POPULATION GENETICS OF *LEBISTES (POECILIA) RETICULATUS*
PETERS (POECILIIDAE; PISCES). I. EFFECTS OF RADIATION-
INDUCED MUTATIONS ON THE SEGREGATION RATIO
IN POSTIRRADIATION F₂

JOHANNES HORST SCHRÖDER AND SEBASTIAN HOLZBERG

*Institute of Biology, Association for Radiation and Environmental Research,
D-8042 Neuherberg near Munich, Germany¹*

ABSTRACT

Wild-type germ cells of the viviparous fish, *Lebistes (Poecilia) reticulatus*, were irradiated in the stages of oögonia, spermatogonia or spermatozoa with 1000 R of X rays at a dose-rate of 200–220 R/min. The grown-up fish were mated after irradiation or sham treatment to unirradiated mutant fish homozygous for three recessive gene loci, causing albinism, lack of yellow and reddish pigmentation ("blue" constitution), and diminution of the size of melanophores ("blond" constitution). As compared to the control series, the phenotypic segregation ratios in the adult F₂ generation after irradiation was changed significantly in favour of wild-type and blue fish, whereas the proportion of albinotic, blond and "white" (= blond + blue) guppies was lowered. This unidirectional effect in postirradiation F₂ was greatest after exposure of spermatozoa with 2×500 R (24 hr apart), followed by single doses of 1000 R to spermatogonia and oögonia. These results were explained in terms of a synergistic interaction of recessive radiation-induced mutations in the heterozygous state with those genotypes of the guppy which affect the formation of melanophoric patterns and the synthesis of melanins.

SYNERGISTIC interactions between certain genotypes and newly arisen mutations are known from experiments with *Drosophila* (DOBZHANSKY, SPASSKY and TIDWELL 1963; KITAGAWA 1967; MUKAI 1969). As far as fish are concerned, papers dealing with differences of mutational response to radiation referred only to the different degree of homozygosity which was already present in the fish prior to irradiation (SCHRÖDER 1967 and 1969). Thus, the present paper is the first report on synergistic interaction of distinct genotypes of teleosts with recessive mutations induced by irradiation with X rays.

MATERIALS AND METHODS

An outbreeding technique was chosen which allowed to distinguish between F₂ animals derived from replicas of irradiated P germ cells and those derived from untreated genomes. Wild-type guppies ($a^+a^+ b^+b^+ r^+r^+$) of the "Istanbul" inbred stock were irradiated as neonatal (corresponding to an irradiation of spermatogonia or oögonia, respectively) or adult male fish (exposure of spermatozoa). In the latter case, the irradiated males were mated to untreated females immediately after the second exposure for a limited period of 20 days to ensure that

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FIGURE 1.—The breeding scheme as applied for the detection of possible differences in the F_2 segregation ratio after irradiation.

only irradiated spermatozoa fertilized the eggs. The fish irradiated as neonatal ones were mated to untreated animals after reaching their sexual maturity at about 90 days (3 months) of age. As unirradiated mates, always "albino-white" guppies were used which were not related to the wild-type Istanbul stock. The albino-white fish are homozygous for three recessive genes ($aa\ bb\ rr$), situated on three non-homologous autosomes. Albino-white guppies do not produce yellow and reddish colour because of their homozygosity for "blue" (rr). Because of albinism (aa), they possess red eyes and lack most of the blackish pigmentation patterns. The remaining melanophores are reduced in size as a result of their "blond" constitution (bb). Apart from the possible occurrence of specific locus mutations in the F_1 generation, all offspring of the cross

$$a+a+b+b+r+r+ \times aa\ bb\ rr$$

must be wild-type fish ($a+a+b+b+r+r$). In the F_2 , however, a segregation ratio of 27 wild-type : 15 albino : 9 blue : 9 blond : 3 white (= blond + blue) : 1 albino-white was expected to appear as shown in Figure 1.

The fish were classified for the different phenotypes at adolescence, i.e., at an age of about 90 days. For the search of anomalies, however, the guppies were examined and counted both at birth and at sexual maturity. They were sexed when about three months old. The fish assigned for further breeding were separated into females and males as early as possible, i.e., immediately after beginning of the transformation of the anal fin into the male gonopodium. Each female was then mated to one male, and couples remained isolated for the duration of the breeding period.

