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

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(With 59 Text-figures and 3 Plates)

CONTENTS

INTRODUCTION

The fact that irradiation of amphibian gametes produces remarkable developmental abnormalities has been reported by many investigators since more than 70 years ago. BARDEEN (1907, 1909, 1911) exposed spermatozoa or ripe ova of a toad or frog to intense X-rays and observed that irradiation gave rise to abnormal development during the embryonic stage. MCGREGOR (1908) obtained similar results by exposing male or female frogs to moderately strong X-rays. The phenomenon known as the paradoxical effect of HERTWIG or simply HERTWIG effect was reported by O. HERTWIG (1911) and G. HERTWIG (1911). While the former author exposed *Rana esculenta* or *R. temporaria* sperm to radium radiation for different periods of time, the latter treated unfertilized eggs of *Rana temporaria* in a similar way. As the result of fertilizing these irradiated gametes by normal gametes, they found that severe radiation-sickness in embryos caused by short exposures was paradoxically reduced by long exposures. These authors confirmed that a pronucleus damaged completely by heavy irradiation does not disturb the development of the egg. The gynogenetic development of frog or toad eggs inseminated with irradiated sperm was further corroborated by G. HERTWIG (1913) and P. HERTWIG (1913).

The paradoxical effect of HERTWIG was also confirmed by SIMON (1930) and DALCQ (1930) in *Rana temporaria* eggs and sperm irradiated with X-rays, ultraviolet rays or radium radiation. The same effect of X-rays upon gametes of American frogs, *Rana pipiens* and *R. catesbeiana,* was reported by RUGH (1939), RUGH and EXNER (1940), HENSHAW (1943), SAUNDERS and RUGH (1943), ROLLASON (1949), and BRIGGS, GREEN and KING (1951). Recently, POGANY (1971, 1973, 1976) re-examined HERTWIG effect by inseminating *Rana pipiens* eggs with UV-irradiated sperm and showed that the most impaired survival occurring at short exposure of sperm to irradiation was concomitant with aneuploid chromosomal conditions. GURDON (1960) and HAMILTON (1967) exposed fertilized eggs of *Xenopus laevis* to UV-rays in order to obtain androgenetic haploids by complete inactivation of the female pronucleus.

The effects of irradiation on chromosomes were examined in urodeles, too. RUGH (1950a) exposed adult males of a Japanese newt species, *Cynops pyrrhogaster,* to large doses of X-rays and showed that the chromosomes lost their identity and became clumped together. SELMAN (1958) produced a high proportion of gynogenetic haploids by inseminating eggs with UV-irradiated sperm in three *Triturus* species, *T. palmatus, T. vulgaris* and *T. alpestris.* Fertilized eggs of *Pleurodeles waltl* were extensively used by GALLIEN and his colleagues in order to elucidate the cause of abnormalities induced by irradiation. At the beginning, GALLIEN and LABROUSSE (1962) observed various abnormalities appearing at the embryonic stage after fertilized eggs were irradiated with neutrons at the stage between the second maturation division and the second polar body formation. Chromosome aberrations were then observed by GALLIEN, LABROUSSE and LACROIX (1963, 1966), GALLIEN, LABROUSSE, PICHERAL and LACROIX (1965) and LABROUSSE (1967) in abnormal embryos raised from fertilized eggs which had been irradiated with γ-rays from radioactive cobalt.

The effects of irradiation on frog gametes have become one of the main subjects of researches in our laboratory since the early part of the 1960's. SAMBUICHI (1964, 1966) exposed oviducal eggs of *Rana nigromaculata* to γ-rays from ^{60}Co and fertilized them with normal sperm. As a result, he obtained androgenetic haploids, a few of which became metamorphosed frogs. Experiments producing gynogenetic diploids in *Rana nigromaculata* by refrigeration of eggs after insemination with UV-irradiated sperm were repeated every year from 1964 to 1968 in this laboratory, and a great number of normally metamorphosed frogs were obtained (KAWAMURA and NISHIOKA, 1977a). Gynogenetic diploids which were more abundant than those of *Rana nigromaculata* were also produced in *Rana brevipoda* during four years from 1966 to 1972 by the same method. Moreover, numerous gynogenetic diploids were similarly obtained in *Rana japonica, R. tsushimensis, R. rugosa, Hyla arborea japonica* and *Bombina orientalis,* too (KAWAMURA and NISHIOKA, 1977a). SAWADA (1967) who was transferred to the Department of Radiation Biology, Research Institute for Nuclear Medicine and Biology after working on the reproduction of Japanese newts in our laboratory exposed oviducal eggs, sperm or fertilized eggs of *Rana nigromaculata* or *R. japonica* to X-rays or neutrons and examined the doses for producing 50% lethal embryos at the hatching stage. A similar experiment to obtain 50% normal survivors at the hatching stage after X- or neutron-irradiation of fertilized *Rana nigromaculata* or *R. japonica* eggs was performed by TAKESHITA and SAWADA (1974).

On the other hand, studies on nucleo-cytoplasmic hybrids have been carried out in our laboratory since 1961. This year, KAWAMURA and NISHIOKA (1963a, b) produced some nucleo-cytoplasmic hybrids between *Rana japonica* and *R. ornativentris* as well as between *Rana nigromaculata* and *R. brevipoda*. Thereafter, first-, second-, third-, fourth- and fifth-generation offspring were obtained from three male nucleo-cytoplasmic hybrids consisting of *Rana ornativentris* cytoplasm and *R. japonica* nuclei by mating repeatedly with the nuclear species during the five years from 1962 to 1966 (KAWAMURA and NISHIOKA, 1972), while the first-, second- and third-generation offspring were produced from two female nucleo-cytoplasmic hybrids consisting of *Rana brevipoda* cytoplasm and *R. nigromaculata* nuclei by mating repeatedly with the nuclear species during the seven years from 1962 to 1968 (NISHIOKA, 1972). In the former case, the majority of the offspring at each of the five generations died before metamorphosis after they had revealed various kinds of abnormalities in their external characters. Most of the normally shaped tadpoles examined at the second, third or fourth generation were not normal in karyotype. A similar situation was also found in the offspring of the three generations derived from the nucleo-cytoplasmic hybrids between *Rana brevipoda* and *R. nigromaculata*. The descendants of both kinds of nucleo-cytoplasmic hybrids were mostly males at the sexually mature stage in contrast with a 1 : 1 sex ratio in the control frogs. On the basis of these abnormalities found in the descendants of nucleo-cytoplasmic hybrids, it occurred to both KAWAMURA and the present author that radiation might give a similar effect upon frog gametes as foreign cytoplasm did upon a transplanted nucleus. Then, in 1967 they began a study on effects of X- or neutron-irradiation of *Rana nigromaculata* gametes to the descendants derived from the latter (KAWAMURA and NISHIOKA, 1973, 1978). Color mutations induced by this irradiation have been reported by the present author (1977). In the next year, the present author commenced a similar study in *Rana japonica* which is systematically remote from *Rana nigromaculata,* in order to confirm the conclusion that would be attained from the researches in *Rana nigromaculata*. The results obtained from the experiments performed in *Rana japonica* during the five years from 1968 to 1972 are reported in detail in this paper.

MATERIALS AND METHODS

Twenty males and twenty females of Japanese brown frogs, *Rana japonica* GÜNTHER, were collected from the suburbs of Hiroshima in October, 1967. In January of the following year, unfertilized eggs obtained from the females after pituitary transplantation and sperm suspensions from the males were irradiated with X-rays or neutrons. The irradiation was carried out at the Research Institute for Nuclear Medicine and Biology attached to Hiroshima University.

The X-ray equipment was Toshiba KXC–18–2 Type which was operated at

180 KVp, 25 mA with the conversion coefficient and exposure dose rate being 0.95 rad/r and ca. 20 rads/min, respectively. Unfertilized eggs received a total dose of 150, 250 or 350 rads, while sperm suspensions a total dose of 150 or 250 rads. The neutron equipment was Toshiba (d.n) He Reaction Type, producing fast neutron having energy of 14.1 MeV. The conversion coefficient was 6.7×10^{-9} rads/n/sec/4π and the exposure dose rate was about 10 rads/min. Unfertilized eggs were irradiated with a total dose of 150 or 250 rads, while sperm suspensions a total dose of 100, 150 or 250 rads.

Artificial fertilization was performed to obtain the first-generation offspring between these irradiated eggs or spermatozoa and untreated gametes of the same species. The fertilized eggs were divided into two groups; one group was utilized for observing their developmental capacity, while the other was utilized for examining their chromosomal abnormality. All the embryos and tadpoles were reared indoors at 15∼17°C for 50 days and then transferred into concrete tanks placed in the open air.

Observation of chromosomes was made in morphologically abnormal embryos as well as in 50-day-old tadpoles with a normal appearance. The chromosomes of the abnormal embryos were examined on the preparations made from their whole bodies by MAKINO and NISHIMURA's squash method (1952) with aceto-orcein after pretreatment with colchicine for 15∼18 hours, while those of the tadpoles were done in their tail-tips by the same method. However, chromosomal analysis was performed when there were more than ten analyzable metaphase plates in each individual. In each individual, chromosomes of more than three metaphase plates were analyzed to establish their karyotypes by making use of photographs enlarged 4500 times, although six to ten metaphase spreads were analyzed in each of chromosomal mosaics.

When tadpoles metamorphosed completely and became young frogs, some of them were killed for the purpose of examining their sex, while the remaining frogs were continuously reared to observe their developmental capacity. At the stage of sexual maturity, some frogs were killed in order to observe the inner structure of their gonads.

Males and females which developed normally and attained sexual maturity were mated with normal frogs collected from the field to obtain second-generation offspring. The viability and chromosomes of these offspring were observed at embryonic and tadpole stages. Their sex was examined at the stage shortly after metamorphosis or of sexual maturity. From male and female second-generation offspring, third-generation offspring were also produced by mating with normal field-caught frogs. By the same way, fourth-generation offspring were produced from the third-generation offspring. The viability, chromosomes and sex of these third- and fourth-generation offspring were examined.

The developmental stages reported in this paper follow those of *Rana pipiens* established by SHUMWAY (1940) and TAYLOR and KOLLROS (1946) for convenience' sake.

The following abbreviations are used.

SX–150♂ — Sperm exposed to 150 rads of X-rays after removed from a male
SX–250♂ — Sperm exposed to 250 rads of X-rays after removed from a male
SN–100♂ — Sperm exposed to 100 rads of neutrons after removed from a male
SN–150♂ — Sperm exposed to 150 rads of neutrons after removed from a male
SN–250♂ — Sperm exposed to 250 rads of neutrons after removed from a male
EX–150♀ — Eggs exposed to 150 rads of X-rays after removed from a female
EX–250♀ — Eggs exposed to 250 rads of X-rays after removed from a female
EX–350♀ — Eggs exposed to 350 rads of X-rays after removed from a female
EN–150♀ — Eggs exposed to 150 rads of neutrons after removed from a female
EN–250♀ — Eggs exposed to 250 rads of neutrons after removed from a female
J♀ — Untreated eggs of a female J♂ — Untreated sperm of a male
J. W68 — Untreated gametes (Control) of the frog(s) caught from the field in 1968

TABLE 1

Developmental capacity of the offspring raised

Parents: Female	Parents: Male	No. of eggs	No. of normally cleaved eggs	No. of gastrulae: Normal	No. of gastrulae: Abnormal
J.W68, Nos. 1～20	J.W68, Nos. 1～20	1635	1281 (78.3%)	1264 (77.3%)	17 (1.0%)
J.W68, Nos. 1～20	SX–150, Nos. 1～20	2258	1825 (80.8%)	1814 (80.3%)	11 (0.5%)
	SX–250, Nos. 1～20	2319	1838 (79.3%)	1809 (78.0%)	29 (1.3%)
EX–150, Nos. 1～20	J.W68, Nos. 1～20	2119	1786 (84.3%)	1752 (82.7%)	34 (1.6%)
EX–250, Nos. 1～20		2260	1927 (85.3%)	1881 (83.2%)	26 (1.2%)
EX–350, Nos. 1～20		2338	1988 (85.0%)	1946 (83.2%)	46 (2.0%)
J.W68, Nos. 1～20	SN–100, Nos. 1～20	2188	1781 (81.4%)	1766 (80.7%)	15 (0.7%)
	SN–150, Nos. 1～20	2204	1767 (80.2%)	1736 (78.8%)	31 (1.4%)
	SN–250, Nos. 1～20	2214	1727 (78.0%)	1701 (76.8%)	26 (1.2%)
EN–150, Nos. 1～20	J.W68, Nos. 1～20	2406	1957 (81.3%)	1943 (80.8%)	14 (0.6%)
EN–250, Nos. 1～20		2275	1834 (80.6%)	1798 (79.0%)	36 (1.6%)

OBSERVATION

I. First-generation offspring raised from irradiated gametes

In order to produce first-generation offspring, 20 males and 20 females of *Rana japonica* were used as parents. The sperm of each male was divided into 11 parts. Five of these parts were exposed to 150 and 250 rads of X-rays and 100, 150 and 250 rads of neutrons, while the other six parts were not irradiated and used for matings with irradiated eggs and the control. The eggs of each female were divided into eleven parts. Five of the latter were exposed to 150, 250 and 350 rads of X-rays and 150 and 250 rads of neutrons, and inseminated with untreated sperm. Five other parts were inseminated with the five kinds of irradiated sperm described above. The remaining part was inseminated with untreated sperm as the control. Thus, the eggs of each female were utilized for the following eleven kinds of matings: J♀ ×J♂ (control), J♀ ×SX–150♂, J♀ ×SX–250♂, J♀ × SN–100♂, J♀ ×SN–150♂, J♀ ×SN–250♂, EX–150♀ ×J♂, EX–250♀ ×J♂, EX–350♀ ×J♂, EN–150♀ ×J♂ and EN–250♀ ×J♂. The eggs of the 20 females used for these control and experimental matings totalled 31920. Of these eggs, 7704 that were about half the number of eggs from six large females (Nos. 1 ~ 6) were used in observing chromosomes.

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 metamor-phosing frogs	No. of metamor-phosed frogs
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal			
1241	23	1209	32	1189	20	1093	1008	942
(75.9%)	(1.4%)	(73.9%)	(2.0%)	(72.7%)	(1.2%)	(66.9%)	(61.7%)	(57.6%)
1753	61	1652	101	1138	514	724	609	585
(77.6%)	(2.7%)	(73.2%)	(4.5%)	(50.4%)	(22.8%)	(32.1%)	(27.0%)	(25.9%)
1745	64	1577	168	822	755	389	300	294
(75.2%)	(2.8%)	(68.0%)	(7.2%)	(35.4%)	(32.6%)	(16.8%)	(12.9%)	(12.7%)
1688	64	1518	170	1032	486	579	470	304
(79.7%)	(3.0%)	(71.6%)	(8.0%)	(48.7%)	(22.9%)	(27.3%)	(22.2%)	(14.3%)
1787	94	1549	238	613	936	241	197	175
(79.1%)	(4.2%)	(68.5%)	(10.5%)	(27.1%)	(41.4%)	(10.7%)	(8.7%)	(7.7%)
1824	122	1496	328	304	1192	90	57	50
(78.0%)	(5.2%)	(64.0%)	(14.0%)	(13.1%)	(51.0%)	(3.8%)	(2.4%)	(2.1%)
1705	61	1542	163	938	604	659	578	542
(77.9%)	(2.8%)	(70.5%)	(7.4%)	(42.9%)	(27.6%)	(30.1%)	(26.4%)	(24.8%)
1641	95	1430	211	690	740	363	296	285
(74.5%)	(4.3%)	(64.9%)	(9.6%)	(31.3%)	(33.6%)	(16.5%)	(13.4%)	(12.9%)
1606	95	1385	221	623	762	268	146	107
(72.5%)	(4.3%)	(62.6%)	(10.0%)	(28.1%)	(34.4%)	(12.1%)	(6.6%)	(4.8%)
1862	81	1712	150	917	795	467	377	310
(77.4%)	(3.4%)	(71.2%)	(6.2%)	(38.1%)	(33.0%)	(19.4%)	(15.7%)	(12.9%)
1703	95	1479	224	622	857	231	149	136
(74.9%)	(4.2%)	(64.8%)	(9.8%)	(27.3%)	(37.7%)	(10.2%)	(6.5%)	(6.0%)

1. Developmental capacity

The development of eggs from cleavage to metamorphosis in each of the 11 kinds of matings is presented in Table 1. The survival curves of the first-generation offspring produced from each mating are shown in Fig. 1. Although there were no differences in the fertilization rate of eggs between the control and experimental series, the embryos and tadpoles produced from irradiated eggs or sperm were distinctly inferior to the control in viability. In the experimental series, the mortality of embryos and tadpoles became higher in proportion to the irradiation dose. There was an evident difference in viability between the individuals produced from eggs and spermatozoa irradiated with the same dose of X-rays, that is, eggs were more sensitive than spermatozoa to irradiation with X-rays. In contrast to this, eggs and spermatozoa were nearly the same in the sensitivity to neutrons.

