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Abnormalities in the Descendants of *Rana nigromaculata* Produced from Irradiated Eggs or Sperm

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(With 32 Text-figures and 8 Plates)

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INTRODUCTION

The effects of irradiation on the developmental capacity of amphibian gametes were first studied by BARDEEN (1907) who exposed toad spermatozoa to X-rays and observed the development of eggs inseminated with irradiated spermatozoa. While such eggs were at first apparently normal or even better than the controls in development, they began to retard in development after the gastrula stage and to reveal remarkable deformities at the time of hatching. Soon thereafter, BARDEEN (1909, 1911) reported that exposure of spermatozoa or ripe ova of a frog or toad to intense X-rays for a considerable period of time did not seem to affect markedly the fertilizing power. Exposure of a female toad to X-rays and subsequent fertilization of her eggs with normal sperm caused the eggs to produce remarkable abnormalities. The same phenomena were also observed by McGREGOR (1908). HERTWIG, O. (1911) exposed *Rana esculenta* or *R. temporaria* sperm to radium radiation for different periods of time and fertilized normal eggs of the same species with the irradiated sperm, while HERTWIG, G. (1911) exposed unfertilized eggs of *Rana temporaria* to radium radiation and fertilized them with normal sperm. Their experiments showed that the severity of radiation-sickness in embryos caused by short exposures was paradoxically reduced by long exposures. This phenomenon is known as the paradoxical effect of HERTWIG or simply HERTWIG effect. According to them, a pronucleus damaged completely by heavy irradiation does not disturb the development of the egg, while a pronucleus damaged partially by a short exposure joins with the other sound pronucleus and severely disturbs the development. The gynogenetic development of frog or toad eggs inseminated with irradiated sperm was further ascertained by G. HERTWIG (1913) and P. HERTWIG (1913).

The experiments performed by the HERTWIGS were conducted by many investigators with the use of various kinds of radiation in various amphibian species. SIMON (1930) and DALCQ (1930) confirmed HERTWIG effect in *Rana temporaria* eggs and sperm irradiated with X-rays, ultraviolet rays or radium radiation. The same effect of X-rays upon gametes of *Rana pipiens* or *R. catesbeiana* has been reported by RUGH (1939), RUGH and EXNER (1940), HENSHAW (1943), SAUNDERS and RUGH (1943), ROLLASON (1949), and BRIGGS, GREEN and KING (1951). In urodeles, RUGH (1950a) exposed adult male *Cynops pyrrhogaster* to large doses of X-rays to examine the effects of irradiation on chromosomes, while SELMAN (1958) exposed sperm of *Triturus palmatus*, *T. vulgaris* or *T. alpestris* to ultraviolet rays in order to obtain a high proportion of gynogenetic haploids. GURDON (1960) irradiated fertilized eggs of *Xenopus laevis* with small doses of ultraviolet rays and obtained androgenetic haploids by complete inactivation of the female pronucleus. This method of enucleation in the eggs of *Xenopus laevis* was utilized by HAMILTON (1967) in her studies on the development of the haploid syndrome in anurans.

GALLIEN and LABROUSSE (1962) exposed fertilized eggs of *Pleurodeles waltl* to neutrons, and found that the eggs irradiated at the stage between the second maturation division and the second polar body formation showed various abnormalities during the embryonic stage, although they were far less injured than those irradiated at the first cleavage. Chromosome aberrations were observed by GALLIEN and his colleagues in abnormal embryos raised from fertilized *Pleurodeles waltl* eggs irradiated by γ-rays from radioactive cobalt (GALLIEN, LABROUSSE and LACROIX, 1963, 1966; GALLIEN, LABROUSSE, PICHERAL and LACROIX, 1965; LABROUSSE, 1966). They reported that many of the abnormal embryos at stages from tail-bud to hatching had distinct chromosome aberrations, such as breakage, deletions, translocations and presence of two centromeres per chromosome. Larvae having chromosome aberrations could be raised up to sexual maturity and to lay eggs. Recently, POGANY (1971, 1973, 1976) investigated again the mechanisms of the paradoxical effect of HERTWIG by inseminating *Rana pipiens* eggs with UV-irradiated sperm. He naturally took a great interest in elucidating the cause of the reduction in survival at short exposures of sperm to irradiation, and reached to a conclusion that the most impaired survival was concomitant with aneuploid chromosomal conditions (POGANY, 1976).

In Japan, SAMBUICHI (1964) in our laboratory exposed oviducal eggs of *Rana nigromaculata* to about 780 r of γ-rays from ^{60}Co and fertilized them with normal sperm. As a result, a remarkable effect of irradiation was first found at the neurula stage. He could count the haploid number (13) of chromosomes in abnormal embryos produced. In order to obtain gynogenetic diploids abundantly in various anuran species, *Rana nigromaculata*, *R. brevipoda*, *R. japonica*, *R.*

tsushimensis, *R. rugosa*, *Hyla arborea japonica* and Korean *Bombina orientalis*, the present authors (1977a) have utilized the method of inactivating sperm nuclei by ultraviolet rays since 1964. SAWADA (1967) exposed oviducal eggs, spermatozoa or fertilized eggs of *Rana nigromaculata* or *Rana japonica* to 48～800 rads of X-rays or 22～350 rads of neutrons, and examined the doses for producing 50% lethal embryos at the hatching stage. TAKESHITA and SAWADA (1974) later carried out a similar experiment to obtain 50% normal survivors at the same stage by X- or neutron-irradiation of fertilized eggs of the same two species.

Owing to the extensive studies performed by the above enumerated researchers, the cause and developmental process of various abnormalities found in individuals raised from irradiated gametes of amphibians have been elucidated to a large extent. However, studies on the genetic effects of irradiation upon descendants of the frogs raised from irradiated gametes have rarely been reported hitherto. On the basis of the results obtained by the present authors from their studies on the descendants of nucleo-cytoplasmic hybrids between *Rana japonica* and *R. ornativentris* (KAWAMURA and NISHIOKA, 1963a, 1972, 1977b) or *Rana nigromaculata* and *Rana brevipoda* (NISHIOKA, 1972), they assumed that some deficiency like low viability or sex reversal of genetic females might appear in the descendants derived from irradiated gametes. Then they began a study in 1967 to corroborate this assumption by making use of *Rana nigromaculata* as the experimental animal and of X-rays and neutrons as the kinds of radiation. This paper describes in detail the results obtained during the subsequent seven years. Some of the results have already been reported in three other papers (KAWAMURA and NISHIOKA, 1973, 1977a; NISHIOKA, 1977). A similar study performed by NISHIOKA (1978) by making use of *Rana japonica* is described in this volume.

MATERIALS AND METHODS

In the present experiments, Japanese pond frogs *Rana nigromoculata* HALLOWELL collected from the suburbs of Hiroshima were utilized as material. Eggs and spermatozoa were obtained from ten females and ten males, respectively, in the breeding season of 1967. Ovulation of the females was accelerated by injecting suspension of frog pituitaries. The irradiation of eggs and spermatozoa with X-rays and neutrons was performed by using the radiation sources of the Research Institute for Nuclear Medicine and Biology, Hiroshima University. The eggs of each female as well as the spermatozoa of each male were divided into thirteen parts. Six of these thirteen parts were exposed to three kinds of doses of X-rays or neutrons, while the other seven parts were not exposed. The three kinds of doses of X-rays or neutrons were selected as those which permitted about 60, 40 and 25% respectively of the fertilized eggs derived from irradiated eggs or spermatozoa to be alive normally at the hatching stage.

X-irradiation was carried out by using an X-ray machine (Toshiba KXC–18–2 Type) operated at 180 KVp and 25 mA. Three parts of eggs obtained from each female were exposed to 90, 145 and 200 rads, respectively, while three parts of spermatozoa obtained from each male were exposed to 90, 170 and 240 rads, respectively, at an average dose rate of 20 rads per minute. The conversion factor of rads was 0.95 rad/R. Neutron-irradiation was performed with fast neutrons from a neutron-generating apparatus with the T(d.n) reactions (Toshiba) operated at 14.1 MeV. Three parts of eggs or spermatozoa of each female or male were exposed to 50, 90 and 130 rads, respectively, at an average dose rate of 10 rads per minute. The conversion factor of rads was 6.7×10^{-9} rad/n. The control eggs and spermatozoa were placed under a similar condition as the irradiated eggs and spermatozoa were and left until the longest time of irradiation of the latter elapsed.

The eggs and spermatozoa irradiated with X-rays or neutrons as well as the control eggs and spermatozoa were fertilized with normal spermatozoa and eggs of frogs collected from the field to produce the first-generation offspring. Fertilized eggs were reared in enamel pans (33.5 cm $\times$ 23.5 cm $\times$ 5.5 cm) at room temperature (20~25°C) until they attained the early tadpole stage. Forty to 50 days after the insemination, tadpoles were transferred from the enamel pans to concrete acquaria (90 cm $\times$ 60 cm $\times$ 20 cm) placed outdoors. After metamorphosis, the frogs were reared again in enamel pans, each of which was covered with a wire gauze. Tadpoles fed on boiled spinach or chard leaves, while frogs fed on flies, bagworms or crickets.

Male and female frogs which matured sexually in the laboratory were mated with each other or with normal females and males collected from the field to produce second-generation offspring. Some of the latter were also reared to the stage of sexual maturity. Third-generation offspring were produced from male and female second-generation offspring by mating with each other or with normal females and males collected from the field.

First-, second- and third-generation offspring were examined in terms of developmental capacity, chromosome abnormalities, sex and reproductive capacity. Metaphase chromosomes were observed in clipped tail-tips of tadpoles by the squash method with aceto-orcein fluid after pretreatment with distilled water, in accordance with MAKINO and NISHIMURA (1952). These tadpoles had been kept in 0.005% colchicine solution for about 18 hours until their tail-tips were clipped.

In the present paper the following signs are utilized.

N or N.W67(69~73)—*Rana nigromaculata* collected from the field in 1967 (1969~1973)

SX–90(170 or 240)——Spermatozoa exposed to 90 (170 or 240) rads of X-rays

EX–90(145 or 200)——Eggs exposed to 90 (145 or 200) rads of X-rays

SN–50(90 or 130)——Spermatozoa exposed to 50 (90 or 130) rads of neutrons

EN–50(90 or 130)——Eggs exposed to 50 (90 or 130) rads of neutrons

OBSERVATION

I. First-generation offspring raised from irradiated gametes

1. Developmental capacity

The body length of ten females (Nos. 1~10) used in the present experiments and the number and diameter of their eggs are presented in Table 1. These females were 77.0~85.5 mm in body length and had 2560~3953 eggs. Fifty eggs taken out at random of the spawn of each female were 1.87±0.01~2.17 ±0.01 mm in mean diameter. The body lengths and the testes sizes of ten males (Nos. 1~10) used in the present experiments are presented in Table 2. They were 60.5~71.0 mm in body length. Their testes were from 3.0 to 4.0 mm in length and 2.0 to 2.5 mm in width.

TABLE 1

Ten field-caught female frogs used for mating experiments in 1967

Kind	Individual no.	Body length (mm)	No. of eggs	Mean diameter of 50 eggs (mm)
N.W67	1	82.5	2560	2.13±0.01
	2	83.5	3953	2.10±0.01
	3	80.0	3692	2.02±0.01
	4	81.0	3491	2.16±0.01
	5	85.0	3750	1.87±0.01
	6	77.0	2775	1.92±0.01
	7	81.5	3570	2.17±0.01
	8	85.5	3912	1.90±0.01
	9	84.0	3474	2.13±0.01
	10	84.5	3756	2.04±0.01

The present experiments were classified into twelve experimental series which were different from one another in the kind and dosage of irradiation. In

TABLE 2

Ten field-caught male frogs used for mating experiments in 1967

Kind	Individual no.	Body length (mm)	Size of the testes		Inner structure
			Left (mm)	Right (mm)	
N.W67	1	67.5	3.0×2.0	3.0×2.0	Type 1
	2	65.5	4.0×2.5	4.0×2.5	Type 1
	3	65.0	3.5×2.0	3.5×2.5	Type 1
	4	63.5	3.5×2.0	3.5×2.0	Type 1
	5	67.0	4.0×2.0	4.0×2.0	Type 1
	6	66.0	4.0×2.5	4.0×2.5	Type 1
	7	60.5	3.0×2.0	3.0×2.0	Type 1
	8	71.0	3.5×2.0	3.5×2.5	Type 1
	9	67.5	4.0×2.5	3.5×2.0	Type 1
	10	65.5	3.5×2.5	3.5×2.5	Type 1

addition, the control series was prepared. Each of the control and the twelve experimental series consisted of ten matings, in which eggs of a female were inseminated with spermatozoa of a male of the same individual number as that of the female. The results of the ten matings in each of the twelve experimental series and the control series are presented in Table 3 and shown in Fig. 1. The first-generation offspring derived from gametes irradiated with larger doses of X-rays or neutrons were clearly inferior in developmental capacity to those derived from gametes irradiated with smaller doses. X-irradiation of eggs had a worse effect on the development of the first-generation offspring than that of spermatozoa, while neutron-irradiation of eggs did not remarkably differ in effectiveness from that of spermatozoa. X- or neutron-irradiation slightly prevented the normal cleavages of fertilized eggs, although larger doses of irradiations did more distinctly than smaller ones. A small number of normally cleaved eggs derived from irradiated eggs or spermatozoa died at gastrula and neurula stages. Nearly all the inviable gastrulae were abnormal in invagination, having no relation to the kind or dosage of irradiation. The inviable neurulae were abnormal in the formation of neural folds or tubes. Most of the embryos which died at stages from tail-bud to hatching were edematous, blistery, microcephalous or revealing curvature of the body or ill-formation of external gills. A few others were bicephalous or of cauda bifida. The tadpoles which died by

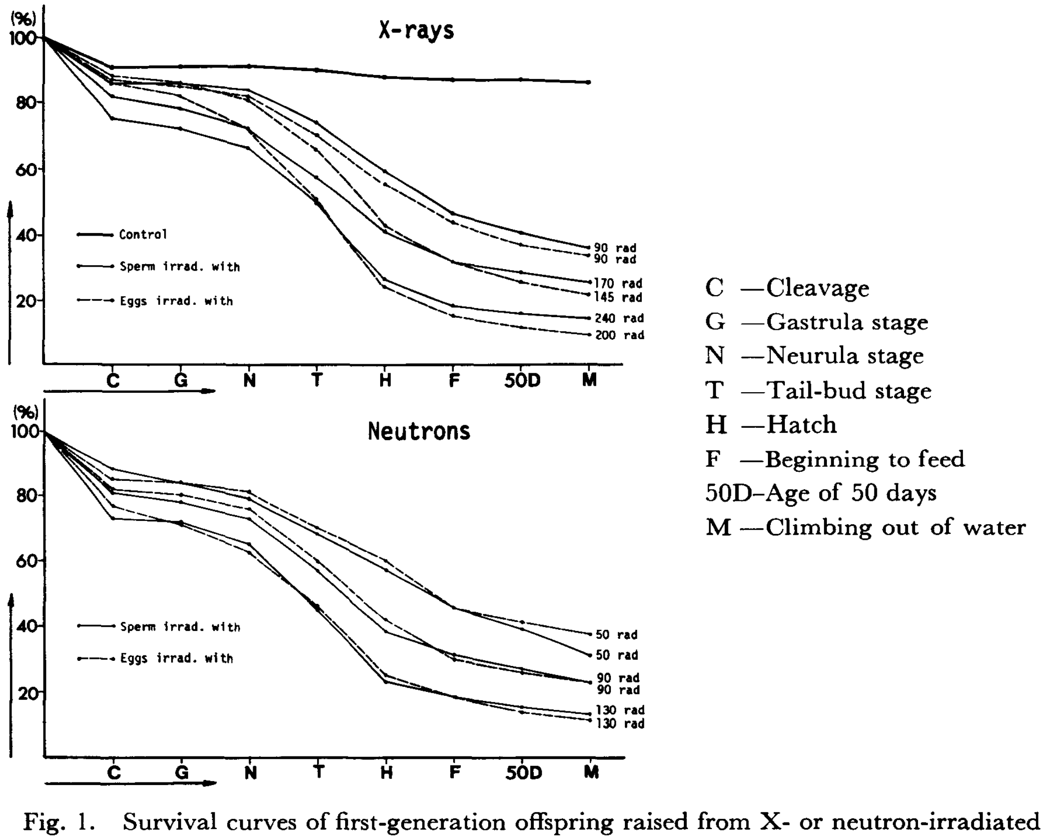


Fig. 1. Survival curves of first-generation offspring raised from X- or neutron-irradiated sperm or oviducal eggs. Sperm was exposed to 90, 170 or 240 rads of X-rays, or 50, 90 or 130 rads of neutrons. Oviducal eggs were exposed to 90, 145 or 200 rads of X-rays, or 50, 90 or 130 rads of neutrons. The survival curve of the control is shown by a thick line.

TABLE 3

Developmental capacity of the offspring raised from

Parents		No. of eggs	No. of cleaved eggs	No. of gastrulae		No. of neurulae	
Female	Male			Normal	Abnormal	Normal	Abnormal
N.W67, Nos. 1~10	N.W67, Nos. 1~10	1103	1004 (91.0%)	1002 (90.8%)	2	1002 (90.8%)	0
N.W67, Nos. 1~10	SX–90, Nos. 1~10	1667	1438 (86.3%)	1432 (85.9%)	6	1400 (84.0%)	32
	SX–170, Nos. 1~10	1756	1434 (81.7%)	1370 (78.0%)	64	1266 (72.1%)	104
	SX–240, Nos. 1~10	1743	1298 (74.5%)	1251 (71.8%)	47	1151 (66.0%)	100
EX–90, Nos. 1~10	N.W67, Nos. 1~10	1770	1533 (86.6%)	1500 (84.7%)	33	1447 (81.8%)	53
EX–145, Nos. 1~10		1791	1599 (89.3%)	1544 (86.2%)	39	1446 (80.7%)	98
EX–200, Nos. 1~10		2033	1756 (86.4%)	1663 (81.8%)	93	1456 (71.6%)	207
N.W67, Nos. 1~10	SN–50, Nos. 1~10	1778	1564 (88.0%)	1494 (84.0%)	70	1408 (79.2%)	86
	SN–90, Nos. 1~10	1678	1360 (81.0%)	1309 (78.0%)	51	1217 (72.5%)	92
	SN–130, Nos. 1~10	1575	1155 (73.3%)	1127 (71.6%)	28	1021 (64.8%)	106
EN–50, Nos. 1~10	N.W67, Nos. 1~10	1722	1471 (85.4%)	1453 (84.4%)	18	1396 (81.1%)	57
EN–90, Nos. 1~10		1946	1600 (82.2%)	1557 (80.0%)	43	1472 (75.6%)	85
EN–130, Nos. 1~10		2053	1575 (76.7%)	1456 (70.9%)	89	1271 (61.9%)	185

the metamorphosing stage were mostly underdeveloped or edematous.

As there were considerable differences in sensitivity to irradiation among individual frogs, the results of ten matings in each series are described in detail with special reference to such differences.

a. Control series

N♀ × N♂

In ten matings Nos. 1 ~ 10 performed by using ten normal females Nos. 1 ~ 10 and ten normal males Nos. 1 ~ 10, 82.2 ~ 99.1%, 91.0% on the average, of the respective total number of eggs cleaved normally, and 79.5 ~ 97.2%, 88.3% on the average, hatched normally (Table 3, Fig. 1). Almost all the tadpoles developed normally and eventually 78.2 ~ 96.2%, 85.7% on the average, became normal, metamorphosed frogs. Thus, only 5.3% of normally cleaved eggs died before the completion of metamorphosis. The cause of their death was variable in each mating, and there were no special abnormalities.

b. Experimental series from X-irradiated sperm

i) N♀ × SX–90♂, Nos. 1 ~ 10

Eggs of the same ten females Nos. 1 ~ 10 as those used in the control series were inseminated with spermatozoa of ten males Nos. 1 ~ 10 after the spermatozoa were exposed to 90 rads of X-rays. In ten matings Nos. 1 ~ 10, 71.0 ~

irradiated sperm or eggs of ten parental frogs in 1967

No. of tail-bud embryos		No. of hatched tadpoles		No. of feeding tadpoles		No. of 50-day-old tadpoles		No. of metamor-phosed frogs
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
988 (89.6%)	14	974 (88.3%)	14	962 (87.2%)	12	954 (86.5%)	8	945 (85.7%)
1227 (73.6%)	173	988 (59.3%)	239	760 (45.6%)	228	677 (40.6%)	83	600 (36.0%)
1003 (57.1%)	263	727 (41.4%)	276	543 (30.9%)	184	488 (27.8%)	55	434 (24.7%)
851 (48.8%)	300	450 (25.8%)	401	320 (18.4%)	130	278 (15.9%)	42	242 (13.9%)
1231 (69.5%)	216	974 (55.0%)	257	759 (42.9%)	215	646 (36.5%)	113	578 (32.7%)
1173 (65.5%)	273	761 (42.5%)	412	556 (31.0%)	205	444 (24.8%)	112	380 (21.2%)
1007 (49.5%)	449	489 (24.1%)	518	300 (14.8%)	189	230 (11.3%)	70	181 (8.9%)
1210 (68.1%)	198	1019 (57.3%)	191	808 (45.4%)	211	688 (38.7%)	120	554 (31.2%)
951 (56.7%)	266	637 (38.0%)	314	511 (30.5%)	126	448 (26.7%)	63	389 (23.2%)
712 (45.2%)	309	369 (23.4%)	343	283 (18.0%)	86	235 (14.9%)	48	202 (12.8%)
1213 (70.4%)	183	1028 (59.7%)	185	809 (47.0%)	219	710 (41.2%)	99	638 (37.0%)
1172 (60.2%)	300	811 (41.7%)	361	580 (29.8%)	231	498 (25.6%)	82	455 (23.4%)
949 (46.2%)	322	515 (25.1%)	434	367 (17.9%)	148	259 (12.6%)	108	217 (10.6%)

96.0%, 86.3% on the average, of the respective total number of eggs cleaved normally (Table 3, Fig. 1). While a small number of the normally cleaved eggs gradually died, most of them developed normally into embryos; 40.9～78.0%, 59.3% on the average, hatched normally. At the age of 50 days, 12.7～62.9%, 40.6% on the average, remained alive, and eventually, 9.5～49.8%, 36.0% on the average, became normal, metamorphosed frogs. The inviable individuals were mostly edematous, blistery, microcephalous, bicephalous or of curved bodies or some other abnormalities. There was a considerable difference in the survival ratio of fertilized eggs between different matings.

ii) N♀×SX–170♂, Nos. 1～10

Eggs of normal females Nos. 1～10 were inseminated with spermatozoa of ten males Nos. 1～10 after the spermatozoa were exposed to 170 rads of X-rays. In ten matings Nos. 1～10, 43.6～96.1%, 81.7% on the average, of the respective total number of eggs cleaved normally, and 34.1～63.2%, 41.4% on the average, hatched normally. Many individuals died of various abnormalities at various embryonic stages. At the age of 50 days, 4.3～41.1%, 27.8% on the average, became normal, feeding tadpoles, and 2.9～38.0%, 24.7% on the average, became normal, metamorphosed frogs. The tadpoles which died during the tadpole stage were ill-developed or edematous.

iii) N♀×SX–240♂, Nos. 1～10

Eggs of normal females Nos. 1～10 were inseminated with spermatozoa of the ten males after the spermatozoa were exposed to 240 rads of X-rays. In ten matings Nos. 1～10, 23.4～92.7%, 74.5% on the average, of the respective total number of eggs cleaved normally, and 5.4～40.6%, 25.8% on the average, hatched normally. Most of the normally cleaved eggs died of various abnormalities at various embryonic stages. At the age of 50 days, 2.4～30.7%, 15.9% on the average, remained alive, and eventually 1.6～25.6%, 13.9% on the average, became normal, metamorphosed frogs.

c. Experimental series from X-irradiated eggs

i) EX-90♀×N♂, Nos. 1～10

Eggs of ten females Nos. 1～10 were exposed to 90 rads of X-rays and then inseminated with spermatozoa of the same ten normal males Nos. 1～10 as those used in the control series. In ten matings Nos. 1～10, 65.8～97.0%, 86.6% on the average, of the respective total number of eggs cleaved normally. While many embryos died of various abnormalities at various stages, 29.4～80.6%, 55.0% on the average, hatched normally. At the age of 50 days, 22.9～48.4%, 36.5% on the average, remained alive, and eventually 20.5～41.6%, 32.7% on the average, became normal, metomorphosed frogs, while the others died mostly of edema or ill-development.

ii) EX-145♀×N♂, Nos. 1～10

Eggs of ten females Nos. 1～10 were exposed to 145 rads of X-rays and then inseminated with spermatozoa of normal males Nos. 1～10. In ten matings Nos. 1～10, 57.7～96.9%, 89.3% on the average, of the respective total number of eggs cleaved normally. While more than half of the normally cleaved eggs died of microcephaly, curvature of the body, blisters or some other abnormalities at various embryonic stages, 21.4～68.1%, 42.5% on the average, hatched normally. After about half the number of the hatched tadpoles died of underdevelopment or edema during the tadpole stages, 11.3～30.2%, 21.2% on the average, became normal, metamorphosed frogs, although 12.5～35.0%, 24.8% on the average, remained alive at the age of 50 days.

iii) EX-200♀×N♂, Nos. 1～10

Eggs of ten females Nos. 1～10 were exposed to 200 rads of X-rays and then inseminated with spermatozoa of normal males Nos. 1～10. In ten matings Nos. 1～10, 33.1～96.2%, 86.4% on the average, of the respective total number of eggs cleaved normally. Most of the normally cleaved eggs died of microcephaly, curvature of the body, blisters, edema or some other abnormalities at various embryonic stages, while 6.3～44.2%, 24.1% on the average, hatched normally. At the age of 50 days, 5.8～30.8%, 11.3% on the average, remained alive. Only 4.4～24.8%, 8.9% on the average, metamorphosed normally and the remaining tadpoles died of underdevelopment or edema.

d. Experimental series from neutron-irradiated sperm

i) N♀×SN-50♂, Nos. 1～10

Eggs of the same ten normal females Nos. 1～10 as those used in the control series were inseminated with spermatozoa of ten males after the spermatozoa were exposed to 50 rads of neutrons. In ten matings Nos. 1～10, 80.2～97.5%, 88.0% on the average, of the respective total number of eggs cleaved normally, and then 42.2～73.9%, 57.3% on the average, hatched normally. The remaining normally cleaved eggs died of microcephaly, bicephaly, edema, blisters or curvature of the body. At the age of 50 days, 19.0～54.7%, 38.7% on the average, remained alive, and eventually 9.5～43.8%, 31.2% on the average, became normal, metamorphosed frogs.

ii) N♀×SN-90♂, Nos. 1～10

Eggs of normal females Nos. 1～10 were inseminated with spermatozoa of ten males Nos. 1～10 after the spermatozoa had been exposed to 90 rads of neutrons. In ten matings Nos. 1～10, 45.8～93.9%, 81.0% on the average, of the respective total number of eggs cleaved normally. More than half of the normally cleaved eggs died of various abnormalities before the hatching stage and only 14.5～53.5%, 38.0% on the average, hatched normally. At the age of 50 days, 7.6～42.0%, 26.7% on the average, remained alive, and eventually only 0～36.5%, 23.2% on the average, became normal, metamorphosed frogs, while the remaining frogs died of ill-development or edema.

iii) N♀×SN-130♂, Nos. 1～10

Eggs of normal females Nos. 1～10 were inseminated with spermatozoa exposed to 130 rads of neutrons. In ten matings Nos. 1～10, 28.5～89.6%, 73.3% on the average, of the respective total number of eggs cleaved normally. Only 9.5～38.2%, 23.4% on the average, hatched normally, while most of the normally cleaved eggs died of various abnormalities at various embryonic stages. At the age of 50 days, 4.0～28.8%, 14.9% on the average, remained alive, and eventually 0～23.3%, 12.8% on the average, attained completion of metamorphosis. The other tadpoles died mostly of edema.

e. Experimental series from neutron-irradiated eggs

i) EN-50♀×N♂, Nos. 1～10

Eggs obtained from ten females Nos. 1～10 were exposed to 50 rads of neutrons and then inseminated with spermatozoa of the same ten normal males as those used in the control series. In ten matings Nos. 1～10, 75.9～98.1%, 85.4% on the average, of the respective total number of eggs cleaved normally. Many of the normally cleaved eggs died of various abnormalities at various embryonic stages, while 36.1～82.8%, 59.7% on the average, hatched normally. Many tadpoles died of edema, underdeveloped teeth or some other abnormalities before the age of 50 days; at this age 23.2～60.2%, 41.2% on the average, remained alive. Afterwards, many tadpoles died similarly of underdevelopment, edema etc., while 21.8～57.7%, 37.0% on the average, became normal, metamorphosed frogs.

ii) EN-90♀×N♂, Nos. 1～10

In ten matings Nos. 1～10, 67.7～98.4%, 82.2% on the average, of the respective total number of eggs irradiated with 90 rads of neutrons cleaved normally.

Many of the normally cleaved eggs died of microcephaly, edema, curvature of the body and other abnormalities at various embryonic stages, while 22.8~70.9%, 41.7% on the average, hatched normally. At the age of 50 days, 14.7~41.2%, 25.6% on the average, remained alive, while the others died of under-development, edema or some other abnormalities. After some tadpoles gradually died, 12.7~38.0%, 23.4% on the average, became normal, metamorphosed frogs.

iii) EN-130♀ × N♂, Nos. 1~10

In ten matings Nos. 1~10, 44.2~97.3%, 76.7% on the average, of the respective total number of eggs irradiated with 130 rads of neutrons cleaved normally. While most of the normally cleaved eggs died of microcephaly, bicephaly, curvature of the body, edema or some other abnormalities, 7.2~49.8%, 25.1% on the average, hatched normally. After hatching, most of the tadpoles died of edema, abnormal gills and some other abnormalities; 5.8~18.7%, 12.6% on the average remained alive at the age of 50 days. Eventually, only 5.5~15.3%, 10.6% on the average, attained completion of metamorphosis.

2. Chromosome aberrations

The karyotypes of normally shaped tadpoles at the ages of 40~50 days in the twelve experimental series and the control series were examined by the squash

TABLE 4

Developmental capacity of the offspring raised from irradiated sperm of males Nos. 3 and 5 or eggs of females Nos. 3 and 5

Parents		No. of eggs	No. of normal cleavages (%)	No. of normal tail-bud embryos (%)	No. of normally hatched tadpoles (%)	No. of 50-day-old tadpoles (%)	No. of metamorphosed frogs (%)
Female	Male						
N.W67, No. 3	N.W67, No. 3	153	132 (86.3)	131 (85.6)	129 (84.3)	127 (83.0)	127 (83.0)
N.W67, No. 5	N.W67, No. 5	120	113 (94.2)	113 (94.2)	113 (94.2)	107 (89.2)	107 (89.2)
N.W67, No. 3	SX-90, No. 3	235	210 (89.4)	203 (86.4)	156 (66.4)	123 (52.3)	111 (47.2)
N.W67, No. 5	SX-90, No. 5	259	223 (86.1)	216 (83.4)	202 (78.0)	163 (62.9)	129 (49.8)
N.W67, No. 3	SX-170, No. 3	245	224 (91.4)	137 (55.9)	104 (42.4)	74 (30.2)	67 (27.3)
N.W67, No. 5	SX-170, No. 5	258	231 (89.5)	216 (83.7)	163 (63.2)	106 (41.1)	98 (38.0)
N.W67, No. 3	SX-240, No. 3	264	242 (91.7)	147 (55.7)	101 (38.3)	81 (30.7)	67 (25.4)
N.W67, No. 5	SX-240, No. 5	219	203 (92.7)	121 (55.3)	87 (39.7)	63 (28.8)	56 (25.6)
EX-90, No. 3	N.W67, No. 3	217	197 (90.8)	148 (68.2)	106 (48.8)	98 (45.2)	81 (37.3)
EX-90, No. 5	N.W67, No. 5	238	228 (95.8)	216 (90.8)	191 (80.3)	114 (47.9)	99 (41.6)
EX-145, No. 3	N.W67, No. 3	251	223 (88.8)	140 (55.8)	98 (39.0)	81 (32.3)	72 (28.7)
EX-145, No. 5	N.W67, No. 5	213	200 (93.9)	183 (85.9)	145 (68.1)	74 (34.7)	64 (30.0)
EX-200, No. 3	N.W67, No. 3	234	206 (88.0)	159 (67.9)	94 (40.2)	72 (30.8)	58 (24.8)
EX-200, No. 5	N.W67, No. 5	286	257 (89.9)	197 (68.9)	126 (44.1)	57 (19.9)	44 (15.4)
N.W67, No. 3	SN-50, No. 3	224	201 (89.7)	152 (67.9)	123 (54.9)	95 (42.4)	83 (37.1)
N.W67, No. 5	SN-50, No. 5	222	213 (95.9)	166 (74.8)	160 (72.1)	119 (53.6)	95 (42.8)
N.W67, No. 3	SN-90, No. 3	252	229 (90.9)	139 (55.2)	105 (41.7)	76 (30.2)	63 (25.0)
N.W67, No. 5	SN-90, No. 5	241	223 (92.5)	184 (76.3)	128 (53.1)	101 (41.9)	86 (35.7)
N.W67, No. 3	SN-130, No. 3	240	204 (85.0)	119 (49.6)	80 (33.3)	69 (28.8)	49 (20.4)
N.W67, No. 5	SN-130, No. 5	275	246 (89.5)	164 (59.6)	105 (38.2)	66 (24.0)	64 (23.3)
EN-50, No. 3	N.W67, No. 3	273	249 (91.2)	209 (76.6)	158 (57.9)	114 (41.8)	85 (31.1)
EN-50, No. 5	N.W67, No. 5	280	263 (93.9)	249 (88.9)	230 (82.1)	168 (60.0)	132 (47.1)
EN-90, No. 3	N.W67, No. 3	220	172 (78.2)	93 (42.3)	73 (33.2)	47 (21.4)	43 (19.5)
EN-90, No. 5	N.W67, No. 5	311	278 (89.4)	254 (81.7)	177 (56.9)	114 (36.7)	64 (20.6)
EN-130, No. 3	N.W67, No. 3	308	136 (44.2)	66 (21.4)	34 (11.0)	18 (5.8)	17 (5.5)
EN-130, No. 5	N.W67, No. 5	326	288 (88.3)	233 (71.5)	162 (49.7)	61 (18.7)	46 (14.1)

method. As two matings Nos. 3 and 5 of each series were the largest in the number of eggs among the ten, the tadpoles derived from these matings were used for karyological examination (Table 4). The survival curve of the first-generation offspring in each of these matings was generally in accord with the average survival curve in their experimental series. Although numerous abnormal embryos and tadpoles were produced in the matings Nos. 3 and 5, their karyotypes were not examined.

The results of chromosomal analyses of normally shaped tadpoles in the experimental and the control series are presented in Tables 5 and 6. The number and rate of normal and abnormal mitoses found in the tadpoles of each series are presented in these tables. These data clearly show that there is a close correlation between the amount of irradiation and the rate of chromosome aberrations. In accordance with the increase of dosage, normal diploids de-

TABLE 5

Chromosomal analysis of normally shaped tadpoles raised from irradiated sperm or eggs

Parents		No. of analyzed tadpoles	Number of tadpoles			
			With normal cells only	With abnormal cells only		With normal and abnormal cells
Female	Male			Pure	Mosaics	
N.W67, No. 3	N.W67, No. 3	25	23 (92.0%)	1 (1)	0	1
N.W67, No. 5	N.W67, No. 5	20	20 (100 %)	0	0	0
N.W67, No. 3	SX–90, No. 3	24	11 (45.8%)	2	3	8 (1)
N.W67, No. 5	SX–90, No. 5	37	11 (29.7%)	1 (1)	9	16
N.W67, No. 3	SX–170, No. 3	16	6 (37.5%)	2	2	6
N.W67, No. 5	SX–170, No. 5	24	7 (29.2%)	3	8	6
N.W67, No. 3	SX–240, No. 3	28	8 (28.6%)	4 (1)	10 (7)	6 (1)
N.W67, No. 5	SX–240, No. 5	18	4 (22.2%)	0	7	7 (2)
EX–90, No. 3	N.W67, No. 3	24	17 (70.8%)	1 (1)	2 (1)	4
EX–90, No. 5	N.W67, No. 5	33	23 (69.7%)	1 (1)	3	6
EX–145, No. 3	N.W67, No. 3	20	10 (50.0%)	3 (2)	2 (1)	5 (1)
EX–145, No. 5	N.W67, No. 5	38	19 (50.0%)	1 (1)	8	10
EX–200, No. 3	N.W67, No. 3	28	10 (35.7%)	4 (2)	6 (1)	8
EX–200, No. 5	N.W67, No. 5	14	4 (28.6%)	5	4	1 (1)
N.W67, No. 3	SN–50, No. 3	20	11 (55.0%)	2 (1)	1	6
N.W67, No. 5	SN–50, No. 5	38	28 (73.7%)	1	3	6
N.W67, No. 3	SN–90, No. 3	13	6 (46.2%)	3 (2)	2 (1)	2
N.W67, No. 5	SN–90, No. 5	20	9 (45.0%)	2 (1)	5	4
N.W67, No. 3	SN–130, No. 3	28	7 (25.0%)	4 (4)	12 (2)	5 (2)
N.W67, No. 5	SN–130, No. 5	20	6 (30.0%)	3	6	5
EN–50, No. 3	N.W67, No. 3	34	15 (44.1%)	4 (4)	3	12 (3)
EN–50, No. 5	N.W67, No. 5	22	12 (54.5%)	0	4	6
EN–90, No. 3	N.W67, No. 3	24	9 (37.5%)	4 (3)	7 (2)	4 (2)
EN–90, No. 5	N.W67, No. 5	24	10 (41.7%)	3 (1)	4	7
EN–130, No. 3	N.W67, No. 3	20	6 (30.0%)	6 (4)	4 (2)	4 (2)
EN–130, No. 5	N.W67, No. 5	20	7 (35.0%)	0	9	4

Parentheses indicate the number of 3n or hyper- or hypotriploid tadpoles.

TABLE 6

Chromosome aberrations in normally shaped

Parents		No. of tadpoles	No. of analyzed mitoses	
Female	Male			2n−1
N.W67, No. 3	N.W67, No. 3	25	76	0
N.W67, No. 5	N.W67, No. 5	20	65	0
N.W67, No. 3	SX–90, No. 3	24	97	2 (2.1%)
N.W67, No. 5	SX–90, No. 5	37	179	10 (5.6%)
N.W67, No. 3	SX–170, No. 3	16	72	3 (4.2%)
N.W67, No. 5	SX–170, No. 5	24	92	0
N.W67, No. 3	SX–240, No. 3	28	107	3 (2.8%)
N.W67, No. 5	SX–240, No. 5	18	82	2 (2.4%)
EX–90, No. 3	N.W67, No. 3	24	86	2 (2.3%)
EX–90, No. 5	N.W67, No. 5	33	117	7 (6.0%)
EX–145, No. 3	N.W67, No. 3	20	73	0
EX–145, No. 5	N.W67, No. 5	38	155	17 (11.0%)
EX–200, No. 3	N.W67, No. 3	28	78	3 (3.8%)
EX–200, No. 5	N.W67, No. 5	14	46	0
N.W67, No. 3	SN–50, No. 3	20	84	6 (7.1%)
N.W67, No. 5	SN–50, No. 5	38	173	6 (3.5%)
N.W67, No. 3	SN–90, No. 3	13	54	3 (5.6%)
N.W67, No. 5	SN–90, No. 5	20	72	0
N.W67, No. 3	SN–130, No. 3	28	103	7 (6.8%)
N.W67, No. 5	SN–130, No. 5	20	73	7 (9.6%)
EN–50, No. 3	N.W67, No. 3	34	164	16 (9.8%)
EN–50, No. 5	N.W67, No. 5	22	84	9 (10.7%)
EN–90, No. 3	N.W67, No. 3	24	89	3 (3.4%)
EN–90, No. 5	N.W67, No. 5	24	84	0
EN–130, No. 3	N.W67, No. 3	20	76	3 (3.9%)
EN–130, No. 5	N.W67, No. 5	20	79	7 (8.9%)

creased in percentage among normally shaped tadpoles produced from eggs or spermatozoa irradiated with X-rays or neutrons.

a. Control series

N♀×N♂, Nos. 3 and 5

In mating No. 3, the karyotypes of 25 tadpoles were examined (Tables 5 and 6). Of these tadpoles, 23 were normal diploids which had only diploid mitoses (Fig. 2). Another tadpole was a triploid, while the remaining was a mosaic consisting of normally and abnormally diploid cells. The latter had a chromosome with a deletion.

In mating No. 5, all the 20 tadpoles examined were normal diploids (Tables 5 and 6).

b. Experimental series from X-irradiated sperm

i) N♀×SX–90♂, Nos. 3 and 5

In mating No. 3, 11 of 24 tadpoles analyzed in terms of karyotypes had normally diploid mitoses only (Tables 5 and 6). Two others were abnormal diptadpoles raised from irradiated sperm or eggs

Number of mitoses				With minute	With frag-ment	With ring
2n normal	2n abnorm.	2n+1	3n±α*			
71 (93.4%)	2 (2.6%)	0	3 (3.9%)	0	0	0
65 (100 %)	0	0	0	0	0	0
58 (59.8%)	31 (32.0%)	3 (3.1%)	3 (3.1%)	0	0	0
89 (49.7%)	73 (40.8%)	4 (2.2%)	3 (1.7%)	0	0	3
35 (48.6%)	25 (34.7%)	3 (4.2%)	6 (8.3%)	0	15	0
40 (43.5%)	52 (56.5%)	0	0	0	0	3
38 (35.5%)	30 (28.0%)	6 (5.6%)	30 (28.0%)	0	1	4
34 (41.5%)	40 (48.8%)	0	6 (7.3%)	0	0	0
54 (62.8%)	21 (24.4%)	3 (3.5%)	6 (7.0%)	0	0	0
85 (72.6%)	22 (18.8%)	0	3 (2.6%)	0	0	0
44 (60.3%)	16 (21.9%)	0	13 (17.8%)	0	0	0
81 (52.3%)	42 (27.1%)	7 (4.5%)	8 (5.2%)	0	0	3
37 (47.4%)	22 (28.2%)	3 (3.8%)	13 (16.7%)	0	0	0
15 (32.6%)	28 (60.9%)	0	3 (6.5%)	0	0	0
50 (59.5%)	9 (10.7%)	16 (19.0%)	3 (3.6%)	0	3	3
94 (54.3%)	65 (37.6%)	8 (4.6%)	0	0	0	0
24 (44.4%)	12 (22.2%)	3 (5.6%)	12 (22.2%)	6	0	1
38 (52.8%)	31 (43.1%)	0	3 (4.2%)	0	0	0
36 (35.0%)	41 (39.8%)	0	19 (18.4%)	2	4	3
31 (42.5%)	29 (39.7%)	6 (8.2%)	0	0	2	2
87 (53.0%)	43 (26.2%)	3 (1.8%)	15 (9.1%)	0	0	0
52 (61.9%)	17 (20.2%)	6 (7.1%)	0	0	0	0
34 (38.2%)	26 (29.2%)	5 (5.6%)	21 (23.6%)	0	1	1
47 (56.0%)	34 (40.5%)	0	3 (3.6%)	0	0	5
24 (31.6%)	21 (27.6%)	3 (3.9%)	25 (32.9%)	2	0	2
36 (45.6%)	33 (41.8%)	3 (3.8%)	0	0	0	0

* $\alpha=0\sim2$

loids; in all the metaphase plates of one of the two, there was a large chromosome (No. 2) with a deletion in the long arm, while in those of the other, there was always a small chromosome (No. 5) with a deletion in the short arm. Another tadpole was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) cells. Two other tadpoles were mosaics of abnormally diploid mitoses, in which there was a chromosome with a translocation or deletion. The remaining eight tadpoles were mosaics of normally diploid and abnormal mitoses. One of them had triploid mitoses, while the others had abnormally diploid mitoses, that is, there was a chromosome with a translocation or deletion.

In mating No. 5, 11 of 37 tadpoles had normally diploid mitoses alone (Tables 5 and 6). Another was a triploid, in which one of No. 4 chromosomes was a ring. Two were mosaics of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses. Seven had no normal mitoses, although they were diploids. In each of them, there were different kinds of mitoses; some mitoses had a chromosome with a translocation, while the others had a chromosome with a deletion. The remaining 16 tadpoles were mosaics of normally and abnormally diploid mitoses.

ii) N♀ × SX-170♂, Nos. 3 and 5

In mating No. 3, six of 16 analyzed tadpoles consisted of normally diploid mitoses alone. One of the other tadpoles was an abnormal diploid whose mitoses contained a chromosome No. 10 with a deletion in the long arm. One had a chromosome No. 3 with a deletion. A tadpole was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses. In these mitoses, there was a loss or an addition of a chromosome No. 9 (Fig. 3). A tadpole had a chromosome No. 2 with a translocation or deletion in the long arm (Fig. 4). The remaining six tadpoles were mosaics of normally diploid and abnormal mitoses; two of them were mosaics of diploid and triploid mitoses, while the other four were those of normally and abnormally diploid mitoses.

In mating No. 5, 7 of 24 analyzed tadpoles had normally diploid mitoses alone. Three other tadpoles were abnormal diploids, in which the metaphase plates of each tadpole were the same in karyotype. One of these tadpoles had a chromosome No. 4 with a deletion in the short arm. Another had a chromosome No. 5 with a translocation in the long arm. The third had a chromosome No. 9 with a deletion in the short arm. Eight tadpoles were abnormal diploids which had no normally diploid mitoses. The metaphase plates of each tadpole were not the same in karyotype. One of these eight tadpoles had a chromosome No. 1 which was ring in shape. The remaining six of the 24 tadpoles were mosaics of

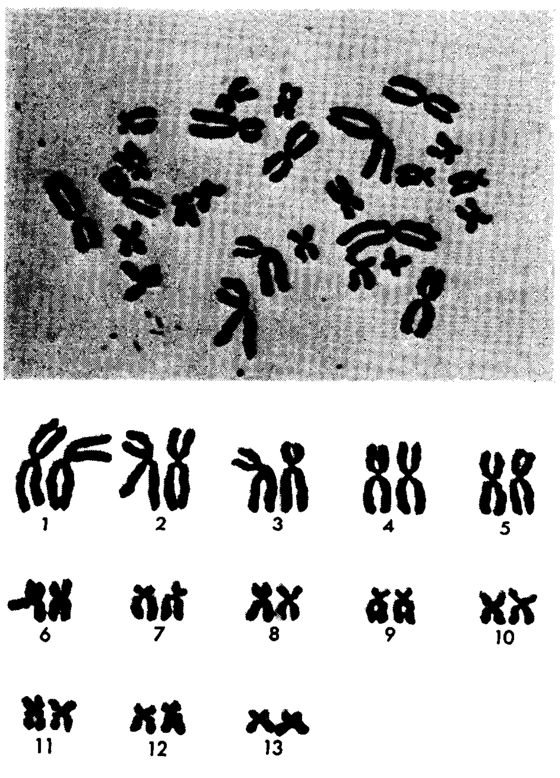


Fig. 2. Normal mitotic chromosomes and the karyotype of a control *Rana nigromaculata*. ×1500

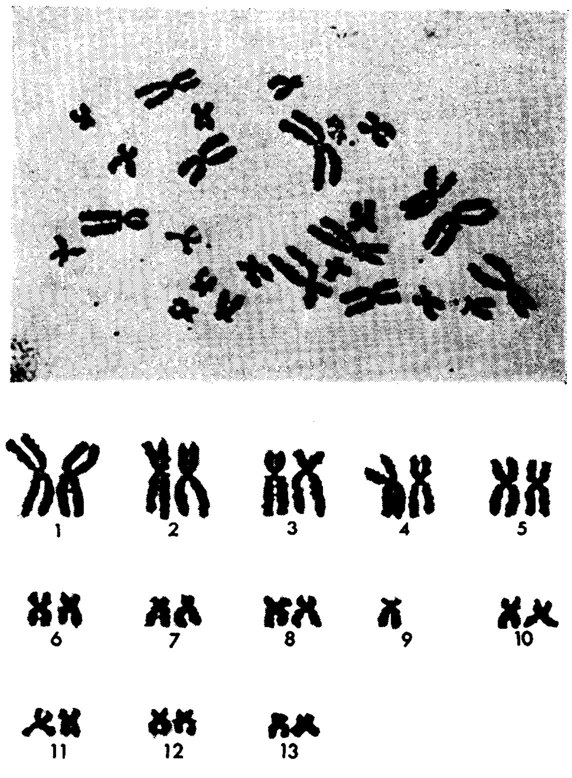


Fig. 3. Chromosome aberration in a normally shaped tadpole produced from a mating, N♂ × SX-170♂, No. 3. A chromosome No. 9 is lost. ×1500

normally and abnormally diploid mitoses.

iii) N♀ × SX-240♂, Nos. 3 and 5

In mating No. 3, only eight of 28 analyzed tadpoles were normal diploids. Each of four other tadpoles consisted of a kind of abnormal mitoses; one was a triploid having 39 chromosomes, one was a hyperdiploid having an additional chromosome No. 10, one had a chromosome No. 5 whose long arm had a deletion, and the last had a chromosome No. 7 whose long arm had a deletion.

Seven of ten other tadpoles were constructed of triploid, hypertriploid or hypotriploid mitoses. Two of them were mosaics of hypertriploid (3n+1) and hypotriploid (3n−1) mitoses, in which there was an addition or a loss of chromosome No. 12. A tadpole was also a mosaic of hypertriploid (3n+1) and hypotriploid (3n−1) mitoses, in which there was an addition or a loss of a chromosome No. 13. A tadpole was a mosaic of regularly triploid and hypertriploid mitoses, which had a ring chromosome derived from a chromosome No. 2 and a fragment. A tadpole was a mosaic of regularly and irregularly triploid mitoses; the latter had a ring chromosome derived from a chromosome No. 4. Two tadpoles were also mosaics of regularly and irregularly triploid mitoses; in one of them, a chromosome No. 5 had a deletion in the long arm, while in the other, a chromosome No. 2 had a deletion in the long arm. Three of the ten tadpoles were mosaics of abnormally diploid mitoses which had a chromosome with a translocation or deletion.

The remaining six tadpoles were mosaics of normally diploid and abnormal mitoses. One of them was a mosaic of diploid and triploid mitoses, while the others were those of normally and abnormally diploid mitoses.

In mating No. 5, four of 18 analyzed tadpoles were normal diploids. A tadpole was a mosaic of hyperdiploid and abnormally diploid mitoses. In the latter, a chromosome had a translocation. Six tadpoles were mosaics of abnormally diploid mitoses, in which a chromosome had a translocation or deletion. Two tadpoles were mosaics of normally diploid and regularly triploid mitoses. The remaining five were mosaics of normally and abnormally diploid mitoses.

c. Experimental series from X-irradiated eggs

i) EX-90♀ × N♂, Nos. 3 and 5

In mating No. 3, 17 of 24 tadpoles whose karyotypes were analyzed were normal diploids. A tadpole was a triploid. One of two other tadpoles was a mosaic of triploid and abnormally diploid mitoses, while the other was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses. The remaining four tadpoles were mosaics of normally and abnormally diploid mitoses.

In mating No. 5, 23 of 33 analyzed tadpoles were normal diploids. A tadpole was a triploid. One of three other tadpoles was a mosaic of abnormally diploid mitoses, whose metaphase plates had a chromosome with a translocation or deletion, while the other two were mosaics of hypodiploid (2n−1) and abnormally diploid mitoses. The remaining six tadpoles were mosaics of normally and abnormally diploid mitoses.

ii) EX–145♀ × N♂, Nos. 3 and 5

In mating No. 3, ten of 20 analyzed tadpoles were normal diploids. Two of three other tadpoles were triploids, while the other was an abnormal diploid whose metaphase plates had three chromosomes Nos. 1, 2 and 5, each of which had a translocation or deletion. Two other tadpoles were mosaics of abnormal mitoses; one consisted of hypertriploid (3n+1) and hypotriploid (3n−1) mitoses, while the other consisted of triploid and abnormally diploid mitoses. One of the remaining five tadpoles was a mosaic of normally diploid and irregularly triploid mitoses, while the other four were mosaics of normally and abnormally diploid mitoses.

In mating No. 5, 19 of 38 analyzed tadpoles were normal diploids. A tadpole was a triploid. Two of eight other tadpoles were mosaics of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, while the other six were mosaics of abnormally diploid and hypodiploid (2n−1) mitoses. The remaining ten tadpoles were mosaics of normally and abnormally diploid mitoses. One of them had a ring chromosome in each of three abnormal mitoses.

iii) EX–200♀ × N♂, Nos. 3 and 5

In mating No. 3, ten of 28 analyzed tadpoles were normal diploids. Two of four other tadpoles were triploids. The other two were abnormal diploids;

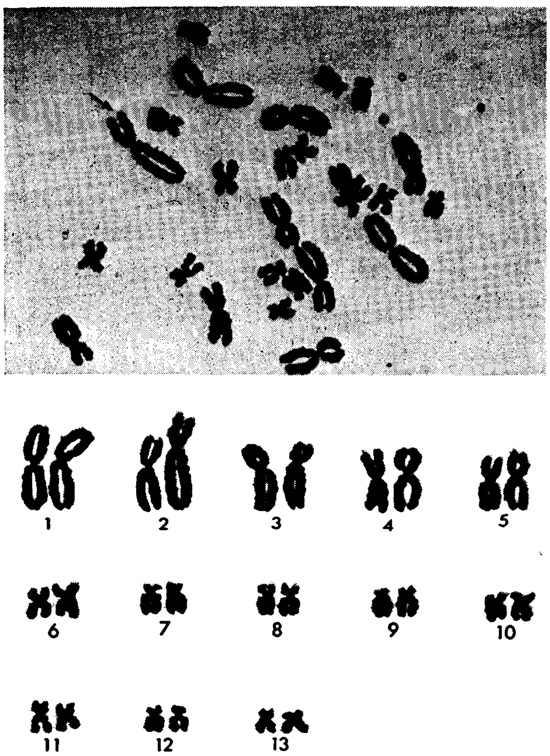


Fig. 4. Chromosome aberration in a normally shaped tadpole produced from a mating, N♀ × SX–170♂, No. 3. An arrow indicates a translocation in a chromosome No. 2.
×1500

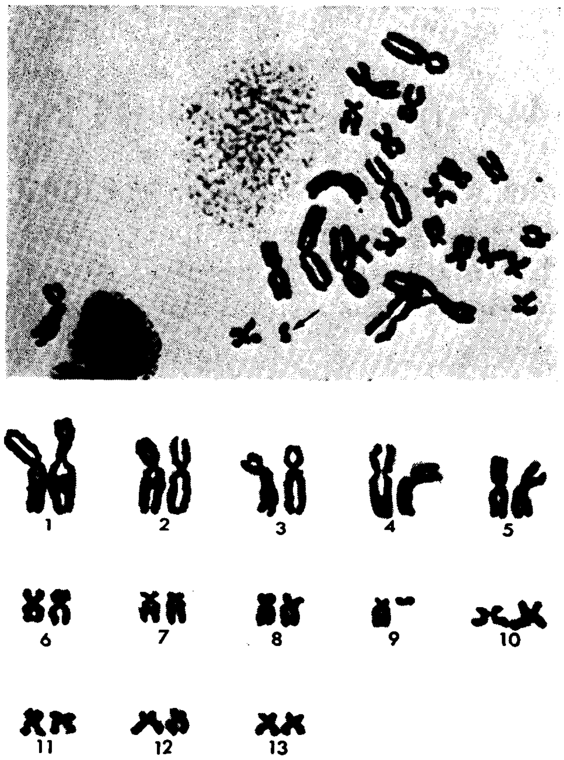


Fig. 5. Chromosome aberration in a normally shaped tadpole produced from a mating, N♀ × SN–50♂, No. 5. An arrow indicates a deletion in the long arm of a chromosome No. 9.
×1500

one had a chromosome No. 4 with a deletion in the long arm, while the other had a chromosome No. 5 with a deletion in the long arm. Six tadpoles were mosaics of abnormal mitoses; one consisted of hypertriploid (3n+1) and hypotriploid (3n−1) mitoses, another did of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, and the remaining four were abnormal diploids consisting of a mixture of different kinds of abnormally diploid mitoses. In the latter, there was a chromosome with a translocation or deletion. The remaining eight tadpoles were mosaics of normally and abnormally diploid mitoses.

In mating No. 5, four of 14 analyzed tadpoles were normal diploids. Five tadpoles were abnormal diploids, each of which was constant in karyotype. The metaphase plates of each tadpole had one or two chromosomes with a translocation or deletion. Four tadpoles were mosaics consisting of a mixture of different kinds of abnormally diploid mitoses. The remaining one was a mosaic of normally diploid and regularly triploid mitoses.

d. Experimental series from neutron-irradiated sperm

i) N♀ × SN–50♂, Nos. 3 and 5

In mating No. 3, eleven of 20 analyzed tadpoles were normal diploids. One of two other tadpoles was an irregular triploid with a ring and a fragment derived from a chromosome No. 2, while the other was a hyperdiploid with an additional small chromosome. Another tadpole was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses. The remaining six were mosaics of normally diploid and abnormal mitoses; one of them had hypodiploid (2n−1), two had hyperdiploid (2n+1) and the other three had abnormally diploid mitoses, in which there was a chromosome with a translocation and deletion.

In mating No. 5, 28 of 38 analyzed tadpoles were normal diploids. Another tadpole had a chromosome No. 9 with a deletion in the long arm (Fig. 5). Two of three other tadpoles were mosaics of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, while the other consisted of a mixture of abnormally diploid mitoses, having a chromosome with a translocation or deletion. The remaining six tadpoles were mosaics of normally and abnormally diploid mitoses.

ii) N♀ × SN–90♂, Nos. 3 and 5

In mating No. 3, six of 13 analyzed tadpoles were normal diploids. One of three other tadpoles was a triploid with a minute chromosome in each metaphase plate, another was a hypotriploid (3n−1), and the remainder was an abnormal diploid, having a minute chromosome. Two others were mosaics of abnormal mitoses; one consisted of hypertriploid (3n+1) and hypotriploid (3n−1) mitoses, while the other consisted of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses. The remaining two tadpoles were mosaics of normally and abnormally diploid mitoses. One of these tadpoles had a metaphase plate with a ring chromosome.

In mating No. 5, nine of 20 analyzed tadpoles were normal diploids. One of two other tadpoles was a hypertriploid (3n+1), having an additional chromosome No. 12, while the other was an abnormal diploid, whose metaphase plates had a chromosome No. 7 with a deletion in the long arm. Five other tadpoles were mosaics of abnormally diploid mitoses which contained a chromosome with a translocation or deletion. The remaining four tadpoles were mosaics of normally and abnormally diploid mitoses.

iii) N♀ × SN-130♂, Nos. 3 and 5

In mating No. 3, seven of 28 analyzed tadpoles were normal diploids. Three of four other tadpoles were regular triploids, while the other was a triploid whose metaphase plates had a ring derived from a chromosome No. 2 (Fig. 6). Twelve other tadpoles were mosaics of abnormal mitoses; two consisted of hypertriploid ($3n+1$) and hypotriploid ($3n-1$) mitoses, two, of hypodiploid ($2n-1$) and abnormally diploid mitoses, having a chromosome with a translocation, and the other eight, of abnormally diploid mitoses, having a chromosome with a translocation or deletion. The remaining five tadpoles were mosaics of normally diploid and abnormal mitoses; two had triploid mitoses, while the other three had abnormally diploid ones, having a minute or a fragment chromosome.

In mating No. 5, six of 20 analyzed tadpoles were normal diploids. Three other tadpoles were abnormal diploids; two of them had a chromosome No. 3 or 5 with a deletion in the short arm, while the other had a chromosome No. 9 with a translocation of a segment which came from the long arm of a chromosome No. 10. Six other tadpoles were mosaics of abnormal mitoses; two consisted of hyperdiploid ($2n+1$) and hypodiploid ($2n-1$) mitoses, while the other

Fig. 6. Chromosome aberration (triploidy) in a normally shaped tadpole produced from a mating, N♀ × SN-130♂, No. 3. An arrow indicates a ring derived from a chromosome No. 2. × 1500

four consisted of abnormally diploid mitoses whose metaphase plates had a chromosome with a translocation or deletion. The remaining five tadpoles were mosaics of normally and abnormally diploid mitoses. One of these tadpoles had a ring and a fragment in place of two chromosomes.

e. Experimental series from neutron-irradiated eggs

i) EN-50♀ ×N♂, Nos. 3 and 5

In mating No. 3, 15 of 34 analyzed tadpoles were normal diploids. Four were regular triploids. Three other tadpoles were mosaics of abnormal mitoses; one consisted of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, in which there was an addition or a loss of a chromosome No. 13 (Figs. 7 and 8), while the other two consisted of abnormally diploid mitoses. The remaining twelve tadpoles were mosaics of normally diploid and abnormal mitoses; three contained regular triploid, three others consisted of hypodiploid (2n−1) mitoses and the other six of abnormally diploid mitoses.

In mating No. 5, twelve of 22 analyzed tadpoles were normal diploids. Four other tadpoles were mosaics of abnormal mitoses; two consisted of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, one, of hypodiploid (2n−1) and abnormally diploid mitoses having a chromosome with a translocation, and the other, of abnormally diploid mitoses having a chromosome with a translocation

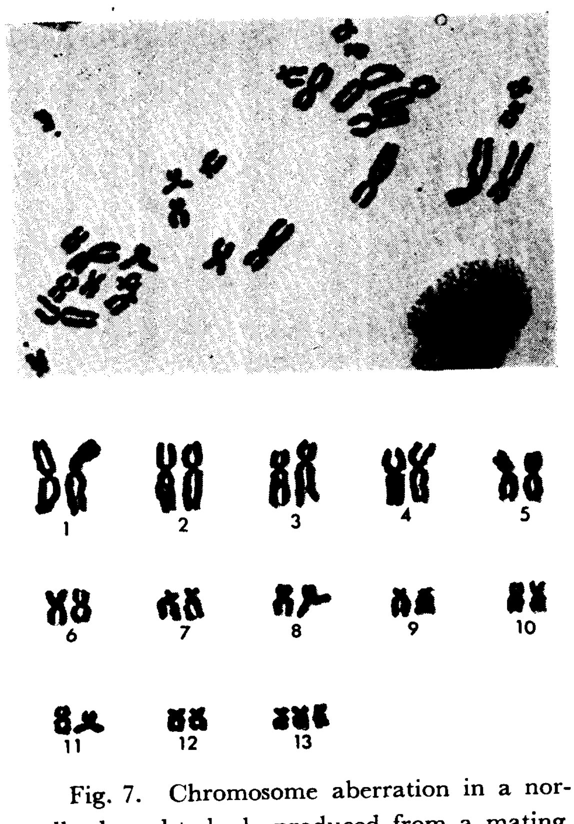


Fig. 7. Chromosome aberration in a normally shaped tadpole produced from a mating, EN-50♀ ×N♂, No. 3. There is an additional chromosome No. 13. ×1500

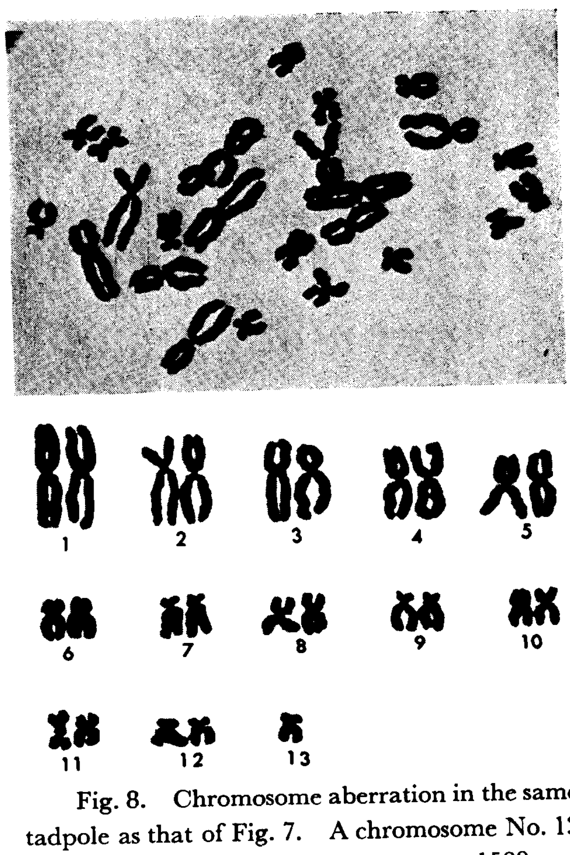


Fig. 8. Chromosome aberration in the same tadpole as that of Fig. 7. A chromosome No. 13 is lost. ×1500

or deletion. The remaining six tadpoles were mosaics of normally and abnormally diploid mitoses.

ii) EN–90♀ × N♂, Nos. 3 and 5

In mating No. 3, nine of 24 analyzed tadpoles were normal diploids. Three of four other tadpoles were regular triploids, while the other was an abnormal diploid, whose metaphase plates were the same in karyotype. Seven other tadpoles were mosaics of abnormal mitoses; two consisted of hypertriploid (3n+1) and hypotriploid (3n−1) mitoses, one, of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, and the other four, of abnormally diploid mitoses having one or two chromosomes with a translocation or deletion. The remaining four tadpoles were mosaics of normally diploid and abnormal mitoses. One of these tadpoles contained hypertriploid (3n+1) mitoses, having a ring and a fragment in place of two chromosomes. Another had regularly triploid mitoses, while the other had abnormally diploid ones.

In mating No. 5, ten of 24 analyzed tadpoles were normal diploids. One of three other tadpoles was a triploid, having a ring derived from a chromosome No. 2, while the other two were abnormal diploids; one had a chromosome No. 4 with a deletion in the short arm, while the other had a chromosome No. 7 with a deletion in the long arm. Four other tadpoles were mosaics of abnormally diploid mitoses, having a chromosome with a translocation or deletion. The remaining seven tadpoles were mosaics of normally and abnormally diploid mitoses. One of them had metaphase plates containing a ring derived from a chromosome No. 5, while the others had those containing a chromosome with a translocation or deletion.

iii) EN–130♀ × N♂, Nos. 3 and 5

In mating No. 3, only six of 20 analyzed tadpoles were normal diploids. Four of six other tadpoles were regular triploids, while the other two were abnormal diploids whose metaphase plates were the same in karyotype; one had a chromosome No. 3 with a deletion in the long arm and a chromosome No. 4 with a translocation in the long arm (Fig. 9), while the other had a chromosome No. 9 with a deletion in the long arm (Fig. 10). Four other tadpoles were mosaics of abnormal mitoses; two consisted of hypertriploid (3n+1) and hypotriploid (3n−1) mitoses, one, of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, and the other, of abnormally diploid mitoses which were not the same in karyotype. The remaining four tadpoles were mosaics of normally diploid and abnormal mitoses; two had regularly triploid mitoses, while the other two had abnormally diploid ones. One of the latter tadpoles had a minute chromosome, while the other had a fragment in place of a chromosome.

In mating No. 5, only seven of 20 analyzed tadpoles were normal diploids. Nine tadpoles were mosaics of abnormal mitoses; one consisted of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, while the other eight consisted of different kinds of abnormally diploid mitoses. The remaining four tadpoles were mosaics of normally diploid and abnormal mitoses; one contained hypodiploid (2n−1), while the other three contained abnormally diploid mitoses.

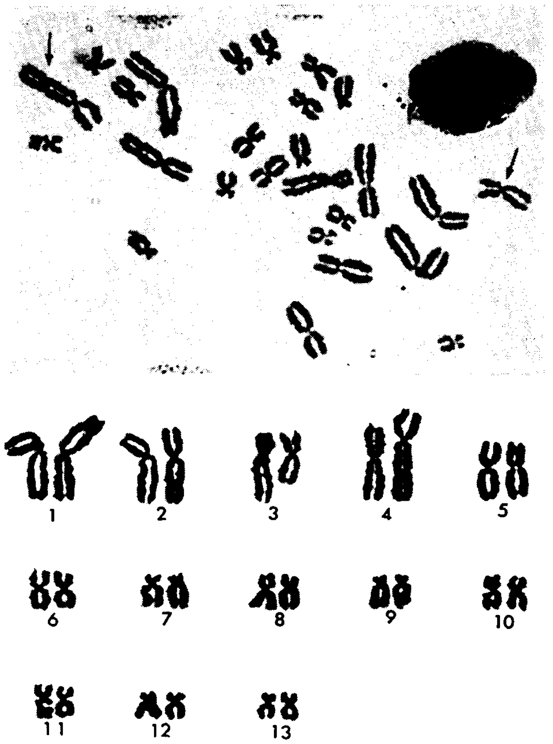


Fig. 9. Chromosome aberration in a normally shaped tadpole produced from a mating, EN–130♀×N♂, No. 3. Two arrows indicate a deletion in a chromosome No. 3 and a translocation in a chromosome No. 4. ×1500

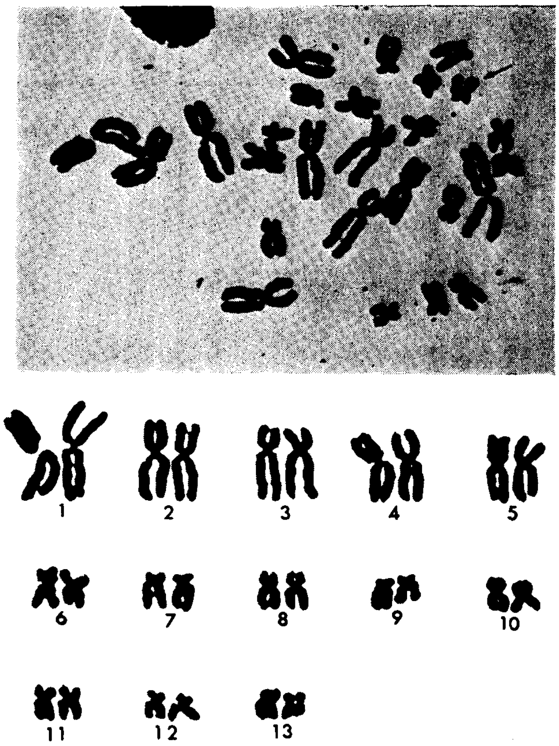


Fig. 10. Chromosome aberration in a normally shaped tadpole produced from a mating, EN–130♀×N♂, No. 3. An arrow indicates a deletion in the long arm of a chromosome No. 9. ×1500

3. Viability and sex of metamorphosed frogs

In the control series, 945 tadpoles climbed out of water at the age of 62.8 days on the average (Table 7). In the twelve experimental series, 181~638 tadpoles climbed out of water at the ages of 62.7~68.7 days on the average. However, 181~600 tadpoles in eleven of the twelve experimental series climbed out of water at the ages of 64.0~68.7 days on the average. In other words, the tadpoles in the experimental series were generally delayed in metamorphosis, as compared with the controls. There were no distinct differences in the age of tadpoles at metamorphosis between different experimental series, except the series of neutron-irradiated eggs.

A total of 900 frogs removed at random immediately after completion of metamorphosis in the control series were 19.2 ± 0.1 mm in body length (Table 7). In the twelve experimental series, all the frogs at the same developmental stage in each of the twelve experimental series were 18.3 ± 0.2~19.3 ± 0.2 mm in body length. In other words, there were no distinct differences in body length between the frogs of the control and the experimental series, as well as between the frogs of different experimental series.

In order to produce mature frogs, 50 young frogs were left alive from among those produced from each of matings Nos. 3 and 5 in each of the twelve ex-

TABLE 7

Number, size and sex of metamorphosed

Parents: Female	Parents: Male	Age at the time of climbing out of water (days)	No. of metamor-phosed frogs	Body length immediately after metamorphosis (mm)	No. of frogs
N.W67, Nos. 1∼10	N.W67, Nos. 1∼10	56∼80 (62.8)	945	19.2±0.1	867
N.W67, Nos. 1∼10	SX–90, Nos. 1∼10	58∼97 (65.0)	600	18.3±0.1	431
	SX–170, Nos. 1∼10	56∼85 (64.2)	434	18.5±0.2	332
	SX–240, Nos. 1∼10	58∼88 (64.0)	242	19.1±0.2	140
EX–90, Nos. 1∼10	N.W67, Nos. 1∼10	56∼90 (64.7)	578	19.3±0.2	457
EX–145, Nos. 1∼10		56∼91 (64.7)	380	19.0±0.2	258
EX–200, Nos. 1∼10		57∼83 (65.7)	181	18.4±0.1	83
N.W67, Nos. 1∼10	SN–50, Nos. 1∼10	57∼83 (64.9)	554	19.2±0.2	463
	SN–90, Nos. 1∼10	58∼91 (65.0)	389	18.5±0.1	241
	SN–130, Nos. 1∼10	58∼91 (65.3)	202	18.9±0.2	75
EN–50, Nos. 1∼10	N.W67, Nos. 1∼10	57∼90 (62.7)	638	18.6±0.1	538
EN–90, Nos. 1∼10		57∼96 (66.8)	455	18.3±0.2	299
EN–130, Nos. 1∼10		57∼94 (68.7)	217	19.1±0.2	153

$♀_N$—Females with normal ovaries $♀_U$—Females with underdeveloped ovaries

perimental series. They were at the stage about one month after completion of metamorphosis. These two matings were those, in which the most numerous eggs were used and consequently the most numerous frogs were obtained among the ten matings of each experimental series. When less than 50 frogs were living in matings No. 3 or 5, the shortage was replenished with frogs of the other matings. However, a total of 80, 80 and 60 frogs were left alive in three experimental series, EX–200♀×N♂, N♀×SN–50♂, and EN–130♀×N♂, respectively, as there were less than 100 frogs in each of these series. In the control series, 30 frogs were left alive among those produced from each of matings Nos. 3 and 5. All the other young frogs in the experimental and the control series were killed to examine their sex.