Fry, mated couples, dead and newborn fish were routinely checked by daily examination of all the basins. Because some broods took several hours to be born and several females were occasionally cannibalistic, all newborn fish were removed from their parents as they were born. They were provisionally kept in small glass vessels and later transferred to permanent aquaria. In addition, water plants such as submersed moss were grown in the basins where births were anticipated, to enable newborn fish to hide from their parents. The deaths occurring between birth and the age of 90 days have been taken into account for estimating post-natal mortality. Juve-

nile and adult fish that disappeared within the postnatal 90-days-interval without leaving dead bodies were excluded.

The whole-body irradiation of the fish was carried out with a 300 kVp X-ray machine. During exposures, the fish were kept in small plastic dishes of 16 cm diameter, containing Munich fresh water at a depth of 2 cm at a temperature between 21 and 24°C. The radiation conditions were as follows: 300 kVp; 10 mA with 0.6-mm copper and 1.0-mm aluminum filtration; dose-rate 200–220 R/min, measured directly in the irradiated water by Victoreendosimeter. The focus-target distance was 30 cm. The X-ray doses of 1000 R to oogonia or spermatogonia and of 2×500 R (24 hr apart) to spermatozoa, respectively, were applied to unnarcotized fish in groups of 10–20 individuals, all from the same litter. With the exception of irradiation, the sham-treated controls were handled in the same manner. After irradiation or sham treatment, the animals were kept in aquaria of 25 or 50 l water content at a room temperature of $27 \pm 3^\circ\text{C}$. The fish lived in slightly brackish water of about 0.1% sea-salt concentration, obtained by addition of an artificial marine salt-mixture to Munich tap water. The illumination was set automatically to a 12-hr rhythm by daylight imitation.

The following four treatment groups could be distinguished from each other: Sham-treated controls (C), 1000 R to oogonia (OOG), 1000 R to spermatogonia (SPG), and 2×500 R to spermatozoa (SPZ). To evaluate the significance of differences between these treatment groups, some calculations were performed. For the comparison of the six phenotypes occurring in F_2 and of the litter size in F_1 and F_2 , analysis of variance was used according to WEBER (1967). The sex ratios in the different treatment groups were compared by the application of chi square in a 2×2 table according to GUILFORD (1956). All the further traits such as the relative incidence of certain phenotypes or of viability characters (stillbirths, postnatal deaths, anomalies) were compared using the chi square method with or without YATES' correction.

RESULTS

Segregation ratios in postirradiation F_2 generations: The expected segregation ratio in F_2 with respect to the six different phenotypes was 42.19% wild type ($a^+ b^+ r^+$) : 23.44% albino ($aa b^+ r^+$; $aa bb r^+$; $aa b^+ rr$) : 14.06% blue ($a^+ b^+ rr$) : 14.06% blond ($a^+ bb r^+$) : 4.69% white ($a^+ bb rr$) : 1.56% albino-white ($aa bb rr$) fish (Figure 1). The difference between theoretical and control F_2 segregation ratios was highly significant ($\chi^2 = 89.7$; d.f. = 5; $P \ll 10^{-10}$ for females and males; $\chi^2 = 65.1$; d.f. = 5; $P < 10^{-10}$ for females only; and $\chi^2 = 44.2$; d.f. = 5; $P = 2 \times 10^{-8}$ for males only). This was mainly due to a considerably lower proportion of albinotic fish in control F_2 than expected from theoretical segregation ratio. There is no doubt that albinotic guppies of each genotype are less viable than non-albinotic sibs (Figure 2).

After irradiation of oogonia, spermatogonia or spermatozoa with 1000 R of X rays, the F_2 segregation ratio was changed in favour of wild-type fish as compared to the controls (Table 1). In the same ratio as the proportion of wild-type fish increased after irradiation, the percentage of albinotic guppies decreased (cf. the upper two lines of Table 1). A rank of increasing effectiveness in benefitting the wild-type guppies and lowering the proportion of albinotic ones could be observed from oogonia via spermatogonia to spermatozoa. The influence of ancestral irradiation upon phenotypic segregation ratios in F_2 thus depended on the stage of gametogenesis under investigation. It could be expressed both by the relative increase of wild-type and blue fish and by the complementary decrease of albinotic, blond and white fish. By this means the proportion of all the fish

FIGURE 2.—The frequency distribution for the six F_2 phenotypes as expected according to the Mendelian laws, as obtained for the controls and after irradiation of oogonia, spermatogonia and spermatozoa with 1000 R of X rays.

carrying mutant genes which affect the synthesis of melanins (albino, blond and white fish) decreased while that of wild-type and blue fish without any influence on the synthesis of melanins increased. However, these shifts of splitting into the six phenotypes were somewhat different for females and males (Figure 2, Table 1).

