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A new breeding system using gynogenesis and sex-reversal for fast inbreeding in carp

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Summary. A new breeding technique is demonstrated using gynogenesis and sex-reversal. The essence of this method is an adequate alternation of gynogenesis and sibmating with a maximal increase in the coefficient of inbreeding. The rate of this increase considerably exceeds that characteristic of sibmating. The change in the coefficient of inbreeding (F) and in the degree of genotypic identity (I) were determined in the carp in which 9 recombination probabilities are known. In the GS system the value of F exceeds 0.9 in the fifth generation, increasing above 0.99 while in the 12th generation.

Key words: Gynogenesis – Carp – Inbreeding – Sex-reversal – Recombination

Introduction

Like others (Nace 1968; Purdom 1969; Stanley 1973; Cherfas 1975), we have also studied the method of artificial gynogenesis in order to obtain, substantially faster than by sibmating, inbred strains indispensable for use in certain genetic studies as well as in practical breeding. The method of mass gynogenesis of the carp has already been developed (Nagy et al. 1978) and applied in solving genetic problems (Nagy et al. 1979). The changes of the various parameters during gynogenesis were studied (Nagy and Csányi 1982), and it was stated that the degree of gynogenetic identity (I) increases rapidly in the successive gynogenetic generations. However, the rate of increase of the coefficient of inbreeding (F) largely depends on the distribution of the recombination probabilities of the genes. Although its value in the first ten gynogenetic generations can be expected to exceed the F values obtained by sibmating, during subsequent generations the coefficient of inbreeding of sibmating surpasses that of gynogenesis. The larger the proportion of genes of high recombination probability, the smaller the rate of progress of the F value. The initial heterozygous state of genes of a recombination probability of 1.00 is not changed by a series of gynogenesis as opposed to the propagation of sexual forms.

Using successive gynogenetic generations no genetically homogeneous strains can be produced. This paper presents an inbreeding technique capable of essentially increasing the rate of inbreeding using – in addition to gynogenesis – sibmating by males obtained by sex-reversal from gynogenetic females as opposed to those inbreeding systems which apply either sibmating or gynogenesis separately.

Results

As was demonstrated in our previous study (Nagy and Csányi 1982) in the case of artificial gynogenesis produced by the retention of the second polar body the coefficient of inbreeding (F_{Gi}) as well as the coefficient of genotypic identity for measuring isogeneity (I_{Gi}) largely depend on the distribution of recombination probabilities. The values of these are as follows:

$$F_{Gi} = 1 - \int_0^1 x^i f(x)\,dx = 1 - M(r^i) \tag{1}$$

$$\begin{aligned} I_{Gi} &= \int_0^1 (1 - \tfrac{1}{2}x^{i-1} - x^i + \tfrac{3}{2}x^{i+1})\, f(x)\,dx \\ &= 1 - \tfrac{1}{2}M(r^{i-1}) - M(r^i) + \tfrac{3}{2}M(r^{i+1}) \end{aligned} \tag{2}$$

where $f(x)$ is the density function of the recombination probabilities and $M(r^i)$ is the i-th moment of this random variable.