About 6% of metamorphosed frogs in the experimental series died within a week after climbing out of water. Most of them died of ill-formation of the forelegs or of edema. While edematous frogs were produced from either eggs or spermatozoa irradiated, the ill-formation of the forelegs occurred almost frogs raised from irradiated sperm or eggs

Sex of frogs killed within one month after metamorphosis					No. of frogs removed and reared	Sex of 2-year-old mature frogs			Sex of all frogs examined		
♀N	♀U	⚥	♂N	♂ (%)*		No. of frogs	♀	♂	Total	♀	♂ (%)*
410	21	28	408	(50.3)	60	49	24	25	916	455	461 (50.3)
166	21	42	202	(56.6)	100	52	25	27	483	212	271 (56.1)
131	9	36	156	(57.8)	100	23	12	11	355	152	203 (57.2)
54	5	8	73	(57.9)	100	7	3	4	147	62	85 (57.8)
169	30	65	193	(56.5)	100	28	14	14	485	213	272 (56.1)
76	15	20	147	(64.7)	100	32	15	17	290	106	184 (63.4)
45	7	9	22	(37.3)	80	15	6	9	98	58	40 (40.8)
185	19	21	238	(55.9)	80	22	12	10	485	216	269 (55.5)
90	17	25	109	(55.6)	100	19	10	9	260	117	143 (55.0)
26	6	7	36	(57.3)	100	27	13	14	102	45	57 (55.9)
215	24	40	259	(55.6)	100	15	7	8	553	246	307 (55.5)
105	20	36	138	(58.2)	100	22	9	13	321	134	187 (58.3)
48	20	16	69	(55.6)	60	25	12	13	178	80	98 (55.1)

⚥—Hermaphrodites ♂N—Males with normal testes * Including hermaphrodites

exclusively in the frogs derived from irradiated eggs.

Some frogs died of edema or hypertrophy of the liver within one month after metamorphosis. A smaller number of frogs died of some diseases, such as stomatitis or skin disease, although they were normal in shape. The frogs in the experimental series were far lower in resistance to diseases than those in the control series.

While a part of males matured by the breeding season of the next year, the other part of males and all the females matured by the year after next. In the breeding season of the third year, 49 mature frogs remained alive from among the 60 frogs in the control series. In the three experimental series derived from X-irradiated spermatozoa, 52, 23 and 7 mature frogs remained alive respectively from among 100 young frogs which came from spermatozoa irradiated with 90, 170 or 240 rads. However, in the other experimental series, frogs derived from gametes which were irradiated with smaller doses were not always superior in viability to those which came from gametes irradiated with larger doses. The inverse relationship to that in the experimental series derived from X-irradiated spermatozoa was found in the experimental series derived from neutron-irradiated eggs. In any case, most of the frogs in the experimental series died before the breeding season of the third year; only 287 of 1120 young frogs remained alive, while 49 of 60 young frogs did live in the control series.

a. Control series

N♀ × N♂, Nos. 1 ~ 10

Within about one month after completion of metamorphosis, 18 of 945 frogs died. Of the other living frogs, 867 were soon killed to examine their sex; 431 were females, 28 hermaphrodites and 408 males (Table 7). Twenty-one of the females had underdeveloped ovaries, while the others had normal ones. All the hermaphrodites had gonads transforming from ovaries into testes. When the hermaphrodites were counted as males, as they would surely become males within a few months, 50.3% of the frogs examined were males. At the age of two years, 49 of 60 frogs left behind were living; 24 were females and 25 males. Of a total of 916 immature and mature frogs, 455 were females and 461 (50.3%) were males including 28 hermaphrodites.

b. Experimental series from X-irradiated sperm

i) N♀ × SX–90♂, Nos. 1 ~ 10

Of 600 metamorphosed frogs, 69 died within about one month after completion of metamorphosis, and 431 of the other living frogs were killed to examine their sex; 187 were females, 42 hermaphrodites and 202 males. When the hermaphrodites were counted as males, 56.6% were males. Although 100 frogs had been left alive, only 52 of them were living at the age of two years; 25 were females and 27 males.

ii) N♀ × SX–170♂, Nos. 1 ~ 10

Only two of 434 metamorphosed frogs died within about one month after completion of metamorphosis. Of the remaining frogs, 332 were killed to examine their sex; 140 were females, 36 hermaphrodites and 156 males. When the hermaphrodites were counted as males, 57.8% were males. At the age of two years, 23 of the remaining 100 frogs were living: twelve were females and eleven males.

iii) N♀ × SX–240♂, Nos. 1 ~ 10

Only two of 242 metamorphosed frogs died within about one month after completion of metamorphosis. Of the remaining frogs, 140 were soon killed to examine their sex; 59 were females, eight hermaphrodites and 73 males. When the hermaphrodites were counted as males, 57.9% were males. Only seven of the remaining 100 frogs were living at the age of two years; three were females and four males.

c. Experimental series from X-irradiated eggs

i) EX–90♀ × N♂, Nos. 1 ~ 10

Twenty-one of 578 metamorphosed frogs died within about one month after completion of metamorphosis. Of the other living frogs, 457 were killed to examine their sex; 199 were females, 65 hermaphrodites and 193 males. When the hermaphrodites were counted as males, 56.5% were males. Of the remaining 100 frogs, 28 were living at the age of two years; 14 were females and 14 males.

ii) EX–145♀ × N♂, Nos. 1～10

Twenty-two of 380 metamorphosed frogs died within about one month after completion of metamorphosis. Of the other living frogs, 258 were soon killed to examine their sex; 91 were females, 20 hermaphrodites and 147 males. When the hermaphrodites were counted as males, 64.7% were males. Of the remaining 100 frogs, 32 were living at the age of two years; 15 were females and 17 males.

iii) EX–200♀ × N♂, Nos. 1～10

Eighteen of 181 metamorphosed frogs died within about one month after completion of metamorphosis. Of the other living frogs, 83 were soon killed; 52 were females, nine hermaphrodites and 22 males. When the hermaphrodites were counted as males, 37.3% were males. Of 80 frogs left behind, only 15 were living at the age of two years; six were females and nine males.

d. Experimental series from neutron-irradiated sperm

i) N♀ × SN–50♂, Nos. 1～10

Eleven of 554 metamorphosed frogs died within about one month after completion of metamorphosis. Of the other living frogs, 463 were soon killed to examine their sex; 204 were females, 21 hermaphrodites and 238 males. When the hermaphrodites were counted as males, 55.9% were males. Of the remaining 80 frogs, 22 were living at the age of two years; 12 were females and ten males.

ii) N♀ × SN–90♂, Nos. 1～10

Of 389 metamorphosed frogs, 48 died within about one month after completion of metamorphosis. Of the remaining frogs, 241 were soon killed to examine their sex; 107 were females, 25 hermaphrodites and 109 males. When the the hermaphrodites were counted as males, 55.6% were males. Of the remaining 100 frogs, only 19 were living at the age of two years; ten were females and nine males.

iii) N♀ × SN–130♂, Nos. 1～10

Twenty-seven of 202 metamorphosed frogs died within about one month after completion of metamorphosis. Of the other living frogs, 75 were soon killed; 32 were females, seven hermaphrodites and 36 males. When the hermaphrodites were counted as males, 57.3% were males. Of the remaining 100 frogs, 27 were living at the age of two years; 13 were females and 14 males.

e. Experimental series from neutron-irradiated eggs

i) EN–50♀ × N♂, Nos. 1～10

All the 638 metamorphosed frogs were alive about one month after completion of metamorphosis. Of these frogs, 538 were soon killed to examine their sex;

239 were females, 40 hermaphrodites and 259 were males. When the hermaphrodites were counted as males, 55.6% were males. Of the remaining 100 frogs, only 15 were living at the age of two years; seven were females and eight males.

ii) EN-90♀×N♂, Nos. 1~10

Of 455 metamorphosed frogs, 56 died within about one month after completion of metamorphosis. Of the other living frogs, 299 were soon killed; 125 were females, 36 hermaphrodites and 138 males. When the hermaphrodites were counted as males, 58.2% were males. Of the remaining 100 frogs, 22 were living at the age of two years; nine were females and 13 males.

iii) EN-130♀×N♂, Nos. 1~10

Four of 217 metamorphosed frogs died within about one month after completion of metamorphosis. Of the other living frogs, 153 were soon killed to examine their sex; 68 were females, 16 hermaphrodites and 69 males. When the hermaphrodites were counted as males, 55.6% were males. Of the remaining 60 frogs, 25 were living at the age of two years; 12 were females and 13 males.

II. Second-generation offspring derived from irradiated gametes by passing over male first-generation offspring

1. Male parents

Male first-generation offspring obtained in 1967 from eggs or spermatozoa which were irradiated with X-rays or neutrons were mated by artificial fertilization in 1969 with ten normal females collected from the field. The reproductive ability of the 2-year-old first-generation offspring as well as the developmental capacity of the second-generation offspring produced from them were examined (Table 14, Figs. 11~13). Ten normal females Nos. 1~10 were 61.0~67.0 mm in body length and had 2022~2566 eggs (Table 8). Fifty eggs removed at random from among those of each female were 1.70±0.01~2.01±0.01 mm in diameter. The male first-generation offspring used for matings were those pre-

TABLE 8

Eggs of ten field-caught female frogs used for mating experiments in 1969

Kind	Individual no.	Body length (mm)	No. of eggs	Mean diameter of 50 eggs (mm)
N.W69	1	61.0	2350	1.78±0.01
	2	61.5	2503	1.92±0.01
	3	64.5	2454	1.84±0.01
	4	62.0	2271	1.74±0.01
	5	65.0	2022	1.98±0.01
	6	63.5	2437	1.73±0.01
	7	61.5	2309	1.87±0.01
	8	67.0	2566	2.01±0.01
	9	65.0	2400	1.70±0.01
	10	66.0	2545	1.71±0.01

sented in Tables 9 ~ 13. They were produced from matings Nos. 3 and 5 of both the experimental and control series in 1967. Ten males obtained from the two matings of the control series were 44.5 ~ 52.5 mm, 49.3 mm on the average, in body length and 5.0 × 2.0 ~ 6.5 × 3.5 mm in the length and width of the testes (Table 9). Ninety-six male first-generation offspring obtained from the two matings of each of the twelve experimental series were 37.0 ~ 62.0 mm, 49.0 mm on the average, in body length. Their testes were mostly 4.0 × 3.0 ~ 5.5 × 3.5 mm in length and width.

TABLE 9

Testes of ten control male frogs used for mating experiments in 1969

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes Left (mm)	Size of the testes Right (mm)	Inner structure
(N·N), No. 3	1	2	47.5	5.0 × 3.0	5.0 × 3.0	Type 1
	2	2	52.0	6.5 × 3.5	6.5 × 3.5	Type 1
	3	2	48.0	5.0 × 2.5	5.0 × 2.5	Type 1
	4	2	44.5	5.0 × 2.0	5.0 × 2.0	Type 1
	5	2	47.0	6.0 × 3.0	5.5 × 3.0	Type 1
(N·N), No. 5	6	2	52.5	5.0 × 2.5	5.0 × 3.0	Type 1
	7	2	50.0	6.0 × 3.0	5.5 × 3.0	Type 1
	8	2	48.5	5.0 × 3.0	5.5 × 3.0	Type 1
	9	2	51.0	5.5 × 3.5	5.0 × 3.0	Type 1
	10	2	52.0	5.0 × 3.0	5.0 × 3.5	Type 1

(N·N), No. 3 or 5: Males produced from mating between a field-caught female (N.W67, No. 3 or 5) and a field-caught male (N.W67, No. 3 or 5)

The testes of the 2-year-old males in the experimental and control series were partly removed from the body and preserved in order to examine their inner structure. The remaining parts of the testes were used for producing their offspring. The number and the body-length of males used for this purpose as well as the size and inner structure of their testes are presented in Tables 9 ~ 13. The testes were divided into 5 types on the basis of abnormality in inner structure as follows:

Type 1. The testis is quite normal in inner structure (Plate I, 1). Seminal tubules are filled with bundles of normal spermatozoa. Abnormal spermatozoa and pycnotic nuclei are very scarce.

Type 2. Although the testis is normal in inner structure, bundle-forming spermatozoa are somewhat fewer than those in type 1 (Plate I, 2). A few abnormal spermatozoa and pycnotic nuclei are found here and there.

Type 3. Numerous spermatozoa and spermatocytes are found along the walls of seminal tubules (Plate I, 3). Although spermatozoa form bundles, they are distinctly fewer than those in types 1 and 2. Abnormal spermatozoa and pycnotic nuclei are usually more numerous than those in types 1 and 2.

Type 4. Spermatozoa are very few and sparsely distributed in seminal tubules (Plate I, 4). Besides normal spermatozoa, a few abnormal spermatozoa and pycnotic nuclei are found. There are abundant spermatogonia and spermatocytes along the walls of seminal tubules.

Type 5. There are no normal spermatozoa, while there are a few abnormal ones (Plate I, 5). The seminal tubules are filled with spermatogonia, spermatocytes and pycnotic nuclei. The pycnotic nuclei are those which have degenerated before completion of the first meiotic division.

All the ten males in the control series had testes of type 1 (Table 9). In the twelve experimental series, males having testes of type 1 were rather rare. Of eight males in experimental series, N♀ × SX–90♂, Nos. 3 and 5, four had testes of type 1, two had those of type 2, one had those of type 3, and the remaining one had those of type 4 (Table 10). In experimental series, N♀ × SX–170♂, Nos. 3 and 5, the testes of two of eight mature males were of type 2, those of five were of type 3 and those of the remaining one were of type 4. There were no males with testes of type 1. In experimental series, N♀ × SX–240♂, Nos. 3 and 5, two of four mature males had testes of type 2, while the other

TABLE 10

Testes of twenty male frogs raised from X-irradiated sperm and used for mating experiments in 1969

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes: Left (mm)	Size of the testes: Right (mm)	Inner structure
N·SX–90, No. 3	1	2	49.5	4.0×2.0	4.0×2.0	Type 1
	2	2	45.0	4.0×2.0	4.0×2.0	Type 3
	3	2	45.5	5.0×2.5	4.5×2.5	Type 2
	4	2	50.0	5.0×2.5	5.0×3.0	Type 1
N·SX–90, No. 5	5	2	45.0	4.0×2.0	—	Type 4
	6	2	50.0	5.5×3.0	5.0×2.5	Type 1
	7	2	43.0	6.5×2.5	1.0×0.5	Type 2
	8	2	37.0	5.0×2.5	2.0×0.7	Type 1
N·SX–170, No. 3	1	2	53.0	3.5×2.0	4.0×2.0	Type 3
	2	2	49.0	5.0×2.0	4.0×2.5	Type 3
	3	2	46.0	4.0×2.5	4.5×2.5	Type 4
	4	2	41.0	3.0×2.0	3.5×2.0	Type 2
N·SX–170, No. 5	5	2	53.0	6.0×2.5	5.5×3.0	Type 3
	6	2	44.0	4.0×3.0	4.0×3.0	Type 3
	7	2	43.0	—	9.5×5.0	Type 3
	8	2	40.5	3.0×2.0	5.0×3.0	Type 2
N·SX–240, No. 3	1	2	51.0	4.5×4.0	4.0×3.0	Type 3
	2	2	53.0	5.0×2.5	5.0×2.0	Type 3
	3	2	48.0	5.0×3.0	4.5×3.5	Type 2
N·SX–240, No. 5	4	2	45.5	4.0×2.5	5.0×2.0	Type 2

N·SX–90, –170 or –240, No. 3 or 5: Males raised from eggs of a field-caught female (N.W67, No. 3 or 5) by fertilization with sperm No. 3 or 5 exposed to 90, 170 or 240 rads of X-rays two had those of type 3.

In experimental series, EX–90♀ ×N♂, Nos. 3 and 5, only one of 13 mature males had testes of type 1 (Table 11). Six males had testes of type 2, five had those of type 3, and the remaining one had those of type 4. In experimental series, EX–145♀ ×N♂, Nos. 3 and 5, only one of 12 mature males had testes of type 1. Five males had testes of type 2, three had those of type 3, and the remaining three had those of type 4. In experimental series, EX–200♀ ×N♂, No. 5, one of five mature males had testes of type 2, while the other four had those of type 3. No males had testes of type 1 in this series.

In experimental series, N♀ ×SN–50♂, Nos. 3 and 5, three of six mature males had testes of type 2, two had those of type 3 and the remaining one had those of

TABLE 11

Testes of thirty male frogs raised from X-irradiated eggs and used for mating experiments in 1969

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure
				Left (mm)	Right (mm)	
EX–90·N, No. 3	1	2	60.0	4.0×3.5	4.0×3.5	Type 2
	2	2	62.0	6.0×6.0	6.0×3.0	Type 3
	3	2	57.0	4.0×2.0	5.5×3.0	Type 3
	4	2	48.0	4.5×2.5	5.0×2.5	Type 2
EX–90·N, No. 5	5	2	60.0	5.5×3.0	5.0×3.0	Type 2
	6	2	46.5	3.5×2.0	3.5×2.0	Type 2
	7	2	54.5	5.5×2.0	5.0×2.0	Type 2
	8	2	47.5	4.0×3.5	4.0×3.0	Type 2
	9	2	46.0	4.0×2.5	4.5×3.0	Type 3
	10	2	45.5	4.0×1.5	4.0×2.0	Type 1
	11	2	50.0	5.0×3.0	4.5×3.0	Type 3
	12	2	45.0	5.0×3.0	4.5×3.0	Type 4
	13	2	52.0	4.5×2.5	4.5×3.0	Type 3
EX–145·N, No. 3	1	2	50.0	3.0×3.0	4.0×2.5	Type 3
	2	2	52.0	2.0×1.5	2.5×1.5	Type 4
	3	2	48.5	4.5×3.0	3.0×2.5	Type 2
EX–145·N, No. 5	4	2	47.5	4.5×3.5	4.0×3.0	Type 3
	5	2	52.0	4.0×2.0	4.0×2.0	Type 2
	6	2	48.5	3.0×2.0	1.0×1.0	Type 4
	7	2	46.0	4.5×3.0	4.0×3.0	Type 3
	8	2	53.0	5.5×3.5	5.0×3.5	Type 1
	9	2	50.5	5.0×4.0	5.0×4.0	Type 4
	10	2	37.5	3.0×2.0	3.0×2.0	Type 2
	11	2	41.0	8.0×5.5	—	Type 2
	12	2	47.0	7.5×5.5	1.0×0.5	Type 2
EX–200·N, No. 5	1	2	48.0	4.5×2.0	4.0×2.0	Type 3
	2	2	44.0	4.5×2.5	4.5×2.5	Type 3
	3	2	48.0	4.0×2.0	4.0×2.5	Type 2
	4	2	47.5	5.0×2.0	5.0×3.0	Type 3
	5	2	48.0	4.0×2.0	4.0×2.5	Type 3

EX–90, –145 or –200·N, No. 3 or 5: Males raised from eggs No. 3 or 5 exposed to 90, 145 or 200 rads of X-rays by fertilization with sperm of a field-caught male (N.W67, No. 3 or 5) type 4 (Table 12). In experimental series, N♀ × SN–90♂, Nos. 3 and 5, three of seven mature males had testes of type 2, three had those of type 3 and the remaining one had those of type 5. In experimental series, N♀ × SN–130♂, Nos. 3 and 5, two of eight mature males had testes of type 2, five had those of type 3, and the remaining one had those of type 5. There were no males with testes of type 1 in the experimental series derived from neutron-irradiated spermatozoa.

TABLE 12

Testes of twenty-one male frogs raised from neutron-irradiated sperm and used for mating experiments in 1969

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes: Left (mm)	Size of the testes: Right (mm)	Inner structure
N·SN–50, No. 3	1	2	54.0	5.5×2.0	—	Type 3
	2	2	43.5	4.0×2.0	3.0×2.0	Type 2
	3	2	40.5	4.0×2.0	5.5×2.0	Type 2
N·SN–50, No. 5	4	2	49.0	4.5×2.5	3.0×3.0	Type 2
	5	2	45.0	7.0×3.0	—	Type 3
	6	2	43.0	4.5×2.0	3.0×2.0	Type 4
N·SN–90, No. 3	1	2	55.0	4.5×3.0	5.0×2.5	Type 3
	2	2	46.5	5.0×2.5	5.5×3.0	Type 2
	3	2	48.0	4.0×3.0	3.5×3.0	Type 2
	4	2	49.5	2.0×2.5	3.0×2.5	Type 2
N·SN–90, No. 5	5	2	48.0	5.0×2.5	4.5×3.0	Type 5
	6	2	50.0	5.0×3.0	5.0×3.0	Type 3
	7	2	49.5	—	7.5×5.0	Type 3
N·SN–130, No. 3	1	2	57.0	5.0×3.5	5.0×3.0	Type 3
	2	2	47.5	5.5×3.0	5.5×3.0	Type 2
	3	2	58.0	6.5×3.0	6.0×3.0	Type 3
	4	2	53.5	3.0×2.5	7.0×3.0	Type 2
N·SN–130, No. 5	5	2	56.5	4.5×3.5	4.5×3.0	Type 3
	6	2	52.5	5.5×3.0	5.5×3.5	Type 3
	7	2	49.0	5.0×3.0	4.5×3.0	Type 3
	8	2	53.0	5.5×3.0	5.0×3.0	Type 5

N·SN–50, –90 or –130, No. 3 or 5: Males raised from eggs of a field-caught female (N·W67, No. 3 or 5) by fertilization with sperm No. 3 or 5 exposed to 50, 90 or 130 rads of neutrons

In experimental series, EN–50♀ × N♂, Nos. 3 and 5, four of six mature males had testes of type 2, while the other two had those of type 3 (Table 13). In experimental series, EN–90♀ × N♂, Nos. 3 and 5, only one of 12 mature males had testes of type 1, seven had those of type 2, three had those of type 3, and the remaining one had those of type 4. In experimental series, EN–130♀ × N♂, Nos. 3 and 5, four of seven mature males had testes of type 2, while the other three had those of type 3. Thus, only one of 25 males produced from neutron-irradiated eggs had testes of type 1.

In the control series, the right and left testes of each male were very similar in size, while in the experimental series, there were some males whose right and

TABLE 13

Testes of twenty-five male frogs raised from neutron-irradiated eggs and used for mating experiments in 1969

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure
				Left (mm)	Right (mm)	
EN–50·N, No. 3	1	2	54.0	4.0×3.5	4.0×3.0	Type 2
EN–50·N, No. 5	2	2	50.5	4.5×3.0	4.5×2.5	Type 2
	3	2	52.5	4.5×3.5	4.0×3.0	Type 2
	4	2	52.0	4.0×3.0	4.0×3.0	Type 3
	5	2	53.0	4.0×3.0	4.0×2.5	Type 3
	6	2	52.5	5.5×3.5	5.0×3.0	Type 2
EN–90·N, No. 3	1	2	51.5	5.0×3.0	5.0×3.0	Type 3
	2	2	51.0	5.5×3.0	5.0×3.5	Type 2
	3	2	60.0	5.0×3.0	5.0×3.0	Type 2
	4	2	53.5	5.5×4.0	5.0×3.5	Type 2
EN–90·N, No. 5	5	2	52.5	5.0×3.0	4.5×3.0	Type 2
	6	2	45.5	4.5×2.5	5.0×3.0	Type 2
	7	2	53.5	4.0×3.5	4.0×3.0	Type 4
	8	2	50.0	4.5×3.0	4.5×2.5	Type 1
	9	2	46.0	7.5×3.0	—	Type 2
	10	2	42.5	5.0×2.5	—	Type 2
	11	2	49.5	5.0×3.5	5.0×3.5	Type 3
	12	2	41.5	6.0×2.5	1.0×0.5	Type 3
EN–130·N, No. 3	1	2	50.5	4.0×3.0	4.0×3.0	Type 3
	2	2	46.0	5.5×3.0	5.0×3.0	Type 3
	3	2	46.5	5.0×3.0	5.0×3.0	Type 3
	4	2	46.0	4.0×3.5	4.0×3.0	Type 2
EN–130·N, No. 5	5	2	45.0	5.5×3.5	5.5×3.0	Type 2
	6	2	46.5	5.5×3.5	5.0×3.5	Type 2
	7	2	46.5	4.0×3.0	5.0×3.0	Type 2

EN–50, –90 or –130·N, No. 3 or 5: Males raised from eggs No. 3 or 5 exposed to 50, 90 or 130 rads of neutrons by fertilization with sperm of a field-caught male (N.W67, No. 3 or 5)

left testes differed distinctly. A few males had the testis on one side, while there was no trace of a gonad on the other side. In most of these cases, the testes were unusually large.

2. Developmental capacity

The male first-generation offspring were remarkably inferior in fertilizing ability than the control males. The second-generation offspring produced from the former were also remarkably inferior in developmental capacity to those from the latter. As a matter of fact, the males whose testes were more abnormal in inner structure were more inferior in reproductive capacity. However, there was no intimate relationship between the dosages of irradiation given to the eggs or spermatozoa and the developmental capacities of the second-generation offspring, in contrast with the condition in the first-generation offspring.

Numerous embryos and tadpoles in the experimental series died of various kinds of abnormalities. Among the abnormalities found at the gastrula stage, formation of exogastrulae attributable to an incomplete invagination and interruption of an invagination were most frequently observed. At stages from tail-bud to hatching, edema was the main cause of death. Blisters, microcephaly and curvature of the body were the second cause of death. Some embryos died of cauda bifida or bicephaly, too. Two or three of these kinds of abnormalities often occurred simultaneously in an embryo. At the tadpole stage, the principal cause of death was ill-formation of the gills or edema. The second cause was underdevelopment of the body or ill-formation of the legs.

a. Control series

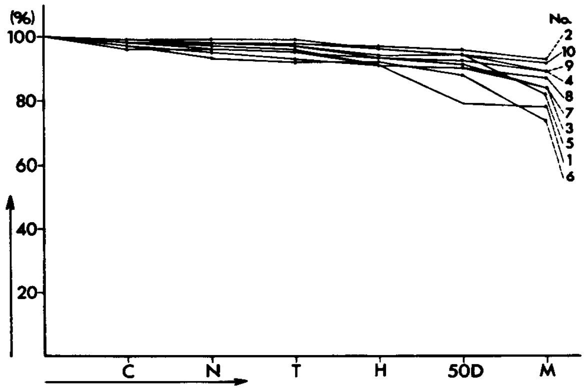
N♀×(N♀×N♂)♂, Nos. 1～10

Ten field-caught females Nos. 1～10 were mated by artificial fertilization with ten 2-year-old males that were produced as the controls of the experiments performed in 1967 (Table 14, Fig. 11). The developmental capacity of the offspring of each male is presented in Table 14, together with the percentage of normally cleaved eggs. The survival curves of the offspring are shown in Fig. 11. In control matings Nos. 1～10, 95.8～99.4%, 98.2% on the average,

TABLE 14

Developmental capacity of the offspring of

Parents		No. of eggs	No. of cleaved eggs	
Female	Male		Normal	Abnormal
N.W69, Nos. 1～10	(N·N), Nos. 1～10	1814	1782 (98.2%)	0
N.W69, Nos. 1～3	(N·SX–90), Nos. 1～8	1417	1167 (82.4%)	15
N.W69, Nos. 4～7	(N·SX–170), Nos. 1～8	1559	1286 (82.5%)	41
N.W69, Nos. 8～10	(N·SX–240), Nos. 1～4	1048	907 (86.5%)	3
N.W69, Nos. 1～3	(EX–90·N), Nos. 1～13	2930	2486 (84.8%)	15
N.W69, Nos. 4～7	(EX–145·N), Nos. 1～12	2677	1973 (73.7%)	134
N.W69, Nos. 8～10	(EX–200·N), Nos. 1～5	742	661 (89.1%)	30
N.W69, Nos. 1～3	(N·SN–50), Nos. 1～6	1024	912 (89.1%)	23
N.W69, Nos. 4～7	(N·SN–90), Nos. 1～7	1530	1173 (76.7%)	42
N.W69, Nos. 8～10	(N·SN–130), Nos. 1～8	1950	1602 (82.2%)	0
N.W69, Nos. 1～3	(EN–50·N), Nos. 1～6	1509	1489 (98.7%)	6
N.W69, Nos. 4～7	(EN–90·N), Nos. 1～12	1144	1059 (92.6%)	50
N.W69, Nos. 8～10	(EN–130·N), Nos. 1～7	1175	1032 (87.8%)	44



C —Cleavage
N —Neurula stage
T —Tail-bud stage
H —Hatch
50D-Age of 50 days
M —Climbing out of water

Fig. 11. Survival curves of control second-generation offspring derived from untreated grandparental gametes by matings, N♀ × (N♀ × N♂) ♂, Nos. 1∼10.

of the respective total number of eggs cleaved normally. Most of the normally cleaved eggs developed into tadpoles; 90.5∼96.7%, 93.5% on the average, hatched normally, and 74.3∼93.0%, 86.4% on the average, became normal, metamorphosed frogs.

males raised from irradiated sperm or eggs

No. of gastrulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of 50-day-old tadpoles		No. of metamorphosed frogs
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
1758 (96.9%)	24	1743 (96.1%)	15	1696 (93.5%)	47	1660 (91.5%)	36	1567 (86.4%)
1152 (81.3%)	15	1125 (79.4%)	27	1046 (73.8%)	79	964 (68.0%)	82	766 (54.1%)
1077 (69.1%)	209	1009 (64.7%)	68	871 (55.9%)	138	668 (42.8%)	109	498 (31.9%)
886 (84.5%)	21	859 (82.0%)	27	765 (73.0%)	94	567 (54.1%)	123	424 (40.5%)
2404 (82.0%)	82	1842 (62.9%)	562	1574 (53.7%)	268	1258 (42.9%)	166	1059 (36.1%)
1896 (70.8%)	77	1772 (66.2%)	124	1408 (52.6%)	364	1222 (45.6%)	145	1026 (38.3%)
610 (82.2%)	51	472 (63.6%)	138	405 (54.6%)	67	344 (46.4%)	61	252 (34.0%)
756 (73.8%)	156	728 (71.1%)	28	673 (65.7%)	55	660 (64.5%)	13	358 (35.0%)
1151 (75.2%)	22	1114 (72.8%)	37	884 (57.8%)	230	745 (48.7%)	139	529 (34.6%)
1589 (81.5%)	13	1574 (80.7%)	15	988 (50.7%)	586	840 (43.1%)	148	617 (31.6%)
1473 (97.6%)	16	1457 (96.6%)	16	1017 (67.4%)	440	911 (60.4%)	106	577 (38.2%)
1030 (90.0%)	29	993 (86.8%)	37	908 (79.4%)	85	723 (63.2%)	152	542 (47.4%)
784 (66.7%)	248	708 (60.3%)	76	661 (56.3%)	47	616 (52.4%)	45	434 (36.9%)

b. Experimental series from X-irradiated sperm

i) N♀ × (N♀ × SX-90♂)♂, Nos. 1~8

The same three normal females Nos. 1~3 as those used in the control series were mated by artificial fertilization with each of eight males Nos. 1~8 that were obtained from normal eggs fertilized with spermatozoa exposed to 90 rads

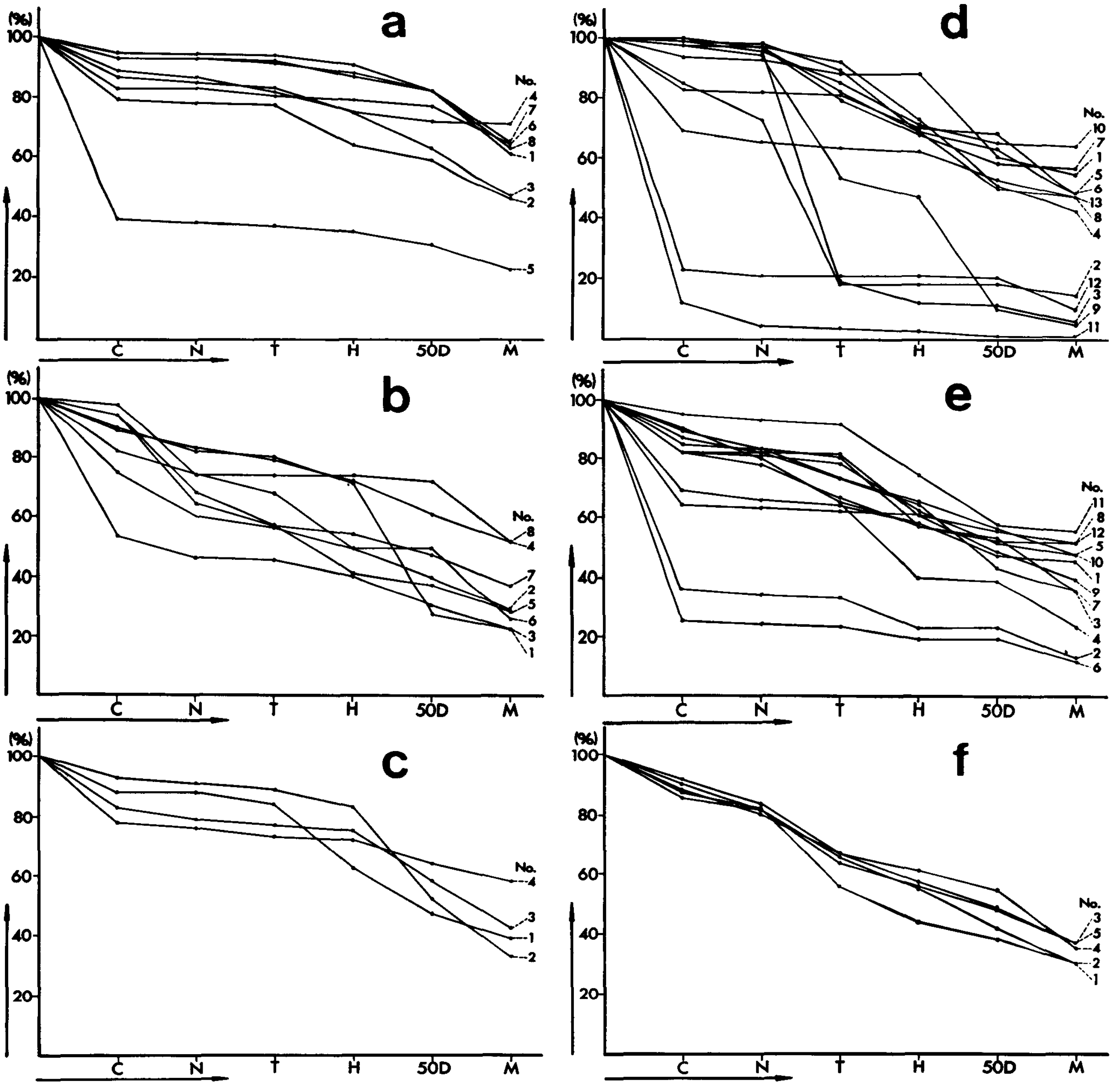


Fig. 12. Survival curves of second-generation offspring derived from X-irradiated grandparental gametes by passing over male first-generation offspring.

a. N♀ × (N♀ × SX-90♂)♂, Nos. 1~8
b. N♀ × (N♀ × SX-170♂)♂, Nos. 1~8
c. N♀ × (N♀ × SX-240♂)♂, Nos. 1~4
d. N♀ × (EX-90♀ × N♂)♂, Nos. 1~13
e. N♀ × (EX-145♀ × N♂)♂, Nos. 1~12
f. N♀ × (EX-200♀ × N♂)♂, Nos. 1~5

C —Cleavage
N —Neurula stage
T —Tail-bud stage
H —Hatch
50D–Age of 50 days
M —Climbing out of water

of X-rays (Table 14, Fig. 12). In eight matings Nos. 1~8, 39.0~95.1%, 82.4% on the average, of the respective total number of eggs cleaved normally. If mating No. 5 was excluded, normal cleavages occurred in more than 78.6% of eggs. The male used in mating No. 5 had testes of type 4 (Table 10). Some of the normally cleaved eggs in the eight matings died of various abnormalities at the embryonic or tadpole stage; 34.5~90.8%, 73.8% on the average, hatched normally, and 23.2~70.9%, 54.1% on the average, became normal, metamorphosed frogs.

ii) N♀×(N♀×SX–170♂)♂, Nos. 1~8

The same four normal females Nos. 4~7 as those used in the control series were mated with each of eight males Nos. 1~8 that were raised from spermatozoa exposed to 170 rads of X-rays. In eight matings Nos. 1~8, 53.0~98.1%, 82.5% on the average, of the respective total number of eggs cleaved normally. Many of the normally cleaved eggs died of various kinds of abnormalities, such as exogastrulation, edema, blisters, microcephaly, curvature or underdevelopment of the body, etc. during the embryonic or tadpole stage. No definite abnormalities appeared at any developmental stage. Eventually, 39.7~74.0%, 55.9% on the average, of the respective total number of eggs hatched normally, and afterwards 21.5~51.0%, 31.9% on the average, became normal, metamorphosed frogs.

iii) N♀×(N♀×SX–240♂)♂, Nos. 1~4

The same three normal females Nos. 8~10 as those used in the control series were mated with each of four males Nos. 1~4 that were raised from spermatozoa exposed to 240 rads of X-rays. In four matings Nos. 1~4, 78.2~92.5%, 86.5% on the average, of the respective total number of eggs cleaved normally. After some of the normally cleaved eggs died of various abnormalities, 62.4~83.2%, 73.0% on the average, hatched normally. After the hatching stage, more numerous individuals died of edema, underdevelopment of the body or ill-formation of the legs before completion of metamorphosis; 31.5~57.6%, 40.5% on the average, became normal, metamorphosed frogs.

c. Experimental series from X-irradiated eggs

i) N♀×(EX–90♀×N♂)♂, Nos. 1~13

Three normal females Nos. 1~3 were mated by artificial fertilization with each of 13 males Nos. 1~13 that were raised from eggs exposed to 90 rads of X-rays (Table 14, Fig. 12). In 13 matings Nos. 1~13, 11.5~99.6%, 84.8% on the average, of the respective total number of eggs cleaved normally. If matings Nos. 11 and 12 were excluded, normal cleavages occurred in more than 68% of the eggs. Many of the normally cleaved eggs died of various abnormalities at various developmental stages. Especially in matings Nos. 2 and 3, a great majority of individuals died at the neurula stage. The main cause of death at the embryonic stage was edema in matings Nos. 4, 6, 8 and 9, while it was edema, microcephaly, blisters, curvature of the body or some other abnormalities in the remaining matings. In the 13 matings, 2.7~87.5%, 53.7% on the average, hatched normally, and afterwards 1.1 ~ 64.4%, 36.1% on the average, became normal, metamorphosed frogs. During the tadpole stage, many individuals died of edema or underdevelopment.

ii) N♀ × (EX–145♀ × N♂)♂, Nos. 1 ~ 12

Four normal females Nos. 4 ~ 7 were mated with each of 12 males Nos. 1 ~ 12 that were raised from eggs exposed to 145 rads of X-rays. In 12 matings Nos. 1 ~ 12, 24.9 ~ 94.5%, 73.7% on the average, of the respective total number of eggs cleaved normally. If matings Nos. 2 and 6 were excluded, normal cleavages occurred in more than 64.3% of the eggs. Some of the normally cleaved eggs died at various embryonic stages. The main cause of death was edema in matings Nos. 4 and 8, while it was various abnormalities such as edema, blisters, microcephaly and curvature of the body in the remaining matings. Eventually, 18.7 ~ 73.7%, 52.6% on the average, hatched normally. During the tadpole stage, many individuals died of edema or underdevelopment; 11.6 ~ 55.3%, 38.3% on the average, metamorphosed normally.

iii) N♀ × (EX–200♀ × N♂)♂, Nos. 1 ~ 5

Three normal females Nos. 8 ~ 10 were mated with each of five males Nos. 1 ~ 5 that were raised from eggs exposed to 200 rads of X-rays. In five matings Nos. 1 ~ 5, 86.0 ~ 92.1%, 89.1% on the average, of the respective total number of eggs cleaved normally. Many of the normally cleaved eggs died at various embryonic stages. The main cause of death was edema in matings Nos. 1 and 5, while it was edema, blisters, microcephaly or curvature of the body in the remainings. Eventually, 43.7 ~ 60.6%, 54.6% on the average, hatched normally. After many individuals died of edema, underdevelopment of the body or ill-formation of the legs during the tadpole stage, 29.8 ~ 36.6%, 34.0% on the average, became normal, metamorphosed frogs.

d. Experimental series from neutron-irradiated sperm

i) N♀ × (N♀ × SN–50♂)♂, Nos. 1 ~ 6

The same three females Nos. 1 ~ 3 as those used in the control series were mated by artificial fertilization with each of six males that were raised from spermatozoa exposed to 50 rads of neutrons (Table 14, Fig. 13). In six matings Nos. 1 ~ 6, 84.1 ~ 96.5%, 89.1% on the average, of the respective total number of eggs cleaved normally. Some of the normally cleaved eggs died of various abnormalities at various embryonic stages; 42.7 ~ 78.6%, 65.7% on the average, hatched normally. Some individuals gradually died of edema, underdevelopment of the body or ill-formation of the legs during the tadpole stage; eventually 26.0 ~ 57.6%, 35.0% on the average, became normal, metamorphosed frogs.

ii) N♀ × (N♀ × SN–90♂)♂, Nos. 1 ~ 7

The same four females Nos. 4 ~ 7 as those used in the control series were mated with each of seven males that were raised from spermatozoa exposed to 90 rads of neutrons. While one (No. 5) of the males was almost sterile, the other six gave rise to numerous cleavages; 88.1 ~ 95.7% of the respective total number of eggs cleaved normally. On the average of the seven matings, 76.7% of eggs

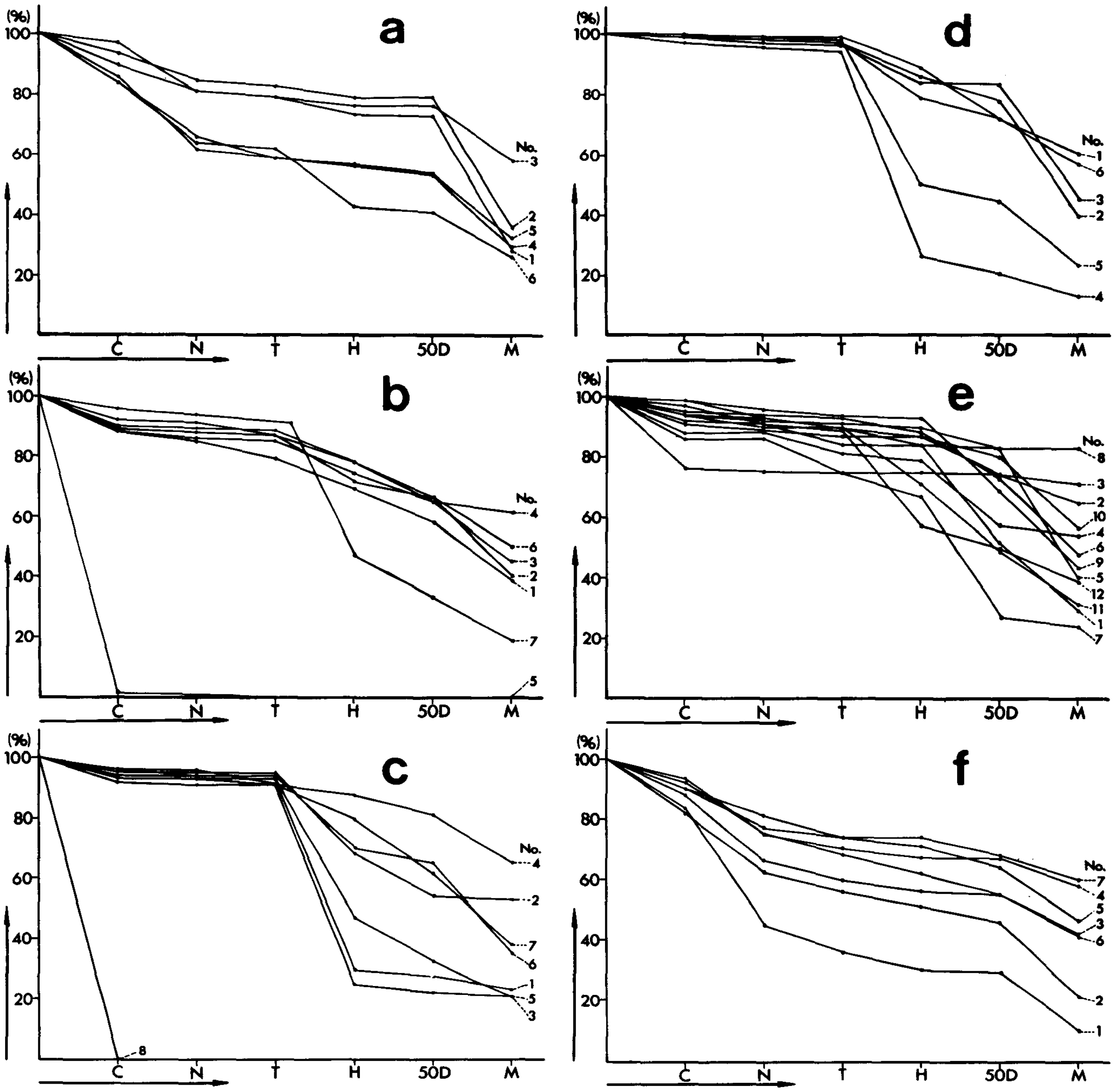


Fig. 13. Survival curves of second-generation offspring derived from neutron-irradiated grandparental gametes by passing over male first-generation offspring.

a. N♀ × (N♀ × SN-50♂)♂, Nos. 1~6
b. N♀ × (N♀ × SN-90♂)♂, Nos. 1~7
c. N♀ × (N♀ × SN-130♂)♂, Nos. 1~8
d. N♀ × (EN-50♀ × N♂)♂, Nos. 1~6
e. N♀ × (EN-90♀ × N♂)♂, Nos. 1~12
f. N♀ × (EN-130♀ × N♂)♂, Nos. 1~7

C —Cleavage
N —Neurula stage
T —Tail-bud stage
H — Hatch
50D-Age of 50 days
M —Climbing out of water

cleaved normally. About half of the normally cleaved eggs in mating No. 7 died simultaneously of edema at the hatching stage. In mating No. 5, no embryos hatched normally, although there were a few normally cleaved eggs. In the remaining five matings, Nos. 1~4 and 6, many embryos died of various abnormalities at various embryonic stages; 68.5~78.2% hatched normally. During the tadpole stage, many individuals also died of edema or underdevelopment; 18.6~60.6% became normal, metamorphosed frogs.

iii) N♀×(N♀×SN–130♂)♂, Nos. 1~8

The same three females Nos. 8~10 as those used in the control series were mated with each of eight males Nos. 1~8 that were raised from spermatozoa exposed to 130 rads of neutrons. One (No. 8) of the males was quite sterile; the testes of this male were of type 5 in inner structure. In the other matings Nos. 1~7, 92.1~96.3%, 94.4% on the average, of the respective total number of eggs cleaved normally. However, 179 of 263, 126 of 173 and 129 of 259 embryos died simultaneously of edema at the hatching stage, and only 29.8%, 24.9% and 46.8% hatched normally in matings Nos. 1, 3 and 5, respectively. In matings Nos. 2, 4, 6 and 7, some of the normally cleaved eggs also died of microcephaly, blisters, curvature of the body and some other abnormalities at the hatching stage; 67.7~88.3% hatched normally. On the average of the seven matings Nos. 1~7, 58.2% hatched normally. During the tadpole stage, some individuals died of edema, underdevelopment of the body or ill-formation of teeth or legs; 20.5~65.4%, 36.3% on the average, metamorphosed normally.

e. Experimental series from neutron-irradiated eggs

i) N♀×(EN–50♀×N♂)♂, Nos. 1~6

The same three females Nos. 1~3 as those used in the control series were mated by artificial fertilization with each of six males Nos. 1~6 that were raised from eggs exposed to 50 rads of neutrons (Table 14, Fig. 13). In six matings, Nos. 1~6, 96.5~99.6%, 98.7% on the average, of the respective total number of eggs cleaved normally. Only a few of the normally cleaved eggs died of exogastrulation at the gastrula stage, and of edema, microcephaly or some other abnormalities at the tail-bud stage. At the hatching stage, many embryos died. Especially in matings Nos. 4 and 5, 193 of 265 and 126 of 256 embryos died about the same time of edema. In the other matings, a comparatively small number of embryos died of microcephaly, edema, blisters and some other abnormalities. In the six matings, 25.5~89.4%, 67.4% on the average, hatched normally. During the tadpole stage, some individuals died of edema, underdevelopment or other abnormalities; 22.5~60.7%, 38.2% on the average, became normal, metamorphosed frogs.

ii) N♀×(EN–90♀×N♂)♂, Nos. 1~12

Four females Nos. 4~7 were mated with each of 12 males Nos. 1~12 that were raised from eggs exposed to 90 rads of neutrons. In 12 matings Nos. 1~12, 76.0~99.0%, 92.6% on the average, of the respective total number of eggs cleaved normally. Some of the normally cleaved eggs died of various abnormalities at various embryonic stages; 56.9~93.3%, 79.4% on the average, hatched normally. In mating No. 11, 25 of 119 embryos died about the same time of edema at the hatching stage, while all the others were normal. During the tadpole stage, some individuals died of edema or underdevelopment; eventually 24.0~83.1%, 47.4% on the average, became normal, metamorphosed frogs.

iii) N♀×(EN–130♀×N♂)♂, Nos. 1~7

Three females Nos. 8 ~ 10 were mated with each of seven males that were raised from eggs exposed to 130 rads of neutrons. In seven matings Nos. 1 ~ 7, 82.4 ~ 94.4%, 87.8% on the average, of the respective total number of eggs cleaved normally. Many of the normally cleaved eggs died of various abnormalities at various embryonic stages; 30.1 ~ 73.7%, 56.3% on the average, hatched normally. Some tadpoles died of edema or some other abnormalities; 10.2 ~ 59.9%, 36.9% on the average, metamorphosed normally.

3. Chromosome aberrations

The karyotypes of normally shaped tadpoles that were 50 ~ 60 days old and of stages V ~ XII in TAYLOR and KOLLROS' table were examined in the tail tips by the squash method after pretreatment with distilled water (Table 15). In the control series, the chromosomes of 92 tadpoles obtained from seven matings Nos. 1 ~ 7 were observed. All the mitoses in each of these tadpoles were diploid, and no chromosome aberrations were found. Differing from this condition in the control series, there were many tadpoles having abnormal mitoses in the experimental series. However, there was no intimate relationship between the dosages of irradiation and the percentages of normal diploids in the second-generation offspring, while there was an inverse proportion in the first-generation offspring.

The abnormal mitoses found in tadpoles of the experimental series had hyperdiploid, hypodiploid or abnormally diploid chromosomes. These abnormal chromosome complements always consisted of a combination of a normal and an

TABLE 15

Chromosomal analysis of normally shaped tadpoles derived from irradiated grandparental sperm or eggs

Parents		No. of analyzed tadpoles	Number of tadpoles			
			With normal cells only	With abnormal cells only		With normal and abnormal cells
Female	Male			Pure	Mosaic	
N.W69, Nos. 1 ~ 3	(N·N), Nos. 1 ~ 3	42	42 (100 %)	0	0	0
N.W69, Nos. 4 ~ 7	(N.N), Nos. 4 ~ 7	50	50 (100 %)	0	0	0
N.W69, Nos. 1 ~ 3	(N·SX–90), No. 1	39	28 (71.8%)	5	2	4
N.W69, Nos. 4 ~ 7	(N·SX–170), No. 8	30	22 (73.3%)	3	2	3
N.W69, Nos. 8 ~ 10	(N.SX–240), No. 4	25	21 (84.0%)	0	1	3
N.W69, Nos. 1 ~ 3	(EX–90·N), No. 10	40	32 (80.0%)	2	3	3
N.W69, Nos. 4 ~ 7	(EX–145·N), No. 11	30	24 (80.0%)	1	2	3
N.W69, Nos. 8 ~ 10	(EX–200·N), No. 3	25	19 (76.0%)	3	1	2
N.W69, Nos. 1 ~ 3	(N.SN–50), No. 3	38	27 (71.1%)	3	3	5
N.W69, Nos. 4 ~ 7	(N·SN–90), No. 4	27	21 (77.8%)	0	3	3
N.W69, Nos. 8 ~ 10	(N·SN–130), No. 4	23	18 (78.3%)	2	1	2
N.W69, Nos. 1 ~ 3	(EN–50·N), No. 1	42	33 (78.6%)	4	2	3
N.W69, Nos. 4 ~ 7	(EN–90·N), No. 8	34	28 (82.4%)	2	0	4
N.W69, Nos. 8 ~ 10	(EN–130·N), No. 7	20	15 (75.0%)	2	2	1

abnormal set of haploid chromosomes. In the latter, there were various kinds of abnormalities, such as an addition or a loss of one or two chromosomes, or existence of a chromosome with a deletion or translocation, or of a ring, fragment, dicentric or tricentric chromosome. In the experimental series, there were four types of tadpoles by the kind of mitoses. The first type was a normal diploid. The second type was an abnormal diploid consisting of one kind of abnormal mitoses. The third type was an abnormal tadpole consisting of a mixture of two or more kinds of abnormal mitoses. The last type consisted of a mixture of normal and abnormal mitoses.

a. Control series N♀×(N♀×N♂)♂, Nos. 1∼7

All the 92 tadpoles whose karyotypes were clearly analyzed were normal diploids and had no other than normally diploid mitoses.

b. Experimental series from X-irradiated sperm

i) N♀×(N♀×SX-90♂)♂, No. 1

In the mating between normal females Nos. 1∼3 from the field and male No. 1 raised from a spermatozoon exposed to 90 rads of X-rays, 39 of 50 normally shaped tadpoles were analyzable in karyotype. Twenty-eight of them were normal diploids which contained no abnormal mitoses. Five tadpoles consisted of a kind of abnormal mitoses. Two of them were hyperdiploids (2n+1) having an additional small chromosome. Two others were abnormal diploids; one had a deletion in the short arm of a large chromosome No. 4, while the other had a deletion in the short arm of a small chromosome. The remaining was a hyperdiploid (2n+1) with an additional small chromosome and had a deletion in a chromosome No. 5. Two tadpoles were mosaics of abnormally diploid mitoses; one consisted of a mixture of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, while the other consisted of a mixture of mitoses having a chromosome with a translocation and those having a chromosome with a deletion.

The remaining four tadpoles were mosaics of normally diploid and abnormal mitoses. In one of them, some mitoses were hypodiploid (2n−1) having a dicentric chromosome and occasionally a fragment. Another tadpole had abnormally diploid mitoses, in which a chromosome had a translocation or a deletion. The other two had hyperdiploid (2n+1) or hypodiploid (2n−1) mitoses.

ii) N♀×(N♀×SX-170♂)♂, No. 8

In the mating between normal females Nos. 4∼7 from the field and male No. 8 raised from a spermatozoon exposed to 170 rads of X-rays, 30 of 50 normally shaped tadpoles examined were analyzable in karyotype.

Of the 30 tadpoles, 22 were normal diploids. Three consisted of a kind of abnormal mitoses; a tadpole was an abnormal diploid with a ring chromosome, the second a hyperdiploid with an additional small chromosome, and the third an abnormal diploid having a large chromosome with a deletion. Two tadpoles were mosaics of abnormally diploid mitoses; one of them had a mixture of mitoses containing a chromosome with a translocation and those containing a chromosome with a deletion. The other tadpole had a mixture of mitoses containing a chromosome with a deletion and those containing a dicentric chromosome and a fragment.

The remaining three tadpoles were mosaics of normally diploid and abnormal mitoses. One of them had triploid mitoses, the second had hyperdiploid (2n+1) mitoses, and the third had hypodiploid (2n−1) mitoses with a dicentric chromosome.

iii) N♀ × (N♀ × SX–240♂)♂, No. 4

In the mating between normal females Nos. 8 ~ 10 from the field and male No. 4 raised from a spermatozoon exposed to 240 rads of X-rays, 25 of 50 tadpoles examined were analyzable in karyotype. Of these 25 tadpoles, 21 were normal diploids. One of the other four tadpoles was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses. The remaining three were mosaics of normally diploid and abnormal mitoses; one contained hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, while the other two contained abnormally diploid mitoses having a chromosome with a translocation or a deletion.

c. Experimental series from X-irradiated eggs

i) N♀ × (EX–90♀ × N♂)♂, No. 10

In the mating between normal females Nos. 1 ~ 3 from the field and male No. 10 raised from an egg exposed to 90 rads of X-rays, 40 of 50 tadpoles examined were analyzable. Thirty-two of them were normal diploids. Two tadpoles consisted of a kind of abnormally diploid mitoses having a chromosome with a deletion. Three other tadpoles were mosaics of abnormal mitoses; two of them consisted of a mixture of mitoses having a chromosome with a translocation and those having a chromosome with a deletion, and the other consisted of a mixture of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses.

The remaining three tadpoles were mosaics of normally and abnormally diploid mitoses. The latter mitoses contained a chromosome with a translocation or a deletion.

ii) N♀ × (EX–145♀ × N♂)♂, No. 11

In the mating between normal females Nos. 4 ~ 7 from the field and male No. 11 raised from an egg exposed to 145 rads of X-rays, 30 of 50 tadpoles examined were analyzable in karyotype. Twenty-four of them were normal diploids. Another tadpole was a hyperdiploid having a ring chromosome and a fragment. Two tadpoles were mosaics of abnormal mitoses; one consisted of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, while the other consisted of a mixture of mitoses having a chromosome with a translocation and those having a chromosome with a deletion.

The remaining three were mosaics of normally diploid and abnormal mitoses. One of them had triploid mitoses, while the other two had mitoses having a chromosome with a translocation or a deletion.

iii) N♀ × (EX–200♀ × N♂)♂, No. 3

In the mating between normal females Nos. 8 ~ 10 from the field and male No. 3

raised from an egg exposed to 200 rads of X-rays, 25 of 50 tadpoles examined were analyzable. Of the 25 tadpoles, 19 were normal diploids. Three of the others consisted of a kind of abnormal mitoses; one was an abnormal diploid having a chromosome with a translocation and a chromosome with a deletion, another was a hyperdiploid (2n+1) having an additional small chromosome, and the remaining was an abnormal diploid having a ring derived from a large chromosome. A tadpole was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses. In these mitoses, there was an addition or a loss of a small chromosome.

The remaining two tadpoles were mosaics of normally and abnormally diploid mitoses; one had a dicentric chromosome, while the other had a chromosome with a translocation or a deletion.

d. Experimental series from neutron-irradiated sperm

i) N♀×(N♀×SN–50♂)♂, No. 3

In the mating between normal females Nos. 1~3 from the field and male No. 3 raised from a spermatozoon exposed to 50 rads of neutrons, 38 of 50 tadpoles examined were analyzable in karyotype. Twenty-seven of them were normal diploids. Three others consisted of a kind of abnormal mitoses; one was an abnormal diploid having a chromosome with a translocation, another an abnormal diploid having a chromosome with a deletion, and the remaining a hyperdiploid (2n+1) with an additional small chromosome. Three tadpoles were mosaics of abnormal mitoses; one consisted of a mixture of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, and the other two consisted of a mixture of mitoses having a chromosome with a translocation and those having a chromosome with a deletion.

The remaining five tadpoles were mosaics of normally diploid and abnormal mitoses; two contained mitoses having a chromosome with a translocation or a deletion, another had hypotriploid (3n−1) mitoses, and the other two had triploid mitoses.

ii) N♀×(N♀×SN–90♂)♂, No. 4

In the mating between normal females Nos. 4~7 from the field and male No. 4 raised from a spermatozoon exposed to 90 rads of neutrons, 27 of 50 tadpoles examined were analyzable. Twenty-one of them were normal diploids. Three others were mosaics of abnormal mitoses; one of them consisted of a mixture of mitoses having a dicentric chromosome and a fragment and those having a chromosome with a deletion, while the other two consisted of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses.

The remaining three tadpoles were mosaics of normally and abnormally diploid mitoses; one had a ring chromosome, while the other had a chromosome with a deletion.

iii) N♀×(N♀×SN–130♂)♂, No. 4

In the mating between normal females Nos. 8~10 from the field and male No. 4 raised from a spermatozoon exposed to 130 rads of neutrons, 23 of 50 tadpoles examined were analyzable in karyotype. Eighteen of them were normal diploids, one was a triploid and one was an abnormal diploid having a chromosome with a translocation and a chromosome with a deletion. A tadpole was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses.

The remaining two tadpoles were mosaics of normally and abnormally diploid mitoses. In the latter mitoses there was a chromosome with a translocation or a deletion.

e. Experimental series from neutron-irradiated eggs

i) N♀ × (EN–50♀ × N♂)♂, No. 1

In the mating between normal females Nos. 1～3 from the field and male No. 1 raised from an egg exposed to 50 rads of neutrons, 42 of 50 tadpoles examined were analyzable in karyotype. Thirty-three of them were normal diploids. Four consisted of a kind of abnormal mitoses; one of them was a triploid, the second was a hyperdiploid (2n+1) having an additional small chromosome, the third was an abnormal diploid having a large chromosome with a deletion and the last was an abnormal diploid having a large chromosome with a translocation and a large chromosome with a deletion. Two tadpoles were mosaics of abnormal mitoses; one consisted of hyperdiploid (2n+1) and hypodiploid (2n−1), and the other consisted of a mixture of abnormal diploid mitoses having a dicentric chromosome and a fragment and those having a chromosome with a translocation or a deletion.

The remaining three tadpoles were mosaics of normally diploid and abnormal mitoses; one contained hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, another had a chromosome with a translocation or a deletion, and the last had triploid mitoses.

ii) N♀ × (EN–90♀ × N♂)♂, No. 8

In the mating between normal females Nos. 4～7 from the field and male No. 8 raised from an egg exposed to 90 rads of neutrons, 34 of 50 tadpoles examined were analyzable in karyotype. Twenty-eight of them were normal diploids and two were abnormal diploids. One of the latter had a chromosome with a translocation, while the other had a chromosome with a deletion and a chromosome with a translocation.

The remaining four were mosaics of normally diploid and abnormal mitoses; one contained hypodiploid (2n−1) mitoses having a dicentric chromosome, another had hyperdiploid (2n+1) mitoses, and the other two contained abnormally diploid mitoses having a chromosome with a translocation or a deletion.

iii) N♀ × (EN–130♀ × N♂)♂, No. 7

In the mating between females Nos. 8～10 from the field and male No. 7 raised from an egg exposed to 130 rads of neutrons, 20 of 40 tadpoles examined were analyzable in karyotype. Fifteen of them were normal diploids. Two others consisted of a kind of abnormal mitoses; one was an abnormal diploid having a ring chromosome, while the other was a hyperdiploid (2n+1) having an additional small chromosome. Two tadpoles were mosaics of abnormal mitoses;

one consisted of a mixture of mitoses having a chromosome with a translocation and those having a chromosome with a deletion, while the other consisted of a mixture of abnormally diploid mitoses having a chromosome with a translocation or a deletion and hypodiploid (2n—1) mitoses having a dicentric chromosome.

The remaining tadpole was a mosaic of normal and abnormal mitoses. The abnormal mitoses had a chromosome with a translocation.

4. Viability and sex of metamorphosed frogs

In the control series, 1567 tadpoles obtained from ten matings climbed out of water at the ages of 83～109 days, 90.5 days on the average (Table 16). From each of the ten matings, 50 frogs were selected at random; their body length was measured immediately after completion of metamorphosis. The 500 frogs in total averaged 21.5±0.1 mm in length.

In the twelve experimental series, 252～1059 tadpoles obtained from 4～13 matings climbed out of water at the ages of 83～122 days, 89.2～97.0 days on the

TABLE 16

Number, size and sex of metamorphosed frogs

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed frogs	Body length immediately after metamorphosis (mm)	
Female	Male				No. of frogs
N.W69, Nos. 1～10	(N·N), Nos. 1～10	83～109 (90.5)	1567	21.5±0.1	1507
N.W69, Nos. 1～3	(N·SX–90), Nos. 1～8	83～111 (96.3)	766	21.2±0.1	469
N.W69, Nos. 4～7	(N·SX–170), Nos. 1～8	83～112 (95.5)	498	21.4±0.1	280
N.W69, Nos. 8～10	(N·SX–240), Nos. 1～4	84～122 (97.0)	424	20.9±0.2	258
N.W69, Nos. 1～3	(EX–90·N), Nos. 1～13	84～114 (95.3)	1059	21.1±0.1	826
N.W69, Nos. 4～7	(EX–145·N), Nos. 1～12	83～114 (95.2)	1026	21.0±0.1	963
N.W69, Nos. 8～10	(EX–200·N), Nos. 1～5	84～98 (89.2)	252	19.4±0.2	114
N.W69, Nos. 1～3	(N·SN–50), Nos. 1～6	85～104 (92.1)	358	21.5±0.2	146
N.W69, Nos. 4～7	(N·SN–90), Nos. 1～7	85～111 (93.0)	529	20.9±0.2	452
N.W69, Nos. 8～10	(N·SN–130), Nos. 1～8	85～119 (95.6)	617	21.1±0.2	515
N.W69, Nos. 1～3	(EN–50·N), Nos. 1～6	83～118 (93.2)	577	20.7±0.1	405
N.W69, Nos. 4～7	(EN–90·N), Nos. 1～12	85～116 (93.6)	542	21.3±0.1	480
N.W69, Nos. 8～10	(EN–130·N), Nos. 1～7	85～120 (95.6)	434	20.6±0.2	381

♀$_N$—Females with normal ovaries ♀$_U$—Females with underdeveloped ovaries average in each mating (Table 16). When an experimental series, N♀ × (EX-200♀ × N♂)♂, was excluded, the tadpoles of each experimental series were somewhat older in the average age of climbing out than those of the control.

From the second-generation offspring produced from each of the 4∼13 matings, 50 frogs were removed at random and measured immediately after the completion of metamorphosis. When there were less than 50 frogs in a mating, all the frogs were measured and the shortage was replenished with frogs produced from another mating of the same series. Accordingly, about 200∼600 frogs were measured in each experimental series, except that all the 252 frogs were measured in the series derived from eggs irradiated with 200 rads of X-rays. The frogs in the twelve experimental series averaged 19.4±0.2∼21.5±0.2 mm in body length. There was no distinct difference in body length between the frogs of the experimental series and those of the control, as well as between the frogs of different experimental series. However, there was a remarkable difference in the viability of frogs during three months after completion of metamorphosis

derived from irradiated grandparental sperm or eggs

Sex of frogs killed about three months after metamorphosis					No. of frogs removed and reared	Sex of 2-year-old mature frogs			Sex of all frogs examined		
♀$_N$	♀$_U$	⚥	♂$_N$	♂ (%)*		No. of frogs	♀	♂	Total	♀	♂ (%)*
760	1	3	743	(49.5)	50	32	18	14	1539	779	760 (49.4)
320	13	9	127	(29.0)	50	18	12	6	487	345	142 (29.2)
162	1	3	114	(41.8)	50	12	9	3	292	172	120 (41.1)
129	1	2	126	(49.6)	50	11	6	5	269	136	133 (49.4)
420	4	9	393	(48.7)	50	27	16	11	853	440	413 (48.4)
523	2	2	436	(45.5)	50	23	10	13	986	535	451 (45.7)
76	2	3	33	(31.6)	50	38	27	11	152	105	47 (30.9)
97	5	3	41	(30.1)	50	36	28	8	182	130	52 (28.6)
272	11	4	165	(37.4)	50	36	14	22	488	297	191 (39.1)
290	6	3	216	(42.5)	50	33	24	9	548	320	228 (41.6)
241	0	0	164	(40.5)	50	16	11	5	421	252	169 (40.1)
277	2	0	201	(41.9)	50	12	8	4	492	287	205 (41.7)
265	0	2	114	(30.4)	50	23	12	11	404	277	127 (31.4)

⚥—Hermaphrodites ♂$_N$—Males with normal testes * Including hermaphrodites between the control and most of the experimental series.

Three months after metamorphosis, 50 frogs were removed from the most prolific mating of each experimental series as well as of the control series. These frogs were continuously reared until the mature stage, while all the other frogs were killed and preserved to examine their sex. The sex of these 3-month-old frogs as well as the number and sex of 2-year-old frogs in each series are presented in Table 16. The frogs in one-third of the twelve experimental series were not inferior in viability to the control frogs. Among the twelve experimental series, the frogs derived from X-irradiated spermatozoa were inferior to those from X-irradiated eggs, while the frogs derived from neutron-irradiated eggs were inferior to those from neutron-irradiated spermatozoa. There was no definite difference in viability of frogs between larger and smaller doses of irradiation to which the gametes were exposed.