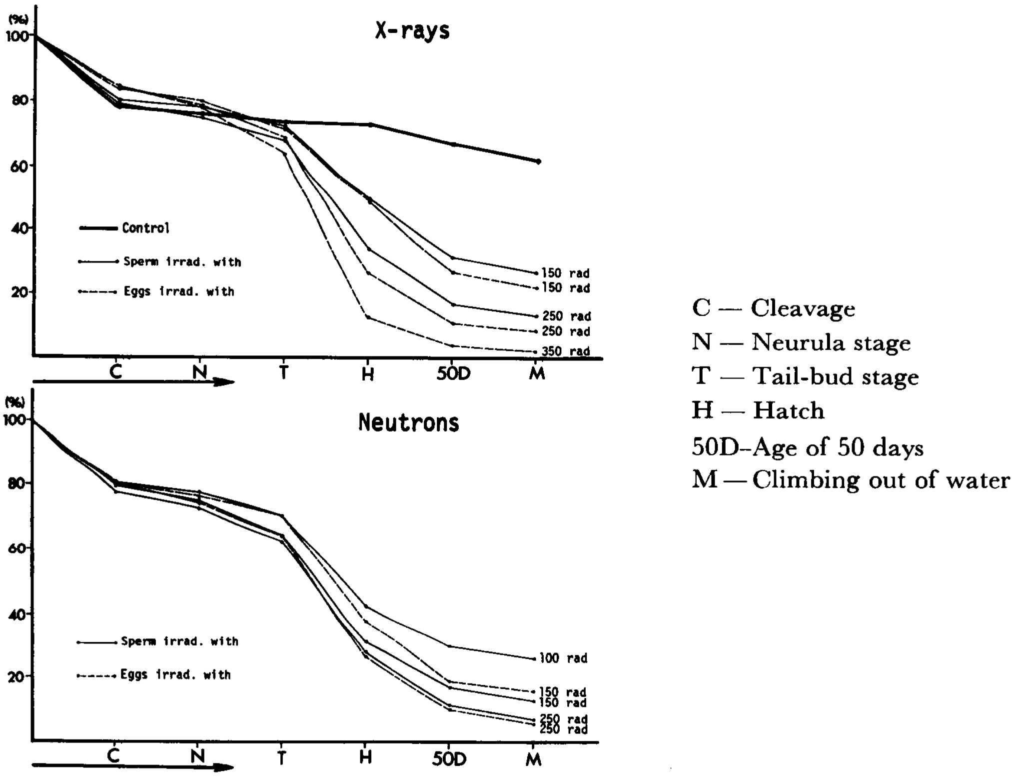


Fig. 1. Survival curves of first-generation offspring raised from X- or neutron-irradiated sperm or oviducal eggs.

Sperm was exposed to 150 or 250 rads of X-rays, or 100, 150 or 250 rads of neutrons. Oviducal eggs were exposed to 150, 250 or 350 rads of X-rays, or 150 or 250 rads of neutrons. The survival curve of the controls is shown by a thick line.

All the individuals which died during development revealed various morphological abnormalities, although the latter had no definite relation to the kind or amount of the dosage with which the eggs or sperm were irradiated. Most of the embryos which died at the gastrula stage were remarkably abnormal in invagination. The individuals which died at the embryonic stage from the beginning of neurulation to the completion of hatching were edematous, acephalous, microcephalous or bicephalous, or their bodies were hypoplastic, severely curved or had irregular protuberances, wrinkles or blisters on the surfaces. Many of normally shaped tadpoles at the hatching stage afterwards became underdeveloped or edematous and died within 50 days after the beginning of development. The underdevelopment of tadpoles at the feeding stage was usually attributable to ill-development of the teeth. Edemata occurred even in the tadpoles with teeth normal enough to take food. Various abnormalities occurred most frequently in tadpoles at the hatching stage. In the other stages, abnormalities occurred more frequently at the first half of the tadpole stage than those at the neurula and tail-bud stages. At the second half of the tadpole stage, abnormalities became remarkably fewer, although tadpoles which were edematous or had ill-developed forelegs were more frequently found than in the controls.

After the completion of metamorphosis, underdeveloped or edematous frogs appeared more frequently in the experimental series than in the controls. Mature frogs decreased in number with increase in the irradiation dose given to the gametes from which they were raised.

2. Chromosome aberrations in abnormal embryos

Chromosome aberrations were examined in abnormal embryos at various stages from the early tail-bud (stage 17) to the hatching (stage 20). These embryos were those raised from sperm or unfertilized eggs irradiated with X-rays or neutrons by fertilization with untreated gametes. All the eggs used were obtained from the largest (No. 1) among the 20 females.

a. Control series

Of 26 abnormal embryos which were squashed and stained with aceto-orcein after pretreatment with colchicine, 20 had more than ten analyzable metaphase spreads (Table 2). While 12 of these embryos were quite normal in the number and shape of chromosomes (Fig. 2) in spite of their abnormal appearance, the other eight had some chromosome aberrations. Two of the latter had no diploid mitoses; one was a pure haploid, that is, 13 in chromosome number, and the other was purely 37 in chromosome number. In this hypotriploid embryo, chromosomes Nos. 3 and 12 were lost from the triploid complement.

Three others of the eight abnormal embryos also had no normally diploid metaphase spreads. They were chromosomal mosaics consisting of two kinds of abnormal cells. One of them was constructed of a mixture of hypertriploid and hyperdiploid cells. In the metaphase spreads of the hypertriploid cells, there were 13 triplets of chromosomes, except one of the three No. 3 chromosomes formed a ring and there was an additional No. 10 chromosome. The hyperdiploid cells were 27 in chromosome number, as there was an additional No. 6 chromosome. Another embryo was a mosaic of hyperdiploid and hyperhaploid

TABLE 2

Chromosomal analysis of abnormal embryos at stage 17~20 raised from irradiated sperm or eggs

Parents		No. of analyzed embryos	Number of embryos			
Female	Male		With normal cells only	With abnormal cells only		With normal and abnormal cells
				Pure	Mosaics	
J.W68, No. 1	J.W68, No. 1	20	12 (60.0%)	2 (10.0%)	3 (15.0%)	3 (15.0%)
J.W68, No. 1	SX–150, No. 1	32	8 (25.0%)	8 (25.0%)	6 (18.8%)	10 (31.3%)
J.W68, No. 1	SX–250, No. 1	41	4 (9.8%)	15 (36.6%)	10 (24.4%)	12 (29.3%)
EX–150, No. 1	J.W68, No. 1	44	12 (27.3%)	10 (22.7%)	14 (31.8%)	8 (18.2%)
EX–250, No. 1	J.W68, No. 1	47	5 (10.6%)	20 (42.6%)	12 (25.5%)	10 (21.3%)
EX–350, No. 1	J.W68, No. 1	50	2 (4.0%)	20 (40.0%)	19 (38.0%)	9 (18.0%)
J.W68, No. 1	SN–100, No. 1	37	6 (16.2%)	10 (27.0%)	16 (43.2%)	5 (13.5%)
J.W68, No. 1	SN–150, No. 1	35	3 (8.6%)	13 (37.1%)	17 (48.6%)	2 (5.7%)
J.W68, No. 1	SN–250, No. 1	31	3 (9.7%)	10 (32.3%)	14 (45.2%)	4 (12.9%)
EN–150, No. 1	J.W68, No. 1	35	5 (14.3%)	11 (31.4%)	15 (42.9%)	4 (11.4%)
EN–250, No. 1	J.W68, No. 1	42	2 (4.8%)	15 (35.7%)	20 (47.6%)	5 (11.9%)

Stage 17 — early tail-bud embryos
Stage 20 — hatching embryos (gill circulation)

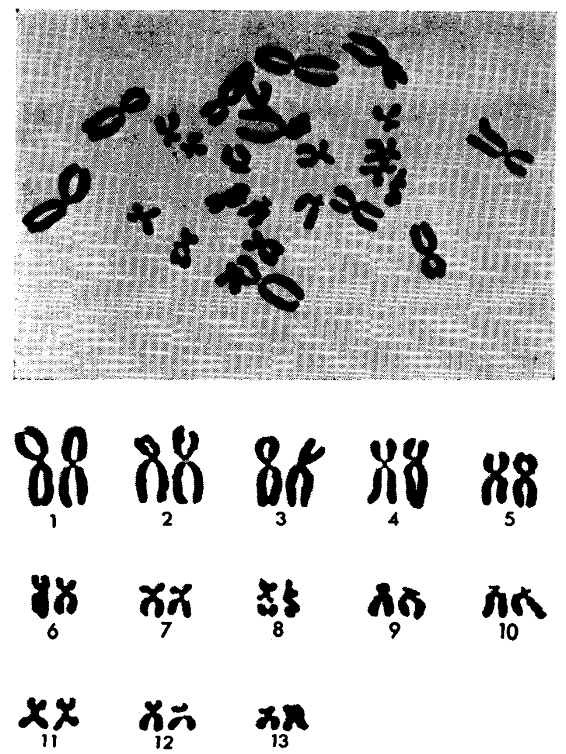


Fig. 2. Normal mitotic chromosomes and karyotype of a control *Rana japonica*. ×1500

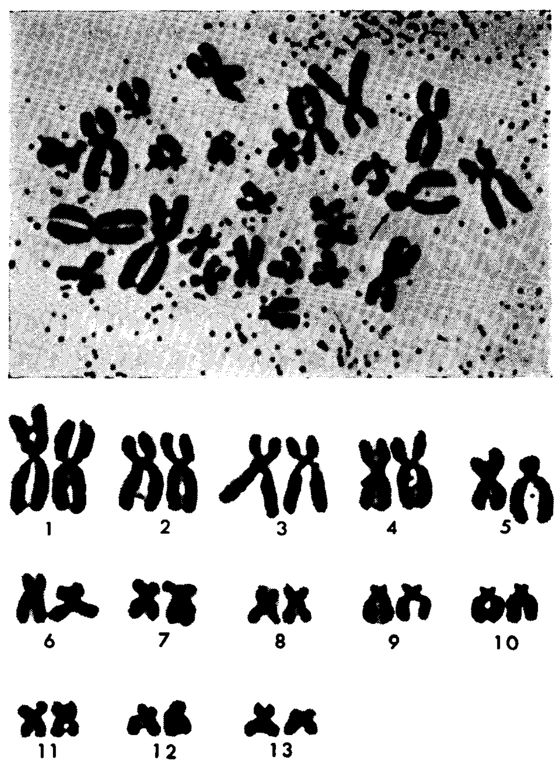


Fig. 3. Chromosome aberration in an abnormal embryo produced from a mating, J♀×SX–150♂. An arrow indicates a pericentric inversion in chromosome No. 5. ×1500

cells. The hyperdiploid cells were 29 in chromosome number, having additional Nos. 5, 9 and 11 chromosomes, while the hyperhaploid cells had 14 chromosomes. The third embryo was a mosaic consisting of haploid and pentaploid cells.

The remaining three abnormal embryos were mosaics of normally diploid and abnormal cells. One of them consisted of a mixture of haploid, diploid and triploid cells, another of a mixture of diploid and pentaploid cells, and the remainder of a mixture of diploid, triploid and tetraploid cells.

b. Experimental series derived from X-irradiated sperm

i) J♀ × SX-150♂

Of 40 abnormal embryos produced from normal eggs by insemination with sperm irradiated with a dose of 150 rads of X-rays, 32 were analyzable in karyotype (Table 2). Eight of these embryos were normal diploids. All their metaphase spreads contained 26 typical chromosomes. Eight other embryos had no normally diploid metaphase spreads. However, all the mitoses of each embryo were the same in karyotype. Two of these eight embryos had a pericentric inversion in a chromosome, although they were 26 in chromosome number (Fig. 3). Two and three embryos were 25 and 24 in chromosome number, respectively. The remaining one had a giant dicentric chromosome, although it was 24 in chromosome number.

Six abnormal embryos were mosaics constructed of two kinds of abnormal cells. One of them consisted of a mixture of hypertriploid and hypotriploid cells, which were 42 and 38 in chromosome number, respectively. Another was a mosaic of haploid and triploid cells. The third one was a mosaic of hyperdiploid and hypodiploid cells, that is, 27 and 25 in chromosome number, respectively. The remaining three had 25 or less chromosomes and consisted of a mixture of two or more kinds of abnormal cells containing dicentric, fragment, giant or minute chromosomes.

The other ten abnormal embryos were mosaics of normally diploid and abnormal cells. In most of them, these two kinds of metaphase spreads were found in nearly an equal number. One embryo consisted of a mixture of haploid, diploid and triploid cells. One consisted of a mixture of diploid and triploid cells. Four consisted of a mixture of normally diploid cells and abnormally diploid ones which had a chromosome with a deletion or translocation or had a ring chromosome. One consisted of a mixture of diploid and hypodiploid cells which were 25 in chromosome number and contained a giant dicentric chromosome. Two consisted of a mixture of diploid and hypodiploid cells which were 25 in chromosome number and the remaining one consisted of a mixture of diploid and hypodiploid cells which were 25 in chromosome number, owing to joining of two chromosomes.

ii) J♀ × SX-250♂

There were 41 analyzable embryos among 50 abnormal ones raised from sperm irradiated with 250 rads of X-rays. Only four of these embryos were normal diploids, while the other 37 had abnormal metaphase spreads. In each of 15 of the latter embryos, all the metaphase spreads were the same in karyotype. Two embryos consisted of triploid cells, seven consisted of abnormally diploid cells having a deletion in a chromosome, two consisted of abnormally diploid cells having a deletion and a translocation, two consisted of hyperdiploid cells having an additional small chromosome, and the remaining two consisted of hypodiploid cells which were 25 in chromosome number due to absence of a small chromosome (Fig. 4).

Ten embryos were chromosomal mosaics which had no normally diploid mitoses. One of them consisted of hypertriploid (3n+1) and hypotriploid (3n−1) cells. Another was constructed of two kinds of triploid cells, that is, those having a ring chromosome and those having a deletion in a chromosome. Two embryos were mosaics of hyperdiploid (2n+1) and hypodiploid (2n−1) cells. Two embryos were mosaics of hypodiploid cells containing a giant dicentric chromosome formed by joining of two large chromosomes and diploid cells containing a deletion or translocation in a chromosome. One embryo was a mosaic of haploid and triploid cells. The other three embryos were constructed of a mixture of various kinds of abnormally diploid cells which had a translocation or deletion in a chromosome or a fragment of a chromosome at the metaphase.