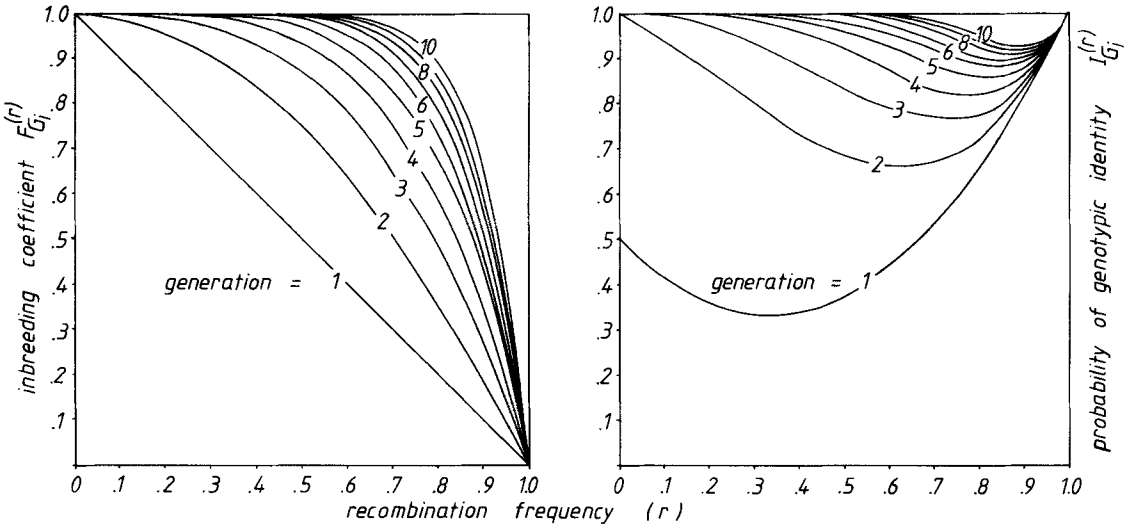

Fig. 1. Dependance of the inbreeding coefficient A and genotypic identity B on recombination probabilities

As far as an individual locus is concerned its coefficient of inbreeding and genotypic identity are the function of its recombination probability (r). For successively applied gynogenesis these functions are shown in Fig. 1. The larger the recombination probability, the smaller the rate of the progress of the F value. The genotypic identity has a minimum in the interval $\frac{1}{3} \leqq r \leqq 1$, depending on the generation number. It can be concluded that gynogenesis itself does not enable the production of homozygous strains to any great extent because of the possible presence of loci having large recombination frequencies.

For improving the inbreeding ability of gynogenesis a breeding system is suggested (Fig. 2). In the proposed system gynogenesis and sibmating are used alternately. Since the carp has female homogametic sex-determination, the sibmating is made possible by sex-reversal (Nagy et al. 1981) of the gynogenetic offspring. The two generations gained by gynogenesis, then by sibmating of gynogenetic offspring, represent as much genetic progress in inbreeding as a generation obtained by selfing. If, therefore, the procedure is cyclically repeated a serial selfing scheme of double-generation time is obtained. Since it was previously demonstrated that genetic progress in case of gynogenesis itself largely depends on the distribution of recombination probabilities, the more important genetic parameters of the new breeding plan will be as follows.

Using the symbols of Fig. 2, the coefficient of inbreeding of populations originating from sibmating S_{m+2i+2} should first be determined. Starting with a mother denoted G_m, let us suppose that its coefficient of inbreeding is F_{Gm}. Starting from the animal G_m, the

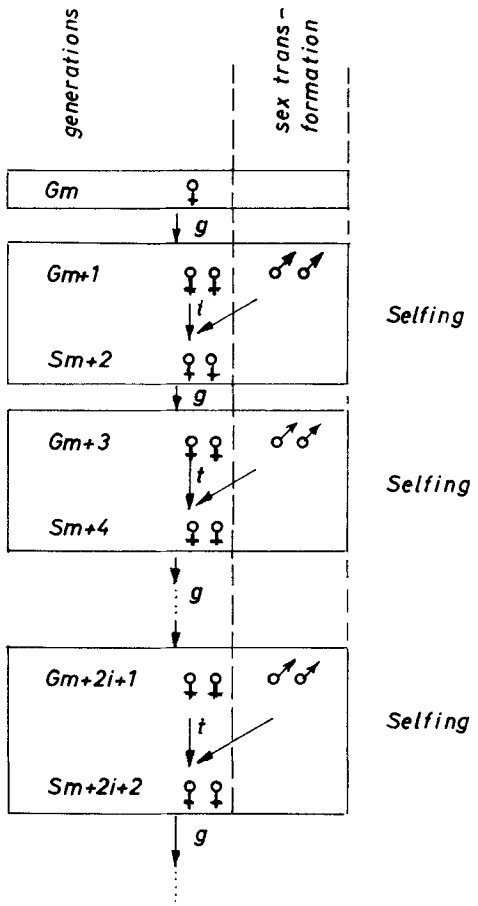

Fig. 2. Protocol of the GS system of inbreeding. g = gynogenesis, t = sibmating

generation of S_{m+2i+2} is obtained by a "selfing" cycle of a number of i + 1, thus the coefficient of inbreeding of this generation is

