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Differential Response to Irradiation in Offspring of Freshwater and Seawater Substrains of *Poecilia (Lebistes) reticulata* Peters in the "Guppy Male Courtship Activity Test"*

O.H. Spieser
Abteilung für Toxikologie

J.H. Schröder
Institut für Biologie, Gesellschaft für Strahlen- und Umweltforschung, Munich (FRG)

Summary. An inbred strain ("Istanbul") of guppies was divided into two substrains, one of which was maintained and bred in seawater. The offspring of irradiated animals of both substrains (1000 R X-rays to spermatogonia and oogonia of neonatal fish) were tested in a new "gruppy male courtship activity test" and compared with control groups. The postirradiation response in the seawater substrain was more intense than in the freshwater substrain.

Key words: Courtship-behaviour - *Poecilia reticulata* - Mutagenicitytesting

Introduction

In the framework of an extensive study dealing with the radiation-induced mutability of polygenically determined morphological characters of teleostean fish (Schröder 1969a, b), the mutagenic response of quantitative behavioural patterns to ionizing radiation was also examined. Previous results on mutational changes concerning the agonistic behaviour of convict cichlids (Holzberg and Schröder 1975) and laboratory mice (Schröder 1977a, b) encouraged the present authors to investigate the mutability of the courtship behaviour of poeciliid fish, known to be determined by polygenes (Franck 1970). However, the quantitative characters of teleosts are not only of a hereditary nature, but are also influenced by environmental factors such as temperature and salinity (Schmidt 1919, 1920). Thus, the average number of dorsal fin rays decreased with decreasing temperature in *Poecilia reticulata* (Schmidt 1919), and the mean vertebral number also decreased with decreasing salinity in *Zoarces viviparus* (Schmidt 1920). The question was asked by the present investigators whether the manifestation of mutations of quantitative behavioural traits depends in a similar manner on the environmental salinity. Thus, a comparison was made between the offspring of irradiated and control guppies of the same strain under seawater and freshwater conditions.

Courtship activity of male guppies has proven to be influenced by several factors. Seghers (1974) and Farr (1975) found it to be correlated with the presence of different types of predators, and Farr and Herrnkind (1974) observed a correlation with population density. Lang (1970) described an influence of lunar periodicity on colour-sensitivity. So it seemed necessary to find out a quick method for testing courtship behaviour of isolated male guppies (Guppy Male Courtship Activity Test) which could also be used for testing mutagenicity.

Material and Methods

Origin of Fish

For these experiments, the inbred strain "Istanbul" was used. This guppy strain has been bred and maintained by "collective breeding" since the early fifties when it was imported from Istanbul to Hamburg by Prof. Dr. Dr. h.c. mult. C. Kosswig. Though only random mating occurred, the strain was reduced from time to time to only one or a few pairs of founder fish, thus ensuring a relatively high degree of inbreeding.

The strain was divided into two subtrains: One substrain was maintained in normal Munich tap water ("freshwater guppies"), whereas the other one was bred and maintained in artificially obtained seawater of a salt concentration of 33.3‰ ("seawater gup-

* Dedicated to Prof.Dr.Dr.h.c.mult. Curt Kosswig on the occasion of his 75th birthday

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pies"). Compared with the freshwater guppies, the seawater substrain was a poor breeder and produced less young per brood.

The guppy strain "Istanbul" is characterized by a normal wildtype coloration of all fish as well as by two gonosomal dominant marker genes; the Y-linked gene 'double-sword', Ds, causing the dorsal and ventral elongation of the caudal fin, and the X-linked marker, Cp, which is responsible for the pigmentation of the caudal fin and caudal peduncle. If together in the same genotype ($X_{Cp}Y_{Ds}$), both markers interact in forming the typical fan-shaped pigmented fail fin of this strain. Cp can be exchanged by crossing-over between the Y and X chromosomes resulting in the Y-linkage (Y_{Cp+Ds}) of this character (Dzwillo 1959).

Maintainance of the Fish

Apart from the fresh- and seawater conditions, all fish were bred and maintained in the same environment. The predominantly artificial illumination was set automatically on a 12-12-hour photoperiod, and temperature varied between 22-24°C. All broods were raised separately in tanks of 10 or 25 liters of water without any gravel, but provided with floating water plants. All aquaria were artificially aerated and pH was about 7.0. The fish were fed *Artemia salina, Tubifex* worms, and TETRAMIN(R).