The remaining 12 embryos having abnormal metaphase spreads were mosaics

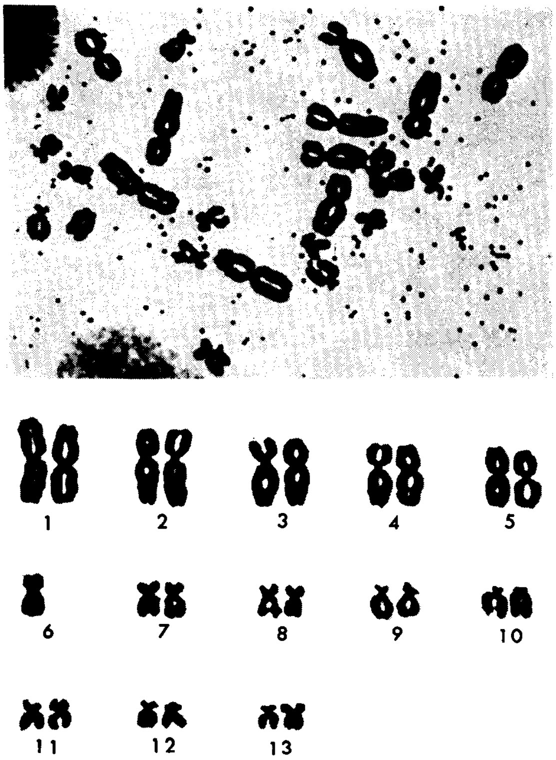


Fig. 4. Chromosome aberration in an abnormal embryo produced from a mating, J♀ × SX-250♂. A chromosome No. 6 is lost. ×1500

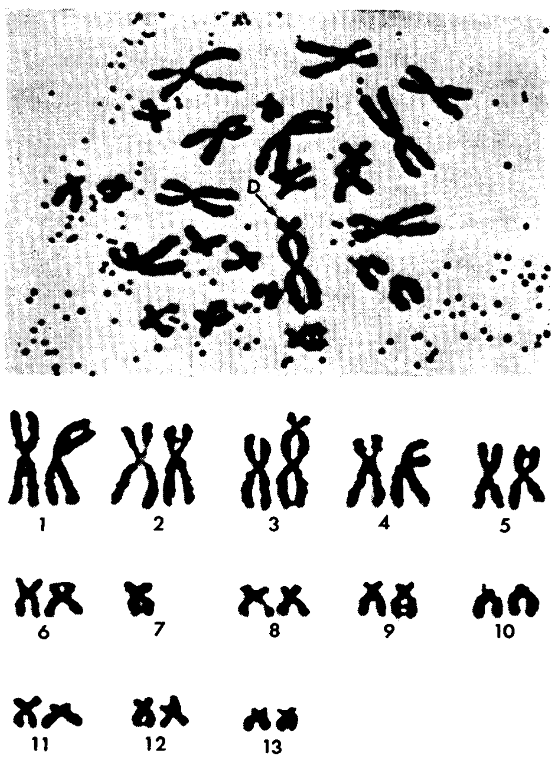


Fig. 5. Chromosome aberration in an abnormal embryo produced from a mating, EX-150♀ ×J♂. An arrow indicates a dicentric chromosome produced by translocation of chromosome No. 7 to the short arm of chromosome No. 3. ×1500

of normally diploid and abnormal cells. In each of these embryos there were nearly an equal number of normally diploid and abnormal metaphase plates. Two embryos were mosaics of diploid and triploid cells. Five others were mosaics of normally and abnormally diploid cells; the latter cells had a deletion or translocation in a chromosome. Three others were mosaics of normally diploid and hypodiploid (2n−1) cells having a giant dicentric chromosome. The remaining two embryos were mosaics of normally and abnormally diploid cells; the latter contained a ring together with a fragment or a ring alone.

c. Experimental series derived from X-irradiated eggs

i) EX-150♀ ×J♂

Forty-four of 50 abnormal embryos at the 17∼20 stages were analyzable (Table 2). Twelve of them had normally diploid metaphase spreads alone, while ten others had no such mitoses, although all the mitoses in each embryo were the same in karyotype. One of these embryos was hypertriploid (3n+1); there was an additional No. 3 chromosome. One embryo was a triploid. Another embryo was also a triploid, although one of the three No. 2 chromosomes was a ring. One embryo was a hyperdiploid (2n+1); one of the two No. 2 chromosomes was a ring and there was an additional No. 12 chromosome. Another embryo was an abnormal diploid; there were one No. 1 chromosome and three No. 9 chromosomes. Three embryos were hypodiploids (2n−1); there was a dicentric chromosome formed by joining of two chromosomes (Fig. 5). One embryo was 21 in chromosome number; five chromosomes were lost from a genome. The remainder was also 21 in chromosome number and had a giant, dicentric chromosome.

Fourteen embryos were mosaics constructed of two or more kinds of abnormal mitoses. One embryo was a mosaic of hypertriploid (3n+1) and hypotriploid (3n−1) cells. Another embryo was a mosaic of haploid and triploid cells. One embryo was a mosaic of abnormally diploid cells containing a ring or a deletion and those containing a translocation. Two embryos were mosaics of hyperdiploid (2n+1) and hypodiploid (2n−1) cells. One embryo was a mosaic of hypodiploid (2n−1) cells containing a dicentric chromosome and abnormally diploid cells containing a deletion. Two embryos were constructed of a mixture of abnormally diploid cells containing different deletions or translocations. Six embryos were mosaics of abnormal cells which varied in chromosome number and always had a giant, dicentric or tricentric chromosome, and hypodiploid cells which were 25 or less in chromosome number and contained one or more fragments.

The remaining eight embryos were mosaics of normally diploid and abnormal cells. Two of them were those of diploid and triploid cells, while another was a mosaic of haploid and diploid cells. Two embryos were mosaics of normally diploid cells and hypodiploid (2n−1) ones containing a dicentric chromosome. One embryo was a mosaic of normally diploid and hypodiploid (2n−2) cells; in the latter two chromosomes were lost from a genome. The remaining two embryos were mosaics of normally diploid and hyperdiploid (2n+1) cells.

ii) EX-250♀×J♂

Of 50 abnormal embryos at the 17~20 stages, 47 were analyzable in regard to their karyotypes. Only five of them were normal diploids.

Twenty embryos had no normally diploid cells, although all the mitoses in each embryo were the same in karyotype. Three embryos were triploids. Another embryo was also a triploid, although one of the three No. 3 chromosomes had a deletion. One embryo was a hypertriploid (3n+1). Another was a hyperdiploid (2n+1); all the metaphase spreads had an additional No. 9 chromosome. Six embryos were abnormal diploids; each of them had one kind of abnormality in the chromosome complements, such as translocation of a part of the long arm of a No. 2 chromosome to the long arm of a No. 7 chromosome, translocation of a part of the long arm of a No. 5 chromosome to the long arm of a No. 4 chromosome, a deletion in a No. 3, No. 5 or No. 9 chromosome or formation of a ring from a No. 5 chromosome. Four embryos were hypodiploids (2n−1); in each of them, there was formation of a dicentric chromosome by joining of a No. 8 and a No. 5 chromosome, or absence of a No. 9, No. 11 or No. 12 chromosome. One embryo was 24 in chromosome number; the metaphase spreads revealed loss of a No. 6 chromosome and formation of a dicentric by translocation of a No. 9 chromosome to the long arm of a No. 7 chromosome. Two other embryos were also 24; two chromosomes were missing from a genome. The other embryo was 23 in chromosome number; three chromosomes were missing from a genome (Fig. 6).

Twelve embryos were mosaics of two or more kinds of abnormal cells. Two of them were those of hypertriploid (3n+1) and hypotriploid (3n−1) cells. One embryo was a mosaic of triploid cells containing a ring chromosome and of those having no ring. Another embryo was a mosaic of haploid and triploid cells. One embryo was a mosaic of hyperdiploid (2n+1) cells having a dicentric and a fragment and abnormally diploid cells having a deletion in a chromosome. Four embryos were mosaics of hyperdiploid (2n+1) and hypodiploid (2n−1) cells; the additional or missing chromosomes were Nos. 9, 12 and 13 in one, two and one of these embryos, respectively. Two embryos were mosaics of two kinds of abnormally diploid cells which had a deletion or translocation. In the chromosome complements of one of them, there was translocation of a segment from a No. 4 chromosome to a No. 3 chromosome which resulted in a giant dicentric, while in those of the other embryo, there was a deletion in a No. 5 chromosome and translocation of a segment from a No. 3 chromosome to a No. 4 chromosome. The remaining embryo was a mosaic of two kinds of hypodiploid cells; one kind was 25 in chromosome number, owing to the loss of a No. 6 chromosome, while the other was 24, owing to the loss of two chromosomes Nos. 7 and 9.

The remaining ten of the 50 abnormal embryos were mosaics of normally diploid and abnormal cells. One of them was a mosaic of haploid and diploid cells, while another was that of diploid and triploid cells. One embryo was a mosaic of diploid cells and hypodiploid (2n−2) cells in which chromosomes Nos. 1 and

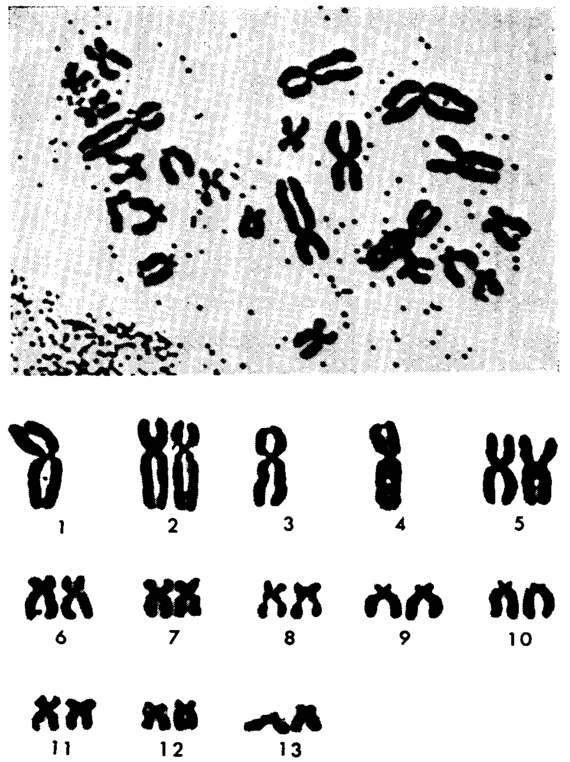


Fig. 6. Chromosome aberration in an abnormal embryo produced from a mating, EX–250♀ ×J♂. Three chromosomes, No. 1, No. 3 and No. 4, are lost. ×1500

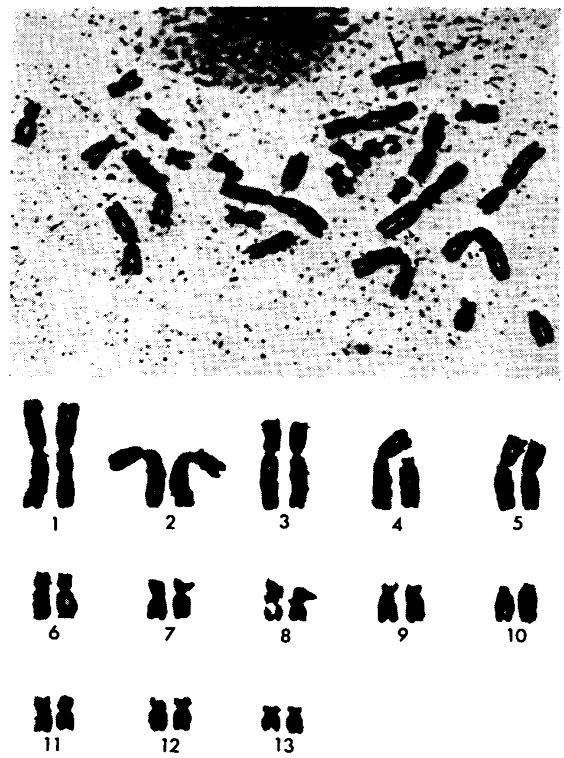


Fig. 7. Chromosome aberration in an abnormal embryo produced from a mating, EX–350♀ ×J♂. An arrow indicates a deletion in the short arm of chromosome No. 4. ×1500

7 were lost, and a ring was formed from a No. 3 chromosome. Three embryos were mosaics of diploid and hypodiploid (2n−1) cells; the latter had a dicentric chromosome. One embryo was a mosaic of diploid and hypodiploid (2n−1) cells, while another was that of diploid and hyper- (2n+1) or hypodiploid (2n−1) cells. The remaining two embryos were mosaics of normally and abnormally diploid cells; the latter had deletions in two or more large chromosomes.

iii) EX–350♀ ×J♂

All the 50 abnormal embryos at the 17∼20 stages were analyzable in regard to their karyotypes. Only two of them were normal diploids, while the others had no normally diploid cells or were mosaics of normally diploid and abnormal cells.

There were 20 embryos which had no normally diploid mitoses, although the metaphase spreads of each of them were the same in karyotype. Eight of them were triploids, although two had a ring chromosome, one had a chromosome with a deletion, and one had a chromosome with a translocation. Four embryos were hyperdiploids (2n+1). Three embryos were abnormal diploids; in one of them the short arm of a No. 4 chromosome had a deletion (Fig. 7), in another the long arm of a No. 4 chromosome was attached to the long arm of a No. 3 chromosome, and in the remainder the long arm of a No. 10 chromosome was lost. Four embryos were hypodiploids (2n−1); one of them had a dicentric chromosome and the other three were deprived of a No. 3, No. 6 and No. 12, respectively. The remainder was a hypodiploid (2n—2); a No. 5 and a No. 1 chromosome had a translocation, and a No. 6 and a No. 8 chromosome were lost.

Nineteen other embryos were mosaics of abnormal cells. One of them was a mosaic of hypertriploid (3n+1) and hypotriploid (3n—1) cells. Another was that of haploid and triploid cells. Five embryos were mosaics of hypodiploid and nearly triploid cells. Three were mosaics of hyperdiploid (2n+1) and hypodiploid (2n—1) cells. Nine were constructed of a mixture of abnormal cells which were various in chromosome number and contained a ring, dicentric, giant, minute or fragment chromosome.

The remaining nine embryos were mosaics of normally diploid and abnormal cells. One of them was a mosaic of haploid and diploid cells. Two were mosaics of diploid and hypodiploid (2n—1) cells. One embryo was a mosaic of normally diploid and abnormally diploid cells having a ring. Two were mosaics of normally diploid cells and abnormally diploid cells which had a dicentric and a fragment chromosome. The remaining three embryos were mosaics of diploid cells and hypodiploid (2n—2) cells in which two chromosomes were lost from a genome.

d. Experimental series derived from neutron-irradiated sperm

i) J♀×SN-100♂

Among 40 abnormal embryos at the 17∼20 stages which were squashed and stained, there were 37 analyzable ones (Table 2). Only six of the latter were quite normal in the number and shape of chromosomes; all their metaphase spreads were normally diploid.

Ten embryos had no normally diploid cells, although all the metaphase spreads of each of them were the same in karyotype. One of these embryos was a hypertriploid (3n+1). Two were hyperdiploids (2n+1); an additional No. 5 chromosome existed in one embryo, while an additional No. 10 existed in the other. Five had a deletion or translocation in their metaphase spreads, although they were 26 in chromosome number. The remaining two were 25 in chromosome number and had a dicentric chromosome.

Sixteen embryos were mosaics of abnormal cells. One of them was a mosaic of haploid and triploid cells. Four were mosaics of hyperdiploid (2n+1) and hypodiploid (2n—1) cells; the additional or missing chromosome was No. 3 in one embryo, No. 5 in two others and No. 6 in the remainder. Seven embryos were mosaics of various kinds of abnormally diploid cells, which contained a deletion or translocation. The remaining four embryos were mosaics of abnormal cells having a giant, ring or dicentric chromosome and those having a minute or fragment chromosome. The constituent cells of these embryos were varied in chromosome number.

The remaining five of the 40 embryos were mosaics of normally diploid and abnormal cells. One of them was a mosaic of diploid cells and hyperdiploid (2n+2) cells which had additional chromosomes Nos. 4 and 9. Another was a mosaic of normally diploid cells and abnormally diploid ones which had a ring chromosome. Three embryos were mosaics of diploid and hypodiploid (2n−1) cells. In one of them, the hypodiploid cells contained a dicentric chromosome.

ii) J♀ ×SN-150♂

Of 40 abnormal embryos squashed and stained, 35 were analyzable in regard to their karyotypes. Only three of them were 26 in chromosome number and all the metaphase spreads were normal in karyotype. Thirty embryos had no normally diploid mitoses, while the other two were mosaics of normally diploid and abnormal cells.

Among the 30 embryos which had no normal mitoses, there were 13, each of which had one kind of karyotype. Three of them were hyperdiploids (2n+1); one had an additional No. 3, another had an additional No. 8 and the remainder had an additional No. 13 chromosome. Three others were abnormal diploids; one was deprived of the long arm of a No. 4 chromosome (Fig. 8), another had the translocation of a part of a No. 2 to a No. 5 chromosome, and the remainder had a No. 5 chromosome which formed a ring. The other seven were hypodiploids (2n−1). Four of them had a dicentric chromosome, although one lost a No. 3, another a No. 7 (Fig. 9) and the remainder a No. 9 chromosome. Seventeen of the 30 abnormal embryos were mosaics of abnormal cells. Three of them were mosaics of hyperdiploid (2n+1) and hypodiploid (2n−1) cells. Nine others were mosaics of abnormally diploid cells which had a ring, minute or giant chromosome. The other five were mosaics of abnormal cells whose

Fig. 8. Chromosome aberration in an abnormal embryo produced from a mating, J♀ ×SN-150♂. An arrow indicates a deletion in the long arm of chromosome No. 4. ×1500

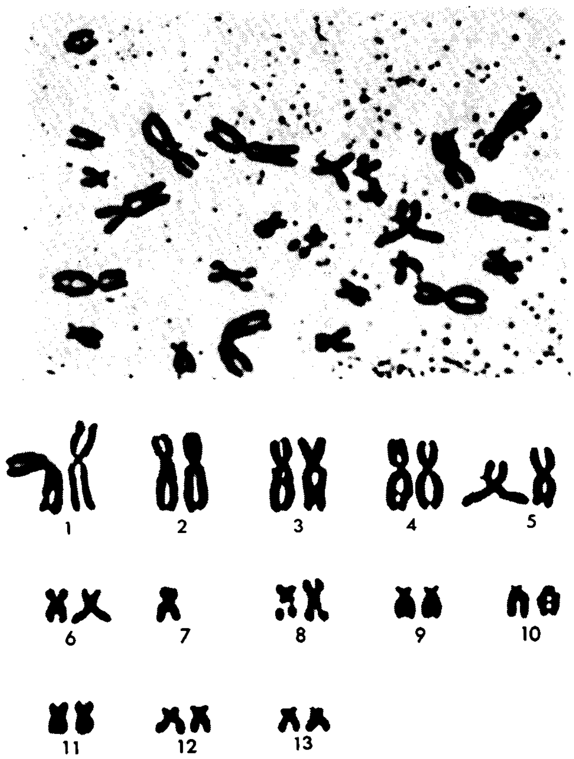


Fig. 9. Chromosome aberration in an abnormal embryo produced from a mating, J♀ × SN-150♂. A chromosome No. 7 is lost. ×1500

metaphase spreads were varied in chromosome number and had one or more dicentric, giant, minute or fragment chromosomes produced by deletion, translocation or fragmentation.