All the males and almost all the females in the experimental and control series matured sexually at the age of two years. There was a slight difference in the degree of maturity between the females in the experimental series and those in the control. While four of five control females ovulated normally after pituitary injection, none of the largest six females in the twelve experimental series did, although their ovaries appeared to be filled with mature ova. Differing from females, all the males in the experimental and control series had thumb-pads. Thus, males were utilized to produce third-generation offspring at the age of two years in 1971, while females were not used until the age of three years in 1972.

a. Control series

N♀ × (N♀ × N♂)♂, Nos. 1 ~ 10

Three months after completion of metamorphosis, 1557 of 1567 metamorphosed frogs were living (Table 16). Fifty frogs produced from mating No. 4 were left alive and reared continuously, while all the other 1507 frogs were killed to examine their sex. It was found that 49.5% of them were males when three hermaphrodites were counted as males, as such juvenile hermaphrodites became males sooner or later. Of the 50 frogs left alive, 18 died before the stage of sexual maturity, while the other 32 attained sexual maturity at the age of two years; 18 were females and 14 males.

b. Experimental series from X-irradiated sperm

i) N♀ × (N♀ × SX–90♂)♂, Nos. 1 ~ 8

Within three months after the completion of metamorphosis, 247 of 766 metamorphosed frogs died (Table 16). Fifty frogs produced from mating No. 4 were left alive and reared continuously, while the other 469 were killed to examine their sex. When nine hermaphrodites were counted as males, 29.0% of the killed frogs were males. Of the 50 frogs left alive, 32 died afterwards, while 18 became two years old. Twelve of the latter were females and six males.

ii) N♀ × (N♀ × SX–170♂)♂, Nos. 1 ~ 8

Of 498 metamorphosed frogs, 168 died within three months after completion of metamorphosis. Of the remaining 330 frogs, 50 produced from mating No. 8 were left alive and the other 280 were killed to examine their sex. As a result, 41.8% of them were males when three hermaphrodites were counted as males. Of the remaining 50 frogs, 38 died afterwards, while 12 became two years old; nine of the latter were females and three males.

iii) N♀×(N♀×SX–240♂)♂, Nos. 1~4

Of 424 metamorphosed frogs, 116 died within three months after completion of metamorphosis. Fifty frogs produced from mating No. 4 were left alive and reared, while the other 258 were killed to examine their sex. When two hermaphrodites were counted as males, 49.6% of the killed frogs were males. Of the 50 frogs left alive, 39 died afterwards, while 11 became two years old. Six of the latter were females and five males.

c. Experimental series from X-irradiated eggs

i) N♀×(EX–90♀×N♂)♂, 1~13

Of 1059 metamorphosed frogs, 183 died within three months after completion of metamorphosis (Table 16). Fifty frogs produced from mating No. 10 were left alive and reared continuously. All the other 826 frogs were killed; 48.7% of them were males when nine hermaphrodites were counted as males. Of the remaining 50 frogs, 23 died and 27 became two years old; 16 of the latter were females and 11 males.

ii) N♀×(EX–145♀×N♂)♂, Nos. 1~12

Only 13 of 1026 metamorphosed frogs died within three months after the completion of metamorphosis. Fifty frogs produced from mating No. 11 were left alive and reared. All the other 963 frogs were killed to examine their sex; 45.5% of them were males when two hermaphrodites were counted as males. Of the remaining 50 frogs, 27 died afterwards and 23 became two years old; ten of the latter were females and 13 males.

iii) N♀×(EX–200♀×N♂)♂, Nos. 1~5

Of 252 metamorphosed frogs, 88 died within three months after completion of metamorphosis. Fifty frogs produced from mating No. 3 were left alive. All the other 114 frogs were killed; 31.6% of them were males when three hermaphrodites were counted as males. Of the 50 frogs left alive, 12 died afterwards and 38 became two years old; 27 of the latter were females and 11 males.

d. Experimental series from neutron-irradiated sperm

i) N♀×(N♀×SN–50♂)♂, Nos. 1~6

Within three months after the completion of metamorphosis, 162 of 358 died (Table 16). Fifty frogs produced from mating No. 3 were left alive and reared continuously. All the other 146 frogs were killed to examine their sex. The results showed that 30.1% of them were males when three hermaphrodites were counted as males. Of the 50 frogs left alive, 14 died and the other 36 became two years old. Of the latter frogs, 28 were females and eight males.

ii) N♀ × (N♀ × SN–90♂)♂, Nos. 1 ~ 7

Of 529 metamorphosed frogs, 27 died within three months after completion of metamorphosis. Fifty frogs produced from mating No. 4 were left alive and reared, while all the other 452 were killed; 37.4% of them were males when four hermaphrodites were counted as males. Of the 50 frogs left alive, 14 died afterwards, while 36 became two years old; 14 of the latter were females and 22 males.

iii) N♀ × (N♀ × SN–130♂)♂, Nos. 1 ~ 8

Of 617 metamorphosed frogs, 52 died within three months after completion of metamorphosis. Fifty frogs produced from mating No. 4 were left alive, while all the other 515 were killed; 42.5% of them were males when three hermaphrodites were counted as males. Of the 50 frogs left alive, 17 died and 33 became two years old. Of the latter frogs 24 were females and nine males.

e. Experimental series from neutron-irradiated eggs

i) N♀ × (EN–50♀ × N♂)♂, Nos. 1 ~ 6

Of 577 metamorphosed frogs, 122 died within three months after completion of metamorphosis (Table 16). Fifty frogs produced from mating No. 1 were left alive and reared. All the other 405 were killed to examine their sex; 40.5% of them were males. Of the 50 frogs left alive, 34 died and the others became two years old; 11 were females and five males.

ii) N♀ × (EN–90♀ × N♂)♂, Nos. 1 ~ 12

Of 542 metamorphosed frogs, 12 died within three months after completion of metamorphosis. Fifty frogs produced from mating No. 8 were left alive and reared, while all the other 480 were killed to examine their sex; 41.9% of them were males. Thirty-eight of the 50 frogs left alive died afterwards, while only 12 became two years old. Eight of the latter were females and four males.

iii) N♀ × (EN–130♀ × N♂)♂, Nos. 1 ~ 7

Only three of 434 metamorphosed frogs died within three months after completion of metamorphosis. Fifty frogs produced from mating No. 7 were left alive and reared, while all the other 381 were killed to examine their sex; 30.4% of them were males, when two hermaphrodites were counted as males. Of the 50 frogs left alive, 27 died afterwards, while 23 became two years old. Twelve of them were females and 11 males.

III. Second-generation offspring derived from irradiated gametes by passing over female first-generation offspring

1. Female parents

Female first-generation offspring which were obtained in 1967 from eggs or spermatozoa irradiated with X-rays or neutrons by insemination with normal gametes matured in the breeding season of 1970. In this season, many female first-generation offspring, three years old, were mated by artificial fertilization with five normal males collected from the field (Table 17).

TABLE 17

Testes of field-caught male frogs used for mating experiments in 1970

Kind	Individual no.	Body length (mm)	Size of the testes		Inner structure
			Left (mm)	Right (mm)	
N.W70	1	65.0	4.5×2.5	4.5×2.5	Type 1
	2	64.5	5.0×2.5	5.0×2.5	Type 1
	3	67.0	5.0×3.0	5.0×3.0	Type 1
	4	62.5	4.0×2.5	4.5×2.5	Type 1
	5	65.5	5.0×3.0	5.0×3.0	Type 1

TABLE 18

Eggs of control female frogs used for mating experiments in 1970

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs			Mean diameter of 50 eggs (mm)
				Total	Normal	White	
(N·N), No. 5	1	3	53.0	915	915	0	1.71±0.01
	2	3	57.5	1043	1043	0	1.77±0.01
	3	3	60.5	1720	1720	0	1.70±0.01
	4	3	59.5	1534	1534	0	1.82±0.01
	5	3	60.0	1660	1660	0	1.89±0.01

(N·N), No. 5: Females produced from mating between a field-caught female (N.W67, No. 5) and a field-caught male (N.W67, No. 5)

TABLE 19

Eggs of female frogs raised from X-irradiated sperm and used for mating experiments in 1970

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs			Mean diameter of 50 eggs (mm)
				Total	Normal	White	
N·SX–90, No. 5	1	3	50.5	544	544	0	1.84±0.02
	2	3	45.0	523	523	0	1.89±0.03
	3	3	52.5	89	89	0	1.91±0.02
	4	3	50.0	408	408	0	1.84±0.01
	5	3	40.0	359	359	0	2.09±0.02
	6	3	60.0	692	692	0	1.83±0.02
	7	3	68.0	619	619	0	2.00±0.01
	8	3	68.0	267	267	0	1.99±0.03
	9	3	57.0	643	643	0	1.70±0.03
	10	3	67.5	0	0	0	—
N·SX–170, No. 5	1	3	52.0	157	157	0	1.70±0.03
	2	3	49.5	452	452	0	1.77±0.02
	3	3	53.0	141	141	0	1.84±0.03
	4	3	46.5	448	448	0	1.95±0.03
	5	3	59.0	475	475	0	1.86±0.03
	6	3	65.0	518	518	0	2.02±0.03
	7	3	67.0	473	320	0	1.79±0.01
					153	0	1.52±0.01
	8	3	55.5	482	482	0	1.71±0.01
	9	3	62.0	0	0	0	—
	10	3	64.0	0	0	0	—

TABLE 19 Continued

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs			Mean diameter of 50 eggs (mm)
				Total	Normal	White	
N·SX–240, No. 5	1	3	57.0	363	248	0	1.78±0.02
					115	0	1.23±0.02
	2	3	59.0	435	22	172	2.05±0.02
					241	0	1.20±0.01
	3	3	60.5	0	0	0	—

N·SX–90, –170 or –240, No. 5: Females raised from eggs of a field-caught female (N.W67, No. 5) by fertilization with sperm No. 5 exposed to 90, 170 or 240 rads of X-rays

TABLE 20

Eggs of female frogs raised from X-irradiated eggs and used for mating experiments in 1970

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs			Mean diameter of 50 eggs (mm)
				Total	Normal	White	
EX–90·N, No. 5	1	3	63.0	387	195	128	2.11±0.02
					64	0	1.53±0.02
	2	3	57.5	546	546	0	1.96±0.02
	3	3	42.0	404	115	203	1.99±0.02
					86	0	1.46±0.01
	4	3	67.5	687	617	70	1.68±0.02
	5	3	60.0	569	163	0	2.24±0.02
					215	0	1.70±0.02
					191	0	1.45±0.01
	6	3	59.0	237	121	116	2.01±0.02
	7	3	60.5	415	415	0	1.73±0.02
	8	3	55.0	396	396	0	1.77±0.02
	9	3	52.0	0	0	0	—
	10	3	45.0	0	0	0	—
EX–145·N, No. 5	1	3	64.0	816	415	401	1.80±0.02
	2	3	62.5	790	790	0	1.79±0.02
	3	3	63.0	530	237	0	1.72±0.01
					0	293	2.00±0.01
	4	3	55.0	632	632	0	1.68±0.02
	5	3	54.5	419	419	0	1.73±0.01
	6	3	54.0	311	136	0	2.04±0.02
					175	0	1.55±0.01
	7	3	54.0	367	367	0	1.71±0.01
	8	3	53.5	364	364	0	1.78±0.01
	9	3	53.0	406	406	0	1.84±0.02
	10	3	56.0	0	0	0	—
EX–200·N, No. 5	1	3	59.5	264	135	129	2.01±0.01
	2	3	60.0	411	411	0	1.77±0.02

EX–90, –145 or –200·N, No. 5: Females raised from eggs No. 5 exposed to 90, 145 or 200 rads of X-rays by fertilization with sperm of a field-caught male (N.W67, No. 5)

The body length of each female and the color and mean diameter of her eggs in the experimental and control series are presented in Tables 18~22. Five females in the control series were those produced from control mating No. 5

between a normal male and a normal female in 1967. They were 53.0~60.5 mm, 58.1 mm on the average, in body length, and laid 915~1720 eggs, 1374.4 eggs on the average, after pituitary injection. In 12 experimental series, there were a total of 91 females produced from mating No. 5 of each series. These females were 40.0~69.0 mm, 57.3 mm on the average, in body length. Fourteen of them did not spawn after pituitary injection. The eggs laid by the other 77 females were 89~1041, 494.3 on the average, in number. Twelve of these females laid white eggs which were accompanied by normally colored eggs in most cases. The mean diameter of eggs was measured on 50 eggs removed at random from among those laid by each female. The eggs of the five females in the control series were $1.70 \pm 0.01 \sim 1.89 \pm 0.01$ mm, while those of the 77 females in the experimental series were $1.20 \pm 0.01 \sim 2.24 \pm 0.02$ mm. While only one of 43 females raised from irradiated spermatozoa laid white eggs, eleven of 34 females

TABLE 21

Eggs of female frogs raised from neutron-irradiated sperm and used for mating experiments in 1970

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs			Mean diameter of 50 eggs (mm)
				Total	Normal	White	
N·SN–50, No. 5	1	3	51.0	567	567	0	1.97±0.02
	2	3	65.5	634	634	0	2.12±0.02
	3	3	62.5	929	929	0	1.68±0.03
	4	3	63.0	1041	1041	0	1.80±0.02
	5	3	54.5	623	623	0	1.76±0.02
	6	3	54.0	254	254	0	1.73±0.02
	7	3	55.0	534	534	0	1.71±0.02
	8	3	63.5	769	769	0	1.89±0.01
	9	3	64.0	342	342	0	2.01±0.02
	10	3	64.0	485	485	0	1.90±0.02
N·SN–90, No. 5	1	3	63.0	463	463	0	2.01±0.02
	2	3	52.5	592	592	0	1.69±0.02
	3	3	49.0	554	554	0	1.68±0.02
	4	3	61.0	615	615	0	1.90±0.02
	5	3	57.0	414	414	0	2.03±0.02
	6	3	57.5	498	498	0	1.82±0.02
	7	3	59.0	0	0	0	—
	8	3	60.5	0	0	0	—
N·SN–130, No. 5	1	3	65.0	755	755	0	2.11±0.02
	2	3	62.5	495	495	0	1.88±0.02
	3	3	59.0	714	714	0	1.84±0.02
	4	3	60.5	219	219	0	1.96±0.02
	5	3	49.0	510	289	0	1.94±0.02
					221	0	1.48±0.01
	6	3	46.5	490	490	0	1.90±0.02
	7	3	45.5	456	456	0	2.01±0.02
	8	3	47.5	341	341	0	1.83±0.02
	9	3	53.5	0	0	0	—
	10	3	54.0	0	0	0	—

N·SN–50, –90 or –130, No. 5: Females raised from eggs of a field-caught female (N.W67, No. 5) by fertilization with sperm No. 5 exposed to 50, 90 or 130 rads of neutrons

TABLE 22

Eggs of female frogs raised from neutron-irradiated eggs and used for mating experiments in 1970

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs			Mean diameter of 50 eggs (mm)
				Total	Normal	White	
EN-50·N, No. 5	1	3	67.0	572	572	0	2.03±0.02
	2	3	65.5	595	595	0	1.99±0.02
	3	3	66.0	490	490	0	2.07±0.03
	4	3	68.5	578	578	0	2.01±0.03
	5	3	69.0	647	647	0	1.92±0.02
	6	3	55.0	596	596	0	1.73±0.03
	7	3	54.5	610	610	0	1.69±0.04
EN-90·N, No. 5	1	3	58.5	536	536	0	1.41±0.01
	2	3	62.5	470	470	0	1.80±0.03
	3	3	45.0	503	113	0	1.32±0.02
					328	0	1.67±0.02
					0	62	2.04±0.02
	4	3	43.0	439	439	0	1.69±0.03
	5	3	43.0	412	245	167	1.88±0.03
	6	3	56.0	0	0	0	—
	7	3	60.5	0	0	0	—
EN-130·N, No. 5	1	3	58.5	379	166	213	1.92±0.02
	2	3	60.0	503	503	0	2.02±0.02
	3	3	61.5	419	224	195	1.99±0.02
	4	3	60.5	0	0	0	—

EN-50, -90 or -130·N, No. 5: Females raised from eggs No. 5 exposed to 50, 90 or 130 rads of neutrons by fertilization with sperm of a field-caught male (N.W67, No. 5)

raised from irradiated eggs did so. On the other hand, while seven of 19 females raised from X-irradiated eggs laid white eggs, four of 15 females raised from neutron-irradiated eggs did so. The white eggs were nearly the same in size as normal eggs of the same female, or rather larger than the latter. Four of 43 females raised from X- or neutron-irradiated spermatozoa laid two kinds of eggs, that is, large and small ones. Six of 34 females raised from X- or neutron-irradiated eggs laid two or three kinds of eggs that were different in size.

2. Developmental capacity

a. Control series

Five females Nos. 1～5 produced from the control mating in 1967 were mated by artificial fertilization with five field-caught males (Nos. 1～5). The body length as well as the size of the testes of these males are presented in Table 17. Their testes were quite normal, that is, of type 1 in inner structure. The eggs of the five females were inseminated with a mixture of spermatozoa obtained from the five males (Table 23, Fig. 14).

In five matings Nos. 1～5, 85.1～97.4%, 92.4% on the average, of the respective total number of eggs cleaved normally. Only a few of the normally cleaved eggs died of various abnormalities during various embryonic stages; 83.3～

95.8%, 89.3% on the average, hatched normally. In the tadpole stage, a few individuals died of edema or underdevelopment; 77.7~94.1%, 84.4% on the average, metamorphosed normally.

b. Experimental series from X-irradiated sperm

i) (N♀×SX–90♂)♀×N♂, Nos. 1~9

Nine of ten females derived from spermatozoa exposed to 90 rads of X-rays spawned after pituitary injection. The remaining one was found to be quite sterile on autopsy. The ovaries of this female looked like those of a triploid. The eggs of nine females Nos. 1~9 were inseminated with a mixture of spermatozoa obtained from the same five males Nos. 1~5 as those used in the control series (Table 23, Fig. 15).

In nine matings Nos. 1~9, 54.7~98.7%, 88.9% on the average, of the respective total number of eggs cleaved normally. At the gastrula stage, nearly all the cleaved eggs produced from mating No. 3 died of incomplete invagination. In the other matings, a considerable number of eggs died of the same abnormality at the gastrula stage, too. At later embryonic stages, only a few individuals died of various abnormalities, such as microcephaly, blisters and edema in most of the matings; in the nine matings, 1.1~79.9%, 62.8% on the average, hatched normally. After the hatching stage, many tadpoles produced from matings Nos. 1, 4, 8 and 9 died of various abnormalities, such as microcephaly, ill-formation of teeth, edema or underdevelopment. In the other matings, a smaller number of tadpoles died of similar abnormalities. Most of the other tadpoles died shortly before completion of metamorphosis. Eventually, 0~62.5%, 39.5% on the average, attained completion of metamorphosis.

ii) (N♀×SX–170♂)♀×N♂, Nos. 1~8

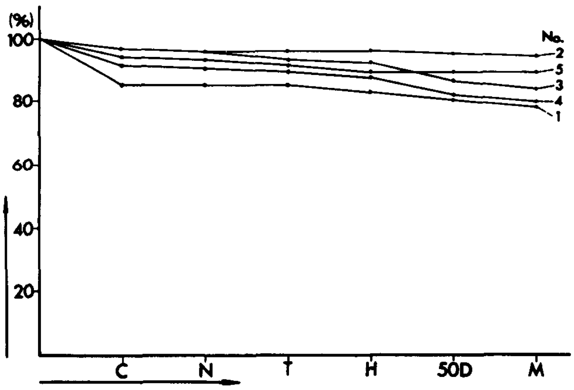
Ten females that derived from spermatozoa exposed to 170 rads of X-rays were injected with pituitary suspension. Ovulation occurred in eight of them. The ovaries of the other two females looked like those of triploids. The eggs of the eight females were inseminated with a mixture of spermatozoa obtained from males Nos. 1~5.

In eight matings Nos. 1~8, 17.2~98.9%, 80.9% on the average, of the respective total number of eggs cleaved normally. While the rates of normal cleavages were very high in matings Nos. 3~8, they were very low in Nos. 1 and 2, as shown in Fig. 15. It was remarkable that many of the normally cleaved eggs produced from matings Nos. 4 and 6 died of incomplete gastrulation, and that many embryos produced from mating No. 3 died of edema immediately before hatching. In the other matings, a small number of the normally cleaved eggs gradually died of various abnormalities at various embryonic stages; 1.3~84.5%, 55.9% on the average, hatched normally. While only a few died of various abnormalities at early tadpole stages, comparatively numerous individuals died of edema shortly before or during the metamorphosis stage, except matings Nos. 1 and 2. In the eight matings, 0.6~56.3%, 39.2% on the average, became normal, metamorphosed frogs.

TABLE 23

Developmental capacity of the offspring of

Parents		No. of eggs	No. of cleaved eggs		No. of gastrulae	
Female	Male		Normal	Abnormal	Normal	Abnormal
(N·N), Nos. 1∼5	N.W70, Nos. 1∼5	909	840 (92.4%)	0	839 (92.3%)	1
(N·SX–90), Nos. 1∼9	N.W70, Nos. 1∼5	3062	2722 (88.9%)	10	2258 (73.7%)	451
(N·SX–170), Nos. 1∼8		1700	1376 (80.9%)	105	1186 (69.8%)	190
(N·SX–240), Nos. 1, 2		405	294 (72.6%)	59	288 (71.1%)	6
(EX–90·N), Nos. 1∼8	N.W70, Nos. 1∼5	1581	1249 (79.0%)	9	1201 (76.0%)	37
(EX–145·N), Nos. 1∼9		2169	933 (43.0%)	265	856 (39.5%)	75
(EX–200·N), Nos. 1, 2		408	315 (77.2%)	0	283 (69.4%)	28
(N·SN–50), Nos. 1∼10	N.W70, Nos. 1∼5	2142	1897 (88.6%)	5	1779 (83.1%)	108
(N·SN–90), Nos. 1∼6		1264	1080 (85.4%)	15	972 (76.9%)	96
(N·SN–130), Nos. 1∼8		1798	1532 (85.2%)	0	1447 (80.5%)	85
(EN–50·N), Nos. 1∼7	N.W70, Nos. 1∼5	1764	1628 (92.3%)	5	1566 (88.8%)	52
(EN–90·N), Nos. 1∼5		1015	614 (60.5%)	32	589 (58.0%)	7
(EN–130·N), Nos. 1∼3		533	436 (81.8%)	27	406 (76.2%)	11



C — Cleavage
N — Neurula stage
T — Tail-bud stage
H — Hatch
50D–Age of 50 days
M — Climbing out of water

Fig. 14. Survival curves of control second-generation offspring derived from untreated grandparental gametes by matings, (N♀ × N♂) ♀ × N♂, Nos. 1∼5.

iii) (N♀ × SX–240♂) ♀ × N♂, Nos. 1 and 2

Three females that derived from spermatozoa exposed to 240 rads of X-rays were injected with pituitary suspension. It was found that ovulation occurred in females raised from irradiated sperm or eggs

No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of 50-day-old tadpoles	No. of metamorphosed frogs
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal		
833 (91.6%)	6	823 (90.5%)	10	812 (89.3%)	11	784 (86.2%)	767 (84.4%)
2150 (70.2%)	108	2056 (67.1%)	94	1922 (62.8%)	134	1721 (56.2%)	1211 (39.5%)
1148 (67.5%)	38	1069 (62.9%)	79	950 (55.9%)	119	914 (53.8%)	666 (39.2%)
279 (68.9%)	9	256 (63.2%)	23	188 (46.4%)	68	165 (40.7%)	124 (30.6%)
1162 (73.5%)	39	1102 (69.7%)	60	975 (61.7%)	127	915 (57.9%)	708 (44.8%)
819 (37.8%)	37	743 (34.3%)	76	576 (26.6%)	167	502 (23.1%)	427 (19.7%)
229 (56.1%)	54	220 (53.9%)	9	173 (42.4%)	47	145 (35.5%)	97 (23.8%)
1684 (78.6%)	95	1588 (74.1%)	96	1543 (72.0%)	45	1480 (69.1%)	1278 (59.7%)
947 (74.9%)	25	871 (68.9%)	76	810 (64.1%)	61	772 (61.1%)	619 (49.0%)
1390 (77.3%)	57	1339 (74.5%)	51	1244 (69.2%)	95	1131 (62.9%)	868 (48.3%)
1546 (87.6%)	20	1490 (84.5%)	56	1399 (79.3%)	91	1345 (76.2%)	966 (54.8%)
576 (56.7%)	13	548 (54.0%)	28	525 (51.7%)	23	479 (47.2%)	347 (34.2%)
396 (74.3%)	10	308 (57.8%)	88	231 (43.3%)	77	220 (41.3%)	161 (30.2%)

two of them. The ovaries of the other female looked like those of a triploid. The eggs of two females Nos. 1 and 2 were inseminated with a mixture of spermatozoa of males Nos. 1 ~ 5.

The eggs of female No. 1 were assorted in size into two clear-cut groups; 248 were of normal size, about 1.8 mm in diameter, while the other 115 were smaller, about 1.2 mm. However, these two groups were nearly the same in the percentage of cleavages. By making use of 101 of the large eggs and 110 of the small ones, 182 (86.3%) normally cleaved eggs were obtained. After a few of them died of edema during the embryonic stage, about one-third of the remaining eggs became simultaneously edematous at the hatching stage, while 119 (56.4%) hatched normally. During the tadpole stage, one-seventh of the individuals died of edema or ill-formation of legs (Plate V, 28). Eventually, 102 (48.3%) became normal, metamorphosed frogs. Several frogs had a large solid swelling on the back.

The eggs of female No. 2 were also divided in size into two groups; 194 were about 2.1 mm and 241 were 1.2 mm in diameter. Of the normal-sized eggs, 22 were of normal color, while 62 were white and 110 brownish white (Table 19).

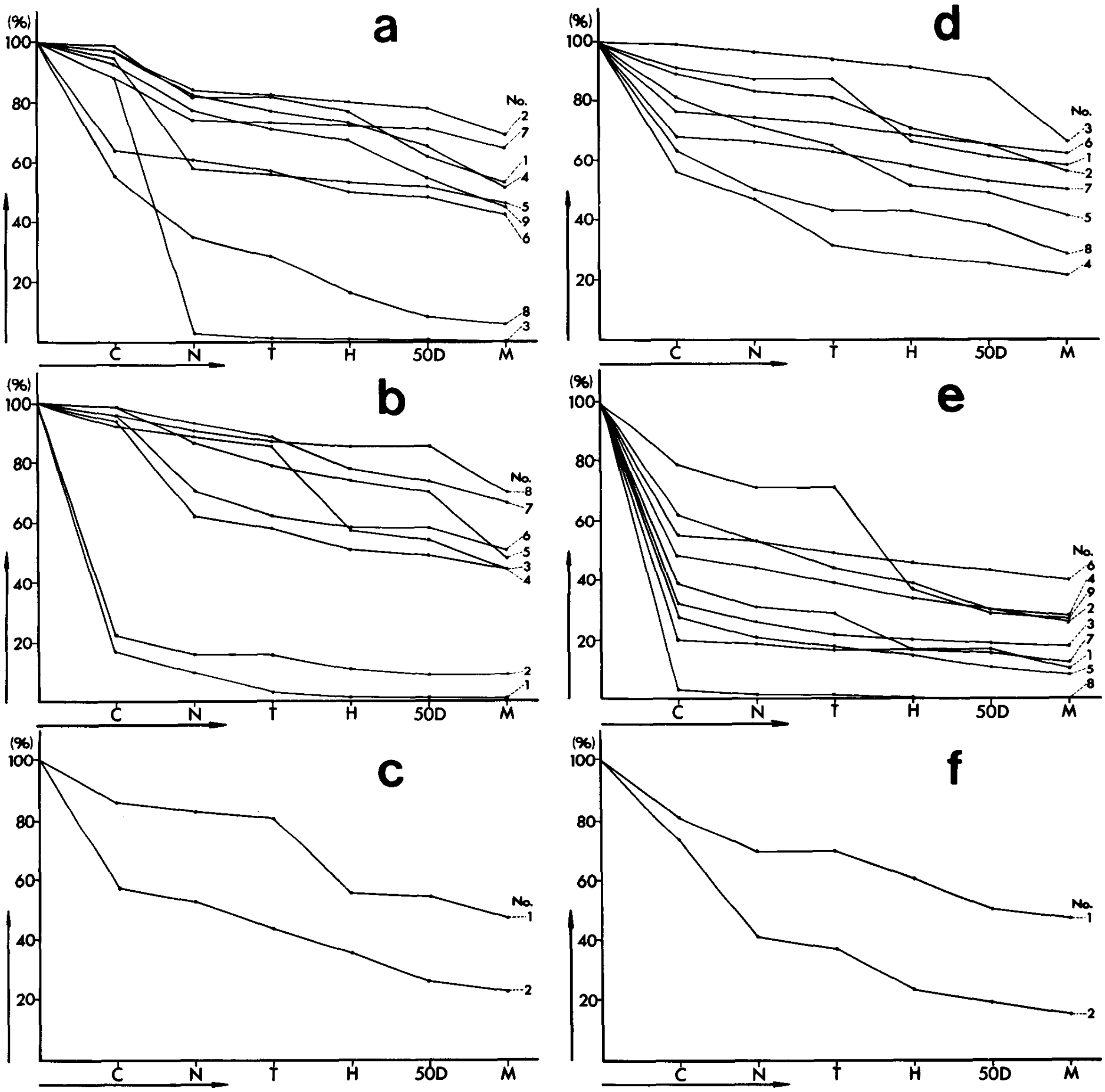


Fig. 15. Survival curves of second-generation offspring derived from X-irradiated grandparental gametes by passing over female first-generation offspring.

a.	(N♀ × SX–90♂) ♀ × N♂, Nos. 1∼9	C — Cleavage
b.	(N♀ × SX–170♂) ♀ × N♂, Nos. 1∼8	N — Neurula stage
c.	(N♀ × SX–240♂) ♀ × N♂, Nos. 1 and 2	T — Tail-bud stage
d.	(EX–90♀ × N♂) ♀ × N♂, Nos. 1∼8	H — Hatch
e.	(EX–145♀ × N♂) ♀ × N♂, Nos. 1∼9	50D–Age of 50 days
f.	(EX–200♀ × N♂) ♀ × N♂, Nos. 1 and 2	M — Climbing out of water

There was scarcely any interrelation between color and developmental capacity of eggs. Of the normal-sized eggs, 112 (57.7%) cleaved normally. Many of the normally cleaved eggs gradually died of edema, curvature of the body, blisters or microcephaly at various embryonic stages (Plate III, 14); 35.6% of the eggs hatched normally. After the hatching stage, the white eggs became tadpoles of normal coloration. Forty-four (22.7%) tadpoles metamorphosed normally. On the average of two matings Nos. 1 and 2, 72.6%, 46.4% and 30.6% of a total number of eggs normally cleaved, hatched and metamorphosed, respectively.

c. Experimental series from X-irradiated eggs

i) (EX–90♀ × N♂)♀ × N♂, Nos. 1～8

Ten females derived from eggs exposed to 90 rads of X-rays were injected with pituitary suspension. Two of them did not ovulate; their ovaries looked like those of triploids in structure. The eggs of the other eight females Nos. 1～8 were inseminated with a mixture of spermatozoa obtained from the same five males Nos. 1～5 as those used in the control series (Table 23, Fig. 15). About half of the eggs of females Nos. 3 and 6, about one-third of those of No. 1 and a little more than 10% of those of No. 4 were white on the whole surface (Table 20). The normal-colored eggs obtained from females Nos. 1, 3 and 5 were assorted in size into two or three groups. The white eggs of each of the above four females were always normal and uniform in size.

The percentage of normally cleaved eggs had no interrelation with the color and size of eggs; 56.2～98.6%, 79.0% on the average, of the respective total number of eggs cleaved normally in the eight matings. However, the white eggs were slightly inferior to the normal ones in the subsequent development, that is, many of them revealed a haploid syndrome at the tail-bud stage or became edematous at the hatching stage, except that many embryos developed from white eggs in No. 1 died of curvature of the body at the hatching stage (Plate IV, 22). The embryos developed from normal-colored eggs scarcely died during the embryonic stage.

In matings Nos. 4, 6 and 7, the main cause of death at various embryonic stages was edema (Plate II, 8), except for incomplete invagination at the gastrula stage, while in Nos. 2, 3, 5 and 8, it was microcephaly, curvature of the body, blisters, edema or a mixture of two or more abnormalities. In the eight matings, 27.9～90.9%, 61.7% on the average of the respective total number of eggs hatched normally. In the embryos produced from white eggs, melanin formation began to occur immediately before the hatching stage. The tadpoles became normal in coloration at early tadpole stages III～IV.

Several tadpoles produced from each mating died mainly of edema or underdevelopment after the hatching stage. The main cause for the death of some tadpoles shortly before the completion of metamorphosis was edema, ascites, hypertrophy of the liver or abnormality of the legs. Eventually, 15.7～60.8%, 44.8% on the average, became normal, metamorphosed frogs.

ii) (EX–145♀ × N♂)♀ × N♂, Nos. 1～9

Ten females derived from eggs exposed to 145 rads of X-rays were injected with pituitary suspension. As a result, ovulation occurred in nine of them. The ovaries of the remaining frog looked like those of a triploid in structure. The eggs of the nine females Nos. 1～9 were inseminated with a mixture of spermatozoa of males Nos. 1～5. About half of the eggs obtained from each of females

Nos. 1 and 3 were white on the whole surface (Table 20). The eggs of each of Nos. 3 and 6 were assorted in size into two groups, that is, large and small eggs.

The percentage of normal cleavages had no interrelation with the color and size of eggs. However, it was generally low in this series; 3.1～79.2%, 43.0% on the average, of the respective total number of eggs cleaved normally. White eggs were very inferior to normal ones in the subsequent development; no individuals developed normally further than the hatching stage. In the eight matings, 1.3～45.9%, 26.6% on the average, hatched normally. In mating No. 9, nearly half of the embryos died of edema during the hatching stage (Plate IV, 20). It was characteristic of this experimental series that a comparatively small number of tadpoles died during the metamorphosis stage; 0～40.2%, 19.7% on the average, metamorphosed normally.

About half of the tadpoles obtained from matings Nos. 3 and 9 had one or more blisters on the tail-fin at the later tadpole stage. Such blisters became large and knotty, and were filled with a mixture of lymph and blood containing huge leucocytes (Plate VIII, 41, 42). During metamorphosis the blisters disappeared simultaneously with absorption of the tail.

iii) (EX–200♀ × N♂)♀ × N♂, Nos. 1 and 2

Two females derived from eggs exposed to 200 rads of X-rays were injected with pituitary suspension. As ovulation occurred in them, eggs were inseminated with a mixture of spermatozoa obtained from males Nos. 1～5. While the eggs of female No. 2 were normal in color, about half of those of the other female No. 1 were white (Table 20).

In the two matings Nos. 1 and 2, 80.9% and 73.1%, 77.2% on the average, of the respective total number of eggs cleaved normally. There was no difference in the percentage of cleavages between normal and white-colored eggs. However, many of the normally cleaved eggs died of various abnormalities at various embryonic stages. The main cause of death in mating No. 1 including white eggs was edema, while that in mating No. 2 was incomplete invagination at the gastrula stage, or bicephaly or cauda bifida at the tail-bud stage. Some embryos had an abnormally short tail and looked like haploids (Plate II, 10). At the hatching stage, some embryos died of microcephaly or curvature of the body. In the two matings, 59.5% and 23.3%, 42.4% on the average, hatched normally. During metamorphosis, many tadpoles produced from No. 1 died of edema, while most of the tadpoles from No. 2 were abnormal in the development of forelegs or hind legs (Plate V, 27); 32.1% and 14.6%, 23.8% on the average, metamorphosed normally in the two matings.

d. Experimental series from neutron-irradiated sperm

i) (N♀ × SN–50♂)♀ × N♂, Nos. 1～10

Ten females derived from spermatozoa exposed to 50 rads of neutrons were injected with pituitary suspension. As ovulation occurred in all the females, their eggs were inseminated with a mixture of spermatozoa of the same males

Nos. 1∼5 as used in the control series (Table 23, Fig. 16). In ten matings Nos. 1∼10, 75.4∼98.3%, 88.6% on the average, of the respective total number of eggs cleaved normally. Some of the normally cleaved eggs died of various abnormalities at various embryonic stages. There was no predominant kind of abnormality except that many eggs in mating No. 1 died of incomplete invagination at the gastrula stage; 38.3∼91.0%, 72.0% on the average, hatched normally.

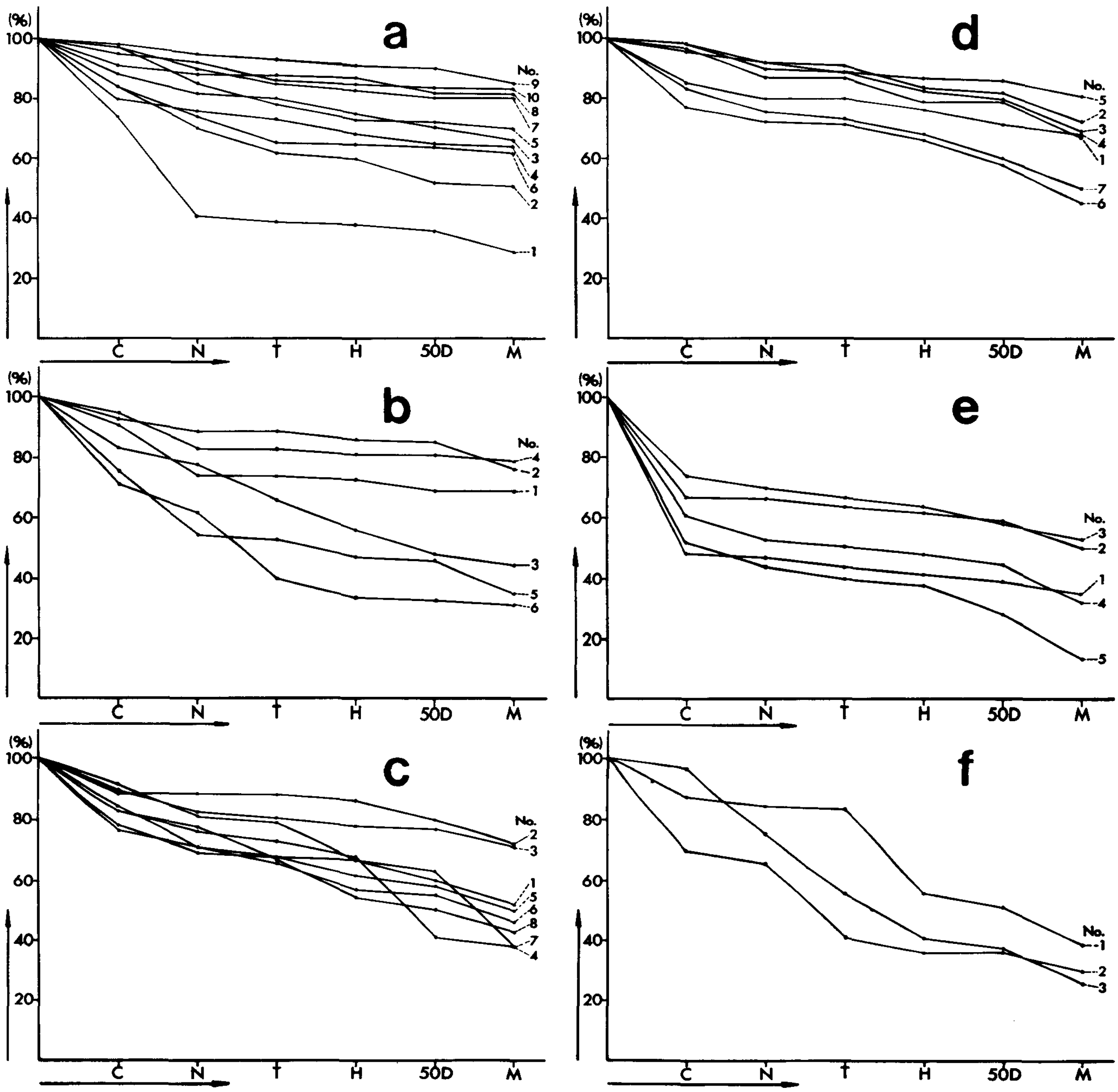


Fig. 16. Survival curves of second-generation offspring derived from neutron-irradiated grandparental gametes by passing over female first-generation offspring.

a.	(N♀ × SN-50♂) ♀ × N♂, Nos. 1∼10	C — Cleavage
b.	(N♀ × SN-90♂) ♀ × N♂, Nos. 1∼6	N — Neurula stage
c.	(N♀ × SN-130♂) ♀ × N♂, Nos. 1∼8	T — Tail-bud stage
d.	(EN-50♀ × N♂) ♀ × N♂, Nos. 1∼7	H — Hatch
e.	(EN-90♀ × N♂) ♀ × N♂, Nos. 1∼5	50D-Age of 50 days
f.	(EN-130♀ × N♂) ♀ × N♂, Nos. 1∼3	M — Climbing out of water

A small number of tadpoles died afterwards of edema or underdevelopment; 26.7 ~ 82.9%, 59.7% on the average, became normal, metamorphosed frogs.

ii) (N♀ × SN–90♂) ♀ × N♂, Nos. 1 ~ 6

Eight females derived from spermatozoa exposed to 90 rads of neutrons were injected with pituitary suspension. The results showed that ovulation occurred in six of them. The other two looked like triploids in structure of their ovaries. The eggs obtained from the six females were inseminated with a mixture of spermatozoa of males Nos. 1 ~ 5.

In six matings Nos. 1 ~ 6, 70.6 ~ 95.2%, 85.4% on the average, of the respective total number of eggs cleaved normally. While many of the normally cleaved eggs obtained from matings Nos. 1, 4 and 5 died of incomplete invagination at the gastrula stage, only a very small number of eggs died of various abnormalities such as microcephaly, edema, blisters or bicephaly at the late embryonic stage. In matings Nos. 3 and 6, many of the normally cleaved eggs died of various abnormalities at various embryonic stages. A good many embryos from No. 3 were especially rough in body surface and died. The normally cleaved eggs in mating No. 2 were comparatively normal in development during the embryonic stage. In the six matings, 34.2 ~ 86.4%, 64.1% on the average, hatched normally. Some individuals died of edema, underdevelopment of the body or hypertrophy of the liver during the tadpole stage; 27.8 ~ 70.0%, 49.0% on the average, metamorphosed normally.

iii) (N♀ × SN–130♂) ♀ × N♂, Nos. 1 ~ 8

Ten females that derived from spermatozoa exposed to 130 rads of neutrons were injected with pituitary suspension. It was found that ovulation occurred in eight of them. The other two females looked like triploids in structure of their ovaries. The eggs of the eight females were inseminated with a mixture of spermatozoa of males Nos. 1 ~ 5.

In eight matings Nos. 1 ~ 8, 76.2 ~ 92.4%, 85.2% on the average, of the respective total number of eggs cleaved normally. Some of the normally cleaved eggs died of edema, microcephaly or blisters at various embryonic stages (Plate III, 16). A few embryos were bicephalous (Plate II, 7). While inviable embryos were few in matings Nos. 2 and 3, those were comparatively numerous in the other matings; 53.9 ~ 87.0%, 69.2% on the average, hatched normally. Many tadpoles produced from matings Nos. 1 ~ 4 died of edema immediately before or during metamorphosis (Plate V, 24, 25). Many from Nos. 5 and 7 died of hypertrophy of the liver at the metamorphosis stage. A few tadpoles from No. 7 had a swelling on the back or abnormal legs. In the latter case, the forelegs and hind legs were underdeveloped or there were additional hind legs (Plate VI, 29 ~ 32). A few tadpoles from Nos. 6 and 8 died also at the metamorphosis stage without showing any abnormal appearance. In the eight matings, 23.3 ~ 66.8%, 48.3% on the average, became normally metamorphosed frogs.

e. Experimental series from neutron-irradiated eggs

i) (EN–50♀ × N♂) ♀ × N♂, Nos. 1 ~ 7

Seven females that had been derived from eggs exposed to 50 rads of neutrons were injected with pituitary suspension. As ovulation occurred in all of them, their eggs were inseminated with a mixture of spermatozoa of the same males Nos. 1～5 as those used in the control series (Table 23, Fig. 16). All the eggs were normal in color.

In seven matings Nos. 1～7, 77.2～98.3%, 92.3% on the average, of the respective total number of eggs cleaved normally. A small number of the normally cleaved eggs died of various abnormalities, such as edema, microcephaly or curvature of the body at various embryonic stages; 65.7～86.8%, 79.3% on the average, hatched normally. At the late tadpole stage, comparatively many individuals obtained from matings Nos. 1, 2, 3, 6 and 7 died of edema, while a small number of tadpoles from Nos. 4 and 5 died of underdevelopment of the legs and hypertrophy of the liver. A few tadpoles from these two matings died of edema, too. Eventually, 33.1～65.1%, 54.8% on the average, metamorphosed normally in the seven matings.

ii) (EN-90♀×N♂)♀×N♂, Nos. 1～5

Seven females derived from eggs exposed to 90 rads of neutrons were injected with pituitary suspension. As a result, five of them ovulated. The other two looked like triploids in structure of their ovaries. The eggs obtained from the five females were inseminated with a mixture of spermatozoa of males Nos. 1～5.

In five matings Nos. 1～5, 48.2～73.6%, 60.5% on the average, of the respective total number of eggs cleaved normally. The eggs of a female (No. 3) were assorted in size into three groups, which were 2.0, 1.7 and 1.3 mm in diameter of eggs. All the largest eggs were white, while the eggs of the other groups were of normal color. The eggs of another female (No. 5) were divided by color into two groups, normal and white, although they were uniform in size (Table 22). In mating No. 3, 13 of 20 white eggs and 146 of 196 normal-colored ones cleaved normally. Some of the normally cleaved eggs died mainly of edema at various embryonic stages. Embryos developed from white eggs began to synthesize melanin at the late embryonic stage, and the normal coloration appeared at stages III～IV (Taylor and Kollros, 1946) after the embryos hatched; 13 white eggs and 125 normal-colored eggs hatched normally. About one-fifth of the normally cleaved eggs that were normal in color died during the tadpole stage. Eventually, 12 white and 98 normal-colored eggs attained completion of metamorphosis. In mating No. 5, 30 of 61 white eggs and 40 of 73 normal-colored ones cleaved normally. Many of the normally cleaved eggs died mainly of edema at various embryonic stages; 19 white and 32 normal-colored eggs hatched normally. Only 5 white and 14 normal-colored eggs became afterwards normal, metamorphosed frogs, while the others died of edema (Plate V, 26). In five matings Nos. 1～5, 38.1～63.9%, 51.7% on the average, hatched normally, and afterwards 14.2～50.9%, 34.2% on the average, became normal, metamorphosed frogs. In mating No. 2, many tadpoles died of hypertrophy of the liver shortly before or during metamorphosis (Plate VII, 37, 38). Their swollen abdomens were filled with blood and lymph. In mating No. 4, a good many tadpoles were characteristic of ill-formation of hind legs or underdevelopment of forelegs immediately before or during metamorphosis. A few tadpoles had only one hind leg, while some others had one or two additional hind legs (Plate VI, 33, 34).

iii) (EN–130♀ × N♂)♀ × N♂, Nos. 1 ~ 3

Four females derived from eggs exposed to 130 rads of neutrons were injected with pituitary suspension. It was found that ovulation occurred in three of them. The other female looked like a triploid in structure of the ovaries. The eggs of the three females were inseminated with a mixture of spermatozoa of males Nos. 1 ~ 5. Nearly half of the eggs obtained from each of two females (Nos. 1 and 3) were white. There was no difference in the percentage of normally cleaved eggs and the subsequent development between white and normal-colored eggs.

In three matings Nos. 1 ~ 3, 88.4%, 69.5% and 97.2%, 81.8% on the average of the respective total number of eggs cleaved normally. A great many of the normally cleaved eggs died of edema or some other abnormalities (Plate II, 11, 12); 56.1%, 36.2% and 40.7%, 43.3% on the average, hatched normally. At the tadpole stage, many individuals gradually died of edema (Plate IV, 21) or blisters on the tail-fin (Plate VIII, 43, 44); 36.0%, 28.8% and 25.4%, 30.2% on the average, became normal, metamorphosed frogs.

3. Chromosome aberrations

Chromosomes were observed in only 100 normally shaped tadpoles obtained from four experimental and control series. Twenty normally shaped tadpoles, 50 days old, were removed at random from each series. When a metaphase spread had ten large and sixteen small chromosomes, it was called normal for the time being, without observing the constitution of each chromosome. Accordingly, deletion or translocation of a small segment was overlooked here. In the control series, none of 20 tadpoles had chromosome aberrations.

a. Experimental series from X-irradiated sperm

Twenty tadpoles were removed from the offspring obtained from mating No. 2 of the series, (N♀ × SX–90♂)♀ × N♂, to examine their chromosomes. It was found that 16 of them were nearly normal diploids and the other four consisted of abnormal mitoses. Two of the four tadpoles were hyperdiploid ($2n+1$); one had a ring chromosome and a fragment in each metaphase spread, while the other had an additional small chromosome. The remaining two tadpoles were abnormal diploids whose mitoses had a chromosome with a translocation or a deletion.

b. Experimental series from X-irradiated eggs

Twenty tadpoles were removed from the offspring produced from mating No. 3 of the series, (EX–90♀ × N♂)♀ × N♂, and examined. Fourteen of them were nearly normal diploids, while the other six had abnormal mitoses. A tadpole was an abnormal diploid having a ring chromosome. Two were abnormal diploids having a chromosome with a deletion. Another tadpole was also an abnormal diploid having a chromosome with a translocation or deletion. The remaining two were mosaics containing hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses. In these mitoses, there was an addition or a loss of a small chromosome.

c. Experimental series from neutron-irradiated sperm

Twenty tadpoles were removed from the offspring produced from mating No. 9 of the series, (N♀ × SN–50♂)♀ × N♂, and examined. While 18 of them were nearly normal diploids, the other two were abnormal diploids. They had a chromosome with a deletion.

d. Experimental series from neutron-irradiated eggs

Twenty tadpoles were removed from the offspring produced from mating No. 5 of the series, (EN–50♀ × N♂)♀ × N♂, and examined. Fifteen of them were normal diploids. A tadpole was a hyperdiploid (2n+1) having an additional small chromosome. Two tadpoles were abnormal diploids; one had a large chromosome with a deletion in the long arm, while the other had a small chromosome with a deletion in the short arm. Another tadpole consisted of a mixture of mitoses having a chromosome with a translocation and those having a chromosome with a deletion. The remaining tadpole was a mosaic of normally and abnormally diploid mitoses. In the latter mitoses, one chromosome had a deletion.

4. Viability and sex of metamorphosed frogs

In the control series, 767 tadpoles produced from five matings climbed out of water at the age of 65～81 days, 71.5 days on the average (Table 24). Fifty frogs were at random removed from the offspring produced from each of five matings immediately after the completion of metamorphosis. A total of 250 frogs were 24.6±0.1 mm in body length. In 12 experimental series, 7472 tadpoles obtained from 2～10 matings climbed out of water at the ages of 64～93 days, 72.0～78.5 days on the average of each experimental series (Table 24). Thus, it was clear that the tadpoles of each experimental series were more or less older in the average age of climbing out than the controls. However, such an age had scarcely any relationship to the kind or amount of irradiation, or to the kind of gametes irradiated.

From the second-generation offspring produced from each of 2～10 matings, 50 frogs were removed at random, and their body length was measured immediately after completion of metamorphosis. When there were less than 50 frogs in a mating, all the frogs were measured, and the shortage was covered with frogs produced from another mating of the same series. When there were a little more than 50 frogs in a mating, all the frogs were measured. Eventually, 97～500 frogs were measured in each experimental series; they were 24.0±0.1～25.3±0.1 mm on the average.

There was no distinct difference in the body length immediately after the completion of metamorphosis between the frogs of the experimental series and the

TABLE 24

Number, size and sex of metamorphosed frogs derived

Parents: Female	Parents: Male	Age at the time of climbing out of water (days)	No. of metamorphosed frogs	Body length immediately after metamorphosis (mm)	Sex of frogs dead one month after: No. of frogs	♀N	♀U
(N·N), Nos. 1∼5	N.W70, Nos. 1∼5	65∼81 (71.5)	767	24.6±0.1	505	238	16
(N·SX–90), Nos. 1∼9	N.W70, Nos. 1∼5	69∼88 (75.6)	1211	24.2±0.1	680	190	74
(N·SX–170), Nos. 1∼8		65∼86 (73.3)	666	24.0±0.1	418	157	24
(N·SX–240), Nos. 1, 2		67∼84 (76.0)	124	25.2±0.1	44	22	1
(EX–90·N), Nos. 1∼8	N.W70, Nos. 1∼5	70∼88 (76.3)	708	25.1±0.1	431	130	52
(EX–145·N), Nos. 1∼9		66∼92 (75.7)	427	24.7±0.1	221	90	14
(EX–200·N), Nos. 1, 2		66∼88 (76.0)	97	25.3±0.1	50	19	6
(N·SN–50), Nos. 1∼10	N.W70, Nos. 1∼5	70∼90 (74.8)	1278	24.9±0.1	1071	530	71
(N·SN–90), Nos. 1∼6		67∼88 (75.9)	619	24.5±0.1	481	196	35
(N·SN–130), Nos. 1∼8		67∼92 (78.5)	868	24.4±0.1	582	284	61
(EN–50·N), Nos. 1∼7	N.W70, Nos. 1∼5	71∼93 (72.3)	966	24.6±0.1	653	315	23
(EN–90·N), Nos. 1∼5		66∼93 (74.9)	347	24.3±0.1	216	96	17
(EN–130·N), Nos. 1∼3		64∼83 (72.0)	161	24.2±0.1	99	39	7

♀N—Females with normal ovaries ♀U—Females with underdeveloped ovaries

controls. There was also no definite relationship between the kind or amount of irradiation, or the kind of irradiated gametes and the body length of frogs.

One month after metamorphosis, 33∼250 frogs with a normal appearance were left alive in each of twelve experimental and the control series to be reared continuously. The other frogs were killed at once to examine their sex. While in each of six experimental series, 200 or 250 frogs were left, only 33∼100 were in each of the remaining six, owing to paucity of healthy frogs with normal appearance. All the other frogs were killed in order to examine their sex, except a small number of them which happened to be lost without being preserved.

a. Control series

(N♀×N♂)♀×N♂, Nos. 1∼5

Of 767 metamorphosed frogs, 112 died within one month after metamorphosis. A total of 150 frogs, 30 from each of five matings (Nos. 1∼5), were from irradiated grandparental sperm or eggs

or killed about metamorphosis			No. of frogs removed and reared	Sex of frogs dead within five months after metamorphosis					Sex of 2-year-old mature frogs			Sex of all frogs examined		
⚥	♂N	♂ (%)*		No. of frogs	♀N	♀U	⚥	♂N	No. of frogs	♀	♂	Total	♀	♂ (%)*
10	241	(49.7)	150	28	14	0	0	14	120	57	63	653	325	328 (50.2)
60	356	(61.2)	200	50	17	1	3	29	142	58	84	872	340	532 (61.0)
43	194	(56.7)	200	57	22	1	6	28	111	39	72	586	243	343 (58.5)
1	20	(47.7)	78	19	9	1	2	7	46	24	22	109	57	52 (47.7)
42	207	(57.8)	200	92	38	3	9	42	93	30	63	616	253	363 (58.9)
23	94	(52.9)	200	69	28	8	3	30	111	52	59	401	192	209 (52.1)
6	19	(50.0)	33	19	8	5	1	5	14	6	8	83	44	39 (47.0)
42	428	(43.9)	100	64	40	5	6	13	25	13	12	1160	659	501 (43.2)
31	219	(52.0)	100	78	24	6	7	41	20	17	3	579	278	301 (52.0)
46	191	(40.7)	250	65	35	8	5	17	155	91	64	802	479	323 (40.3)
17	298	(48.2)	200	105	36	3	13	53	73	33	40	831	410	421 (50.7)
19	84	(47.7)	100	34	14	2	1	17	53	28	25	303	157	146 (48.2)
8	45	(53.5)	45	16	6	2	0	8	29	15	14	144	69	75 (52.1)

⚥—Hermaphrodites ♂N—Males with normal testes * Including hermaphrodites

removed from the remaining 655 and reared continuously, while all the other frogs were killed and preserved. Twenty-eight of the 150 frogs died within five months after metamorphosis, and two others died afterwards. At the ages of two years, there were 120 frogs.

Of 505 young frogs whose sex was examined, 254 were females, 10 hermaphrodites and 241 males (Table 24). When the hermaphrodites were counted as males, 49.7% of the young frogs were males. Among 28 frogs which died within 5 months after metamorphosis, there were 14 females and 14 males. Of the 120 two-year-old frogs, 57 were females and 63 (52.5%) males.

b. Experimental series from X-irradiated sperm

i) (N♀ × SX–90♂)♀ × N♂, Nos. 1∼9

Within one month after metamorphosis, 331 of 1211 normally metamorphosed frogs died of edema, ascites, hypertrophy of the liver or abnormality of the foreleg or hind leg. There were only 880 living frogs one month after metamorphosis. A total of 200 frogs, 40 from each of five matings Nos. 1, 2, 5, 6 and 7, were removed from the living frogs and reared continuously, while all the others were killed and preserved. Within five months, one year, and two years after metamorphosis, 50, 5, and 3 of the 200 frogs died, respectively; 142 frogs became two years old.

The sex of 680 frogs which were killed one month after metamorphosis was examined (Table 24). As a result, 264 were females, 60 hermaphrodites and 356 males. When the hermaphrodites were counted as males, 61.2% of the total frogs were males. The percentages of males were somewhat different with matings. In matings Nos. 2, 4, 6, 7 and 9, they were remarkably higher than 50%, that is, 59%, 68%, 69%, 64% and 79% were males, respectively. Differing from these matings, there was nearly an equal number of males and females in matings Nos. 1, 5 and 8. Of 50 frogs which died within five months after metamorphosis, 18 were females, three hermaphrodites and 29 males. At the age of two years, 58 frogs were females and 84 (59.2%) males.

ii) (N♀ × SX–170♂)♀ × N♂, Nos. 1 ~ 8

Of 666 normally metamorphosed frogs, 415 were living one month after metamorphosis, while the others died. Especially numerous frogs produced from matings Nos. 5, 6 and 7 died of edema or ascites within one month after metamorphosis. Although a small number of frogs from matings Nos. 3 and 4 died, they were rather of a normal appearance. A total of 200 frogs, that is, 20 from each of matings Nos. 3 and 6 and 40 from each of matings Nos. 4, 5, 7 and 8, were left alive, while all the remaining were killed to examine their sex. Of the 200 frogs, 57, 16 and 16 died within five months, one year and two years after metamorphosis, respectively. At the age of two years, 111 frogs were living.

A total of 418 frogs which were dead or killed within one month after metamorphosis consisted of 181 females, 43 hermaphrodites and 194 males. When the hermaphrodites were counted as males, 56.7% were males. Such a predominance of males was attributable to those among the frogs from matings Nos. 3, 4 and 8, in which 74%, 62% and 64% were males, respectively. A similar preponderance of males was also found among two-year-old frogs from these matings; there were two females and 14 males in No. 3, 11 females and 23 males in No. 4 and no females and five males in No. 8. In the other matings Nos. 5, 6 and 7, there were nearly an equal number of males and females. In all the six matings Nos. 3 ~ 8, 23 of 57 frogs which died during the period between one and five months after metamorphosis were females, 6 hermaphrodites and 28 males. At the age of two years, 39 of 111 were females and 72 (65.0%) males.

iii) (N♀ × SX–240♂)♀ × N♂, Nos. 1 and 2

Of 124 normally metamorphosed frogs, 122 were living one month after metamorphosis. A total of 78 frogs, that is, 40 from mating No. 1 and 38 from No. 2, were left alive and reared, while the other 44 were killed. Twenty-three of the latter were females, one hermaphrodite and 20 males. When the hermaphrodite was counted as a male, 47.7% were males. Within five months, one year and two years after metamorphosis, 19, 6 and 7 of the 78 frogs died, respectively. Of 19 frogs which died during the period between one and five months after metamorphosis, ten were females, two hermaphrodites and seven males. At the age of two years, 24 were females and 22 (47.8%) males.

c. Experimental series from X-irradiated eggs

i) (EX–90♀×N♂)♀×N♂, Nos. 1~8

Of 708 normally metamorphosed frogs, 612 were living one month after metamorphosis, while the others died mainly of edema, ascites or hypertrophy of the liver (Table 24). A total of 200 frogs, that is, 50 from each of matings Nos. 1 and 6, 30 from each of matings Nos. 2 and 3 and 40 from No. 7, were left alive and reared. All the other frogs were killed to examine their sex. Within five months, one year and two years after metamorphosis, 92, 9 and 6 of the 200 frogs died, respectively; 93 were living at the age of two years.

A total of 431 frogs which were dead or killed one month after metamorphosis were examined in terms of their sex; 182 were females, 42 hermaphrodites and 207 males. When the hermaphrodites were counted as males, 57.8% were males. Such a predominance of males was attributable to those among the frogs from matings Nos. 1~3, in which 66%, 58% and 73% were males, respectively. In the other matings Nos. 4~8, there was nearly an equal number of males and females.

Of 92 frogs which died during the period between one and five months after metamorphosis, 41 were females, nine hermaphrodites and 42 males. At the age of two years, 30 frogs were females and 63 (67.7%) males.

ii) (EX–145♀×N♂)♀×N♂, Nos. 1~9

Of 427 normally metamorphosed frogs, 362 were living one month after metamorphosis, while the others died mainly of edema or ascites (Table 24). A total of 200 frogs, that is, 20 from mating No. 1, 40 from each of matings Nos. 2, 4 and 9 and 30 from each of matings Nos. 3 and 6, were left alive and reared. All the others were killed to examine their sex. Of the 200 frogs left alive, 69, 4 and 16 died within five months, one year and two years after metamorphosis, respectively. At the age of two years, there were 111 frogs.

Of 221 frogs which were dead or killed one month after metamorphosis, 104 were females, 23 hermaphrodites and 94 males. When the hermaphrodites were counted as males, 52.9% were males. Although the predominance of males was not remarkable, 77% and 63% of the frogs produced from matings Nos. 1 and 3 were males. On the other hand, 40% of frogs from No. 4 were males, and there were nearly the same number of males and females in each of the other five matings. Of 69 frogs which died during the period between one and five months after metamorphosis, 36 were females, three hermaphrodites and 30 males. At the age of two years, 52 were females and 59 (53.2%) males. As in the young frogs, there were two females and 12 males in mating No. 1, and three females and eight males in mating No. 3, while there were 19 females and 12 males in mating No. 4. In each of the other matings, there was nearly an equal number of males and females.

iii) (EX–200♀ ×N♂)♀ ×N♂, Nos. 1 and 2

Forty-five of 97 normally metamorphosed frogs were living one month after metamorphosis, while most of the frogs produced from mating No. 1 became edematous and died. A total of 33 frogs, that is, 30 from mating No. 1 and three from No. 2, were left alive and reared. Of these frogs, four and 15 died within five months and one year after metamorphosis, respectively. At the age of two years, 14 frogs were living.

A total of 50 frogs which were dead or killed one month after metamorphosis were examined in terms of their sex. The results showed that 25 were females, six hermaphrodites and 19 males. When the hermaphrodites were counted as males, 50% were males. Of 19 other frogs which died within five months after metamorphosis, 13 were females, one hermaphrodite and five males. At the age of two years, six were females and eight males.

d. Experimental series from neutron-irradiated sperm

i) (N♀ ×SN–50♂)♀ ×N♂, Nos. 1∼10

Of 1278 normally metamorphosed frogs, 1171 were living one month after metamorphosis, while the others died of edema or some other abnormalities (Table 24). Twenty-five frogs produced from each of four matings Nos. 2∼5 were left alive and reared. All the others were killed to examine their sex. Of the 100 frogs left alive, 64, 7 and 4 died within five months, one year and two years after metamorphosis, respectively. At the age of two years, only 25 frogs were living.

A total of 1071 frogs which were killed one month after metamorphosis were examined in terms of their sex. It was found that 601 were females, 42 hermaphrodites and 428 males. When the hermaphrodites were counted as males, 43.9% were males. Such a comparatively low percentage of males was attributable to the fact that only 14% and 10% of the respective total number of frogs were males in matings Nos. 4 and 5, respectively. All the frogs produced from these two matings died without attaining the age of two years. There was nearly an equal number of males and females among the frogs produced from each of the other eight matings.

Of 64 frogs which died afterwards within five months after metamorphosis, 45 were females, six hermaphrodites and 13 males. At the age of two years, 13 frogs were females and 12 males.

ii) (N♀ ×SN–90♂)♀ ×N♂, Nos. 1∼6

Of 619 normally metamorphosed frogs, 481 were living one month after metamorphosis. The others died of edema, ascites or hypertrophy of the liver. Twenty-five frogs produced from each of four matings Nos. 1∼4 were left alive and reared, while all the others were killed to examine their sex. Of the 100 frogs left alive, 78, one and one died within five months, one year and two years after metamorphosis, respectively. At the age of two years, only 20 frogs were living.

The sex of 481 frogs which were dead or killed one month after metamorphosis was examined; 231 were females, 31 hermaphrodites and 219 males. When the hermaphrodites were counted as males, 52.0% were males. There was no distinct difference in number between males and females among the frogs produced from each of matings Nos. 1～6. Of 78 frogs which died within five months, 30 were females, seven hermaphrodites and 41 males. At the age of two years, 17 were females and three males. This scarcity of males was attributable to the death of many males during hibernation.

iii) (N♀ × SN-130♂)♀ × N♂, Nos. 1～8

Of 868 normally metamorphosed frogs, 663 were living one month after metamorphosis, while the others died of edema, ascites or hypertrophy of the liver. A total of 250 frogs, that is, 40 from mating No. 6 and 30 from each of the other seven matings, were left alive and reared, while all the others were killed to examine their sex. Of the 250 frogs, 65, 14 and 16 died within five months, one year and two years after metamorphosis, respectively. At the age of two years, 155 frogs were living.

The sex of 582 frogs which were dead or killed one month after metamorphosis was examined; 345 were females, 46 hermaphrodites and 191 males. When the hermaphrodites were counted as males, 40.7% were males. This comparatively low percentage of males was attributable to those in four matings; 40%, 41%, 28% and 37% were males in Nos. 1, 2, 3 and 5, respectively. On the other hand, 73% were males in No. 4. There was nearly an equal number of males and females in each of the other three matings Nos. 6～8.

Of 65 frogs which died within five months after metamorphosis, 43 were females, five hermaphrodites and 17 males. At the age of two years, 91 were females and 64 (41.3%) males.

e. Experimental series from neutron-irradiated eggs

i) (EN-50♀ × N♂)♀ × N♂, Nos. 1～7

Of 966 normally metamorphosed frogs, 748 were living one month after metamorphosis, while the others died mainly of edema (Table 24). A total of 200 frogs, that is, 40 from each of five matings Nos. 1～5, were left alive and reared. All the others were killed to examine their sex. Of the 200 frogs left alive, 105, 20 and two died within five months, one year and two years, respectively. At the age of two years, 73 frogs were living.

The sex of 653 frogs which were dead or killed one month after metamorphosis was examined; 338 were females, 17 hermaphrodites and 298 males. When the hermaphrodites were counted as males, 48.2% were males. Of 105 frogs which died within five months after metamorphosis, 39 were females, 13 hermaphrodites and 53 males. At the age of two years, 33 were females and 40 (54.8%) males.

ii) (EN-90♀ × N♂)♀ × N♂, Nos. 1～5

Of 347 normally metamorphosed frogs, 144 were living one month after metamorphosis, while the others died of edema, ascites or hypertrophy of the liver. A total of 100 frogs, that is, 30 from each of matings Nos. 1 and 3 and 20 from each of matings Nos. 2 and 4, were left alive and reared. All the others were killed to examine their sex. Of the 100 frogs left alive, 34, 9 and 4 died within five months, one year and two years after metamorphosis, respectively. At the age of two years, 53 frogs were living.

The sex of 216 frogs which were killed one month after metamorphosis was as follows: 113 were females, 19 hermaphrodites and 84 males. When the hermaphrodites were counted as males, 47.7% were males. Of 34 frogs which died within five months after metamorphosis, 16 were females, one hermaphrodite and 17 males. At the age of two years, 28 were females and 25 (47.2%) males.

iii) (EN–130♀ × N♂)♀ × N♂, Nos. 1 ~ 3

Of 161 normally metamorphosed frogs, 144 were living one month after metamorphosis. A total of 45 frogs, that is, 15 from mating No. 1, 20 from No. 2 and 10 from No. 3, were left alive and reared, while all the others were killed to examine their sex. Of the 45 frogs left alive, 16 died within five months after metamorphosis, respectively. At the age of two years, 29 frogs were living.

The sex of 99 frogs which were killed one month after metamorphosis was as follows: 46 were females, eight hermaphrodites and 45 males. When the hermaphrodites were counted as males, 53.5% were males. Of 16 frogs which died within five months after metamorphosis, eight were females and eight males. At the age of two years, 15 were females and 14 (48.3%) males.

IV. Third-generation offspring derived from irradiated gametes by passing over male first- and second-generation offspring

1. Male parents

Male second-generation offspring derived from eggs or spermatozoa irradiated with X-rays or neutrons were mated with normal females in the breeding season of 1971. These males were produced in 1969 from matings between normal females collected from the field and male first-generation offspring of irradiated

TABLE 25

Eggs of twelve field-caught female frogs used for mating experiments in 1971

Kind	Individual no.	Body length (mm)	No. of eggs	Mean diameter of 50 eggs (mm)
N.W71	1	60.0	2032	1.69±0.01
	2	64.0	2357	1.83±0.01
	3	64.0	2104	1.96±0.01
	4	62.5	2411	1.73±0.01
	5	63.0	2108	1.90±0.01
	6	61.0	2243	1.91±0.01
	7	61.0	2062	1.82±0.01
	8	61.5	2023	1.69±0.01
	9	61.0	2100	1.72±0.01
	10	65.0	2541	1.91±0.01
	11	64.0	1978	1.68±0.01
	12	60.0	2323	1.71±0.01

eggs or spermatozoa. Mating experiments of 46 male second-generation offspring with 12 normal females collected from the field were performed by artificial insemination in 1971 to produce the third-generation offspring. As the controls of the male second-generation offspring, three males produced from mating No. 4 in the control series of 1969 were used.

Twelve normal females Nos. 1∼12 collected from the field were 60.0∼65.0 mm in body length (Table 25). After pituitary injection, they laid 1978∼2541 eggs, 2190.2 eggs on the average. Fifty eggs removed at random from each female

TABLE 26

Testes of twenty-six male frogs derived from X-irradiated grandparental sperm or eggs and those of three controls used for mating experiments in 1971

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure
				Left (mm)	Right (mm)	
N(N·N), No. 4	1	2	56.0	5.0×2.5	5.0×2.5	Type 1
	2	2	61.5	5.5×2.5	5.5×3.0	Type 1
	3	2	59.0	5.0×3.0	5.5×2.5	Type 1
N(N·SX–90), No. 4	1	2	59.5	4.0×2.0	3.5×2.5	Type 1
	2	2	61.0	4.5×2.0	6.0×2.5	Type 2
	3	2	62.0	5.0×2.5	4.5×2.5	Type 1
N(N·SX–170), No. 8	1	2	57.5	6.0×3.0	5.5×3.0	Type 1
	2	2	61.5	5.0×2.5	5.0×2.5	Type 1
N(N·SX–240), No. 4	1	2	58.0	5.0×2.5	5.0×2.5	Type 1
N(EX–90·N), No. 10	1	2	61.0	4.5×2.5	5.5×2.5	Type 1
	2	2	59.0	5.5×3.0	5.5×3.0	Type 1
	3	2	60.5	5.5×2.5	5.5×3.0	Type 1
N(EX–145·N), No. 11	1	2	59.5	6.0×2.5	5.0×2.5	Type 2
	2	2	58.5	5.0×2.0	4.0×2.5	Type 2
	3	2	60.0	6.0×2.5	6.0×2.5	Type 1
	4	2	60.0	5.5×2.5	5.5×2.5	Type 1
	5	2	59.0	5.0×3.0	5.0×2.5	Type 1
	6	2	58.5	5.0×2.5	4.0×2.5	Type 1
N(EX–200·N), No. 3	1	2	60.5	5.5×3.0	5.5×4.0	Type 3
	2	2	59.0	5.5×3.0	5.0×3.0	Type 1
	3	2	60.0	5.0×2.5	5.0×2.5	Type 1
	4	2	62.0	6.0×3.0	5.0×2.5	Type 3
	5	2	61.5	5.0×2.5	4.0×2.0	Type 1
	6	2	58.5	5.0×2.0	4.5×2.5	Type 3
	7	2	58.5	4.5×2.0	4.5×2.0	Type 2
	8	2	63.0	6.5×3.0	6.0×3.5	Type 2
	9	2	62.0	6.0×3.0	5.5×3.0	Type 2
	10	2	59.5	5.0×2.5	5.0×3.0	Type 3
	11	2	57.5	5.0×2.5	5.0×2.5	Type 2

N(N·N), No. 4: Males obtained by N.W69♀, Nos. 1∼10×(N·N)♂, No. 4
N(N·SX–90), No. 4: Males obtained by N.W69♀, Nos. 1∼3×(N·SX–90)♂, No. 4
N(N·SX–170), No. 8: Males obtained by N.W69♀, Nos. 4∼7×(N·SX–170)♂, No. 8
N(N·SX–240), No. 4: Male obtained by N.W69♀, Nos. 8∼10×(N·SX–240)♂, No. 4
N(EX–90·N), No. 10: Males obtained by N.W69♀, Nos. 1∼3×(EX–90·N)♂, No. 10
N(EX–145·N), No. 11: Males obtained by N.W69♀, Nos. 4∼7×(EX–145·N)♂, No. 11
N(EX–200·N), No. 3: Males obtained by N.W69♀, Nos. 8∼10×(EX–200·N)♂, No. 3

were 1.68±0.01 ~ 1.96±0.01 mm in mean diameter.

The three control males were 56.0 ~ 61.5 mm, 58.8 mm on the average, in body length (Table 26). Their testes were 5.0×2.5 ~ 5.5×3.0 mm in length and width. All these testes were of type 1 in inner structure, that is, the seminal tubules were filled with bundles of spermatozoa.

