

PROSPECTS OF PRODUCING INBRED LINES FOR CONSOLIDATION OF GROWTH PERFORMANCE

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SUMMARY

When developing inbred lines through brother-sister matings, along with selection for pan-size bodyweight, no difference in growth performance to the outbred control was observed in second generation inbred lines ($F = 0.375$). The inbreeding depression in viability also turns out much lower, than in the first inbreeding generation ($F = 0.25$). Gynogenesis, as an alternative inbreeding method, could fasten inbreeding process. There is the possibility of producing homozygous diploid gynogenetic rainbow trout. Apparently the techniques necessary to inhibit the first mitotic division need further improvement in order to be of practical use, as the 3 one-year-old homozygous diploid gynogenetic fish produced indicate. The significance of inbred lines for the production performance of rainbow trout can only be estimated after further inbreeding through test crossing of lines.

INTRODUCTION

Crossbreeding of selected inbred lines proved a very effective breeding strategy in maize (Eberhart, 1977), while its success in farm animals has been much less notable, mainly due to genetic and economic constraints in producing highly inbred lines in numbers sufficient for testing. In rainbow trout this breeding scheme has not yet been investigated, although large numbers of offspring and special techniques, such as gynogenesis and sex reversal, could help to establish highly inbred lines. Therefore the production of inbred lines through full-sib matings, along with selection for body weight was initiated, using different populations of trout as a genetic basis for inbreeding. In addition, as an alternative breeding method, the techniques for producing homozygous diploid gynogenetic trout were evaluated. The preliminary results of these experiments will be presented in this paper.

MATERIAL AND METHODS

The inbreeding studies were based on 7 European populations of spring spawning rainbow trout, which had shown significant differences in pan-size body weight (Morkramer *et al.*, 1985). Each population consisted of 3 to 4 full-sib families, not related to each other. 48 spawners (3 year old), derived from all families after within-family selection of the heaviest fish ($i = 1.4$), were used as parents for the 24 brother-sister matings in the first inbreeding generation. In the control groups, one spawner out of each family was randomly mated with another. The same procedure was used to establish second generation inbred families ($F = 0.375$) and controls. While inbred parents ($F = 0.25$) were selected for high body weight through within-family selection ($i = 1.4$), the control line was continued by random sampling and mating spawners of the first generation of control fish. While inbred families could be identified throughout the whole testing procedure, single-pair matings of controls were kept together after hatching. Comparisons of viability and growth performance of inbreds and controls

were carried out according to the Relliehausen standard testing procedure (Morkramer et al., 1985) with slight modifications concerning stocking densities. Due to high losses during the egg and fry stage, observed in some inbred families, an additional stocking density, with an appropriate control class was maintained. All trout were fattened in the silo system only. After a 3-month fattening period during the second generation, high numbers of fish were lost due to technical problems in the system. Survivors were restocked, along with appropriate numbers of other rainbow trout, 3 days later and fattening was continued.

To induce gynogenesis 25 females and 10 males were sampled from spawners, used to begin inbreeding through full-sib matings. UV-treatment (Chourrout, 1982) was used in order to inactivate the parental genome. Since in preceding investigations, inhibition of the first mitotic division could not be achieved by the method of Chourrout (1984), modifications of his method, including higher and longer pressure shocks, were analysed with regard to a successful diploidisation. Haploid controls, chromosome preparations (Chourrout and Happe, 1986) and genetic markers (Diebig et al., 1979) served as a control for successful treatments.

RESULTS

Starting with 24 inbred families ($F = 0.25$), 10 families had been lost by the beginning of the rearing phase, partly due to insufficient numbers of survivors required for the testing procedure used. The average rearing weight of all inbred fish ($N = 1346$) in the standard density class is significantly lower with 9.4 g, when compared to the weights of the control ($N = 359$). In the class with the lower density, this difference increases to as much as 41 g. Pan-size weight of inbred fish shows equal inbreeding depression [calculated as $((\text{weight of inbreds} - \text{weight of controls}) / \text{weight of controls}) * 100$] of - 27%, regardless of the density class the fish belonged to during the rearing phase. The inbreeding depression for both weights differs significantly between lines. An inbred line can also have different ranks with regard to the inbreeding depression, observed for its rearing and fattening weights.

Since not all first generation spawners of the 14 inbred families showed simultaneous maturity, only 10 inbred families have been produced in the second generation. From these 10 families ($F = 0.375$), 6 have finished the rearing and fattening phase (Table 1). Losses within families ($F = 0.375$) during the fry and fingerling stage, are about 1/3 lower than in families of the first generation ($F = 0.25$). The average rearing weight of inbreds ($F = 0.375$) has been 27.7 g ($N = 1250$), versus 26.8 g ($N = 244$) of the controls, with inbred lines showing significantly higher (6 g) or lower (- 7 g) rearing weights than the control (standard density class). Even though only a limited number of observations has been available for the fattening weight, due to the technical failure of the silo, inbreds ($N = 115$) and controls ($N = 21$) have similar weights of 187.3 g and 184.2 g, respectively.