The remaining two embryos were mosaics of normally diploid and abnormal cells. One of them was a mosaic of diploid and triploid cells, while the other was that of diploid cells and hypodiploid (2n−3) cells which had a dicentric chromosome (Fig. 10).

iii) J♀ × SN-250♂

Thirty-one of 40 abnormal embryos were analyzable. Three of them were 26 in chromosome number and normal in karyotype. Twenty-four consisted of abnormal cells alone, and the remaining four were mosaics of normally diploid and abnormal cells.

In ten of the 24 abnormal embryos, all the metaphase spreads of each one were the same in karyotype. Five of them were abnormal diploids; three had a deletion in one or more chromosomes, while the other two had a deletion and a translocation. The remaining five of the ten were hypodiploids (2n−1); one chromosome was lost in four embryos, while in the other there was a dicentric.

Fourteen abnormal embryos were mosaics of two or more kinds of abnormal cells. Two of them were mosaics of hypodiploid (2n−2) and abnormally diploid cells; in the former two large chromosomes were joined to form a dicentric and

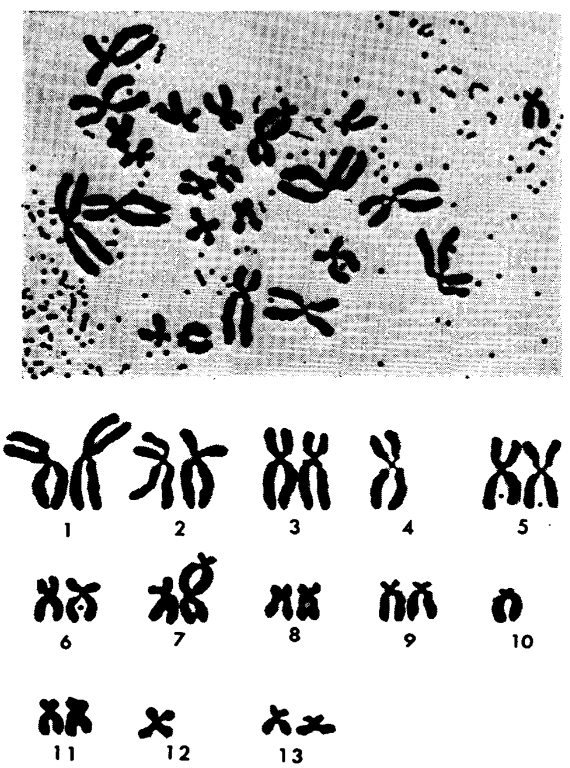


Fig. 10. Chromosome aberration in an abnormal embryo produced from a mating, J♀ × SN-150♂. An arrow indicates a dicentric chromosome found in hypodiploid (2n−3) metaphase. ×1500

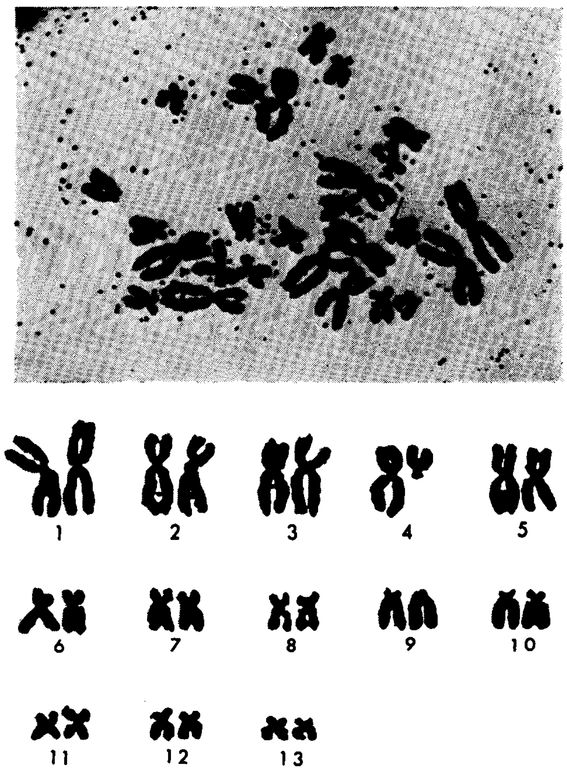


Fig. 11. Chromosome aberration in an abnormal embryo produced from a mating, EN-250♀ × J♂. An arrow indicates a deletion in the long arm of chromosome No. 4. ×1500

a small chromosome was lost, while in the latter, there was a deletion in one or more chromosomes. One embryo consisted of a mixture of diploid cells containing a dicentric and hypodiploid (2n−1) or diploid cells containing a fragment. Three were mosaics of hyperdiploid (2n+1) and hypodiploid (2n−1) cells. Two were mosaics of two or more kinds of abnormally diploid cells which contained one or more deletions or translocations. The remaining six consisted of a mixture of various kinds of hypodiploid cells, which contained a dicentric, tricentric, deficient, minute, fragment, or an additional chromosome.

Among the four abnormal embryos consisting of normally diploid and abnormal cells, there were a mosaic of diploid and triploid cells, two mosaics of diploid cells and hypodiploid (2n−1) cells with a dicentric chromosome and a mosaic of diploid cells and hypodiploid (2n−1) cells having a ring.

e. Experimental series derived from neutron-irradiated eggs

i) EN-150♀ ×J♂

There were 35 analyzable embryos among 40 abnormal ones squashed and stained at the 17～20 stages (Table 2). Only five of them consisted of normally diploid cells, while 26 consisted of abnormal cells and the remaining four were mosaics of normally diploid and abnormal cells.

Among the 26 embryos with abnormal cells alone, there were 11, each of which had the same karyotype in all their metaphase spreads. Of these 11 embryos, one was a triploid, three were also triploids having a ring chromosome, one was a hypotriploid (3n−1), one was a hyperdiploid (2n+1) having a ring and a fragment, two were hyperdiploids (2n+1) having an additional chromosome, one was a diploid having a chromosome with a translocation, and the remaining two were hypodiploids (2n−1) in which one chromosome was missing.

Fifteen embryos were mosaics of two or more kinds of abnormal cells. One was a mosaic of hypertriploid (3n+1) and hypotriploid (3n−1) cells, two were mosaics of triploid cells having a ring and those having a fragment, one was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) cells, six were mosaics of abnormally diploid cells whose metaphase spreads had one or more chromosomes having a deletion or translocation, and the other five were mosaics of abnormal cells whose metaphase spreads were varied in chromosome number and contained a dicentric, minute or fragment chromosome.

Of the four embryos consisting of a mixture of normally diploid and abnormal cells, one was a mosaic of diploid and triploid cells, another was a mosaic of diploid and hyperdiploid (2n+1) cells, still another was that of diploid and hypodiploid (2n−1) cells and the remainder was a mosaic of diploid cells and hypodiploid (2n−2) cells having two giant dicentric chromosomes.

ii) EN-250♀ ×J♂

There were 42 analyzable embryos among 50 abnormal ones squashed and stained. Only two of them were normal diploids, while 35 consisted of abnormal cells and the other five consisted of a mixture of normally diploid and abnormal cells.

Fifteen of the 35 abnormal embryos having abnormal cells alone had the same karyotype in all the metaphase spreads of each embryo. Among them, there were one hypertriploid (3n+1), three triploids, two hypotriploids (3n−1), five abnormal diploids (Fig. 11), three hypodiploids (2n−1) and one hypodiploid (2n−2) (Fig. 12). One of the three triploids had a ring chromosome. In the

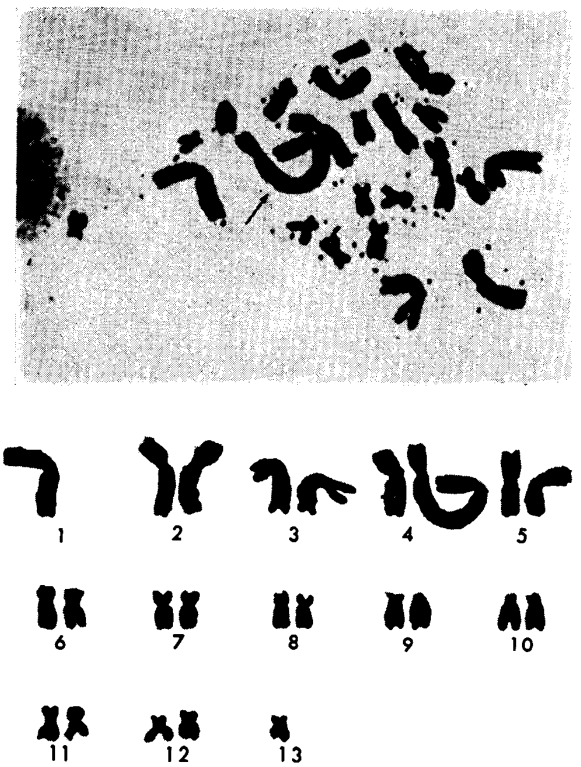


Fig. 12. Chromosome aberration in an abnormal embryo produced from a mating, EN-250♀ ×J♂. An arrow indicates a dicentric produced by translocation of chromosome No. 4 to chromosome No. 1. A chromosome No. 13 is lost. ×1500

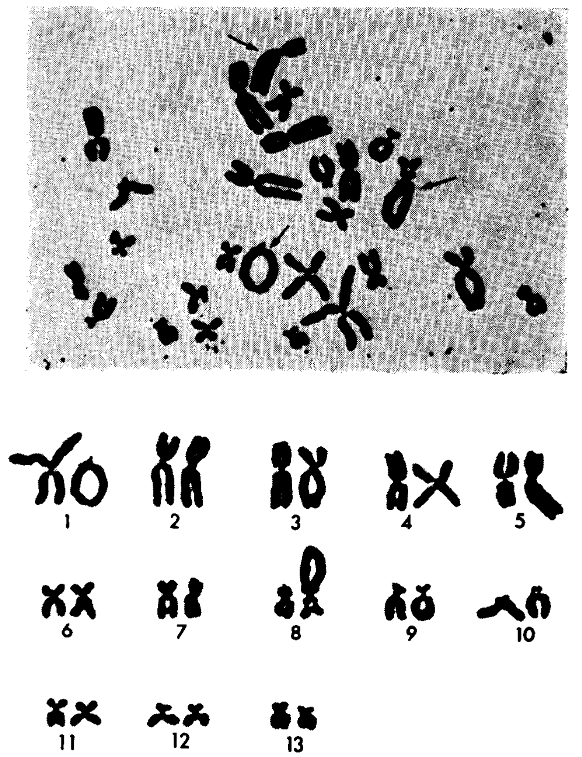


Fig. 13. Chromosome aberration in an abnormal embryo produced from a mating, EN-250♀ ×J♂. Three arrows indicate a deletion in chromosome No. 1 and translocations in chromosomes No. 5 and No. 8. ×1500

abnormal diploids, one or more chromosomes had a deletion or translocation (Fig. 13). Of the other 20 embryos having abnormal cells alone, one was a mosaic of haploid and triploid cells and two were mosaics of hypertriploid (3n+1) and hypotriploid (3n−1) cells. Four others were mosaics of hyperdiploid (2n+1) and hypodiploid (2n−1) cells. The hyperdiploid metaphase spreads of one of the latter four embryos had a ring and a fragment, and the hypodiploid of the same embryo had a dicentric chromosome, while the hyper- and hypodiploid metaphase spreads of the other three embryos had neither a ring nor a dicentric chromosome. The other 13 embryos consisted of a mixture of various kinds of abnormal cells which were varied in chromosome number and had a dicentric, tricentric, ring, minute or fragment chromosome or a chromosome having a deletion.

Among the remaining five embryos constructed of a mixture of normally diploid and abnormal cells, there were two mosaics of diploid and triploid cells, a mosaic of diploid and hyperdiploid (2n+1) cells and two mosaics of diploid and hypodiploid (2n−2) cells.

3. Chromosome aberrations in normally shaped tadpoles

Chromosome aberrations were found in normally shaped tadpoles raised from X- or neutron-irradiated sperm or unfertilized eggs by fertilization with normal, untreated gametes. These tadpoles were 50 days old and at the V∼X stages. All the eggs used were obtained from the largest (No. 1) among the 20 females.

a. Control series

Squash preparations were made from the tail-tips of 30 normally shaped tadpoles at the V∼X stages. Twenty-five of them were analyzable in regard to their karyotypes; all of them were normal diploids, that is, they were 26 in chromosome number and had no abnormal metaphase spreads (Table 3).

TABLE 3

Chromosomal analysis of normally shaped 50-day-old tadpoles raised from irradiated sperm or eggs

Parents		No. of analyzed tadpoles	Number of tadpoles			
Female	Male		With normal cells only	With abnormal cells only: Pure	With abnormal cells only: Mosaics	With normal and abnormal cells
J.W68, No. 1	J.W68, No. 1	25	25 (100 %)	0	0	0
J.W68, No. 1	SX–150, No. 1	22	12 (54.5%)	3 (13.6%)	2 (9.1%)	5 (22.7%)
J.W68, No. 1	SX–250, No. 1	20	6 (30.0%)	5 (25.0%)	2 (10.0%)	7 (35.0%)
EX–150, No. 1	J.W68, No. 1	38	25 (65.8%)	4 (10.5%)	2 (5.3%)	7 (18.4%)
EX–250, No. 1	J.W68, No. 1	31	14 (45.2%)	4 (12.9%)	3 (9.7%)	10 (32.3%)
EX–350, No. 1	J.W68, No. 1	24	6 (25.0%)	5 (20.8%)	4 (16.7%)	9 (37.5%)
J.W68, No. 1	SN–100, No. 1	24	14 (58.3%)	2 (8.3%)	1 (4.2%)	7 (29.2%)
J.W68, No. 1	SN–150, No. 1	32	13 (40.6%)	3 (9.4%)	1 (3.1%)	15 (46.9%)
J.W68, No. 1	SN–250, No. 1	23	10 (43.5%)	9 (39.1%)	2 (8.7%)	2 (8.7%)
EN–150, No. 1	J.W68, No. 1	20	12 (60.0%)	3 (15.0%)	1 (5.0%)	4 (20.0%)
EN–250, No. 1	J.W68, No. 1	23	12 (52.3%)	2 (8.7%)	3 (13.0%)	6 (26.1%)

b. Experimental series derived from X-irradiated sperm

i) J♀ × SX–150♂

Among 25 normally shaped tadpoles whose tail-tips were squashed and stained at the V∼X stages, there were 22 which were analyzable in regard to their karyotypes (Table 3). While 12 of the latter were normal diploids, five others had no normally diploid metaphase spreads, and the remaining five consisted of a mixture of normally diploid and abnormal cells.

Of the five tadpoles which had no normally diploid cells, each of three was of the same karyotype in all the metaphase spreads, while the other two were mosaics of different kinds of abnormal cells. One of the former three was a triploid, another was a diploid having a deletion in a chromosome, and the other was a trisomic, that is, it had an additional No. 12 chromosome. One of the two mosaics was that of hyperdiploid (2n+1) and hypodiploid (2n—1) cells in which a No. 12 chromosome was additional or missing. The other was a mosaic of diploid cells having a deletion and a translocation and hypodiploid (2n—1) cells (Figs. 14, 15).

