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Changes of Genetic Parameters in Successive Gynogenetic Generations and Some Calculations for Carp Gynogenesis

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Summary. In artificial gynogenesis diploidization of the sperm activated zygote is achieved by retention of the second polar body and by this method perfectly normal and fully viable broods can easily be reared. Studying this form of propagation, we determined the coefficient of inbreeding (F), the fixation index (P) and the degree of genotypic identity (I) introduced for characterizing the isogeneity of gynogenetic populations with the help of the parameters of the probability distribution of meiotic recombination between the centromere and the genes.

All three parameters were linear functions of the moments of the distribution of recombination probabilities. The theoretical relationships were investigated in the artificial gynogenesis of the carp.

It appeared without exception that the progress of inbreeding made by gynogenesis largely depends on the distribution of the recombination probabilities of the genes. The rate of increase of the F value was more rapid only in the first few generations (2–5) compared to sibmating. The increase of the F value slowed in subsequent generations and fell significantly below the values obtained by sibmating. The degree of genotypic identity (I) is not as sensitive to the type of the distribution of recombination probability. In the case of gynogenesis, the value of I abruptly converges to 1 much more abruptly than in the case of sibmating.

Used alone, gynogenesis does not enable the production of homozygous strains to any great extent. However, partly heterozygous, but isogenic, linea can be rapidly produced.

Key words: Gynogenesis – Carp – Inbreeding – Recombination in gynogenesis

Introduction

Gynogenesis is a special type of parthenogenesis in the course of which penetration of the spermatozoon into the ovum merely activates the development of the zygote without incorporation its genetic material into the zygote. Each cell of the developing embryo contains chromosomes of exclusively maternal origin. Artificial gynogenesis produced by spermatozoa inactivated by large doses of X-ray radiation was first described by Hertwig in the frog (Hertwig 1911). It has so far been used for determining karyotype (Parmenter 1933), for sex-determination studies (Kawamura 1939) and gene mapping (Volpe 1970; Nace et al. 1970) and for producing inbred strains (Nace 1968, Nagy et al. 1978).

The zygote arising during artificial gynogenesis is usually haploid and is viable only until hatching.

During artificial gynogenesis it seldomly occurs that viable diploid zygotes also arise, either by abnormal cleavage or by the retention of the second polar body, and both maternal chromosome series participate in forming the nucleus of the zygote. Various methods are used for increasing the proportion of diploid individuals: cold shock (Moriwaki 1957), heat shock (Volpe and Dasgupta 1962) and high pressure (Dasgupta 1962). Artificial gynogenesis is particularly effective in the case of the fish (Purdom 1969; Cherfas 1975; Stanley et al. 1975; Nagy et al. 1978).

The genetic homogeneity of the gynogenetic offspring usually increases as follows: let us assume that a given locus in a female providing all chromosomes of the offspring is heterozygous. If the given homologous loci split during the first meiotic division, the diploid produced by the retention of the polar body will be a homozygote corresponding to either of the homologous loci. If, however, splitting of the sister loci occurs, the diploid zygote will be a heterozygote. Splitting of the sister loci is due to the crossing over between the given locus and the centromere. Consequently, the frequency of a heterozygous genotype (assuming that the female has been a heterozygote), in a given gene in a gynogenetic population, is a direct marker of the probability of meiotic recombination between that gene and the centromere (Volpe and Dasgupta 1962). It is obvious, therefore, that determination of the genetic parameters characteristic of an individual or popula-

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tion (e.g. coefficient of inbreeding, fixation index, etc.) requires knowledge of the recombination probabilities involving a greater number of genes.

Though Nace and co-workers, studying the gynogenesis of *Rana pipiens*, attempted to estimate these parameters from data referring to 2–3 genes, their procedure can be criticized from several aspects. They assumed that in measuring chromosomal distances in Morgan's metric the genes are uniformly distributed. Furthermore, lacking the total genome size of the frog, this value was estimated on the basis of measurements accepted for the mouse (Nace et al. 1970).

For the genetic characterization of gynogenetic generations we have chosen a different method which does not rely on the strong assumptions used by Nace and co-workers. Our calculations have been based on the use of the distribution of the recombination probability of genes selected by chance from the genome as a random variable. This distribution can be well estimated with a recombination probability measured in a sufficient number of marker genes. In the carp the recombination probability of 9 genes has so far been described (Cherfas 1977, 1978; Nagy et al. 1978, 1979).