The 46 male second-generation offspring belonged to four groups each of which consisted of three experimental series. They were 57.5 ~ 63.5 mm, 59.9 mm on the average, in body length (Tables 26 and 27). Three of six males belonging to the first group were produced from mating No. 4 in the experimental series derived from spermatozoa exposed to 90 rads of X-rays. They were 59.5 ~ 62.0 mm, 60.8 mm on the average, in body length. Their testes were 4.0×2.0 ~ 6.0×2.5 mm in length and width. While the testes of two males Nos. 1 and 3 were of type 1 in inner structure, those of the other male No. 2 were of type 2 (cf. p. 29). Two other males were produced from mating No. 8 in the experimental series that derived from spermatozoa exposed to 170 rads of X-rays. They were 57.5 and 61.5 mm, 59.5 mm on the average, in body length. Their

TABLE 27

Testes of twenty male frogs derived from neutron-irradiated grandparental sperm or eggs and used for mating experiments in 1971

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure
				Left (mm)	Right (mm)	
N(N·SN–50), No. 3	1	2	57.5	4.0×2.5	4.0×2.5	Type 1
	2	2	60.5	4.5×2.0	4.5×2.5	Type 1
N(N·SN–90), No. 4	1	2	59.0	5.0×3.0	5.0×2.5	Type 1
	2	2	61.0	5.0×2.5	4.0×2.5	Type 1
	3	2	58.5	5.0×2.5	4.0×3.0	Type 2
N(N·SN–130), No. 4	1	2	63.0	5.0×2.5	5.0×3.0	Type 3
	2	2	57.5	5.0×3.0	5.0×2.0	Type 1
	3	2	61.0	4.5×2.0	5.5×3.0	Type 1
	4	2	59.5	4.5×4.0	4.5×2.0	Type 1
	5	2	59.0	4.0×2.5	4.0×2.5	Type 1
	6	2	63.5	5.0×2.5	5.0×2.5	Type 1
N(EN–50·N), No. 1	1	2	61.0	4.0×3.0	5.0×2.0	Type 1
	2	2	58.0	5.0×2.5	4.5×2.0	Type 1
	3	2	59.5	5.5×2.5	5.0×2.5	Type 1
	4	2	60.0	5.0×2.0	5.0×3.5	Type 3
N(EN–90·N), No. 8	1	2	62.0	4.5×2.5	4.0×2.5	Type 3
	2	2	57.5	4.0×3.0	4.0×3.0	Type 1
	3	2	57.5	4.5×2.5	4.5×2.5	Type 2
N(EN–130·N), No. 7	1	2	58.0	4.0×2.0	4.0×2.5	Type 1
	2	2	63.0	5.5×3.0	5.5×2.5	Type 1

N(N·SN–50), No. 3: Males obtained by N.W69♀, Nos. 1 ~ 3× (N·SN–50)♂, No. 3
N(N·SN–90), No. 4: Males obtained by N.W69♀, Nos. 4 ~ 7× (N·SN–90)♂, No. 4
N(N·SN–130), No. 4: Males obtained by N.W69♀, Nos. 8 ~ 10× (N·SN–130)♂, No. 4
N(EN–50·N), No. 1: Males obtained by N.W69♀, Nos. 1 ~ 3× (EN–50·N)♂, No. 1
N(EN–90·N), No. 8: Males obtained by N.W69♀, Nos. 4 ~ 7× (EN–90·N)♂, No. 8
N(EN–130·N), No. 7: Males obtained by N.W69♀, Nos. 8 ~ 10× (EN–130·N)♂, No. 7

testes were 5.0×2.5∼6.0×3.0 mm, and of type 1. The remaining male was the only mature one that was produced from mating No. 4 in the experimental series derived from spermatozoa exposed to 240 rads of X-rays. Each of the testes was 5.0×2.5 mm and of type 1.

Three of twenty males belonging to the second group were produced from mating No. 10 in the experimental series derived from eggs exposed to 90 rads of X-rays. They were 59.0∼61.0 mm, 60.2 mm on the average, in body length. Their testes were 4.5×2.5∼5.5×3.0 mm in length and width and of type 1 in inner structure. Six other males were produced from mating No. 11 in the experimental series derived from eggs exposed to 145 rads of X-rays. They were 58.5∼60.0 mm, 59.3 mm on the average, in body length. Their testes were 4.0×2.5∼6.0×2.5 mm. The testes of two males (Nos. 1 and 2) were of type 2, while those of the other four (Nos. 3∼6) were of type 1. The remaining eleven males were produced from mating No. 3 in the experimental series derived from eggs exposed to 200 rads of X-rays. They were 57.5∼63.0 mm, 60.2 mm on the average, in body length. Their testes were 4.0×2.0∼6.5×3.0 mm in length and width. Of these males, three (Nos. 2, 3 and 5) were of type 1, four (Nos. 7, 8, 9 and 11) of type 2 and the remaining four (Nos. 1, 4, 6 and 10) of type 3 in inner structure of the testes.

Two of 11 males belonging to the third group were produced from mating No. 3 in the experimental series derived from spermatozoa exposed to 50 rads of neutrons. They were 57.5 and 60.5 mm, 59.0 mm on the average, in body length. Their testes were 4.0×2.5∼4.5×2.5 mm in length and width, and of type 1 in inner structure. Three other males were produced from mating No. 4 in the experimental series derived from spermatozoa exposed to 90 rads of neutrons. They were 58.5∼61.0 mm, 59.5 mm on the average, in body length. Their testes were 4.0×2.5∼5.0×3.0 mm in length and width. While two (Nos. 1 and 2) of the three males were of type 1 in inner structure of the testes, the other was of type 2. The remaining six males were produced from mating No. 4 in the experimental series derived from spermatozoa exposed to 130 rads of neutrons. They were 57.5∼63.5 mm, 60.6 mm on the average, in body length. Their testes were 4.0×2.5∼5.5×3.0 mm in length and width. While the testes of a male (No. 1) were of type 3 in inner structure, those of the other five (Nos. 2∼6) were of type 1.

Four of nine males belonging to the fourth group were produced from mating No. 1 in the experimental series derived from eggs exposed to 50 rads of neutrons. They were 58.0∼61.0 mm, 59.6 mm on the average, in body length. Their testes were 4.5×2.0∼5.0×3.5 mm in length and width. While three (Nos. 1∼3) of the four males were of type 1 in inner structure of the testes, the other (No. 4) was of type 3. Three other males were produced from mating No. 8 in the experimental series derived from eggs exposed to 90 rads of neutrons. They were 57.5∼62.0 mm, 59.0 mm on the average, in body length. Their testes were 4.0×2.5∼4.0×3.0 mm in length and width. One (No. 2) of the three males was of type 1, another (No. 3) of type 2 and the remaining (No. 1) of type 3 in inner structure of the testes. The remaining two males were produced from mating No. 7 in the experimental series derived from eggs exposed to 130 rads of neutrons. They were 58.0 and 63.0 mm, 60.5 mm on the average, in body length. Their testes were $4.0 \times 2.0 \sim 5.5 \times 3.0$ mm in length and width and of type 1 in inner structure.

2. Developmental capacity

a. Control series

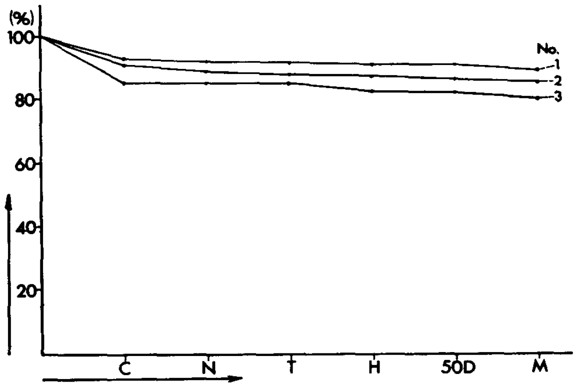
N♀ × {N♀ × (N♀ × N♂) ♂} ♂, Nos. 1~3

In three matings Nos. 1~3 between 12 normal females Nos. 1~12 collected from the field and three males produced in the laboratory as controls, 92.7%, 90.9% and 84.7%, 89.9% on the average, of the respective total number of eggs cleaved normally (Table 28, Fig. 17). While only a few of the normally cleaved eggs died of abnormalities during the embryonic stage, 91.0%, 87.2% and 82.2%, 87.4% on the average, hatched normally. During the tadpole stage, only a few individuals also died of various abnormalities; 88.8%, 84.8% and 80.3%, 85.2% on the average, metamorphosed normally.

TABLE 28

Developmental capacity of the offspring of male frogs

Parents		No. of eggs	No. of cleaved eggs		No. of gastrulae	
Female	Male		Normal	Abnormal	Normal	Abnormal
N.W71, Nos. 1~12	N(N·N), Nos. 1~3	554	498 (89.9%)	2	494 (89.2%)	4
N.W71, Nos. 1~3	N(N·SX-90), Nos. 1~3	596	444 (74.5%)	14	433 (72.7%)	11
	N(N·SX-170), Nos. 1, 2	356	286 (80.3%)	14	275 (77.2%)	11
	N(N·SX-240), No. 1	216	212 (98.1%)	0	186 (86.1%)	26
N.W71, Nos. 4~6	N(EX-90·N), Nos. 1~3	526	358 (68.1%)	9	339 (64.4%)	19
	N(EX-145·N), Nos. 1~6	2076	1629 (78.5%)	79	1607 (77.4%)	22
	N(EX-200·N), Nos. 1~11	3387	1957 (57.8%)	48	1937 (57.2%)	20
N.W71, Nos. 7~9	N(N·SN-50), Nos. 1, 2	415	391 (94.2%)	2	381 (91.8%)	10
	N(N·SN-90), Nos. 1~3	527	412 (78.2%)	0	394 (74.8%)	18
	N(N·SN-130), Nos. 1~6	1074	771 (71.8%)	14	766 (71.3%)	5
N.W71, Nos. 10~12	N(EN-50·N), Nos. 1~4	545	366 (67.2%)	6	354 (65.0%)	9
	N(EN-90·N), Nos. 1~3	410	278 (67.8%)	4	262 (63.9%)	11
	N(EN-130·N), Nos. 1, 2	426	303 (71.1%)	11	296 (69.5%)	7



C — Cleavage
N — Neurula stage
T — Tail-bud stage
H — Hatch
50D–Age of 50 days
M — Climbing out of water

Fig. 17. Survival curves of control third-generation offspring derived from untreated great-grandparental gametes by matings, N♀ × {N♀ × (N♀ × N♂) ♂} ♂, Nos. 1 ~ 3.

b. Experimental series from X-irradiated sperm

i) N♀ × {N♀ × (N♀ × SX–90♂) ♂} ♂, Nos. 1 ~ 3

Each of three male second-generation offspring (Nos. 1 ~ 3) derived from a spermatozoon exposed to 90 rads of X-rays was mated with the same three females

derived from irradiated grandparental sperm or eggs, I

No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of 50-day-old tadpoles	No. of metamorphosed frogs
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal		
494 (89.2%)	0	491 (88.6%)	3	484 (87.4%)	7	482 (87.0%)	472 (85.2%)
433 (72.7%)	0	431 (72.3%)	2	415 (69.6%)	16	407 (68.3%)	343 (57.6%)
265 (74.4%)	10	252 (70.8%)	13	242 (68.0%)	10	225 (63.2%)	219 (61.5%)
162 (75.0%)	24	153 (70.8%)	9	95 (44.0%)	58	86 (39.8%)	84 (38.9%)
311 (59.1%)	28	280 (53.2%)	31	220 (41.8%)	60	210 (39.9%)	204 (38.8%)
1605 (77.3%)	2	1586 (76.4%)	19	1368 (65.9%)	218	979 (47.2%)	696 (33.5%)
1935 (57.1%)	2	1920 (56.7%)	15	1436 (42.4%)	484	1174 (34.7%)	840 (24.8%)
363 (87.5%)	18	350 (84.3%)	13	319 (76.9%)	31	303 (73.0%)	287 (69.2%)
384 (72.9%)	10	359 (68.1%)	25	330 (62.6%)	29	266 (50.5%)	247 (46.9%)
763 (71.0%)	3	752 (70.0%)	11	722 (67.2%)	30	682 (63.5%)	563 (52.4%)
330 (60.6%)	24	312 (57.2%)	18	285 (52.3%)	27	266 (48.8%)	242 (44.4%)
238 (58.0%)	24	222 (54.1%)	16	207 (50.5%)	15	201 (49.0%)	186 (45.4%)
279 (65.5%)	17	262 (61.5%)	17	239 (56.1%)	23	235 (55.2%)	228 (53.5%)

Nos. 1～3 as those used in the control series to produce the third-generation offspring (Table 28, Fig. 18). In three matings Nos. 1～3, 83.3%, 51.3% and 87.7% of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to those of the control series at least until the stage of 50-day-old tadpoles. Many tadpoles died of edema before metamorphosis; 63.3%, 39.3% and 69.2%, 57.6% on the average, became normal, metamorphosed frogs.

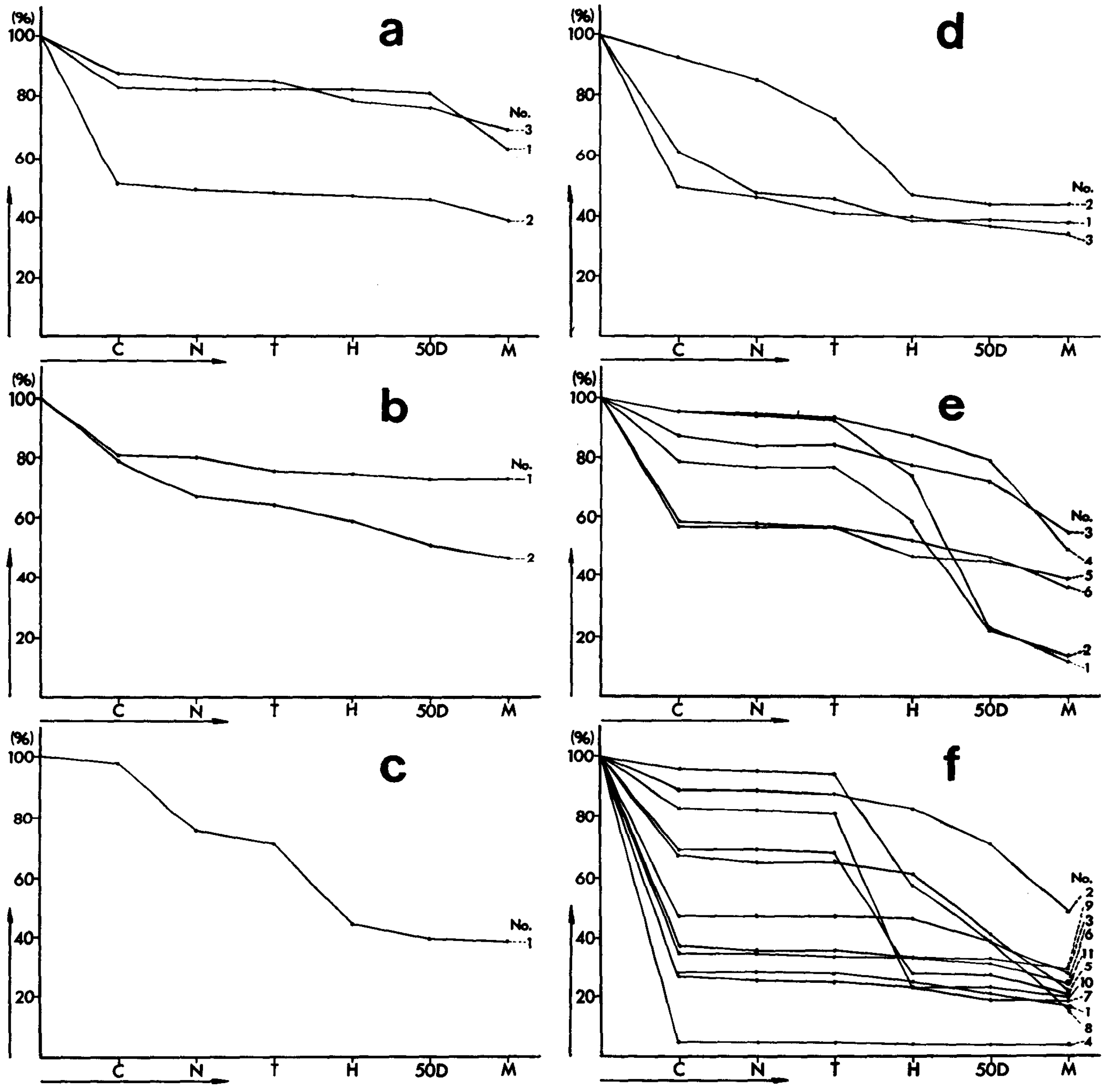


Fig. 18. Survival curves of third-generation offspring derived from X-irradiated great-grandparental gametes by passing over male first- and second-generation offspring.

a. N♀ × {N♀ × (N♀ × SX-90♂) ♂} ♂, Nos. 1～3
b. N♀ × {N♀ × (N♀ × SX-170♂) ♂} ♂, Nos. 1 and 2
c. N♀ × {N♀ × (N♀ × SX-240♂) ♂} ♂, No. 1
d. N♀ × {N♀ × (EX-90♀ × N♂) ♂} ♂, Nos. 1～3
e. N♀ × {N♀ × (EX-145♀ × N♂) ♂} ♂, Nos. 1～6
f. N♀ × {N♀ × (EX-200♀ × N♂) ♂} ♂, Nos. 1～11

C — Cleavage
N — Neurula stage
T — Tail-bud stage
H — Hatch stage
50D–Age of 50 days
M — Climbing out of water

ii) N♀ × {N♀ × (N♀ × SX–170♂) ♂} ♂, Nos. 1 and 2

Each of two male second-generation offspring Nos. 1 and 2 derived from a spermatozoon exposed to 170 rads of X-rays was mated with females Nos. 1 ~ 3. In two matings Nos. 1 and 2, 81.2% and 79.2%, 80.3% on the average, of the respective total number of eggs cleaved normally. A small number of normally cleaved eggs died afterwards of various abnormalities; 75.1% and 59.1%, 68.0% on the average, hatched normally. Some individuals died gradually of underdevelopment, edema or some other abnormalities during the tadpole stage; 73.1% and 47.2%, 61.5% on the average, metamorphosed normally.

iii) N♀ × {N♀ × (N♀ × SX–240♂) ♂} ♂, No. 1

The single male second-generation offspring derived from a spermatozoon exposed to 240 rads of X-rays was mated with females Nos. 1 ~ 3. In this mating, 98.1% of the total number of eggs cleaved normally. Nearly half of the normally cleaved eggs died of various abnormalities, such as microcephaly, curvature of the body or bicephaly at various embryonic stages (Plate III, 18); 44.0% hatched normally. Some individuals died of underdevelopment, edema or some other abnormalities during the tadpole stage; 38.9% metamorphosed normally.

c. Experimental series from X-irradiated eggs

i) N♀ × {N♀ × (EX–90♀ × N♂) ♂} ♂, Nos. 1 ~ 3

Each of three male second-generation offspring Nos. 1 ~ 3 derived from an egg exposed to 90 rads of X-rays was mated with the same normal females Nos. 4 ~ 6 as those used in the control series (Table 28, Fig. 18). In three matings Nos. 1 ~ 3, 61.8%, 92.7% and 50.0%, 68.1% on the average, of the respective total number of eggs cleaved normally. Some of the normally cleaved eggs died of various abnormalities, such as edema, microcephaly, curvature of the body or bicephaly at various embryonic stages; 39.2%, 47.0% and 40.0%, 41.8% on the average, hatched normally. During the tadpole stage, a few individuals died of edema; 38.2%, 43.9% and 34.0%, 38.8% on the average, metamorphosed normally.

ii) N♀ × {N♀ × (EX–145♀ × N♂) ♂} ♂, Nos. 1 ~ 6

Each of six male second-generation offspring Nos. 1 ~ 6 derived from an egg exposed to 145 rads of X-rays was mated with females Nos. 4 ~ 6. In six matings Nos. 1 ~ 6, most eggs cleaved normally. Only a few of the normally cleaved eggs died of various abnormalities before the tail-bud stage. Some embryos died of abnormalities, such as edema, curvature of the body, or microcephaly at the hatching stage; 46.7 ~ 86.5%, 65.9% on the average, hatched normally. Many individuals died of edema or underdevelopment during the tadpole stage; 12.2 ~ 54.6%, 33.5% on the average, became normal, metamorphosed frogs.

iii) N♀ × {N♀ × (EX–200♀ × N♂) ♂} ♂, Nos. 1 ~ 11

Each of 11 male second-generation offspring derived from an egg exposed to 200 rads of X-rays was mated with females Nos. 4 ~ 6. In 11 matings Nos. 1 ~ 11, 4.7 ~ 95.9%, 57.8% on the average, of the respective total number of eggs cleaved normally. Of the normally cleaved eggs, only a few died of various abnormalities before the hatching stage. However, at this stage, 132 of 226, 198 of 276 and 106 of 274 embryos died of various abnormalities, such as edema or curvature of the body, in matings Nos. 5, 7 and 8, respectively. In the other matings, a small number of embryos died at this stage. In the 11 matings, 4.1~83.1%, 42.4% on the average, hatched normally. During the tadpole stage, numerous individuals produced from matings Nos. 2, 8 and 11 died of edema or underdevelopment, while a comparatively small number of tadpoles died in the other matings; 3.5~47.5%, 24.8% on the average, became metamorphosed frogs, including those with abnormal forelegs. In matings Nos. 2 and 3, 92 of 171 and 43 of 108 frogs had abnormal forelegs.

d. Experimental series from neutron-irradiated sperm

i) N♀ × {N♀ × (N♀ × SN–50♂) ♂} ♂, Nos. 1 and 2

Each of two male second-generation offspring Nos. 1 and 2 derived from a spermatozoon exposed to 50 rads of neutrons was mated with the same three normal females Nos. 7~9 as those used in the control series (Table 28, Fig. 19). In two matings Nos. 1 and 2, 96.8% and 91.2%, 94.2% on the average, of the respective total number of eggs cleaved normally. Of the normally cleaved eggs produced from mating No. 1, less than one-tenth died of various abnormalities during the embryonic stage; 89.1% hatched normally. In mating No. 2, numerous embryos died of various abnormalities (Plate III, 17); 62.9% hatched normally. After the hatching stage, numerous tadpoles also died of edema or underdevelopment. Eventually, 86.9% and 49.0%, 69.2% on the average, metamorphosed normally in matings Nos. 1 and 2, respectively.

ii) N♀ × {N♀ × (N♀ × SN–90♂) ♂} ♂, Nos. 1~3

Each of three male second-generation offspring Nos. 1~3 derived from a spermatozoon exposed to 90 rads of neutrons was mated with females Nos. 7~9. In three matings Nos. 1~3, 79.8%, 62.2% and 93.3%, 78.2% on the average, of the respective total number of eggs cleaved normally. Some of the normally cleaved eggs died of various abnormalities, such as curvature of the body, microcephaly or bicephaly; 68.1%, 50.6% and 68.0%, 62.6% on the average, hatched normally. After the hatching stage, a small number of tadpoles died of edema in matings Nos. 1 and 2, while more than half the number of tadpoles produced from mating No. 3 died about the same time of the same sickness; 62.9%, 42.7% and 28.7%, 46.9% on the average, became normal, metamorphosed frogs in matings Nos. 1, 2 and 3, respectively.

iii) N♀ × {N♀ × (N♀ × SN–130♂) ♂} ♂, Nos. 1~6

Each of six male second-generation offspring (Nos. 1~6) derived from a spermatozoon exposed to 130 rads of neutrons was mated with females Nos. 7~9. In six matings Nos. 1~6, 45.1~94.1%, 71.8% on the average, of the respective total number of eggs cleaved normally. A small number of normally cleaved eggs died of various abnormalities at various embryonic stages; 42.1~82.8%, 67.2% on the average, hatched normally. After the hatching stage, comparatively numerous tadpoles died of edema or underdevelopment; 31.1~63.1%,

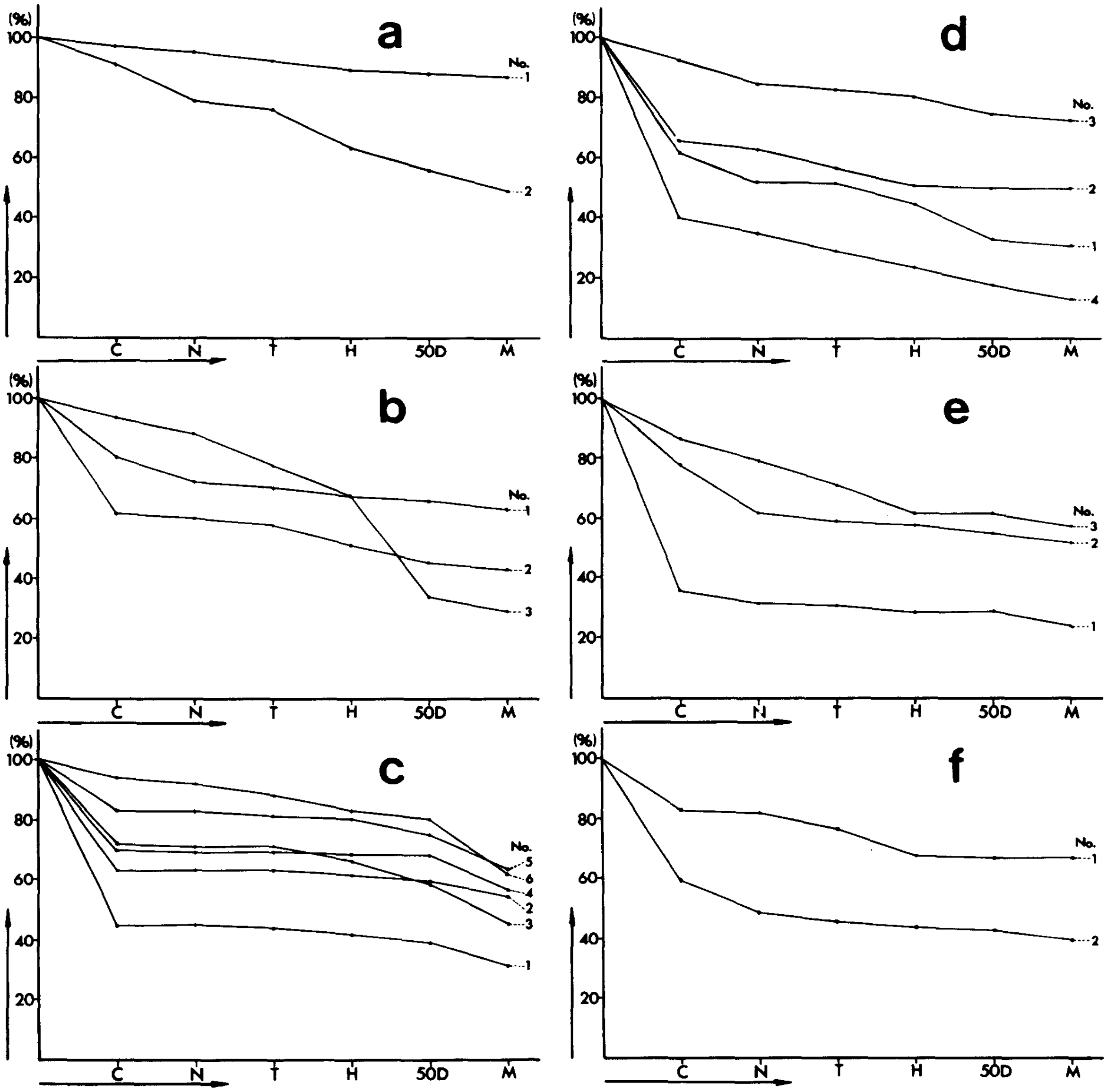


Fig. 19. Survival curves of third-generation offspring derived from neutron-irradiated great-grand-parental gametes by passing over male first- and second-generation offspring.

a. N♀ × {N♀ × (N♀ × SN-50♂) ♂} ♂, Nos. 1 and 2	C — Cleavage
b. N♀ × {N♀ × (N♀ × SN-90♂) ♂} ♂, Nos. 1∼3	N — Neurula stage
c. N♀ × {N♀ × (N♀ × SN-130♂) ♂} ♂, Nos. 1∼6	T — Tail-bud stage
d. N♀ × {N♀ × (EN-50♀ × N♂) ♂} ♂, Nos. 1∼4	H — Hatch
e. N♀ × {N♀ × (EN-90♀ × N♂) ♂} ♂, Nos. 1∼3	50D-Age of 50 days
f. N♀ × {N♀ × (EN-130♀ × N♂) ♂} ♂, Nos. 1 and 2	M — Climbing out of water

52.4% on the average, became normal, metamorphosed frogs.

e. Experimental series from neutron-irradiated eggs

i) N♀ × {N♀ × (EN-50♀ × N♂) ♂} ♂, Nos. 1∼4

Each of four male second-generation offspring Nos. 1∼4 derived from an egg exposed to 50 rads of neutrons was mated with the same three normal females

Nos. 10~12 as those used in the control series (Table 28, Fig. 19). In four matings Nos. 1~4, 40.0~93.1%, 67.2% on the average, of the respective total number of eggs cleaved normally. Some of the normally cleaved eggs died gradually of various abnormalities at various embryonic stages; 23.8~81.3%, 52.3% on the average, hatched normally. After the hatching stage, some tadpoles also died of edema or underdevelopment; 13.3~72.9%, 44.4% on the average, metamorphosed normally.

ii) N♀×{N♀×(EN-90♀×N♂)♂}♂, Nos. 1~3

Each of three second-generation offspring Nos. 1~3 derived from an egg exposed to 90 rads of neutrons was mated with females Nos. 10~12. In three matings Nos. 1~3, 36.3%, 77.6% and 86.2%, 67.8% on the average, of the respective total number of eggs cleaved normally. Some of the normally cleaved eggs died afterwards of various abnormalities; 29.0%, 57.7% and 62.3%, 50.5% on the average, hatched normally, and 24.2%, 51.9% and 57.7%, 45.4% on the average, metamorphosed normally.

iii) N♀×{N♀×(EN-130♀×N♂)♂}♂, Nos. 1 and 2

Each of two male second-generation offspring Nos. 1 and 2 derived from an egg exposed to 130 rads of neutrons was mated with females Nos. 10~12. In two matings Nos. 1 and 2, 89.9% and 59.0%, 71.1% on the average, of the respective total number of eggs cleaved normally. A small number of normally cleaved eggs died of various abnormalities, such as edema, bicephaly, microcephaly or curvature of the body; 68.1% and 43.8%, 56.1% on the average, hatched normally. After the hatching stage, a few tadpoles died of edema; 67.1% and 39.5%, 53.5% on the average, became normal, metamorphosed frogs.

3. Chromosome aberrations

Chromosomes were observed in 10~25 normally shaped 40-day-old tadpoles obtained from one mating in each of the following eight experimental and the control series. When a metaphase spread had ten large and 16 small chromosomes, it was called normal for the time being, without observing the constitution of each chromosome in detail. In the control series, no chromosome aberrations were found in each of 25 tadpoles that were analyzable in karyotype.

a. Experimental series from X-irradiated sperm

i) N♀×{N♀×(N♀×SX-90♂)♂}♂, No. 3

Twenty tadpoles removed from the third-generation offspring that were produced from mating No. 3 of this experimental series were analyzable in karyotype. Seventeen of them were normal diploids. One of the other three was a mosaic of normal diploid, hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses. In these hyper- and hypodiploid mitoses, a small chromosome was added to or lost from the diploid complement. Another tadpole was an abnormal diploid having a large chromosome with a deletion and a ring chromosome. The remaining tadpole was also an abnormal diploid having a chromosome with a deletion.

ii) N♀×{N♀×(N♀×SX-170♂)♂}♂, No. 1

Ten tadpoles removed from the third-generation offspring that were produced from mating No. 1 of this experimental series were analyzed. Seven of them were normal diploids, while a tadpole was a triploid having a ring chromosome, another was an abnormal diploid having a chromosome with a deletion and the remainder was a hypodiploid having a dicentric chromosome.

b. Experimental series from X-irradiated eggs

i) N♀ × {N♀ × (EX–90♀ × N♂) ♂} ♂, No. 2

Eighteen tadpoles removed from the third-generation offspring that were produced from mating No. 2 of this experimental series were analyzed. Fifteen of them were normal diploids. One of the other three was a mosaic of hyper-diploid (2n+1) and hypodiploid (2n−1) mitoses. Another was a hyperdiploid having an additional small chromosome. The remaining tadpole was a mosaic of hypertriploid (3n+1) and hypotriploid (3n−1) mitoses.

ii) N♀ × {N♀ × (EX–200♀ × N♂) ♂} ♂, No. 2

Twenty-five tadpoles removed from the third-generation offspring that were produced from mating No. 2 of this experimental series were analyzed. Nineteen of them were normal diploids. Another was a triploid. Two others were abnormal diploids; one had a dicentric chromosome and a fragment, while the other had a chromosome with a deletion. The remaining three tadpoles were mosaics; one consisted of diploid and triploid mitoses, another of normal and hyperdiploid (2n+1) mitoses, and the remainder of normal diploid, hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses. In these hyperdiploid and hypodiploid mitoses, a small chromosome was added to or lost from the diploid complement.

c. Experimental series from neutron-irradiated sperm

i) N♀ × {N♀ × (N♀ × SN–50♂) ♂} ♂, No. 1

Eighteen tadpoles removed from the third-generation offspring that were produced from mating No. 1 of this experimental series were analyzed. Sixteen of them were normal diploids. One of the remaining two was a mosaic of normally and abnormally diploid mitoses. The latter mitoses contained a ring chromosome. The other tadpole was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses.

ii) N♀ × {N♀ × (N♀ × SN–130♂) ♂} ♂, No. 6

Twenty-one tadpoles removed from the third-generation offspring that were produced from mating No. 6 of this experimental series were analyzed. Sixteen of them were normal diploids. Four of the other five tadpoles were abnormal diploids; two had a chromosome with a deletion, another had a ring chromosome and the remainder had a dicentric chromosome and a fragment. A tadpole was a hyperdiploid having an additional small chromosome.

d. Experimental series from neutron-irradiated eggs

i) N♀ × {N♀ × (EN–50♀ × N♂) ♂} ♂, No. 3

Twenty-five tadpoles removed from the third-generation offspring that were produced from mating No. 3 of this experimental series were analyzed. Twenty-three of them were normal diploids. Another was a mosaic of haploid and triploid mitoses. The remainder was an abnormal diploid having a small chromosome in which the short arm was lost.

ii) N♀ × {N♀ × (EN–90♀ × N♂)♂}♂, No. 3

Thirteen tadpoles removed from the third-generation offspring that were produced from mating No. 3 of this experimental series were analyzed. Eleven of them were normal diploids. Another was a triploid, while the remainder was an abnormal diploid having a ring chromosome.

4. Viability and sex of metamorphosed frogs

In the control series, 472 tadpoles produced from three matings climbed out of water at the age of 80～96 days, 90.2 days on the average (Table 29). In the 12 experimental series, a total of 4139 tadpoles produced from 46 matings climbed out of water at the age of 78～116 days, 91.5～93.4 days on the

TABLE 29

Number, size and sex of metamorphosed frogs derived

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed frogs	Body length immediately after metamorphosis (mm)	
Female	Male				No. of frogs
N.W71, Nos. 1～12	N(N·N), Nos. 1～3	80～96 (90.2)	472	19.7±0.1	415
N.W71, Nos. 1～3	N(N·SX–90), Nos. 1～3	81～102 (92.3)	343	20.3±0.1	291
	N(N·SX–170), Nos. 1, 2	80～100 (91.5)	219	19.4±0.2	170
	N(N·SX–240), No. 1	80～101 (92.6)	84	20.1±0.4	42
N.W71, Nos. 4～6	N(EX–90·N), Nos. 1～3	81～113 (93.4)	204	19.5±0.2	162
	N(EX–145·N), Nos. 1～6	81～109 (93.1)	696	19.0±0.2	477
	N(EX–200·N), Nos. 1～11	80～105 (92.5)	840	19.1±0.2	708
N.W71, Nos. 7～9	N(N·SN–50), Nos. 1, 2	80～106 (92.0)	287	19.3±0.3	216
	N(N·SN–90), Nos. 1～3	79～110 (92.3)	247	20.2±0.2	203
	N(N·SN–130), Nos. 1～6	79～116 (93.0)	563	19.3±0.2	521
N.W71, Nos. 10～12	N(EN–50·N), Nos. 1～4	81～105 (92.2)	242	19.6±0.2	210
	N(EN–90·N), Nos. 1～3	78～111 (92.7)	186	19.2±0.2	129
	N(EN–130·N), Nos. 1, 2	81～107 (91.9)	228	20.0±0.2	196

♀$_{N}$—Females with normal ovaries ♀$_{U}$—Females with underdeveloped ovaries average of each experimental series. Thus, the tadpoles in the experimental series were a little retarded in metamorphosis as a whole, as compared with the controls. However, there was no distinct difference in the mean age of tadpoles at the time of climbing out of water among the 12 experimental series, which were different from one another in the kind of gametes irradiated or the kind and amount of irradiation.

In the control series, 50 frogs were at random removed from the offspring that were produced from each of the three matings immediately after completion of metamorphosis. A total of 150 frogs were 19.7 ± 0.1 mm in body length. In the experimental series, 50 frogs were also at random removed from the third-generation offspring that were produced from each mating immediately after completion of metamorphosis and measured. When the frogs produced from a mating were less than 50 in number, all the frogs were measured. The frogs measured in each experimental series were $19.0 \pm 0.2 \sim 20.3 \pm 0.1$ mm in body length. There was no remarkable difference in body length between the frogs of

from irradiated great-grandparental sperm or eggs, I

Sex of frogs killed about one month after metamorphosis					No. of frogs removed and reared	Sex of 2-year-old mature frogs			Sex of all frogs examined		
♀$_N$	♀$_U$	⚥	♂$_N$	♂ (%)*		No. of frogs	♀	♂	Total	♀	♂ (%)*
204	7	12	192	(49.2)	30	26	12	14	441	223	218 (49.4)
104	24	38	125	(56.0)	30	11	5	6	302	133	169 (56.0)
71	12	18	69	(51.2)	30	14	7	7	184	90	94 (51.1)
16	3	3	20	(54.8)	30	8	3	5	50	22	28 (56.0)
73	7	12	70	(50.6)	30	10	5	5	172	85	87 (50.6)
207	31	26	213	(50.1)	30	14	7	7	491	245	246 (50.1)
305	22	15	366	(53.8)	30	8	3	5	716	330	386 (53.9)
88	14	10	104	(52.8)	30	20	9	11	236	111	125 (53.0)
82	9	13	99	(55.2)	30	4	1	3	207	92	115 (55.6)
210	17	21	273	(56.4)	30	8	3	5	529	230	299 (56.5)
74	15	6	115	(57.6)	30	8	2	6	218	91	127 (58.3)
42	12	14	61	(58.1)	30	9	3	6	138	57	81 (58.7)
75	21	9	91	(51.0)	30	12	5	7	208	101	107 (51.4)

⚥ —Hermaphrodites ♂$_N$—Males with normal testes * Including hermaphrodites the experimental and the control series. There was also no definite interrelation between the kind or amount of irradiation, or the kind of gametes irradiated and the body length of frogs.

A small number of normally metamorphosed frogs in the 12 experimental and the control series died within one month after metamorphosis.

a. Control series

N♀ × {N♀ × (N♀ × N♂)♂}♂, Nos. 1~3

Of 472 metamorphosed frogs, 445 (94.3%) were living one month after metamorphosis. Thirty frogs were removed from the offspring produced from mating No. 3 and reared continuously, as these offspring appeared most viable among those from three matings. All the other 415 frogs were killed to examine their sex (Table 29). It was found that 211 were females, 12 hermaphrodites and 192 males. When the hermaphrodites were counted as males, 49.2% of the total number of frogs were males. Of the 30 frogs left alive, four died and 26 were living at the age of two years; 12 were females and 14 males.

b. Experimental series from X-irradiated sperm

i) N♀ × {N♀ × (N♀ × SX–90♂)♂}♂, Nos. 1~3

Twenty-two of 343 metamorphosed frogs died and 321 (93.6%) were living one month after metamorphosis. Thirty of the living frogs produced from mating No. 3 were left alive, and the other 291 were killed to examine their sex (Table 29). Of these frogs, 128 were females, 38 hermaphrodites and 125 males. When the hermaphrodites were counted as males, 56.0% were males. Eleven of the 30 frogs left alive were living at the age of two years, while 19 died. Five of the 11 frogs were females and six males.

ii) N♀ × {N♀ × (N♀ × SX–170♂)♂}♂, Nos. 1 and 2

One month after metamorphosis, 200 (91.3%) of 219 metamorphosed frogs were living, while the other 19 died. As the offspring produced from mating No. 2 were better in viability than those from No. 1, 30 of them were left alive. The other 170 frogs were all killed to examine their sex. Of these frogs, 83 were females, 18 hermaphrodites and 69 males. When the hermaphrodites were counted as males, 51.2% were males. At the age of two years, 14 of the 30 frogs left alive were still living; seven were females and seven males.

iii) N♀ × {N♀ × (N♀ × SX–240♂)♂}♂, No. 1

Of 84 metamorphosed frogs, 12 died and 72 (85.7%) were living one month after metamorphosis. Thirty of the latter were left alive, while the other 42 were killed to examine their sex. Of these frogs, 19 were females, three hermaphrodites and 20 males. When the hermaphrodites were counted as males, 54.8% were males. Only eight of the 30 frogs left alive were living at the age of two years; three were females and five males.

c. Experimental series from X-irradiated eggs

i) N♀ × {N♀ × (EX–90♀ × N♂)♂}♂, Nos. 1~3

Of 204 metamorphosed frogs, 192 (94.1%) were living one month after metamorphosis. Thirty of the offspring produced from mating No. 2 were left alive and reared, while all the other 162 were killed to examine their sex (Table 29). Of the latter, 80 were females, 12 hermaphrodites and 70 males. When the hermaphrodites were counted as males, 50.6% were males. Ten of the 30 frogs left alive were living at the age of two years; five were females and five males.

ii) N♀ × {N♀ × (EX–145♀ × N♂) ♂} ♂, Nos. 1 ~ 6

Of 696 metamorphosed frogs, 189 died and the other 507 (72.8%) were living one month after metamorphosis. Thirty of the offspring produced from mating No. 3 were left alive, while all the other 477 were killed to examine their sex. Of the latter, 238 were females, 26 hermaphrodites and 213 males. When the hermaphrodites were counted as males, 50.1% were males. Of the 30 frogs left alive, 14 were living at the age of two years; seven were females and seven males.

iii) N♀ × {N♀ × (EX–200♀ × N♂) ♂} ♂, Nos. 1 ~ 11

Of 840 metamorphosed frogs, 738 (87.9%) were living one month after metamorphosis, although 62 of them had abnormal forelegs. Thirty normally shaped frogs of the offspring produced from mating No. 2 were left alive, while all the other 708 were killed to examine their sex. Of the killed frogs, 327 were females, 15 hermaphrodites and 366 males. When the hermaphrodites were counted as males, 53.8% were males. Only eight of the 30 frogs left alive were living at the age of two years. Three of the eight frogs were females and five males.

d. Experimental series from neutron-irradiated sperm

i) N♀ × {N♀ × (N♀ × SN–50♂) ♂} ♂, Nos. 1 and 2

Of 287 metamorphosed frogs, 246 (85.7%) were living one month after metamorphosis. Thirty of the offspring produced from mating No. 1 were left alive, while the other 216 were killed to examine their sex (Table 29). Of the latter frogs, 102 were females, ten hermaphrodites and 104 males. When the hermaphrodites were counted as males, 52.8% were males. Twenty of the 30 frogs left alive were living at the age of two years; nine were females and 11 males.

ii) N♀ × {N♀ × (N♀ × SN–90♂) ♂} ♂, Nos. 1 ~ 3

Of 247 metamorphosed frogs, 233 (94.3%) were living one month after metamorphosis. Thirty of the offspring produced from mating No. 1 were left alive, while all the other 203 frogs were killed to examine their sex. Of these killed frogs, 91 were females, 13 hermaphrodites and 99 males. When the hermaphrodites were counted as males, 55.2% were males. Only four of the 30 frogs left alive were living at the age of two years; one was a female and three males.

iii) N♀ × {N♀ × (N♀ × SN–130♂) ♂} ♂, Nos. 1 ~ 6

Of 563 metamorphosed frogs, 551 (97.9%) were living one month after metamorphosis. As the offspring produced from mating No. 5 were the most vigorous among those from the six matings, 30 of them were left alive, while all the other 521 frogs were killed to examine their sex. Of the latter, 227 were females, 21 hermaphrodites and 273 males. When the hermaphrodites were counted as males, 56.4% were males. Only eight of the 30 frogs left alive were living at the age of two years; three were females and five males.

e. Experimental series from neutron-irradiated eggs

i) N♀ × {N♀ × (EN–50♀ × N♂) ♂} ♂, Nos. 1 ∼ 4

Only two of 242 metamorphosed frogs died, while the other 240 (99.2%) were living one month after metamorphosis. Thirty of the offspring produced from mating No. 3 were left alive and reared. All the other 210 frogs were killed to examine their sex (Table 29). As a result, 89 were females, six hermaphrodites and 115 males. When the hermaphrodites were counted as males, 57.6% were males. Only eight of the 30 frogs left alive were living at the age of two years; two were females and six males.

ii) N♀ × {N♀ × (EN–90♀ × N♂) ♂} ♂, Nos. 1 ∼ 3

Of 186 metamorphosed frogs, 159 (85.5%) were living one month after metamorphosis. Thirty of the offspring produced from mating No. 3 were left alive and reared. All the other 129 frogs were killed to examine their sex. Of these frogs, 54 were females, 14 hermaphrodites and 61 males. When the hermaphrodites were counted as males, 58.1% were males. Only nine of the 30 frogs left alive were living at the age of two years; three were females and six males.

iii) N♀ × {N♀ × (EN–130♀ × N♂) ♂} ♂, Nos. 1 and 2

Only two of 228 metamorphosed frogs died, while the other 226 (99.1%) were living one month after metamorphosis. Thirty of the offspring produced from mating No. 1 were left alive and reared. All the other 196 frogs were killed to examine their sex. Of these frogs, 96 were females, nine hermaphrodites and 91 males. When the hermaphrodites were counted as males, 51.0% were males. Twelve of the 30 frogs left alive were living at the age of two years; five were females, seven males.

V. Third-generation offspring derived from irradiated gametes by passing over male first-generation and female second-generation offspring

1. Female parents

As female second-generation offspring derived from eggs or spermatozoa irradiated with X-rays or neutrons matured in the breeding season of 1972, they were mated by artificial insemination with three normal males collected from the field to produce the third-generation offspring (Tables 30 ∼ 34). These females were produced in 1969 from male first-generation offspring by mating with normal females collected from the field. As the control of the female second-generation offspring, six females produced from the mating No. 4 of the control series in 1969 were used.

The three normal males Nos. 1 ∼ 3 used in 1972 were 65.0 ∼ 70.0 mm in body length. Their testes were 5.0 × 3.0 ∼ 5.5 × 3.5 mm in length and width and

TABLE 30

Testes of three field-caught male frogs used for mating experiments in 1972

Kind	Individual no.	Body length (mm)	Size of the testes		Inner structure
			Left (mm)	Right (mm)	
N.W72	1	65.0	5.0×3.0	5.0×3.0	Type 1
	2	68.5	5.5×3.5	5.5×3.5	Type 1
	3	70.0	5.5×3.0	5.0×3.0	Type 1

quite normal in inner structure (Table 30). The six control females Nos. 1～6, three years old, were 62.5～67.0 mm in body length. Five of them spawned after pituitary injection. Each female laid 1059～1991 eggs, 1537.0 on the average. In order to calculate the mean diameter of the eggs of each female, 100 eggs were taken out at random and measured for convenience' sake. The eggs of each female were found to be $1.77 \pm 0.02 \sim 2.01 \pm 0.02$ mm in diameter.

A total of 92 female second-generation offspring belonged to four groups each

TABLE 31

Eggs of eighteen female frogs derived from X-irradiated grandparental sperm and those of six controls used for mating experiments in 1972

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 100 eggs (mm)
N(N·N), No. 4	1	3	64.0	1892	1.77±0.02
	2	3	62.5	1278	1.91±0.02
	3	3	65.0	1465	1.92±0.02
	4	3	62.5	1059	2.01±0.02
	5	3	67.0	1991	1.89±0.02
	6	3	66.0	0	—
N(N·SX-90), No. 4	1	3	62.0	762	1.92±0.02
	2	3	65.5	679	1.79±0.03
	3	3	62.5	551	2.03±0.04
	4	3	65.0	211	1.74±0.04
	5	3	61.0	326	1.94±0.03
	6	3	64.0	121	1.69±0.02
	7	3	60.0	0	—
	8	3	62.5	0	—
N(N·SX-170), No. 8	1	3	64.0	1231	1.70±0.02
	2	3	61.5	1497	1.67±0.02
	3	3	65.5	521	1.92±0.04
	4	3	61.5	329	1.88±0.03
	5	3	67.0	673	1.84±0.04
	6	3	67.5	129	1.93±0.03
	7	3	61.0	0	—
N(N·SX-240), No. 4	1	3	65.0	537	1.78±0.03
	2	3	63.5	426	1.89±0.03
	3	3	62.0	0	—

N(N·N), No. 4: Females obtained by N.W69♀, Nos. 1～10×N.W69♂, No. 4
N(N·SX-90), No. 4: Females obtained by N.W69♀, Nos. 1～3×(N·SX-90)♂, No. 4
N(N·SX-170), No. 8: Females obtained by N.W69♀, Nos. 4～7×(N·SX-170)♂, No. 8
N(N·SX-240), No. 4: Females obtained by N.W69♀, Nos. 8～10×(N·SX-240)♂, No. 4

of which consisted of three experimental series. All of them were three years old and 60.0~70.0 mm, 64.8 mm on the average, in body length. After injection of frog pituitaries, 68 of them spawned; each female laid 120~1952 eggs, 650.6 on the average and the mean diameter of 100 eggs was 1.63±0.02~2.04 ±0.02 mm (Tables 31~34). Eight of 18 females belonging to the first group were produced from mating No. 4 of the experimental series derived from spermatozoa exposed to 90 rads of X-rays (Table 31). They were 60.0~65.5 mm, 62.8 mm on the average, in body length. Ovulation occurred in six (Nos. 1~6) of the eight females after pituitary injection. Each female laid 121~762 eggs, 441.7 on the average, and the mean diameter of 100 eggs was 1.69±0.02~2.03 ±0.04 mm. Seven other females were produced from mating No. 8 of the experimental series derived from spermatozoa exposed to 170 rads of X-rays. They were 61.0~67.5 mm, 64.0 mm on the average, in body length. Six (Nos. 1~6) of them spawned after pituitary injection. Each female laid 129~1497

TABLE 32

Eggs of twenty-six female frogs derived from X-irradiated grandparental eggs and used for mating experiments in 1972

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 100 eggs (mm)
N(EX-90·N), No. 10	1	3	67.0	652	1.71±0.02
	2	3	66.5	790	1.83±0.04
	3	3	61.5	418	1.85±0.03
	4	3	61.5	575	1.82±0.03
	5	3	63.0	667	2.01±0.02
	6	3	63.5	319	1.84±0.03
N(EX-145·N), No. 11	1	3	60.0	938	1.83±0.02
	2	3	67.0	546	1.75±0.03
	3	3	65.5	794	1.91±0.03
	4	3	64.0	264	1.71±0.02
	5	3	66.0	415	2.02±0.02
	6	3	61.0	350	1.94±0.03
	7	3	61.5	537	1.68±0.02
	8	3	60.0	712	1.73±0.03
	9	3	62.0	0	—
	10	3	68.0	0	—
N(EX-200·N), No. 3	1	3	61.0	629	1.78±0.03
	2	3	65.5	927	1.92±0.02
	3	3	63.0	1052	1.84±0.03
	4	3	65.0	481	1.74±0.04
	5	3	65.0	802	1.83±0.03
	6	3	67.0	120	1.76±0.03
	7	3	64.5	160	1.87±0.04
	8	3	66.5	0	—
	9	3	67.0	0	—
	10	3	60.0	0	—

N(EX-90·N), No. 10: Females obtained by N.W69♀, Nos. 1~3×(EX-90·N)♂, No. 10
N(EX-145·N), No. 11: Females obtained by N.W69♀, Nos. 4~7×(EX-145·N)♂, No. 11
N(EX-200·N), No. 3: Females obtained by N.W69♀, Nos. 8~10×(EX-200·N)♂, No. 3

eggs, 730.0 on the average, and the mean diameter of 100 eggs was 1.67±0.02~1.93±0.03 mm. The remaining three females were produced from mating No. 4 of the experimental series derived from spermatozoa exposed to 240 rads of X-rays. They were 62.0~65.0 mm, 63.5 mm on the average, in body length. Two of them ovulated after pituitary injection and laid 537 and 426 eggs, 481.5 on the average; 100 eggs of each female were 1.78±0.03 mm or 1.89±0.03 mm in the mean diameter.

Six of 26 females belonging to the second group were produced from mating No. 10 of the experimental series derived from eggs exposed to 90 rads of X-rays (Table 32). They were 61.5~67.0 mm, 63.8 mm on the average, in body length and laid 319~790 eggs, 570.2 on the average, after pituitary injection. The mean diameter of 100 eggs was 1.71±0.02~2.01±0.02 mm. Ten other females were produced from mating No. 11 of the experimental series derived from eggs exposed to 145 rads of X-rays. They were 60.0~68.0 mm, 63.5 mm on the average, in body length. After pituitary injection, eight of them laid 264~938 eggs, 569.5 on the average, which were 1.68±0.02~2.02±0.02 mm in mean diameter. The remaining ten females were produced from mating No. 3 of the experimental

TABLE 33

Eggs of twenty-three female frogs derived from neutron-irradiated grandparental sperm and used for mating experiments in 1972

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 100 eggs (mm)
N(N·SN–50), No. 3	1	3	62.5	703	1.92±0.02
	2	3	66.0	695	1.94±0.03
	3	3	65.0	990	1.98±0.02
	4	3	60.5	927	1.84±0.03
	5	3	67.0	1244	1.69±0.02
	6	3	65.5	621	1.74±0.04
	7	3	65.5	0	—
	8	3	63.5	0	—
N(N·SN–90), No. 4	1	3	69.0	1070	1.81±0.02
	2	3	64.5	639	1.93±0.03
	3	3	68.5	937	2.01±0.02
	4	3	65.0	582	1.94±0.03
	5	3	67.5	0	—
	6	3	65.5	0	—
	7	3	62.0	0	—
N(N·SN–130), No. 4	1	3	66.0	1420	1.76±0.03
	2	3	67.5	628	1.93±0.03
	3	3	67.0	575	1.87±0.03
	4	3	63.5	550	1.93±0.03
	5	3	68.5	982	2.04±0.02
	6	3	65.0	0	—
	7	3	67.0	0	—
	8	3	67.5	0	—

N(N·SN–50), No. 3: Females obtained by N.W69 ♀, Nos. 1~3 × (N·SN–50) ♂, No. 3
N(N·SN–90), No. 4: Females obtained by N.W69 ♀, Nos. 4~7 × (N·SN–90) ♂, No. 4
N(N·SN–130), No. 4: Females obtained by N.W69 ♀, Nos. 8~10 × (N·SN–130) ♂, No. 4

series derived from eggs exposed to 200 rads of X-rays. They were 60.0 ~ 67.0 mm, 64.5 mm on the average, in body length. Seven of them laid 120 ~ 1052 eggs, 595.9 on the average, after pituitary injection; these eggs were 1.74±0.04 ~ 1.92±0.02 mm in mean diameter.

Eight of 23 females belonging to the third group were produced from mating No. 3 of the experimental series derived from spermatozoa exposed to 50 rads of neutrons (Table 33). They were 60.5 ~ 67.0 mm, 64.4 mm on the average, in body length. After pituitary injection, six of them laid 621 ~ 1244 eggs, 863.3 on the average. The mean diameter of 100 eggs was 1.69±0.02 ~ 1.98±0.02 mm. Seven other females were produced from mating No. 4 of the experimental series derived from spermatozoa exposed to 90 rads of neutrons. They were 62.0 ~ 69.0 mm, 66.0 mm on the average, in body length. Four of them laid 582 ~ 1070 eggs, 807.0 on the average, after pituitary injection; the mean diameter of 100 eggs was 1.81±0.02 ~ 2.01±0.02 mm. The remaining eight females were produced from mating No. 4 of the experimental series derived from spermotozoa exposed

TABLE 34

Eggs of twenty-five female frogs derived from neutron-irradiated grandparental eggs and used for mating experiments in 1972

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 100 eggs (mm)
N(EN–50·N), No. 1	1	3	65.0	436	1.76±0.03
	2	3	64.5	362	1.91±0.02
	3	3	67.0	591	1.99±0.02
	4	3	69.0	957	1.93±0.03
	5	3	67.0	564	1.91±0.03
	6	3	68.5	230	1.79±0.03
	7	3	65.0	349	1.86±0.03
	8	3	67.0	208	1.94±0.04
	9	3	65.5	0	—
	10	3	68.0	0	—
N(EN–90·N), No. 8	1	3	70.0	1952	1.92±0.02
	2	3	67.0	650	1.83±0.03
	3	3	63.0	592	1.79±0.02
	4	3	65.0	0	—
	5	3	64.5	0	—
N(EN–130·N), No. 7	1	3	65.5	1030	1.85±0.03
	2	3	67.5	473	1.83±0.03
	3	3	63.0	552	1.74±0.03
	4	3	68.5	975	1.82±0.02
	5	3	62.0	561	1.81±0.02
	6	3	65.0	559	1.63±0.02
	7	3	63.5	496	1.99±0.03
	8	3	64.0	0	—
	9	3	66.5	0	—
	10	3	67.5	0	—

N(EN–50·N), No. 1: Females obtained by N.W69♀, Nos. 1 ~ 3 × (EN–50·N)♂, No. 1
N(EN–90·N), No. 8: Females obtained by N.W69♀, Nos. 4 ~ 7 × (EN–90·N)♂, No. 8
N(EN–130·N), No. 7: Females obtained by N.W69♀, Nos. 8 ~ 10 × (EN–130·N)♂, No. 7

to 130 rads of neutrons. They were 63.5 ~ 68.5 mm, 66.5 mm on the average, in body length. After pituitary injection, five of them laid 550 ~ 1420 eggs, 831.0 on the average, which were $1.76 \pm 0.03 \sim 2.04 \pm 0.02$ mm in mean diameter.

Ten of 25 females belonging to the fourth group were produced from mating No. 1 of the experimental series derived from eggs exposed to 50 rads of neutrons. They were 64.5 ~ 69.0 mm, 66.7 mm on the average, in body length (Table 34). After pituitary injection, eight of them laid 208 ~ 957 eggs, 462.1 on the average; the mean diameter of 100 eggs was $1.76 \pm 0.03 \sim 1.99 \pm 0.02$ mm. Five others of the 25 females were produced from mating No. 8 of the experimental series derived from eggs exposed to 90 rads of neutrons. Ovulation occurred in three of them by pituitary injection. They were 63.0 ~ 70.0 mm, 65.9 mm on the average, in body length. These frogs laid 592 ~ 1952 eggs, 1064.7 on the average, which were $1.79 \pm 0.02 \sim 1.92 \pm 0.02$ mm in mean diameter. The remaining ten females were produced from mating No. 7 of the experimental series derived from eggs exposed to 130 rads of neutrons. They were 62.0 ~ 68.5 mm, 65.3 mm on the average, in body length. Seven of them laid 473 ~ 1030 eggs, 663.7 on the average, after pituitary injection. These eggs were $1.63 \pm 0.02 \sim 1.99 \pm 0.03$ mm in mean diameter.

2. Developmental capacity

a. Control series

{N♀ × (N♀ × N♂) ♂} ♀ × N♂, Nos. 1 ~ 5

Each of the five control females Nos. 1 ~ 5 whose ovulation was accelerated by pituitary injection was mated by artificial insemination with three normal males Nos. 1 ~ 3 collected from the field (Table 35, Fig. 20). In the five matings Nos. 1 ~ 5, 86.5 ~ 93.0% of the respective total number of eggs or an average of 90.1% cleaved normally. While a few percent of the normally cleaved eggs died of various abnormalities at various embryonic stages, 80.1 ~ 89.4%, 85.8% on the average, hatched normally. During the tadpole stage a few individuals also died; 76.9 ~ 85.8%, 81.3% on the average, metamorphosed normally.

b. Experimental series derived from X-irradiated sperm

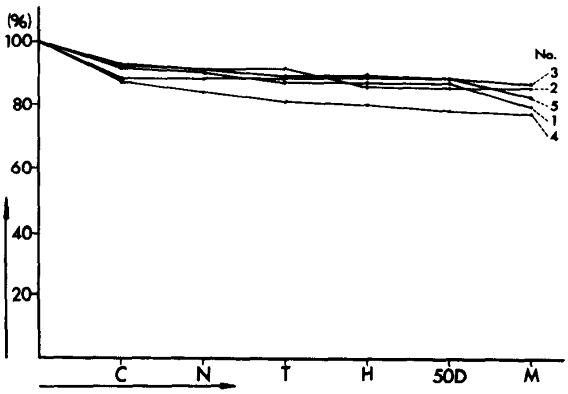
i) {N♀ × (N♀ × SX–90♂) ♂} ♀ × N♂, Nos. 1 ~ 5

Although ovulation occurred after pituitary injection in six second-generation offspring derived from a spermatozoon exposed to 90 rads of X-rays, five of these females were mated by artificial insemination with the same three normal males Nos. 1 ~ 3 as those used in the control series (Table 35, Fig. 21). In five matings Nos. 1 ~ 5, 27.6 ~ 89.0%, 69.3% on the average, of the respective total number of eggs cleaved normally. Some of the normally cleaved eggs gradually died of various abnormalities at various embryonic stages. Especially in mating No. 3, 102 of 145 embryos died simultaneously of edema at the tail-bud stage, although the eggs were highest in the percentage of normal cleavages. A comparatively large number of embryos produced from mating No. 4 died before the hatching stage. In the five matings, 20.7 ~ 80.0%, 48.6% on the average, hatched nor-

TABLE 35

Developmental capacity of the offspring of females

Parents		No. of eggs	No. of cleaved eggs	
Female	Male		Normal	Abnormal
N(N·N), Nos. 1∼5	N.W72, Nos. 1∼3	675	608 (90.1%)	4 (0.6%)
N(N·SX–90), Nos. 1∼5	N.W72, Nos. 1∼3	825	572 (69.3%)	13 (1.6%)
N(N·SX–170), Nos. 1∼5		797	567 (71.1%)	7 (0.9%)
N(N·SX–240), Nos. 1, 2		478	314 (65.7%)	0
N(EX–90·N), Nos. 1∼5	N.W72, Nos. 1∼3	783	587 (75.0%)	9 (1.1%)
N(EX–145·N), Nos. 1∼5		929	709 (76.3%)	13 (1.4%)
N(EX–200·N), Nos. 1∼5		850	715 (84.1%)	12 (1.4%)
N(N·SN–50), Nos. 1∼5	N.W72, Nos. 1∼3	867	697 (80.4%)	15 (1.7%)
N(N·SN–90), Nos. 1∼4		735	603 (82.0%)	7 (1.0%)
N(N·SN–130), Nos. 1∼5		846	650 (76.8%)	21 (2.5%)
N(EN–50·N), Nos. 1∼5	N.W72, Nos. 1∼3	683	472 (69.1%)	31 (4.5%)
N(EN–90·N), Nos. 1∼3		537	460 (85.7%)	34 (6.3%)
N(EN–130·N), Nos. 1∼5		877	644 (73.4%)	48 (5.5%)



C — Cleavage
N — Neurula stage
T — Tail-bud stage
H — Hatch
50D–Age of 50 days
M — Climbing out of water

Fig. 20. Survival curves of control third-generation offspring derived from untreated great-grandparental gametes by matings, {N♀ × (N♀ × N♂) ♂} ♀ × N♂, Nos. 1∼5.

derived from irradiated grandparental sperm or eggs, I

No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of 50-day-old tadpoles	No. of metamorphosed frogs
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal		
599	8	588	11	579	9	571	549
(88.7%)	(1.2%)	(87.1%)	(1.6%)	(85.8%)	(1.3%)	(84.6%)	(81.3%)
554	14	436	118	401	35	386	372
(67.2%)	(1.7%)	(52.8%)	(14.3%)	(48.6%)	(4.2%)	(46.8%)	(45.1%)
551	10	518	33	437	81	420	391
(69.1%)	(1.3%)	(65.0%)	(4.1%)	(54.8%)	(10.2%)	(52.7%)	(49.1%)
302	7	287	15	263	24	257	249
(63.2%)	(1.5%)	(60.0%)	(3.1%)	(55.0%)	(5.0%)	(53.8%)	(52.1%)
564	18	556	8	535	21	520	505
(72.0%)	(2.3%)	(71.0%)	(1.0%)	(68.3%)	(2.7%)	(66.4%)	(64.5%)
634	20	622	12	585	37	491	436
(68.2%)	(2.2%)	(67.0%)	(1.3%)	(63.0%)	(4.0%)	(52.9%)	(46.9%)
677	29	652	25	603	49	468	420
(79.6%)	(3.4%)	(76.7%)	(2.9%)	(70.9%)	(5.8%)	(55.1%)	(49.4%)
677	17	646	31	614	32	584	564
(78.1%)	(2.0%)	(74.5%)	(3.6%)	(70.8%)	(3.7%)	(67.4%)	(65.1%)
590	9	576	14	515	61	493	463
(80.3%)	(1.2%)	(78.4%)	(1.9%)	(70.1%)	(8.3%)	(67.1%)	(63.0%)
548	7	536	12	490	46	452	425
(64.8%)	(0.8%)	(63.4%)	(1.4%)	(57.9%)	(5.4%)	(53.4%)	(50.2%)
432	17	397	35	357	40	339	310
(63.3%)	(2.5%)	(58.1%)	(5.1%)	(52.3%)	(5.9%)	(49.6%)	(45.4%)
392	22	366	26	334	32	313	300
(73.0%)	(4.1%)	(68.2%)	(4.8%)	(62.2%)	(6.0%)	(58.3%)	(55.9%)
534	29	495	40	409	86	400	373
(60.9%)	(3.3%)	(56.4%)	(4.6%)	(46.6%)	(9.8%)	(45.6%)	(42.5%)

mally. During the tadpole stage some individuals died of edema or underdevelopment; 12.8～77.1%, 45.1% on the average, metamorphosed normally.

ii) {N♀ ×(N♀ ×SX–170♂)♂}♀ ×N♂, Nos. 1～5

Although six of seven female second-generation offspring derived from a spermatozoon exposed to 170 rads of X-rays ovulated by pituitary injection, five (Nos. 1～5) of them were mated with normal males Nos. 1～3. In matings Nos. 1～5, 22.5～93.4%, 71.1% on the average, of the respective total number of eggs cleaved normally. Comparatively many of the normally cleaved eggs in mating Nos. 2 and 5 died of edema or blisters during the stages from tail-bud to hatching, that is, 47 of 142 from No. 2 and 32 of 132 from No. 5 died during these stages. A few embryos died during these stages in each of the other matings. In the five matings, 18.1～83.6%, 54.8% on the average, hatched normally. After the hatching stage, some tadpoles died of various abnormalities; 11.3～82.0%, 49.1% on the average, became normally metamorphosed frogs.

iii) {N♀ ×(N♀ ×SX–240♂)♂}♀ ×N♂, Nos. 1 and 2

Two (Nos. 1 and 2) of three female second-generation offspring derived from a spermatozoon exposed to 240 rads of X-rays ovulated by pituitary injec-

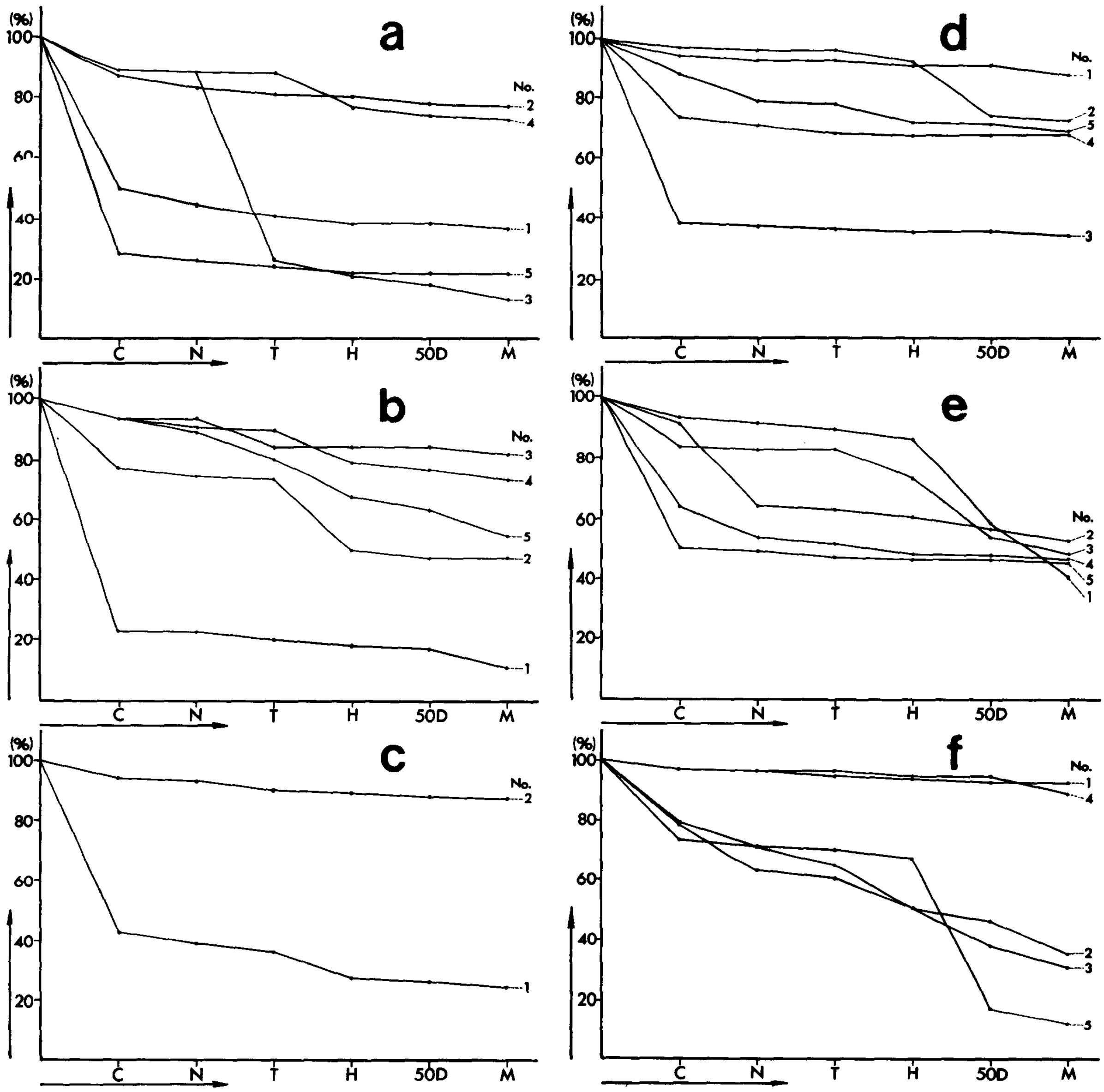


Fig. 21. Survival curves of third-generation offspring derived from X-irradiated great-grandparental gametes by passing over male first- and female second-generation offspring.

a. {N♀ × (N♀ × SX-90♂) ♂} ♀ × N♂, Nos. 1∼5
b. {N♀ × (N♀ × SX-170♂) ♂} ♀ × N♂, Nos. 1∼5
c. {N♀ × (N♀ × SX-240♂) ♂} ♀ × N♂, Nos. 1 and 2
d. {N♀ × (EX-90♀ × N♂) ♂} ♀ × N♂, Nos. 1∼5
e. {N♀ × (EX-145♀ × N♂) ♂} ♀ × N♂, Nos. 1∼5
f. {N♀ × (EX-200♀ × N♂) ♂} ♀ × N♂, Nos. 1∼5

C — Cleavage
N — Neurula stage
T — Tail-bud stage
H — Hatch
50D-Age of 50 days
M — Climbing out of water

tion. They were mated with normal males Nos. 1∼3. In two matings Nos. 1 and 2, 43.0% and 93.5%, 65.7% on the average, of the respective total number of eggs cleaved normally. Many of the normally cleaved eggs in mating No. 1 died of various abnormalities at various embryonic stages, while only a few eggs died at the same stages in mating No. 2; 27.4% and 88.8%, 55.0% on the average, hatched normally in the two matings. During the tadpole stage, some individuals died of edema or underdevelopment; eventually, 23.6% and 87.0%, 52.1% on the average, metamorphosed normally.

c. Experimental series derived from X-irradiated eggs

i) {N♀ × (EX–90♀ × N♂)♂}♀ × N♂, Nos. 1 ~ 5

All the six second-generation offspring derived from an egg exposed to 90 rads of X-rays ovulated by pituitary injection. Five (Nos. 1 ~ 5) of them were mated with normal males Nos. 1 ~ 3 (Table 35, Fig. 21). In five matings Nos. 1 ~ 5, 37.8 ~ 93.8%, 75.0% on the average, of the respective total number of eggs cleaved normally. Some of the normally cleaved eggs died gradually of various abnormalities at various embryonic stages; 34.9 ~ 90.3%, 68.3% on the average, hatched normally. While comparatively numerous tadpoles produced from mating No. 2 died of edema or underdevelopment at the early tadpole stage, only a few tadpoles from the other matings died at the same stage. Eventually, 33.7 ~ 86.8%, 64.5% on the average, became normal, metamorphosed frogs.