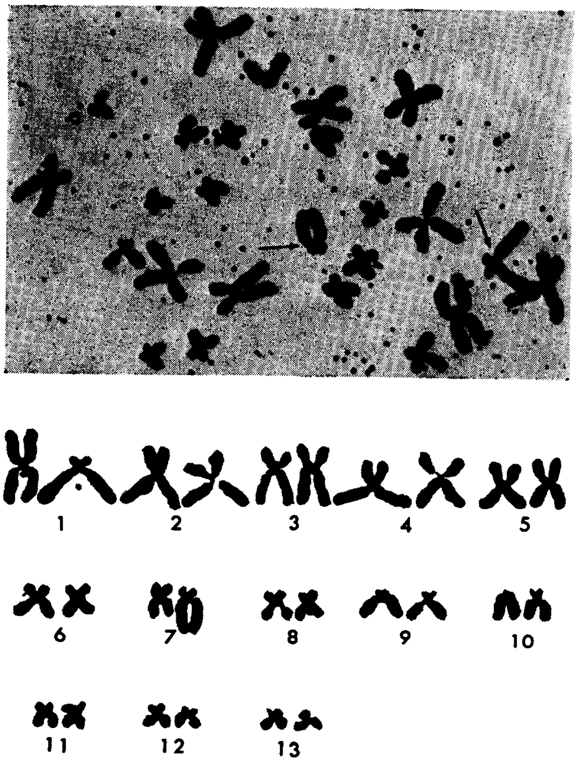


Fig. 14. Chromosome aberration in a normally shaped tadpole produced from a mating, J♀ × SX-150♂. Two arrows indicate a deletion in chromosome No. 1 and a translocation in chromosome No. 7. × 1500

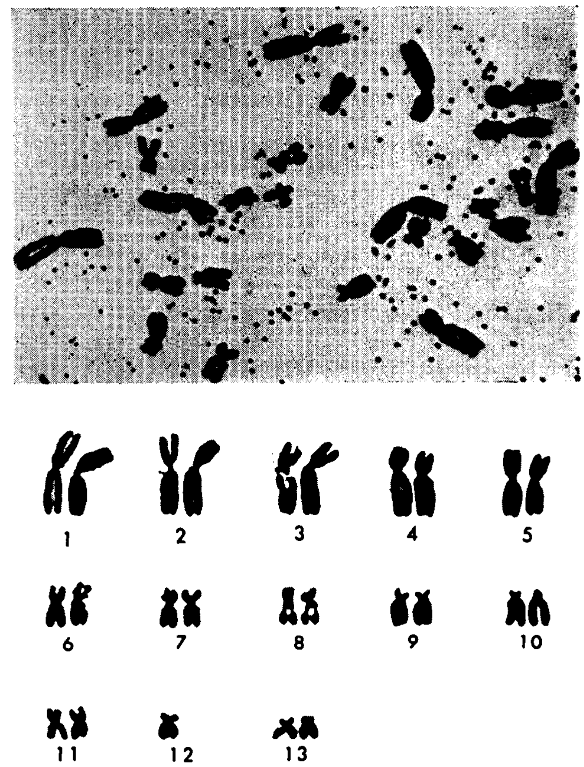


Fig. 15. Chromosome aberration in the same tadpole as that of Fig. 14. A chromosome No. 12 is lost. × 1500

One of the five tadpoles constructed of a mixture of normally diploid and abnormal cells was a mosaic of diploid and triploid cells. Another was a mosaic of diploid cells and hyperdiploid cells which had an additional No. 10 chromosome. Still another tadpole consisted of a mixture of normally diploid cells and abnormally diploid cells having a ring. The other two were mosaics of normally diploid cells and abnormally diploid cells having a deletion or translocation.

ii) J♀ × SX-250♂

Twenty of 25 normally shaped tadpoles whose tail-tips were squashed and stained were analyzable in regard to their karyotypes. While six of them were normal diploids, seven others had no normal metaphase spreads and the other seven were mosaics of normally diploid and abnormal cells.

In five of the seven tadpoles having no normally diploid cells, all the metaphase spreads of each tadpole were the same in karyotype. One tadpole was a triploid, another was a hyperdiploid (2n+1) having an additional No. 13 chromosome, and the other three were abnormal diploids having a deletion or translocation in a chromosome. The remaining two of the seven tadpoles were mosaics containing different kinds of karyotypes. One of these two consisted of hyperdiploid (2n+1) and hypodiploid (2n−1) cells; there was an addition or loss of a No. 12 chromosome in each cell. The other tadpole was a mosaic of abnormally diploid cells having a deletion in the long arm of a No. 4 chromosome and those having a translocation of a segment from the No. 4 chromosome to the long arm of a No. 5 chromosome.

Of the seven mosaics of normally diploid and abnormal cells, one consisted of diploid and triploid cells, one of diploid and hyperdiploid (2n+1) cells, two of normally diploid and abnormally diploid cells having a deletion and a translocation, one of normally diploid and abnormally diploid cells having a ring, and the remaining two of normally diploid and abnormally diploid cells having a deletion.

c. Experimental series derived from X-irradiated eggs

i) EX–150♀×J♂

Of 40 normally shaped tadpoles that developed from eggs irradiated with 150 rads of X-rays, 38 were analyzable in regard to their karyotypes (Table 3). Twenty-five of them were normal diploids, six others had no normally diploid cells and the other seven were mosaics of normally diploid and abnormal cells.

In four of the six tadpoles having no normally diploid cells, all the metaphase spreads of each tadpole were the same in karyotype, while the other two were mosaics. One of the former four was a triploid, another was a hyperdiploid having an additional No. 11 chromosome, still another was an abnormal diploid in which a segment of the long arm of a No. 2 chromosome was translocated to the long arm of a No. 5 chromosome, and the other was an abnormal diploid having a deletion in the long arm of a No. 4 chromosome. The remaining two of the six tadpoles were mosaics of abnormal cells. One consisted of a mixture of hyperdiploid (2n+1) and hypodiploid (2n−1) cells in which a No. 12 chromosome was added or lost. The other was a mosaic of hypodiploid (2n−1) cells having a dicentric chromosome and those not having a dicentric chromosome (Figs. 16, 17).

Among the seven tadpoles that consisted of a mixture of normally diploid and abnormal cells, there were a mosaic of diploid and triploid cells, a mosaic of haploid and diploid cells, two mosaics of diploid and hyperdiploid (2n+1) cells, a mosaic of normally diploid and abnormally diploid cells having a ring, and two mosaics of normally diploid and abnormally diploid cells having a deletion or translocation.

ii) EX–250♀×J♂

Thirty-one of 35 normally shaped tadpoles whose tail-tips were squashed and stained were analyzable in regard to their karyotypes. While 14 of them

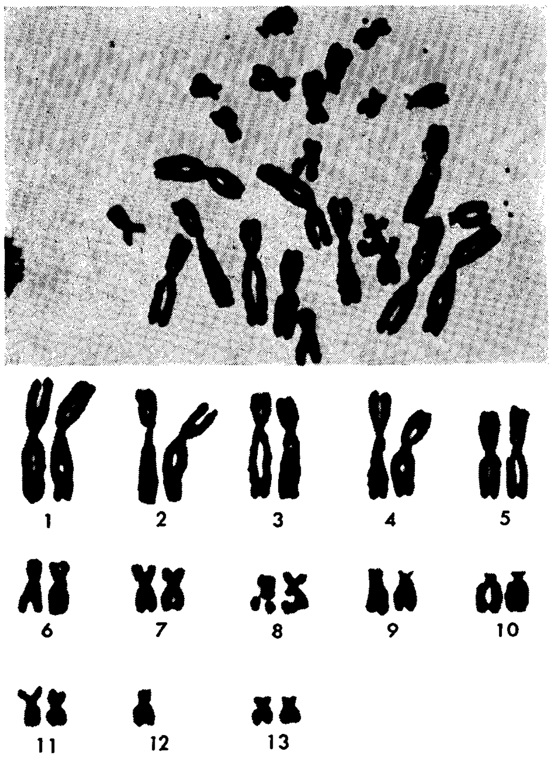


Fig. 16. Chromosome aberration in a normally shaped tadpole produced from a mating, EX-150♀ ×J♂. A chromosome No. 12 is lost. ×1500

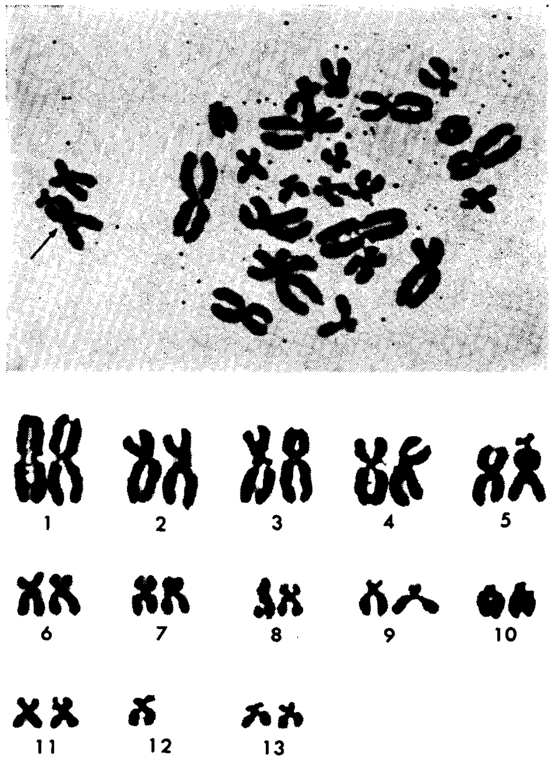


Fig. 17. Chromosome aberration in a normally shaped tadpole produced from a mating, EX-150♀ ×J♂. An arrow indicates a dicentric derived from chromosome No. 5. A chromosome No. 12 is lost. ×1500

were normal diploids, seven others did not have normally diploid metaphase spreads and the other ten were mosaics of normally diploid and abnormal cells.

In four of the seven tadpoles which had no normally diploid cells, all the mitoses of each tadpole were the same in karyotype, while the other three were mosaics. One of the former four was a triploid (Fig. 18), another was a hyperdiploid (2n+1) having an additional No. 13 chromosome, still another was an abnormal diploid in which a No. 5 chromosome was a ring (Fig. 19), and the remainder was an abnormal diploid in which a segment of the long arm of a No. 4 chromosome was translocated to the long arm of a No. 5 chromosome. Of the three mosaics, one was that of hypertriploid (3n+1) and hypotriploid (3n−1) cells formed by addition or loss of a No. 11 chromosome. Another was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) cells formed by addition or loss of a No. 13 chromosome. The remainder was a mosaic of diploid cells having a deletion and those having a translocation.

Of the ten mosaics of normally diploid and abnormal cells, two were those of diploid and triploid cells. One was a mosaic of diploid cells and hyperdiploid (2n+1) cells having an additional No. 12 chromosome. Six were mosaics of normally and abnormally diploid cells. In one of these six tadpoles, the abnormally diploid cells had a ring, while in the other five there was a deletion or translocation. The remaining tadpole was a mosaic of diploid cells and

Fig. 18. Chromosome aberration (triploidy) in a normally shaped tadpole produced from a mating, EX-250♀ ×J♂. ×1500

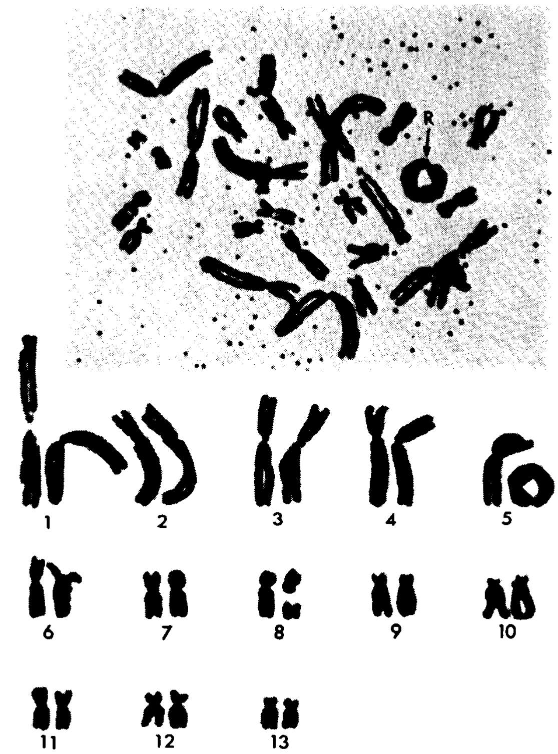


Fig. 19. Chromosome aberration in a normally shaped tadpole produced from a mating, EX-250♀ ×J♂. An arrow indicates a ring derived from chromosome No. 5. ×1500

hypodiploid (2n−1) cells having a dicentric chromosome.

iii) EX-350♀ ×J♂

Twenty-four of 25 normally shaped tadpoles whose tail-tips were squashed and stained were analyzable. While six of them were normal diploids, nine others had no normally diploid cells and the remaining nine were mosaics of normally diploid and abnormal cells.

In five of the nine tadpoles having no normally diploid cells, all the mitoses of each tadpole were the same in karyotype. Two of them were triploid, another was a trisomic having an additional No. 11 chromosome, still another was a diploid in which a segment of the long arm of a No. 9 chromosome was translocated to the long arm of a No. 10 chromosome, and the remainder was a diploid having a deletion in the long arm of a No. 10 chromosome. The other four of the nine tadpoles were mosaics of different kinds of abnormal cells. One of them was a mosaic of hypertriploid (3n+1) and hypotriploid (3n−1) cells, another was a mosaic of triploid cells having a deletion and those having a ring, and the remaining two were mosaics of diploid cells having a deletion and those having a ring.

Among the remaining nine tadpoles constructed of a mixture of normally diploid and abnormal cells, there were two mosaics of diploid and triploid cells, one of diploid and hyperdiploid (2n+1) cells, four of normally diploid cells and abnormally diploid cells having a deletion or translocation, and two of diploid cells and hypodiploid (2n—1) cells in which a No. 8 or 11 chromosome was lost (Figs. 20, 21).

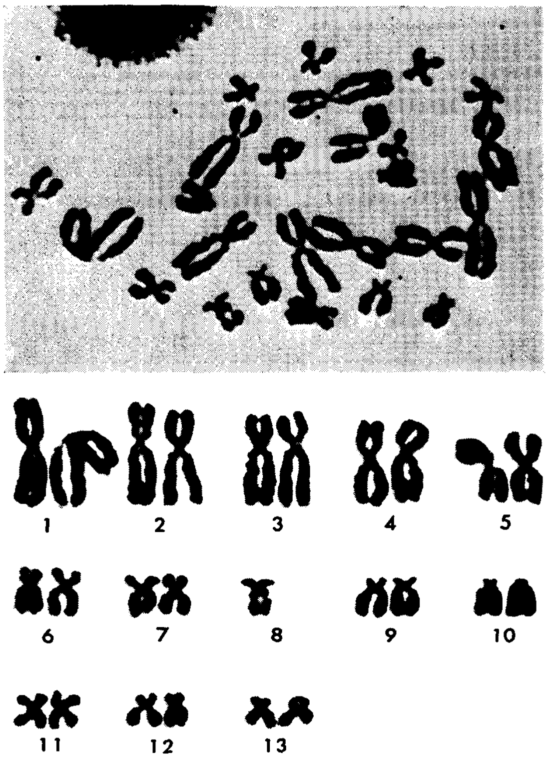


Fig. 20. Chromosome aberration in a normally shaped tadpole produced from a mating, EX-350♀ ×J♂. A chromosome No. 8 is lost. ×1500

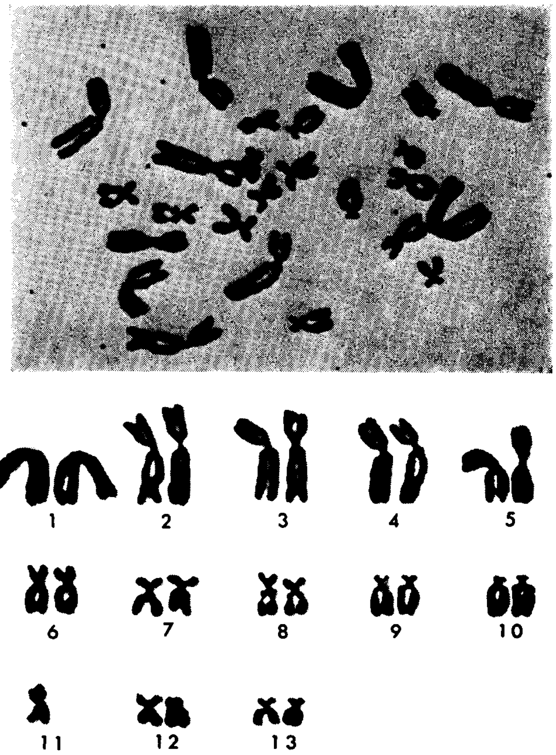


Fig. 21. Chromosome aberration in a normally shaped tadpole produced from a mating, EX-350♀ ×J♂. A chromosome No. 11 is lost. ×1500

d. Experimental series derived from neutron-irradiated sperm

i) J♀ ×SN-100♂

Of 25 normally shaped tadpoles whose tail-tips were squashed and stained, 24 were analyzable (Table 3). While 14 of the latter were normal diploids, three others had abnormal mitoses alone and the remaining seven were mosaics of normally diploid and abnormal cells.