In this study, three genetic characteristics of successive gynogenetic generations, i.e. the coefficient of inbreeding, the fixation index and the degree of genotypic identity have been described.

Results

Definitions of the Studied Parameters

Coefficient of Inbreeding

This coefficient gives the probability that the alleles of any gene of an offspring are identical, i.e. homozygous. If this coefficient is examined in the case of a given gene, this gives the coefficient of inbreeding for the gene. The coefficient of inbreeding of the i-th gynogenetic generation is denoted by F_{Gi}. When referring to single gene of recombination probability r, this denotation will be $F_{Gi}^{(r)}$.

Fixation Index

The fixation index of an offspring generation gives the probability of the exclusive occurrence of only one allele of a given gene. The fixation index of the i-th gynogenetic generation is denoted by P_{Gi}. When referring to a single gene characterized by recombination probability r, it is denoted by $P_{Gi}^{(r)}$.

Degree of Genotypic Identity

This parameter represents the probability with which an identical genotype occurs in a given gene of 2 given individuals of an offspring generation. The degree of genotypic identity of the i-th generation is denoted by I_{Gi}, and $I_{Gi}^{(r)}$ if referring to a locus of recombination probability r.

A Change of the Coefficient of Inbreeding in Successive Gynogenetic Generations

The probability for a gene of recombination frequency r to be heterozygous in the i-th gynogenetic generation is r^i, hence

$$F_{Gi}^{(r)} = 1 - r^i . \tag{1}$$

Let us consider the recombination probability as a random variable, and let us suppose that the density functions of recombination probabilities belonging to the genes is any f(x) function defined on the (0.1) closed interval. Then obviously

$$F_{Gi} = \int_0^1 F_{Gi}^{(x)} f(x)\,dx = \int_0^1 (1 - x^i)\, f(x)\,dx = 1 - \int_0^1 x^i f(x)\,dx = 1 - M(r^i) \tag{2}$$

where $M(r^i)$ is the i-th moment of the random variable of the r recombination probabilities.

B Change of the Fixation Index in Successive Gynogenetic Generations

Fixation will occur in the i-th gynogenetic generation in those genes where the female used for producing the generation was a homozygote. Thus, in the case of a gene of recombination probability r this is

$$P_{Gi}^{(r)} = F_{G(i-1)}^{(r)} = 1 - r^{i-1} \tag{3}$$

and

$$P_{Gi} = \int_0^1 P_{Gi}^{(x)} f(x)\,dx = \int_0^1 (1 - x^{i-1})\, f(x)\,dx = 1 - \int_0^1 x^{i-1} f(x)\,dx = 1 - M(r^{i-1}) . \tag{4}$$

C Change of Genotypic Identity in Successive Gynogenetic Generations

Two individuals selected from the i-th gynogenetic generation in a gene with a given r-recombination probability are of the same genotype if fixation has already occurred in the gene. The probability of this is $P_{Gi}^{(r)}$. If the given gene has still not been fixed (the probability of this is $1 - P_{Gi}^{(r)}$, then both allelic variations (A, a) of the gene occur. The frequency of the genotypes AA, Aa and aa is $\left(\frac{1-r}{2}\right)$, r and $\left(\frac{1-r}{2}\right)$, respectively.

Thus, the degree of genotypic identity of the two selected individuals is

$$\tfrac{1}{2}(1 - r)^2 + r^2 = \tfrac{1}{2} - r + \tfrac{3}{2} r^2 . \tag{5}$$

So

$$I_{Gi}^{(r)} = P_{Gi}^{(r)} + (1 - P_{Gi}^{(r)}) \left(\tfrac{1}{2} - r + \tfrac{3}{2} r^2\right) . \tag{6}$$

From (3)

$$I_{Gi}^{(r)} = 1 - \frac{1}{2} r^{i-1} - r^i + \frac{3}{2} r^{i+1} \quad (7)$$

and

$$I_{Gi}^{(r)} = \int_0^1 I_{Gi}^{(x)} f(x)\,dx = \int_0^1 (1 - \frac{1}{2} x^{i-1} - x^i + \frac{3}{2} x^{i+1}) f(x)\,dx$$
$$= 1 - \frac{1}{2} M(r^{i-1}) - M(r^i) + \frac{3}{2} M(r^{i+1}). \quad (8)$$

$I_{Gi}^{(r)}$, as the function of r (in the case of fixed i), has a minimum in the open interval (0.1), namely

$$r^i = \frac{i + \sqrt{4i^2 - 3}}{3(i+1)}. \quad (9)$$

This means that the value of I^{Gi} in the i-th gynogenetic generation, would be minimal if the recombination probabilities of all the genes equalled r_i.