$$F_{S_{m+2i+2}} = F_{G_m} + (1 - F_{G_m})\frac{2^{i+1} - 1}{2^{i+1}}$$

$$= 1 - \frac{1 - F_{G_m}}{2^{i+1}} \quad (i = 0, 1, \ldots). \tag{3}$$

Calculation of the coefficient of inbreeding of population G_{m+2i+1} is different. Suppose that the coefficient of inbreeding referring to a gene of a recombination probability r of a G_m mother is $F^{(r)}_{G_m}$. The assumption of the dependence on r is needed for the purpose of generalization, thus allowing G_m to be gynogenetic. If G_m is not gynogenetic, and it has no gynogenetic ancestor on which the coefficient of inbreeding referring to the individual genes depended on the recombination probability of the gene, the value of $F^{(r)}_{G_m}$ is a constant, independent of r, being equal to F_{G_m}. The probability that a gene of a recombination probability of r remains heterozygote in generation G_{m+2i+1} is obviously

$$(1 - F^{(r)}_{G_m})(\tfrac{1}{2})^i r, \tag{4}$$

since generation G_{m+2i+1} has been produced by i "selfing" cycles and by one gynogenesis. Hence, its coefficient of inbreeding is

$$F_{G_{m+2i+1}} = 1 - \int_0^1 (1 - F^{(x)}_{G_m})\,(\tfrac{1}{2})^i\, x\, f(x)\, dx, \tag{5}$$

where f is the density function of recombination values. If $F^{(r)}_{G_m} = F_{G_m}$, i.e. it is independent of r, then

$$F_{G_{m+2i+1}} = 1 - \frac{(1 - F_{G_m})\, M(r)}{2^i}. \tag{6}$$

Where M(r) is the mean of recombination frequencies.

Description and characterization of a GS system of inbreeding

a) Coefficient of inbreeding

If G_m is an animal originating from an m-th gynogenetic generation, $F^{(r)}_{G_m} = 1 - r^m$ and hence from (5) we derive

$$F_{G_{m+2i+1}} = 1 - \int_0^1 x^{m+1}\,(\tfrac{1}{2})^i\, f(x)\, dx = 1 - \frac{M(r^{m+1})}{2^i} \tag{7}$$

where $M(r^{m+1})$ is the m + 1-th moment of r.

The coefficient of inbreeding of the S_{m+2i+2} offspring generation is from (1):

$$F_{S_{m+2i+2}} = 1 - \frac{M(r^m)}{2^i}. \tag{8}$$

During the first gynogenetic propagation in the cycles of the GS system it is primarily the homogeneity of genes of low recombination probability that is increased, the second sibmated generation increases the proportion of homozygous states primarily in genes of high recombination probability. It is not necessarily the regularly alternating system which induces maximal increase of the coefficient of inbreeding. This can, however, be determined by considering the distribution probabilites of the recombination frequencies.

Let us suppose that the G_m animal is an m-th generation gynogenetic, the "selfing" cycle has been switched to after an m number of successive gynogenesises. Let c be a sufficiently large fixed number, so that the c-eth generation is derived from sibmating $c = m + 2i + 2$. The number of "selfing" cycles used up to the c-th generation is thus $i = \frac{c - m - 2}{2}$, and the coefficient of inbreeding of this generation from (8) is

$$F_c = 1 - \frac{M(r^m)}{2^{\frac{c-m}{2}}}. \tag{9}$$

The important question is what m value of F will be maximal, where $0 < m > c$. Suppose that F_c, as the function of m, is defined not only on the integers but on the [0, c] closed interval.

The first derivative of F_c, according to m, is obviously zero for those m values belonging to the (0, c) open interval, where F_c has its extreme (if it has any).

That is

$$F'_c = -\frac{M(r^m \ln r)\, 2^{\frac{c-m}{2}} - M(r^m)(-2^{\frac{c-m}{2}} \ln \sqrt{2})}{2^{c-m}} = 0 \tag{10}$$

with rearrangement it takes up the following form:

$$F'_c = -\frac{M(\ln(\sqrt{2}\, r)^{r^m}}{2^{\frac{c-m}{2}}}. \tag{11}$$

This is zero if the numerator is zero, i.e. in an integral form:

$$\int_0^1 \ln(\sqrt{2}\, x)^{x^m} f(x)\, dx = 0. \tag{12}$$

Suppose that this equation holds for $m = m_s$ (0, c). From this expression, on the basis of simple reasoning the following can be stated:

1. F_c, as the extreme of the function of m is independent of c (c is sufficiently large and comes from the sibmated c-th generation).

2. Within the [0, c] closed interval one m_s value at the most may be found. Here the F_c function has its maximum.

3. It follows from statements 1 and 2 that in all S_{m_s+2i+2} generations the coefficient of inbreeding takes up its maximal value.