Irradiation

All fish were irradiated or sham-treated on the day of birth, and upon becoming sexually mature, they were individually mated to sibs of the same brood. Because irradiated females were mated to irradiated brothers, the genetically effective radiation dose in irradiated F_1 (I - F_1) was the sum of the paternal and maternal radiation dose; i.e., 1000 + 1000 R of X-rays. To produce the corresponding irradiated F_2 (I - F_2), I - F_1 fish of the same litter were mated to each other. For the control series, however, unirradiated fish were sib-mated in the same manner to obtain control-F_1 (C - F_1) and control-F_2 (C - F_2) offspring (Fig.1).

The whole-body irradiation of the fish was accomplished with a 300 kvp X-ray machine. During irradiation exposure, the fish were kept in small plastic dishes of 16 cm diameter, containing Munich tap water or artificially produced seawater (33‰ salinity) to 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 aluminium filtration; doserate 200 - 220 R/min, measured directly in the irradiated water by Victoreen-dosimeter. The focus-target distance was 30 cm. The X-ray dose of 1000 R was applied to unnarcotized fishes in groups of 10 - 20 guppies, all from the same brood. Except for irradiation, the sham-treated controls were handled in the same way as the irradiated fish.

Testing Male Courtship Activity

For the guppy male courtship activity test, the fish were placed into cuboidal aquaria with a side length of 20 cm, each tank containing only one pair. They were allowed to adapt to this situation for at least one day. Each of the males was observed for about twelve sessions at a temperature of 27°C. Only one session per day was conducted with each animal, each session lasting 10 minutes. Two hours before each session, the female was removed from the aquarium; i.e., the test male was isolated for two hours. During the session, a test female was then placed into a glass ball (5 cm in diameter) which was fixed in the middle of the aquarium containing the male to be tested (Fig.2). Several females were used throughout all the test series, but the same female was offered to both I and C males of a given series.

By this means, visual, but no olfactory and gustatory contacts between the female and the male to be

1000 R to		1000 R to	Sham-treatment of		
P♀	×	P♂	P♀	×	P♂
I - F_1♀	×	I - F_1♂	C - F_1♀	×	C - F_1♂
I - F_2♀	×	I - F_2♂	C - F_2♀	×	C - F_2♂

Fig.1. Origin of the F_1 and F_2 generations of the irradiated and control series

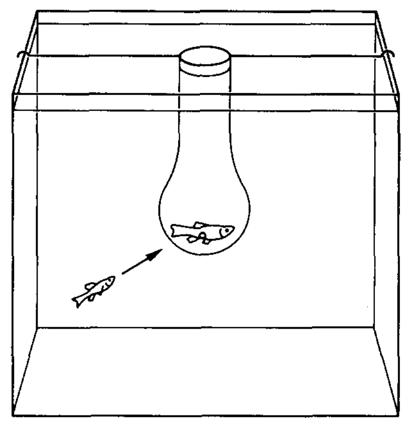

Fig.2. The test arrangement. In the center a glass vessel containing the test female. Male movements directed towards the female; "approaching"

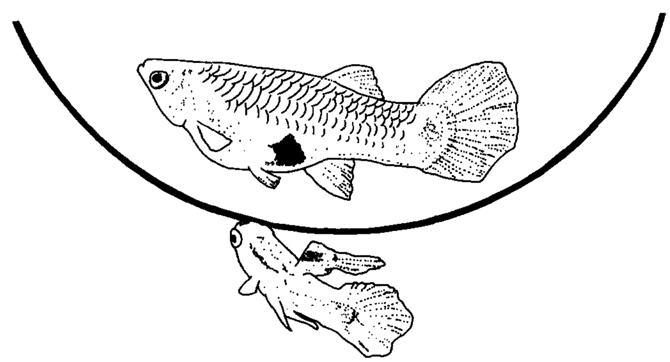

Fig.3. Male attempts to enter the glass vessel containing the female; "penetrating"

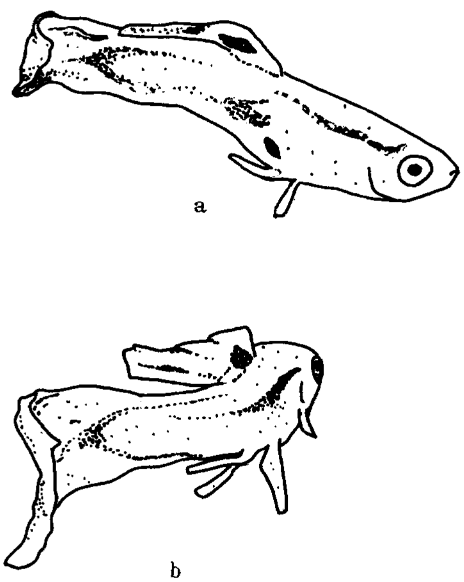

Fig.4. S-curving of the male vertebral column; "sigmoidal position". a) closed display, b) open display