If one compares the 4 treatment groups (C, OOG, SPG, SPZ) to each other by analysis of variance referring to adult animals, significant differences between any irradiated line and the control were obtained (Table 2). Considering the deviation between the three treated lines to each other, significant differences were found to have established between the irradiation of oogonia and spermatogonia or spermatozoa. However, no significant difference was determined between 1000 R to spermatogonia and 2×500 R to spermatozoa.

Viability characters in F_1 and F_2 : As to viability characters, no significant differences between the various treatment groups were found in F_1 . This refers to the frequency of stillbirths, anomalies, postnatal mortality, and sex-ratio (Table 3).

TABLE 1

The segregation ratios of the six phenotypes in F_2 of the cross wild type ($a^+a^+ b^+b^+ r^+r^+$) $\times$ albino-white ($aa\ bb\ rr$) guppy as obtained for the four different treatment groups

Phenotypic constitution	Treatment group							
	Control		1000 R to oogonia		1000 R to spermatogonia		500 + 500 R to spermatozoa	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
FEMALES and MALES								
wild type	342a,b	52.86	266	61.57	1159a	69.82	93b	73.81
albino	52c	8.04	34	7.87	113	6.81	3c	2.38
blue	98d	15.15	72	16.67	283	17.05	29d	23.02
blond	113ef	17.47g	43f	9.95	55e	3.31	0	0.00g
white	34h,i	5.26j	11h	2.55	16i	0.96	0	0.00j
albino-white	8	1.24	6	1.39	34	2.05	1	0.79
FEMALES								
wild type	176k	58.09	140	66.67	613k	74.67	39	70.91
albino	21l	6.93	17	8.10	33l	4.02	1	1.82
blue	28m,n	9.24	25	11.90	133m	16.20	15n	27.27
blond	58o,p	19.14q	21o	10.00	24p	2.92	0	0.00q
white	17r,s	5.61	4r	1.90	6s	0.73	0	0.00
albino-white	3	0.99	3	1.43	12	1.46	0	0.00
MALES								
wild type	166t,u	48.26	126	56.76	546t	65.08	54u	76.06
albino	31	9.01	17	7.66	80	9.54	2	2.82
blue	70	20.35	47	21.17	150	17.88	14	19.72
blond	55v,w	15.99	22	9.91	31v	3.69	0w	0.00
white	17x	4.94	7	3.15	10x	1.19	0	0.00
albino-white	5	1.45	3	1.35	22	2.62	1	1.41

a — x: significant differences ($P < 0.05$).

In F_2 , however, the percentage of stillbirths increased significantly after 2×500 R to spermatozoa. The postnatal mortality was significantly higher after 1000 R to oogonia and spermatogonia as compared to that of the control F_2 . The overall sex ratio was not affected in either case (Table 4).

If one compares the figures in Tables 1 and 4, some inconsistency may be observed. This is because some of the experimental series were not yet analyzed

TABLE 2

Test of significance for the differences between the F_2 segregation ratios of the four different treatment groups (Control, 1000 R to oogonia, 1000 R to spermatogonia, and 2×500 to spermatozoa of the parental generation) as performed by an analysis of variance

Results of the analysis of variance	Compared treatment groups					
	OOG vs. C	SPG vs. C	SPZ vs. C	OOG vs. SPG	OOG vs. SPZ	SPG vs. SPZ
F-value	12.312	87.319	23.637	69.167	7.990	1.183
P-value	<0.005	$\ll 0.005$	$\ll 0.005$	$\ll 0.005$	<0.005	>0.05

TABLE 3

The frequency of viability characters (stillbirths, postnatal deaths, anomalies and sex-ratio) in F_1 as obtained for the four different treatment groups

Viability character	Treatment group (F_1)			
	Control	1000 R to oogonia	1000 R to spermatogonia	500 + 500 R (24 hr apart) to spermatozoa
Stillbirths				
Number	1/111	0/25	0/239	0/94
Percent*	0.90	0.00	0.00	0.00
Postnatal mortality				
Number	0/111	0/25	10/239	0/94
Percent†	0.00	0.00	4.18	0.00
Anomalies				
Number (♀ ♀ + ♂ ♂)	0/56	0/23	0/220	0/68
Percent (♀ ♀ + ♂ ♂)‡	0.00	0.00	0.00	0.00
Anomalies				
Number (♀ ♀)	0/32	0/13	0/111	0/34
Percent (♀ ♀)§	0.00	0.00	0.00	0.00
Anomalies				
Number (♂ ♂)	0/24	0/10	0/109	0/34
Percent (♂ ♂)¶	0.00	0.00	0.00	0.00
Sex ratio				
Number of ♀ ♀	32	13	111	34
Number of ♂ ♂	24	10	109	34
♂ ♂				
♀ ♀ + ♂ ♂	0.4286	0.4348	0.4955	0.5000

* Stillbirths in percent of living neonatal fish.