ii) {N♀ × (EX–145♀ × N♂)♂}♀ × N♂, Nos. 1 ~ 5

Ovulation occurred by pituitary injection in eight of ten female second-generation offspring that derived from an egg exposed to 145 rads of X-rays. Five of them were mated with normal males Nos. 1 ~ 3. In five matings Nos. 1 ~ 5, 50.0 ~ 92.6%, 76.3% on the average, of the respective total number of eggs cleaved normally. Many of the normally cleaved eggs in matings Nos. 2 and 4 died of incomplete invagination at the gastrula stage. After this stage, comparatively numerous embryos produced from No. 3 died of edema at the hatching stage. A small number of normally cleaved eggs in the other two matings Nos. 1 and 5 died also of various abnormalities at various embryonic stages. In the five matings, 46.2 ~ 85.7%, 63.0% on the average, hatched normally. After the hatching stage, many tadpoles produced from matings Nos. 1 and 3 died of edema or underdevelopment, while nearly all the tadpoles from Nos. 4 and 5 completed metamorphosis. In the five matings, 41.1 ~ 53.1%, 46.9% on the average, became normal, metamorphosed frogs.

iii) {N♀ × (EX–200♀ × N♂)♂}♀ × N♂, Nos. 1 ~ 5

Ovulation occurred by pituitary injection in seven of ten female second-generation offspring derived from an egg exposed to 200 rads of X-rays. Five (Nos. 1 ~ 5) of them were mated with normal males Nos. 1 ~ 3. In the five matings, 73.0 ~ 97.1%, 84.1% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs in matings Nos. 1 and 4 did not differ in developmental capacity from the controls; 92.9% and 94.1% hatched normally, and afterwards 91.7% and 89.4% metamorphosed normally, respectively. In the other matings, most of the normally cleaved eggs died before the stage of metamorphosis. Especially in Nos. 2 and 3, they died gradually of various abnormalities at various embryonic and tadpole stages; 50.0% and 50.3% hatched normally and 34.7% and 30.2% became normal, metamorphosed frogs, respectively. Although the normally cleaved eggs from No. 5 developed almost normally until the hatching stage, most of them died of underdevelopment at the feeding tadpole stage. Immediately before metamorphosis, many tadpoles died of edema, too. Eventually, 50.0~94.1%, 70.9% on the average, hatched normally, and 11.4~91.7%, 49.4% on the average, metamorphosed normally in the five matings.

d. Experimental series derived from neutron-irradiated sperm

i) {N♀ × (N♀ × SN-50♂)♂}♀ × N♂, Nos. 1~5

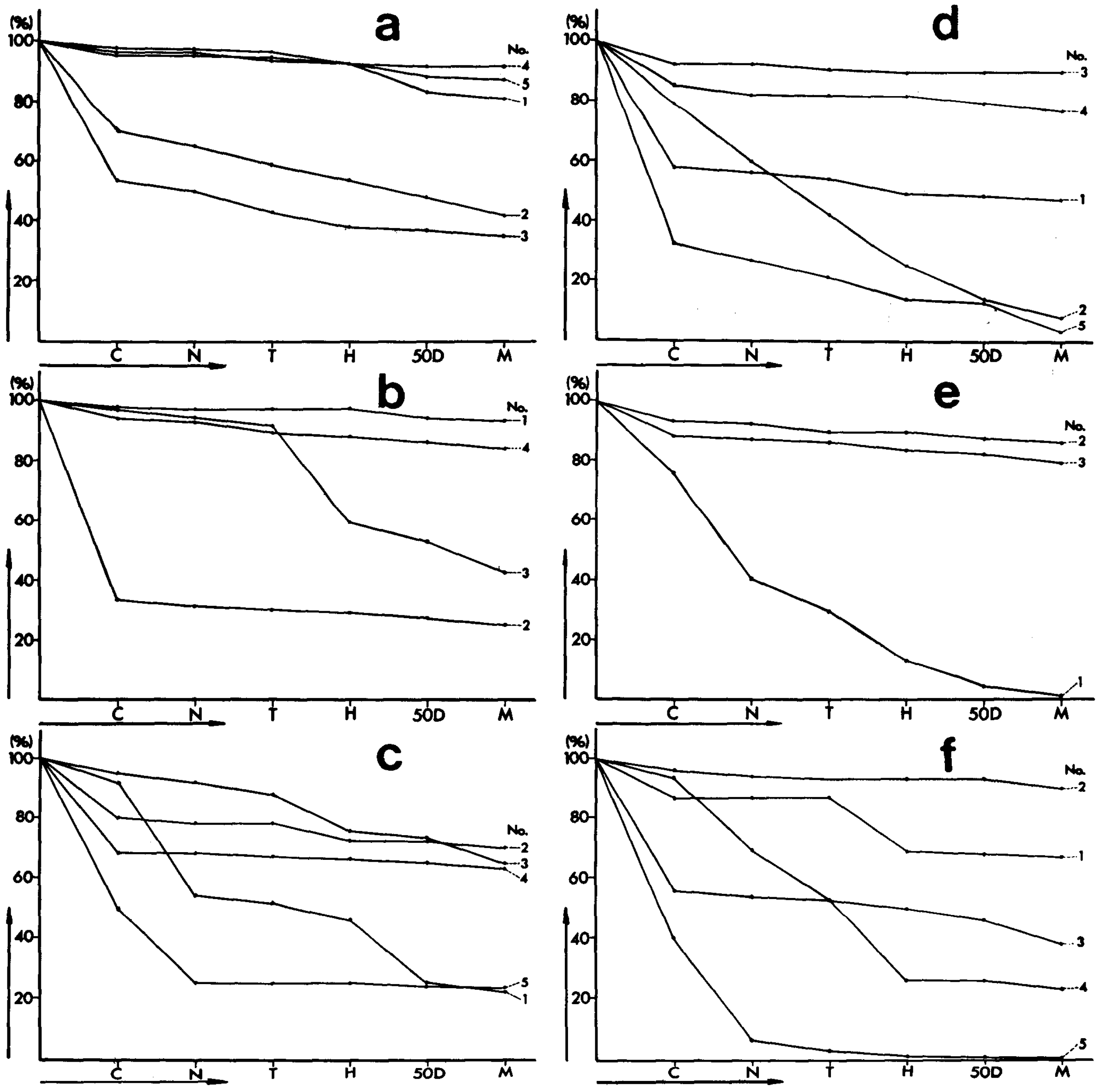


Fig. 22. Survival curves of third-generation offspring derived from neutron-irradiated great-grand-parental gametes by passing over male first- and female second-generation offspring.

a. {N♀ × (N♀ × SN-50♂)♂} ♀ × N♂, Nos. 1~5 C — Cleavage
b. {N♀ × (N♀ × SN-90♂)♂} ♀ × N♂, Nos. 1~4 N — Neurula stage
c. {N♀ × (N♀ × SN-130♂)♂} ♀ × N♂, Nos. 1~5 T — Tail-bud stage
d. {N♀ × (EN-50♀ × N♂)♂} ♀ × N♂, Nos. 1~5 H — Hatch
e. {N♀ × (EN-90♀ × N♂)♂} ♀ × N♂, Nos. 1~3 50D–Age of 50 days
f. {N♀ × (EN-130♀ × N♂)♂} ♀ × N♂, Nos. 1~5 M — Climbing out of water

Although six of eight female second-generation offspring derived from a spermatozoon exposed to 50 rads of neutrons ovulated by pituitary injection, five females Nos. 1～5 were mated with normal males Nos. 1～3 (Table 35, Fig. 22). In matings Nos. 1～5, 53.7～97.4%, 80.4% on the average, of the respective total number of eggs cleaved normally. Especially in Nos. 1, 4 and 5, more than 95% cleaved normally. The normally cleaved eggs in these matings were normal in developmental capacity; 91.6～91.7% hatched and 81.1～90.6% metamorphosed normally. In the other two matings Nos. 2 and 3, many of the normally cleaved eggs died gradually of various abnormalities at embryonic and tadpole stages. Eventually, 38.0～91.7%, 70.8% on the average, hatched normally, and 35.2～90.6%, 65.1% on the average, became normal, metamorphosed frogs in the five matings.

ii) {N♀ × (N♀ × SN–90♂)♂}♀ × N♂, Nos. 1～4

As four (Nos. 1～4) of seven female second-generation offspring derived from a spermatozoon exposed to 90 rads of neutrons ovulated by pituitary injection, they were mated with normal males Nos. 1～3. In three (Nos. 1, 3 and 4) of four matings, 97.5%, 97.1% and 93.8% of the respective total number of eggs cleaved normally, while 32.9% did in the other mating No. 2. In these four matings, 82.0% on the average cleaved normally. The normally cleaved eggs in matings Nos. 1, 2 and 4 did not differ in developmental capacity from the controls. In mating No. 3, about one-third of the embryos died about the same time of edema at the hatching stage, and moreover, many individuals died of edema during the tadpole stage. In the four matings, 28.7～96.5%, 70.1% on the average, hatched normally, and 25.0～92.6%, 63.0% on the average, became normal, metamorphosed frogs.

iii) {N♀ × (N♀ × SN–130♂)♂}♀ × N♂, Nos. 1～5

Five (Nos. 1～5) of eight female second-generation offspring derived from a spermatozoon exposed to 130 rads of neutrons were mated with normal males Nos. 1～3, as they ovulated by pituitary injection. In five matings Nos. 1～5, 49.5～94.7%, 76.8% on the average, of the respective total number of eggs cleaved normally. A large number of normally cleaved eggs, that is, 51 of 124 in mating No. 1 and 42 of 85 in No. 5 died of incomplete invagination at the gastrula stage. Many embryos produced from the matings Nos. 1～3 became edematous and died at the hatching stage. In the five matings, 25.0～75.2%, 57.9% on the average, hatched normally. After the hatching stage, about half the number of tadpoles produced from mating No. 1 died of edema. Eventually, 22.2～69.5%, 50.2% on the average, became normal, metamorphosed frogs in the five matings.

e. Experimental series derived from neutron-irradiated eggs

i) {N♀ × (EN–50♀ × N♂)♂}♀ × N♂, Nos. 1～5

Eight of ten female second-generation offspring derived from an egg exposed to 50 rads of neutrons ovulated by pituitary injection. Five (Nos. 1～5) of them were mated with normal males Nos. 1～3 (Table 35, Fig. 22). In five matings

Nos. 1 ~ 5, 32.7 ~ 91.8%, 69.1% on the average, of the respective total number of eggs cleaved normally. Almost all the normally cleaved eggs in mating No. 3 developed normally and attained the completion of metamorphosis. In matings Nos. 1 and 4, about one-tenth of the normally cleaved eggs died gradually of various abnormalities at embryonic and tadpole stages. In mating No. 2, a large number of normally cleaved eggs died of incomplete invagination at the gastrula stage, while a smaller number of them in No. 5 died of the same abnormality. Many embryos produced from these two matings (Nos. 2 and 5) died at the late embryonic stage; comparatively numerous ones died simultaneously at the hatching stage. Moreover, 7 of 17 tadpoles in No. 2 and 16 of 20 in No. 5 had underdeveloped forelegs. Eventually, 14.0 ~ 89.0%, 52.3% on the average, hatched normally, and 2.7 ~ 89.0%, 45.4% on the average, became normally metamorphosed frogs in the five matings.

ii) {N♀ × (EN–90♀ × N♂)♂}♀ × N♂, Nos. 1 ~ 3

As three of five female second-generation offspring derived from an egg exposed to 90 rads of neutrons ovulated by pituitary injection, they were mated with normal males Nos. 1 ~ 3. In three matings Nos. 1 ~ 3, 76.0 ~ 92.9%, 85.7% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs in matings Nos. 2 and 3 were almost normal in developmental capacity until the completion of metamorphosis, while those in No. 1 were mostly very abnormal, that is, 63 of 133 eggs died of incomplete invagination at the gastrula stage, and afterwards almost all the remainders died of edema before the hatching stage. In the three matings, 13.1%, 88.8% and 83.4%, 62.2% on the average, hatched normally and 1.1%, 85.8% and 79.3%, 55.9% on the average, metamorphosed normally.

iii) {N♀ × (EN–130♀ × N♂)♂}♀ × N♂, Nos. 1 ~ 5

Seven of ten female second-generation offspring derived from an egg exposed to 130 rads of neutrons ovulated by pituitary injection. Five (Nos. 1 ~ 5) of them were mated with normal males Nos. 1 ~ 3. In five matings Nos. 1 ~ 5, 40.2 ~ 96.3%, 73.4% on the average, cleaved normally. The normally cleaved eggs in mating No. 2 alone were normal in developmental capacity until the completion of metamorphosis. In mating No. 1, 30 of 145 embryos died simultaneously of edema. In No. 3, a small number of embryos died gradually of edema and blisters before the hatching stage; about one-sixth of metamorphosing tadpoles had underdeveloped forelegs. In No. 4, most of the normally cleaved eggs died of incomplete invagination at the gastrula stage as well as of edema at the neurula, tail-bud and hatching stages. In No. 5, 61 of 72 normally cleaved eggs died of incomplete invagination at the gastrula stage. Eventually, 1.1 ~ 92.6%, 46.6% on the average, hatched normally, and 0.6 ~ 90.1%, 42.5% on the average, became normal, metamorphosed frogs in the five matings.

3. Viability and sex of metamorphosed frogs

In the control series, 549 tadpoles produced from the five matings climbed out of water at the age of 72 ~ 95 days, 78.7 days on the average (Table 36). In the 12 experimental series a total of 4808 frogs produced from 54 matings climbed out of water at the age of 80 ~ 108 days, 87.4 ~ 95.3 days on the average of each experimental series. Thus, the tadpoles in the experimental series were somewhat retarded in metamorphosis as a whole, as compared with the controls. There were no definite differences in the average age of tadpoles at the time of climbing out of water among the 12 experimental series which differed from one another in the kind of irradiated gametes or the kind or amount of irradiation.

In the control series, the body length of all the frogs from the five matings was measured immediately after the completion of metamorphosis (Table 36). A total of 549 frogs were 19.3 ± 0.1 mm in mean body length. In the 12 experimental series, all the third-generation offspring were also measured; they were $18.4 \pm 0.2 \sim 19.5 \pm 0.2$ mm in the mean body length of each experimental series. There were no remarkable differences in the body length of frogs between the experimental and control series or between different experimental series.

About one month after metamorphosis, all the living frogs were killed to examine their sex (Table 36).

a. Control series

{N♀ × (N♀ × N♂)♂}♀ × N♂, Nos. 1 ~ 5

Of 549 normally metamorphosed frogs, 20 died within one month after metamorphosis. The other 529 were killed; 266 were females, 14 hermaphrodites and 249 males. When the hermaphrodites were counted as males, 49.7% were males.

b. Experimental series derived from X-irradiated sperm

i) {N♀ × (N♀ × SX–90♂)♂}♀ × N♂, Nos. 1 ~ 5

Twenty-one of 372 normally metamorphosed frogs died within one month after metamorphosis. The other 351 frogs were killed to examine their sex; 173 were females, 36 hermaphrodites and 142 males. When the hermaphrodites were counted as males, 50.7% were males.

ii) {N♀ × (N♀ × SX–170♂)♂}♀ × N♂, Nos. 1 ~ 5

Seventeen of 391 normally metamorphosed frogs died within one month after metamorphosis. The other 374 frogs were killed; 188 were females, 33 hermaphrodites and 153 males. When the hermaphrodites were counted as males, 49.7% were males.

iii) {N♀ × (N♀ × SX–240♂)♂}♀ × N♂, Nos. 1 and 2

Of 249 normally metamorphosed frogs, 33 died within one month after metamorphosis. The other 216 frogs were killed; 110 were females, nine hermaphrodites and 97 males. When the hermaphrodites were counted as males, 49.1% were males.

c. Experimental series derived from X-irradiated eggs

i) {N♀ × (EX–90♀ × N♂)♂}♀ × N♂, Nos. 1 ~ 5

Twenty-two of 505 normally metamorphosed frogs died within one month after metamorphosis. The other 483 frogs were killed; 253 were females, 62

TABLE 36

Number, size and sex of metamorphosed frogs derived

Parents: Female	Parents: Male	Age at the time of climbing out of water (days)	No. of metamorphosed frogs
N(N·N), Nos. 1～5	N.W72, Nos. 1～3	72～95 (78.7)	549
N(N·SX–90), Nos. 1～5	N.W72, Nos. 1～3	83～101 (89.3)	372
N(N·SX–170), Nos. 1～5		83～108 (87.4)	391
N(N·SX–240), Nos. 1, 2		85～94 (88.6)	249
N(EX–90·N), Nos. 1～5	N.W72, Nos. 1～3	85～94 (86.7)	505
N(EX–145·N), Nos. 1～5		89～104 (93.1)	436
N(EX–200·N), Nos. 1～5		86～101 (92.4)	420
N(N·SN–50), Nos. 1～5	N.W72, Nos. 1～3	81～101 (90.1)	564
N(N·SN–90), Nos. 1～4		88～105 (92.0)	463
N(N·SN–130), Nos. 1～5		83～92 (87.9)	425
N(EN–50·N), Nos. 1～5	N.W72, Nos. 1～3	80～98 (85.9)	310
N(EN–90·N), Nos. 1～3		83～101 (89.5)	300
N(EN–130·N), Nos. 1～5		90～102 (95.3)	373

♀$_N$—Females with normal ovaries ♀$_U$—Females with underdeveloped ovaries

hermaphrodites and 168 males. When the hermaphrodites were counted as males, 47.6% were males.

ii) {N♀ × (EX–145♀ × N♂) ♂} ♀ × N♂, Nos. 1～5

Twenty-four of 436 normally metamorphosed frogs died within one month after metamorphosis. The other 412 frogs were killed; 191 were females, 27 hermaphrodites and 194 males. When the hermaphrodites were counted as males, 53.6% were males.

iii) {N♀ × (EX–200♀ × N♂) ♂} ♀ × N♂, Nos. 1～5

Of 420 normally metamorphosed frogs, 45 died within one month after metamorphosis. The other 375 frogs were killed; 185 were females, 37 hermaphrodites and 153 males. When the hermaphrodites were counted as males, 50.7% were males.

d. Experimental series derived from neutron-irradiated sperm

i) {N♀ × (N♀ × SN–50♂) ♂} ♀ × N♂, Nos. 1～5

Of 564 normally metamorphosed frogs, 42 died within one month after from irradiated great-grandparental sperm or eggs, II

Body length immediately after metamorphosis (mm)	Sex of frogs killed about one month after metamorphosis					
	No. of frogs	♀N	♀U	⚥	♂N	♂ (%)*
19.3±0.1	529	253	13	14	249	(49.7)
18.7±0.1	351	144	29	36	142	(50.7)
19.2±0.1	374	166	22	33	153	(49.7)
19.5±0.2	216	99	11	9	97	(49.1)
18.8±0.2	483	217	36	62	168	(47.6)
19.1±0.1	412	175	16	27	194	(53.6)
18.9±0.2	375	161	24	37	153	(50.7)
19.0±0.2	522	236	24	35	227	(50.2)
19.1±0.2	411	174	20	17	200	(52.8)
18.4±0.2	402	177	15	21	189	(52.2)
18.7±0.2	302	133	14	12	143	(51.3)
18.5±0.2	293	122	26	23	122	(49.5)
18.9±0.2	347	127	20	27	173	(57.6)

⚥—Hermaphrodites ♂N—Males with normal testes * Including hermaphrodites

metamorphosis. The other 522 frogs were killed; 260 were females, 35 hermaphrodites and 227 males. When the hermaphrodites were counted as males, 50.2% were males.

ii) {N♀×(N♀×SN–90♂)♂}♀×N♂, Nos. 1∼4

Of 463 normally metamorphosed frogs, 52 died within one month after metamorphosis. The other 411 frogs were killed; 194 were females, 17 hermaphrodites and 200 males. When the hermaphrodites were counted as males, 52.8% were males.

iii) {N♀×(N♀×SN–130♂)♂}♀×N♂, Nos. 1∼5

Twenty-three of 425 normally metamorphosed frogs died within one month after metamorphosis. The other 402 frogs were killed; 192 were females, 21 hermaphrodites and 189 males. When the hermaphrodites were counted as males, 52.2% were males.

e. Experimental series derived from neutron-irradiated eggs

i) {N♀×(EN–50♀×N♂)♂}♀×N♂, Nos. 1∼5

Only eight of 310 normally metamorphosed frogs died within one month after metamorphosis. The other 302 frogs were killed; 147 were females, 12 hermaphrodites and 143 males. When the hermaphrodites were counted as males, 51.3% were males.

ii) {N♀ × (EN–90♀ × N♂) ♂} ♀ × N♂, Nos. 1 ~ 3

Only seven of 300 normally metamorphosed frogs died within one month after metamorphosis. The other 293 frogs were killed; 148 were females, 23 hermaphrodites and 122 males. When the hermaphrodites were counted as males, 49.5% were males.

iii) {N♀ × (EN–130♀ × N♂) ♂} ♀ × N♂, Nos. 1 ~ 5

Of 373 normally metamorphosed frogs, 26 died within one month after metamorphosis. The other 347 frogs were killed; 147 were females, 27 hermaphrodites and 173 males. When the hermaphrodites were counted as males, 57.6% were males.

VI. Third-generation offspring derived from irradiated gametes by passing over female first-generation and male second-generation offspring

1. Male parents

Male second-generation offspring produced from female first-generation offspring by mating with normal males collected from the field in 1970 matured in the breeding season of 1972 (Tables 37 ~ 40). The female first-generation offspring were produced from irradiated spermatozoa or eggs by fertilization with gametes of normal males or females collected from the field. The male second-generation offspring were mated by artificial insemination with normal females collected from the field (Table 41).

Five normal females Nos. 1 ~ 5 were used for producing third-generation offspring. They were 61.0 ~ 67.0 mm, 64.8 mm on the average, in body length and laid 2055 ~ 2400 eggs after pituitary injection (Table 37). The eggs were 1.67 ± 0.01 ~ 1.89 ± 0.01 mm in mean diameter when 50 eggs taken out at random from each female were measured.

In the control series, five males Nos. 1 ~ 5 produced from mating No. 1 were 50.5 ~ 53.0 mm, 51.8 mm on the average, in body length (Table 38). Their

TABLE 37

Eggs of five field-caught female frogs used for mating experiments in 1972

Kind	Individual no.	Body length (mm)	No. of eggs	Mean diameter of 50 eggs (mm)
N.W72	1	67.0	2345	1.70 ± 0.01
	2	65.5	2400	1.69 ± 0.01
	3	65.5	2236	1.77 ± 0.01
	4	65.0	2314	1.67 ± 0.01
	5	61.0	2055	1.89 ± 0.01

TABLE 38

Testes of five control male frogs used for mating experiments in 1972

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure
				Left (mm)	Right (mm)	
(N·N)N, No. 1	1	2	50.5	5.0×2.5	4.5×3.0	Type 1
	2	2	51.5	4.5×3.0	4.5×2.5	Type 1
	3	2	51.5	5.0×2.5	5.0×2.5	Type 1
	4	2	53.0	5.0×2.5	4.5×2.5	Type 1
	5	2	52.5	5.0×2.5	5.0×2.5	Type 1

(N·N)N, No. 1: Males obtained by (N·N) ♀, No. 1×N.W70 ♂, Nos. 1∼5

TABLE 39

Testes of twenty-seven male frogs derived from X-irradiated grandparental sperm or eggs and used for mating experiments in 1972

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure
				Left (mm)	Right (mm)	
(N·SX–90)N, No. 2	1	2	52.5	4.0×2.0	4.5×2.5	Type 2
	2	2	51.0	3.5×3.0	4.5×2.0	Type 3
	3	2	47.0	4.0×2.0	3.0×2.0	Type 2
	4	2	48.5	5.0×2.5	4.5×2.5	Type 1
	5	2	53.0	4.0×2.5	4.0×2.5	Type 3
(N·SX–170)N, No. 4	1	2	51.5	4.5×2.5	4.5×2.5	Type 3
	2	2	50.0	5.5×3.0	4.0×3.0	Type 2
	3	2	49.5	5.0×2.5	5.0×3.0	Type 3
	4	2	49.0	1.5×1.0	7.5×3.5	Type 1
	5	2	53.0	4.0×2.0	4.0×2.0	Type 2
(N·SX–240)N, No. 1	1	2	52.0	4.0×2.5	4.0×2.5	Type 1
	2	2	48.5	7.5×3.0	—	Type 3
	3	2	49.5	5.0×2.0	4.0×2.5	Type 3
	4	2	51.0	5.0×3.0	1.5×1.0	Type 2
	5	2	52.0	4.5×2.5	4.5×2.5	Type 2
	6	2	49.0	8.0×3.5	7.0×4.0	Type 5
(EX–90·N)N, No. 6	1	2	55.0	5.5×2.0	4.5×2.5	Type 3
	2	2	53.0	4.5×2.5	4.5×2.5	Type 3
	3	2	52.5	5.5×3.0	3.0×1.5	Type 2
	4	2	47.5	7.0×3.0	—	Type 1
	5	2	49.0	6.0×3.0	1.5×1.0	Type 1
(EX–145·N)N, No. 4	1	2	51.5	4.0×2.0	4.0×2.0	Type 1
	2	2	50.5	4.5×2.5	4.5×2.5	Type 1
	3	2	48.0	5.0×2.5	3.5×2.0	Type 1
	4	2	47.5	5.0×2.5	4.0×2.5	Type 1
	5	2	53.0	5.5×2.5	4.0×2.5	Type 2
(EX–200·N)N, No. 1	1	2	52.0	4.0×3.0	4.0×3.0	Type 2

(N·SX–90)N, No. 2: Males obtained by (N·SX–90) ♀, No. 2×N.W70 ♂, Nos. 1∼5
(N·SX–170)N, No. 4: Males obtained by (N·SX–170) ♀, No. 4×N.W70 ♂, Nos. 1∼5
(N·SX–240)N, No. 1: Males obtained by (N·SX–240) ♀, No. 1×N.W70 ♂, Nos. 1∼5
(EX–90·N)N, No. 6: Males obtained by (EX–90·N) ♀, No. 6×N.W70 ♂, Nos. 1∼5
(EX–145·N)N, No. 4: Males obtained by (EX–145·N) ♀, No. 4×N.W70 ♂, Nos. 1∼5
(EX–200·N)N, No. 1: Male obtained by (EX–200·N) ♀, No. 1×N.W70 ♂, Nos. 1∼5

testes were 4.5 × 2.5 ~ 4.5 × 3.0 mm in length and width and quite normal in inner structure; the seminal tubules were filled with close bundles of spermatozoa.

In the experimental series, 53 males were used for producing third-generation offspring. They were produced in 12 experimental series of four groups. All of them were two years old and 47.0 ~ 55.0 mm, 51.2 mm on the average, in body length (Tables 39 and 40). Five of 16 males belonging to the first group were produced from mating No. 2 of the experimental series derived from spermatozoa exposed to 90 rads of X-rays. They were 47.0 ~ 53.0 mm, 50.4 mm on the average, in body length (Table 39). Their testes were 3.0 × 2.0 ~ 5.0 × 2.5 mm in length and width. While a male was of type 1 in inner structure of the testes, two others were of type 2 and the remaining two were of type 3 (cf. p. 29). Five other males belonging to the first group were produced from mating No. 4 of the experimental series derived from spermatozoa exposed to 170 rads of X-rays. They were 49.0 ~ 53.0 mm, 50.6 mm on the average, in body length. The testes of four of these males were 4.0 × 2.0 ~ 5.5 × 3.0 mm in length and width, while those of the remainder were extremely asymmetric, the left being 1.5 × 1.0 and the right 7.5 × 3.5 mm in length and width, although they were of type 1 in inner structure. The testes of the other four males were of type 2 or 3. The remaining six males of the first group were produced from mating No. 1 of the experimental series that derived from spermatozoa exposed to 240 rads of X-rays. They were 48.5 ~ 52.0 mm, 50.3 mm on the average, in body length. The testes of four of them were 4.0 × 2.5 ~ 7.0 × 4.0 mm in length and width. Another male had only one testis which was 7.5 × 3.0 mm, while the remainder had remarkably asymmetric testes, the left being 5.0 × 3.0 mm and the right 1.5 × 1.0 mm. In inner structure of the testes, one of the six males was of type 1, two of type 2, two of type 3 and the last one of type 5.

Five of 11 males belonging to the second group were produced from mating No. 6 of the experimental series derived from eggs exposed to 90 rads of X-rays. They were 47.5 ~ 55.0 mm, 51.4 mm on the average, in body length. The testes of three males were 3.0 × 1.5 ~ 5.5 × 3.0 mm in length and width. Another male had only one testis which was 7.0 × 3.0 mm, while the remainder had remarkably asymmetric testes, the left being 6.0 × 3.0 mm and the right 1.5 × 1.0 mm. In inner structure of the testes, two males were of type 1, another of type 2 and the remaining two of type 3. Five other males of the second group were produced from mating No. 4 of the experimental series derived from eggs exposed to 145 rads of X-rays. They were 47.5 ~ 53.0 mm, 50.1 mm on the average, in body length. Their testes were 3.5 × 2.0 ~ 5.5 × 2.5 mm in length and width. In inner structure of the testes, four of the five males were of type 1, while the other was of type 2. The remaining male of the second group was produced from mating No. 1 of the experimental series that derived from eggs exposed to 200 rads of X-rays. This male was 52.0 mm in body length, and had testes which were 4.0 × 3.0 mm in length and width. These testes were of type 2 in inner structure.

Five of 11 males belonging to the third group were produced from mating No. 2 of the experimental series derived from spermatozoa exposed to 50 rads of neutrons. They were 47.0～53.5 mm, 51.5 mm on the average, in body length (Table 40). Their testes were 3.5×2.0～6.0×3.0 mm in length and width. While the testes of four males were of type 1 in inner structure, those of the other was of type 3. Another male of the third group was produced from mating No. 4 of the experimental series derived from spermatozoa exposed to 90 rads of neutrons. This frog was 51.0 mm in body length. The testes were 5.0×2.5 mm and 6.0×3.5 mm in length and width, and of type 2 in inner structure. The remaining five males of the third group were produced from mating No. 2 of the experimental series that derived from spermatozoa exposed to 130 rads of neutrons. They were 51.0～54.5 mm, 52.7 mm on the average, in body length. Their testes were 4.5×2.0～5.5×3.0 mm in length and width. In inner structure of

TABLE 40

Testes of twenty-six male frogs derived from neutron-irradiated grandparental sperm or eggs and used for mating experiments in 1972

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure
				Left (mm)	Right (mm)	
(N·SN–50)N, No. 2	1	2	53.0	4.5×3.0	4.0×2.5	Type 1
	2	2	53.5	5.0×2.5	5.0×3.0	Type 1
	3	2	51.5	4.0×2.0	3.5×2.0	Type 1
	4	2	52.5	5.0×2.5	5.0×2.0	Type 1
	5	2	47.0	5.5×3.0	6.0×3.0	Type 3
(N·SN–90)N, No. 4	1	2	51.0	5.0×2.5	6.0×3.5	Type 2
(N·SN–130)N, No. 2	1	2	53.5	5.5×3.0	5.5×3.0	Type 3
	2	2	54.5	4.5×2.5	4.5×2.5	Type 1
	3	2	53.0	5.0×2.5	4.0×2.5	Type 1
	4	2	51.0	5.0×2.5	5.0×2.5	Type 1
	5	2	51.5	4.5×2.0	4.0×2.5	Type 2
(EN–50·N)N, No. 2	1	2	51.0	4.5×2.0	4.5×2.0	Type 1
	2	2	53.5	5.0×2.5	5.0×2.5	Type 1
	3	2	51.0	4.5×2.0	4.5×2.5	Type 1
	4	2	52.5	4.0×2.0	4.0×2.0	Type 1
	5	2	51.5	5.5×3.0	4.5×3.0	Type 2
(EN–90·N)N, No. 3	1	2	52.0	5.0×3.0	5.0×2.5	Type 1
	2	2	51.0	5.0×2.5	5.0×2.5	Type 1
	3	2	52.5	4.0×2.5	4.0×2.5	Type 1
	4	2	54.0	6.0×3.0	5.5×2.5	Type 4
	5	2	50.5	5.5×3.0	5.0×2.5	Type 2
(EN–130·N)N, No. 1	1	2	47.5	4.0×2.5	4.5×3.0	Type 3
	2	2	53.0	5.0×3.0	5.0×3.0	Type 2
	3	2	51.5	5.0×2.5	5.0×3.0	Type 1
	4	2	49.0	4.5×2.5	4.0×2.5	Type 1
	5	2	48.0	4.0×2.0	4.0×2.5	Type 1

(N·SN–50)N, No. 2: Males obtained by (N·SN–50) ♀, No. 2×N.W70♂, Nos. 1～5
(N·SN–90)N, No. 4: Male obtained by (N·SN–90) ♀, No. 4×N.W70♂, Nos. 1～5
(N·SN–130)N, No. 2: Males obtained by (N·SN–130) ♀, No. 2×N.W70♂, Nos. 1～5
(EN–50·N)N, No. 2: Males obtained by (EN–50·N) ♀, No. 2×N.W70♂, Nos. 1～5
(EN–90·N)N, No. 3: Males obtained by (EN–90·N) ♀, No. 3×N.W70♂, Nos. 1～5
(EN–130·N)N, No. 1: Males obtained by (EN–130·N) ♀, No. 1×N.W70♂, Nos. 1～5

the testes, three were of type 1, one of type 2 and the remainder of type 3.

Five of 15 males belonging to the fourth group were produced from mating No. 2 of the experimental series derived from eggs exposed to 50 rads of neutrons. They were 51.0~53.5 mm, 51.9 mm on the average, in body length. Their testes were 4.0×2.0~5.5×3.0 mm in length and width. In inner structure of the testes, four males were of type 1, while the other was of type 2. Five other males of the fourth group were produced from mating No. 3 of the experimental series that derived from eggs exposed to 90 rads of neutrons. They were 50.5~54.0 mm, 52.0 mm on the average, in body length. Their testes were 4.0×2.5~6.0×3.0 mm in length and width. In inner structure of the testes, three males were of type 1, one of type 2 and the remainder of type 4. The remaining five males of the fourth group were produced from mating No. 1 of the experimental series derived from eggs exposed to 130 rads of neutrons. They were 47.5~53.0 mm, 49.8 mm on the average, in body length. Their testes were 4.0×2.0~5.0×3.0 mm in length and width. In inner structure of the testes, three males were of type 1, one of type 2 and the remainder of type 3.

TABLE 41

Developmental capacity of the offspring of male frogs

Parents		No. of eggs	No. of cleaved eggs	
Female	Male		Normal	Abnormal
N.W72, Nos. 1~5	(N·N)N, Nos. 1~5	470	453 (96.4%)	0
N.W72, Nos. 1~5	(N·SX-90)N, Nos. 1~5	431	224 (52.0%)	94 (21.8%)
	(N·SX-170)N, Nos. 1~5	355	201 (56.6%)	55 (15.5%)
	(N·SX-240)N, Nos. 1~5	408	171 (41.9%)	78 (19.1%)
N.W72, Nos. 1~5	(EX-90·N)N, Nos. 1~5	499	213 (42.7%)	34 (6.8%)
	(EX-145·N)N, Nos. 1~5	474	314 (66.2%)	62 (13.1%)
	(EX-200·N)N, No. 1	134	95 (70.9%)	10 (7.5%)
N.W72, Nos. 1~5	(N·SN-50)N, Nos. 1~5	467	326 (69.8%)	34 (7.3%)
	(N·SN-90)N, No. 1	170	95 (55.9%)	3 (1.8%)
	(N·SN-130)N, Nos. 1~5	592	426 (72.0%)	46 (7.8%)
N.W72, Nos. 1~5	(EN-50·N)N, Nos. 1~5	476	346 (72.7%)	35 (7.4%)
	(EN-90·N)N, Nos. 1~5	495	276 (55.8%)	26 (5.3%)
	(EN-130·N)N, Nos. 1~5	464	260 (56.0%)	23 (5.0%)

2. Developmental capacity

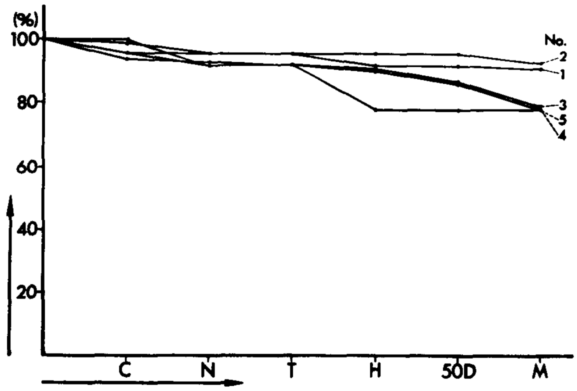
a. Control series

N♀ × {(N♀ × N♂)♀ × N♂}♂, Nos. 1～5

Five matings were made between five normal females Nos. 1～5 collected from the field and five male second-generation offspring Nos. 1～5 derived from a control frog used in 1967, to produce the third-generation offspring. In each of the matings, a female and a male of the same individual number were paired (Table 41, Fig. 23). In the five matings Nos. 1～5, 94.0～100%, 96.4% on the average, of the respective total number of eggs cleaved normally. Three, eleven, two and fifteen of 453 normally cleaved eggs died of various abnormalities at the gastrula, neurula, tail-bud and hatching stages, respectively; 78.4～96.2%, 89.8% on the average, hatched normally. During the tadpole stage, 38 individuals died of edema and 78.4～92.5%, 81.7% on the average, became normal, metamorphosed frogs.

derived from irradiated grandparental sperm or eggs, II

No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of 50-day-old tadpoles	No. of metamorphosed frogs
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal		
439 (93.4%)	11 (2.3%)	437 (93.0%)	2 (0.4%)	422 (89.8%)	15 (3.2%)	413 (87.9%)	384 (81.7%)
191 (44.3%)	22 (5.1%)	185 (42.9%)	5 (1.2%)	165 (38.3%)	20 (4.6%)	143 (33.2%)	91 (21.1%)
179 (50.4%)	11 (3.1%)	171 (48.2%)	8 (2.3%)	163 (45.9%)	8 (2.3%)	123 (34.6%)	83 (23.4%)
139 (34.1%)	8 (2.0%)	137 (33.6%)	2 (0.5%)	133 (32.6%)	4 (1.0%)	101 (24.8%)	67 (16.4%)
205 (41.1%)	8 (1.6%)	201 (40.3%)	4 (0.8%)	198 (39.7%)	3 (0.6%)	135 (27.1%)	100 (20.0%)
297 (62.7%)	17 (3.6%)	295 (62.2%)	2 (0.4%)	292 (61.6%)	3 (0.6%)	225 (47.5%)	178 (37.6%)
64 (47.8%)	20 (14.9%)	40 (29.9%)	24 (17.9%)	37 (27.6%)	3 (2.2%)	33 (24.6%)	31 (23.1%)
297 (63.6%)	18 (3.9%)	295 (63.2%)	2 (0.4%)	290 (62.1%)	5 (1.1%)	217 (46.5%)	184 (39.4%)
83 (48.8%)	9 (5.3%)	81 (47.6%)	2 (1.2%)	70 (41.2%)	11 (6.5%)	61 (35.9%)	57 (33.5%)
391 (66.0%)	11 (1.9%)	388 (65.5%)	3 (0.5%)	366 (61.8%)	22 (3.7%)	255 (43.1%)	209 (35.3%)
297 (62.4%)	17 (3.6%)	278 (58.4%)	19 (4.0%)	276 (58.0%)	2 (0.4%)	212 (44.5%)	149 (31.3%)
261 (52.7%)	13 (2.6%)	258 (52.1%)	3 (0.6%)	255 (51.5%)	3 (0.6%)	238 (48.1%)	223 (45.1%)
257 (55.4%)	2 (0.4%)	253 (54.5%)	4 (0.9%)	252 (54.3%)	1 (0.2%)	237 (51.1%)	188 (40.5%)



C —Cleavage
N —Neurula stage
T —Tail-bud stage
H —Hatch
50D–Age of 50 days
M —Climbing out of water

Fig. 23. Survival curves of control third-generation offspring derived from untreated great-grandparental gametes by matings, N♀ × {(N♀ × N♂) ♀ × N♂} ♂, Nos. 1∼5.

b. Experimental series derived from X-irradiated sperm

i) N♀ × {(N♀ × SX–90♂) ♀ × N♂} ♂, Nos. 1∼5

Five male second-generation offspring Nos. 1∼5 derived from a spermatozoon exposed to 90 rads of X-rays were mated with the same five normal females Nos. 1∼5 as those in the control series (Table 41, Fig. 24). In five matings Nos. 1∼5, 22.8∼72.6%, 52.0% on the average, of the respective total number of eggs cleaved normally. A small number of normally cleaved eggs died of various abnormalities, such as incomplete invagination at the gastrula stage or microcephaly, blisters, curvature of the body or edema at various embryonic stages; 17.8∼58.0%, 38.3% on the average, hatched normally. During the tadpole stage, more than half the number of individuals produced from each of matings Nos. 1, 3 and 5 died of edema or underdevelopment, while a very small number of tadpoles died of the same abnormalities in the remaining matings Nos. 2 and 4. Eventually, 12.2∼49.4%, 21.1% on the average, metamorphosed normally.

ii) N♀ × {(N♀ × SX–170♂) ♀ × N♂} ♂, Nos. 1∼5

Five male second-generation offspring Nos. 1∼5 derived from a spermatozoon exposed to 170 rads of X-rays were mated with normal females Nos. 1∼5. In five matings Nos. 1∼5, 47.5∼66.7%, 56.6% on the average, of the respective total number of eggs cleaved normally. Some of the normally cleaved eggs died of incomplete invagination at the gastrula stage or of various abnormalities such as blisters, edema, curvature of the body or microcephaly at the late embryonic stage; 35.6∼52.8%, 45.9% on the average, hatched normally. Numerous tadpole died of edema or underdevelopment before the completion of metamorphosis. In mating No. 1, more than half the number of tadpoles died of edema immediately before metamorphosis, while in matings Nos. 2, 4 and 5, many tadpoles died of underdevelopment or edema at various stages from the stage shortly after hatching to metamorphosis. Eventually, 20.3∼32.8%, 23.4% on the average, in the five matings became normally metamorphosed frogs.

iii) N♀ × {(N♀ × SX–240♂) ♀ × N♂} ♂, Nos. 1∼5

Although there were six male second-generation offspring derived from a

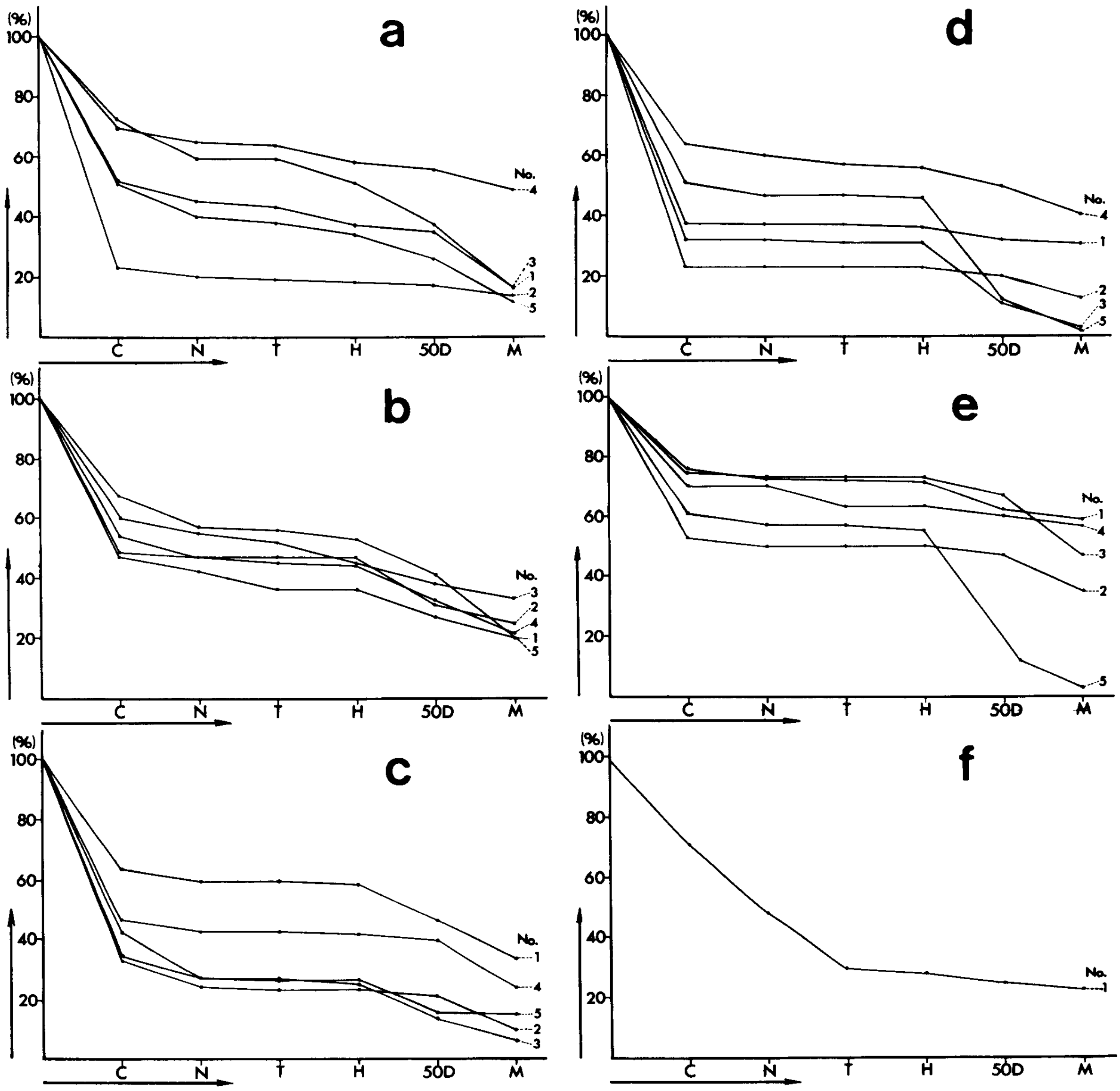


Fig. 24. Survival curves of third-generation offspring derived from X-irradiated great-grandparental gametes by passing over female first- and male second-generation offspring.

a. N♀ × {(N♀ × SX-90♂)♀ × N♂} ♂, Nos. 1~5	C —Cleavage
b. N♀ × {(N♀ × SX-170♂)♀ × N♂} ♂, Nos. 1~5	N —Neurula stage
c. N♀ × {(N♀ × SX-240♂)♀ × N♂} ♂, Nos. 1~5	T —Tail-bud stage
d. N♀ × {(EX-90♀ × N♂)♀ × N♂} ♂, Nos. 1~5	H —Hatch
e. N♀ × {(EX-145♀ × N♂)♀ × N♂} ♂, Nos. 1~5	50D–Age of 50 days
f. N♀ × {(EX-200♀ × N♂)♀ × N♂} ♂, No. 1	M —Climbing out of water

spermatozoon exposed to 240 rads of X-rays, five (Nos. 1~5) of them were mated with normal females Nos. 1~5. The remaining male was quite sterile; their testes were deprived of normal spermatozoa. In five matings Nos. 1~5, 32.5~62.7%, 41.9% on the average, of the respective total number of eggs cleaved normally. Some of the normally cleaved eggs died of incomplete invagination at the gastrula stage or of edema, blisters, microcephaly or curvature of the body at various embryonic stages; 22.9 ~ 57.6%, 32.6% on the average, hatched normally. Many tadpoles produced from matings Nos. 1, 3 and 5 died of edema or underdevelopment at the early tadpole stage. At the metamorphosis stage, many tadpoles from matings Nos. 1 ~ 4 also died of the same abnormalities; 5.6 ~ 33.9%, 16.4% on the average, became normal, metamorphosed frogs.

c. Experimental series derived from X-irradiated eggs

i) N♀ × {(EX–90♀ × N♂)♀ × N♂}♂, Nos. 1 ~ 5

Five male second-generation offspring Nos. 1 ~ 5 derived from an egg exposed to 90 rads of X-rays were mated with the same five normal females Nos. 1 ~ 5 as those used in the control series (Table 41, Fig. 24). In five matings Nos. 1 ~ 5, 22.6 ~ 63.6%, 42.7% on the average, of the respective total number of eggs cleaved normally. Of the normally cleaved eggs in four of the five matings, only a few died at various embryonic stages; 22.6 ~ 56.2%, 39.7% on the average, hatched normally in the five matings. However, many individuals died of underdevelopment after the hatching stage. Especially in matings Nos. 3 and 5, only a few tadpoles attained the metamorphosis stage. Eventually, 2.1 ~ 41.3%, 20.0% on the average, metamorphosed normally in the five matings.

ii) N♀ × {(EX–145♀ × N♂)♀ × N♂}♂, Nos. 1 ~ 5

Five male second-generation offspring Nos. 1 ~ 5 derived from an egg exposed to 145 rads of X-rays were mated with normal females Nos. 1 ~ 5. In five matings Nos. 1 ~ 5, 52.6 ~ 75.6%, 66.2% on the average, of the respective total number of eggs cleaved normally. A very small number of normally cleaved eggs died at various embryonic stages; 49.5 ~ 73.0%, 61.6% on the average, hatched normally. Many tadpoles produced from mating No. 1 died of edema at the early tadpole stage, while many from Nos. 2 and 3 became edematous at the metamorphosis stage and died by the completion of metamorphosis. Most of the tadpoles produced from mating No. 5 could not eat owing to ill-formation of the teeth, and consequently they died of underdevelopment. Eventually, 2.6 ~ 58.5%, 37.6% on the average, metamorphosed normally in the five matings.

iii) N♀ × {(EX–200♀ × N♂)♀ × N♂}♂, No. 1

A male second-generation offspring derived from an egg exposed to 200 rads of X-rays was mated with the same normal female No. 1 as that used in the control series. In this mating, 95 (70.9%) of 134 eggs cleaved normally. Seven normally cleaved eggs became partial blastulae, four died of incomplete invagination at the gastrula stage, and twenty, twenty-four and three died of edema, blisters or some other abnormalities at the neurula, tail-bud and hatching stages, respectively, while 37 (27.6%) hatched normally. Six individuals died of edema during the tadpole stage; 31 (23.1%) became normally metamorphosed frogs.

d. Experimental series derived from neutron-irradiated sperm

i) N♀ × {(N♀ × SN–50♂)♀ × N♂}♂, Nos. 1 ~ 5

Five male second-generation offspring Nos. 1 ~ 5 derived from a spermatozoon exposed to 50 rads of neutrons were mated with the same five normal females

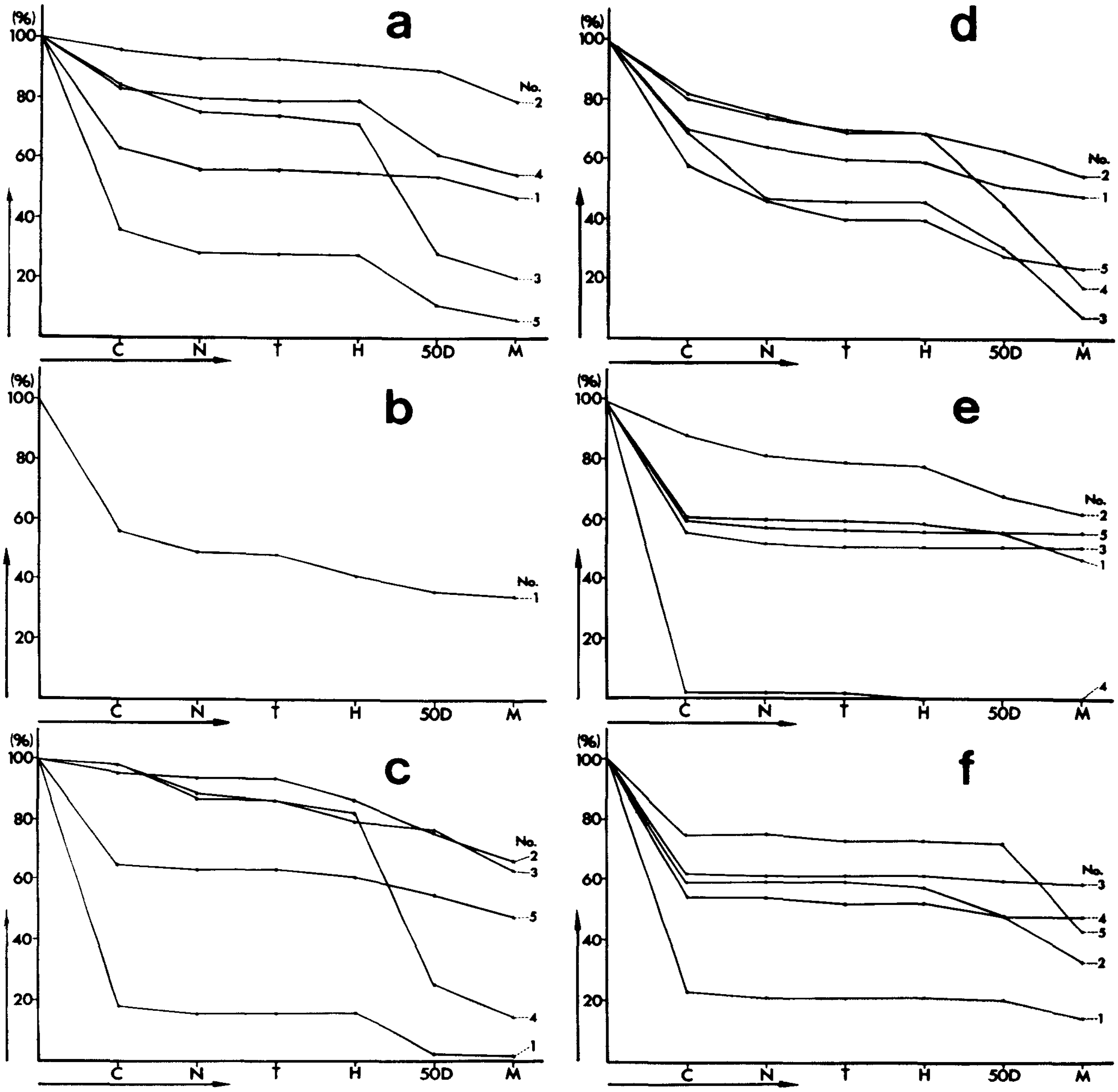


Fig. 25. Survival curves of third-generation offspring derived from neutron-irradiated great-grand-parental gametes by passing over female first- and male second-generation offspring.

a. N♀ × {(N♀ × SN–50♂)♀ × N♂} ♂, Nos. 1∼5
b. N♀ × {(N♀ × SN–90♂)♀ × N♂} ♂, No. 1
c. N♀ × {(N♀ × SN–130♂)♀ × N♂} ♂, Nos. 1∼5
d. N♀ × {(EN–50♀ × N♂)♀ × N♂} ♂, Nos. 1∼5
e. N♀ × {(EN–90♀ × N♂)♀ × N♂} ♂, Nos. 1∼5
f. N♀ × {(EN–130♀ × N♂)♀ × N♂} ♂, Nos. 1∼5

C —Cleavage
N —Neurula stage
T —Tail-bud stage
H —Hatch
50D–Age of 50 days
M —Climbing out of water

Nos. 1∼5 as those used in the control series (Table 41, Fig. 25). In five matings Nos. 1∼5, 63.3%, 96.3%, 83.8%, 83.2% and 36.0%, 69.8% on the average, of the respective total number of eggs cleaved normally. While a small number of normally cleaved eggs died of various abnormalities, 55.1%, 91.3%, 71.3%, 78.9% and 27.2%, 62.1% on the average, hatched normally. Many individuals died of underdevelopment or edema during the tadpole stage. Especially in mating No. 3, 35 of 57 tadpoles became edematous and died at the early tadpole stage. Six others died of edema shortly before metamorphosis, while 16 (20.0%) metamorphosed normally. In mating No. 4, 23 of 75 tadpoles became thin owing to ill-formation of the teeth and died; 52 (54.7%) metamorphosed normally. Of 31 tadpoles produced from mating No. 5, 24 died of edema during the tadpole stage; only 7 (6.1%) became normally metamorphosed frogs. In the other matings Nos. 1 and 2, 46 (46.9%) and 63 (78.8%) metamorphosed normally, respectively. On the average, 39.4% of the total number of eggs in the five matings became normally metamorphosed frogs.

ii) N♀×{(N♀×SN–90♂)♀×N♂}♂, No. 1

A male second-generation offspring derived from a spermatozoon exposed to 90 rads of neutrons was mated with the same normal female No. 1 as that used in the control series. In this mating, 95 (55.9%) of 170 eggs cleaved normally. Three and nine normally cleaved eggs died of abnormalities at the gastrula and neurula stage, respectively. Two and eleven died of edema at the tail-bud and hatching stage, respectively, while 70 (41.2%) hatched normally. Thirteen tadpoles died of edema or underdevelopment by the metamorphosis stage; 57 (33.5%) metamorphosed normally.

iii) N♀×{(N♀×SN–130♂)♀×N♂}♂, Nos. 1∼5

Five male second-generation offspring Nos. 1∼5 derived from a spermatozoon exposed to 130 rads of neutrons were mated with the same five normal females Nos. 1∼5 as those used in the control series. In five matings Nos. 1∼5, 16.7%, 95.3%, 98.3%, 97.7% and 65.0%, 72.0% on the average, of the respective total number of eggs cleaved normally. At various embryonic stages, a small number of normally cleaved eggs died of edema, blisters, microcephaly, curvature of the body or some other abnormalities; 14.5%, 85.9%, 79.2%, 82.2% and 60.0%, 61.8% on the average, hatched normally. Almost all the tadpoles produced from mating No. 1 hardly ate and gradually died of underdevelopment; only one (0.7%) became a normally metamorphosed frogs. A small number of tadpoles produced from No. 2 died of edema, while 56 (65.9%) metamorphosed normally. A small number of tadpoles produced from matings Nos. 3 and 5 became thin and gradually died owing to ill-formation of the teeth; 76 (63.3%) and 58 (48.3%) metamorphosed normally, respectively. Most of the tadpoles produced from No. 4 became edematous immediately after hatching; only 32 of 106 began to take food. Some of these tadpoles gradually died of underdevelopment, and eventually 18 (14.0%) metamorphosed normally. In the five matings, 35.3% of the total number of eggs became normal, metamorphosed frogs.

e. Experimental series derived from neutron-irradiated eggs

i) N♀×{(EN–50♀×N♂)♀×N♂}♂, Nos. 1∼5

Five male second-generation offspring Nos. 1∼5 derived from an egg exposed to 50 rads of neutrons were mated with the same five normal females Nos. 1∼5 as those used in the control series (Table 41, Fig. 25). In five matings Nos. 1∼5, 58.4∼82.0%, 72.7% on the average, cleaved normally. Some of the normally cleaved eggs died of various abnormalities, such as blisters and edema; 40.4∼

69.4%, 58.0% on the average, hatched normally. Many individuals died afterwards of underdevelopment. Especially in mating No. 3, more than half the number of tadpoles died of edema immediately before metamorphosis, while in No. 4 most tadpoles became thin and died owing to ill-formation of the teeth. Eventually, 7.8～55.1%, 31.3% on the average, metamorphosed normally in the five matings.

ii) N♀×{(EN–90♀×N♂)♀×N♂}♂, Nos. 1～5

Five male second-generation offspring Nos. 1～5 derived from an egg exposed to 90 rads of neutrons were mated with normal females Nos. 1～5. In four of five matings Nos. 1～5, 56.1～87.7% of the respective total number of eggs cleaved normally, while in the remaining No. 4, only 2.4% of the eggs did so. On the average in the five matings, 55.8% cleaved normally. All the normally cleaved eggs in mating No. 4 died before the hatching stage, while 51.0～78.3% hatched normally in the other four matings. The individuals produced from two matings Nos. 3 and 5 scarcely died during the period from the neurula stage to the completion of metamorphosis. In the four matings, 51.0～62.3% became normally metamorphosed frogs.

iii) N♀×{(EN–130♀×N♂)♀×N♂}♂, Nos. 1～5

Five male second-generation offspring Nos. 1～5 derived from an egg exposed to 130 rads of neutrons were mated with normal females Nos. 1～5. In five matings Nos. 1～5, 23.4～75.0%, 56.0% on the average, of the respective total number of eggs cleaved normally. A few of the normally cleaved eggs died of various abnormalities such as edema and blisters; 20.8～73.1%, 54.3% on the average, hatched normally. In matings Nos. 2 and 5, 14 of 42 and 30 of 75 tadpoles died of edema, immediately before or during metamorphosis, respectively. Eventually, 14.3～57.8%, 40.5% on the average, metamorphosed normally in the five matings.

3. Viability and sex of metamorphosed frogs

In the control series, 384 tadpoles produced from five matings climbed out of water at the age of 79～90 days, 80.2 days on the average (Table 42). In the 12 experimental series, 1560 tadpoles produced from 52 matings climbed out of water at the age of 80～113 days, 84.9～96.0 days on the average in each experimental series. Thus, the tadpoles in the experimental series were retarded as a whole in metamorphosis as compared with the controls. In three groups of experimental series derived from X-irradiated spermatozoa and eggs as well as neutron-irradiated spermatozoa, the tadpoles from gametes exposed to larger doses were distinctly behind those from gametes exposed to smaller doses in metamorphosis. However, in the experimental series derived from neutron-irradiated eggs, the tadpoles from gametes exposed to smaller doses were behind those from gametes exposed to larger doses.

The body length of all the individuals in the experimental and control series was measured immediately after completion of metamorphosis (Table 42). It was found that the frogs in the experimental series averaged 20.5±0.1～24.7±0.1 mm

TABLE 42

Number, size and sex of metamorphosed frogs derived

Parents: Female	Parents: Male	Age at the time of climbing out of water (days)	No. of metamorphosed frogs
N.W72, Nos. 1～5	(N·N)N, Nos. 1～5	79～90 (80.2)	384
N.W72, Nos. 1～5	(N·SX–90)N, Nos. 1～5	80～104 (85.3)	91
	(N·SX–170)N, Nos. 1～5	80～113 (87.7)	83
	(N·SX–240)N, Nos. 1～5	88～108 (92.6)	67
N.W72, Nos. 1～5	(EX–90·N)N, Nos. 1～5	82～104 (85.9)	100
	(EX–145·N)N, Nos. 1～5	83～95 (87.9)	178
	(EX–200·N)N, No. 1	91～109 (96.0)	31
N.W72, Nos. 1～5	(N·SN–50)N, Nos. 1～5	81～98 (85.1)	184
	(N·SN–90)N, No. 1	87～109 (91.6)	57
	(N·SN–130)N, Nos. 1～5	83～111 (93.5)	209
N.W72, Nos. 1～5	(EN–50·N)N, Nos. 1～5	87～111 (92.5)	149
	(EN–90·N)N, Nos. 1～5	81～98 (88.7)	223
	(EN–130·N)N, Nos. 1～5	81～92 (84.9)	188

$♀_N$—Females with normal ovaries $♀_U$—Females with underdeveloped ovaries

in body length in each experimental series, while the control frogs were 23.1 ±0.1 mm. There was no remarkable difference in body length of young frogs between the experimental and control series. There were also no definite differences among the 12 experimental series that differed from one another in kind of irradiated gametes as well as in agent or amount of irradiation. However, there was a tendency in the experimental series for the frogs showing much delay in metamorphosis to be somewhat larger as a whole. All the frogs in the experimental and control series were killed about one month after metamorphosis to examine their sex (Table 42).

a. Control series

N♀×{(N♀×N♂)♀×N♂}♂, Nos. 1～5

Two of 384 normally metamorphosed frogs produced from five matings died within one month after metamorphosis. The remaining 382 frogs were killed; 192 were females, 3 hermaphrodites and 187 males. When the hermaphrodites were counted as males, 49.7% were males.

from irradiated great-grandparental sperm or eggs, III

Body length immediately after metamorphosis (mm)	Sex of frogs killed about one month after metamorphosis					
	No. of frogs	♀N	♀U	⚥	♂N	♂ (%)*
23.1±0.1	382	185	7	3	187	(49.7)
21.3±0.1	86	32	7	9	38	(54.7)
23.2±0.1	81	29	11	10	31	(50.6)
23.6±0.1	67	27	6	9	25	(50.7)
20.5±0.1	92	41	8	12	31	(46.7)
21.9±0.1	154	56	19	21	58	(51.3)
24.7±0.1	31	10	3	4	14	(58.1)
20.7±0.1	182	70	17	14	81	(52.2)
23.4±0.1	57	24	4	3	26	(50.9)
21.0±0.1	203	75	23	26	79	(51.7)
24.1±0.1	145	76	2	5	62	(46.2)
22.3±0.1	214	81	21	27	85	(52.3)
23.4±0.1	175	69	14	16	76	(52.6)

⚥—Hermaphrodites ♂N—Males with normal testes * Including hermaphrodites

b. Experimental series derived from X-irradiated sperm

i) N♀×{(N♀×SX-90♂)♀×N♂}♂, Nos. 1∼5

Five of 91 normally metamorphosed frogs produced from five matings died within one month after metamorphosis. The remaining 86 frogs were killed; 39 were females, 9 hermaphrodites and 38 males. When the hermaphrodites were counted as males, 54.7% of the total number of frogs examined were males.

ii) N♀×{(N♀×SX-170♂)♀×N♂}♂, Nos. 1∼5

Two of 83 normally metamorphosed frogs produced from five matings died within one month after metamorphosis. Of the remaining 81 frogs, 40 were females, 10 hermaphrodites and 31 males. When the hermaphrodites were counted as males, 50.6% were males.

iii) N♀×{(N♀×SX-240♂)♀×N♂}♂, Nos. 1∼5

All the 67 normally metamorphosed frogs produced from five matings were living one month after metamorphosis. Of these frogs, 33 were females, 9 hermaphrodites and 25 males. When the hermaphrodites were counted as males, 50.7% were males.

c. Experimental series derived from X-irradiated eggs

i) N♀ × {(EX–90♀ × N♂)♀ × N♂}♂, Nos. 1 ~ 5

Eight of 100 normally metamorphosed frogs produced from five matings died within one month after metamorphosis. Of the remaining 92 frogs, 49 were females, 12 hermaphrodites and 31 males. When the hermaphrodites were counted as males, 46.7% were males.

ii) N♀ × {(EX–145♀ × N♂)♀ × N♂}♂, Nos. 1 ~ 5

Of 178 normally metamorphosed frogs produced from five matings, 24 died within one month after metamorphosis. Of the remaining frogs, 75 were females, 21 hermaphrodites and 58 males. When the hermaphrodites were counted as males, 51.3% were males.

iii) N♀ × {(EX–200♀ × N♂)♀ × N♂}♂, No. 1

All the 31 normally metamorphosed frogs produced from one mating were living one month after metamorphosis; 13 were females, 4 hermaphrodites and 14 males. When the hermaphrodites were counted as males, 58.1% were males.

d. Experimental series derived from neutron-irradiated sperm

i) N♀ × {(N♀ × SN–50♂)♀ × N♂}♂, Nos. 1 ~ 5

Two of 184 normally metamorphosed frogs produced from five matings died within one month after metamorphosis. Of the remaining 182 frogs, 87 were females, 14 hermaphrodites and 81 males. When the hermaphrodites were counted as males, 52.2% were males.

ii) N♀ × {(N♀ × SN–90♂)♀ × N♂}♂, No. 1

All the 57 normally metamorphosed frogs produced from one mating were living one month after metamorphosis. Of these frogs, 28 were females, three hermaphrodites and 26 males. When the hermaphrodites were counted as males, 50.9% were males.

iii) N♀ × {(N♀ × SN–130♂)♀ × N♂}♂, Nos. 1 ~ 5

Six of 209 normally metamorphosed frogs produced from five matings died within one month after metamorphosis. Of the remaining frogs, 98 were females, 26 hermaphrodites and 79 males. When the hermaphrodites were counted as males, 51.7% were males.

e. Experimental series derived from neutron-irradiated eggs

i) N♀ × {(EN–50♀ × N♂)♀ × N♂}♂, Nos. 1 ~ 5

Four of 149 normally metamorphosed frogs produced from five matings died within one month after metamorphosis. Of the remaining 145 frogs, 78 were females, 5 hermaphrodites and 62 males. When the hermaphrodites were counted as males, 46.2% were males.

ii) N♀ × {(EN–90♀ × N♂)♀ × N♂}♂, Nos. 1 ~ 5

Nine of 223 normally metamorphosed frogs produced from five matings died within one month after metamorphosis. Of the remaining 214, 102 were females, 27 hermaphrodites and 85 males. When the hermaphrodites were counted as males, 52.3% were males.

iii) N♀ × {(EN–130♀ × N♂)♀ × N♂}♂, Nos. 1～5

Of 188 normally metamorphosed frogs, 13 died within one month after metamorphosis. The remaining 175 were killed; 83 were females, 16 hermaphrodites and 76 males. When the hermaphrodites were counted as males, 52.6% were males.

VII. Third-generation offspring derived from irradiated gametes by passing over female first- and second-generation offspring

1. Female parents

Female second-generation offspring produced from female first-generation offspring by mating with normal males collected from the field in 1970 matured by the breeding season of 1973. The female first-generation offspring were produced from X- or neutron-irradiated eggs or spermatozoa by fertilization with normal gametes. In 1973, the female second-generation offspring were mated by artificial insemination with five normal males collected from the field in order to produce third-generation offspring (Table 49).

Five normal males Nos. 1～5 collected from the field were 60.0～67.0 mm, 62.8 mm on the average, in body length (Table 43). Their testes were 4.5 × 2.5～5.5 × 3.0 mm in length and width, and of type 1 in inner structure, that is, the seminal tubules were filled with close bundles of normal spermatozoa. In the control series, six female second-generation offspring produced from control

TABLE 43

Testes of five field-caught male frogs used for mating experiments in 1973

Kind	Individual no.	Body length (mm)	Size of the testes		Inner structure
			Left (mm)	Right (mm)	
N.W73	1	62.0	4.5 × 2.5	4.5 × 2.5	Type 1
	2	60.0	5.0 × 3.0	5.0 × 3.0	Type 1
	3	61.5	5.0 × 2.5	5.0 × 3.0	Type 1
	4	63.5	5.0 × 2.5	4.5 × 2.5	Type 1
	5	67.0	5.5 × 3.0	5.5 × 3.0	Type 1

TABLE 44

Eggs of six control female frogs used for mating experiments in 1973

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 100 eggs (mm)
(N·N)N, No. 1	1	3	65.0	1624	1.82 ± 0.02
	2	3	67.5	1772	1.81 ± 0.02
	3	3	68.0	1835	1.87 ± 0.02
	4	3	70.0	2067	1.89 ± 0.02
	5	3	65.0	1472	1.84 ± 0.02
	6	3	64.5	1721	1.80 ± 0.02

(N·N)N, No. 1: Females obtained by (N·N)♀, No. 1 × N.W70♂, Nos. 1～5 mating No. 1 were used to produce the third-generation offspring. These females were 64.5 ~ 70.0 mm, 66.7 mm on the average, in body length. They discharged 1472 ~ 2067 eggs, 1748.5 on the average, after their ovulation was accelerated by pituitary injection. The mean diameter of 100 eggs was 1.80 ± 0.02 ~ 1.89 ± 0.02 mm, 1.84 ± 0.02 mm on the average (Table 44).