Hence,

$$\min_{f(x)} I_{Gi} = I_{Gi}^{(r_i)}$$
$$= 1 - \frac{1}{2}\left(\frac{i + \sqrt{4i^2 - 3}}{3(i+1)}\right)^{i-1} - \left(\frac{i + \sqrt{4i^2 - 3}}{3(i+1)}\right)^{i}$$
$$+ \frac{3}{2}\left(\frac{i + \sqrt{4i^2 - 3}}{3(i+1)}\right)^{i+1}. \quad (10)$$

This $\min_{f(x)} I_{Gi}$, as the function of i, depending on F(x), is the lower envelope of all possible I_{Gi} functions. In the i-th gynogenetic generation with any distribution of the recombination probabilities, the degree of genotypic identity is larger or equal to the value of the function taken here (10).

D Estimation of the Studied Parameters in Case of the Gynogenesis of the Carp

Based on the above equations, using the moments of the recombination probability as the random variable, the coefficient of inbreeding, the fixation index and the degree of genotypic identity characteristic of the gynogenetic generations can be determined.

In the carp, so far, the recombination probability of nine different loci have been determined (Cherfas 1977, 1978; Nagy et al. 1978, 1979). These are shown in Table 1. In the case of repeated measurings of individual genes, the average of the measurements for characterizing the recombination probability of the gene was used. Since only nine values are available, the genetic parameters cannot be accurately estimated. However, it is still worthwhile to compare the genetic parameters calculated from the distribution of the value of this small sample with values calculated from three different arbitrarily selected distributions.

The density functions of the selected distributions of the recombination probabilities are of type $f(x) = J\,x^{J-1}$, where J = 0.5, J = 1 and J = 2. The shape, as well as the distribution, of the 9 measured recombination probabilities are shown in Fig. 1. The parameters of F_{Gi}, P_{Gi} and of I_{Gi} corresponding to the individual distributions in the first thirty generations are shown in Figs. 2, 3 and 4, respectively. For comparison, parameters corresponding to selfing and sibmating are given. The case of selfing is a special case based on the

Table 1. Recombination probabilities of some known genes of the carp

Phenotype	Locus	Recombination Probability	Average of measurements
Transferrin	Tr	0.063[a]	
		0.13[b]	0.081
		0.05[d]	
Colour	P	0.12[b]	
	R	0.12[b]	
Scale	S	0.11[b]	
		0.048[d]	0.079
	N	0.973[c]	0.973
Colour	–	0.739[d]	0.739
Shape	–	0.700[d]	0.700
Esterase	S	0.091[d]	0.091
	F	0.284[d]	0.284
			$\bar{r} = 0.354$