One of the three tadpoles having abnormal cells alone was a diploid having a deletion in the long arm of a No. 3 chromosome. Another was a diploid having an additional segment translocated to the long arm of a No. 5 chromosome. The remainder was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n—1) cells. In these cells there was addition or loss of a small No. 12 chromosome.

Of the seven tadpoles constructed of a mixture of normally diploid and abnormal cells, one was a mosaic of diploid and triploid cells, two were mosaics of diploid cells and hyperdiploid (2n+1) cells having an additional small chromosome, three were mosaics of normally diploid and abnormally diploid cells having a deletion or translocation, and the remainder was a mosaic of diploid cells and hypodiploid (2n—1) cells having a dicentric chromosome.

ii) J♀ ×SN–150♂

Thirty-two of 35 normally shaped tadpoles from which metaphase spreads were obtained were analyzable in regard to their karyotypes. Among them, there were 13 normal diploids, four individuals having no normally diploid cells and 15 mosaics of normally diploid and abnormal cells.

In three of the four tadpoles having no normally diploid cells, all the metaphase spreads of each tadpole were the same in karyotype; one was a trisomy having an additional small chromosome, another was a diploid having a deletion in the short arm of a No. 10 chromosome, and the remainder was a diploid in which a segment of the long arm of a No. 7 chromosome was translocated to the long arm of a No. 5 chromosome. The other of the four tadpoles was a mosaic of diploid cells having a translocation and hyperdiploid (2n+1) cells.

Of the 15 tadpoles constructed of a mixture of normally diploid and abnormal cells, two were mosaics of diploid cells and hyperdiploid (2n+1) ones having an additional small chromosome. Eight were mosaics of normally and abnormally diploid cells; one of them had a ring, five had a deletion or both a deletion and a translocation, and the other two had a dicentric and a fragment chromosome in the abnormal metaphase spreads. Two other tadpoles were mosaics of diploid and triploid cells. The remaining three were a mosaic of diploid cells and hypodiploid (2n−1) cells in which a small chromosome was lost.

iii) J♀ ×SN–250♂

Of 25 normally shaped tadpoles whose tail-tips were squashed and stained, 23 were analyzable. While ten of them were normal diploids, 11 others had no normally diploid mitoses and the remaining two were constructed of a mixture of normally diploid, hyperdiploid (2n+1) and hypodiploid (2n−1) cells.

In nine of the 11 tadpoles having no normally diploid cells, all the mitoses fo each tadpole were the same in karyotype, while the other two were mosaics. One of the nine was a triploid, another was a hyperdiploid (2n+1) having an additional small chromosome, and the remaining seven were abnormal diploids, that is, two had a ring and five had a deletion or translocation. One of the two mosaics consisted of a mixture of diploid cells having a dicentric chromosome and hypodiploid (2n−1) cells (Figs. 22, 23).

The other was a mosaic of hyperdiploid (2n+1) cells having a ring and a fragment and those having a deletion.

e. Experimental series derived from neutron-irradiated eggs

i) EN–150♀ ×J♂

Twenty of 25 normally shaped tadpoles whose tail-tips were squashed and stained were analyzable (Table 3). Twelve of them were normal diploids, four others had no normally diploid mitoses, and the remaining four consisted of a mixture of normally diploid and abnormal cells.

Of the four tadpoles having no normally diploid cells, one was a triploid, another was a hyperdiploid (2n+1) having an additional small chromosome, still another was a diploid having a deletion in a chromosome, and the remainder was a mosaic

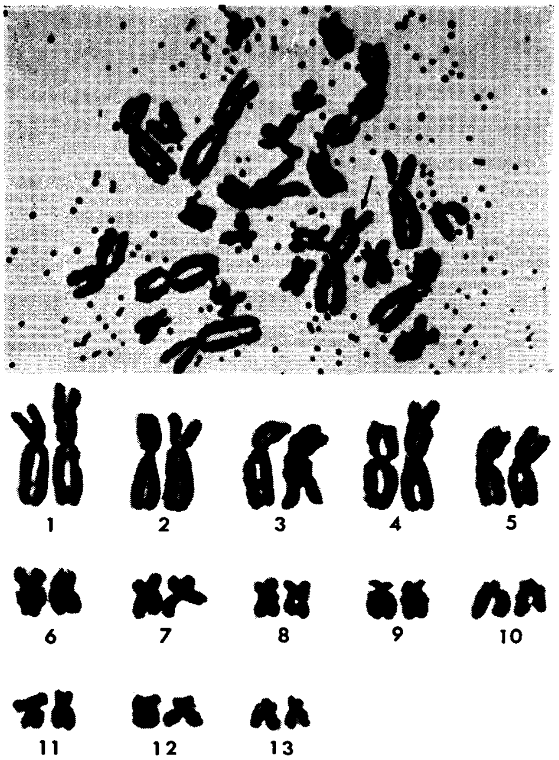


Fig. 22. Chromosome aberration in a normally shaped tadpole produced from a mating, J♀ × SN-250♂. An arrow indicates a dicentric derived from chromosome No. 4. × 1500

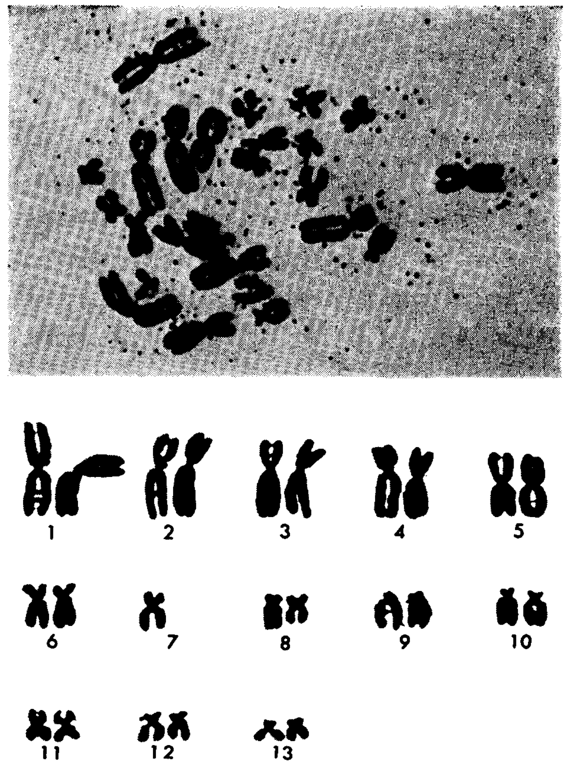


Fig. 23. Chromosome aberration in the same tadpole as that of Fig. 22. A chromosome No. 7 is lost. × 1500

of hyperdiploid (2n+1) and hypodiploid (2n−1) cells in which a small chromosome was added or lost.

Among the four tadpoles consisting of a mixture of normally diploid and abnormal cells, there were a mosaic of diploid and triploid cells, two mosaics of diploid cells and hyperdiploid (2n+1) cells having an additional small chromosome and a mosaic of normally diploid cells and abnormally diploid cells having a ring chromosome.

ii) EN-250♀ ×J♂

Twenty-three of 25 normally shaped tadpoles were analyzable in regard to their karyotypes. Twelve of them were normal diploids, five others were constructed of abnormal cells alone and the remaining six were mosaics of normally diploid and abnormal cells.

In two of the five tadpoles having no normally diploid cells, all the mitoses of each tadpole were the same in karyotype, that is, one tadpole was a triploid and the other was a hyperdiploid (2n+1) having an additional small chromosome. The remaining three were mosaics of abnormal cells. One of these tadpoles was a mosaic of hypertriploid (3n+1) and hypotriploid (3n−1) cells. Another was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) cells, in which a small chromosome was added to or lost from a genome (Figs. 24, 25). The remainder was a mosaic of diploid cells having a deletion in a chromosome and

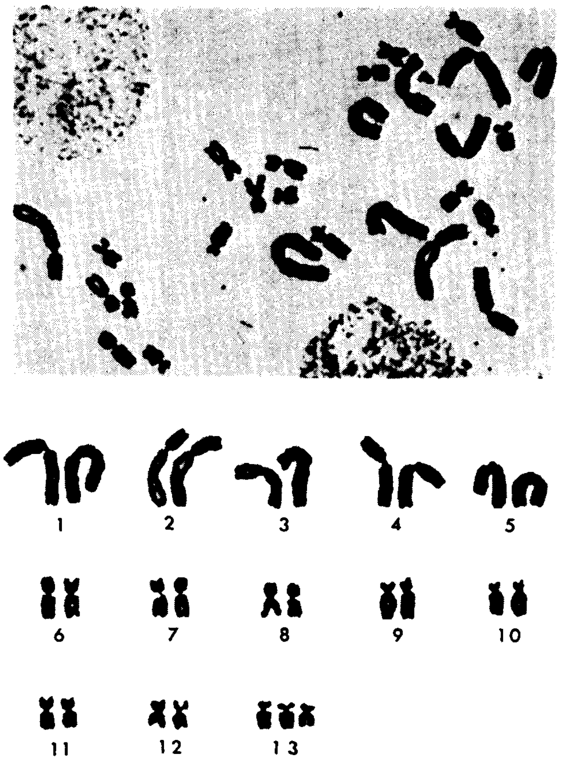


Fig. 24. Chromosome aberration in a normally shaped tadpole produced from a mating, EN-250♀×J♂. There is an additional chromosome No. 13. ×1500

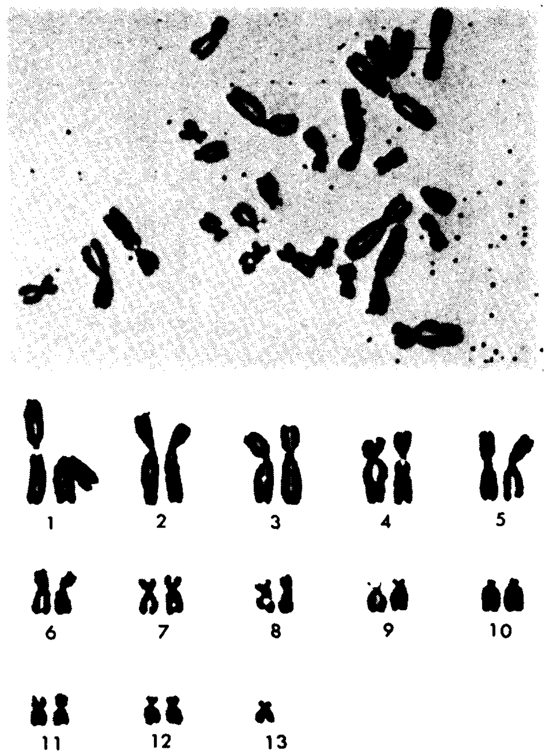


Fig. 25. Chromosome aberration in the same tadpole as that of Fig. 24. A chromosome No. 13 is lost. ×1500

hypodiploid (2n−1) cells having a dicentric.

Of the six tadpoles consisting of normally diploid and abnormal cells, two were mosaics of diploid and triploid cells, one was a mosaic of diploid cells and hyperdiploid (2n+1) cells having an additional small chromosome, and the remaining three were mosaics of normally diploid cells and abnormally diploid cells having a deletion or translocation.

4. Viability and sex of metamorphosed frogs

a. Viability

The days from fertilization to metamorphosis in the individuals of the experimental and control series are presented in Table 4. When sperm suspensions were irradiated with X-rays or neutrons, there were no remarkable differences in the length of the period from fertilization to metamorphosis between different dosages. In contrast with this, this period was somewhat longer when eggs had been irradiated with larger doses of X-rays or neutrons. It was also remarkable that the individuals which developed from eggs irradiated with a dose of 350 rads of X-rays were smaller in body length immediately after metamorphosis than those irradiated with lower doses of X-rays or neutrons.

The frogs produced from irradiated eggs or sperm were feeble as a whole and comparatively slow in activity as compared with the controls. Many frogs

TABLE 4

Number, size and sex of metamorphosed frogs

Parents		Age at the time of climbing out of water (days)	No. of metamor-phosed frogs	Mean body length of 100 frogs immediately after metamorphosis (mm)	
Female	Male				No. of frogs
J.W68, Nos. 1~20	J.W68, Nos. 1~20	96~106 (100.6)	942	19.3±0.2	106
J.W68, Nos. 1~20	SX-150, Nos. 1~20	104~117 (108.3)	585	19.1±0.2	172
	SX-250, Nos. 1~20	100~115 (106.1)	294	18.7±0.2	161
EX-150, Nos. 1~20	J.W68, Nos. 1~20	91~114 (95.3)	304	18.9±0.2	103
EX-250, Nos. 1~20		97~118 (102.7)	175	18.6±0.2	77
EX-350, Nos. 1~20		113~120 (116.5)	50	16.3±0.3*	32
J.W68, Nos. 1~20	SN-100, Nos. 1~20	96~114 (103.9)	542	18.5±0.2	219
	SN-150, Nos. 1~20	106~119 (110.3)	282	19.0±0.2	82
	SN-250, Nos. 1~20	109~115 (107.3)	107	18.7±0.2	78
EN-150, Nos. 1~20	J.W68, Nos. 1~20	100~110 (106.0)	310	19.1±0.2	183
EN-250, Nos. 1~20		106~119 (111.5)	136	18.6±0.2	36

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

suffered from abnormalities in the backbone, jaw-bones or some other bones, ill-development of forelegs, or from edematous abdomen or legs. In addition to these frogs, there were a fairly large number of frogs which suddenly died in spite of their normal appearance. Many of edematous or normally shaped frogs which died by the stage of sexual maturity had abnormal kidneys.

One month after metamorphosis, 100 frogs were left alive and continuously reared until their sexual maturity in each experimental series and the controls, while the others were killed and preserved for later examination of the inner structure of their gonads. In the series having less than 100 frogs one month after metamorphosis, all of them were reared until their sexual maturity.

All the frogs which lived for one year after fertilization attained sexual maturity. The number of living frogs at the breeding season one year after fertilization in the experimental and control series is shown in Table 4. The frogs which lived during the period from one month after metamorphosis to sexual maturity distinctly decreased in number when larger doses were given to the eggs or sperm. The mortality of frogs was remarkably higher in the series from irradiated eggs than that in the series from irradiated spermatozoa.

raised from irradiated sperm or eggs

Sex of frogs dead or killed about one month after metamorphosis					No. of frogs removed and reared	Sex of mature frogs			Sex of all frogs examined		
♀$_N$	♀$_U$	⚥	♂$_N$	♂ (%)†		No. of frogs	♀	♂	Total	♀	♂ (%)†
55	2	0	49	(46.2)	100	92	45	47	198	102	96 (48.5)
82	30	11	49	(34.9)	100	89	35	54	261	147	114 (43.7)
65	24	12	60	(44.7)	100	34	14	20	195	103	92 (47.2)
37	20	3	43	(44.7)	100	15	7	8	118	64	54 (45.8)
30	17	3	27	(39.0)	98	8	2	6	85	49	36 (42.4)
15	4	4	9	(40.6)	18	0	0	0	32	19	13 (40.6)
85	37	15	82	(44.3)	100	44	20	24	263	142	121 (46.0)
35	13	6	28	(41.5)	100	28	13	15	110	61	49 (44.5)
28	16	10	24	(43.6)	13	4	0	4	82	44	38 (46.3)
60	49	14	60	(40.4)	100	16	7	9	199	116	83 (41.7)
12	8	3	13	(44.4)	100	10	5	5	46	25	21 (45.7)

⚥—Hermaphrodites ♂$_N$—Males with normal testes

* Mean body length of 50 frogs † Including hermaphrodites

b. Sex

The sex of young frogs killed one month after metamorphosis is presented in Table 4. In the control series, where 106 frogs in total were examined, there were 49 (46.2%) males with normal testes, two females with underdeveloped ovaries and 55 females with normal ovaries. In two experimental series from X-irradiated sperm and three experimental series from neutron-irradiated sperm, a total of 333 and 379 frogs were examined, respectively. In the former series, there were 109 (33%) males with normal testes, 23 hermaphrodites, 54 females with underdeveloped ovaries and 147 females with normal ovaries, while in the latter, there were 134 (35%) males with normal testes, 31 hermaphrodites, 66 females with underdeveloped ovaries and 148 females with normal ovaries. On the other hand, in three experimental series from X-irradiated eggs and two experimental series from neutron-irradiated eggs, a total of 212 and 219 frogs were examined, respectively. In the former series, there were 79 (37%) males with normal testes, 10 hermaphrodites, 41 females with underdeveloped ovaries and 82 females with normal ovaries, while in the latter, there were 73 (33%) males with normal testes, 17 hermaphrodites, 57 females with underdeveloped ovaries and 72 females with normal ovaries.