In four groups consisting of 12 experimental series, 105 females in total were used to produce the third-generation offspring. These females were three years old, being 57.5 ~ 71.5 mm, 63.7 mm on the average, in body length (Tables 45 ~ 48). Ten of 30 females belonging to the first group were produced from mating No. 2 of the experimental series derived from spermatozoa exposed to 90 rads

TABLE 45

Eggs of thirty female frogs derived from X-irradiated grandparental sperm and used for mating experiments in 1973

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 100 eggs (mm)
(N·SX-90)N, No. 2	1	3	65.0	969	2.02 ± 0.02
	2	3	67.0	1253	2.01 ± 0.02
	3	3	71.5	2136	2.08 ± 0.02
	4	3	67.0	1674	1.92 ± 0.02
	5	3	69.0	1955	1.92 ± 0.02
	6	3	68.0	740	1.73 ± 0.03
	7	3	70.5	812	1.68 ± 0.03
	8	3	67.0	0	—
	9	3	69.5	0	—
	10	3	65.5	0	—
(N·SX-170)N, No. 4	1	3	71.0	2133	1.79 ± 0.02
	2	3	67.5	1211	1.92 ± 0.02
	3	3	68.0	1428	1.98 ± 0.02
	4	3	68.5	1274	1.83 ± 0.02
	5	3	66.0	754	2.02 ± 0.02
	6	3	65.0	1149	1.93 ± 0.02
	7	3	62.5	1372	1.72 ± 0.02
	8	3	65.5	1186	1.83 ± 0.02
	9	3	67.0	0	—
	10	3	67.5	0	—
(N·SX-240)N, No. 1	1	3	65.5	1324	1.74 ± 0.02
	2	3	67.0	1975	1.78 ± 0.02
	3	3	69.0	1770	2.03 ± 0.02
	4	3	67.5	1433	1.91 ± p.02
	5	3	65.0	1549	2.13 ± 0.02
	6	3	65.5	1742	2.01 ± 0.02
(N·SX-240)N, No. 2	7	3	67.0	1225	1.69 ± 0.02
	8	3	68.5	1754	1.82 ± 0.02
	9	3	67.0	0	—
	10	3	70.0	0	—

(N·SX-90)N, No. 2: Females obtained by (N·SX-90) ♀, No. 2 × N.W70 ♂, Nos. 1 ~ 5
(N·SX-170)N, No. 4: Females obtained by (N·SX-170) ♀, No. 4 × N.W70 ♂, Nos. 1 ~ 5
(N·SX-240)N, No. 1 or 2: Females obtained by (N·SX-240) ♀, No. 1 or 2 × N.W70 ♂, Nos. 1 ~ 5

of X-rays. They were 65.0～71.5 mm, 68.0 mm on the average, in body length. After pituitary injection seven of them discharged 740～2136 eggs, 1362.7 on the average, which were 1.68±0.03～2.08±0.02 mm in diameter (Table 45). Ten other females were produced from mating No. 4 of the experimental series derived from spermatozoa exposed to 170 rads of X-rays. They were 62.5～71.0 mm, 66.9 mm on the average, in body length. Eight of them laid 754～2133 eggs, 1313.4 on the average, which were 1.72±0.02～2.02±0.02 mm in diameter. The remaining ten females were produced from matings Nos. 1 and 2 of the experimental series derived from spermatozoa exposed to 240 rads of X-rays. They were 65.0～70.0 mm, 67.2 mm on the average, in body length. Eight of them laid 1225～1975 eggs, 1596.5 on the average, which were 16.9±0.02～2.13±0.02 mm in diameter.

Ten of 22 females belonging to the second group were produced from mating No. 6 of the experimental series derived from eggs exposed to 90 rads of X-rays (Table 46). They were 57.5～65.5 mm, 61.9 mm on the average, in body length. After pituitary injection seven of them laid 1099～1743 eggs, 1362.4 on the average, which were 1.71±0.02～1.89±0.02 mm in diameter. Nine other fe-

TABLE 46

Eggs of twenty-two female frogs derived from X-irradiated grandparental eggs and used for mating experiments in 1973

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 100 eggs (mm)
(EX-90·N)N, No. 6	1	3	65.0	1285	1.71±0.02
	2	3	65.5	1740	1.78±0.02
	3	3	60.0	1129	1.89±0.02
	4	3	59.5	1254	1.72±0.03
	5	3	63.5	1743	1.71±0.02
	6	3	61.5	1099	1.72±0.02
	7	3	61.5	1287	1.76±0.02
	8	3	60.0	0	—
	9	3	65.0	0	—
	10	3	57.5	0	—
(EX-145·N)N, No. 4	1	3	62.0	1295	1.90±0.02
	2	3	60.0	1743	1.86±0.02
	3	3	59.5	1032	1.82±0.02
	4	3	59.0	1120	1.92±0.02
	5	3	67.5	934	1.91±0.02
	6	3	64.5	1573	1.79±0.02
	7	3	64.5	993	1.87±0.02
	8	3	59.0	757	1.73±0.02
	9	3	63.5	0	—
(EX-200·N)N, No. 1	1	3	62.0	1264	1.82±0.02
	2	3	65.5	1355	1.87±0.02
	3	3	67.0	0	—

(EX-90·N)N, No. 6: Females obtained by (EX-90·N) ♀, No. 6×N.W70♂, Nos. 1～5
(EX-145·N)N, No. 4: Females obtained by (EX-145·N) ♀, No. 4×N.W70♂, Nos. 1～5
(EX-200·N)N, No. 1: Females obtained by (EX-200·N) ♀, No. 1×N.W70♂, Nos. 1～5

males were produced from mating No. 4 of the experimental series derived from eggs exposed to 145 rads of X-rays. They were 59.0～67.5 mm, 62.2 mm on the average, in body length. Eight of them laid 757～1743 eggs, 1180.9 on the average, which were 1.73±0.02～1.92±0.02 mm in diameter. The remaining three of the 22 females were produced from mating No. 1 of the experimental series derived from eggs exposed to 200 rads of X-rays. They were 62.0～67.0 mm, 64.8 mm on the average, in body length. Two of them discharged 1264 and 1355 eggs, 1309.5 on the average, which were 1.82±0.02 mm and 1.87±0.02 mm in diameter, respectively.

Five of 25 females belonging to the third group were produced from mating No. 2 of the experimental series derived from spermatozoa exposed to 50 rads of neutrons (Table 47). They were 58.0～65.0 mm, 61.2 mm on the average, in body length. Three of them laid 1029～1642 eggs, 1234.7 on the average, which were 1.74±0.02～18.2 ±0.02 mm in diameter. Ten other females were produced from matings Nos. 1 and 4 of the experimental series derived from sper-

TABLE 47

Eggs of twenty-five female frogs derived from neutron-irradiated grandparental sperm and used for mating experiments in 1973

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 100 eggs (mm)
(N·SN–50)N, No. 2	1	3	65.0	1642	1.74±0.02
	2	3	61.5	1033	1.79±0.02
	3	3	59.0	1029	1.82±0.02
	4	3	58.0	0	—
	5	3	62.5	0	—
(N·SN–90)N, No. 4	1	3	61.0	1749	1.92±0.02
	2	3	61.5	1175	1.73±0.02
	3	3	67.0	1579	1.83±0.02
	4	3	59.5	1050	2.03±0.02
	5	3	63.5	1121	1.81±0.02
(N·SN–90)N, No. 1	6	3	57.5	1053	1.72±0.02
	7	3	60.0	1536	1.77±0.02
	8	3	64.0	1522	1.89±0.02
	9	3	66.0	0	—
	10	3	61.0	0	—
(N·SN–130)N, No. 2	1	3	65.5	1188	1.72±0.02
	2	3	62.5	1467	1.75±0.02
	3	3	59.0	1076	1.78±0.02
	4	3	57.5	1094	1.75±0.02
	5	3	58.5	997	2.03±0.02
	6	3	64.0	981	1.72±0.02
	7	3	63.0	1793	1.76±0.02
	8	3	66.5	1063	1.79±0.02
	9	3	61.0	0	—
	10	3	68.0	0	—

(N·SN–50)N, No. 2: Females obtained by (N·SN–50) ♀, No. 2×N.W70 ♂, Nos. 1～5
(N·SN–90)N, No. 1 or 4: Females obtained by (N·SN–90) ♀, No. 1 or 4×N.W70 ♂, Nos. 1～5
(N·SN–130)N, No. 2: Females obtained by (N·SN–130) ♀, No. 2×N.W70 ♂, Nos. 1～5

matozoa exposed to 90 rads of neutrons. They were 57.5～67.0 mm, 62.1 mm on the average, in body length. After pituitary injection eight of them laid 1050～1749 eggs, 1348.1 on the average, which were 1.72±0.02～2.03±0.02 mm in diameter. The remaining ten females were produced from mating No. 2 of the experimental series derived from spermatozoa exposed to 130 rads of neutrons. They were 57.5～68.0 mm, 62.6 mm on the average, in body length. Eight of them laid 981～1793 eggs, 1207.4 on the average, which were 1.72±0.02～2.03±0.02 mm in diameter.

Ten of 28 females belonging to the fourth group were produced from mating No. 2 of the experimental series derived from eggs exposed to 50 rads of neutrons (Table 48). They were 58.0～67.5 mm, 62.7 mm on the average, in body length. After pituitary injection eight of them laid 980～1821 eggs, 1353.5 on the average,

TABLE 48

Eggs of twenty-eight female frogs derived from neutron-irradiated grandparental eggs and used for mating experiments in 1973

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 100 eggs (mm)
(EN-50·N)N, No. 2	1	3	62.5	980	1.71±0.02
	2	3	67.0	1329	1.92±0.02
	3	3	61.5	1055	1.96±0.02
	4	3	62.0	1563	1.93±0.02
	5	3	58.0	1339	1.82±0.02
	6	3	59.0	1099	1.66±0.02
	7	3	65.5	1642	1.75±0.02
	8	3	63.0	1821	1.72±0.02
	9	3	61.0	0	—
	10	3	67.5	0	—
(EN-90·N)N, No. 3	1	3	65.0	1677	1.77±0.02
	2	3	62.0	1635	1.89±0.02
	3	3	60.5	1447	1.93±0.02
	4	3	58.0	973	1.82±0.02
	5	3	59.0	1270	1.92±0.02
	6	3	57.5	1066	1.76±0.02
	7	3	66.0	1538	1.72±0.02
	8	3	62.0	1429	1.73±0.02
(EN-130·N)N, No. 1	1	3	63.0	976	1.91±0.02
	2	3	65.5	1370	1.91±0.02
	3	3	62.5	1184	1.73±0.02
	4	3	59.0	1071	1.75±0.02
	5	3	58.5	0	—
	6	3	61.5	0	—
	7	3	63.0	0	—
	8	3	66.5	0	—
(EN-130·N)N, No. 2	9	3	62.0	1548	1.91±0.02
	10	3	65.0	0	—

(EN-50·N)N, No. 2: Females obtained by (EN-50·N) ♀, No. 2×N.W70 ♂, Nos. 1～5
(EN-90·N)N, No. 3: Females obtained by (EN-90·N) ♀, No. 3×N.W70 ♂, Nos. 1～5
(EN-130·N)N, No. 1 or 2: Females obtained by (EN-130·N) ♀, No. 1 or 2×N.W70 ♂, Nos. 1～5

which were 1.66±0.02∼1.96±0.02 mm in diameter. Eight other females were produced from mating No. 3 of the experimental series derived from eggs exposed to 90 rads of neutrons. They were 57.5∼66.0 mm, 61.3 mm on the average, in body length. These females all discharged 973∼1677 eggs, 1379.4 on the average, which were 1.72±0.02∼1.93±0.02 mm in diameter. The remaining ten females were produced from matings Nos. 1 and 2 of the experimental series derived from eggs exposed to 130 rads of neutrons. They were 58.5∼66.5 mm, 62.7 mm on the average, in body length. Five of them laid 976∼1548 eggs, 1229.8 on the average, which were 1.73±0.02∼1.91±0.02 mm in diameter.

In summary, each of 80 of 105 mature females in the experimental series discharged 740∼2136 eggs, 1332.3 on the average, after pituitary injection. These eggs were 1.66±0.02∼2.13±0.02 mm, 1.84±0.02 mm on the average, in mean diameter. Each of six control females discharged 1748.5 eggs on the average, which were 1.84±0.02 mm in mean diameter.

2. Developmental capacity

a. Control series

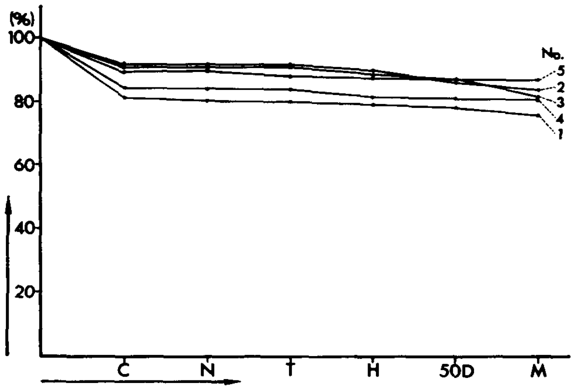
{(N♀×N♂)♀×N♂}♀×N♂, Nos. 1∼5

TABLE 49

Developmental capacity of the offspring of female frogs

Parents		No. of eggs	No. of cleaved eggs	
Female	Male		Normal	Abnormal
(N·N)N, Nos. 1∼5	N.W73, Nos. 1∼5	602	529 (87.9%)	3 (0.5%)
(N·SX-90)N, Nos. 1∼5	N.W73, Nos. 1∼5	539	331 (61.4%)	0
(N·SX-170)N, Nos. 1∼5		483	408 (84.5%)	0
(N·SX-240)N, Nos. 1∼5		598	451 (75.4%)	3 (0.5%)
(EX-90·N)N, Nos. 1∼5	N.W73, Nos. 1∼5	651	516 (79.3%)	2 (0.3%)
(EX-145·N)N, Nos. 1∼5		725	463 (63.9%)	81 (11.2%)
(EX-200·N)N, Nos. 1,2		241	75 (31.1%)	35 (14.5%)
(N·SN-50)N, Nos. 1∼3	N.W73, Nos. 1∼5	398	331 (83.2%)	8 (2.0%)
(N·SN-90)N, Nos. 1∼5		701	445 (63.5%)	8 (1.1%)
(N·SN-130)N, Nos. 1∼5		599	433 (72.3%)	16 (2.7%)
(EN-50·N)N, Nos. 1∼5	N.W73, Nos. 1∼5	708	517 (73.0%)	13 (1.8%)
(EN-90·N)N, Nos. 1∼5		558	435 (78.0%)	3 (0.5%)
(EN-130·N)N, Nos. 1∼4		714	455 (63.7%)	3 (0.4%)

Although all six females discharged more than 1400 eggs after pituitary injection, five (Nos. 1~5) of them were mated with five field-caught males Nos. 1~5 by artificial insemination to produce control third-generation offspring (Table 49, Fig. 26). In five matings Nos. 1~5, 81.4~92.0%, 87.9% on the average, of



C —Cleavage
N —Neurula stage
T —Tai-bud stage
H —Hatch
50D–Age of 50 days
M —Climbing out of water

Fig. 26. Survival curves of control third-generation offspring derived from untreated great-grandparental gametes by matings, {(N♀ × N♂) ♀ × N♂} ♀ × N♂, Nos. 1~5.

derived from irradiated grandparental sperm or eggs, II

No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of 50-day-old tadpoles	No. of meta-morphosed frogs
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal		
526 (87.4%)	3 (0.5%)	523 (86.9%)	3 (0.5%)	514 (85.4%)	9 (1.5%)	505 (83.9%)	492 (81.7%)
318 (59.0%)	13 (2.4%)	315 (58.4%)	3 (0.6%)	314 (58.3%)	1 (0.2%)	283 (52.5%)	262 (48.6%)
400 (82.8%)	8 (1.7%)	398 (82.4%)	2 (0.4%)	392 (81.2%)	6 (1.2%)	285 (59.0%)	246 (50.9%)
423 (70.7%)	24 (4.0%)	411 (68.7%)	12 (2.0%)	382 (63.9%)	29 (4.8%)	308 (51.5%)	255 (42.6%)
490 (75.3%)	22 (3.4%)	489 (75.1%)	1 (0.2%)	482 (74.0%)	7 (1.1%)	410 (63.0%)	368 (56.5%)
444 (61.2%)	18 (2.5%)	443 (61.1%)	1 (0.1%)	409 (56.4%)	34 (4.7%)	397 (54.8%)	389 (53.7%)
51 (21.2%)	21 (8.7%)	38 (15.8%)	13 (5.4%)	31 (12.9%)	7 (2.9%)	23 (9.5%)	15 (6.2%)
328 (82.4%)	3 (0.8%)	315 (79.1%)	13 (3.3%)	241 (60.6%)	74 (18.6%)	210 (52.8%)	166 (41.7%)
426 (60.8%)	19 (2.7%)	366 (52.2%)	60 (8.6%)	343 (48.9%)	23 (3.3%)	315 (44.9%)	300 (42.8%)
420 (70.1%)	12 (2.0%)	415 (69.3%)	5 (0.8%)	380 (63.4%)	35 (5.8%)	360 (60.1%)	328 (54.8%)
494 (69.8%)	21 (3.0%)	467 (66.0%)	27 (3.8%)	395 (55.8%)	72 (10.2%)	376 (53.1%)	291 (41.1%)
388 (69.5%)	39 (7.0%)	364 (65.2%)	24 (4.3%)	347 (62.2%)	17 (3.0%)	316 (56.6%)	304 (54.5%)
438 (61.3%)	16 (2.2%)	415 (58.1%)	23 (3.2%)	332 (46.5%)	83 (11.6%)	273 (38.2%)	252 (35.3%)

the respective total number of eggs cleaved normally. Only a few of the normally cleaved eggs died of various abnormalities at various embryonic stages; 79.4~90.0%, 85.4% on the average, hatched normally. After the hatching stage a few tadpoles also died of underdevelopment or edema; 75.5~86.6%, 81.7% on the average, became normally metamorphosed frogs.

b. Experimental series derived from X-irradiated sperm

i) {(N♀ ×SX-90♂)♀ ×N♂}♀ ×N♂, Nos. 1~5

Ovulation occurred in seven female second-generation offspring after pituitary injection; five (Nos. 1~5) of them were mated by artificial insemination with the same five field-caught males Nos. 1~5 as those used in the control series to produce third-generation offspring (Table 49, Fig. 27). In five matings Nos. 1~5, 37.4%, 13.3%, 75.0%, 97.0% and 97.1%, 61.4% on the average, of the respective total number of eggs cleaved normally. Almost all the normally cleaved eggs developed normally until the hatching stage, except that a somewhat large number of eggs in matings Nos. 3 and 4 died by the neurula stage; 35.8%, 12.0%, 68.1%, 91.1% and 95.2%, 58.3% on the average, hatched normally. Most of the tadpoles produced from mating No. 2 died of underdevelopment or edema by the metamorphosis stage. In mating No. 3, 17 of 49 tadpoles did not take food and seven others died of underdevelopment without metamorphosis. A few tadpoles produced from the other three matings died of underdevelopment or edema, too. Eventually, 29.1%, 3.6%, 34.7%, 86.1% and 91.3%, 48.6% on the average, metamorphosed normally.

ii) {(N♀ ×SX-170♂)♀ ×N♂}♀ ×N♂, Nos. 1~5

Although ovulation occurred in eight of ten female second-generation offspring, five females Nos. 1~5 were used for producing third-generation offspring. These females were mated with field-caught males Nos. 1~5. In five matings Nos. 1~5, 58.2~93.8%, 84.5% on the average, of the respective total number of eggs cleaved normally. A few of the normally cleaved eggs died of various abnormalities at various embryonic stages; 58.2~91.2%, 81.2% on the average, hatched normally. However, most of the individuals produced from matings Nos. 4 and 5 died afterwards of edema. During the first half of the tadpole stage, 46 of 116 individuals from No. 4 and 58 of 114 from No. 5 became edematous and died. Shortly before metamorphosis, 18 tadpoles from No. 4 and 16 from No. 5 became also edematous and died. In the other matings, only a few tadpoles died of underdevelopment or some other abnormalities. Eventually, 32.0~74.4%, 50.9% on the average, became normal, metamorphosed frogs.

iii) {(N♀ ×SX-240♂)♀ ×N♂}♀ ×N♂, Nos. 1~5

All six female second-generation offspring produced from mating No. 1 and two of the four produced from mating No. 2 ovulated normally after pituitary injection. Five (Nos. 1~5) of the former were used for producing third-generation offspring. They were mated with field-caught males Nos. 1~5. While 81.3~94.9% of the respective total number of eggs cleaved normally in matings Nos. 2~5, only 4.1% did so in No. 1; on the average in five matings

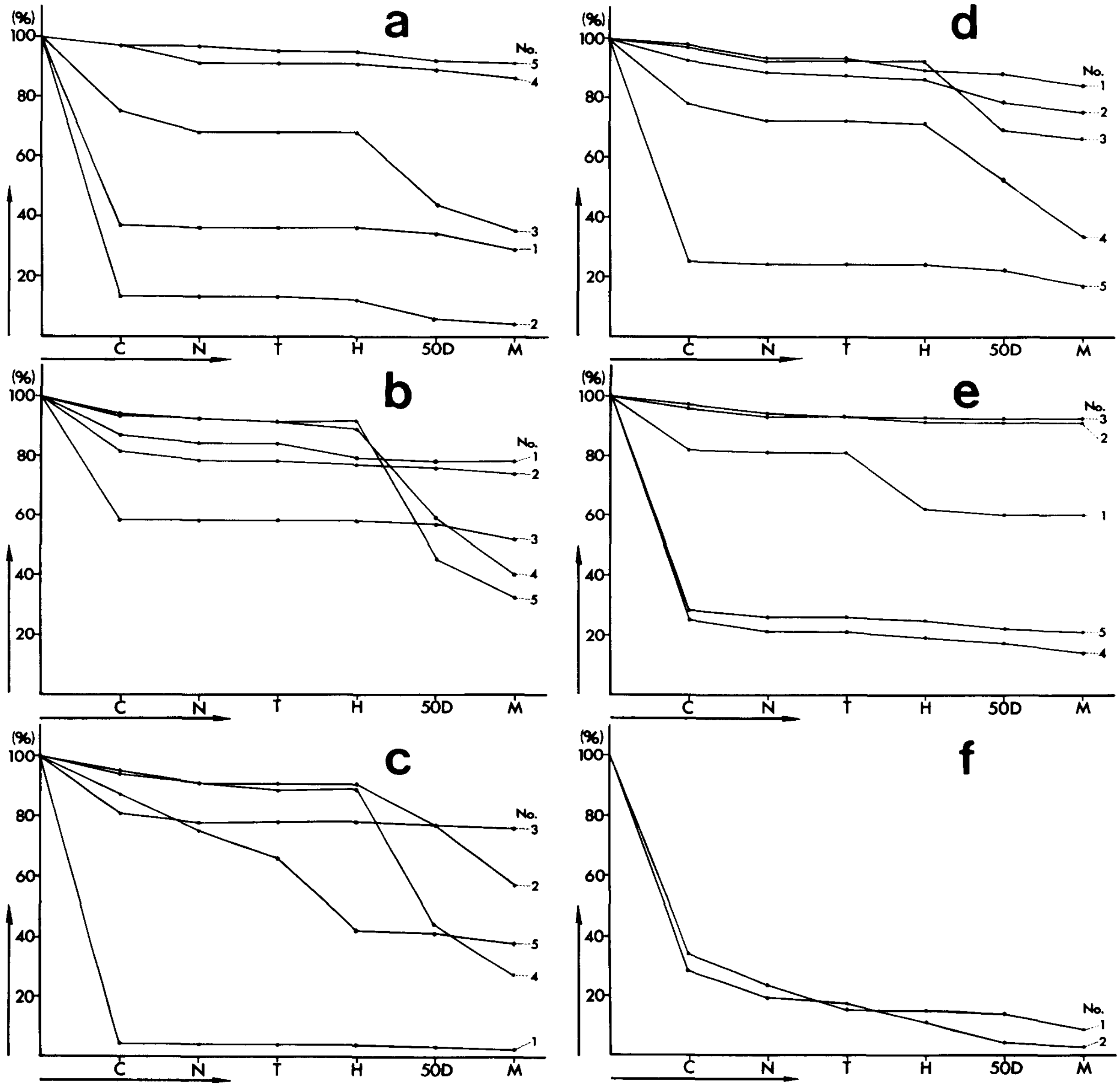


Fig. 27. Survival curves of third-generation offspring derived from X-irradiated great-grandparental gametes by passing over female first- and second-generation offspring.

a.	{(N♀×SX–90♂)♀×N♂} ♀×N♂, Nos. 1～5	C —Cleavage
b.	{(N♀×SX–170♂)♀×N♂} ♀×N♂, Nos. 1～5	N —Neurula stage
c.	{(N♀×SX–240♂)♀×N♂} ♀×N♂, Nos. 1～5	T —Tail-bud stage
d.	{(EX–90♀×N♂)♀×N♂} ♀×N♂, Nos. 1～5	H —Hatch
e.	{(EX–145♀×N♂)♀×N♂} ♀×N♂, Nos. 1～5	50D–Age of 50 days
f.	{(EX–200♀×N♂)♀×N♂} ♀×N♂, Nos. 1 and 2	M —Climbing out of water

Nos. 1～5, 75.4% cleaved normally. Many of the normally cleaved eggs in mating No. 5 died of various abnormalities at various embryonic stages, while only a few died in the other matings; 4.1～91.3%, 63.9% hatched normally. During the first half of the tadpole stage, 18 of 115 individuals produced from mating No. 2 and 52 of 104 from No. 4 became edematous and died. Shortly before metamorphosis, 25 tadpoles from No. 2 and 21 from No. 4 died of edema, too. Several tadpoles in the other matings died of underdevelopment or edema. Eventually, 2.1∼75.5%, 42.6% on the average, metamorphosed normally in the five matings.

c. Experimental series derived from X-irradiated eggs

i) {(EX-90♀×N♂)♀×N♂}♀×N♂, Nos. 1∼5

Seven of ten female second-generation offspring ovulated normally after pituitary injection; five (Nos. 1∼5) of them were used for producing third-generation offspring by mating with field-caught males Nos. 1∼5 (Table 49, Fig. 27). In five matings Nos. 1∼5, 97.8%, 91.5%, 96.5%, 77.6% and 24.8%, 79.3% on the average, of the respective total number of eggs cleaved normally. While a few of the normally cleaved eggs died of various abnormalities, the others developed normally; 89.1%, 86.0%, 92.3%, 71.2% and 23.9%, 74.0% on the average, hatched normally. However, 33 of 132 tadpoles produced from mating No. 3 died of underdevelopment. Of 89 tadpoles from No. 4, 23 and 24 died of edema during the first half of the tadpole stage and shortly before metamorphosis, respectively. The tadpoles produced from matings Nos. 1 and 2 scarcely differed in developmental capacity from those of the control series. In mating No. 5, only a few tadpoles became abnormal in the development of the forelegs. Eventually, 83.9%, 75.2%, 66.4%, 32.8% and 17.1%, 56.5% on the average, metamorphosed normally.

ii) {(EX-145♀×N♂)♀×N♂}♀×N♂, Nos. 1∼5

Although eight of nine female second-generation offspring ovulated after pituitary injection, five (Nos. 1∼5) of them were used for producing third-generation offspring by mating with field-caught males Nos. 1∼5. In five matings Nos. 1∼5, 82.3%, 96.4%, 97.0%, 24.7% and 27.6%, 63.9% on the average, of the respective total number of eggs cleaved normally. While the normally cleaved eggs in matings Nos. 2∼5 scarcely differ in developmental capacity from those of the control series during the embryonic stage, 26 of 114 individuals produced from mating No. 1 became edematous and died at the hatching stage; 62.4%, 91.4%, 92.6%, 19.2% and 24.5%, 56.4% on the average, hatched normally. During the tadpole stage, a very small number of individuals died of edema or underdevelopment; 59.6%, 90.7%, 91.9%, 13.7% and 20.9%, 53.7% on the average, became normal, metamorphosed frogs.

iii) {(EX-200♀×N♂)♀×N♂}♀×N♂, Nos. 1 and 2

As two of three female second-generation offspring ovulated after pituitary injection, they were mated with the same two males Nos. 1 and 2 as those used in the control series to produce third-generation offspring. In two matings Nos. 1 and 2, 33.9% and 28.1%, 31.1% on the average, of the respective total number of eggs cleaved normally. Many of the normally cleaved eggs died of various abnormalities, such as edema, blisters, microcephaly or curvature of the body; 15.0% and 10.5%, 12.9% on the average, hatched normally. Many individuals died afterwards of edema or underdevelopment; only 9.4% and 2.6%, 6.2% on the average, became normal, metamorphosed frogs.

d. Experimental series derived from neutron-irradiated sperm

i) {(N♀ ×SN-50♂)♀ ×N♂}♀ ×N♂, Nos. 1～3

As three (Nos. 1～3) of five female second-generation offspring ovulated after pituitary injection, they were mated with the same three males, Nos. 1～3 as those used in the control series for producing third-generation offspring (Table 49, Fig. 28). In three matings Nos. 1～3, 93.1%, 79.2% and 80.2%, 83.2% on the average, of the respective total number of eggs cleaved normally. While the nor-

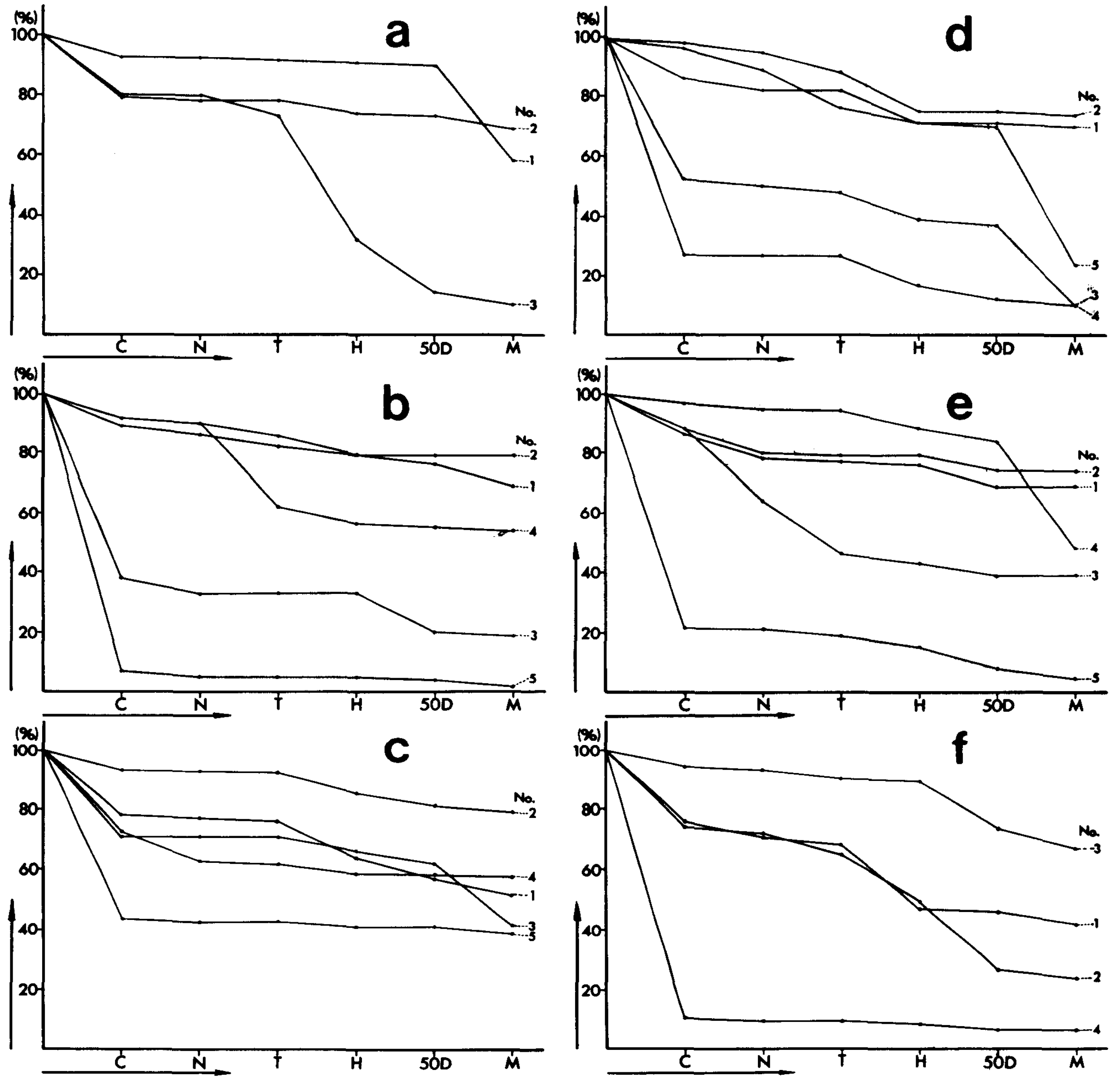


Fig. 28. Survival curves of third-generation offspring derived from neutron-irradiated great-grand-parental gametes by passing over female first- and second-generation offspring.

a. {(N♀ ×SN-50♂)♀ ×N♂} ♀ ×N♂, Nos. 1～3
b. {(N♀ ×SN-90♂)♀ ×N♂} ♀ ×N♂, No 1～5
c. {(N♀ ×SN-130♂)♀ ×N♂} ♀ ×N♂, Nos. 1～5
d. {(EN-50♀ ×N♂)♀ ×N♂} ♀ ×N♂, Nos. 1～5
e. {(EN-90♀ ×N♂)♀ ×N♂} ♀ ×N♂, Nos. 1～5
f. {(EN-130♀ ×N♂)♀ ×N♂} ♀ ×N♂, Nos. 1～4

C —Cleavage
N —Neurula stage
T —Tail-bud stage
H —Hatch
50D-Age of 50 days
M —Climbing out of water

mally cleaved eggs in matings Nos. 1 and 2 scarcely differed in developmental capacity from those of the control series, 68 of 121 embryos produced from mating No. 3 died simultaneously of edema at the hatching stage; 91.1%, 73.8% and 31.7%, 60.6% on the average, hatched normally. Many tadpoles produced from No. 3 became edematous and died at the first half of the tadpole stage. Although nearly all the individuals produced from No. 1 developed normally until the stage immediately before metamorphosis, 32 of 91 tadpoles became abnormal in the development of the forelegs at the metamorphosis stage. In mating No. 2, six of 96 tadpoles died during the tadpole stage. Eventually, 58.4%, 69.2% and 10.7%, 41.7% on the average, metamorphosed normally.

ii) {(N♀ × SN-90♂)♀ × N♂}♀ × N♂, Nos. 1 ~ 5

Although ovulation occurred in all five female second-generation offspring produced from mating No. 4 as well as in three of the five produced from No. 1, the former five females Nos. 1 ~ 5 were used for producing third-generation offspring by mating with the same five males Nos. 1 ~ 5 as those used in the control series. In five matings Nos. 1 ~ 5, 92.4%, 88.7%, 38.4%, 91.8% and 6.9%, 63.5% on the average, of the respective total number of eggs cleaved normally. Only a small number of normally cleaved eggs in matings Nos. 1, 2, 3 and 5 became abnormal at various embryonic stages, while 51 and 12 of 165 embryos produced from No. 4 became edematous and died at the tail-bud and hatching stage, respectively; 79.0%, 79.0%, 33.0%, 55.7% and 5.4%, 48.9% on the average, hatched normally. In the mating No. 3, 21 of 53 tadpoles took no food after the hatching stage and died. Comparatively many tadpoles produced from No. 1 died of edema during the metamorphosis stage. In the other matings, only a few tadpoles died of underdevelopment or edema. Eventually, 68.6%, 79.0%, 18.9%, 53.6% and 1.5%, 42.8% on the average, became normal,

183 normally cleaved eggs in No. 3 became edematous before the hatching stage. In the four matings, 47.3%, 49.4%, 79.0% and 8.2%, 46.5% on the average, hatched normally. In the first half of the tadpole stage, many of the tadpoles produced from matings Nos. 2 and 3 died of edema or underdevelopment, while a few tadpoles died of edema or some other abnormalities in mating No. 1. In mating No. 4, 5 of 15 tadpoles died gradually before completion of metamorphosis. Eventually, 42.4%, 24.4%, 66.7% and 5.5%, 35.3% on the average, metamorphosed normally in the four matings.

3. Viability and sex of metamorphosed frogs

In the control series, 492 tadpoles produced from five matings climbed out of water at the age of 80 ~ 97 days, 83.7 days on the average (Table 50). In the 12 experimental series, 3176 tadpoles produced from 54 matings climbed out of water at the age of 77 ~ 127 days, 81.2 ~ 95.4 days on the average in each series. Although the tadpoles of the experimental series as a whole metamorphosed later than those in the control series, the tadpoles in two

TABLE 50

Eight of ten female second-generation offspring ovulated normally after pituitary injection. Five (Nos. 1 ~ 5) of them were mated with field-caught males Nos. 1 ~ 5 for producing third-generation offspring (Table 49, Fig. 28). In five matings Nos. 1 ~ 5, 85.8%, 98.4%, 51.7%, 27.3% and 96.2%, 73.0% on the average, of the respective total number of eggs cleaved normally. In matings Nos. 1 ~ 4, 16 of 121, 24 of 161, 13 of 70 and 13 of 35 embryos died of edema at the hatching stage, respectively. Of 93 normally cleaved eggs in mating No. 5, 13 and six became edematous and died at the tail-bud and hatching stages, respectively. In the five matings, 70.9%, 74.9%, 39.3%, 17.2% and 71.2%, 55.8% on the average, hatched normally. Almost all the tadpoles produced from matings Nos. 1 and 2 afterwards developed normally into metamorphosed frogs. Of 57 tadpoles produced from No. 3, 11 and 32 died of edema at the first and second half of the tadpole stage, respectively. In mating No. 4, nine of 22 tadpoles became edematous and died during the tadpole stage. In mating No. 5, 48 of 73 tadpoles showed abnormal development of the forelegs. Eventually, 70.3%, 73.8%, 9.6%, 10.2% and 24.0%, 41.1% on the average, became normal, metamorphosed frogs in the five matings.

ii) {(EN–90♀ × N♂)♀ × N♂}♀ × N♂, Nos. 1 ~ 5

Ovulation occurred in all eight female second-generation offspring. Five of them were used in producing third-generation offspring by mating with field-caught males Nos. 1 ~ 5. In five matings Nos. 1 ~ 5, 86.7%, 88.9%, 88.5%, 96.8% and 21.2%, 78.0% on the average, of the respective total number of eggs cleaved normally. Some of the normally cleaved eggs died of various abnormalities at various embryonic stages. Especially in mating No. 3, many embryos died of edema at the neurula and tail-bud stages. In the five matings, 76.0%, 79.0%, 43.3%, 87.9% and 15.2%, 62.2% on the average, hatched normally. In the first half of the tadpole stage, a small number of individuals produced from each mating died of edema or underdevelopment. In the second half, all the tadpoles produced from matings Nos. 1 ~ 3 developed normally into normal, metamorphosed frogs, while nearly half of the tadpoles from No. 4 died of edema during metamorphosis. Ten of 15 tadpoles produced from No. 5 died of edema by the completion of metamorphosis after hatching. In the five matings, 69.3%, 74.1%, 38.5%, 47.6% and 5.1%, 54.5% on the average, metamorphosed normally.

iii) {(EN–130♀ × N♂)♀ × N♂}♀ × N♂, Nos. 1 ~ 4

Although normal ovulation occurred in four of eight second-generation offspring produced from mating No. 1 as well as in one of the two from No. 2, the former four females (Nos. 1 ~ 4) were used in order to produce third-generation offspring by mating with the same four males as those used in the control series. In four matings Nos. 1 ~ 4, 75.8%, 74.4%, 93.8% and 10.4%, 63.7% on the average, cleaved normally. Of 125 normally cleaved eggs in mating No. 1, eight, five and 34 died of edema at the neurula, tail-bud and hatching stages, respectively. In mating No. 2, four, 12 and 26 of 128 normally cleaved eggs died of edema at the neurula, tail-bud and hatching stages, respectively. Thirty of

183 normally cleaved eggs in No. 3 became edematous before the hatching stage. In the four matings, 47.3%, 49.4%, 79.0% and 8.2%, 46.5% on the average, hatched normally. In the first half of the tadpole stage, many of the tadpoles produced from matings Nos. 2 and 3 died of edema or underdevelopment, while a few tadpoles died of edema or some other abnormalities in mating No. 1. In mating No. 4, 5 of 15 tadpoles died gradually before completion of metamorphosis. Eventually, 42.4%, 24.4%, 66.7% and 5.5%, 35.3% on the average, metamorphosed normally in the four matings.

3. Viability and sex of metamorphosed frogs

In the control series, 492 tadpoles produced from five matings climbed out of water at the age of 80～97 days, 83.7 days on the average (Table 50). In the 12 experimental series, 3176 tadpoles produced from 54 matings climbed out of water at the age of 77～127 days, 81.2～95.4 days on the average in each series. Although the tadpoles of the experimental series as a whole metamorphosed later than those in the control series, the tadpoles in two

TABLE 50

Number, size and sex of metamorphosed frogs derived

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed frogs
Female	Male		
(N·N)N, Nos. 1～5	N.W73, Nos. 1～5	80～97 (83.7)	492
(N·SX–90)N, Nos. 1～5	N.W73, Nos. 1～5	80～104 (82.5)	262
(N·SX–170)N, Nos. 1～5		81～109 (85.9)	246
(N·SX–240)N, Nos. 1～5		87～104 (93.3)	255
(EX–90·N)N, Nos. 1～5	N.W73, Nos. 1～5	83～100 (84.6)	368
(EX–145·N)N, Nos. 1～5		83～98 (88.1)	389
(EX–200·N)N, Nos. 1, 2		88～98 (93.2)	15
(N·SN–50)N, Nos. 1～3	N.W73, Nos. 1～5	87～126 (95.1)	166
(N·SN–90)N, Nos. 1～5		80～107 (92.6)	300
(N·SN–130)N, Nos. 1～5		81～127 (95.4)	328
(EN–50·N)N, Nos. 1～5	N.W73, Nos. 1～5	84～109 (92.8)	291
(EN–90·N)N, Nos. 1～5		77～91 (81.2)	304
(EN–130·N)N, Nos. 1～4		83～98 (86.7)	252

$♀_N$—Females with normal ovaries $♀_U$—Females with underdeveloped ovaries experimental series climbed out of water at the average ages of 81.2 and 82.5 days, respectively; those in the other experimental series did at the average age of more than 84 days. In the experimental series derived from X-irradiated spermatozoa or eggs, the tadpoles from gametes exposed to smaller doses metamorphosed earlier as a whole. Such a difference was not found in the experimental series derived from neutron-irradiated gametes. There were also no definite differences between the experimental series derived from irradiated spermatozoa and those from irradiated eggs.

In the control series, 50 frogs were at random removed from those produced from each of the five matings immediately after the completion of metamorphosis in order to measure their body length (Table 50). A total of 250 frogs from the five matings were 19.7±0.1 mm in mean body length. In each of the experimental series, 50 frogs were also at random removed from the third-generation offspring produced from each mating, and their body length was measured. When the frogs produced from one of the matings of each experimental series were less than 50 in number, all of them were measured. The frogs in each of

from irradiated great-grandparental sperm or eggs, IV

Body length immediately after metamorphosis (mm)	Sex of frogs killed about one month after metamorphosis					
	No. of frogs	♀N	♀U	⚥	♂N	♂ (%)*
19.7±0.1	476	232	7	2	235	(49.8)
18.6±0.2	261	118	10	12	121	(51.0)
19.2±0.2	240	105	13	16	106	(50.8)
21.3±0.2	246	102	11	29	104	(54.1)
20.0±0.2	355	145	26	31	153	(51.8)
19.5±0.1	382	148	34	29	171	(52.4)
20.4±0.2	15	7	0	0	8	(53.3)
22.7±0.2	160	66	9	8	77	(53.1)
19.8±0.2	293	113	22	27	131	(53.9)
21.1±0.2	324	110	29	34	151	(57.1)
20.0±0.2	290	125	16	7	142	(51.4)
18.5±0.2	300	120	10	11	159	(56.7)
17.9±0.1	250	103	15	16	116	(52.8)

⚥—Hermaphrodites ♂N—Males with normal testes * Including hermaphrodites the 12 experimental series were $17.9 \pm 0.1 \sim 22.7 \pm 0.2$ mm in mean body length. There were no remarkable differences in the body length of frogs among the 12 experimental series and the controls. One month after completion of metamorphosis, all the living frogs in the experimental and control series were killed to examine their sex (Table 50).

a. Control series

{(N♀ × N♂)♀ × N♂}♀ × N♂, Nos. 1 ~ 5

Sixteen of 492 normally metamorphosed frogs died within one month after metamorphosis. The remaining frogs were killed to examine their sex; 239 were females with normal or underdeveloped ovaries, 2 were hermaphrodites with gonads transforming from ovaries into testes and 235 were males with normal testes. When the hermaphrodites were counted as males, 49.8% were males.

b. Experimental series derived from X-irradiated sperm

i) {(N♀ × SX-90♂)♀ × N♂}♀ × N♂, Nos. 1 ~ 5

Only one of 262 metamorphosed frogs died within one month after metamorphosis. All the other frogs were killed; 128 were females with normal or underdeveloped ovaries, 12 were hermaphrodites with gonads transforming from ovaries into testes and 121 were males with normal testes. When the hermaphrodites were counted as males, 51.0% were males.

ii) {(N♀ × SX-170♂)♀ × N♂}♀ × N♂, Nos. 1 ~ 5

Six of 246 metamorphosed frogs died within one month after metamorphosis. The other 240 frogs were killed; 118 were females, 16 hermaphrodites and 106 males. When the hermaphrodites were counted as males, 50.8% were males.

iii) {(N♀ × SX-240♂)♀ × N♂}♀ × N♂, Nos. 1 ~ 5

Nine of 255 metamorphosed frogs died within one month after metamorphosis. The remaining 246 frogs were killed; 113 were females, 29 hermaphrodites and 104 males. When the hermaphrodites were counted as males, 54.1% were males.

c. Experimental series derived from X-irradiated eggs

i) {(EX-90♀ × N♂)♀ × N♂}♀ × N♂, Nos. 1 ~ 5

Thirteen of 368 metamorphosed frogs died within one month after metamorphosis. The other 355 were killed; 171 were females, 31 hermaphrodites and 153 males. When the hermaphrodites were counted as males, 51.8% were males.

ii) {(EX-145♀ × N♂)♀ × N♂}♀ × N♂, Nos. 1 ~ 5

Seven of 389 metamorphosed frogs died within one month after metamorphosis. The other 382 frogs were killed; 182 were females, 29 hermaphrodites and 171 males. When the hermaphrodites were counted as males, 52.4% were males.

iii) {(EX-200♀ × N♂)♀ × N♂}♀ × N♂, Nos. 1 and 2

All the 15 metamorphosed frogs were living one month after metamorphosis. They were killed; seven were females and eight males.

d. Experimental series derived from neutron-irradiated sperm

i) {(N♀ × SN–50♂)♀ × N♂}♀ × N♂, Nos. 1∼3

Six of 166 metamorphosed frogs died within one month after metamorphosis. The other 160 were killed; 75 were females, eight hermaphrodites and 77 males. When the hermaphrodites were counted as males, 53.1% were males.

ii) {(N♀ × SN–90♂)♀ × N♂}♀ × N♂, Nos. 1∼5

Seven of 300 metamorphosed frogs died within one month after metamorphosis. The other 293 were killed; 135 were females, 27 hermaphrodites and 131 males. When the hermaphrodites were counted as males, 53.9% were males.

iii) {(N♀ × SN–130♂)♀ × N♂}♀ × N♂, Nos. 1∼5

Four of 328 metamorphosed frogs died within one month after metamorphosis. The other 324 were killed; 139 were females, 34 hermaphrodites and 151 males. When the hermaphrodites were counted as males, 57.1% were males.

e. Experimental series derived from neutron-irradiated eggs

i) {(EN–50♀ × N♂)♀ × N♂}♀ × N♂, Nos. 1∼5

Only one of 291 metamorphosed frogs died within one month after metamorphosis. The other 290 frogs were killed; 141 were females, seven hermaphrodites and 142 males. When the hermaphrodites were counted as males, 51.4% were males.

ii) {(EN–90♀ × N♂)♀ × N♂}♀ × N♂, Nos. 1∼5

Four of 304 metamorphosed frogs died within one month after metamorphosis. The other 300 frogs were killed; 130 were females, 11 hermaphrodites and 159 males. When the hermaphrodites were counted as males, 56.7% were males.

iii) {(EN–130♀ × N♂)♀ × N♂}♀ × N♂, Nos. 1∼4

Two of 252 metamorphosed frogs died within one month after metamorphosis. The other 250 were killed; 118 were females, 16 hermaphrodites and 116 males. When the hermaphrodites were counted as males, 52.8% were males.

VIII. Fourth-generation offspring derived from irradiated gametes by passing over male first-, second- and third-generation offspring

1. Male parents

As male first-generation offspring raised from irradiated gametes in 1967 matured in 1969, they were mated with normal females collected from the field to produce second-generation offspring. As male second-generation offspring matured in 1971, they were then mated again with normal females collected from the field to produce third-generation offspring. As five males of these third-generation offsprings matured in 1973, they were still again mated with five normal females collected from the field to produce fourth-generation offspring. These normal females were 64.0∼70.5 mm, 67.6 mm on the average, in body length (Table 51). After pituitary injection each of them discharged 2084∼2521 eggs. Fifty eggs removed at random from the spawn of each female were $1.73 \pm 0.01 \sim 1.91 \pm 0.01$ mm in diameter.

TABLE 51

Eggs of five field-caught female frogs used for mating experiments in 1973

Kind	Individual no.	Body length (mm)	No. of eggs	Mean diameter of 50 eggs (mm)
N.W73	1	70.5	2456	1.73±0.01
	2	69.0	2355	1.75±0.01
	3	69.5	2521	1.77±0.01
	4	65.0	2219	1.77±0.01
	5	64.0	2084	1.91±0.01

Five male third-generation offspring Nos. 1～5 produced from mating No. 3 in the control series were utilized as the controls of the male third-generation offspring derived from irradiated gametes (Table 52). They were two years old and 60.5～63.5 mm, 61.5 mm on the average, in body length. Their testes were 4.5×2.5～5.5×3.0 mm in length and width and were quite normal (Type 1) in inner structure.

TABLE 52

Testes of five control male frogs used for mating experiments in 1973

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure
				Left (mm)	Right (mm)	
N{N(N·N)}, No. 3	1	2	60.5	5.0×2.5	5.0×2.5	Type 1
	2	2	60.5	5.0×2.5	5.0×2.5	Type 1
	3	2	61.0	4.5×2.5	4.5×2.5	Type 1
	4	2	62.0	5.5×3.0	5.5×2.5	Type 1
	5	2	63.5	5.5×3.0	5.5×3.0	Type 1

N{N(N·N)}, No. 3: Males obtained by N·W71♀, Nos. 1～12×N(N·N)♂, No. 3

In the experimental series, a total of 57 male third-generation offspring derived from irradiated gametes were used for producing fourth-generation offspring (Tables 53 and 54). All of them were two years old and 56.0～63.5 mm, 59.8 mm on the average, in body length. Their testes were 3.0×2.0～5.5×3.0 mm in length and width. All the testes were quite normal (Type 1) in inner structure. These male third-generation offspring were those produced in 12 experimental series sorted into four groups. Five (Nos. 1～5) of 15 male third-generation offspring belonging to the first group were produced from the mating No. 3 of the experimental series derived from a spermatozoon which had been exposed to 90 rads of X-rays (Table 53). They were 57.5～61.0 mm, 59.1 mm on the average, in body length. Their testes were 4.5×2.0～5.0×2.5 mm in length and width. Five other males (Nos. 1～5) were produced from mating No. 1 of the experimental series derived from a spermatozoon which had been exposed to 170 rads of X-rays. They were 56.5～61.0 mm, 58.8 mm on the average, in body length. Their testes were 3.0×2.0～5.5×2.5 mm in length and width. The remaining five males (Nos. 1～5) were produced from mating No. 1 of the

TABLE 53

Testes of thirty male frogs derived from X-irradiated great-grandparental sperm or eggs and used for mating experiments in 1973

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure
				Left (mm)	Right (mm)	
N{N(N·SX–90)}, No. 3	1	2	58.5	4.5×2.5	4.5×2.5	Type 1
	2	2	60.0	5.0×2.5	5.0×2.0	Type 1
	3	2	61.0	4.5×3.0	4.5×2.5	Type 1
	4	2	58.5	4.5×3.0	4.5×3.0	Type 1
	5	2	57.5	4.5×2.0	4.5×2.0	Type 1
N{N(N·SX–170)}, No. 1	1	2	60.5	5.5×2.5	4.0×2.5	Type 1
	2	2	59.0	5.0×2.5	5.0×2.5	Type 1
	3	2	57.0	3.5×2.0	3.0×2.0	Type 1
	4	2	56.5	4.0×2.0	4.0×3.0	Type 1
	5	2	61.0	5.0×2.5	5.0×2.0	Type 1
N{N(N·SX–240)}, No. 1	1	2	57.0	4.0×2.0	4.0×2.0	Type 1
	2	2	59.5	4.0×2.5	5.0×2.0	Type 1
	3	2	59.0	4.5×2.5	4.5×2.5	Type 1
	4	2	60.0	4.5×2.0	4.5×2.0	Type 1
	5	2	60.0	5.0×3.0	5.0×2.5	Type 1
N{N(EX–90·N)}, No. 2	1	2	61.5	5.0×2.0	5.0×2.0	Type 1
	2	2	62.0	5.0×2.5	5.0×2.5	Type 1
	3	2	57.5	3.5×2.5	4.5×2.5	Type 1
	4	2	57.0	4.5×2.5	4.5×2.5	Type 1
	5	2	60.5	5.0×2.5	5.0×2.5	Type 1
N{N(EX–145·N)}, No. 3	1	2	57.5	4.0×2.0	4.0×2.0	Type 1
	2	2	57.0	4.0×2.5	3.5×2.5	Type 1
	3	2	60.5	5.0×3.0	5.0×2.5	Type 1
	4	2	62.0	5.5×3.0	4.0×3.0	Type 1
	5	2	62.0	4.5×2.5	4.5×2.0	Type 1
N{N(EX–200·N)}, No. 2	1	2	60.0	5.0×2.5	5.0×2.5	Type 1
	2	2	61.5	4.5×2.0	4.5×2.0	Type 1
	3	2	60.5	4.5×2.0	4.5×2.0	Type 1
	4	2	59.0	4.0×2.0	4.0×2.0	Type 1
	5	2	57.5	4.5×2.0	4.0×2.0	Type 1

N{N(N·SX–90)}, No. 3: Males obtained by N·W71 ♀, Nos. 1∼3×N(N·SX–90) ♂, No. 3
N{N(N·SX–170)}, No. 1: Males obtained by N·W71 ♀, Nos. 1∼3×N(N·SX–170) ♂, No. 1
N{N(N·SX–240)}, No. 1: Males obtained by N·W71 ♀, Nos. 1∼3×N(N·SX–240) ♂, No. 1
N{N(EX–90·N)}, No. 2: Males obtained by N·W71 ♀, Nos. 4∼6×N(EX–90·N) ♂, No. 2
N{N(EX–145·N)}, No. 3: Males obtained by N·W71 ♀, Nos. 4∼6×N(EX–145·N) ♂, No. 3
N{N(EX–200·N)}, No. 2: Males obtained by N·W71 ♀, Nos. 4∼6×N(EX–200·N) ♂, No. 2

experimental series derived from a spermatozoon which had been exposed to 240 rads of X-rays. They were 57.0∼60.0 mm, 59.1 mm on the average, in body length. Their testes were 4.0×2.0∼5.0×3.0 mm in length and width.

Five (Nos. 1∼5) of 15 male third-generation offspring belonging to the second group were produced from mating No. 2 of the experimental series derived from an egg which had been exposed to 90 rads of X-rays. They were 57.0∼62.0 mm, 59.7 mm on the average, in body length. Their testes were 3.5×2.5∼5.0×2.5 mm in length and width. Five other males (Nos. 1∼5) were produced from mating No. 3 of the experimental series derived from an egg which had been exposed to 145 rads of X-rays. They were 57.0~62.0 mm, 59.8 mm on the average, in body length. Their testes were 4.0×2.0~5.5×3.0 mm in length and width. The remaining five males (Nos. 1~5) were produced from mating No. 2 of the experimental series derived from an egg which had been exposed to 200 rads of X-rays. They were 57.5~61.5 mm, 59.7 mm on the average, in body length. Their testes were 4.0×2.0~5.0×2.5 mm in length and width.

Five (Nos. 1~5) of 12 male third-generation offspring belonging to the third group were produced from mating No. 1 of the experimental series derived from a spermatozoon which had been exposed to 50 rads of neutrons (Table 54). They were 58.5~60.5 mm, 59.4 mm on the average, in body length. Their

TABLE 54

Testes of twenty-seven male frogs derived from neutron-irradiated great-grandparental sperm or eggs and used for mating experiments in 1973

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes Left (mm)	Size of the testes Right (mm)	Inner structure
N{N(N·SN-50)}, No. 1	1	2	58.5	4.5×2.0	4.5×2.0	Type 1
	2	2	59.0	5.0×2.5	4.5×2.5	Type 1
	3	2	59.0	5.0×2.5	5.0×2.5	Type 1
	4	2	60.0	4.5×2.5	4.5×2.5	Type 1
	5	2	60.5	4.0×2.0	4.0×2.0	Type 1
N{N(N·SN-90)}, No. 1	1	2	62.0	5.0×2.5	5.0×2.5	Type 1
	2	2	61.5	4.5×2.5	4.5×3.0	Type 1
N{N(N·SN-130)}, No. 5	1	2	57.0	4.0×2.0	4.0×2.0	Type 1
	2	2	56.0	4.0×2.0	4.0×2.0	Type 1
	3	2	60.5	4.5×2.5	4.5×2.5	Type 1
	4	2	61.0	4.5×2.0	4.5×2.0	Type 1
	5	2	63.0	5.5×3.0	4.0×1.5	Type 1
N{N(EN-50·N)}, No. 3	1	2	59.0	5.0×2.5	5.0×2.5	Type 1
	2	2	59.0	4.5×2.0	4.5×2.0	Type 1
	3	2	61.5	5.0×2.5	4.0×3.0	Type 1
	4	2	62.0	5.0×2.5	5.0×2.5	Type 1
	5	2	63.5	5.5×3.0	5.5×3.0	Type 1
N{N(EN-90·N)}, No. 3	1	2	57.5	5.0×2.0	5.0×2.0	Type 1
	2	2	59.0	4.5×2.5	4.5×2.5	Type 1
	3	2	60.5	4.5×2.5	4.5×3.0	Type 1
	4	2	59.5	4.5×2.5	4.0×2.5	Type 1
	5	2	61.0	5.0×2.5	5.0×3.0	Type 1
N{N(EN-130·N)}, No. 1	1	2	60.0	4.5×2.5	4.0×2.5	Type 1
	2	2	61.5	4.5×3.0	4.0×3.0	Type 1
	3	2	61.5	4.5×2.5	4.5×2.5	Type 1
	4	2	62.0	5.0×2.5	5.0×2.5	Type 1
	5	2	63.0	5.0×3.0	5.0×2.5	Type 1

N{N(N·SN-50)}, No. 1: Males obtained by N·W71 ♀, Nos. 7~9×N(N·SN-50) ♂, No. 1
N{N(N·SN-90)}, No. 1: Males obtained by N·W71 ♀, Nos. 7~9×N(N·SN-90) ♂, No. 1
N{N(N·SN-130)}, No. 5: Males obtained by N·W71 ♀, Nos. 7~9×N(N·SN-130) ♂, No. 5
N{N(EN-50·N)}, No. 3: Males obtained by N·W71 ♀, Nos. 10~12×N(EN-50·N) ♂, No. 3
N{N(EN-90·N)}, No. 3: Males obtained by N·W71 ♀, Nos. 10~12×N(EN-90·N) ♂, No. 3
N{N(EN-130·N)}, No. 1: Males obtained by N·W71 ♀, Nos. 10~12×N(EN-130·N) ♂, No. 1

testes were 4.0×2.0∼5.0×2.5 mm in length and width. Two other males (Nos. 1 and 2) were produced from mating No. 1 of the experimental series derived from a spermatozoon which had been exposed to 90 rads of neutrons. They were 62.0 and 61.5 mm, 61.8 mm on the average, in body length and had testes which were 4.5×2.5∼4.5×3.0 mm in length and width. The remaining five males (Nos. 1∼5) were produced from the mating No. 5 of the experimental series derived from a spermatozoon which had been exposed to 130 rads of neutrons. They were 56.0∼63.0 mm, 59.5 mm on the average, in body length. Their testes were 4.0×2.0∼5.5×3.0 mm in length and width.

Five (Nos. 1∼5) of 15 male third-generation offspring belonging to the fourth group were produced from mating No. 3 of the experimental series derived from an egg which had been exposed to 50 rads of neutrons. They were 59.0∼63.5 mm, 61.0 mm on the average, in body length. Their testes were 4.5×2.0∼5.5×3.0 mm in length and width. Five other males (Nos. 1∼5) were produced from mating No. 3 of the experimental series derived from an egg which was exposed to 90 rads of neutrons. They were 57.5∼61.0 mm, 59.5 mm on the average, in body length. Their testes were 4.0×2.5∼5.0×3.0 mm in length and width. The remaining five males (Nos. 1∼5) were produced from mating No. 1 of the experimental series derived from an egg which had been exposed to 130 rads of neutrons. They were 60.0∼63.0 mm, 61.6 mm on the average, in body length. Their testes were 4.0×2.5∼5.0×3.0 mm in length and width.

Mating in each of the experimental and control series was performed in the way that eggs of a female were artificially inseminated with spermatozoa of a male of the same number as that of the female.

2. Developmental capacity

a. Control series

N♀×[N♀×{N♀×(N♀×N♂)♂}♂]♂, Nos. 1∼5

Five male third-generation offspring Nos. 1∼5 in the control series were mated with five females Nos. 1∼5 collected from the field to produce fourth-generation offspring (Table 55). In five matings Nos. 1∼5, 82.1∼97.9%, 88.8% on the average, of the respective total number of eggs cleaved normally. Only a few of the normally cleaved eggs died of various abnormalities at various embryonic stages; 76.9∼92.8%, 83.6% on the average, hatched normally. During the tadpole stage, a few individuals died of underdevelopment, edema or some other abnormalities; 74.4∼92.8%, 81.8% on the average, became normal, metamorphosed frogs.

b. Experimental series derived from X-irradiated sperm

i) N♀×[N♀×{N♀×(N♀×SX–90♂)♂}♂]♂, Nos. 1∼5

Five male third-generation offspring Nos. 1∼5 were mated with the same five females as those used in the control series (Table 55). In five matings Nos. 1∼5, 88.8∼99.0%, 92.7% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental

TABLE 55

Developmental capacity of the offspring of male frogs derived

Parents		No. of eggs	No. of cleaved eggs	
Female	Male		Normal	Abnormal
N·W73, Nos. 1～5	N{N(N·N)}, Nos. 1～5	556	494 (88.8%)	2 (0.4%)
N·W73, Nos. 1～5	N{N(N·SX–90)}, Nos. 1～5	920	853 (92.7%)	1 (0.1%)
	N{N(N·SX–170)}, Nos. 1～5	922	832 (90.2%)	3 (0.3%)
	N{N(N·SX–240)}, Nos. 1～5	722	642 (88.9%)	1 (0.1%)
N·W73, Nos. 1～5	N{N(EX–90·N)}, Nos. 1～5	896	800 (89.3%)	2 (0.2%)
	N{N(EX–145·N)}, Nos. 1～5	900	823 (91.4%)	2 (0.2%)
	N{N(EX–200·N)}, Nos. 1～5	757	689 (91.0%)	1 (0.1%)
N·W73, Nos. 1～5	N{N(N·SN–50)}, Nos. 1～5	767	687 (89.6%)	2 (0.3%)
	N{N(N·SN–90)}, Nos. 1, 2	443	366 (82.6%)	0
	N{N(N·SN–130)}, Nos. 1～5	758	659 (86.9%)	2 (0.3%)
N·W73, Nos. 1～5	N{N(EN–50·N)}, Nos. 1～5	651	554 (85.1%)	2 (0.3%)
	N{N(EN–90·N)}, Nos. 1～5	762	677 (88.8%)	3 (0.4%)
	N{N(EN–130·N)}, Nos. 1～5	665	573 (86.2%)	1 (0.2%)

capacity to those in the control series; 82.9～96.4%, 89.2% on the average, hatched normally, and afterwards 80.5～94.3%, 87.0% on the average, metamorphosed normally.

ii) N♀×[N♀×{N♀×(N♀×SX–170♂)♂}♂]♂, Nos. 1～5

Five male third-generation offspring Nos. 1～5 were mated with field-caught females Nos. 1～5. In five matings Nos. 1～5, 87.0～96.1%, 90.2% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to those in the control series; 83.4～90.0%, 86.7% on the average, hatched normally, and afterwards 79.2～88.9%, 84.3% on the average, metamorphosed normally.

iii) N♀×[N♀×{N♀×(N♀×SX–240♂)♂}♂]♂, Nos. 1～5

Five male third-generation offspring Nos. 1～5 were mated with field-caught females Nos. 1～5. In five matings Nos. 1～5, 77.9～95.3%, 88.9% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to those of the control series; 76.0～93.7%, 84.9% on the average, hatched normally, and afterwards 74.0～91.2%, 82.5% on the average, metamorphosed normally.

from irradiated great-grandparental sperm or eggs, I

No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of 50-day-old tadpoles	No. of meta-morphose frogs
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal		
490	4	482	8	465	17	461	455
(88.1%)	(0.7%)	(86.7%)	(1.4%)	(83.6%)	(3.1%)	(82.9%)	(81.8%)
846	3	833	13	821	12	814	800
(92.0%)	(0.3%)	(90.5%)	(1.4%)	(89.2%)	(1.3%)	(88.5%)	(87.0%)
823	5	812	11	799	13	787	777
(89.3%)	(0.5%)	(88.1%)	(1.2%)	(86.7%)	(1.4%)	(85.4%)	(84.3%)
638	1	626	12	613	13	604	596
(88.4%)	(0.1%)	(86.7%)	(1.7%)	(84.9%)	(1.8%)	(83.7%)	(82.5%)
793	6	781	12	767	14	761	745
(88.5%)	(0.7%)	(87.2%)	(1.3%)	(85.6%)	(1.6%)	(84.9%)	(83.1%)
819	3	809	10	795	14	785	776
(91.0%)	(0.3%)	(89.9%)	(1.1%)	(88.3%)	(1.6%)	(87.2%)	(86.2%)
685	3	664	21	654	10	627	602
(90.5%)	(0.4%)	(87.7%)	(2.8%)	(86.4%)	(1.3%)	(82.8%)	(79.5%)
679	6	668	11	656	12	647	631
(88.5%)	(0.8%)	(87.1%)	(1.4%)	(85.5%)	(1.6%)	(84.4%)	(82.3%)
364	2	361	3	353	8	347	335
(82.2%)	(0.5%)	(81.5%)	(0.7%)	(79.7%)	(1.8%)	(78.3%)	(75.6%)
650	8	643	7	623	20	619	608
(85.8%)	(1.1%)	(84.8%)	(0.9%)	(82.2%)	(2.6%)	(81.7%)	(80.2%)
548	5	541	7	515	26	506	493
(84.2%)	(0.8%)	(83.1%)	(1.1%)	(79.1%)	(4.0%)	(77.7%)	(75.7%)
673	3	666	7	646	20	636	631
(88.3%)	(0.4%)	(87.4%)	(0.9%)	(84.8%)	(2.6%)	(83.5%)	(82.8%)
568	4	556	12	534	22	524	516
(85.4%)	(0.6%)	(83.6%)	(1.8%)	(80.3%)	(3.3%)	(78.8%)	(77.6%)

c. Experimental series derived from X-irradiated eggs

i) N♀×[N♀×{N♀×(EX–90♀×N♂)♂}♂]♂, Nos. 1∼5

Five male third-generation offspring Nos. 1∼5 were mated with field-caught females Nos. 1∼5. In five matings Nos. 1∼5, 81.2∼93.7%, 89.3% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to those in the control series; 76.4∼89.9%, 85.6% on the average, hatched normally, and afterwards 75.2∼88.4%, 83.1% on the average, became normal, metamorphosed frogs.

ii) N♀×[N♀×{N♀×(EX–145♀×N♂)♂}♂]♂, Nos. 1∼5

Five male third-generation offspring Nos. 1∼5 were mated with field-caught females Nos. 1∼5. In five matings Nos. 1∼5, 89.3∼93.1%, 91.4% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to those of the control series; 85.3∼91.4%, 88.3% on the average, hatched normally, and afterwards 84.1∼89.7%, 86.2% on the average, metamorphosed normally.

iii) N♀×[N♀×{N♀×(EX–200♀×N♂)♂}♂]♂, Nos. 1∼5

Five male third-generation offspring Nos. 1∼5 were mated with field-caught females Nos. 1～5. In five matings Nos. 1～5, 84.2～98.2%, 91.0% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to those of the control series during the embryonic stage; 76.5～93.9%, 86.4% on the average, hatched normally. During the tadpole stage, only a few individuals produced from matings Nos. 1～4 died; 81.4～92.1% metamorphosed normally. However, 36 of 140 individuals produced from the remaining mating No. 5 died of underdevelopment or edema during the first half of the tadpole stage, while the other 104 (56.8%) metamorphosed normally. On the average, 79.5% became normal, metamorphosed frogs.

d. Experimental series derived from neutron-irradiated sperm

i) N♀×[N♀×{N♀×(N♀×SN-50♂)♂}♂]♂, Nos. 1～5

Five male third-generation offspring Nos. 1～5 were mated with field-caught females Nos. 1～5 (Table 55). In five matings Nos. 1～5, 82.9～96.3%, 89.6% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were nearly the same in developmental capacity as those of the control series; 76.7～88.1%, 85.5% on the average, hatched normally, and afterwards 73.3～87.7%, 82.3% on the average, metamorphosed normally.

ii) N♀×[N♀×{N♀×(N♀×SN-90♂)♂}♂]♂, Nos. 1 and 2

Two male third-generation offspring Nos. 1 and 2 were mated with the same two females Nos. 1 and 2 as those used in the control series. In two matings Nos. 1 and 2, 89.8% and 75.8%, 82.6% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were nearly the same in developmental capacity as those of the control series; 86.1% and 73.6%, 79.7% on the average, hatched normally, and afterwards 81.0% and 70.5%, 75.6% on the average, metamorphosed normally.

iii) N♀×[N♀×{N♀×(N♀×SN-130♂)♂}♂]♂, Nos. 1～5

Five male third-generation offspring Nos. 1～5 were mated with the same five females Nos. 1～5 as those used in the control series. In five matings Nos. 1～5, 83.8～94.1%, 86.9% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to those of the control series; 75.3～87.6%, 82.2% on the average, hatched normally, and afterwards 75.3～87.1%, 80.2% on the average, became normal, metamorphosed frogs.

e. Experimental series derived from neutron-irradiated eggs

i) N♀×[N♀×{N♀×(EN-50♀×N♂)♂}♂]♂, Nos. 1～5

Five male third-generation offspring Nos. 1～5 were mated with field-caught females Nos. 1～5 (Table 55). In five matings Nos. 1～5, 81.9～90.2%, 85.1% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were nearly the same in developmental capacity as those of the control series; 74.3～89.3%, 79.1% on the average, hatched normally, and afterwards 70.5～85.7%, 75.7% on the average, metamorphosed normally.

ii) N♀ × [N♀ × {N♀ × (EN–90♀ × N♂)♂}♂]♂, Nos. 1 ~ 5

Five male third-generation offspring Nos. 1 ~ 5 were mated with field-caught females Nos. 1 ~ 5. In five matings Nos. 1 ~ 5, 78.0 ~ 97.1%, 88.8% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to those of the control series; 75.5 ~ 93.7%, 84.8% on the average, hatched normally, and afterwards 71.7 ~ 92.3%, 82.8% on the average, metamorphosed normally.

iii) N♀ × [N♀ × {N♀ × (EN–130♀ × N♂)♂}♂]♂, Nos. 1 ~ 5

Five male third-generation offspring Nos. 1 ~ 5 were mated with field-caught females Nos. 1 ~ 5. In five matings Nos. 1 ~ 5, 74.6 ~ 97.6%, 86.2% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were nearly the same in developmental capacity as those of the control series; 74.6 ~ 91.1%, 80.3% on the average, hatched normally, and 73.0 ~ 87.0%, 77.6% on the average, became normal, metamorphosed frogs.

3. Viability and sex of metamorphosed frogs

In the control series, 455 tadpoles produced from five matings climbed out of water at the age of 83 ~ 92 days, 86.5 days on the average (Table 56). In the 12 experimental series, 7510 tadpoles produced from 57 matings climbed out of water at the age of 80 ~ 113 days, 83.5 ~ 96.8 days on the average in each series. There was no definite difference in the average age when tadpoles could climb out of water between the experimental and control series, or between different experimental series, except one of the latter. The tadpoles produced from the matings in the experimental series derived from a spermatozoon which had been exposed to 90 rads of X-rays were definitely retarded in metamorphosis, as compared with those of the control series as well as the other 11 experimental series.

In the control series, 50 frogs were at random removed from those produced from each of the five matings immediately after completion of metamorphosis in order to measure their body length; 250 frogs in total were 21.5 ± 0.1 mm in mean body length. In each of the experimental series, 50 frogs were also at random removed from the fourth-generation offspring produced from each mating to measure their body length immediately after completion of metamorphosis. A total of 250 or 100 frogs in each series were 19.1 ± 0.2 ~ 21.6 ± 0.2 mm in mean body length. There were no remarkable differences in body length of frogs among the 12 experimental series as well as between the experimental and control series.

One month after completion of metamorphosis, all the living frogs in the experimental and control series were killed to examine their sex (Table 56).

a. Control series

N♀ × [N♀ × {N♀ × (N♀ × N♂)♂}♂]♂, Nos. 1 ~ 5

Nine of 455 normally metamorphosed frogs died within one month after metamorphosis. The remaining 446 frogs were killed to examine their sex; 224 were females, five hermaphrodites and 217 males. When the hermaphrodites were counted as males, 49.8% were males.