[a] Nagy et al. 1978; [b] Nagy et al. 1979; [c] Cherfas 1977; [d] Cherfas 1978

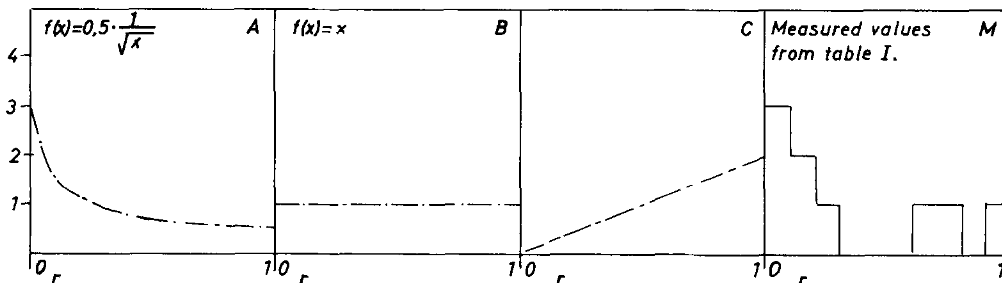

Fig. 1. Density functions of recombination frequencies

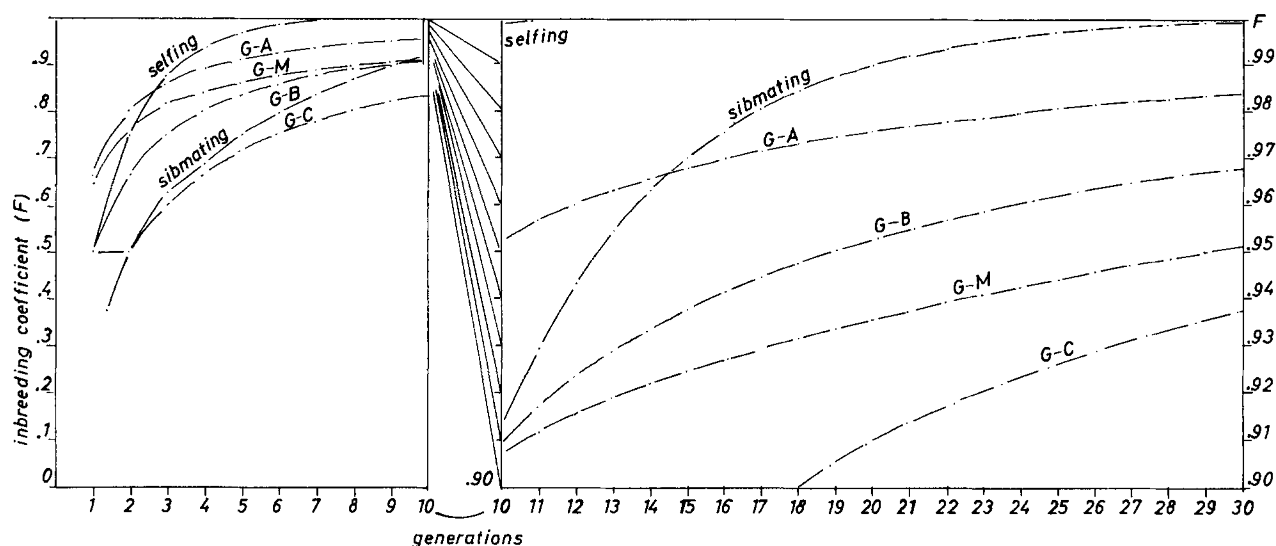

Fig. 2. Change of the coefficient of inbreeding in various breeding plans, as well as in the case of gynogenesis with different distributions of recombination frequencies

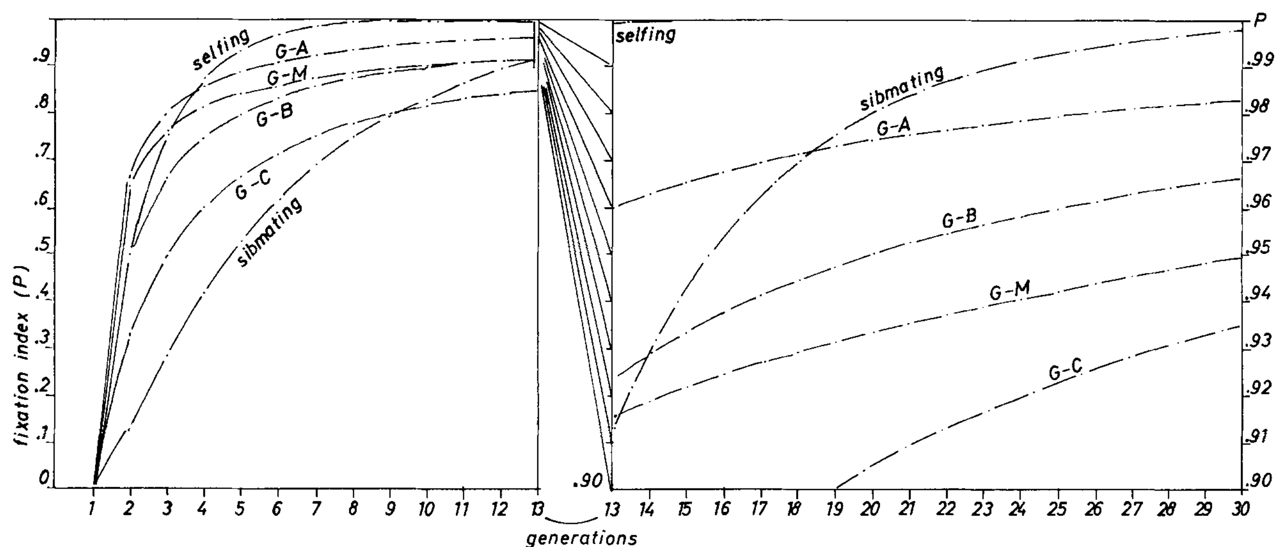

Fig. 3. Change of fixation index in different breeding plans as well as during gynogenesis with different distributions of recombination frequencies

equations $F_i = F_{Gi}^{(0.5)}$ and $I = I_{Gi}^{(0.5)}$. The values belonging to sibmating have been calculated from the coefficient of inbreeding given by Li (1955).