Unlike the 46.2% in the control series, only 33~37% of frogs were males in the four kinds of experimental series. Moreover, in contrast with the absence of hermaphrodites among the 106 control frogs, there were 81 (7.1%) hermaphrodites among a total of 1143 frogs in the experimental series. Even if the number of hermaphrodites is added to that of males, as they were destined to become males, the sum total of males is 476 (41.6%). It was also remarkable that there were many females with underdeveloped ovaries in the experimental series. While only two (1.9%) females had underdeveloped ovaries in the control series, 218 (19.1%) had such ovaries in the experimental series. Thus, it was very clear that the frogs in the experimental series somewhat differed from the controls in the process of sex differentiation.

The sex of mature frogs at the age of one year is presented in Table 4. While there were 47 (51%) males and 45 females among the control frogs, there were 74 (60%) males and 49 females in two experimental series from X-irradiated sperm, and 43 (57%) males and 33 females in three experimental series from neutron-irradiated sperm. On the other hand, 14 (61%) of 23 frogs were males in three experimental series from X-irradiated eggs and 14 (54%) of 26 frogs were males in the two experimental series from neutron-irradiated eggs. The males in these four kinds of experimental series totalled 145 (58%) of 248 mature frogs. Briefly, there was always a male preponderance among the mature frogs

TABLE 5

Testes of mature males raised

Parents		No. of analyzed frogs	Body length (mm)
Female	Male		
J.W68, Nos. 1~20	J.W68, Nos. 1~20	47	28.5~39.0 (33.5)
J.W68, Nos. 1~20	SX-150, Nos. 1~20	54	24.0~40.0 (33.9)
	SX-250, Nos. 1~20	20	26.0~38.5 (32.0)
EX-150, Nos. 1~20	J.W68, Nos. 1~20	8	30.0~35.5 (32.2)
EX-250, Nos. 1~20		6	33.0~39.0 (36.4)
J.W68, Nos. 1~20	SN-100, Nos. 1~20	24	26.5~44.0 (32.1)
	SN-150, Nos. 1~20	15	27.0~43.0 (31.9)
	SN-250, Nos. 1~20	4	28.5~34.5 (31.3)
EN-150, Nos. 1~20	J.W68, Nos. 1~20	9	27.5~38.0 (33.2)
EN-250, Nos. 1~20		5	31.0~39.0 (34.2)

in each of the four kinds of experimental series unlike that among the young frogs. There were no remarkable differences in the male preponderance between X-rays and neutrons, between sperm and eggs irradiated and between higher and lower doses of X-rays or neutrons.

5. Testes of mature males

All the mature frogs at the age of one year in the experimental and control series were killed, and immediately thereafter their body lengths and testis sizes were measured. From a part of these males the left testes were removed and utilized for obtaining offspring by artificial fertilization with eggs of normal females. All the males with their right or both testes were fixed in NAVASHIN's fluid. The preserved testes were cut into sections and stained with HEIDENHAIN's hematoxylin to examine their inner structure. The results of observation are presented in Table 5. The testes were classified into the following five types on the basis of their inner structure.

Type 1. Testes are normal in inner structure as in the controls. The seminal tubules are filled with bundles of numerous normal spermatozoa; there are a few pycnotic nuclei of nearly the same size in the tubules (Plate I, 1, 2).

Type 2. Testes are slightly abnormal in inner structure. The seminal tubules contain abnormal spermatozoa of different sizes and pycnotic nuclei together with bundles of normal spermatozoa. In this type, there are various degrees of abnormality, from that having far more pycnotic nuclei than those in

from irradiated sperm or eggs

Size of testes (Mean)		Inner structure of testes				
Left (mm)	Right (mm)	Type 1	Type 2	Type 3	Type 4	Type 5
2.7×2.2	2.7×2.2	47	0	0	0	0
2.5×1.8	2.4×1.8	24	13	12	3	2
2.6×1.7	2.3×1.5	5	4	4	3	4
2.8×1.9	1.9×1.4	2	2	2	1	1
2.8×1.9	2.2×1.6	2	3	1	0	0
2.4×1.7	2.3×1.6	7	6	4	5	2
2.7×2.1	2.7×2.0	5	3	2	3	2
2.3×1.7	2.3×1.7	1	1	1	0	1
2.9×1.8	2.8×1.8	4	2	1	2	0
2.6×1.8	2.0×1.5	3	1	0	1	0

the testes of type 1 to that having a little more numerous normal spermatozoa than the sum of abnormal spermatozoa and pycnotic nuclei (Plate I, 3).

Type 3. The interstitial tissues are distinctly hypertrophied. In the seminal tubules, there are numerous spermatogonia along the walls, while first and second spermatocytes are very scarce. Normal spermatozoa are remarkably diminished and nearly equal in number to or much less than the sum of abnormal spermatozoa and pycnotic nuclei. The abnormal spermatozoa and pycnotic nuclei are varied in size and shape (Plate I, 4).

Type 4. The general state of the interstitial tissues, spermatogonia and spermatocytes is similar to that of type 3. The seminal tubules are mostly filled with abnormal spermatozoa and pycnotic nuclei. There are a few normal spermatozoa. The abnormal spermatozoa and pycnotic nuclei are varied in size and shape (Plate II, 5, 7).

Type 5. The general state of the interstitial tissues, spermatogonia and spermatocytes is similar to that of types 3 and 4. There are no normal spermatozoa. The seminal tubules are filled with abnormal spermatozoa and pycnotic nuclei, both of which are varied in size and shape (Plate II, 6, 8).

a. Control series

The results of measurements and observation on 47 mature males are presented in Table 5. These males were 28.5~39.0 mm, 33.5 mm on the average, in body length. Between their right and left testes, there was usually no distinct difference in size. In fact, 42 of the 47 males had right and left testes of equal size. The largest testes of them were 3.5 mm in length and 2.5 mm in width, while the smallest were 2.0 mm in length and 1.5 mm in width. In the remaining five males, the left and the right testis were 3.0 mm × 2.0 mm and 2.5 mm × 2.0 mm, 2.5 mm × 1.5 mm and 2.5 mm × 2.0 mm, 2.5 mm × 2.0 mm and 3.0 mm × 2.0 mm, and 3.0 mm × 2.5 mm, 2.5 mm × 2.5 mm or 2.0 mm × 1.5 mm and 2.5 mm × 2.0 mm. The right and left testes of the total 47 males were equal in size on the average, that is, 2.7 mm in length and 2.2 mm in width.

All the testes of these males were of type 1 in inner structure; their seminal tubules were filled with bundles of normal spermatozoa.

b. Experimental series derived from X-irradiated sperm

i) J♀ × SX-150♂

In this series, 54 mature males were observed (Table 5). They were 24.0~40.0 mm, 33.9 mm on the average, in body length. The left and right testes of these males were 2.5 mm × 1.8 mm and 2.4 mm × 1.8 mm on the average, respectively. There was no definite parallelism between their body lengths and testis sizes. However, unlike the testes of the control males, there was a difference in size and shape between the right and left testes in 24 males. While such a difference was slight in 19 of them, it was very remarkable in the other five. The left and right testes of three of the latter were 1.5 mm × 1.0 mm and 2.5 mm × 2.0 mm, 1.0 mm × 0.5 mm and 3.5 mm × 2.5 mm, and 4.0 mm × 3.5 mm and

0.3 mm × 0.2 mm in size, respectively. In the remaining two, the right testis consisted of two lobes; the left testis of one male was 3.0 mm × 2.0 mm and the two lobes of the right testis of the same male were 4.0 mm × 3.5 mm and 0.5 mm × 0.3 mm, while in the other male, the left testis was 3.0 mm × 2.7 mm and the right testis was 3.0 mm × 2.0 mm and 2.3 mm × 1.5 mm in size.

The remaining 30 males had right and left testes of the same size. These testes were from 0.5 mm × 0.2 mm to 4.0 mm × 2.0 mm in size. In some males, the testes were far smaller than those of the control males.

Concerning the inner structure of testes, 30 males had testes of types 2∼5, while the other 24 males had right and left testes of type 1 (Table 5). The testes of thirteen, twelve, three and two males were of type 2, type 3, type 4 and type 5, respectively. Although one male had a left testis of type 5 and a right testis of type 1, the testes of this male were counted as those of type 3 by using an average for convenience' sake. The testes of another male were also counted as those of type 4, by taking the median between the right testis of type 5 and the left testis of type 3. There was no definite relationship between the size or shape and inner structure of testes.

ii) J♀ × SX–250♂

Twenty mature males were 26.0∼38.5 mm, 32.0 mm on the average, in body length. The left and the right testes were 2.6 mm × 1.7 mm and 2.3 mm × 1.5 mm on the average in size, respectively. There was no definite parallelism between body lengths and testis sizes of these males. Eight males had right and left testes which were similar to each other in size and shape. Their testes were from 2.1 mm × 1.0 mm to 4.0 mm × 2.5 mm in size. Eight other males had right and left testes which differed slightly from each other in size. In still two others, both testes differed remarkably in size; they were 1.2 mm × 1.0 mm and 2.0 mm × 1.0 mm, or 2.5 mm × 2.0 mm and 2.0 mm × 1.5 mm in size. The remaining two males had no right testes. Their left testes were 5.0 mm × 2.5 mm or 2.0 mm × 1.0 mm in size.

There was no definite relationship between the size or shape and inner structure of testes. The testes of five, four, four, three and four males were of type 1, type 2, type 3, type 4 and type 5 in inner structure, respectively.

c. Experimental series derived from X-irradiated eggs

i) EX–150♀ × J♂

Eight mature males were 30.0 mm∼35.5 mm, 32.2 mm on the average, in body length (Table 5). Their left testes were 2.8 mm × 1.9 mm and the right testes were 1.9 mm × 1.4 mm on the average in size. There was no definite parallelism between the body lengths and testis sizes. In two males, the right and left testes were equal in size, while there was a slight difference between them in four other males. In the remaining two, there was no right testis. Their left testes were 3.0 mm × 2.0 mm and 4.0 mm × 2.0 mm in size.

There was no definite relationship between the size and inner structure of testes. The testes of two, two, two, one and one males were type 1, type 2, type 3, type 4 and type 5 in inner structure, respectively.

ii) EX–250♀ ×J♂

Six mature males were 33.0 mm ~ 39.0 mm, 36.4 mm on the average, in body length. There was no parallelism between their body lengths and testis sizes. The right and left testes in three males were the same in size and shape. In another male, there was a slight difference between them. In still another male, the left testis was 4.0 mm × 2.5 mm, while the right was 3.0 mm × 2.0 mm in size. In the remaining male, there was no right testis, while the left was 2.5 mm × 2.0 mm in size.

There was no intimate relationship between the size and inner structure of testes. The testes of two, three and one males were of type 1, type 2 and type 3 in inner structure, respectively.

d. Experimental series derived from neutron-irradiated sperm

i) J♀ ×SN–100♂

Twenty-four males were 26.5 mm ~ 44.0 mm, 32.1 mm on the average, in body length (Table 5). There was no parallelism between their body lengths and testis sizes. The left and the right testes of these males were 2.4 mm × 1.7 mm and 2.3 mm × 1.6 mm on the average in size, respectively. In 17 males, the testes of both sides were equal in size; they were 1.5 mm × 1.0 mm to 3.5 mm × 3.0 mm. There was a slight difference in size between the right and left testes in five other males. In the remaining two, there was a remarkable difference in size between both testes; in one male, the left and right testes were 1.5 mm × 1.0 mm and 1.0 mm × 0.5 mm, respectively, while in the other, the left testis was 1.5 mm × 1.0 mm and the right testis consisted of two lobes, 2.0 mm × 1.5 mm and 2.5 mm × 2.0 mm in size.

There was no intimate relationship between the size and inner structure of testes. The testes of seven, six, four, five and two males were of type 1, type 2, type 3, type 4 and type 5 in inner structure, respectively.

ii) J♀ ×SN–150♂

Fifteen mature males were 27.0 ~ 43.0 mm, 31.9 mm on the average, in body length. Their left and the right testes were 2.7 mm × 2.1 mm and 2.7 mm × 2.0 mm on the average, in size, respectively. In ten males, the testes of both sides were the same in size and shape; the largest was 3.5 mm × 2.5 mm and the smallest 2.0 mm × 1.5 mm. The remaining five had right and left testes which differ slightly from each other in size.

In the inner structure of testes, five males were of type 1, three of type 2, two of type 3, three of type 4 and the remaining two of type 5.

iii) J♀ ×SN–250♂

Four mature males were 28.5 ~ 34.5 mm, 31.3 mm on the average, in body length. The right and left testes of these males were 2.3 mm × 1.7 mm on the average in size. Two males had right and left testes of equal size, while in the other two the two testes differed slightly in size. The testes of the four males were of types 1, 2, 3, and 5 in inner structure.

e. Experimental series derived from neutron-irradiated eggs

i) EN–150♀ ×J♂

Nine mature males were 27.5～38.0 mm, 33.2 mm on the average, in body length (Table 5). The left and right testes of them were 2.9 mm × 1.8 mm and 2.8 mm × 1.8 mm on the average in size, respectively. In five males, the testes of both sides were the same in size and shape, while there was a slight difference in the other four.

Four males had testes of type 1, two had those of type 2, one had those of type 3 and the remaining two had those of type 4.

ii) EN–250♀ ×J♂

Five males were 31.0～39.0 mm, 34.2 mm on the average, in body length. The left and right testes of these males were 2.6 mm × 1.8 mm and 2.0 mm × 1.5 mm on the average in size, respectively. The right and left testes of one male were the same in size and shape, while those of three others differed slightly. In the remainder, there was a large difference in size between both testes; the left was 2.5 mm × 2.0 mm and the right 0.5 mm × 0.5 mm.

Three males had testes of type 1, while the other two had testes of type 2 or 4.

6. Kidneys of mature males

It was noteworthy that about one-third of the mature males in the experimental series had distinctly abnormal kidneys. The kidneys of mature males were divided into four types on the basis of their size, shape and inner structure.

Type 1. Kidneys are normal in size, shape and inner structure (Plate III, 9).

Type 2. Kidneys are normal in size and shape. However, the lumens of uriniferous tubules are excessively dilated in one-fourth to half of each kidney (Plate III, 10).

Type 3. Kidneys are small and somewhat irregular in shape. Nearly a half of each kidney is very abnormal in inner structure. This portion is mostly occupied with a few abnormal uriniferous tubules whose lumens are extremely dilated; there are no Malpighian corpuscles (Plate III, 11).

Type 4. Kidneys are small and very irregular in shape. A large portion of each kidney is very abnormal in inner structure. Malpighian corpuscles and uriniferous tubules are irregularly arranged; the lumens of some uriniferous tubules are extremely dilated (Plate III, 12).

The types of the kidneys of mature males in nine experimental and the control series are presented in Table 6. In the control series, 45 of 47 mature males had kidneys of type 1, while the other two had those of type 2. No control males had kidneys of type 3 or 4. In contrast, numerous males had kidneys of type 2, 3, or 4 in the experimental series. As presented in the table, one-fifth to half the number of males in each of the nine experimental series had kidneys of type 3 or 4. Males having kidneys of type 3 or 4 were com-

TABLE 6
Kidney of mature males raised from irradiated sperm or eggs

Parents		No. of analyzed frogs	Inner structure of Kidney			
Female	Male		Type 1	Type 2	Type 3	Type 4
J.W68, Nos. 1~20	J.W68, Nos. 1~20	47	45	2	0	0
J.W68, Nos. 1~20	SX–150, Nos. 1~20	54	20	15	9	10
J.W68, Nos. 1~20	SX–250, Nos. 1~20	20	12	4	2	2
EX–150, Nos. 1~20	J.W68, Nos. 1~20	8	5	0	2	1
EX–250, Nos. 1~20	J.W68, Nos. 1~20	6	2	1	1	2
J.W68, Nos. 1~20	SN–100, Nos. 1~20	24	13	4	3	4
J.W68, Nos. 1~20	SN–150, Nos. 1~20	15	9	2	3	1
J.W68, Nos. 1~20	SN–250, Nos. 1~20	4	2	0	1	1
EN–150, Nos. 1~20	J.W68, Nos. 1~20	9	4	2	2	1
EN–250, Nos. 1~20	J.W68, Nos. 1~20	5	2	0	2	1

paratively numerous in the series from gametes which had been irradiated with a larger dose, except those from X-irradiated spermatozoa. In this exceptional series, 19 of 54 males raised from spermatozoa which had been exposed to 150 rads of X-rays had kidneys of type 3 or 4, while 4 of 20 males raised from spermatozoa which had been exposed to 250 rads had such kidneys. There was no remarkable difference in the percentage of males with kidneys of type 3 or 4 between the series from irradiated spermatozoa and those from irradiated eggs, and between the series from X-irradiated gametes and those from neutron-irradiated ones.