† Postnatal deaths (within 90 days *post natum*) in percent of living neonatal fish.

‡ Abnormal fish (skeletal curvatures, dwarfs and giants, swim-bladder defects, etc.) in percent of adult ♀ ♀ + ♂ ♂.

§ Abnormal ♀ ♀ in percent of adult ♀ ♀.

¶ Abnormal ♂ ♂ in percent of adult ♂ ♂.

quantitatively with regard to the phenotypes of the adult fish, whereas all data are included referring to the newborn ones.

With respect to litter size, comparisons were made between C *versus* OOG, SPG and SPZ (Table 5). A decrease of litter-size could be confirmed statistically for the 2nd litters in F_1 after 1000 R to oogonia and after 2×500 R to spermatozoa in F_2 . For the 3rd litters in F_1 , a significant increase of litter size was observed after 2×500 R to spermatozoa. In the F_2 , however, the litter size was reduced significantly from 17.7 in the controls to 8.92 in SPZ for the 3rd litters. For the 6th litters, a further significant decrease was found in the F_2 of the OOG series. As far as the pooled data of the 1st to 6th litters are concerned, a significant decrease could be found only for the F_2 generation after 2×500 R to spermatozoa. Thus, the maximum effect on the segregation ratio of the six phenotypes after irradiation of spermatozoa was associated with an apparently recessive radiation damage expressed by a significant lowering of the litter size.

TABLE 4

The frequency of viability characters (stillbirths, postnatal deaths, anomalies and sex-ratio) in F₂ as obtained for the four different treatment groups

Viability character	Treatment group (F ₂)			
	Control	1000 R to oögonia	1000 R to spermatogonia	500 + 500 R (24 hr apart) to spermatozoa
Stillbirths				
Number	0/785a	0/532	7/1904	13/595a
Percent*	0.00	0.00	0.37	2.18
Postnatal mortality				
Number	67/785b,c	66/532b	283/1904c	67/595
Percent†	8.54	12.41	14.86	11.26
Anomalies				
Number (♀ ♀ + ♂ ♂)	5/616	2/438	24/1406	5/242
Percent (♀ ♀ + ♂ ♂)‡	0.81	0.46	1.71	2.07
Anomalies				
Number (♀ ♀)	4/284	2/211	14/699	4/106
Percent (♀ ♀)§	1.41	0.95	2.00	3.77
Anomalies				
Number (♂ ♂)	1/332	0/227	10/707	1/136
Percent (♂ ♂)¶	0.30	0.00	1.41	0.74
Sex ratio				
Number of ♀ ♀	284	211	699	106
Number of ♂ ♂	332	227	707	136
♂ ♂				
♀ ♀ + ♂ ♂	0.5390	0.5183	0.5028	0.5620

* Stillbirths in percent of living neonatal fish.

† Postnatal deaths (within 90 days *post natum*) in percent of living neonatal fish.

‡ Abnormal fish (skeletal curvatures, dwarfs and giants, swim-bladder defects, etc.) in percent of adult ♀ ♀ + ♂ ♂.

§ Abnormal ♀ ♀ in percent of adult ♀ ♀.

¶ Abnormal ♂ ♂ in percent of adult ♂ ♂.

a - c: significant differences ($P < 0.05$).

DISCUSSION

Although the question of how to explain the changes of segregation ratios in F₂ after irradiation remains open, two possible mechanisms could be considered to perhaps explain the relative decrease of albinotic, blond and white fish and the complementary increase of wild-type and blue F₂ guppies.

First, it might be suggested that some *heterotic effects* due to overdominance of newly arisen mutant genes favour the wild-type fish when heterozygous for the new mutations as known from experiments with *Drosophila* (MUKAI *et al.* 1954 and 1965; WALLACE 1963). Second, radiation-induced mutations may be assumed to *interfere* in some way with the different F₂ phenotypes causing different survival (DOBZHANSKY *et al.* 1963; KITAGAWA 1967; MUKAI 1969 b).