4. Since m can actually take up only integers, the m value representing the true maximal progress of inbreeding is one of the two integers next to the real number m_s.

5. The necessary, but not sufficient condition for the existence of the m_s extreme on the (0, c) open interval is for

$$\int_0^{\frac{1}{\sqrt{2}}} f(x)\,dx \neq 0 \quad \text{and} \quad \int_{\frac{1}{\sqrt{2}}}^{1} f(x)\,dx \neq 0$$

to hold.

The same in words implies that the necessary condition for the existence of m_s is that the chance of the recombination probability falling below and above $1/\sqrt{2}$ should be qually non-zero.

6. If the recombination probabilities are lower then $1/\sqrt{2}$, the fastest inbreeding can be expected in the case of $m=c$, i.e. successive gynogenesis should be applied throughout.

7. If all recombination probabilities are higher than $1/\sqrt{2}$, the maximal progress of inbreeding can be attained in the case of $m=0$. Breeding should immediately be started by the "selfing" cycle.

The above maximization of the rate of progress of the coefficient of inbreeding refers to the sibmated generation (S_{m+2i+2}) of the "selfing" cycle. The same can be calculated for the gynogenetic generations (G_{m+2i+2}). Then, the $m=m_G$ value representing the maximum of the coefficient of inbreeding is given by the equation:

$$\int_0^1 \ln\left(\sqrt{2}x\right)^{x^{m+1}} f(x)\,dx = 0. \tag{13}$$

Comparing the two integral equations representing the two extremes, m_s and m_G, it can be seen that in the case of the existence of m_G and m_S

$$m_G = m_S - 1. \tag{14}$$

If the coefficient of inbreeding of the gynogenetic generations to be maximized the so-called "selfing" cycle should be started one generation earlier.

b) The degree of genotypic identity in the GS system of inbreeding

S_{m+2i+2} generation. Suppose that in a given parental population, in a given gene, the frequency of heterozygosity (supposing 2 alleles) is q, while that of the two kinds of homozygosity is $(1-q)/2$, respectively. Then the degree of genotypic identity in the biparental offspring generation selected at random from the population is by simple reasoning:

$$1 - q + \tfrac{3}{8}q^2.$$

The S_{m+2i+2} generation derives from G_{m+2i+1} by sibmating. The probability that in a gene of recombination probability r, an individual of a given G_{m+2i+1} population is a heterozygote is obviously

$$q = r^m \left(\tfrac{1}{2}\right)^i r = r^{m+1}\frac{1}{2^i} \tag{15}$$

since heterozygosity persists despite successive gynogenesis, an i number of "selfing" cycles and one more gynogenesis. Thus, in the S_{m+2i+2} offspring generation, the degree of genotypic identity referring to the present gene is

$$I^{(r)}_{S_{m+2i+2}} = 1 - \frac{r^{m+1}}{2^i} + \frac{3}{8}\frac{r^{2m+2}}{2^{2i}}. \tag{16}$$

The same referring to any gene is

$$I_{S_{m+2i+2}} = 1 - \frac{M(r^{m+1})}{2^i} + \frac{3}{8}\frac{M(r^{2m+2})}{2^{2i}}. \tag{17}$$

The degree of the genotypic identity. The G_{m+2i+1} generation is produced by the gynogenetic propagation of one individual of the $S_{m+2(i-1)+2}$ generation. The two individuals selected from the G_{m+2i+1} offspring generation are certainly of the same genotype for a given gene with recombination probability r if the parent is a homozygote. The probability of this is

$$F^{(r)}_{S_{m+2(i-1)+2}} = 1 - r^m\frac{1}{2^i}. \tag{18}$$

If the parent is a heterozygote (probability is 1–(18)), the probability of the genotypic identity of 2 individuals selected from the gynogenetic population is by simple reasoning

$$\tfrac{1}{2} - r + \tfrac{3}{2}r^2. \tag{19}$$

Thus, now without limitations, the degree of genotypic identity in a given gene of r recombination probability is

$$\begin{aligned} I^{(r)}_{G_{m+2i+1}} &= 1 - r^m\frac{1}{2^i} + r^m\frac{1}{2^i}\left(\tfrac{1}{2} - r + \tfrac{3}{2}r^2\right) \\ &= 1 - \frac{1}{2^{i+1}}\left(r^m + 2r^{m+1} - 3r^{m+2}\right). \end{aligned} \tag{20}$$