Discussion

The genetic parameters of successive gynogenetic generations which are of primary importance in describing breeding of a closed system were examined: i.e. the coefficient of inbreeding, the fixation index and the index of genotypic identity introduced for characterizing isogeneity. These three parameters of the gynogenetic generations are the linear functions of moments of the recombination probabilities characteristic of genes. The fixation index of a given gynogenetic generation corresponds to the coefficient of inbreeding of the preceeding generation.

Based on the distribution of recombination probabilities known in the carp, as well as on three different arbitrarily selected distribution functions, it has been revealed that the change of the coefficient of inbreeding shows a rapid increase within the first couple of gynogenetic generations; its rate of progress essen-

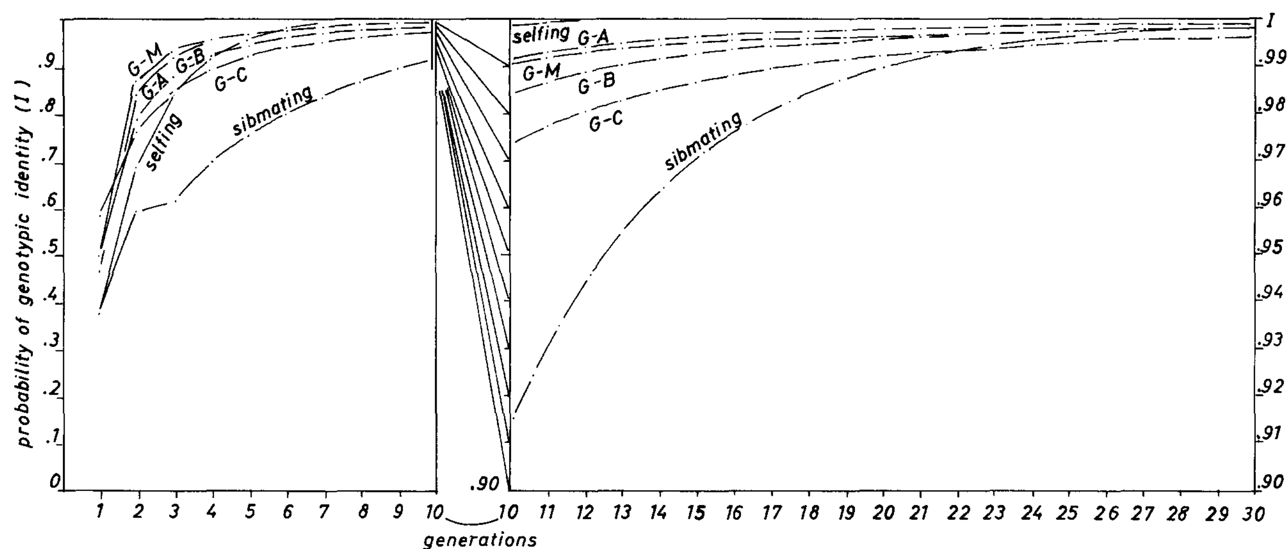

Fig. 4. Change of the probability of genotypic identity (I) in different breeding schemes as well as during gynogenesis with different distributions of recombination frequencies

tially exceeds the values obtained for sibmating. This rate of progress subsequently decreases to a point at which the value of F = 0.9 of the coefficient of inbreeding obtained for sibmating will reach, then surpass, the value characteristic of gynogenesis. As a consequence, if genetically homogeneous strains are to be produced, gynogenesis should be used only in the first few generations. For producing considerably homozygous inbred strains, contrary to foregoing expectations (Stanley and Sneed 1974; Nagy et al. 1978), gynogenezis is not suitable in itself.