TABLE 56

Number, size and sex of metamorphosed frogs derived

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed frogs
Female	Male		
N.W73, Nos. 1∼5	N{N(N·N)}, Nos. 1∼5	83∼92 (86.5)	455
N.W73, Nos. 1∼5	N{N(N·SX–90)}, Nos. 1∼5	86∼113 (96.8)	800
	N{N(N·SX–170)}, Nos. 1∼5	81∼97 (83.5)	777
	N{N(N·SX–240)}, Nos. 1∼5	80∼105 (91.3)	596
N.W73, Nos. 1∼5	N{N(EX–90·N)}, Nos. 1∼5	80∼94 (86.7)	745
	N{N(EX–145·N)}, Nos. 1∼5	85∼98 (90.2)	776
	N{N(EX–200·N)}, Nos. 1∼5	81∼112 (83.6)	602
N.W73, Nos. 1∼5	N{N(N·SN–50)}, Nos. 1∼5	85∼95 (91.3)	631
	N{N(N·SN–90)}, Nos. 1, 2	83∼105 (91.9)	335
	N{N(N·SN–130)}, Nos. 1∼5	83∼107 (91.1)	608
N.W73, Nos. 1∼5	N{N(EN–50·N)}, Nos. 1∼5	83∼109 (91.3)	493
	N{N(EN–90·N)}, Nos. 1∼5	80∼104 (83.6)	631
	N{N(EN–130·N)}, Nos. 1∼5	83∼95 (86.3)	516

$♀_N$—Females with normal ovaries $♀_U$—Females with underdeveloped ovaries

b. Experimental series derived from X-irradiated sperm

i) N♀×[N♀×{N♀×(N♀×SX–90♂)♂}♂]♂, Nos. 1∼5

Of 800 normally metamorphosed frogs, 64 died within one month after metamorphosis. The other 736 frogs were killed; 362 were females, 37 hermaphrodites and 337 males. When the hermaphrodites were counted as males, 50.8% were males.

ii) N♀×[N♀×{N♀×(N♀×SX–170♂)♂}♂]♂, Nos. 1∼5

Twenty-two of 777 normally metamorphosed frogs died within one month after metamorphosis. The other 755 were killed; 373 were females, 35 hermaphrodites and 347 males. When the hermaphrodites were counted as males, 50.6% were males.

iii) N♀×[N♀×{N♀×(N♀×SX–240♂)♂}♂]♂, Nos. 1∼5

Only four of 596 normally metamorphosed frogs died within one month after metamorphosis. The other 592 frogs were killed; 292 frogs were females, 15 hermaphrodites and 285 males. When the hermaphrodites were counted as from irradiated great-great-grandparental sperm or eggs, I

Body length immediately after metamorphosis (mm)	Sex of frogs killed about one month after metamorphosis					
	No. of frogs	♀N	♀U	⚥	♂N	♂ (%)*
21.5±0.1	446	213	11	5	217	(49.8)
21.3±0.2	736	332	30	37	337	(50.8)
19.6±0.2	755	342	31	35	347	(50.6)
19.1±0.2	592	279	13	15	285	(50.7)
20.5±0.2	720	331	22	26	341	(51.0)
19.5±0.2	754	336	33	45	340	(51.1)
20.1±0.2	600	277	20	34	269	(50.5)
19.7±0.2	620	282	26	28	284	(50.3)
20.7±0.1	333	154	9	12	158	(51.1)
19.9±0.2	605	262	18	27	298	(53.7)
20.3±0.1	475	220	14	18	223	(50.7)
21.6±0.2	626	283	24	19	300	(51.0)
21.4±0.1	513	224	29	30	230	(50.7)

⚥—Hermaphrodites ♂N—Males with normal testes * Including hermaphrodites

males, 50.7% were males.

c. Experimental series derived from X-irradiated eggs

i) N♀×[N♀×{N♀×(EX–90♀×N♂)♂}♂]♂, Nos. 1∼5

Twenty-five of 745 normally metamorphosed frogs died within one month after metamorphosis. The other 720 frogs were killed; 353 were females, 26 hermaphrodites and 341 males. When the hermaphrodites were counted as males, 51.0% were males.

ii) N♀×[N♀×{N♀×(EX–145♀×N♂)♂}♂]♂, Nos. 1∼5

Twenty-two of 776 normally metamorphosed frogs died within one month after metamorphosis. The other 754 were killed; 369 were females, 45 hermaphrodites and 340 males. When the hermaphrodites were counted as males, 51.1% were males.

iii) N♀×[N♀×{N♀×(EX–200♀×N♂)♂}♂]♂, Nos. 1∼5

Only two of 602 normally metamorphosed frogs died within one month after metamorphosis. The other 600 frogs were killed; 297 were females, 34 hermaphrodites and 269 males. When the hermaphrodites were counted as males, 50.5% were males.

d. Experimental series derived from neutron-irradiated sperm

i) N♀ × [N♀ × {N♀ × (N♀ × SN–50♂) ♂} ♂] ♂, Nos. 1 ~ 5

Eleven of 631 normally metamorphosed frogs died within one month after metamorphosis. The other 620 frogs were killed; 308 were females, 28 hermaphrodites and 284 males. When the hermaphrodites were counted as males, 50.3% were males.

ii) N♀ × [N♀ × {N♀ × (N♀ × SN–90♂) ♂} ♂] ♂, Nos. 1 and 2

Only two of 335 normally metamorphosed frogs died within one month after metamorphosis. The other 333 frogs were killed; 163 were females, 12 hermaphrodites and 158 males. When the hermaphrodites were counted as males, 51.1% were males.

iii) N♀ × [N♀ × {N♀ × (N♀ × SN–130♂) ♂} ♂] ♂, Nos. 1 ~ 5

Only three of 608 normally metamorphosed frogs died within one month after metamorphosis. The other 605 frogs were killed; 280 were females, 27 hermaphrodites and 298 males. When the hermaphrodites were counted as males, 53.7% were males.

e. Experimental series derived from neutron-irradiated eggs

i) N♀ × [N♀ × {N♀ × (EN–50♀ × N♂) ♂} ♂] ♂, Nos. 1 ~ 5

Eighteen of 493 normally metamorphosed frogs died within one month after metamorphosis. The other 475 frogs were killed; 234 were females, 18 hermaphrodites and 223 males. When the hermaphrodites were counted as males, 50.7% were males.

ii) N♀ × [N♀ × {N♀ × (EN–90♀ × N♂) ♂} ♂] ♂, Nos. 1 ~ 5

Only five of 631 normally metamorphosed frogs died within one month after metamorphosis. The other 626 frogs were killed; 307 were females, 19 hermaphrodites and 300 males. When the hermaphrodites were counted as males, 51.0% were males.

iii) N♀ × [N♀ × {N♀ × (EN–130♀ × N♂) ♂} ♂] ♂, Nos. 1 ~ 5

Only three of 516 normally metamorphosed frogs died within one month after metamorphosis. The other 513 frogs were killed; 253 were females, 30 hermaphrodites and 230 males. When the hermaphrodites were counted as males, 50.7% were males.

IX. Fourth-generation offspring derived from irradiated gametes by passing over male first-generation, male second-generation and female third-generation offspring

1. Female parents

Female third-generation offspring derived from X- or neutron-irradiated spermatozoa or eggs matured in 1973. Eggs of these females were fertilized with sperm of three normal males collected from the field to produce fourth-generation offspring. The female third-generation offspring were produced in 1971 from male second-generation offspring by mating with normal females from the field. These male second-generation offspring were produced in 1969 from male first-generation offspring by mating with normal females collected from the field. The first-generation offspring were obtained in 1967 from irradiated gametes by fertilization with normal gametes.

Three field-caught males Nos. 6～8 which were mated with the female third-generation offspring to produce fourth-generation offspring were 59.5～67.0 mm, 62.2 mm on the average, in body length (Table 57). Their testes were 4.5×2.5～5.0×3.0 mm in length and width and quite normal (Type 1) in inner structure. In the control series, five females Nos. 1～5 were mated with these three males (Table 58). They were 2-year-old third-generation offspring produced from mating No. 3 between a male second-generation offspring and a field-caught

TABLE 57

Testes of three field-caught male frogs used for mating experiments in 1973

Kind	Individual no.	Body length (mm)	Size of the testes		Inner structure
			Left (mm)	Right (mm)	
N.W73	6	67.0	5.0×2.5	5.0×2.5	Type 1
	7	60.0	5.0×3.0	4.5×2.5	Type 1
	8	59.5	4.5×3.0	4.5×3.0	Type 1

TABLE 58

Eggs of five control female frogs used for mating experiments in 1973

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 50 eggs (mm)
N{N(N·N)}, No. 3	1	2	60.5	957	1.83±0.02
	2	2	60.5	986	1.89±0.02
	3	2	64.0	1211	1.83±0.02
	4	2	61.0	874	2.04±0.02
	5	2	61.5	0	—

N{N(N·N)}, No. 3: Females obtained by N.W71 ♀, Nos. 1～3×N(N·N) ♂, No. 3

female. This male second-generation offspring was derived from a male first-generation offspring of a normal male and a normal female collected from the field. The five females that were utilized to produce fourth-generation offspring in the control series were 60.5～64.0 mm, 61.5 mm on the average, in body length. After pituitary injection four of them discharged 874～1211 eggs, 1007 on the average, which were 1.83±0.02～2.04±0.02 mm in diameter.

In the experimental series, a total of 50 females were prepared. They were produced in 12 experimental series of four groups. They were two years old, being 53.0～63.0 mm, 59.4 mm on the average, in body length (Tables 59 and

TABLE 59

Eggs of twenty-nine female frogs derived from X-irradiated great-grandparental sperm or eggs and used for mating experiments in 1973

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 50 eggs (mm)
N{N(N·SX–90)}, No. 3	1	2	56.5	769	1.92±0.03
	2	2	61.0	920	1.96±0.04
	3	2	59.0	946	1.94±0.03
	4	2	60.5	0	—
	5	2	57.0	0	—
N{N(N·SX–170)}, No. 1	1	2	54.0	792	1.83±0.02
	2	2	57.5	564	2.06±0.03
	3	2	57.5	922	2.04±0.03
	4	2	61.0	735	1.93±0.03
	5	2	58.0	0	—
	6	2	59.0	0	—
	7	2	57.5	0	—
N{N(N·SX–240)}, No. 1	1	2	53.0	861	1.93±0.02
	2	2	57.0	747	2.04±0.03
	3	2	57.5	0	—
N{N(EX–90·N)}, No. 2	1	2	60.0	560	1.89±0.02
	2	2	55.5	426	1.84±0.02
	3	2	54.0	0	—
	4	2	59.5	0	—
	5	2	60.5	0	—
N{N(EX–145·N)}, No. 3	1	2	58.5	724	1.72±0.03
	2	2	61.5	527	2.01±0.03
	3	2	59.0	456	2.04±0.02
	4	2	60.0	0	—
	5	2	61.5	0	—
	6	2	57.5	0	—
N{N(EX–200·N)}, No. 2	1	2	60.0	421	2.03±0.02
	2	2	57.5	0	—
	3	2	61.0	0	—

N{N(N·SX–90)}, No. 3: Females obtained by N.W71 ♀, Nos. 1~3 × N(N·SX–90) ♂, No. 3
N{N(N·SX–170)}, No. 1: Females obtained by N.W71 ♀, Nos. 1~3 × N(N·SX–170) ♂, No. 1
N{N(N·SX–240)}, No. 1: Females obtained by N.W71 ♀, Nos. 1~3 × N(N·SX–240) ♂, No. 1
N{N(EX–90·N)}, No. 2: Females obtained by N.W71 ♀, Nos. 4~6 × N(EX–90·N) ♂, No. 2
N{N(EX–145·N)}, No. 3: Females obtained by N.W71 ♀, Nos. 4~6 × N(EX–145·N) ♂, No. 3
N{N(EX–200·N)}, No. 2: Females obtained by N.W71 ♀, Nos. 4~6 × N(EX–200·N) ♂, No. 2

60). Five of 15 females belonging to the first group were produced from mating No. 3 of the experimental series derived from a spermatozoon which had been exposed to 90 rads of X-rays (Table 59). They were 56.5~61.0 mm, 58.5 mm on the average, in body length. After pituitary injection, three of them discharged 769~946 eggs, 878.3 on the average, which were 1.92±0.03~1.96

±0.04 mm in diameter. Seven other females were produced from mating No. 1 of the experimental series derived from a spermatozoon which had been exposed to 170 rads of X-rays. They were 54.0～61.0 mm, 57.8 mm on the average, in body length. After pituitary injection, four of them discharged 564～922 eggs, 753.3 on the average, which were 1.83±0.02～2.06±0.03 mm in diameter. The remaining three females were produced from mating No. 1 of the experimental series derived from a spermatozoon which had been exposed to 240 rads of X-rays. They were 53.0～57.5 mm, 55.8 mm on the average, in body length. After pituitary injection, two of them discharged 861 and 747 eggs, 804 on the average, which were 1.93±0.02 and 2.04±0.03 mm in diameter, respectively.

Five of 14 females belonging to the second group were produced from mating No. 2 of the experimental series derived from an egg which had been exposed to 90 rads of X-rays. They were 54.0～60.5 mm, 57.9 mm on the average, in body length. After pituitary injection, two of them discharged 560 and 426 eggs, 493 on the average, which were 1.89±0.02 and 1.84±0.02 mm in diameter, respectively. Six other females were produced from mating No. 3 of the experimental series derived from an egg which had been exposed to 145 rads of X-rays. They were 57.5～61.5 mm, 59.7 mm on the average, in body length. After pituitary injection, three of them discharged 456～724 eggs, 569 on the average, which were 1.72±0.03～2.04±0.02 mm in diameter. The remaining three females were produced from mating No. 2 of the experimental series derived from an egg which had been exposed to 200 rads of X-rays. They were 57.5～61.0 mm, 59.5 mm on the average, in body length. After pituitary injection, only one of them discharged 421 eggs which were 2.03±0.02 mm in diameter.

Five of 11 females belonging to the third group were produced from mating No. 1 of the experimental series derived from a spermatozoon which had been exposed to 50 rads of neutrons (Table 60). They were 58.5～62.0 mm, 60.4 mm on the average, in body length. After pituitary injection, three of them discharged 563～743 eggs, 643.3 on the average, which were 1.79±0.02～2.03±0.02 mm in diameter. Another female was produced from mating No. 1 of the experimental series derived from a spermatozoon which had been exposed to 90 rads of neutrons. This female discharged 621 eggs which were 1.94±0.02 mm in diameter. The remaining five females were produced from mating No. 5 of the experimental series derived from a spermatozoon which were exposed to 130 rads of neutrons. They were 59.5～62.0 mm, 61.0 mm on the average, in body length. After pituitary injection, three of them discharged 439～554 eggs, 495 on the average, which were 1.83±0.02～2.06±0.02 mm in diameter.

Two of ten females belonging to the fourth group were produced from mating No. 3 of the experimental series derived from an egg which had been exposed to 50 rads of neutrons. They were 63.0 and 61.5 mm, 62.3 mm on the average, in body length. After pituitary injection, one of them discharged 742 eggs which were 1.75±0.02 mm in diameter. Three other females were produced from mating No. 3 of the experimental series derived from an egg which had been exposed to 90 rads of neutrons. They were 59.0～62.5 mm, 61.0 mm on the average, in body

TABLE 60

Eggs of twenty-one female frogs derived from neutron-irradiated great-grandparental sperm or eggs and used for mating experiments in 1973

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 50 eggs (mm)
N{N(N·SN–50)}, No. 1	1	2	58.5	743	1.79±0.02
	2	2	60.0	563	2.03±0.02
	3	2	62.0	624	1.94±0.03
	4	2	60.5	0	—
	5	2	61.0	0	—
N{N(N·SN–90)}, No. 1	1	2	63.0	621	1.94±0.02
N{N(N·SN–130)}, No. 5	1	2	59.5	439	2.06±0.02
	2	2	61.0	554	1.93±0.03
	3	2	62.0	492	1.83±0.02
	4	2	61.0	0	—
	5	2	61.5	0	—
N{N(EN–50·N)}, No. 3	1	2	63.0	742	1.75±0.02
	2	2	61.5	0	—
N{N(EN–90·N)}, No. 3	1	2	59.0	497	1.94±0.03
	2	2	62.5	557	1.81±0.03
	3	2	61.5	0	—
N{N(EN–130·N)}, No. 1	1	2	62.5	485	1.97±0.02
	2	2	60.0	562	1.83±0.02
	3	2	60.0	429	2.02±0.02
	4	2	61.0	0	—
	5	2	59.5	0	—

N{N(N·SN–50)}, No. 1: Females obtained by N.W71 ♀, Nos. 7~9×N(N·SN–50) ♂, No. 1

N{N(N·SN–90)}, No. 1: Female obtained by N.W71 ♀, Nos. 7~9×N(N·SN–90) ♂, No. 1

N{N(N·SN–130)}, No. 5: Females obtained by N.W71 ♀, Nos. 7~9×N(N·SN–130) ♂, No. 5

N{N(EN–50·N)}, No. 3: Females obtained by N.W71 ♀, Nos. 10~12×N(EN–50·N) ♂, No. 3

N{N(EN–90·N)}, No. 3: Females obtained by N.W71 ♀, Nos. 10~12×N(EN–90·N) ♂, No. 3

N{N(EN–130·N)}, No. 1: Females obtained by N.W71 ♀, Nos. 10~12×N(EN–130·N) ♂, No. 1

length. After pituitary injection, two of them discharged 497 and 557 eggs, 527 on the average, which were 1.94±0.03 and 1.81±0.03 mm in diameter, respectively. The remaining five females were produced from mating No. 1 of the experimental series derived from an egg which had been exposed to 130 rads of neutrons. They were 59.5~62.5 mm, 60.5 mm on the average, in body length. After pituitary injection, three of them discharged 429~562 eggs, 492 on the average, which were 1.83 ±0.02~2.02±0.02 mm in diameter.

In summary, 28 of the 50 females in the 12 experimental series each discharged after pituitary injection 421~946 eggs, 631.4 on the average, which were 1.72 ±0.03~2.06±0.03 mm, 1.93±0.02 mm on the average, in mean diameter, while four of the five females in the control series discharged 874~1211 eggs, 1007 on the average, which were 1.90±0.02 mm in mean diameter.

2. Developmental capacity

a. Control series

[N♀×{N♀×(N♀×N♂)♂}♂]♀×N♂, Nos. 1~4

Eggs of four females (Nos. 1~4) in which ovulation had occurred were fertilized with a mixture of sperm of three normal males collected from the field (Table 61). In the four matings (Nos. 1~4), 89.3~98.3%, 93.5% on the average, of the respective total number of eggs cleaved normally. Only a very small number of the normally cleaved eggs died of various kinds of abnormalities during embryonic and tadpole stages; 86.7~95.4%, 89.8% on the average, hatched normally, and then 77.6~94.3%, 86.3% on the average, became normal, metamorphosed frogs.

b. Experimental series derived from X-irradiated sperm

i) [N♀×{N♀×(N♀×SX–90♂)♂}♂]♀×N♂, Nos. 1~3

As ovulation occurred in three females Nos. 1~3, eggs of each of them were inseminated with a mixture of sperm of the same three males as those used in the control series (Table 61). In matings Nos. 1~3, 86.4~90.2%, 88.3% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were very similar in developmental capacity to those of the control series; 78.0~87.6%, 81.7% on the average, hatched normally, and 77.3~85.6%, 80.3% on the average, became normal, metamorphosed frogs.

ii) [N♀×{N♀×(N♀×SX–170♂)♂}♂]♀×N♂, Nos. 1~4

As four (Nos. 1~4) of seven females ovulated normally, they were mated with the same three males as those used in the control series. In matings Nos. 1~4, 84.8~87.0%, 85.9% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were very similar in developmental capacity to those of the control series; 78.6~83.7%, 80.7% on the average, hatched normally, and 76.6~80.7%, 78.5% on the average, became normal metamorphosed frogs.

iii) [N♀×{N♀×(N♀×SX–240♂)♂}♂]♀×N♂, Nos. 1 and 2

Two (Nos. 1 and 2) of three females ovulated normally. They were mated with the same three males as those used in the control series. In two matings Nos. 1 and 2, 85.8% and 50.2%, 67.3% on the average, of the respective total number of eggs cleaved normally. Although the percentage of normally cleaved eggs was low in mating No. 2, they were nearly normal in development. In these two matings, 80.4% and 47.7%, 63.5% on the average, hatched normally, and 78.2% and 45.3%, 61.1% on the average, became normal, metamorphosed frogs.

c. Experimental series derived from X-irradiated eggs

i) [N♀×{N♀×(EX–90♀×N♂)♂}♂]♀×N♂, Nos. 1 and 2

As two (Nos. 1 and 2) of five females discharged eggs, they were mated with

TABLE 61

Developmental capacity of the offspring of female frogs

Parents		No. of eggs	No. of cleaved eggs	
Female	Male		Normal	Abnormal
N{N(N·N)}, Nos. 1∼4	N.W73, Nos. 6∼8	783	732 (93.5%)	3 (0.4%)
N{N(N·SX–90)}, Nos. 1∼3	N.W73, Nos. 6∼8	575	508 (88.3%)	19 (3.3%)
N{N(N·SX–170)}, Nos. 1∼4		647	556 (85.9%)	17 (2.6%)
N{N(N·SX–240)}, Nos. 1, 2		468	315 (67.3%)	8 (1.7%)
N{N(EX–90·N)}, Nos. 1, 2	N.W73, Nos. 6∼8	525	459 (87.4%)	15 (2.9%)
N{N(EX–145·N)}, Nos. 1∼3		751	685 (91.2%)	22 (2.9%)
N{N(EX–200·N)}, No. 1		266	232 (87.2%)	6 (2.3%)
N{N(N·SN–50)}, Nos. 1∼3	N.W73, Nos. 6∼8	608	522 (85.9%)	10 (1.6%)
N{N(N·SN–90)}, No. 1		411	375 (91.2%)	10 (2.4%)
N{N(N·SN–130)}, Nos. 1∼3		634	567 (89.4%)	14 (2.2%)
N{N(EN–50·N)}, No. 1	N.W73, Nos. 6∼8	276	236 (85.5%)	0
N{N(EN–90·N)}, Nos. 1, 2		455	415 (91.2%)	4 (0.9%)
NN{(EN–130·N)}, Nos. 1∼3		658	584 (88.8%)	7 (1.1%)

the same three males as those used in the control series (Table 61). In two matings Nos. 1 and 2, 89.7% and 85.0%, 87.4% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were very similar in developmental capacity to those of the control series; 83.0% and 78.7%, 81.0% on the average, hatched normally, and 81.2% and 76.8%, 79.0% on the average, became normal, metamorphosed frogs.

ii) [N♀ × {N♀ × (EX–145♀ × N♂)♂}♂]♀ × N♂, Nos. 1∼3

As ovulation occurred in three (Nos. 1∼3) of six females, they were mated with the same three males as those used in the control series. In three matings Nos. 1∼3, 90.3∼91.6%, 91.2% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were quite the same in developmental capacity as those of the control series; 84.5∼88.6%, 87.1% on the average, hatched normally, and 83.7∼85.6%, 84.8% on the average, became normal, metamorphosed frogs.

iii) [N♀ × {N♀ × (EX–200♀ × N♂)♂}♂]♀ × N♂, No. 1

Only one (No. 1) of three females ovulated after pituitary injection. Eggs of this female were inseminated with a mixture of sperm of the same three males derived from irradiated great-grandparental sperm or eggs, II

No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of 50-day-old tadpoles	No. of meta-morphosed frogs
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal		
729	3	718	11	703	15	682	676
(93.1%)	(0.4%)	(91.7%)	(1.4%)	(89.8%)	(1.9%)	(87.1%)	(86.3%)
501	7	489	12	470	19	467	462
(87.1%)	(1.2%)	(85.0%)	(2.1%)	(81.7%)	(3.3%)	(81.2%)	(80.3%)
552	4	544	8	522	22	517	508
(85.3%)	(0.6%)	(84.1%)	(1.2%)	(80.7%)	(3.4%)	(79.9%)	(78.5%)
313	2	310	3	297	13	291	286
(66.9%)	(0.4%)	(66.2%)	(0.6%)	(63.5%)	(2.8%)	(62.2%)	(61.1%)
443	6	425	18	425	0	416	415
(84.4%)	(1.1%)	(81.0%)	(3.4%)	(81.0%)		(79.2%)	(79.0%)
671	8	664	7	654	10	643	637
(89.3%)	(1.1%)	(88.4%)	(0.9%)	(87.1%)	(1.3%)	(85.6%)	(84.8%)
230	0	227	3	221	6	220	220
(86.5%)		(85.3%)	(1.1%)	(83.1%)	(2.3%)	(82.7%)	(82.7%)
515	4	507	8	486	21	481	471
(84.7%)	(0.7%)	(83.4%)	(1.3%)	(79.9%)	(3.5%)	(79.1%)	(77.5%)
372	3	371	1	369	2	367	365
(90.5%)	(0.7%)	(90.3%)	(0.2%)	(89.8%)	(0.5%)	(89.3%)	(88.8%)
564	2	549	15	541	8	535	531
(89.0%)	(0.3%)	(86.6%)	(2.4%)	(85.3%)	(1.3%)	(84.4%)	(83.8%)
235	1	231	4	230	1	230	224
(85.1%)	(0.4%)	(83.7%)	(1.4%)	(83.3%)	(0.4%)	(83.3%)	(81.2%)
414	1	406	8	401	5	392	388
(91.0%)	(0.2%)	(89.2%)	(1.8%)	(88.1%)	(1.1%)	(86.2%)	(85.3%)
581	1	568	13	562	6	556	549
(88.3%)	(0.2%)	(86.3%)	(2.0%)	(85.4%)	(0.9%)	(84.5%)	(83.4%)

as those used in the control series. As a result, 87.2% of the eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to those of the control series; 83.1% hatched normally, and afterwards 82.7% became normal, metamorphosed frogs.

d. Experimental series derived from neutron-irradiated sperm

i) [N♀ × {N♀ × (N♀ × SN–50♂)♂}♂]♀ × N♂, Nos. 1∼3

As three females Nos. 1∼3 ovulated normally, they were mated with the same three males as those used in the control series (Table 61). In three matings Nos. 1∼3, 83.9∼88.1%, 85.9% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were very similar in developmental capacity to those of the control series; 77.7∼83.0%, 79.9% on the average, hatched normally, and 76.4∼78.9%, 77.5% on the average, became normal, metamorphosed frogs.

ii) [N♀ × {N♀ × (N♀ × SN–90♂)♂}♂]♀ × N♂, No. 1

As a single female (No. 1) ovulated after pituitary injection, eggs of this female were inseminated with a mixture of sperm of the same three males as those used in the control series. It was found that 91.2% of the eggs cleaved normally. The normally cleaved eggs were rather superior in developmental capacity to those of the control series; 89.3% hatched normally, and 88.8% became normal, metamorphosed frogs.

iii) [N♀ × {N♀ × (N♀ × SN–130♂) ♂} ♂] ♀ × N♂, Nos. 1 ~ 3

As ovulation occurred in three (Nos. 1 ~ 3) of five females, they were mated with the same three males as those used in the control series. In three matings Nos. 1 ~ 3, 87.5 ~ 91.1%, 89.4% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to those of the control series; 80.2 ~ 90.0%, 85.3% on the average, hatched normally, and 79.7 ~ 87.6%, 83.8% on the average, became normal, metamorphosed frogs.

e. Experimental series derived from neutron-irradiated eggs

i) [N♀ × {N♀ × (EN–50♀ × N♂) ♂} ♂] ♀ × N♂, No. 1

One (No. 1) of two females ovulated normally after pituitary injection. Eggs of this female were inseminated with a mixture of sperm of the same three males as used in the control series (Table 61). As a result, 85.5% of the eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to those of the control series; 83.3% hatched normally, and 81.2% became normal, metamorphosed frogs.

ii) [N♀ × {N♀ × (EN–90♀ × N♂) ♂} ♂] ♀ × N♂, Nos. 1 and 2

Two (Nos. 1 and 2) of three females ovulated normally. They were mated with the same three males as those used in the control series. In two matings Nos. 1 and 2, 88.3% and 94.2%, 91.2% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to those of the control series; 87.0% and 89.3%, 88.1% on the average, hatched normally, and 82.6% and 88.0%, 85.3% on the average, became normal, metamorphosed frogs.

iii) [N♀ × {N♀ × (EN–130♀ × N♂) ♂} ♂] ♀ × N♂, Nos. 1 ~ 3

As three (Nos. 1 ~ 3) of five females ovulated normally, they were mated with the same three males as those used in the control series. In three matings Nos. 1 ~ 3, 87.4 ~ 90.7%, 88.8% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to those of the control series; 83.0 ~ 86.9%, 85.4% on the average, hatched normally, and 79.6 ~ 86.0%, 83.4% on the average, became normal, metamorphosed frogs.

3. Viability and sex of metamorphosed frogs

In the control series, 676 tadpoles produced from four matings climbed out of water at the age of 80 ~ 98 days, 90.5 days on the average (Table 62). In the 12 experimental series, 5056 tadpoles produced from 28 matings climbed out of water at the age of 80 ~ 117 days, 84.9 ~ 93.1 days on the average in each series. There was no definite difference in the average age when tadpoles could climb out of water between the experimental and the control series, or between different experimental series.

In the experimental and control series, 50 frogs were at random removed from those produced from each mating immediately after completion of metamorphosis in order to measure their body length. In the control series, a total of 200 tadpoles was 20.5 ± 0.1 mm in mean body length, while a total of 50~200 tadpoles in each experimental series was $19.3 \pm 0.1 \sim 21.7 \pm 0.1$ mm in body length. There were no remarkable differences in the body length of frogs among the 12 experimental series between the experimental and control series.

One month after completion of metamorphosis, all the living frogs in the experimental and control series were killed to examine their sex.

a. Control series

[N♀×{N♀×(N♀×N♂)♂}♂]♀×N♂, Nos. 1~4

Only one of 676 normally metamorphosed frogs produced from four matings died within one month after metamorphosis. All the other frogs were killed in order to examine their sex. As a result, it was found that 337 were females, five hermaphrodites and 333 males. When the hermaphrodites were counted as males, 50.1% were males.

b. Experimental series derived from X-irradiated sperm

i) [N♀×{N♀×(N♀×SX-90♂)♂}♂]♀×N♂, Nos. 1~3

Only two of 462 normally metamorphosed frogs produced from three matings died within one month after metamorphosis. All the other frogs were killed to examine their sex; 227 were females, 17 hermaphrodites and 216 males. When the hermaphrodites were counted as males, 50.7% were males.

ii) [N♀×{N♀×(N♀×SX-170♂)♂}♂]♀×N♂, Nos. 1~4

Only three of 508 normally metamorphosed frogs produced from four matings died within one month after metamorphosis. All the other frogs were killed; 246 were females, 20 hermaphrodites and 239 males. When the hermaphrodites were counted as males, 51.3% were males.

iii) [N♀×{N♀×(N♀×SX-240♂)♂}♂]♀×N♂, Nos. 1 and 2

Twelve of 286 normally metamorphosed frogs produced from two matings died within one month after metamorphosis. The other 274 frogs were killed; 127 were females, seven hermaphrodites and 140 males. When the hermaphrodites were counted as males, 53.6% were males.

c. Experimental series derived from X-irradiated eggs

i) [N♀×{N♀×(EX-90♀×N♂)♂}♂]♀×N♂, Nos. 1 and 2

Five of 415 normally metamorphosed frogs produced from two matings died within one month after metamorphosis. The other 410 frogs were killed; 181 were females, 11 hermaphrodites and 218 males. When the hermaphrodites were counted as males, 55.9% were males.

ii) [N♀×{N♀×(EX-145♀×N♂)♂}♂]♀×N♂, Nos. 1~3

TABLE 62

Number, size and sex of metamorphosed frogs derived

Parents		Age at the time of climbing out of water (days)
Female	Male	
N{N(N·N)}, Nos. 1~4	N.W73, Nos. 6~8	80~98 (90.5)
N{N(N·SX-90)}, Nos. 1~3	N.W73, Nos. 6~8	80~113 (91.4)
N{N(N·SX-170)}, Nos. 1~4		81~104 (84.9)
N{N(N·SX-240)}, Nos. 1, 2		81~105 (85.1)
N{N(EX-90·N)}, Nos. 1, 2	N.W73, Nos. 6~8	83~107 (89.3)
N{N(EX-145·N)}, Nos. 1~3		85~101 (92.5)
N{N(EX-200·N)}, No. 1		86~112 (91.7)
N{N(N·SN-50)}, Nos. 1~3	N.W73, Nos. 6~8	88~108 (90.7)
N{N(N·SN-90)}, No. 1		82~106 (92.9)
N{N(N·SN-130)}, Nos. 1~3		82~117 (93.1)
N{N(EN-50·N)}, No. 1	N.W73, Nos. 6~8	82~106 (85.9)
N{N(EN-90·N)}, Nos. 1, 2		80~107 (92.5)
N{N(EN-130·N)}, Nos. 1~3		80~98 (91.2)

♀$_N$—Females with normal ovaries ♀$_U$—Females with underdeveloped ovaries

Five of 637 normally metamorphosed frogs produced from three matings died within one month after metamorphosis. The other 632 were killed; 301 were females, 20 hermaphrodites and 311 males. When the hermaphrodites were counted as males, 52.4% were males.

iii) [N♀ × {N♀ × (EX-200♀ × N♂) ♂} ♂] ♀ × N♂, No. 1

Six of 220 normally metamorphosed frogs produced from one mating died within one month after metamorphosis. The other 214 frogs were killed; 99 were females, 12 hermaphrodites and 103 males. When the hermaphrodites were counted as males, 53.7% were males.

d. Experimental series derived from neutron-irradiated sperm

i) [N♀ × {N♀ × (N♀ × SN-50♂) ♂} ♂] ♀ × N♂, Nos. 1~3

Only one of 471 normally metamorphosed frogs produced from three matings died within one month after metamorphosis. All the other frogs were killed; 231 were females, 23 hermaphrodites and 216 males. When the hermaphrodites were counted as males, 50.9% were males.

from irradiated great-great-grandparental sperm or eggs, II

No. of metamorphosed frogs	Body length immediately after metamorphosis (mm)	Sex of frogs killed about one month after metamorphosis					
		No. of frogs	♀N	♀U	⚥	♂N	♂ (%)*
676	20.5±0.1	675	330	7	5	333	(50.1)
462	19.5±0.1	460	217	10	17	216	(50.7)
508	19.3±0.1	505	235	11	20	239	(51.3)
286	20.0±0.1	274	118	9	7	140	(53.6)
415	19.8±0.1	410	162	19	11	218	(55.9)
637	20.3±0.1	632	276	25	20	311	(52.4)
220	21.2±0.2	214	89	10	12	103	(53.7)
471	21.7±0.1	470	215	16	23	216	(50.9)
365	20.7±0.2	361	160	11	13	177	(52.6)
531	21.3±0.2	529	241	14	31	243	(51.8)
224	20.6±0.1	220	93	15	14	98	(50.9)
388	19.5±0.2	386	170	17	20	179	(51.6)
549	19.8±0.2	535	233	19	41	242	(52.9)

⚥—Hermaphrodites ♂N—Males with normal testes * Including hermaphrodites

ii) [N♀×{N♀×(N♀×SN-90♂)♂}♂]♀×N♂, No. 1

Four of 365 normally metamorphosed frogs produced from a mating died within one month after metamorphosis. The other 361 frogs were killed; 171 were females, 13 hermaphrodites and 177 males. When the hermaphrodites were counted as males, 52.6% were males.

iii) [N♀×{N♀×(N♀×SN-130♂)♂}♂]♀×N♂, Nos. 1～3

Only two of 531 normally metamorphosed frogs produced from three matings died within one month after metamorphosis. The other frogs were killed; 255 were females, 31 hermaphrodites and 243 males. When the hermaphrodites were counted as males, 51.8% were males.

e. Experimental series derived from neutron-irradiated eggs

i) [N♀×{N♀×(EN-50♀×N♂)♂}♂]♀×N♂, No. 1

Four of 224 normally metamorphosed frogs produced from a mating died within one month after metamorphosis. The other 220 frogs were killed; 108 were females, 14 hermaphrodites and 98 males. When the hermaphrodites were counted as males, 50.9% were males.

ii) [N♀ × {N♀ × (EN–90♀ × N♂) ♂} ♂] ♀ × N♂, Nos. 1 and 2

Only two of 388 normally metamorphosed frogs produced from two matings died within one month after metamorphosis. The other frogs were killed; 187 were females, 20 hermaphrodites and 179 males. When the hermaphrodites were counted as males, 51.6% were males.

iii) [N♀ × {N♀ × (EN–130♀ × N♂) ♂} ♂] ♀ × N♂, Nos. 1～3

Of 549 normally metamorphosed frogs, 14 died within one month after metamorphosis. The other 535 were killed; 252 were females, 41 hermaphrodites and 242 males. When the hermaphrodites were counted as males, 52.9% were males.

X. Summary of the developmental capacity and sex of individuals derived from four types of irradiated gametes

1. Developmental capacity

The developmental capacities of individuals raised from irradiated sperm or oviducal eggs and of their descendants presented in Tables 3, 14, 23, 28, 35, 41, 49, 55 and 61 are shown in Figs. 29～32 in order to facilitate the comparison between different generations as well as among the four types of irradiated gametes. In the control series, more than 80% of the total number of eggs became normally metamorphosed frogs in each of the four generations derived from ten paris of parents.

a. Descendants from X-irradiated sperm

By sperm irradiated with 90, 170 and 240 rads of X-rays, 36%, 25% and 14% of eggs became normally metamorphosed frogs (Table 3). This shows that the deleterious effects of irradiation on the developmental capacity of spermatozoa increased almost linearly with dose. In contrast with this, such a linear decrease in the percentage of normally metamorphosed frog was scarcely found in the second-generation offspring among the three groups of 90 rad, 170 rad and 240 rad irradiation series (Fig. 29). Although the percentages of normally metamorphosed frogs in the six series of second-generation offspring were all low, being 31～54%, and nearly similar to one another, they somewhat increased as compared with those of the first-generation offspring in each of the three groups of irradiation series (Tables 14, 23). In the third generation, the percentages of normally metamorphosed frogs were slightly higher as a whole than those in the second generation, although there was a very low percentage in each of the three groups of irradiation series (Tables 28, 35, 41, 49). On the other hand, there were no remarkable differences in the percentages of normally metamorphosed frogs among these three groups of irradiation series. The fourth-generation offspring differed drastically from the first-, second- and third-generation offspring in this respect (Tables 55, 61). The percentages of normally metamorphosed frogs scarcely differed from those in the control series, except that in one of two

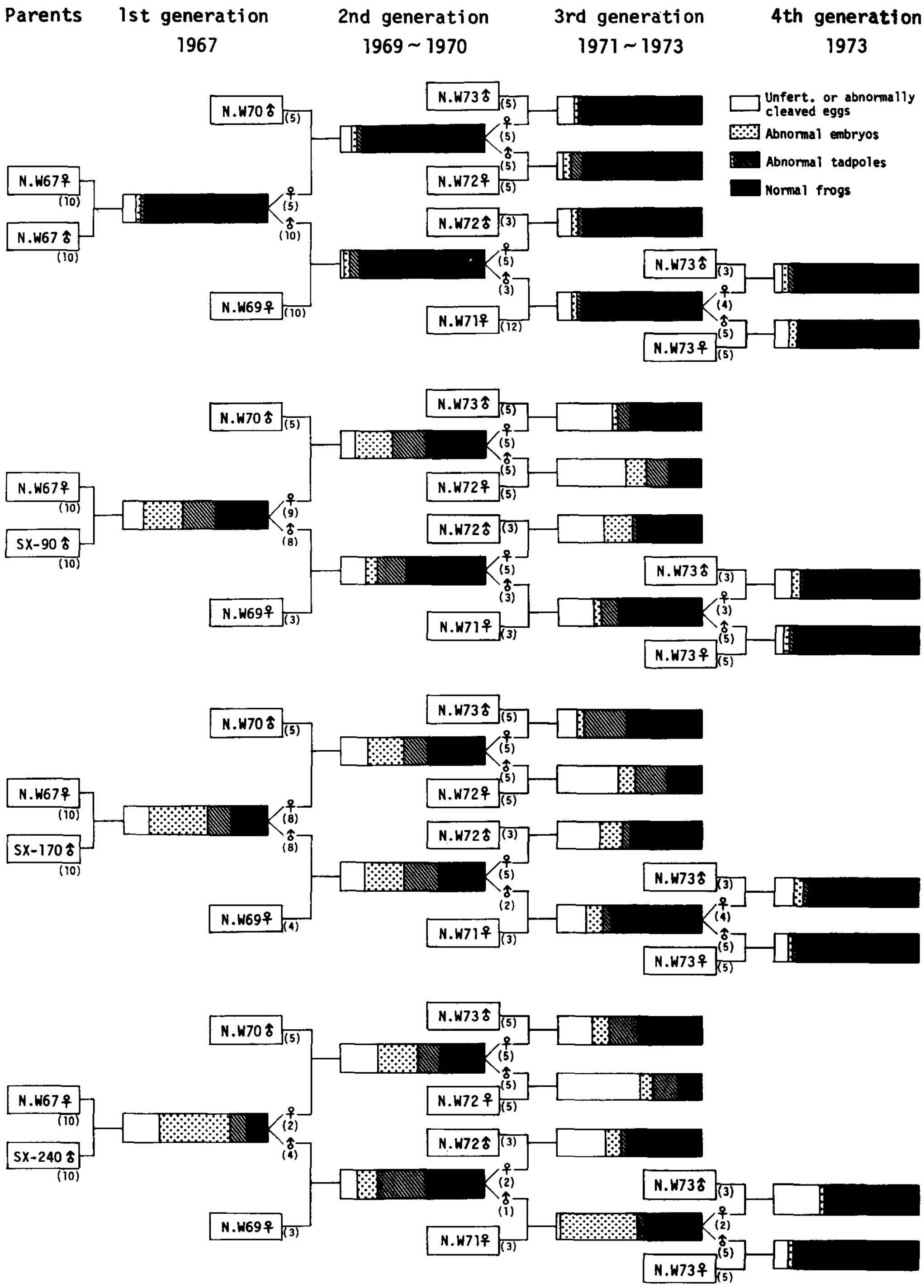


Fig. 29. Diagrammatic representation of the viabilities of first- to fourth-generation offspring derived from X-irradiated sperm and their controls. The number of frogs used for each kind of matings is given in parentheses.

240 rad series only 61% of eggs became normally metamorphosed frogs. In the other series as well as in two 90 rad and two 170 rad series, more than 78% of the respective total number of eggs grew into normally metamorphosed frogs, while 86% and 82% did so in two control series.

The chromosome aberrations induced by X-irradiation are presented in

Tables 5 and 6. Only 22～46% of normally shaped tadpoles raised from irradiated spermatozoa in six experimental series had normally diploid mitoses alone. The percentages of normal diploids in the 90 rad, 170 rad and 240 rad series were nearly in parallel with those of normally metamorphosed frogs in these series (Tables 4, 5). The remainders were abnormal in all or a part of the mitoses analyzed, that is, each of the tadpoles had the same karyotype or was a mosaic consisting of two or more kinds of abnormal mitoses or of normally diploid and abnormal mitoses. It was remarkable that most of them had abnormally diploid mitoses in which a translocation or deletion was found in a chromosome. A small number of tadpoles had hyper- and hypodiploid, triploid or hyper- and hypotriploid mitoses (Table 6). Besides, a dicentric, ring or fragment chromosome was found in the mitoses of a few tadpoles.

Here, it should be noted that the normally diploid mitoses would not be always normal in the strict sense of the word. LACROIX and LOONES (1974) have described that about 70% of chromosome mutations identifiable on the lampbrush chromosomes of oocytes in *Pleurodeles poireti* cannot be detected or characterized on the mitotic chromosomes. Accordingly, it is very probable that even the tadpoles consisting of normally diploid mitoses alone had some undetected chromosome aberrations.

A total of 23 females and a total of 20 males raised from X-irradiated sperm were prepared to produce second-generation offspring, together with five control females and ten control males. After pituitary injection, 19 females laid 89～692 eggs, whereas each of the five control females laid 915～1720 eggs (Tables 18, 19). Three of the females in the experimental series laid normally sized eggs and smaller ones, and one of them laid white eggs besides normally colored ones. While all the ten control males had testes that were normal in spermatogenesis, 16 of 20 males in the three experimental series had testes of types 2, 3 and 4 that were more or less abnormal in spermatogenesis (Table 10). The remaining four males with normal testes were those raised from spermatozoa irradiated with 90 rads of X-rays. The intensity of abnormalities in the number, size and color of eggs as well as in the structure of testes corresponded roughly to the scarceness of normally metamorphosed frogs in the following generation. However, normally metamorphosed frogs produced from two of the four males with normal testes (type 1) were somewhat fewer than those from the control males (Table 10, Figs. 11, 12a). On the other hand, the normally shaped tadpoles produced from a male with normal testes in an experimental series were not always normal in karyotype. Of 39 analyzed tadpoles produced from a male (No. 1) in the 90 rad series, eleven had various kinds of chromosome aberrations (Table 15). Somewhat smaller number of chromosome aberrations were also found in normally shaped tadpoles produced from two males with slightly abnormal testes in the 170 rad and 240 rad series. Among the females raised from X-irradiated spermatozoa, the number of their eggs had scarcely any intimate interrelation to the percentage of normally metamorphosed frogs in their offspring, although the females that laid a very small number of eggs were inferior to the others in the percentage of normally metamorphosed frogs (Table 19, Fig. 15a, b, c). Chromosome aberrations were also observed in four of 20 normally shaped tadpoles produced from a female (No. 2) that laid only 523 eggs in the 90 rad series.

Of 50~200 metamorphosed frogs derived from irradiated grandparental spermatozoa in each of the six experimental series, 22~71% became two-year-old mature ones, while 32 (64%) and 120 (80%) of 50 and 150 metamorphosed frogs respectively did so in the control series (Tables 16, 24). A total of 48 mature females were selected for producing third-generation offspring from the 90 rad, 170 rad and 240 rad series (Tables 31, 45), while a total of 12 were selected from the controls (Tables 31, 44). After pituitary injection, 11 of the 12 control females laid 1059~2067 eggs. In the 90 rad series, four of 18 females laid 1253~2136 eggs, six others laid 551~969 eggs, three still others laid 121~326 eggs and the remaining five laid no eggs. Of 17 females in the 170 rad series, nine laid 1149~2133 eggs, three others laid 521~754 eggs, two others laid 129 and 329 eggs and the remaining three laid no eggs. Of 13 females in the 240 rad series, eight laid 1225~1975 eggs, two others laid 426 and 537 eggs and the remaining three laid no eggs. Although the females produced from female parents raised from X-irradiated spermatozoa generally laid more numerous eggs in 1973 than those from male parents raised from X-irradiated spermatozoa did in 1972, it was evident that such a difference was attributable to that between the rearing conditions in the two years, because a similar difference was observable between the number of eggs laid by the control females in 1972 and 1973. There were no females which laid white eggs. On the other hand, a total of 22 mature males were selected for producing third-generation offspring (Tables 26, 39). As the result of examining their testes, it was found that eight males had normal testes (type 1), 13 others were more or less abnormal in spermatogenesis (type 2 and type 3) and the remainder had no normal spermatozoa in the testes (type 5). The 21 males other than the last one were actually used for obtaining offspring. It was noteworthy that the males produced from female parents raised from X-irradiated spermatozoa were generally much more abnormal in spermatogenesis than were those from male parents raised from X-irradiated spermatozoa, in contrast with the case of females. As observed in the production of second-generation offspring, the numbers of eggs laid by female second-generation offspring had scarcely any intimate interrelation to the percentage of normally metamorphosed frogs produced by these females, although some females that laid a very small number of eggs were not used for the mating experiments to produce the third-generation offspring. Contrarily, the intensity of abnormalities in the structure of testes generally corresponded to the percentage of normally metamorphosed frogs in the third-generation offspring (Tables 26, 39, Figs. 18a, b, c, 24a, b, c). However, it was remarkable that some males with normal testes (type 1) were distinctly inferior in reproductive capacity to the controls.

The karyotypes of normally shaped tadpoles in the third generation from X-irradiated spermatozoa were only examined in the series that passed over male first- and second-generation offspring. In the 90 rad and 170 rad series, six of

30 tadpoles were abnormal in karyotype, although their male parents had apparently normal testes (type 1).

In order to produce fourth-generation offspring, 15 female and 15 male third-generation offspring obtained by passing over male first- and second-generation offspring were prepared. After pituitary injection, nine of the 15 females laid 564～946 eggs, while four of the five controls laid 874～1211 eggs (Tables 58, 59). The 15 males had normal testes (type 1) as five controls had (Tables 52, 53). In each of the 90 rad, 170 rad and 240 rad series, the percentages of normally cleaved eggs were more or less inferior to those of the controls. Especially, one of the two females in the 240 rad series was very low in the percentage of normally cleaved eggs. However, all the normally cleaved eggs in the three experimental series scarcely differed from the controls in the development until metamorphosis (Table 61). The males in the experimental series were not inferior to the controls in reproductive capacity (Table 55).

b. Descendants from X-irradiated eggs

The same effect of X-rays as found on spermatozoa was observed on eggs (Fig. 30). Of the eggs irradiated with 90, 145 and 200 rads of X-rays, 33%, 21% and 9% became metamorphosed frogs, respectively (Table 3, Fig. 1). Such a clear-cut decrease was not found in the second- and third-generation offspring. As in the offspring derived from X-irradiated spermatozoa, the percentages of normally metamorphosed frogs in each of the three groups of irradiation series somewhat increased in the second-generation offspring as compared with those in the first-generation offspring (Tables 14, 23). A slight increment in the percentage of normally metamorphosed frogs was also found as a whole in the third-generation offspring, as compared with those of the second-generation in each group (Tables 28, 35, 41, 49). However, the percentages of normally metamorphosed frogs were distinctly lower than and nearly the same with those of the preceding generation in three and three of the 12 series, respectively. In the fourth-generation offspring derived from eggs irradiated with 90, 145 and 200 rads of X-rays by passing over male first- and second-generation offspring and male and female third-generation offspring, the percentages of normal embryos and tadpoles and normally metamorphosed frogs were nearly the same as those of the control series (Tables 55, 61).

Of normally shaped tadpoles raised from irradiated eggs in the 90 rad, 145 rad and 200 rad series, about 70%, 50% and 33% were normal in karyotype, respectively (Table 5). These percentages were nearly in parallel with those of normally metamorphosed frogs, although the latter differed to some extent with their female parents (Table 4).

Of the mature females raised from X-irradiated eggs, 22 were selected to produce their offspring. After pituitary injection, 19 females laid 237～816 eggs, in contrast with the control females that laid 915～1720 eggs (Tables 18, 20). Four females laid two kinds of eggs and another did three kinds of eggs in size. Three of the former females and four others laid normally colored eggs and white

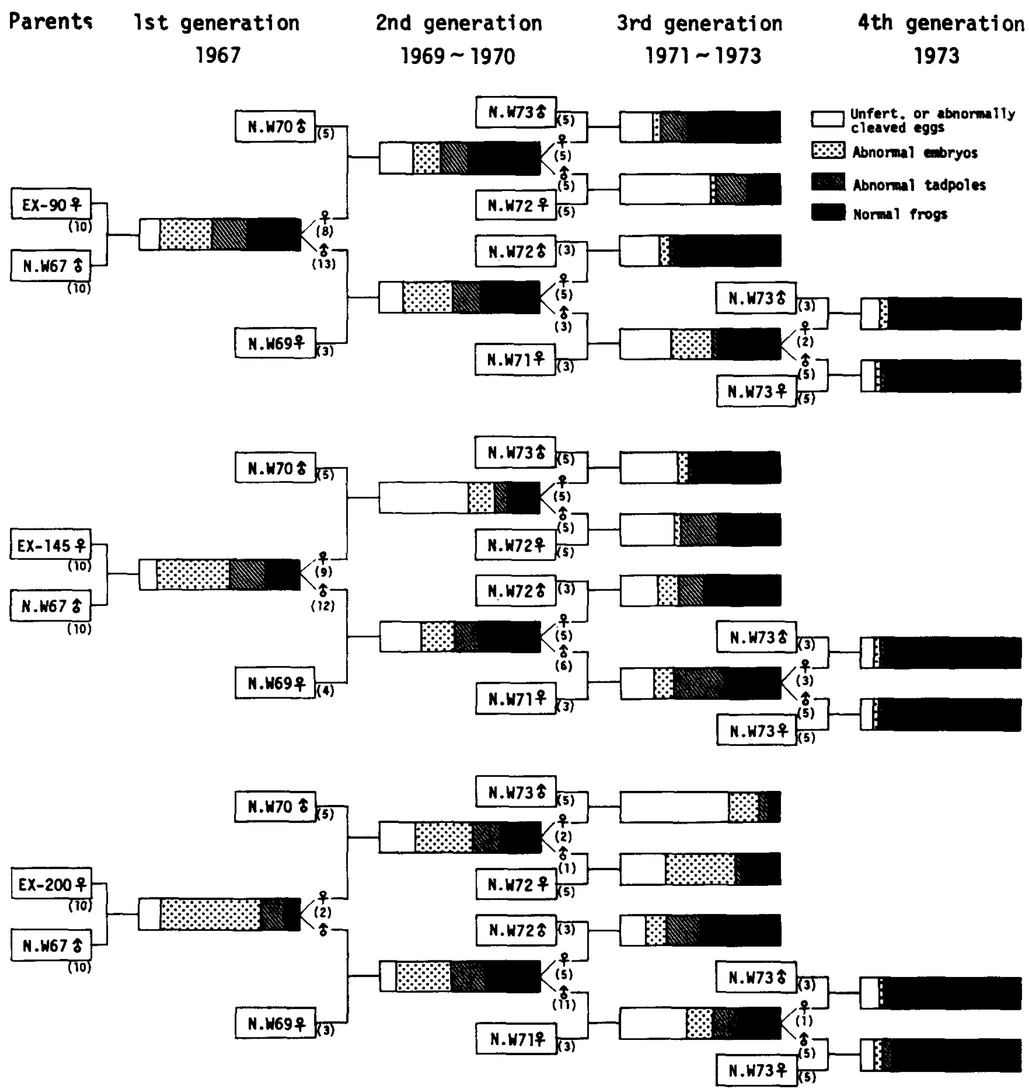


Fig. 30. Diagrammatic representation of the viabilities of first- to fourth-generation offspring derived from X-irradiated oviducal eggs. The number of frogs used for each kind of matings is given in parentheses.

ones. A total of 30 males raised from X-irradiated eggs were used for producing their offspring. While only two of them had normal testes, that is, of type 1, 12 others had those of type 2, 12 still others had those of type 3 and the remaining four had those of type 4. As a matter of course, there was an intimate interrelation between the types of testes and the percentage of normally metamorphosed frogs produced in the following generation (Table 11, Fig. 12d, e, f). Although males with normal or nearly normal testes were superior in reproductive capacity than those with abnormal testes, the two males with type 1 testes were clearly inferior to the controls in reproductive capacity. Actually, the normally shaped tadpoles produced from one of the two males were not always normal in karyotype. Of 40 tadpoles from a male which had been raised from an eggs irradiated with 90 rads of X-rays, eight had various kinds of chromosome aberrations. Of 55 normally shaped tadpoles from two males which had been raised from eggs irradiated with 145 and 200 rads of X-rays and had slightly abnormal testes (type 2), 12 had also various kinds of chromosomal aberrations. Among the females raised from X-irradiated eggs, those that laid two kinds of eggs, large and small or normally colored and white, were not always inferior to the others in reproductive capacity (Table 20, Fig. 15d, e, f). Chromosome aberrations were found in six of 20 normally shaped tadpoles produced from a female (No. 3) which had been raised from an egg exposed to 90 rads of X-rays and had two kinds of eggs in each of size and color (Table 20).

In order to produce third-generation offspring, a total of 48 females and a total of 31 males derived from X-irradiated eggs were selected from the 90 rad, 145 rad and 200 rad series (Tables 26, 32, 39, 46). After pituitary injection, 38 of the females laid 120 ~ 1743 eggs. Of these females, 21 laid 120 ~ 1052 eggs in 1972, while the other 17 laid 757 ~ 1743 eggs in 1973. Each group of females was considerably inferior to the controls as a whole in the number of eggs. There were no females that laid white eggs or two kinds of eggs in size. Of the 31 males, 16 had testes of type 1, nine others had those of type 2 and the remaining six had those of type 3 (Tables 26, 39). Forty-three normally shaped tadpoles produced by two males which had been derived from X-irradiated eggs by passing over male first- and second-generation offspring were grossly examined in terms of their chromosomes. It was found that three of 18 tadpoles derived from an egg exposed to 90 rads as well as six of 25 tadpoles derived from an egg exposed to 200 rads were abnormal in karyotype.

The percentages of normally metamorphosed frogs in the third-generation offspring were closely related as a whole to the type of the testes of their male parents, although they were usually remarkably lower even in the offspring of males with normal testes (type 1) than those in the offspring of the controls (Tables 26, 39, Figs. 18d, e, f, 24d, e, f). There were some males which were very defective in reproductive capacity in spite of the normal appearance of their testes. On the other hand, the number of eggs laid by female second-generation offspring was barely related to the percentage of normally metamorphosed frogs produced by these females (Tables 32, 46, Figs. 21d, e, f, 27d, e, f). Some females were not inferior to the control in reproductive capacity except that they laid far less eggs than the controls did.

Fourth-generation offspring were produced by six females and 15 males which had been derived from X-irradiated eggs by passing over male first-, second- and third-generation offspring. After 14 females in the 90 rad, 145 rad and 200 rad series were injected with pituitaries, only six of them laid 421 ~ 724 eggs (Table 59). The number of these eggs was distinctly smaller than the number of eggs laid by the control females. Some females in the experimental series were also slightly inferior to the controls in the percentage of normally cleaved eggs. However, they did not differ from the latter in the development of the normally cleaved eggs until metamorphosis. The 15 males were not inferior to the controls in reproductive capacity, except one (No. 5) of five male third-generation offspring derived from eggs irradiated with 200 rads of X-rays. Although 84% of eggs cleaved normally by fertilization with sperm of this male, only 57%

became normally metamorphosed frogs. The testes of this male parent were of type 1.

c. Descendants from neutron-irradiated sperm

Normally metamorphosed frogs were obtained from 31%, 23% and 13% of oviducal eggs by sperm irradiated with 50, 90 and 130 rads of neutrons, respectively (Table 3, Fig. 31). As in the descendatns from X-irradiated sperm, such a regular decrease in the percentage of normally metamorphosed frogs with an increase in dosage was not observable in the second-, third- and fourth-generation offspring. In the second generation, the 50 rad, 90 rad and 130 rad series scarcely differed from one another in the percentage of normally metamorphosed frogs. Although these percentages in the second-generation offspring produced from male parents were remarkably lower than those from female parents, each of them was more or less higher than that in the first-generation offspring of the same irradiation group. In the third generation, the percentages of normally metamorphosed frogs somewhat increased as a whole, as compared with those in the second generation. However, curious relations were found between the second- and third-generation offspring derived from spermatozoa irradiated with 50 rads of neutrons (Fig. 31). The percentages of normally metamorphosed frogs in the third generation derived from grandmothers were far lower than that in the parental second generation. Contrarily, those in the third generation derived from grandfathers were far higher than that in the parental second generation. Similar relations were also found in each of the series derived from spermatozoa irradiated with 90 rads and 130 rads of neutrons, although there were a few exceptional cases. The fourth-generation offspring were remarkably higher than the third-generation offspring and similar to the controls in the percentage of normally metamorphosed frogs.

Many of the normally shaped tadpoles raised from neutron-irradiated spermatozoa had various kinds of chromosome aberrations (Tables 5, 6). The percentages of normal diploids distinctly decreased with increase of dosage from 50 rads to 130 rads. They were nearly in parallel with those of normally metamorphosed frogs. Tadpoles with chromosomal aberrations had mostly mitoses that contained a chromosome with a translocation or deletion. Besides, there were some tadpoles containing hypo- or hyperdiploid, or hypo- or hypertriploid mitoses. A dicentric, ring or minute chromosome were found in a few tadpoles.

In contrast to the controls, most of 80 or 100 normally metamorphosed frogs died before sexual maturity in each of the three groups of 50, 90 and 130 rad irradiation. From the remaining frogs, a total of 28 three-year-old females and a total of 21 two-year-old males were selected to produce second-generation offspring in the three groups of irradiation. After pituitary injection, 24 of the females laid 219～1041 eggs which were far fewer than those laid by the control females (Table 21). While a female laid two kinds of eggs in size, none laid white eggs. There were no interrelations between the number of eggs laid by females and the percentage of normally metamorphosed frogs produced by the latter (Table 21,

Fig. 16a, b, c). Four of ten females from the 50 rad series and two of six females from the 90 rad series were scarcely inferior to the controls in this percentage. Karyotypes were examined in 20 normally shaped tadpoles removed from second-generation offspring produced by a female (No. 9 of the 50 rad series) which did not differ from the controls in the production of normally metamorphosed frogs. As a result, two of them were abnormal diploids having a chromosome with a deletion.

None of the 21 males stated above had normal testes of type 1. Eight, ten, one and two males were of type 2, type 3, type 4 and type 5, respectively, in inner structure of the testes. These types of their testes were substantially reflected in their reproductive ability (Table 12, Fig. 13a, b, c). There were no males that were as good as the controls in reproduction. Karyotypes were examined in normally shaped tadpoles which were produced by a male first-generation offspring which was the best in producing normally metamorphosed frogs in each of the 50, 90

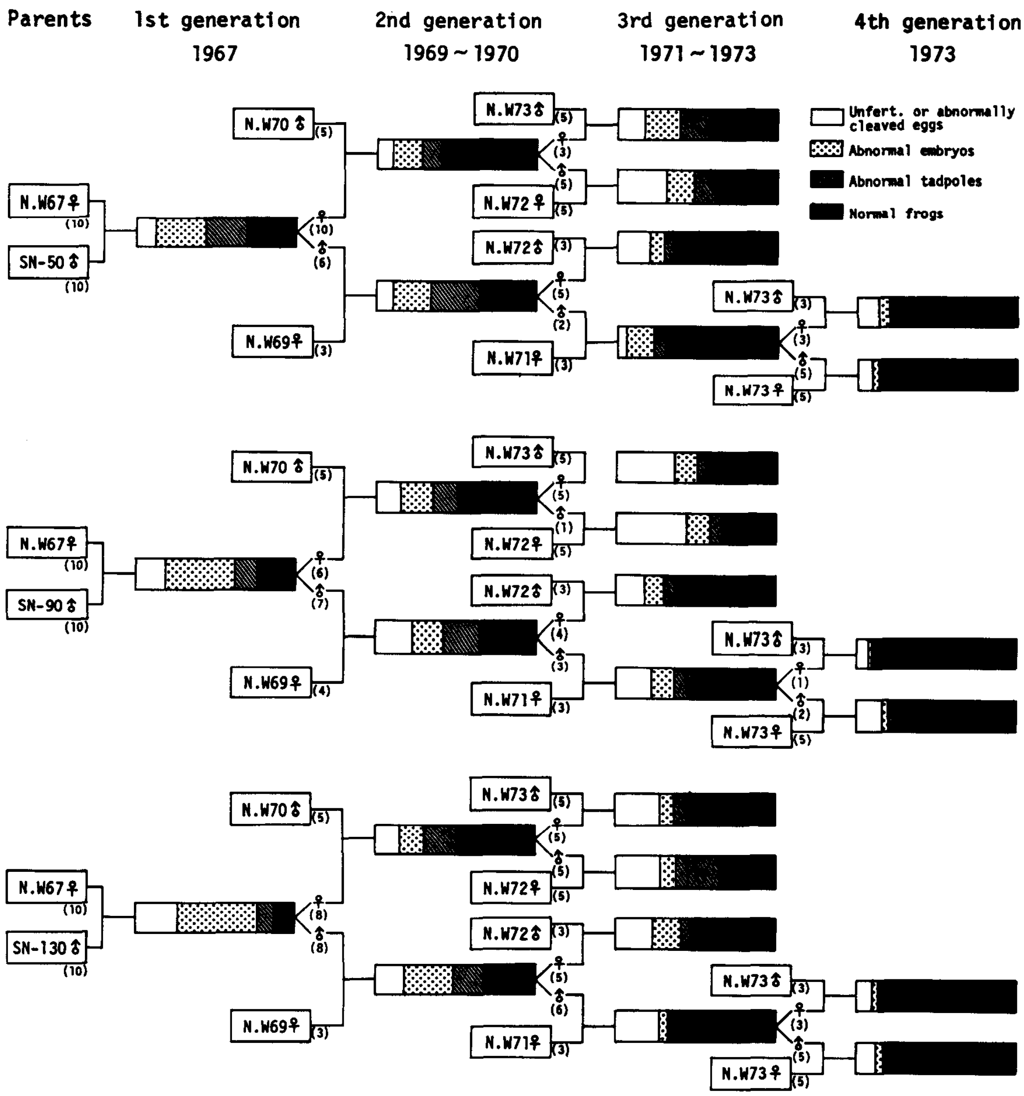


Fig. 31. Diagrammatic representation of the viabilities of first- to fourth-generation offspring derived from neutron-irradiated sperm. The number of frogs used for each kind of matings is given in parentheses.

and 130 rad irradiation series. The results showed that 22 (25%) of 88 tadpoles had various kinds of chromosome aberrations (Table 15).

In order to produce third-generation offspring, a total of 48 females and a total of 22 males were selected from second-generation offspring belonging to six experimental series. Twenty-three of the females were produced by male parents derived from neutron-irradiated spermatozoa, while the other 25 were produced by female parents of the same source. After pituitary injection, 15 of the former and 19 of the latter laid 550～1420 and 981～1793 eggs, respectively (Tables 33, 47). There were no females that laid white eggs or two kinds of eggs in size. The remaining 14 females laid no eggs. The eggs laid by each female were considerably fewer than those laid by each of the control females. However, the number of eggs had no interrelations with their developmental capacities. Of the 19 females produced from female first-generation offspring, 13 were used to produce their offspring. As a result, only one or two belonging to each of the 50, 90 and 130 rad irradiation series were similar to the controls in reproductive capacity, while the others were inferior to various extents (Fig. 28a, b, c). On the other hand, 14 of the 15 females produced from male first-generation offspring were used to produce their offspring. It was found that three belonging to the 50 rad series and two belonging to the 90 rad series were similar to the controls in reproductive capacity. The other nine females were inferior to various extents (Fig. 22a, b, c).

Of the 22 males used for producing third-generation offspring, eleven were obtained from female first-generation offspring, while the other eleven were from male first-generation offspring. Of the former eleven males, seven had testes of type 1, two had those of type 2 and the remaining two had those of type 3 (Table 40). Of the latter eleven males, nine had testes of type 1, while the other two had those of type 2 or 3 (Table 27). The types of their testes were reflected to some extent in the percentages of normally metamorphosed frogs produced by them, although the males with testes of type 1 were mostly inferior to the controls in the percentage of normally metamorphosed frogs produced by them, and moreover, a few of them were very defective in reproductive capacity (Figs. 19a, b, c, 25a, b, c). Karyotypes were examined in 39 normally shaped tadpoles produced by two male second-generation offspring which had been derived from spermatozoa irradiated with 50 and 130 rads of neutrons, respectively. As a result, it was found that seven of them had various kinds of chromosome aberrations.

Fourth-generation offspring were produced by seven female and twelve male third-generation offspring which had been derived from spermatozoa irradiated with 50, 90 or 130 rads of neutrons by passing over male first- and second-generation offspring. All these third-generation offspring were two years old. Although eleven females were injected with pituitaries, only seven of them laid 439～743 eggs, which were remarkably fewer than those laid by the controls (Tables 58, 60). However, these eggs scarcely differed from those of the controls in developmental capacity, except that the eggs of some females were slightly inferior to those of the controls in the percentage of normal cleavages. All the twelve male third-generation offspring had testes of type 1 (Table 54). Although some of them were slightly inferior to the controls in the percentage of normally cleaved eggs, they did not differ from the latter in the subsequent development.

d. Descendants from neutron-irradiated eggs

In the three series derived from eggs exposed to 50, 90 and 130 rads of neutrons, 37%, 23% and 11% of the respective total number of eggs became normally metamorphosed frogs, respectively (Table 3, Fig. 32). In contrast with this, such a clear-cut decrease in the percentage of normally metamorphosed frogs with the increase in the dosage of neutrons was not found in the second- and third-generation offspring. The percentages of normally metamorphosed frogs in the second generation were more or less higher than those in their parental generation. Among the series derived from eggs exposed to 50, 90 and 130 rads of neutrons, there were no remarkable differences in the percentage of normally metamor-

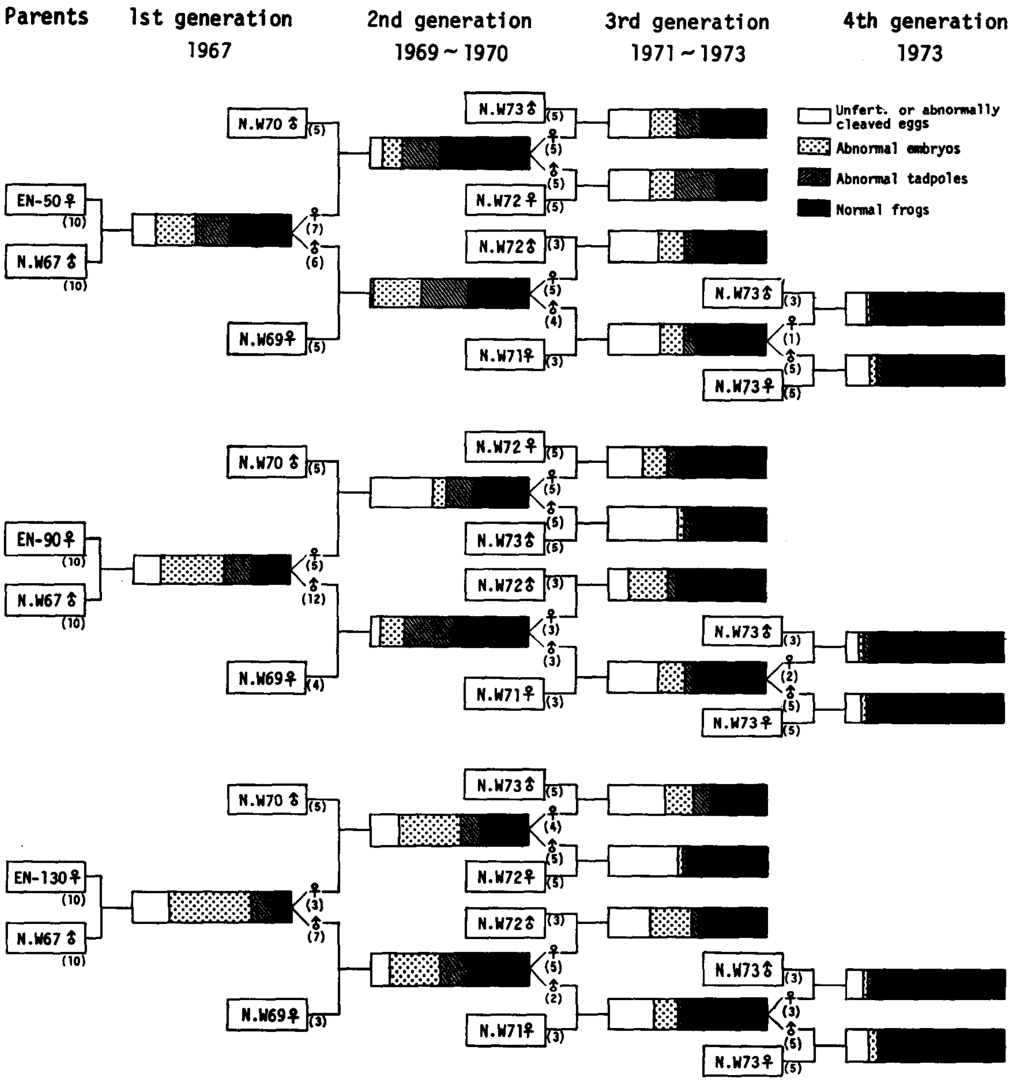


Fig. 32. Diagrammatic representation of the viabilities of first- to fourth-generation offspring derived from neutron-irradiated oviducal eggs. The number of frogs used for each kind of matings is given in parentheses.

phosed frogs. Such percentages in the third generation were somewhat higher as a whole than those in the second generation, although there were a few cases in which the former were remarkably lower than or nearly equal to the latter. The fourth-generation offspring completely differed from the third-generation ones in developmental capacity. The percentages of normally metamorphosed frogs in each experimental series were nearly the same as those in the controls.