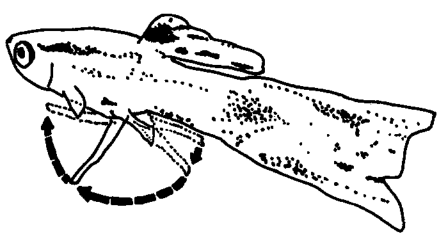

Fig.5. Forward movement of the copulatory organ; "gonopodial swinging"

tested were possible. The test itself consisted of the determination of four behavioural parameters registered by time and event recorders: Male movements directed towards the female ("approaching", Fig.2); attempts to enter the glass vessel of the female ("penetrating", Fig.3); S-curving of the male's vertebral column ("sigmoidal position" according to Baerends, Brouwer and Waterbolk 1955, taking open and closed display as one behavioural trait); and down- and foreward movement of the copulatory organ ("gonopodial swinging" according to Clark and Aronson 1951, Fig.5). Whether these activities actually belong to the courtship display of the male guppy will be discussed in the final section of this paper. The human observers did not know whether the test males belonged to the I or C groups.

Statistical Calculations

The mean values ($\bar{x}$) and standard errors of the mean ($s_{\bar{x}}$) were calculated by the use of a programmed calculator (HP-65). For the determination of the 95% confidence limits, the formula

$$\bar{x} \pm t_{0.05} \cdot s_{\bar{x}}$$

with $t_{0.05} = 2.0$ for n = 50 was applied.

The distribution of the behavioural parameters examined among the different individuals and series was compared by an analysis of variance (Sachs 1974).

Results

Sea Water Guppies

In the F_1, only three males of each series were tested. The frequency of three out of four behavioural parameters in I-F_1 was significantly enhanced as compared to C-F_1, and the duration of "approaching" and "sigmoidal position" was significantly greater. However, we could not be certain whether these changes of mean values reflected radiation-induced alterations in I-F_1 because the behavioural activities of the three I males were also greatly different from each other. In F_2, however, 347 sessions with 17 I males were compared to 209 sessions with 10 C males. There was a significant reduction in the frequency of I-F_2 compared to C-F_2 for the same three behavioural traits which increased in I-F_1 (Table 1). Thus, the ratio of I/C exceeded 2.0 in F_1 and was lower than 0.75 in F_2. Though no significant change was found for "gonopodial swinging" both in F_1 and F_2, its direction of change was the same as observed for the three other parameters. In other words, the courtship activity increased in I-F_1 and decreased in I-F_2, relative to the corresponding controls. The same holds true for the duration of the four traits, with the exception that only "approaching" and "sigmoidal position" increased significantly in I-F_1, whereas all four behavioural traits decreased significantly in I-F_2 (Table 2). If one compares the distribution of the F_2-males according to their mean values, a considerable increase of variability was found in I-F_2, both for the frequency and the duration of the four traits (Fig.6). The analysis of variance revealed a significant difference for the frequency of "approaching", "penetrating", and "sigmoidal position" and for the duration of "approaching" and "penetrating". There was no significant change for "gonopodial swinging" in either case.

Table 1. Courtship activity of male sea water guppies; mean values ± 95%-confidence limits (frequency)

Parameter	F_1 Generation			F_2 Generation		
	I - F_1	C - F_1	I/C	I - F_2	C - F_2	I/C
	3 males 51 sessions	3 males 51 sessions		17 males 347 sessions	10 males 209 sessions	
Approaching	12.25 ± 2.38^a	6.10 ± 1.28^a	2.01^a	7.12 ± 0.76^d	10.48 ± 1.02^d	0.68^d
Penetrating	6.98 ± 2.40^b	2.06 ± 0.98^b	3.39^b	2.83 ± 0.58^e	5.45 ± 0.92^e	0.52^e
Sigmoidal Position	20.73 ± 6.72^c	9.51 ± 3.84^c	2.18^c	7.74 ± 1.32^f	13.49 ± 2.08^f	0.57^f
Gonopodial Swinging	3.73 ± 1.24	1.76 ± 0.84	2.12	1.78 ± 0.50	2.45 ± 0.58	0.73

[a-f] Significant differences ($P < 0.05$)

Table 2. Courtship activity of male seawater guppies; mean values ± 95%-confidence limits (duration)