As to the first assumption, wild-type and mutant F₂ guppies can be distinguished from each other by the number of autosomes exposed in immature or

TABLE 5

The mean litter size of the four different treatment groups for the 1st to 6th litters in F₁ and F₂ generations

Rank of successive litters	Mean litter size of the 4 treatment groups							
	Control		1000 R to oogonia		1000 R to spermatogonia		500 + 500 R (24 hr apart) to spermatozoa	
	F ₁	F ₂	F ₁	F ₂	F ₁	F ₂	F ₁	F ₂
1st litters	13.00	9.21	4.33	12.56	9.50	13.23	10.67	5.55
2nd litters	19.33a	19.67b	6.00a	12.63	26.75	15.00	17.00	8.35b
3rd litters	1.00c	17.70d	—	24.00	18.00	18.18	22.00c	8.92d
4th litters	—	24.89	—	15.25	4.50	18.67	6.00	9.27
5th litters	—	6.50	—	37.00	12.00	24.15	—	9.88
6th litters	—	6.00e	—	2.00e	19.00	18.33	—	6.75
All litters	13.88	16.35f	5.00	17.16	15.93	17.15	13.43	7.83f

a - f: significant differences ($P < 0.05$).

mature germ cells of the P generation. These non-homologous autosomes were represented by three different linkage groups (a^+ , b^+ and r^+). According to the Mendelian laws, about 96% (26 out of 27, cf. Figure 1) of all F₂ wild-type fish were expected to be heterozygous for at least one mutant allele (a , b , or r). Furthermore, the two original guppy stocks which were used as parental generations (P), had been inbred since *ca.* 40 (wild-type "Istanbul" stock) or at least 10 (albino-white stock) generations, respectively. Accordingly, a relatively high degree of heterozygosity was already ensured to occur among the interstrain F₂ offspring without any mutagenic treatment of the P germ cells. Thus, any additional effect on the segregation ratio in postirradiation F₂ generations must be due to the action of radiation-induced mutations under the conditions of a heterozygous genetic background. These radiation-induced mutations could be thought to exert beneficial influences on their heterozygous carriers even when they behave as detrimental or even lethals in the homozygous state. If so, radiation-induced recessive damage must become detectable in postirradiation F₂ as compared to the control F₂. This was found to be true for all the treatment groups, because either the postnatal mortality was enhanced as shown for the exposure of oogonia or spermatogonia, or the mean litter size was reduced as found for spermatozoal irradiation (cf. Tables 4 and 5). However, any heterotic effect is expected to become visible not only in postirradiation F₂ generation, but also to a much higher extent in postirradiation F₁ (cf. Table 3). This is because F₁ fish were expected to possess a higher degree of heterozygosity for radiation-induced mutations than post-irradiation F₂ offspring. Apart from a non-significant increase of litter size after 1000 R to spermatogonia relative to the control (cf. Table 5), no further beneficial effect in post-irradiation F₁ was discovered. Moreover, the increase of blue fish in F₂ after irradiation could not be explained in terms of overdominance of radiation-induced mutations, because blue fish were expected to contain the same average number of replicas of autosomes exposed in P germ-cells as their blond sibs.

Looking at the possible interference of radiation-induced mutations with the viability of the different F_2 phenotypes, at first let us confine ourselves to the ratio of wild-type to blond fish, because this ratio was changed in any case as compared to the controls. The average wild-type guppy in F_2 is expected to be distinguished from its blond (bb) sib by the possibility that replicas of 22 autosomes exposed in P germ cells would combine in wild-type F_2 fish as compared to only 21 autosomes in blond F_2 fish according to the total number of 23 chromosomes of the haploid set of *Lebistes* (Post 1965). Twenty-two of these chromosomes are acting as autosomes while the remaining one is the sex chromosome (X or Y, respectively). Consequently, the probable difference for homozygous lethal and/or detrimental mutations between wild-type and blond F_2 individuals would be too small to be responsible for the differential viability of the two phenotypes. In addition, the proportion of blue F_2 fish did not decrease though this category possessed the same average number of chromosomes derived from irradiated ones as its blond sibling. Accordingly, one must conclude that the different survival of the two F_2 phenotypes in question is likely to be associated with an influence of the radiation-induced mutations on certain genotypes. The synergistic action of the radiation-induced mutations must be strongly restricted to those genotypes which affect the synthesis of melanins or melanophoric patterns (albino, blond and white). Because of the high probability of being heterozygous in F_2 , these radiation-induced mutations are suggested to interfere with the F_2 phenotypes more likely in *heterozygous* than in homozygous condition. In other words, heterozygosity for radiation-induced recessive mutations is assumed to exert a deleterious effect on the viability of albinotic, blond and white F_2 fish, the viability of which is already poor without any mutagenic treatment of their ancestors. If so, even if the average viability of wild-type and blue fish containing replicas of irradiated autosomes is actually decreased, their segregation ratio becomes relatively better than the same genotype in the control.

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