The same referring to any gene is

$$I_{G_{m+2i+1}} = 1 - \frac{1}{2^{i+1}}\left(M(r^m) + 2M(r^{m+1}) - 3M(r^{m+2})\right). \tag{21}$$

Change of the genetic parameters in the GS system

Based on the nine known recombination probabilities of the carp, both the F_G and I_G values in the cases of successive gynogenesis were estimated (Nagy and Csányi 1982). Since only these nine values are available, the genetic parameters cannot be accurately estimated. As was done in the above-mentioned paper, it is worthwhile to compare the genetic parameters calculated from the distribution of the values of this small sample with values calculated from three different, arbitrarily selected distributions. The density functions of these are of the type $f(x) = Jx^{J-1}$, where $J=0.5$, $J=1$ and $J=2$. The functions and the distribution of the nine recombination frequencies measured are shown in Fig. 3. Calculating the values of F'_c ($c=m+2i+2$) with

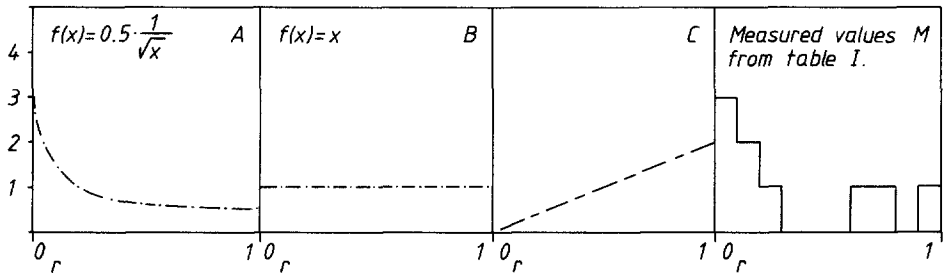

Fig. 3. Density functions of recombination frequencies

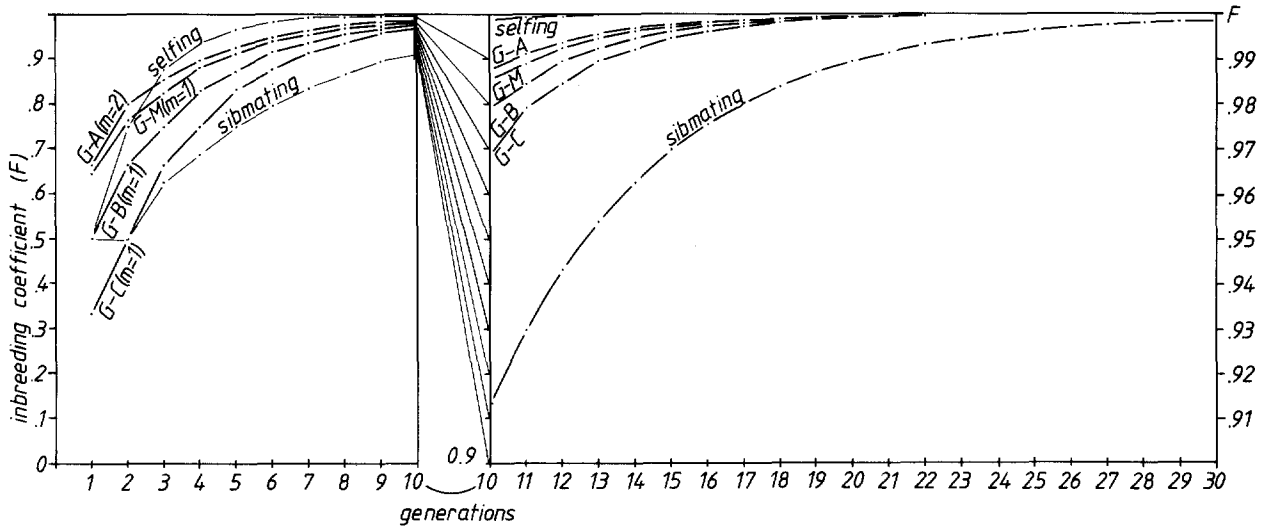

Fig. 4. Variations in the coefficient of inbreeding in various breeding plans, as well as in the case of the GS system with different distributions of recombination frequencies