Parameter	F_1 Generation			F_2 Generation		
	I - F_1	C - F_1	I/C	I - F_2	C - F_2	I/C
	3 males 51 sessions	3 males 51 sessions		17 males 347 sessions	10 males 209 sessions	
Approaching	9.57 ± 1.84^a	4.78 ± 1.08^a	2.00^a	6.10 ± 0.66^c	8.99 ± 0.86^c	0.68^c
Penetrating	35.96 ± 18.82^b	8.94 ± 5.26^b	4.02^b	10.32 ± 2.52^d	17.69 ± 3.48^d	0.58^d
Sigmoidal Position	18.71 ± 6.46	11.10 ± 5.48	1.69	8.16 ± 1.80^e	12.25 ± 1.94^e	0.67^e
Gonopodial Swinging	3.02 ± 1.00	1.61 ± 0.88	1.88	1.57 ± 0.42^f	2.19 ± 0.54^f	0.72^f

[a-f] Significant differences ($P < 0.05$)

Fresh Water Guppies

The frequency of the four parameters determined was not significantly changed both in I - F_1 and I - F_2. Correspondingly, the ratios of I/C were approximately 1.0, with a slight tendency to increase in I - F_1 and decrease in I - F_2 (Table 3). The same tendency was found for the duration of the parameters (Table 4). In the latter case, however, the duration of "sigmoidal position" decreased significantly in I - F_1 in comparison to C - F_2 (Table 4). This situation was also reflected by the distribution of F_2-males according to their mean values (Fig.6) which demonstrates an increase in variability mainly for the duration of "sigmoidal position", but no statistically significant differences in variability.

Comparison of Seawater and Freshwater Guppies

By summerizing the data for the four different behavioural traits, the "overall courtship activity" was obtained (Table 5). This summary confirmed the previous findings; the significant increase of courtship activity in I - F_1 and its significant decrease in I - F_2 both for the frequency and duration of the seawater guppies. For the freshwater fish, however, a significant decrease was found only for the duration of courtship behaviour in I - F_2 compared to C - F_2. If one compares the I/C values between seawater and freshwater guppies, a value near 1.0 was characteristic for the freshwater fish whereas this value exceeded 2.1 for the seawater guppies both for frequency and duration in F_1. The I/C values were close together for the F_2 of both environments (Table 5). Thus, a higher correspondence between irradiated and control series was found in F_2 than in F_1.

The standard deviation within the series for the overall courtship activity was different for seawater and freshwater guppies (Table 6). This value was found to be higher in I - F_1 than in C - F_1 for both frequency and duration in seawater fish and lower in I - F_1 for both frequency and duration in freshwater fish. However, as already mentioned, the behavioural parameters of only three F_1 males from each group were scored for the seawater substrain. In F_2,