Karyotypes were examined in 144 normally shaped tadpoles raised from eggs exposed to 50, 90 or 130 rads of neutrons (Tables 5, 6). Of these tadpoles 85 (59%) were abnormal in karyotype. Normal diploids decreased in number with increase of the dosage of irradiation. Seventeen tadpoles consisted of a single type of abnormal mitoses, while the other 68 consisted of two or more types of mitoses. The chromosome aberrations found most frequently were abnormally diploid mitoses containing a chromosome with a deletion or translocation. Besides, there were hyper- or hypodiploid, triploid, or hyper- or hypotriploid mitoses. In some mitoses, there was a minute, fragment or ring chromosome.

In order to produce second-generation offspring, 25 two-year-old males and 18 three-year-old females were removed from among the frogs raised from eggs exposed to 50, 90 and 130 rads of neutrons. After pituitary injection, 15 of the female frogs laid 379 ~ 647 eggs which were remarkably few, as compared with those of the controls (Tables 18, 22). Four females raised from eggs irradiated with 90 or 130 rads of neutrons laid white eggs besides normal ones. One of these females laid three kinds of eggs in size; the white ones were the largest. Only one of the 15 females was the same as the controls in the percentage of normally metamorphosed frogs produced (Figs. 14, 16a, b, c). This female was that raised from an egg exposed to 50 rads. Four other females raised from eggs irradiated with the same dose were slightly inferior to the controls in this percentage. All the other females raised from neutron-irradiated eggs were remarkably inferior to the controls. There were no interrelations between the number of eggs laid by each female and the percentage of normally metamorphosed frogs produced by her. The male frogs used for producing progeny had more or less abnormal testes except one whose testes were of type 1 (Table 13). The testes of 15, 8 and one males were type 2, type 3 and type 4, respectively. These types were completely reflected in the percentages of normally metamorphosed frogs among the offspring (Fig. 13a, b, c). While a male (No. 8) that had been derived from an egg exposed to 90 rads of neutrons and was the only one having normal testes was similar to the controls (Fig. 11) in percentage of normally metamorphosed frogs, all the other 24 males were more or less inferior to the latter.

The karyotypes of second-generation offspring produced by males which had been raised from neutron-irradiated eggs were examined in 96 normally shaped tadpoles (Table 15). Of these tadpoles, 20 (21%) had various kinds of chromosome aberrations, while the others were normal diploids. It was noteworthy that the male (No. 8) stated above produced six tadpoles with chromosome aberrations among 34 tadpoles, in contrast with the finding that there were no tadpoles with chromosome aberrations among 92 tadpoles produced by the controls. The abnormal mitoses of the six tadpoles had a chromosome with a translocation or deletion, a ring or an additional chromosome. Karyotypes were also examined in 20 normally shaped tadpoles produced by females which had been raised from neutron-irradiated eggs. While 15 of them were normal diploids, the others were abnormal in karyotype. One tadpole was a hyperdiploid, and the other four had abnormal mitoses containing a chromosome with a translocation or deletion.

From among mature second-generation offspring were removed 24 two-year-old males and 53 three-year-old females. After pituitary injection, 39 of the females laid 208～1952 eggs, and 19 of these 39 laid less than 1000 eggs, while eleven of 12 controls laid 1059～2067 eggs (Tables, 31, 34, 44, 48). There were no females which laid white eggs. A total of 27 females which laid 362～1952 eggs were mated with field-caught males in order to produce third-generation offspring (Figs. 22d, e, f, 28d, e, f). Most of these females were slightly or distinctly inferior to the controls (Figs. 20, 26) in the percentage of normally metamorphosed frogs. No interrelation was found between the number of eggs and the percentage of normally metamorphosed frogs produced by each female. Of the 24 males used for producing third-generation offspring, sixteen, four, three and one had testes of type 1, type 2, type 3 and type 4, respectively (Tables 27, 40). All the males were more or less inferior to the controls in the percentage of normally metamorphosed frogs (Figs. 17, 19d, e, f, 23, 25d, e, f). While the testes of type 2 were not always inferior to those of type 1, the testes of types 3 and 4 were very low in this percentage.

The karyotypes of third-generation offspring were examined in 38 normally shaped tadpoles produced by male second-generation offspring derived from eggs exposed to 50 or 90 rads of neutrons. It was found that four of them had various kinds of chromosome aberrations, while the others were normal diploids. One of the four tadpoles was a triploid and the others had haploid or triploid mitoses or abnormally diploid ones containing a ring or deletion-bearing chromosome. In order to produce fourth-generation offspring, 15 males and ten females were removed from among two-year-old third-generation offspring which had been derived from neutron-irradiated eggs by passing over male first- and second-generation offspring (Tables 54, 60). Of the ten females, only six laid 429～742 eggs, while four of five controls did 874～1211 eggs. Each of the females in the experimental series was nearly the same as the controls in the percentage of normally metamorphosed frogs produced by each female. The 15 males stated above had testes of type 1. They scarcely differed from the controls in producing normally metamorphosed frogs, except a few males which were slightly inferior to the controls in this respect.

2. Sex ratio

The sex ratios of the first-, second-, and third-generation offspring from X- or neutron-irradiated spermatozoa or oviducal eggs differed from those of the control frogs (Tables 7, 16, 24, 29, 36, 42, 50), while the sex ratio of the fourth-generation offspring was fairly similar to that of the controls (Tables 56, 62). The sex of juvenile frogs in each generation is presented in Table 63.

a. First-generation offspring from irradiated gametes

There were 1503 (43.3%) females, 325 (9.4%) hermaphrodites and 1642 (47.3%) males among a total of 3470 frogs killed about one month after completion of metamorphosis in the twelve experimental series. Of the females, 1310 (37.8%) had normal ovaries, while the other 193 (5.6%) had underdeveloped ones. When the hermaphrodites were counted as males, 56.7% were males. Eleven of the twelve experimental series were each more than 55% in the sum total of males and hermaphrodites, while the remaining series derived from eggs exposed to 200 rads of X-rays was 37.3%, although the total number of frogs were comparatively few. In the control series, 431 (49.7%) were females, 28 (3.2%) were hermaphrodites and 408 (47.1%) were males; the sum total of males and

TABLE 63

Sex of first-, second-, third- and fourth-generation offspring derived from irradiated gametes

Generation (Group)	Series	No.	♀$_{N}$	♀$_{U}$	⚥	♂$_{N}$	⚥ + ♂$_{N}$
First	Cont.	867	410 (47.2%)	21 (2.4%)	28 (3.2%)	408 (47.1%)	436 (50.3%)
	Exp.	3470	1310 (37.8%)	193 (5.6%)	325 (9.4%)	1642 (47.3%)	1967 (56.7%)
Second (from I ♂)	Cont.	1507	760 (50.4%)	1 (0.1%)	3 (0.2%)	743 (49.3%)	746 (49.5%)
	Exp.	5289	3072 (58.1%)	47 (0.9%)	40 (0.8%)	2130 (40.3%)	2170 (41.0%)
(from I ♀)	Cont.	505	238 (47.1%)	16 (3.2%)	10 (2.0%)	241 (47.7%)	251 (49.7%)
	Exp.	4946	2068 (41.8%)	385 (7.8%)	338 (6.8%)	2155 (43.6%)	2493 (50.4%)
(Total)	Cont.	2012	998 (49.6%)	17 (0.8%)	13 (0.6%)	984 (48.9%)	997 (49.6%)
	Exp.	10235	5140 (50.2%)	432 (4.2%)	378 (3.7%)	4285 (41.9%)	4663 (45.6%)
Third (from I ♂, II ♂)	Cont.	415	204 (49.2%)	7 (1.7%)	12 (2.9%)	192 (46.3%)	204 (49.2%)
	Exp.	3325	1347 (40.5%)	187 (5.6%)	185 (5.6%)	1606 (48.3%)	1791 (53.9%)
(from I ♂, II ♀)	Cont.	529	253 (47.8%)	13 (2.5%)	14 (2.6%)	249 (47.1%)	263 (49.7%)
	Exp.	4488	1931 (43.0%)	257 (5.7%)	339 (7.6%)	1961 (43.7%)	2300 (51.2%)
(from I ♀, II ♂)	Cont.	382	185 (48.4%)	7 (1.8%)	3 (0.8%)	187 (49.0%)	190 (49.7%)
	Exp.	1487	590 (39.7%)	135 (9.1%)	156 (10.5%)	606 (40.8%)	762 (51.2%)
(from I ♀, II ♀)	Cont.	476	232 (48.2%)	7 (1.5%)	2 (0.4%)	235 (49.4%)	242 (50.8%)
	Exp.	3116	1262 (40.5%)	195 (6.3%)	220 (7.1%)	1439 (46.2%)	1659 (53.2%)
(Total)	Cont.	1802	874 (48.5%)	34 (1.9%)	31 (1.7%)	863 (47.9%)	899 (49.9%)
	Exp.	12443	5157 (41.4%)	774 (6.2%)	900 (7.2%)	5612 (45.1%)	6512 (52.3%)
Fourth (from I ♂, II ♂, III ♂)	Cont.	446	213 (47.8%)	11 (2.5%)	5 (1.1%)	217 (48.7%)	222 (49.8%)
	Exp.	7329	3322 (45.3%)	269 (3.7%)	326 (4.4%)	3412 (46.6%)	3738 (51.0%)
(from I ♂, II ♂, III ♀)	Cont.	675	330 (48.9%)	7 (1.0%)	5 (0.7%)	333 (49.3%)	338 (50.1%)
	Exp.	4996	2209 (44.2%)	176 (3.5%)	229 (4.6%)	2382 (47.7%)	2611 (52.3%)
(Total)	Cont.	1121	543 (48.4%)	18 (1.6%)	10 (0.8%)	550 (49.1%)	560 (50.0%)
	Exp.	12325	5531 (44.9%)	445 (3.6%)	555 (4.5%)	5794 (47.0%)	6349 (51.5%)

hermaphrodites was 50.3% of the young frogs examined. At the age of two years, 149 (51.9%) of 287 frogs were males in the experimental series, while 25 (51.0%) of 49 frogs were males in the controls. In the above exceptional series from eggs irradiated with 200 rads of X-rays, there were six females and nine males. When the figures of the two-year-old frogs in the twelve experimental series were added to those of the young frogs stated above, 2116 (56.3%) of 3757 frogs were males or hermaphrodites in the experimental series, while 461 (50.3%) of 916 controls were males. In the series from eggs irradiated with 200 rads of X-rays, 40 (40.8%) of 98 frogs were males or hermaphrodites (Table 7).

b. Second-generation offspring from irradiated gametes

The second-generation offspring from irradiated gametes were sorted into two groups. One group came from first-generation males, while the other came from first-generation females. Among a total of 10235 juveniles in the two groups of second-generation offspring there were 5572 (54.4%) females and 4663 (45.6%) males including 378 (3.7%) hermaphrodites. Among a total of 2012 controls, there were 1015 (50.4%) females and 997 (49.6%) males including only a few hermaphrodites. It was remarkable that the two groups of second-generation offspring distinctively differed from each other in sex ratio. The first group from first-generation males was generally smaller in the rate of males than the second group from first-generation females, when these groups of second-generation offspring were examined at the immature as well as mature frog stage.

i) From first-generation males

A total of 5574 second-generation offspring produced from 96 matings in the 12 experimental series as well as 1539 control frogs were examined in terms of their sex. Among 5289 frogs killed within three months after metamorphosis in the 12 experimental series, there were 3119 females and 2170 (41.0%) males including 40 hermaphrodites. Of the females, 3072 had normal ovaries, while the other 47 had underdeveloped ones. In the control series, 49.5% of immature frogs were males including three hermaphrodites. At the age of two years, a total of 285 frogs in the 12 experimental series were examined; 177 were females and 108 (37.9%) males. In the control series, 18 of 32 were females and 14 males. When the immature frogs were put together with these 2-year-old frogs, 3296 were females and 2278 (40.9%) were males including hermaphrodites in the 12 experimental series, while 779 were females and 760 (49.4%) were males including hermaphrodites in the control series. When hermaphrodites were counted as males, 28.5～31.4% of the respective total number of frogs were males in four of the 12 experimental series, while 39.1～41.7% and 45.7～49.4% were males in the other five and three experimental series, respectively (Table 16).

ii) From first-generation females

A total of 6486 second-generation offsprings produced from 77 matings in the 12 experimental series were examined in terms of their sex (Table 24). One month after metamorphosis 2453 of 4946 frogs were females; 2068 and 385 had normal and underdeveloped ovaries, respectively. The other 2493 (50.4%) were males and hermaphrodites; 2155 had normal testes and 338 (6.8%) had gonads transforming from ovaries to testes. In the control series, 254 were females and 251 (49.7%) were males and hermaphrodites. In five of the 12 experimental series, 40.7～48.2% of the respective total number of frogs were males and hermaphrodites, while 52.0～61.2% were those in six others. In the remaining single experimental series, 50% of 50 frogs were males and hermaphrodites, although the number was very small.

One month after metamorphosis, 1706 frogs were left alive in the 12 experimental series. Of these frogs, 668 were examined in terms of their sex within five months after metamorphosis, as they died; 322 were females and 346 (51.8%)

were males and hermaphrodites, while 14 were females and 14 males in the control series. At the age of two years, 872 frogs were living in the 12 experimental series; 406 were females and 466 (53.4%) males. In the control series, 57 of 120 were females and 63 (52.5%) males. Of the sum total of 6486 immature and mature frogs produced from the 12 experimental series, 51.0% were males and hermaphrodites, while 50.2% of 653 were those in the controls. However, 40.3 ~ 48.2% in five of the experimental series, 52.0 ~ 61.0% in six others and 50.7% in the remaining were males and hermaphrodites (Table 24).

c. Third-generation offspring from irradiated gametes

The third-generation offspring from irradiated gametes were not similar to the controls in sex ratio. Of a total of 12443 juveniles in the four groups of experimental series, 5157 (41.4%) and 774 (6.2%) were females with normal and underdeveloped ovaries, respectively. Of the others, 900 (7.2%) were hermaphrodites transforming from females to males, and 5612 (45.1%) were males with normal testes. When the hermaphrodites were counted as males, 52.3% of the total number of frogs were males. In contrast with these frogs derived from irradiated gametes, 899 (49.9%) of 1802 control frogs were males including hermaphrodites; 863 (47.9%) were males with normal testes and 31 (1.7%) were hermaphrodites. Of the others, 874 (48.5%) and 34 (1.9%) were females with normal and underdeveloped ovaries, respectively.

It was remarkable that two of the four groups of third-generation offspring somewhat differed from the other two in sex ratio. The third-generation offspring obtained by passing over first- and second-generation males or females were higher in the rate of males than those passing over first-generation males and second-generation females or first-generation females and second-generation males. In the former two groups, 53.9% and 53.2% of the respective total number of juveniles were males including a small number of hermaphrodites (Tables 29, 50), while 50.9% and 51.2% were those in the latter two groups (Tables 36, 42). In contrast with the second-generation offspring, 50.1 ~ 58.7% were males including hermaphrodites in 22 of the 24 experimental series of third-generation offspring produced by second-generation males, while 46.2% and 46.7% were those only in the remaining two experimental series.

i) From first- and second-generation males

A total of 3451 third-generation offspring produced from 46 matings in the 12 experimental series were examined in terms of their sex (Table 29). Among 3325 frogs examined one month after metamorphosis, there were 1534 females; 1347 (40.5%) and 187 (5.6%) had normal and underdeveloped ovaries, respectively. The other 1791 (53.9%) frogs were males and hermaphrodites; 1606 (48.3%) had normal testes and 185 (5.6%) had gonads transforming from ovaries into testes. Among 415 control frogs, there were 204 (49.2%) males including 12 (2.9%) hermaphrodites. At the age of two years, 53 of 126 frogs were females and 73 (57.9%) were males. The sex of all the frogs examined in the experimental and control series is presented in Table 29. The hermaphrodites in each series are included in the number of males. Of the sum total of 3451 frogs in the experimental series, 1864 (54.0%) were males, while 218 (49.4%) of 441 controls were males. While 50.1∼51.4% of the respective total number of frogs in four experimental series were males, 53.0% and 53.9% in two others and 55.6∼58.7% in the remaining six were males.

ii) From first-generation males and second-generation females

A total of 4515 third-generation offspring produced from 54 second-generation females were examined in terms of their sex (Table 36). Of these frogs, 2215 were females; 1958 had normal ovaries and 257 had underdeveloped ones. The other 2300 (50.9%) were males or hermaphrodites; 1961 (43.4%) had normal testes, and 339 (7.5%) had gonads transforming from ovaries into testes. Of the 12 experimental series, one was 47.6, six were 49.1∼50.7, one was 51.3, and the remaining four were higher than 52 in percentage of the total sum of males and hermaphrodites. In the control series, 263 (49.7%) of 529 frogs were males and hermaphrodites; 249 (47.1%) had normal testes, and only 14 (2.6%) had gonads transforming from ovaries to testes.

iii) From first-generation females and second-generation males

Of a total of 1487 young frogs examined in the twelve expriemental series, 762 (51.2%) were males and hermaphrodites, while 190 (49.7%) of the controls were males and hermaphrodites. Hermaphrodites were far numerous in the experimental series, as compared with those in the control; there were 156 (10.5%) in the former and only three (0.8%) in the latter. Two of the 12 experimental series were 46.2% and 46.7%, three were 50.6∼50.9%, two were 51.3% and 51.7%, and the remaining five were higher than 52% in the rate of males including hermaphrodites.

iv) From first- and second-generation females

A total of 3116 young frogs were examined in terms of their sex one month after metamorphosis. They were third-generation offspring produced from 54 second-generation females. Of these young frogs, 1457 were females; 1262 had normal ovaries and 195 had underdeveloped ones. The remaining 1659 (53.2%) were males and hermaphrodites; 1439 (46.2%) had normal testes, and 220 (7.1%) had gonads transforming from ovaries into testes. There was no experimental series in which the percentage of the total sum of males and hermaphrodites was lower than that in the control series. Hermaphrodites were usually far numerous in each of the experimental series as compared with those in the controls (Table 50).

d. Fourth-generation offspring from irradiated gametes

In the fourth-generation offspring from irradiated spermatozoa or oviducal eggs, 6349 (51.5%) of a total number of 12325 frogs were males including a small number of hermaphrodites, while 560 (50.0%) of the controls were those, when examined about one month after metamorphosis. Of the total number of fourth-generation offspring, 5531 (44.9%) and 445 (3.6%) were females with normal and underdeveloped ovaries, respectively, while 5794 (47.0%) and 555 (4.5%) were males with normal testes and hermaphrodites, respectively. Of the control frogs, 543 (48.4%) and 18 (1.6%) were females with normal and underdeveloped ovaries, respectively, while 550 (49.1%) and 10 (0.8%) were males with normal testes and hermaphrodites, respectively.

i) From first-, second- and third-generation males

In the 12 experimental series, 50.3 ~ 53.7% of juveniles examined were males including hermaphrodites, while 49.8% were those in the controls. Hermaphrodites were remarkably numerous in the experimental series, in which there were 3412 (46.6%) males and 326 (4.4%) hermaphrodites, while there were 217 (48.7%) males and only five (1.1%) hermaphrodites in the controls. Of the 12 experimental series, 50.3 ~ 50.8% in seven, 51.0 ~ 51.1% in four, and 53.7% in the remainder were males including hermaphrodites.

ii) From first- and second-generation males and third-generation females

In the 12 experimental series, 50.7 ~ 55.9% of young frogs examined were males and hermaphrodites, while 50.1% were those in the control series. Hermaphrodites were comparatively numerous in the experimental series; 229 (4.6%) were hermaphrodites and 2382 (47.7%) were males. In the control series, there were only five (0.7%) hermaphrodites. Of the 12 experimental series, 50.7 ~ 50.9% in three, 51.3 ~ 51.6% in three, 52.4 ~ 52.9% in three, and 53.6 ~ 55.9% in the remaining three were males including hermaphrodites.

DISCUSSION

1. Low viability of descendants

RUGH (1939) has suggested that both dominant and recessive lethal mutations may be produced by X-irradiation of frog sperm chromosomes. According to him, death prior to metamorphosis after gastrulation may be considered to result from induced dominant lethal mutations; the number and the importance of chromosome changes produced by X-irradiation may determine the exact time of death. A remarkable reciprocal translocation was actually found between a chromosome No. 1 and a chromosome No. 12 among 12 pairs of chromosomes in the mitoses of epidermal cells of the caudal fin at the hatching stage as well as among lampbrush chromosomes of oocytes in an urodelous amphibian, *Pleurodeles waltl*, raised from a fertilized egg irradiated with 70 r of γ-rays (GALLIEN, LABROUSSE and LACROIX, 1966; LABROUSSE, 1967; GALLIEN, 1969a, b). On the basis of the fact that the same chromosome aberration was found in both somatic cells and oocytes, they presumed that this chromosome abnormality would be formed in one of the pronuclei of the egg after irradiation. Another female adult raised from a fertilized egg irradiated with the same dosage was a genetic mosaic and had two kinds of chromosome aberrations, a deletion and a translocation, when mitotic figures were observed in epidermal cells of the caudal fin (LABROUSSE, 1967, 1969). Although this female was peculiar in appearance of the skin, she was fertile and produced offspring of two generations. While the chromosome aberrations stated above were not discovered in the mitoses of first-generation offspring, the translocation was found only in a few second-generation offspring (LABROUSSE, 1969).

JAYLET and BACQUIER (1967) exposed the abdomen of male *Pleurodeles waltl* to 250 to 500 r of X-rays and mated them with unirradiated females. Although the proportion of abnormal embryos was very significant, 80 metamorphosed newts were obtained. JAYLET and BACQUIER reported in detail about the chromosome aberrations in each of six individuals which could attained the age of about one year. According to them, three of them had one translocation, another had two translocations and the remaining two had one deletion. These six newts with chromosome aberrations were nearly normal or slightly retarded in development as compared with the controls. LACROIX and LOONES (1971) examined the lampbrush chromosomes of ten *Pleurodeles waltl* which had been raised from sperm X-irradiated by the method utilized by JAYLET and BACQUIER, and found that nine of these newts had chromosome aberrations. CONTER and JAYLET (1974) have reported on a strain of *Pleurodeles waltl* which have a triple translocation involving three chromosomes Nos. 3, 6 and 7. This chromosome aberration was detected in a male offspring which had been produced from an X-irradiated male by mating with a normal female. The only male provided with the chromosome aberration was mated again with a normal female and produced many male and female second-generation offspring. Of the latter, two males and four females which had the same chromosome aberration as that in their father were mated with normal newts to produce the third-generation offspring. Both the males and females showed nine bivalents and one hexavalent in their first meiotic divisions. While about one-fourth of the third-generation offspring were morphologically normal, the remainders were very abnormal and died early in the larval stage with few exceptions. The former had 24 normal chromosomes or 24 chromosomes with the same triple translocation as that found in their parents. In contrast, the latter had various kinds of unbalanced chromosome formulas, which were attributable to multifarious segregation of the hexavalent chromosome at the gametogeneses of their parents. LACROIX and LOONES (1974) made similar researches as those by CONTER and JAYLET in an allied species, *Pleurodeles poireti*. They produced twenty mature males and ten mature females from matings between two normal females and two X-irradiated males. Seven of the mature females were found to have chromosome aberrations as the result of examining their lampbrush chromosomes. While one of them had an inversion and three others had a reciprocal translocation, another had two inversions and one reciprocal translocation. Still another had two translocations involving two and three bivalents and the remainder had a chain of translocations involving five bivalents. These females with chromosome mutations were mated with normal males. While the offspring of the female with an inversion could normally develop into adults, those of each of the other six females were almost lethal in various developmental stages. Only a few adults were produced from among numerous fertilized eggs laid by each of four females with one or two reciprocal translocations. Of the larvae produced from the female with two translocations, 90% were polyploids.

On the other hand, trisomic *Pleurodeles waltl* obtained from crosses between triploid males and diploid females were also fertile (LACROIX, 1967; GUILLEMIN, 1972, 1974). By mating with normal diploids, they produced many inviable embryos which were morphologically and karyologically abnormal in addition to normally shaped diploids and trisomics.

The present research on the effects of irradiation on the developmental capacity of gametes in *Rana nigromaculata* was commenced in 1967. Thereafter, the effects of irradiation were examined from generation to generation until the fourth-generation offspring. Color mutations induced by the same kinds of irradiation have been reported in detail by one of the present authors (NISHIOKA, 1977). As these color mutations are a part of recessive gene mutations, there must have been many other kinds of dominant and recessive gene mutation as well as various kinds of chromosome mutations induced by irradiation at the same time as the color mutations were induced. The inviability or ill-development of the individuals raised from irradiated gametes seems attributable to both gene mutations and chromosome aberrations, since the irradiation of cytoplasm with such dosages as used in the present research does not seem to diminish the developmental capacity of the fertilized eggs.

The deleterious effects of X-rays or neutrons on spermatozoa or oviducal eggs can roughly be shown by the percentages of metamorphosed frogs raised from these irradiated gametes for convenience' sake; 36%, 25% and 14% of eggs by spermatozoa irradiated with 90, 170 and 240 rads of X-rays (Fig. 29), 33%, 21% and 9% of eggs by irradiation with 90, 145 and 200 rads of X-rays (Fig. 30), 31%, 23% and 13% of eggs by spermatozoa irradiated with 50, 90 and 130 rads of neutrons (Fig. 31), and 37%, 23% and 11% of eggs by irradiation with 50, 90 and 130 rads of neutrons (Fig. 32) became normally metamorphosed frogs, respectively. From these figures it was clear that the deleterious effects of irradiation increased approximately in proportion to the increase of dose. In contrast with these first-generation offspring raised from irradiated gametes, the second- and third-generation offspring derived from these gametes scarcely showed such a gradient in the percentage of normally metamorphosed frogs. However, the second- and third-generation offspring were somewhat higher as a whole in this respect than the first- and second-generation offspring, respectively. The fourth-generation offspring were nearly normal in development.

In contrast with the first-generation offspring raised from irradiated gametes, their second- and third-generation offspring produced by mating with field-caught frogs seem to have been burdened with chromosome mutations alone as the cause of inviability or ill-development. The dominant lethal genes induced by irradiation must have been lost by extinction of the first-generation offspring which carried them. The recessive lethal genes induced by irradiation must have had no chance to become homozygous and to function. On the other hand, all the chromosome aberrations induced by irradiation must have been lost at the same time as the dominant lethal genes, as far as they were deleterious to the existence of individuals. However, when the chromosome aberrations of gametes induced by irradiation happened to be balanced like a reciprocal translocation or an inversion, the embryos raised from these gametes by fertilization with normal gametes of field-caught frogs must have been able to become mature frogs. Although the karyotypes of mature frogs raised from irradiated gametes were not directly examined in the present research, it is evident that most of these frogs at least had one or more balanced, chromosome aberrations in their karyotypes. As stated above, chromosome aberrations in mature newts raised from irradiated eggs have been repeatedly observed by GALLIEN, LABROUSSE and LACROIX (1963, 1966) and others. The fact that the offspring of the newts having chromosome aberrations such as one or two translocations are almost lethal owing to various kinds of unbalanced chromosome formulas has been confirmed by CONTER and JAYLET (1974) in *Pleurodeles waltl* and by LACROIX and LOONES (1974) in *Pleurodeles poireti*. It is very probable that similar phenomena occurred in the first-, second- and third-generation offspring derived from irradiated *Rana nigromaculata* gametes.

It is assumed that most of the males and females raised from irradiated spermatozoa or oviducal eggs and used for producing the second-generation offspring by mating with field-caught frogs had one or more reciprocal translocations or an additional chromosome, that is, almost balanced chromosome formulas. While about one-fourth to three-fourths of the normally shaped tadpoles raised from X- or neutron-irradiated spermatozoa or oviducal eggs had chromosome aberrations, only a small part of such tadpoles in the second or third generation derived from these gametes was not normal in karyotype. While normally shaped tadpoles mostly completed metamorphosis (Tables 3, 14, 23, 28), most or about half of the normally metamorphosed frogs could not attain their sexual maturity (Tables 7, 16, 24, 29). In the first-generation offspring, only 287 (26%) of 1120 newly metamorphosed frogs raised from X- or neutron-irradiated spermatozoa or oviducal eggs were alive at the age of two years, while 49 (82%) of 60 control frogs were so (Table 7). In the second-generation offspring produced from males and females which had been raised from X- or neutron-irradiated spermatozoa or oviducal eggs, 1157 (50%) of 2306 newly metamorphosed frogs became two-year-old frogs, while 152 (76%) of 200 controls did so (Tables 16, 24). In the third-generation offspring produced from males which have been derived from the four kinds of irradiated gametes, 126 (35%) of 360 newly metamorphosed frogs were living at the age of two years, while 26 (87%) of 30 controls were alive at the same age (Table 29). Accordingly, it is very probable that all or most of the normally shaped tadpoles having chromosome aberrations died at the tadpole and frog stages, and that the mature frogs had scarcely such chromosome aberrations as those observed at the tadpole stage. At the same time, the mature frogs raised from X- or neutron-irradiated spermatozoa or oviducal eggs as well as those in the offspring of these frogs are mostly presumed to have had a few reciprocal translocations that could not be detected in the metaphase spreads at the tadpole stage. Such reciprocal translocations may be found in the lampbrush chromosomes of oocytes of females. In contrast, a few mature first- and second-generation offspring as well as almost all mature third-generation offspring derived from irradiated gametes seem to have been free from such chromosome aberrations, as they were nearly the same as the controls in reproductive capacity.

The normally shaped tadpoles with chromosome aberrations in the first-, second- or third-generation offspring derived from irradiated gametes were mostly mosaics in karyotype. As these chromosomal mosaics consisted of a mixture of two or more kinds of abnormal karyotypes or a mixture of normal and abnormal karyotypes, they must have been established at the first cleavage or later as results of abnormal divisions of somatic chromosomes like non-disjunction, elimination or fragmentation. This situation seems to show that karyotypes with chromosome aberrations usually prevent the regular progress of both meioses and mitoses, even if they are of balanced chromosome formulas.

2. Abnormality in sex differentiation

In *Rana nigromaculata*, the male heterogamety is well established. This was assumed for the first time by the senior author (KAWAMURA, 1939) on the basis of the sex of parthenogenetically produced frogs; of 23 frogs, 16 were females with normal ovaries, two were females with degenerating ovaries, four were transforming into males and one was a male with an almost typical testis. Of 18 frogs produced parthenogenetically by him in 1940, 16 were females with normal ovaries, one was a female with underdeveloped ovaries and the remainder was a male with almost normal testes (KAWAMURA, 1949). Thereafter, the heterogamety of *Rana nigromaculata* was repeatedly confirmed by the present authors (1977a) by producing diploid frogs gynogenetically. Of 1400 diploid frogs raised gynogenetically from eggs of 25 of 26 field-caught females, 1372 were females, 8 were juvenile hermaphrodites and 20 were males. In contrast with the 25 field-caught females, the remaining one produced many males; while of 18 gynogenetic frogs which died within one year after fertilization, 14 were females, three were hermaphrodites and one was a male, 43 of 44 frogs that lived for one year or more were males and the remainder was a female. All the males and hermaphrodites produced parthenogenetically or gynogenetically were believed to be sex-reversed or sex-reversing genetic females. These males had really some traces of sex reversal from ovaries to testes in the structure of their gonads.

The male heterogamety of the Japanese brown frog, *Rana japonica*, was assumed by KAWAMURA and YOKOTA (1959) from the result of their experiments that the progeny produced by about half the number of males which had been raised from androgen-injected tadpoles were almost exclusively females. It was evident that these male parents were sex-reversed genetic females. The same result was also obtained in *Rana nigromaculata* by the present authors (1977a); of 216 newly metamorphosed frogs produced from two of five males which had been raised from androgen-injected tadpoles, 202 were females and 14 were hermaphrodites, while the other three males produced 154 females, 153 (49.8%) males and no hermaphrodites. It was indubitable that the hermaphrodites in the experimental series became males if they were reared a little longer. Thus, the male heterogamety of *Rana nigromaculata* was reconfirmed by examining the sex of the offspring produced from sex-reversed parents. At the same time, this experiment showed that sex reversal occurred in a few frogs without any extrinsic effect.

The frogs raised from X- or neutron-irradiated spermatozoa or oviducal eggs differed from the controls in that they were not 1: 1 in sex ratio. In eleven of the twelve experimental series, 55% or more frogs were males including a small number of juvenile hermaphrodites, while about 40% were those in the remaining experimental series. Another distinguishable character of the frogs raised from irradiated gametes was that there were comparatively numerous hermaphrodites among the frogs killed within one month after metamorphosis. The second-genertion offspring produced by males raised from irradiated gametes were remarkably different in sex ratio from those produced by females raised from irradiated gametes. In the former case, only 28.6 ~31.4% of the frogs in each of four and 39.1~41.7% of the frogs in each of five of the twelve experimental series were males including a small number of hermaphrodites, respectively. In the remaining three experimental series, 45.7~49.4% were males including hermaphrodites. Of the control frogs, 49.4% were males including a small number of juvenile hermaphrodites. In the latter case where second-generation offspring were produced from female parents, 40.3~48.2% of the frogs in each of five, 52.0~61.0% of the frogs in each of six and 50.7% of the frogs in the remaining of the twelve experimental series were males including a small number of juvenile hermaphrodites, while 50.2% of the controls were males.

The small percentages of males produced by male parents raised from irradiated gametes seems to be attributable to the fact that some of these male parents were sex-reversed genetic females. The fact that 55% or more frogs raised from irradiated gametes were males including a small number of juvenile hermaphrodites in each of the eleven experimental series seemed to show the existence of such sex-reversed genetic females, although these females were never sorted out.

In the third-generation offspring derived from irradiated gametes, there were four groups having different histories; 1) those that passed over first- and second-generation males, 2) those that passed over first-generation males and second-generation females, 3) those that passed over first-generation females and second-generation males and 4) those that passed over first- and second-generation females. In each of the four groups, 50.9~53.9% of frogs examined one or three months after metamorphosis were males including a small number of hermaphrodites, while 49.2~50.8% of the control frogs were males. Such a preponderance of males in the experimental series seems to be attributable to sex reversal occurring in a few genetic females. Besides, it was remarkable that hermaphrodites were far numerous in the experimental series, as compared with the controls. While only 31 (1.7%) of 1802 controls in total were hermaphrodites, 900 (7.2%) of 12443 juveniles examined in the four groups of experimental series were hermaphrodites. Females with underdeveloped ovaries were also more numerous in the experimental series than the controls. While only 34 (1.9%) of the controls had underdeveloped ovaries, 774 (6.2%) of the juveniles in the experimental series had such ovaries.

The fourth-generation offspring from irradiated gametes were considerably similar to the third-generation offspring and not the same as the controls in sex differentiation as well as in sex ratio. While there were 18 (1.6%) females with underdeveloped ovaries and 10 (0.8%) hermaphrodites among 1121 controls, there were 445 (3.6%) females with underdeveloped ovaries and 555 (4.5%) hermaphrodites among 12325 juveniles in the 24 experimental series. When hermaphrodites were counted as males, 51.5% of these juveniles was males, in contrast with 50.0% of the controls. Thus, it was evident that the fourth-generation offspring from irradiated gametes were not completely normal in sex differentiation as well as in sex ratio.

The abundance of hermaphrodites and females with underdeveloped ovaries among the juveniles in each of the four consecutive generations derived from irradiated gametes seems to be attributable to some chromosome aberrations in genetic females, since such a phenomenon was inherited from generation to generation through male or female parents. The smallness in the number of eggs produced from mature first-, second- and third-generation females as well as the abnormality in structure of the testes of mature first- and second-generation males seems also to be attributable to the same cause. The fact that all the mature third-generation males used for producing their offspring were nearly normal in structure of the testes may be explained by minuteness of chromosome aberrations in their karyotypes if they had any chromosome aberrations.

In order to clarify further the effect of irradiation of gametes on the sex differentiation of the descendants, the present authors (1977a) produced gynogenetic diploids from eggs of females raised from X- or neutron-irradiated spermatozoa or oviducal eggs of *Rana nigromaculata*. It was found that about 48% and 18% on the average of frogs derived from X- and neutron-irradiated gametes, respectively, were males, while only 3% of the controls were males. When third-generation offspring were produced from some of these gynogenetic males by mating with field-caught females, about 41% of the frogs derived from X-irradiated gametes were males. As the gynogenetically produced males and their male offspring were all sex-reversed genetic females, these experiments clearly showed that the irradiation of gametes gave rise to presumptive chromosome aberrations inducing sex reversal of genetic females. It was found in these experiments that the larger the dose of X-irradiation, the more frequently the sex reversal occurred, and that X-irradiation of gametes produced sex reversal in females more frequently than neutron-irradiation did.

Remarkable sex reversal has been reported by the present authors (1972, 1977b) in seven consecutive generations of descendants derived from three male nucleo-cytoplasmic hybrids consisting of *Rana ornativentris* cytoplasm and *Rana japonica* nuclei. Sex-reversed genetic females were always accompanied with anatomically and cytologically abnormal embryos and tadpoles and besides they were generally of low viability. The causes for the sex reversal, the anatomical and cytological abnormalities and the low viability seemed to exist in the chromosomes of gametes produced by their parents. A similar phenomenon of sex reversal was reported by NISHIOKA (1972) in the second- and third-generation offspring derived from a female nucleo-cytoplasmic hybrid consisting of *Rana brevipoda* cytoplasm and *Rana nigromaculata* nuclei. Among these offspring, there were significantly numerous females with underdeveloped ovaries and hermaphrodites as compared with the controls, when examined shortly after metamorphosis (Table 13 in NISHIOKA, 1972). Of the respective total number of frogs, 59~71% were males including hermaphrodites. The sex reversal as well as the abundance of hermaphrodites and females with underdeveloped ovaries was accompanied with frequent occurrence of various kinds of chromosome aberrations in normally shaped tadpoles of the same experimental series.

As is generally known, sex reversal from a female to a male arised from various causes besides administration of androgens in several species of *Rana*. Overripeness of oviducal eggs resulted in sex reversal of genetic females (KUSCHAKEWITSCH, 1910; R. HERTWIG, 1921; WITSCHI, 1924). When female tadpoles of *Rana sylvatica* were reared under high temperature, their ovaries were gradually transformed into testes (WITSCHI, 1929). In this case, the medullary portions of the ovaries seemed to make a compensatory growth and to differentiate into rete apparatus as a result of suppression of the normal development of the cortical portions. As the development of the cortical portions of ovaries is probably most sensitively affected by a general abnormality in the metabolism of the body, it is probable that chromosome aberrations bring about such abnormal metabolism of the body and in turn suppress the normal development of the ovarian cortices. When these chromosome aberrations in embryos are large, the latter will sooner or later die of morphological and physiological deficiencies. When they are slight and the genomes are almost balanced, the embryos may be able to develop into mature frogs. However, in the females with such slight, chromosome aberrations, the normal development of the ovaries will be more or less suppressed and result in underdevelopment of the ovaries at the juvenile stage as well as smallness in the number of eggs at the mature stage. In some cases, sex reversal seems to occur in these females by compensatory growth of the medullary portions of the ovaries.

SUMMARY

1. First- to fourth-generation offspring were produced from X- or neutron-irradiated spermatozoa or oviducal eggs of *Rana nigromaculata* by fertilizing with normal gametes in order to clarify the genetic effects of irradiation on the development and sex of amphibians.

2. The number of normally metamorphosed frogs in the first-generation offspring decreased approximately in inverse proportion to increase of radiation

dosage, while such a decrease was not found in the second-, third- and fourth-generation offspring. The number of normally metamorphosed frogs more or less increased in the second-generation offspring as compared with that in the first-generation offspring. A slight increase was also found as a whole in the third-generation offspring. In the fourth-generation offspring, the number of normally metamorphosed frogs as well as those of normal embryos and tadpoles scarcely differed from those in the controls.

3. About one-fourth to three-fourths of normally shaped tadpoles raised from X- or neutron-irradiated spermatozoa or oviducal eggs had chromosome aberrations in all or a part of the metaphase spreads prepared by the squash method. Most of these abnormal mitoses were diploid ones having a chromosome with a translocation or deletion. The number of normal diploids decreased distinctly in inverse proportion to the increase of the irradiation dosage. It was approximately in parallel with the number of normally metamorphosed frogs.

The female frogs raised from X- or neutron-irradiated spermatozoa or oviducal eggs laid remarkably fewer eggs than the controls did, while a few of them did not ovulate after injection of frog pituitaries. A few females laid two or three kinds of eggs which differed in size. Some of the females raised from X- or neutron-irradiated eggs laid white eggs besides normally colored ones. While a female raised from X-irradiated spermatozoa laid a mixture of normal and white eggs, no females from neutron-irradiated spermatozoa laid white eggs. Almost all the males raised from X- or neutron-irradiated spermatozoa or oviducal eggs had testes that were more or less abnormal in spermatogenesis.

4. A small part of the normally shaped tadpoles in the second-generation offspring derived from X- or neutron-irradiated spermatozoa or eggs had various kinds of chromosome aberrations. About one-fourth of the mature females of the same origin did not ovulate after pituitary injection, while most of the remainders laid considerably fewer eggs than the controls did. There were no females that laid white or small eggs besides normal ones. Aproximately one-fourth to three-fourths of the male second-generation offspring derived from irradiated spermatozoa or oviducal eggs had testes that were more or less abnormal in spermatogenesis.

5. A small part of the normally shaped tadpoles in the third-generation offspring derived from X- or neutron-irradiated spermatozoa or oviducal eggs was abnormal in karyotype. Nearly half of the mature females of the same origin did not ovulate after pituitary injection, while one of five controls did not ovulate. Most of the remainders derived from irradiated gametes laid somewhat fewer eggs than the controls did. The mature males of the same origin had all normal testes.

6. While the eggs of female third-generation offspring derived from X- or neutron-irradiated spermatozoa or oviducal eggs were sometimes slightly inferior

to those of the controls in the number of normal cleavages, normally cleaved eggs scarcely differed from the controls in development until metamorphosis. The mature male third-generation offspring derived from X- or neutron-irradiated spermatozoa or oviducal eggs had normal testes and were generally very similar to the controls in producing normally metamorphosed frogs, while a few males were slightly inferior to the latter.

7. The sex ratios of the first-, second-, and third-generation offspring from X- or neutron-irradiated spermatozoa or oviducal eggs differed from those of the control frogs, while the fourth-generation offspring were fairly similar to the controls in sex ratio.

8. In the first-generation offspring raised from irradiated gametes, there were distinctly more hermaphrodites as well as more females with underdeveloped ovaries than those in the controls, when gonads were examined within one month after metamorphosis. Fifty-five percent or more of the sum total of juvenile and mature frogs in eleven of the twelve experimental series were males including a few hermaphrodites, while 50.3% of the controls were males. In the remaining single experimental series, 40.8% of frogs were males including a few hermaphrodites.

9. The second-generation offspring produced by males raised from irradiated gametes differed remarkably in sex ratio from those produced by females of similar origin. Of the offspring of the males, 40.9% were males including juvenile hermaphrodites, while 51.0% of the offspring of the females were males including juvenile hermaphrodites. In the latter case, the percentages of males differed remarkably from one another with the experimental series. In the two control series, 49.4% and 50.2% of the respective number of frogs were males including juvenile hermaphrodites. Hermaphrodites and females with underdeveloped ovaries in the experimental series were generally more numerous than those in the control, when examined at the juvenile stage.

10. The third-generation offspring from irradiated gametes were also not similar to the controls in sex ratio. When hermaphrodites were counted as males, 53.2% of a total number of frogs were males, while 49.9% of the controls were males. In the fourth-generation offspring from irradiated gametes, 51.5% of a total number of frogs were males including a few hermaphrodites, while 50.0% of the controls were males. As found in the first- and second-generation offspring, hermaphrodites as well as females with underdeveloped ovaries were more numerous in the third- and fourth-generation offspring than those of the controls, when examined at the juvenile stage.

11. All the low viability, abnormal sex differentiation and sex reversal in the descendants of the frogs raised from irradiated gametes seem eventually to be attributable to chromosome aberrations induced by irradiation.

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EXPLANATION OF PLATES

PLATE I

Cross-sections of seminal tubules of the testes of mature males, two years old, raised from irradiated gametes of *Rana nigromaculata*. The testes of mature males were divided into five types on the basis of abnormality in inner structure. ×350

1. Type 1 testis of a control, N♀ × N♂, No. 3.
2. Type 2 testis of a male No. 7 produced from a mating, N♀ × SX–90♂, No. 5.
3. Type 3 testis of a male No. 9 produced from a mating, EX–90♀ × N♂, No. 5.
4. Type 4 testis of a male No. 3 produced from a mating, N♀ × SX–170♂, No. 3.
5. Type 5 testis of a male No. 8 produced from a mating, N♀ × SN–130♂, No. 5.

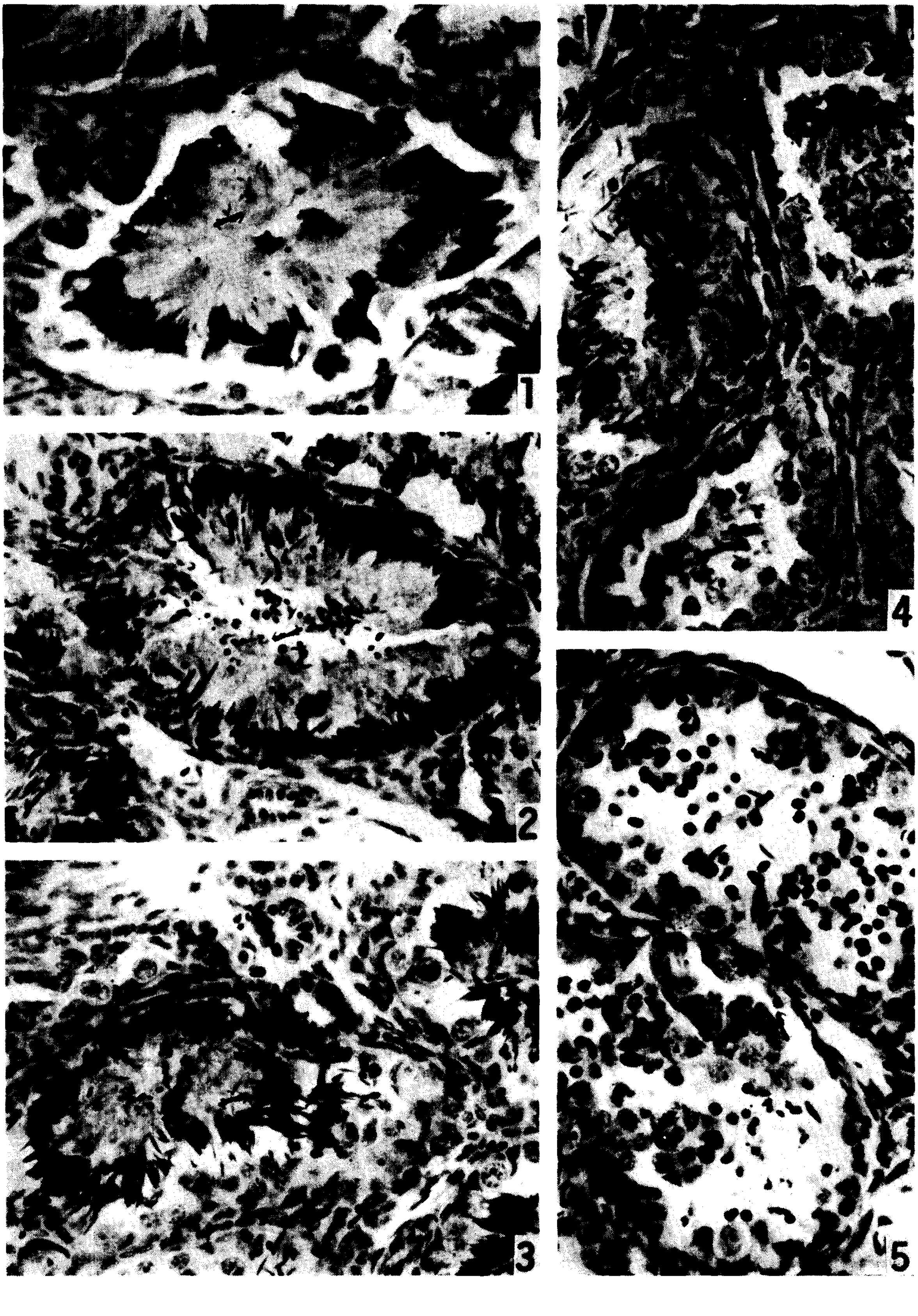
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PLATE II

Abnormal embryos in the second-generation offspring derived from irradiated gametes by passing over female first-generation offspring. ×3.5

6. Controls.
7. (N♀ ×SN–130♂)♀ ×N♂, No. 7.
8. (EX–90♀ ×N♂)♀ ×N♂, No. 4.
9. (EX–145♀ × ♂N)♀ ×N♂, No. 2.
10. (EX–200♀ × ♂N)♀ ×N♂, No. 2.
11. (EN–130♀ × ♂N)♀ ×N♂, No. 2.
12. (EN–130♀ × ♂N)♀ ×N♂, No. 3.

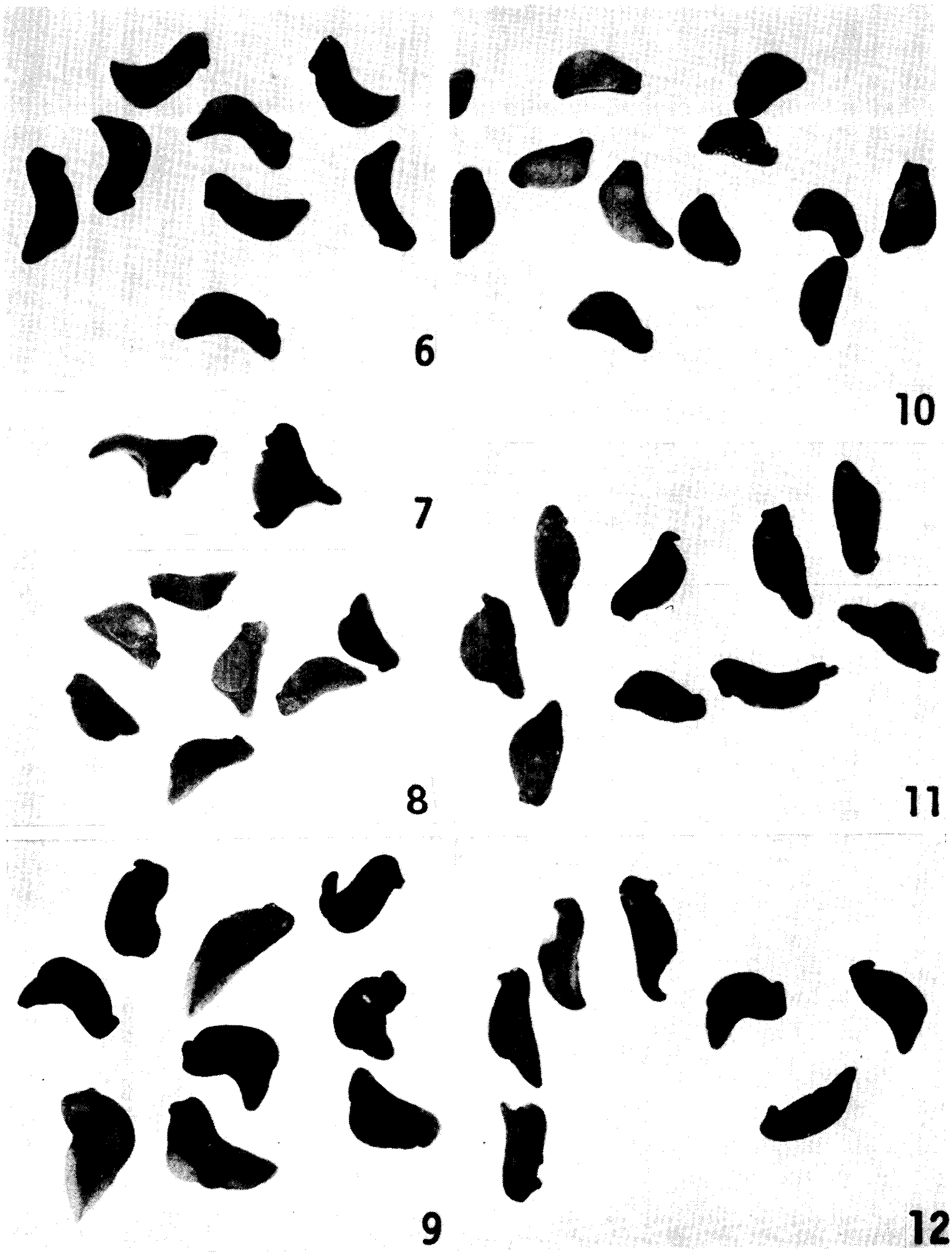
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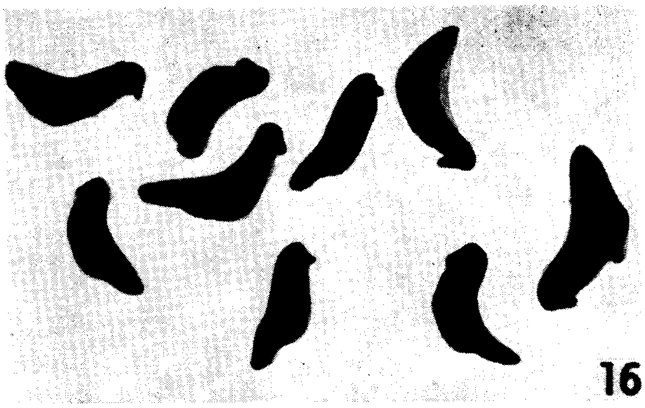
PLATE III

Abnormal individuals at the hatching stage in the second- and third-generation offspring derived from irradiated gametes. ×3.0

13. Controls.
14. (N♀ × SX–240♂)♀ × N♂, No. 2.
15. (EX–145♀ × N♂)♀ × N♂, No. 7.
16. (N♀ × SN–130♂)♀ × N♂, No. 8.
17. N♀ × {N♀ × (N♀ × SN–50♂)♂}♂, No. 2.
18. N♀ × {N♀ × (N♀ × SX–240♂)♂}♂, No. 1.

13



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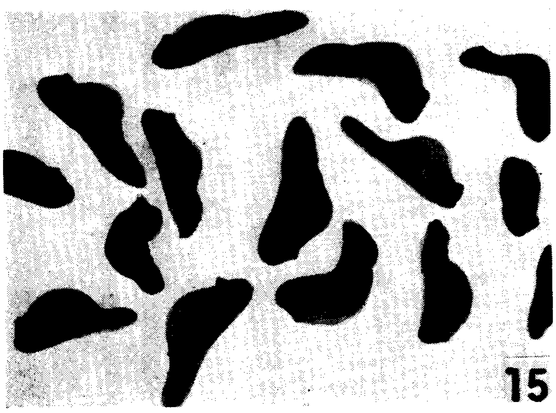

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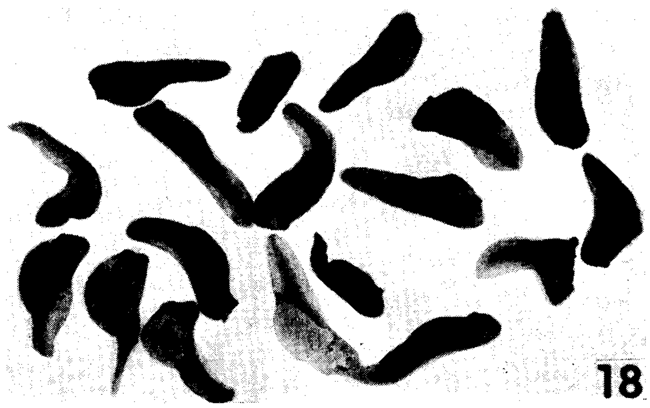

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PLATE IV

Abnormal tadpoles in the second-generation offspring derived from irradiated oviducal eggs by passing over female first-generation offspring. ×4.5

19. Controls.
20. (EX–145 ♀ × N ♂) ♀ × N ♂, No. 9.
21. (EN–130 ♀ × N ♂) ♀ × N ♂, No. 1.
22. (EX–90 ♀ × N ♂) ♀ × N ♂, No. 1.

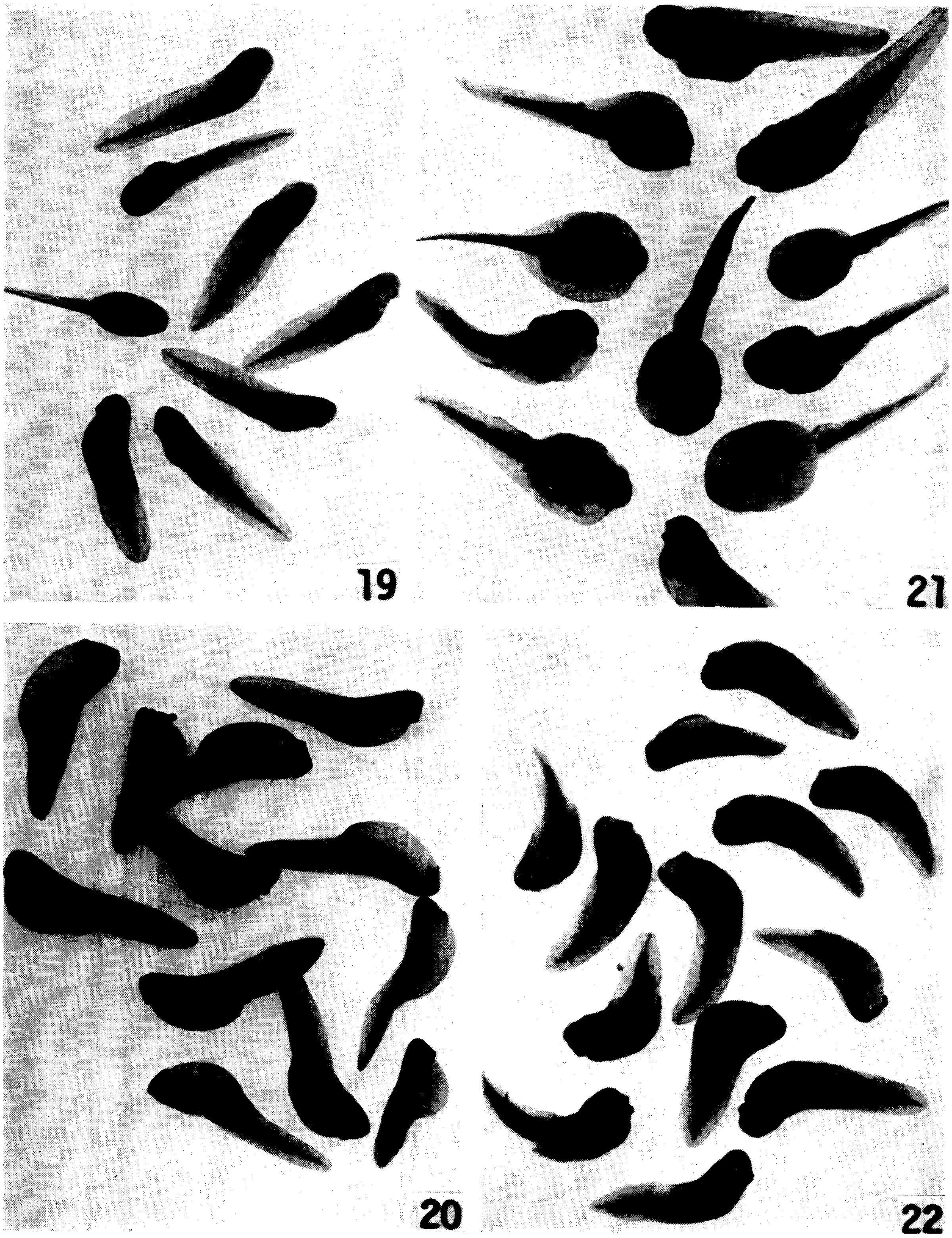
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PLATE V

Abnormal frogs at the stages during or immediately after metamorphosis in the second-generation offspring. ×1.3

23. Controls.
24. (N♀ × SN–130♂)♀ × N♂, No. 4.
25. Ditto.
26. (EN–90♀ × N♂)♀ × N♂, No. 5.
27. (EX–200♀ × N♂)♀ × N♂, No. 2.
28. (N♀ × SX–240♂)♀ × N♂, No. 1.

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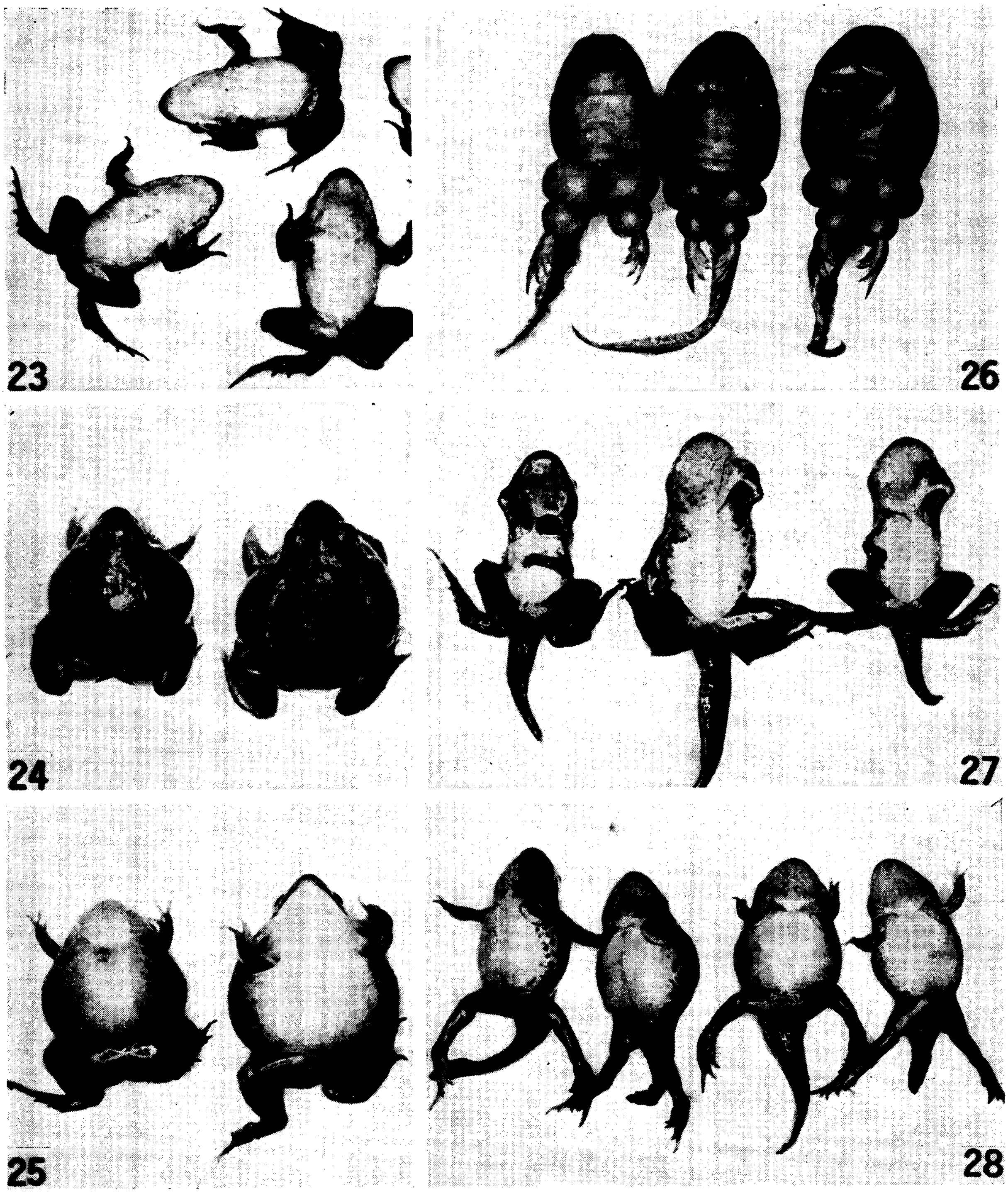

PLATE VI

Frogs with abnormal hind legs in the second-generation offspring. ×1.5

29, 30. (N♀ × SN–130♂)♀ × N♂, No. 7.
31, 32. (N♀ × SN–130♂)♀ × N♂, No. 7.
33, 34. (EN–90♀ × N♂)♀ × N♂, No. 4.

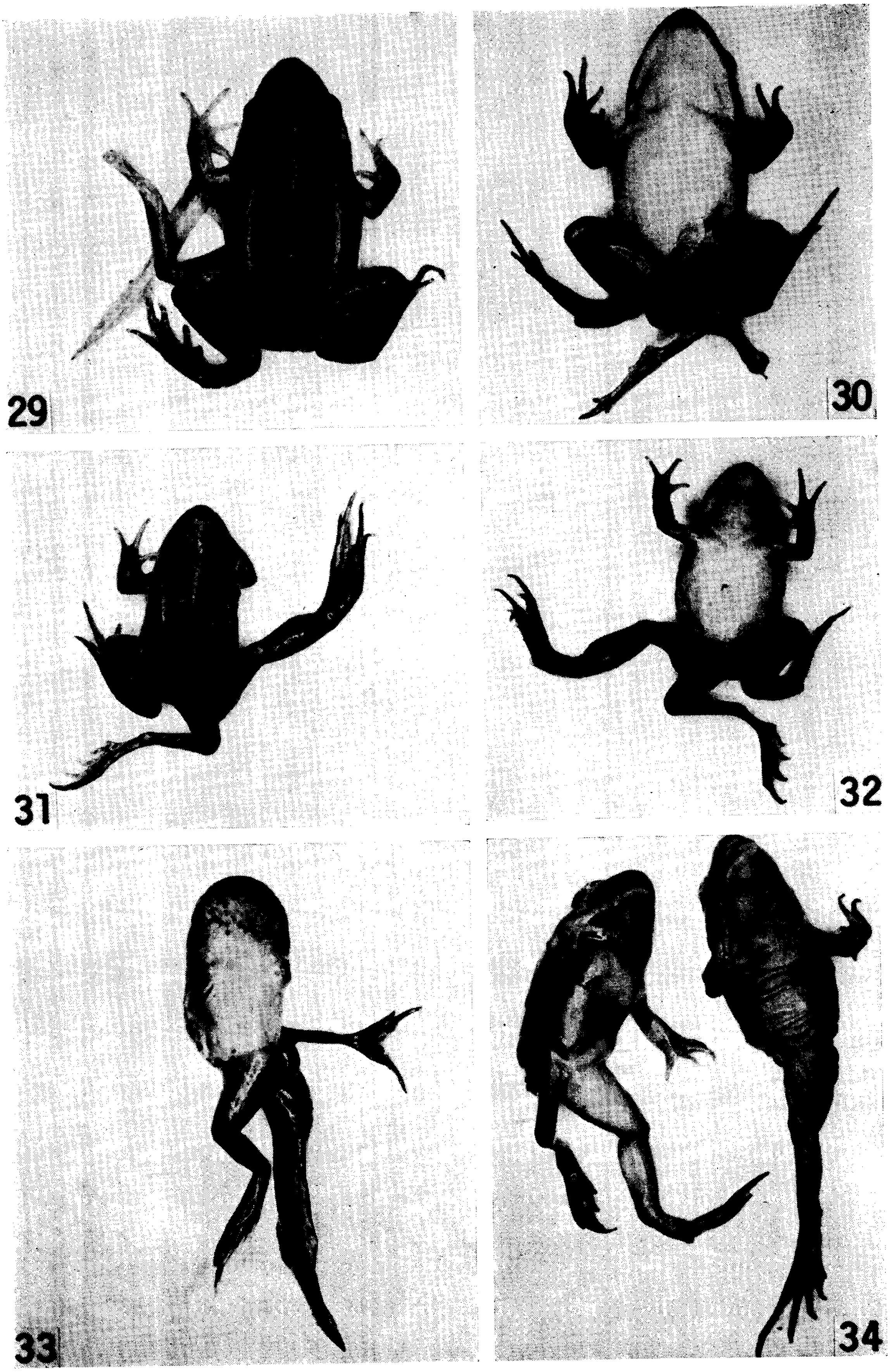
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PLATE VII

A section of a hypertrophic liver of a frog in the second-generation offspring.

35. Liver of a control frog. ×110.
36. Ditto. ×560.
37. Liver of a frog in the second-generation offspring, (EN–90♀ ×N♂)♀ ×N♂, No. 2. ×110.
38. Ditto. ×560.

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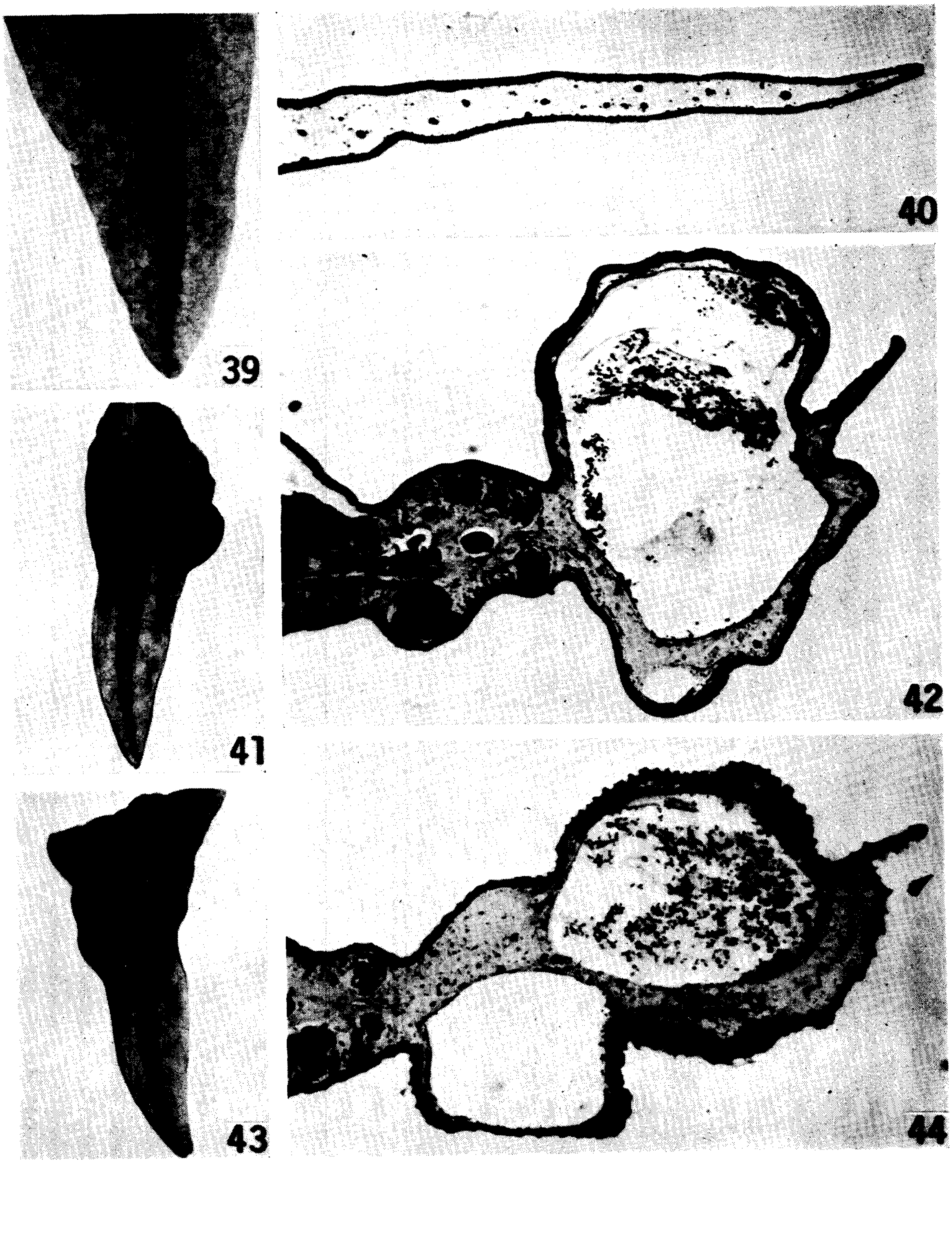
PLATE VIII

Abnormal tail-fins of two tadpoles in the second-generation offspring. Cross sections of the tail-fins in Figs. 39, 41 and 43 are shown in Figs. 40, 42 and 44, respectively.

39, 40.	A control tadpole.	×4.5, ×70.
41, 42.	(EX–145♀×N♂)♀×N♂, No. 9.	×3.5, ×70.
43, 44.	(EN–130♀×N♂)♀×N♂, No. 3.	×3.5, ×70.


39
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41
42
43
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