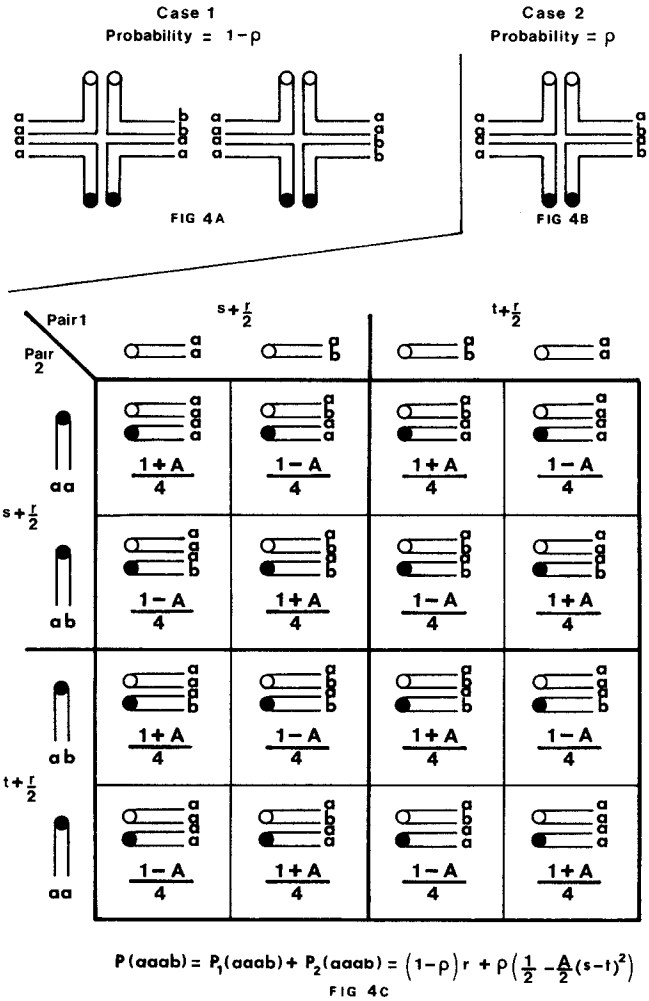

Fig. 4. Frequency of $aa;ab$ genotype in the gynogenetic diploid progey of a female $aa;ab$ in the case of duplicate loci with pseudotetrasomic inheritance (see Appendix 1 for the definition of p, r, s, t and A)

gosity over generations (Nagy et al. 1982). This effect can be measured by comparing the observed curve with that obtained with the same mean residual heterozygosity and no locus variation of r (Fig. 2). In rainbow trout, this deviation represents 40% of the true value after four generations of gynogenesis.

Three different inbreeding schemes can be applied to rainbow trout: (1) full-sib mating, (2) selfing and (3) gynogenesis by retention of the second polar body. Figure 2 reports theoretical evolution of homozygosity in these three inbreeding schemes. Selfing and full-sib mating curves start at generation one because parents are not related at generation 0 and one year is needed to produce hermaphrodite individuals (Chevassus et al. 1979). Accordingly, gynogenesis does not appear to be a very efficient inbreeding method in rainbow trout. The discrepancy between observations and expected heterozygosity, assuming no interference, exceeds 40%. Likewise, gynogenesis takes a longer time than full-sib mating and selfing.

If these results could be confirmed by more extensive studies, it would be more advisable to try other systems of production and management of inbred lines. A breeding scheme where gynogenesis and full-sib mating alternate from one generation to another might be an attractive solution. In such a scheme, designated as "alternate gynogenesis", the $2(n-1)^{th}$ generation has the same inbreeding value as the n^{th} generation in a normal selfing (Appendix 2). This "alternate gynogenesis" is more efficient than full-sib mating and normal gynogenesis in rainbow trout (Fig. 3). It should also be pointed out that the gain in homozygosity occurs at a faster rate in alternate gynogenesis than in any other kind of inbreeding involving both gynogenesis and full-sib mating. Moreover, alternate gynogenesis shows inbreeding nodes at the $2(n-2)^{th}$ generations where all the loci have the same homozygosity level.