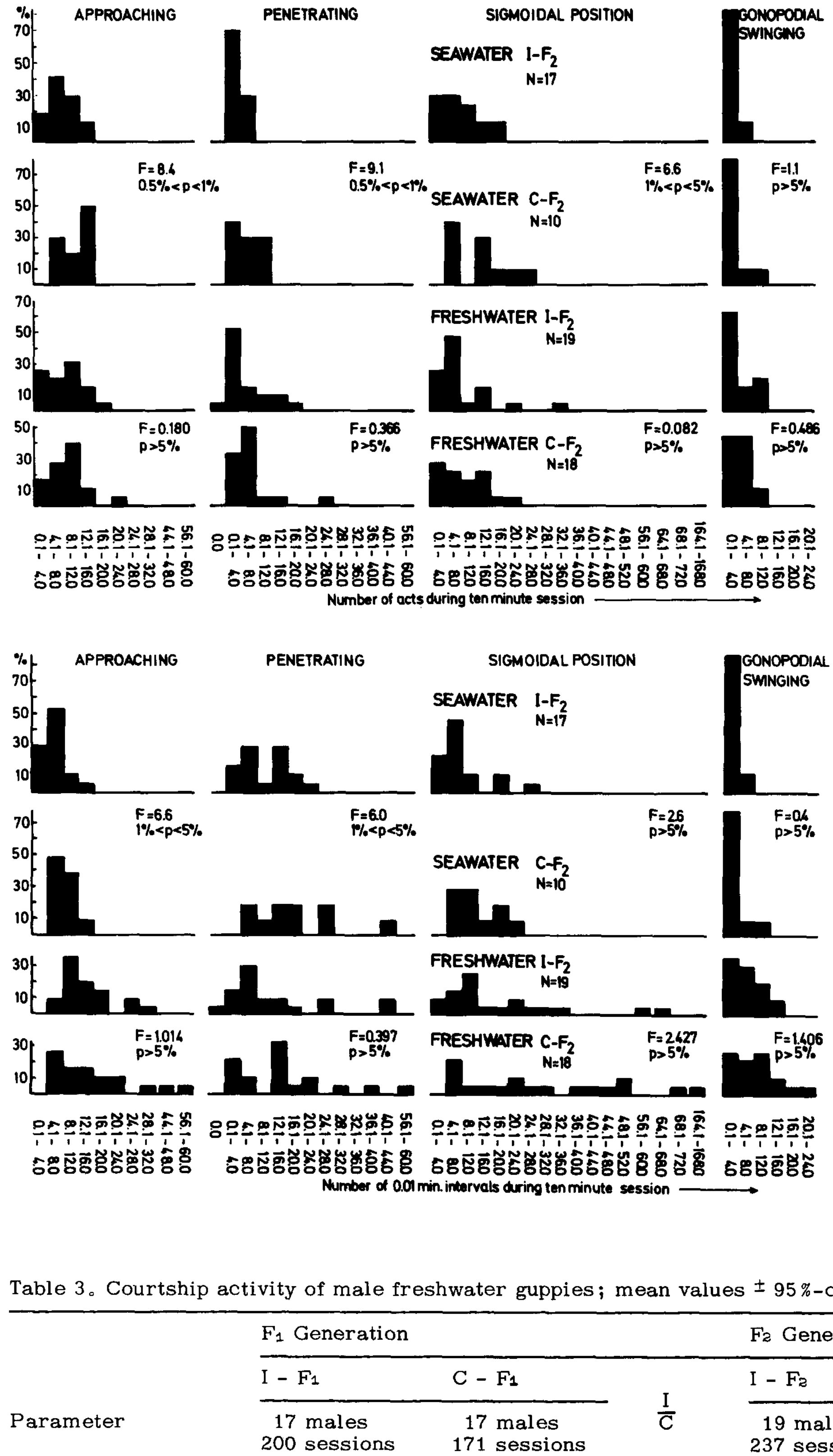

Fig.6. Distribution of the mean values for frequency and duration of four categories of sexual behavior of F_2 - ♂♂ in irradiated ($I - F_2$) and control ($C - F_2$) lines of seawater and freshwater substrains of guppies

Table 3. Courtship activity of male freshwater guppies; mean values ± 95%-confidence limits (frequency)

Parameter	F_1 Generation			F_2 Generation		
	$I - F_1$	$C - F_1$	I/C	$I - F_2$	$C - F_2$	I/C
	17 males 200 sessions	17 males 171 sessions		19 males 237 sessions	18 males 216 sessions	
Approaching	8.00 ± 0.88	7.07 ± 1.04	1.13	8.41 ± 1.05	8.85 ± 1.19	0.95
Penetrating	4.35 ± 0.92	4.56 ± 1.20	0.95	5.35 ± 1.23	6.31 ± 1.87	0.85
Sigmoidal Position	9.34 ± 1.42	9.21 ± 3.20	1.01	8.63 ± 1.42	9.13 ± 1.52	0.95
Gonopodial Swinging	2.82 ± 0.68	2.19 ± 0.66	1.29	4.33 ± 1.06	5.06 ± 0.98	0.86

Table 4. Courtship activity of male freshwater guppies; mean values ± 95 %-confidence limits

Parameter	F_1 Generation			F_2 Generation		
	I - F_1 17 males 200 sessions	C - F_1 17 males 171 sessions	$\frac{I}{C}$	I - F_2 19 males 237 sessions	C - F_2 18 males 216 sessions	$\frac{I}{C}$
Approaching	10.15 ± 1.15	11.87 ± 6.51	0.86	14.44 ± 2.04	18.21 ± 3.03	0.79
Penetrating	13.25 ± 3.23	14.78 ± 4.18	0.90	13.15 ± 3.61	15.88 ± 4.19	0.83
Sigmoidal Position	15.92 ± 3.05	15.21 ± 3.96	1.05	19.46 ± 3.74[a]	34.35 ± 8.37[a]	0.57[a]
Gonopodial Swinging	3.66 ± 0.89	3.05 ± 0.86	1.20	5.98 ± 1.07	7.96 ± 1.66	0.75

[a] Significant difference ($P < 0.05$)

Table 5. Overall courtship activity of male seawater and freshwater guppies; mean values ± 95 %-confidence limits (frequency and duration)