The degree of gynogenetic identity, having been introduced for characterizing isogeneity, also increases rapidly in the first few gynogenetic generations, similar to the coefficient of inbreeding. Its rate of progress substantially surpasses the values obtained for sibmating. The decrease in the rate of progress follows only in much later generations. During sibmating, a high value characteristic of the gynogenetic lines is obtained only after 20 to 25 generations, i.e. over a value of I = 0.99. In the successive gynogenetic generations, the degree of genotypic identity is much less sensitive to the distribution of recombination probabilities than the coefficient of inbreeding. So, for example, based on the estimation obtained according to the nine known recombination probabilities of the carp, there is a genotypic identity of 96.8% between two individuals in the fifth gynogenetic generation. Such an increase of identity is due to the relatively constant proportion of the heterozygous states of the genes of a high recombination during the gynogenesis. Therefore, strains obtained by a series of gynogenesis are homozygotes, having a great probability in genes of low recombination frequencies, and they are heterozygotes in genes of high recombination probability. Since, however, the degree of genotypic identity is high, these strains can be used for several practical purposes, e.g. for producing heterosis hybrids.

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Literature

Cherfas, N.B. (1975): Investigation of radiation induced diploid gynogenesis in the carp (*Cyprinus carpio* L.). 1: Experiments on obtaining the diploid gynogenetic progeny in mass-quantities. Genetika **9/7,** 78–86 (in Russian)

Cherfas, N.B. (1977): Investigation of radiation induced diploid gynogenesis in the carp (*Cyprinus carpio* L.). 2: Segregation with respect to certain morphological characters in gynogenetic progenies. Genetika **13/5,** 811–820 (in Russian)

Cherfas, N.B. (1978): Investigation on radiation induced diploid gynogenesis in the carp (*Cyprinus carpio* L.). 3: Gynogenetic offspring analysis by biochemical markers. Genetika **14/4,** 599–604 (in Russian)

Dasgupta, S. (1962): Induction of triploidity by hydrostatic pressure in the Leopard frog (*Rana pipiens*). J. Exp. Zool. **151,** 105–116

Hertwig, O. (1911): Die Radiumkrankheit tierischer Keimzellen. Arch. Mikrosk. Anat. Entwicklungsmech. **77,** 1–97

Kawamura, T. (1939): Artificial parthenogenesis in the frog. 1: Chromosome numbers and their relation cleavage histories. J. Sci. Hirosima Univ., Ser. B **17,** 39–45

Li, C.C. (1955): Population Genetics. Chicago: Univers. Chicago Press

Moriwaki, T. (1957): Studies on matured parthenogenetic frogs. 1: The development and the reproductive ability. J. Sci. Hirosima Univ. Ser. B1 **17,** 13–22

Nace, G.W. (1968): The Amphibian Facility of the University of Michigan. Bioscience **18/8,** 767–775

Nace, G.W.; Richards, C.M.; Asher, Jr. J.H. (1970): Parthenogenesis and genetic variability. 1: Linkage and inbreeding estimations in the frogs (*Rana pipiens*). Genetica **66,** 349–368

Nagy, A.; Rajki, K.; Horváth, L.; Csányi, V. (1978): Investigation on carps (*Cyprinus carpio* L.) gynogenesis. J. Fish Biol. **13,** 215–224

Nagy, A.; Rajki, K.; Bakos, J.; Csányi, V. (1979): Genetic analysis and mapping by the help of gynogenesis in carp. Heredity **43,** 35–40

Parmenter, C.L. (1933): Haploid, diploid, triploid and tetraploid chromosome numbers and their origin in parthenogenetically developed larvae and frogs on *Rana pipiens* and *Rana palustris.* J. Exp. Zool. **66,** 409

Purdom, C.E. (1969): Radiation-induced Gynogenesis and in fish. Heredity **24,** 431–444

Stanley, G.J.; Sneed, K.E. (1974): Artificial gynogenesis and its application in genetics and selective breeding of fishes. In: Early life history of fish (ed. Blaxter, J.H.S.), pp. 527–536. Berlin, Heidelberg, New York: Springer

Stanley, J.G.; Marti, J.M.; Jones, J.B. (1975): Gynogenesis as a possible method for producing monosex grass carp (*Ctenopharingodon idella*). Prog. Fish Cult. **37,** 25–26

Volpe, E.P. (1970): Chromosme mapping in the leopard frog. Genetics **64,** 11–21

Volpe, E.P.; Dasgupta, S. (1962): Gynogenetic diploids of mutant leopard frogs. J. Exp. Zool. **151,** 287–301

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