Experiments to produce homozygous diploid gynogenetic trout have shown that UV-treatment of sperm resulted in an inactivation of the paternal genetic material, as the controls indicate, in which only embryos with a haploid chromosome set and no hatched fry had been observed. Diploid embryos at the eyed-egg stage have been found in the samples used for chromosome counts in only 10 batches, representing different shock treatments to inhibit the first mitotic division (Table 2). These batches were coming from 5 of the 25 females used. 6 batches have shown sac-fry with an average hatching rate of 1.5% (0.2% - 2.5%). After the first

Table 1: Inbreeding depression (ID) observed for fingerling (FBW)- and pan-size body weight (PZBW) of the six inbred lines tested in both generations (F = 0.25, F = 0.375)

Line	1. Generation (F = 0.25)				2. Generation (F = 0.375)			
	N	ID (%)		PZBW	N	ID (%)		PZBW
		FBW	N			FBW	N	
22	132	- 45.3*	74	- 23.9*	237	- 25.0*	13	- 5.0
23	123	- 29.6*	84	- 17.4*	220	8.5	16	0.6
71	61 ¹	- 48.8*	23	- 47.7*	280	- 1.9	32	1.1
73	124	- 0.9	41	- 18.3*	254	5.2	24	2.4
53	48 ¹	- 29.6*	37	- 7.9	259	22.0*	30	5.0
81	131	- 22.1*	98	- 24.6*	54 ¹	- 2.1	8	3.3

ID % = ((weight of inbreds - weight of controls)/weight of controls) * 100;

* = p < 0.05; ¹ = tested in low density class.

Table 2: Observations of diploid (+) or haploid (-) embryos at the eyed-egg-stage in treatments studied for inhibition of the 1. mitotic division in egg batches activated by radiated sperm

Duration of shock:	7000 psi				8000 psi				8600 psi	
	4 min		5.5 - 6 min		4 min		5.5 - 6 min		4 min	6 min
	wE	+E	wE	+E	wE	+E	wE	+E	wE	
Time* 325	-	-	-	-/+ ¹	-	-/+ ²	-/+ ³	-/+ ⁴	-	+
340	-	-	-	n.t.	-	-	+	n.t.	-	+
400	-	-	n.t.	n.t.	+	-	n.t.	n.t.	n.t.	n.t.

* Start of pressure shock after activation of eggs; n.t. = not yet tested;

¹ = 2 females tested/1 female success; ² = 2 females tested/1 female success;

³ = 2 females tested/1 female success; ⁴ = 9 females tested/ 2 females success;

wE = without ether; +E = additional ether treatment of eggs.

feeding, fry of 5 batches have been left. In total 40 fry from 2733 treated eggs (5 batches) have been raised. 15 fry have reached 4 months of age. There are 3 one-year-old fish, so far. Results of gene marker studies (To, Pgm) by starch gel electrophoresis have given evidence of maternal inheritance only. The homozygous diploid gynogenetic trout have been obtained through a pressure shock of 8600 psi, applied 325 - 340 min after activation of the eggs with radiated sperm.

DISCUSSION

The approach of building up inbred lines through full-sib matings, along with selection for pan-size body weight, has revealed inbred lines ($F = 0.375$), which show equal rearing and fattening weights as the outbred control, and lower inbreeding depression, with regard to viability, as the first inbred generation ($F = 0.25$). It seems possible, therefore, to obtain highly inbred lines, which show an efficient growth performance. Inbreeding depression in the first generation, which was on the average comparable to the ones observed by Kincaid (1976, 1983) and Gjerde *et al.* (1983) for similar traits, resulted in high losses of whole lines, however, mainly during the egg and fry stage. Although the genetic basis was reduced thereby, high variation of inbreeding depression could be observed between the remaining inbred lines. For further inbreeding it is, therefore, advisable, to include more replications within lines.

On the other hand, the possibility of producing homozygous diploid gynogenetic rainbow trout exists, but the required techniques seem to need further improvements in order to be of practical use. The possible effect of the maternal genome on successful treatments should be considered in further studies. Eggs of different mothers may show different suitability for inhibition of first mitosis or may need different treatments in order to success. Both inbreeding systems, gynogenesis and full-sib matings, require high numbers of eggs, due to occasionally extreme losses during the egg and fry stage. With further hatching facilities, this would be financially feasible. The time saving factor undoubtedly favour gynogenesis.

The value of all inbred lines can only be examined by testcrossings, therefore, it is too early to discuss the possible significance of various genetic origins or selection history of lines (including testing procedures used). With regard to body weight, the fast recovery through only 1 generation of moderate within-family selection might indicate that non-additive genetic effects are of minor importance. The only advantage of crossing inbred lines might be the production of fish, which show reduced phenotypic variance (Gjerde, 1988) and reproducible genetic make up.

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