Parameter	Environment	F_1 Generation			F_2 Generation		
		I - F_1	C - F_1	$\frac{I}{C}$	I - F_2	C - F_2	$\frac{I}{C}$
Frequency	Seawater	10.92 ± 2.12[a]	5.10 ± 1.25[a]	2.14[a]	4.87 ± 0.45[c]	7.97 ± 1.04[c]	0.61[c]
	Freshwater	6.13 ± 0.54	5.76 ± 0.93	1.06	6.68 ± 0.61	7.34 ± 0.76	0.91
Duration	Seawater	16.81 ± 5.27[b]	6.60 ± 1.98[b]	2.55[b]	6.54 ± 0.81[d]	10.28 ± 1.10[d]	0.63[d]
	Freshwater	10.74 ± 1.21	11.23 ± 2.21	0.96	13.26 ± 1.45[e]	19.10 ± 2.57[e]	0.69[e]

[a–e] Significant differences ($P < 0.05$)

Table 6. Overall courtship activity of male seawater and freshwater guppies; standard deviation within the series (frequency and duration)

Parameter	Environment	F_1 Generation			F_2 Generation		
		I - F_1	C - F_1	$\frac{I}{C}$	I - F_2	C - F_2	$\frac{I}{C}$
Frequency	Seawater	± 13.77	± 8.47	1.63	± 7.92	± 14.47	0.55
	Freshwater	± 7.17	± 11.88	0.60	± 9.25	± 10.52	0.88
Duration	Seawater	± 35.77	± 13.77	2.60	± 14.82	± 14.87	1.00
	Freshwater	± 16.51	± 28.57	0.58	± 21.89	± 36.66	0.60

Table 7. Courtship activity of male seawater and freshwater guppies; standard deviation within the series (frequency)

Environment	Seawater			Freshwater			Seawater			Freshwater		
Generation	I - F_1	C - F_1	$\frac{I}{C}$	I - F_1	C - F_1	$\frac{I}{C}$	I - F_2	C - F_2	$\frac{I}{C}$	I - F_2	C - F_2	$\frac{I}{C}$
Parameter: Approaching	± 7.41	± 4.54	1.63	±5.35	± 5.79	0.92	± 7.03	± 7.43	0.95	±7.25	± 7.72	0.94
Penetrating	± 6.02	± 3.46	1.74	±5.80	± 5.80	0.84	± 5.42	± 6.70	0.81	±8.37	±12.87	0.65
Sigmoidal Position	±16.81	±13.08	1.29	±9.13	±20.05	0.46	±12.30	±15.04	0.82	±8.29	± 9.86	0.84
Gonopodial Swinging	± 3.76	± 8.03	0.47	±4.36	± 3.58	1.22	± 4.58	± 4.23	1.08	±7.95	± 6.76	1.18

Table 8. Courtship activity of male seawater and freshwater guppies; standard deviation within the series (duration)

Environment		Seawater			Freshwater			Seawater			Freshwater		
Generation		$I-F_1$	$C-F_1$	$\frac{I}{C}$	$I-F_1$	$C-F_1$	$\frac{I}{C}$	$I-F_2$	$C-F_2$	$\frac{I}{C}$	$I-F_2$	$C-F_2$	$\frac{I}{C}$
Parameter	Approaching	± 5.99	± 3.79	1.58	± 7.27	±42.34	0.17	± 6.08	± 6.15	0.99	±14.80	±18.24	0.8[illegible]
	Penetrating	±54.44	±18.55	2.94	±20.26	±24.40	0.83	±23.42	±25.16	0.93	±25.83	±28.65	0.9[illegible]
	Sigmoidal Position	±17.57	±19.26	0.91	±18.98	±20.49	0.93	±16.67	±14.05	1.19	±24.15	±51.47	0.4[illegible]
	Gonopodial Swinging	± 3.11	± 2.95	1.05	± 5.74	± 4.69	1.22	± 3.96	± 3.97	1.00	± 7.69	±11.30	0.6[illegible]

the standard deviation within the series was lower for the frequency and equal for the duration in irradiated than in control seawater guppies. For the freshwater fish, however, this value was higher both for frequency and duration in $C-F_2$ (Table 6).

Considering the standard deviation within the series for the four different traits separately (Tables 7 and 8), the differences were found to be higher in F_1 than in F_2. With the exception of "sigmoidal position", the standard deviations within the series for the frequency (Table 7) of F_1 freshwater guppies were relatively close together. This was not true for seawater F_1: The corresponding values of $I-F_1$ exceeded those of $C-F_1$, except for "sigmoidal position". The I/C values for both "sigmoidal position" and "gonopodial swinging" under seawater and freshwater conditions were reciprocal to each other;, i.e., the standard deviation within the series was higher in $I-F_1$ for seawater and lower for freshwater, whereas this value was lower in $I-F_1$ for sea water and higher in $I-F_1$ for fresh water.