As sex-reversal of gynogenetic individuals is needed to cross gynogenetic strains, which are nearly all female in rainbow trout (Chourrout and Quillet 1982), alternate gynogenesis does not give rise to special practical problems and can be applied without additional work.

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Appendix 1

Pseudotetrasomy. Let us consider a female $\frac{a\,b}{a\,a}$ for two duplicate loci. When tetravalents occur two cases should be considered: (1) allele b is only on pair 1 or pair 2 with probability $1-\varrho$ (Fig. 4A), (2) allele b is present on each pair of homoeologous chromosomes with a probability ϱ (Fig. 4B).

Let us assume r to be probability of post-reduction within a chromosome pair; r is the frequency of a a a b gynogenetic offsprings in case 1. In case 2, let us assume s, r and t to be the respective probabilities of events (1) no allele is exchanged between homologous chromosomes, (2) one allele is exchanged and (3) two alleles are exchanged. Figure 4C gives the frequencies of all possible first meiotic division products. A is a measure of the degree of nonrandom segregation of chromosomes at anaphase I. Then, the frequency of a a a b genotypes is:

$$P(aaab) = r\{1-\varrho\} + \varrho/2\{1-A(s-t)^2\}, \quad -1 < A < +1.$$

When $A=0$ (random segregation), $P(aaab) = (1-\varrho)\,r + \varrho/2$; $P(aaab)$ is $< 1/2$ when $r < 1/2$ and $< r$ when $r > 1/2$.

True tetrasomy. If bivalents and tetravalents occur, this case is the same as the pseudotetrasomy. If no bivalent is formed, the maximal frequency of a a a b individuals is half that of disomy. If chromatids are randomly exchanged, a a a b frequency is given by $C_2^1 \times C_6^3 / C_8^4 = 0.57$ when the locus segregates independantly of its centromere.

Appendix 2

Alternate gynogenesis

Generation n + 1: gynogenesis. After a full-sib mating at generation n, let us assume that the inbreeding coefficient is $F_n = 1 - (1/2)^m$; therefore, the n^{th} generation frequencies of the heterozygous and both homozygous genotypes will be respectively: $f_n(a\,b) = (1/2)^m$ and $f_n(a\,a) = f_n(b\,b) = 1/2 - (1/2)^{m+1}$. Then, after one generation of gynogenesis, we obtain: $f_{n+1}(a\,b) = r \cdot (1/2)^m$ and $f_{n+1}(a\,a) = f_{n+1}(b\,b) = 1/2 - r \cdot (1/2)^{m+1}$.

Generation n + 2: full-sib mating. If generation n + 2 is produced by full-sib mating, the (n + 2)-th generation heterozygotes will be derived from the n^{th} generation heterozygotes. The genotypes frequencies in the gynogenetic progeny of an heterozygous individual are $\frac{1-r}{2}$ a a, r a b and $\frac{1-r}{2}$ b b. Then, a full-sib mating of these off-springs leads to an heterozygote frequency of:

$$2\frac{1-r}{2}\,b\,b \times \frac{1-r}{2}\,a\,a + 2\tfrac{1}{2} \cdot \frac{1-r}{2}(b\,b + a\,a) \times r \cdot a\,b + 1/2 \cdot r \cdot a\,b \times r \cdot a\,b = 1/2\,.$$

So, the heterozygous frequency generation n + 2 will be: $f_{n+2}(a\,b) = (1/2)^{m+1}$.

Generation n + 3: gynogenesis. $f_{n+3}(a\,b) = r \cdot (1/2)^{m+1}$.

So, it can be write in an alternate gynogenesis: $1 - F_n = 1/2 \cdot (1 - F_{n-2})$ with $F_{2n+1} = 1 - r \cdot (1/2)^n$ (gynogenesis generation) and $F_{2n} = 1 - (1/2)^n$ (full-sib mating generation).

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