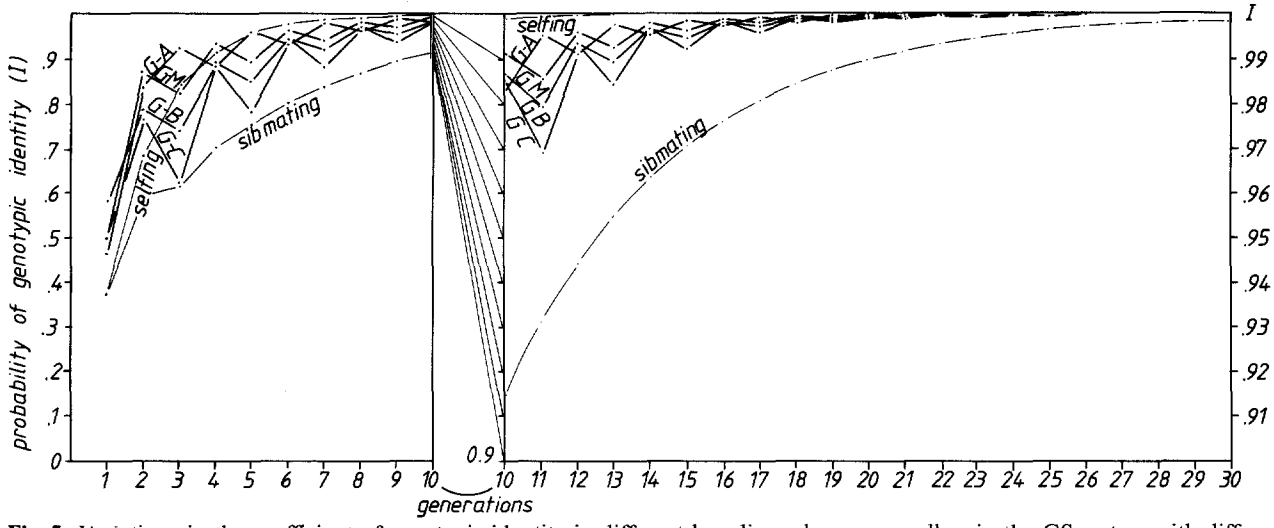

Fig. 5. Variations in the coefficient of genotypic identity in different breeding schemes as well as in the GS system with different distributions of recombination frequencies

these distributions, change sign between m = 1 and m = 2, maximal inbreeding consequently takes place in the case of one of the two values. In both cases, the rates of inbreeding were determined. At three of the four distributions m = 1 represented a somewhat faster progresss, shown in Fig. 4. The degrees of genotypic identity are demonstrated in Fig. 5. They show a characteristic oscillation. The I value of the generations originating from sibmating is lower than that of the preceding gynogenetic generation.

Discussion

Using gynogenesis and sex-reversal, a new breeding technique named GS system has been demonstrated. The essence of the technique is the adequate alternation of gynogenesis and sibmating, for which the coefficient of inbreeding being maximal. This rate considerably exceeds the values characteristic of sibmating and gynogenesis applied separately. Earlier it was shown that no homogeneous strains can be produced solely by gynogenesis. In the first couple of generations, the value of F considerably depends on the recombination probability distributions. Because its value approaches 1 very rapidly, the differences arise from the unlike distributions being significant after the fourth-fifth generation.

As expected, the degree of genotypic identity approaches 1 more slowly in the GS system of inbreeding than in cases of successive gynogenesis. The alternately inserted sibmating causes, namely, Mendelien segregation in the genes remaining heterozygous with high probability by gynogenesis. However, this parameter still exceeds the values obtained for sibmating.

For practical purposes in the GS system, an adequate homogeneity can be probably produced by 6–8 subsequent generations. Nevertheless, it seems that the necessary time can further be decreased. Thus, in the case of most of fish species, including in the carp, there is a significant difference between the time of sexual maturation of males and females. From the males mature sperm can be gathered more earlier than a mature ovum from the female. If the (sex-reversal) gynogenetic males are crossed not with their sisters but backcrossed to the mother, the time of the GS cycle may considerably decrease. A further decrease in the time of the cycle can be obtained by producing hermaphrodites capable of selfing. In our experiment attempting sex-reversal, under certain experimental conditions, hermaphrodite carps could be produced (Nagy et al. 1981). Unfortunately, at present we have no experience whether these hermaphrodites (they were tested at the age of 6 months) will, actually, be capable of selfing. If so, the time of the GS cycle would decrease to the time-span of one generation by their use, since there would be no need of an inserted gynogenesis.

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