The comparison of the standard deviations within the series for the duration of the four behavioural parameters (Table 8) gives a somewhat different picture. Thus, the I/C values of F_1 were quite different from seawater to freshwater conditions for "approaching" and "penetrating": The values being higher for $I-F_1$ in seawater and lower for $I-F_1$ in freshwater environments. The corresponding values were more similar to each other both for "sigmoidal position" and "gonopodial swinging". The I/C values were almost unity for seawater F_2. Larger differences were found for "sigmoidal position" and "gonopodial swinging" in $I-F_2$ compared to $C-F_2$ in freshwater, whereby the standard deviation in $C-F_2$ exceeded that of $I-F_2$ considerably.

The four treatment groups of F_2 were once more compared by use of the analysis of variance (Table 9). As already mentioned, significant differences occurred between $I-F_2$ and $C-F_2$ of seawater and freshwater guppies. However, significant differences appeared also between the control groups of seawater and freshwater guppies as well as between the irradiated groups of seawater and freshwater fish revealing that the different environmental conditions themselves may contribute to differences in the behavioural traits. Thus, the duration of "gonopodial swinging" between seawater and freshwater control guppies differed significantly from each other. Statistically significant differences were also found for the frequency of "gonopodial swinging" and for the duration of "approaching", "sigmoidal position", and "gonopodial swinging" between the irradiated F_2 of seawater and freshwater guppies.

Discussion

According to Baerends, Brouwer and Waterbolk (1955), who made the most comprehensive study of courtship behaviour of the male guppy, several courting activities are distinguishable and occur most frequently in the order of searching; approaching and following the female; posturing in front of her; luring; sigmoidal position; ending either by a display jump or by a copulation attempt. This sequence is believed to correspond to an increase of the intensity of the sexual drive. The order is not rigidly fixed, but influenced to a considerable extent by changes in external factors, particularly by the presence of the female. Thus, we presented the female to the test male inside a glass ball. The fixed position of the female in the centre of

Table 9. Courtship activity of male guppies; comparison by an analysis of variance (F-test)

Comparison		Parameter							
		Approaching		Penetrating		Sigmoidal Posit.		Gonopodial Swing.	
		F	P	F	P	F	P	F	P
C - F_2 vs. I - F_2	Frequency	8.441*	>0.5 %* <1.0 %	9.084*	>0.5 %* <1.0 %	6.601*	>1 %* <5 %	1.054	>5 %
Seawater (SW)	Duration	6.625*	>1 %* <5 %	6.006*	>1 %* <5 %	2.599	>5 %	0.410	>5 %
C - F_2 vs. I - F_2 Freshwater (FW)	Frequency	0.180	>5 %	0.366	>5 %	0.082	>5 %	0.486	>5 %
	Duration	1.014	>5 %	0.397	>5 %	2.427	>5 %	1.406	>5 %
C - F_2 (SW) vs. I - F_2 (SW) vs. C - F_2 (FW) vs. I - F_2 (FW)	Frequency	2.105	>5 %	1.901	>5 %	1.807	>5 %	5.057*	<0.5 %*
	Duration	5.560*	<0.5 %*	1.231	>5 %	4.455*	>0.5 % <1 %*	10.316*	<0.5 %*
C - F_2 (SW) vs. C - F_2 (FW)	Frequency	1.292	>5 %	0.123	>5 %	2.746	>5 %	4.021	>5 %
	Duration	4.107	>5 %	0.225	>5 %	3.417	>5 %	9.648*	<0.5 %*
I - F_2 (SW) vs. I - F_2 (FW)	Frequency	1.210	>5 %	3.411	>5 %	0.207	>5 %	9.440*	<0.5 %*
	Duration	22.629*	<0.5 %*	0.639	>5 %	6.428*	>1 % <5 %*	20.829*	<0.5 %*

* Significant differences (p < 5 %)

the test aquarium allowed only visual interactions between male and female; i.e., there could be no gustatory and olfactory influence on the courtship activity of the test male through substances delivered from the female (Amouriq 1964, 1965, 1967; Gandolfi 1969; Parzefall 1973; and Zeiske 1968). There is clear evidence that the behavioural patterns of the male guppy scored in the present investigation can only represent a portion of the entire repertoire of the male courtship behaviour. To make the test system suitable for the examination of mutagenesis, all female activities influencing the male courtship behaviour were eliminated. Thus, the artificial arrangement of our experiments guaranteed an almost identical procedure for all the males of the different treatment groups. Another study with freshwater guppies of the same strain, derived from either irradiated or control parents, deals with a more natural test system (Werner and Schröder in preparation). Furthermore, the sexual motivation of the male activities in our experiments were not proven experimentally. For instance, "approaching" can also be exploratory behaviour of the male, perhaps including an aggressive component, and the "sigmoidal position" also belongs to the aggressive activities of poeciliid fish (Franck 1970). Other courting activities such as "gonopodial swinging" cannot be distinguished with certainty from displacement activities (Baerends et al. 1955). On the other hand there is no doubt that these innate movements have primary functions in courtship. However, "penetrating" is an artifact of our arrangement, because glass vessels do not exist in nature. In spite of this, male attempts to reach the female include components which would be courtship movements under natural conditions. Accordingly, most of the activities observed should actually be considered as courting activities. Since an isolation of two hours before the onset of the test sessions was used for all our experiments, all males began the sessions under similar physiological conditions. It is known that courtship behaviour is periodic: Series of courtship movements are followed by lulls in courtship, and again by short phases of higher frequency. Previous unpublished experiments have shown that an isolation time lasting longer than one hour caused an increase in the frequency and duration of courtship movements displayed per ten minutes of observation time. After an isolation of more than one day, however, the males behaved very differently during the test. Some of them did not react in any way to the female, whilst other males courted at a very high or very low level. The most uniform results were found after an isolation time of two hours.

The most important finding of the present study was the differential response to irradiation of freshwater and seawater substrains. Though no evidence could be provided that the quantitative behavioural changes were really caused by mutational events, the nature of these changes completely correspond to that which was obtained from quantitative morphological traits after irradiation with the same X-ray dose (Schröder 1969a, b). Furthermore, the tendency for the mean values to increase in the postirradiation F_1 and the means to decrease with increasing variability in postirradiation F_2 was the same for the behavioural changes observed in both freshwater and seawater guppies. Unidirectional shifts of the mean values and increasing variability in F_2 generations after irradiation were also found for morphological characters such as body proportions and vertebral number of the guppy, as well as for changes of male agonistic behaviour of convict cichlids *(Cichlasoma nigrofasciatum)* and mice (Schröder 1977a, b). In the case of cichlids, the reduced aggressiveness of male fish in postirradiation F_1 was proven to be hereditary (Holzberg and Schröder 1975). From these findings we may assume that the quantitative changes of male courting activities of *Poecilia reticulata* described here can also reflect mutational changes perhaps associated with chromosomal rearrangements affecting the central nervous system. The influence of the seawater environment on the manifestation of presumed radiation-induced mutations of behaviour cannot be estimated at the present. Because only a few founder pairs of guppies tolerated the seawater and could be maintained and continuously bred under these artificial conditions, the seawater substrain may represent a higher degree of inbreeding than the corresponding fresh water substrain. If so, a more drastic response to newly arisen mutations would have been expected to occur in seawater than in freshwater guppies (Dobzhansky et al. 1963; Mukai 1969a, b; Mukai et al. 1964, 1965). The higher standard deviation within the series of the postirradiation F_1 of seawater guppies (Table 6 and 7) favours this view. Thus, radiation-induced mutations would preferentially improve viability characters such as behavioural activity in the highly inbred seawater substrain than they would in the less inbred freshwater substrain. However, physiological effects of the seawater itself on the manifestation of metabolic traits might be involved in the phenomena, as shown by the differences between the control groups of seawater and freshwater guppies. Parallel studies on quantitative morphological characters of the same two substrains not yet quantitatively analyzed may help to answer the question of differential response of seawater and freshwater guppies to mutagenic treatment.

Conclusion

The courtship activity of male guppies *(Poecilia reticulata)* to a female conspecific in a ball-like glass vessel was determined by scoring "approaching" attempts to enter the glass vessel ("penetrating"), "sigmoidal position", and "gonopodial swinging". The test males belonged to two substrains derived from the same inbred guppy strain ("Istanbul"). One of these was maintained and bred in seawater ("seawater guppies"), whereas the other was maintained and bred under normal freshwater conditions ("freshwater guppies"). Each substrain was divided into two groups, either derived from irradiated (1000 R X-rays to oogonia and spermatogonia) or control guppies. The mean values of both the frequency and the duration of the four behavioural traits increased in postirradiation F_1 of the seawater substrain but were not changed in postirradiation F_1 of freshwater guppies. In postirradiation F_2, however, the mean values of the same behavioural characters decreased in both seawater and freshwater substrains. The variability of the four traits in postirradiation F_2 of both substrains varied in an irregular manner. These findings are discussed in terms of population genetics.

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Dr. O.H. Spieser
Abteilung für Toxikologie;
Prof. Dr. J.H. Schröder
Institut für Biologie;
Gesellschaft für Strahlen- und Umweltforschung
Ingolstädter Landstr. 1
D-8042 Neuherberg bei München
(FRG)