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

1. Developmental capacity

As the frogs of nine experimental series obtained in 1968 attained sexual maturity in the breeding season of the next year, 27 males of them were mated with 13 normal females collected from the field (Table 7) by the artificial fertili-

TABLE 7
Eggs of field-caught females used for mating experiments in 1969

Kind	Individual no.	Body length (mm)	No. of eggs	Mean diameter of 50 eggs (mm)
J.W69	1	38.0	1034	1.61±0.02
	2	38.0	1238	1.49±0.02
	3	40.0	1402	1.48±0.02
	4	38.5	1136	1.52±0.02
	5	41.0	1442	1.63±0.02
	6	41.5	965	1.68±0.02
	7	37.5	1037	1.69±0.02
	8	37.0	992	1.50±0.02
	9	38.5	1221	1.48±0.02
	10	56.5	2434	1.64±0.02
	11	42.0	1432	1.67±0.02
	12	39.0	1016	1.48±0.02
	13	37.5	998	1.62±0.02

zation method in order to obtain second-generation offspring. Before using these males for the fertilization experiments, their body length and testis size were measured. The left testis of each male was utilized for artificial fertilization, while the right was sectioned and stained for observing the inner structure. When there was no right testis, a half of the left was utilized for artificial fertilization, and the other half was sectioned and stained. The results of measurements and observations on the males in the experimental series are presented in Table 8, together with those on three control males.

The 27 males in the experimental series were 27.0~44.0 mm in body length,

TABLE 8

Testes of males used for mating experiments in 1969

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure (Type)
				Left (mm)	Right (mm)	
(J·J), No. 1	1	1	36.0	3.0×2.5	3.0×2.5	1
	2	1	34.5	3.0×2.5	3.0×2.5	1
	3	1	34.5	2.5×2.0	2.5×2.0	1
(J·SX–150), No. 1	1	1	31.5	1.5×1.0	1.5×1.0	3
	2	1	34.5	2.5×2.0	2.0×1.5	1
	3	1	36.5	3.0×2.5	3.0×2.0	1
(J·SX–250), No. 1	1	1	32.5	2.5×2.0	2.0×1.5	1
	2	1	33.5	5.0×2.5	—	2
	3	1	34.0	2.7×1.5	2.7×1.5	3
(EX–150·J), No. 1	1	1	33.0	2.0×1.5	2.5×2.0	1
	2	1	35.5	2.5×2.0	2.5×2.0	4
	3	1	32.0	3.0×2.0	—	2
(EX–250·J), No. 1	1	1	39.0	4.0×2.5	3.0×2.0	2
	2	1	38.0	2.5×2.0	2.5×2.0	1
	3	1	33.0	2.5×2.0	—	3
(J·SN–100), No. 1	1	1	28.0	1.5×1.0	1.5×1.0	1
	2	1	44.0	3.5×3.0	3.5×3.0	1
	3	1	36.5	2.5×2.0	2.5×2.0	1
(J·SN–150), No. 1	1	1	30.5	2.5×1.5	2.5×2.0	2
	2	1	30.0	2.0×1.5	2.0×1.5	1
	3	1	37.5	2.5×2.5	2.5×2.0	1
	4	1	37.5	2.5×2.0	2.5×2.0	1
(J·SN–250), No. 1	1	1	32.0	2.5×2.0	2.5×1.5	2
	2	1	30.0	2.0×1.5	2.0×2.0	1
(EN–150·J), No. 1	1	1	32.0	2.0×1.5	2.0×1.0	2
	2	1	27.0	2.5×1.0	2.5×1.0	1
	3	1	29.5	2.5×2.0	2.5×2.0	1
(EN–250·J), No. 1	1	1	31.5	2.5×2.0	0.5×0.5	4
	2	1	39.0	3.0×2.0	2.5×2.0	2
	3	1	38.0	3.5×2.0	3.0×2.5	1

(J·J), No. 1: Males obtained by J.W68♀, No. 1×J.W68♂, No. 1
(J·SX–150 or –250), No. 1: Males obtained by J.W68♀, No. 1×SX–150 or –250♂, No. 1
(EX–150 or –250·J), No. 1: Males obtained by EX–150 or –250♀, No. 1×J.W68♂, No. 1
(J·SN–100 or –150), No. 1: Males obtained by J.W68♀, No. 1×SN–100 or –150♂, No. 1
(EN–150 or –250·J), No. 1: Males obtained by EN–150 or –250♀, No. 1×J.W68 ♂, No. 1

while the three controls were 34.5～36.0 mm. However, 23 of the former were 30.0～39.0 mm, 34.2 mm on the average. The other four were 27.0 mm, 28.0 mm, 29.5 mm and 44.0 mm. While the testes of the control males were all of type 1, there were 15 males having testes of type 1, seven having those of type 2, three having those of type 3 and two having those of type 4 in the experimental series.

The results of matings between the field-caught females and the male first-generation offspring and controls are presented in Table 9 and shown in Figs. 26, 27 and 28. While more than 87% of eggs cleaved normally in the control series, less than 81% of eggs did so in most of the experimental series. There was no intimate relationship between the percentage of cleaved eggs and the kind or dosage of irradiation. The embryos and tadpoles in the experimental series were far inferior to the controls in viability. While more than 74% of the control eggs became normally metamorphosed frogs, far smaller percentages of eggs did so in the experimental series. It was remarkable that there was no intimate relationship between the viability of second-generation embryos or tadpoles and the dosage of irradiation. Such a condition distinctly differed from that in the first-generation offspring. Moreover, the type and the appearance time of abnormalities occurring in the embryonic and tadpole stages varied greatly with their male parents. For example, the offspring of a male became simultaneously edematous, those of another male were ill-developed, and those of still another

TABLE 9

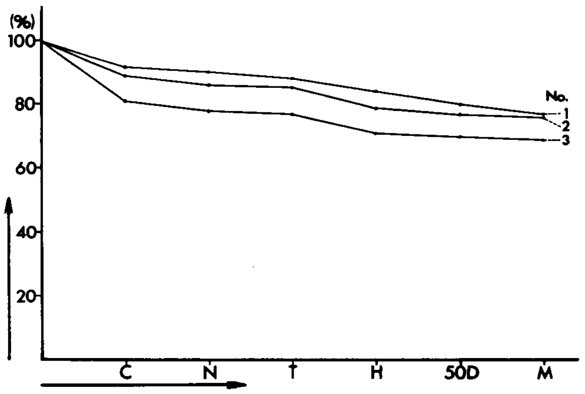
Developmental capacity of the offspring of males

Parents		No. of eggs	No. of cleaved eggs		No. of gastrulae	
Female	Male		Normal	Abnormal	Normal	Abnormal
J.W69, Nos. 1～12	J·J, Nos. 1～3	807	707 (87.7%)	0	705 (87.4%)	2 (0.2%)
J.W69, Nos. 1～3	J·SX–150, Nos. 1～3	1680	1041 (62.0%)	7 (0.4%)	1019 (60.7%)	22 (1.3%)
	J·SX–250, Nos. 1～3	1257	1011 (80.4%)	1 (0.1%)	984 (78.3%)	27 (2.1%)
J.W69, Nos. 4～6	EX–150·J, Nos. 1～3	1736	1169 (67.3%)	17 (1.0%)	1121 (64.6%)	48 (2.8%)
	EX–250·J, Nos. 1～3	1428	1007 (70.5%)	11 (0.8%)	986 (69.0%)	21 (1.5%)
J.W69, Nos. 7～9	J·SN–100, Nos. 1～3	1507	1124 (74.6%)	8 (0.5%)	1116 (74.1%)	8 (0.5%)
	J·SN–150, Nos. 1～4	2882	2312 (80.2%)	11 (0.4%)	2240 (77.7%)	72 (2.5%)
J.W69, No. 13	J·SN–250, Nos. 1,2	954	825 (86.5%)	0	823 (86.3%)	2 (0.2%)
J.W69, Nos. 10～12	EN–150·J, Nos. 1～3	1552	1423 (91.7%)	0	1379 (88.9%)	44 (2.8%)
	EN–250·J, Nos. 1～3	1432	1133 (79.1%)	10 (0.7%)	1067 (74.5%)	66 (4.6%)

male had abnormal forelegs at a definite stage, respectively. The offspring of some other males revealed various kinds of abnormalities at various stages, as found in the first-generation offspring.

a. Control series, J♀ × (J♀ × J♂)♂, Nos. 1 ~ 3

Three male first-generation offspring produced from a control mating in 1968 were mated with 13 normal females Nos. 1 ~ 13 collected from the field. The development and viability of the second-generation offspring as a whole are presented in Table 9 and shown in Fig. 57, while the survival curve of the offspring produced from each of the three males is shown in Fig. 26. In the



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

Fig. 26. Survival curves of control second-generation offspring derived from untreated grandparental gametes by matings, J♀ × (J♀ × J♂)♂, Nos. 1 ~ 3.

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		
686	19	673	13	633	40	615	601
(85.0%)	(2.4%)	(83.4%)	(1.6%)	(78.4%)	(5.0%)	(76.2%)	(74.5%)
996	23	996	0	968	28	880	868
(59.3%)	(1.4%)	(59.3%)		(57.6%)	(1.7%)	(52.4%)	(51.7%)
960	24	951	9	904	47	723	703
(76.4%)	(1.9%)	(75.7%)	(0.7%)	(71.9%)	(3.7%)	(57.5%)	(55.9%)
1092	29	1036	56	932	104	893	552
(62.9%)	(1.7%)	(59.7%)	(3.2%)	(53.7%)	(6.0%)	(51.4%)	(31.8%)
936	50	886	50	738	148	676	578
(65.5%)	(3.5%)	(62.0%)	(3.5%)	(51.7%)	(10.4%)	(47.3%)	(40.5%)
996	120	984	12	913	71	840	794
(66.1%)	(8.0%)	(65.3%)	(0.8%)	(60.6%)	(4.7%)	(55.7%)	(52.7%)
2041	199	2020	21	1920	100	1725	1588
(70.8%)	(6.9%)	(70.1%)	(0.7%)	(66.6%)	(3.5%)	(59.9%)	(55.1%)
815	8	802	13	726	76	701	683
(85.4%)	(0.8%)	(84.1%)	(1.4%)	(76.1%)	(8.0%)	(73.5%)	(71.6%)
1334	45	1177	157	706	471	652	586
(86.0%)	(2.9%)	(75.8%)	(10.1%)	(45.5%)	(30.3%)	(42.0%)	(37.8%)
1046	21	916	130	758	158	699	576
(73.0%)	(1.5%)	(64.0%)	(9.1%)	(52.9%)	(11.0%)	(48.8%)	(40.2%)

three control matings, 92.0%, 88.6% and 81.3%, 87.7% on the average, of the respective total number of eggs cleaved normally. Most of the cleaved eggs developed normally into hatched tadpoles and then into metamorphosed frogs. The numbers of normally hatched tadpoles from the three matings corresponded to 83.5%, 78.9% and 71.4%, 78.4% on the average, of the respective total number of eggs, and those of normally metamorphosed frogs corresponded to 77.1%, 76.3% and 69.4%, 74.5% on the average.

b. Experimental series derived from X-irradiated grandparental sperm

i) J♀ × (J♀ × SX-150♂)♂, Nos. 1∼3

Three males produced from spermatozoa which had been exposed to 150 rads of X-rays were mated with three normal females Nos. 1∼3 collected from the field (Table 9). The survival curve of the second-generation offspring produced from each of the three males is shown in Fig. 27a. Although 17.6%, 75.9% and 72.0%, 62.0% on the average, of the respective total number of eggs in the three matings Nos. 1∼3 cleaved normally, they scarcely differed in development from those in the control series, that is, 15.1%, 71.7% and 66.7%, 57.6%

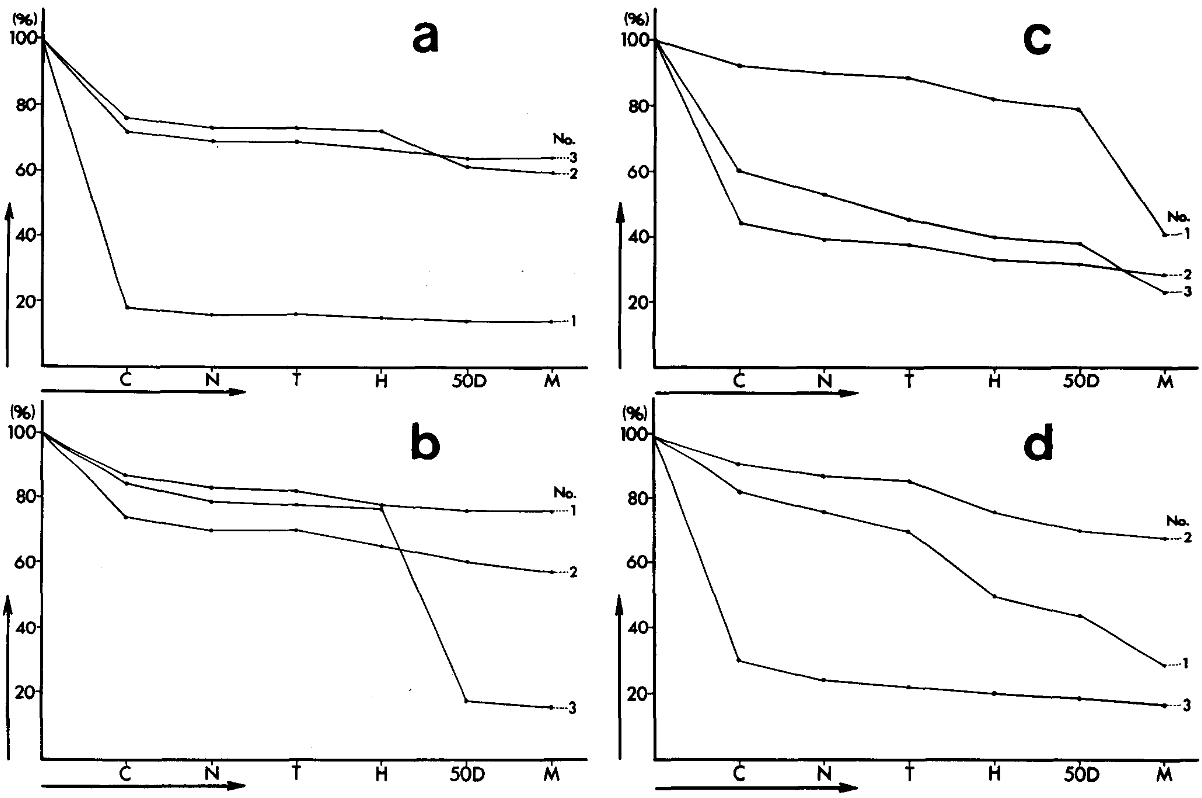


Fig. 27. Survival curves of second-generation offspring derived from X-irradiated grandparental gametes by passing over male first-generation offspring.

a. J♀ × (J♀ × SX-150♂) ♂, Nos. 1∼3
b. J♀ × (J♀ × SX-250♂) ♂, Nos. 1∼3
c. J♀ × (EX-150♀ × J♂) ♂, Nos. 1∼3
d. J♀ × (EX-250♀ × J♂) ♂, Nos. 1∼3

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

on the average, hatched normally. However, in one (No. 2) of the three matings, 63 of 420 feeding tadpoles became thin and died within 50 days after fertilization, while only a few died in the other matings. The numbers of normally metamorphosed frogs from the three matings eventually corresponded to 13.6%, 59.2% and 63.7%, 51.7% on the average, of the respective total number of eggs.

ii) J♀×(J♀×SX-250♂)♂, Nos. 1~3

Three males produced from spermatozoa which had been exposed to 250 rads of X-rays were mated with the same three females as those in the above matings. The survival curve of the second-generation offspring produced from each of the three males is shown in Fig. 27b. The proportions of normal cleavages in the three matings did not distinctly differ from those in the control series, that is, they were 86.1%, 74.1% and 84.1%, 80.4% on the average, of the respective total number of eggs. However, these cleaved eggs were considerably inferior as a whole in development to the controls (Table 9). While in one (No. 1) of the three experimental matings 75.8% of the total number of eggs became normally metamorphosed frogs, 56.7% and 15.9% did so in the other two (Nos. 2 and 3) matings. In No. 1, a few embryos and tadpoles died of various kinds of abnormalities at various stages, just as in the control mating. In No. 2, 65.1% of the total number of eggs hatched normally, although a small number of embryos mostly died of microcephaly and edema. Some of the hatched tadpoles died of edema afterwards. In No. 3, 76.6% of the total number of eggs hatched normally and became swimming tadpoles. However, as a great majority of the latter had underdeveloped teeth, they could not eat and eventually 141 of 183 tadpoles died 50 days after fertilization. Of the remaining 42, 38 became normally metamorphosed frogs.

c. Experimental series derived from X-irradiated grandparental eggs

i) J♀×(EX-150♀×J♂)♂, Nos. 1~3

Three males produced from eggs which had been exposed to 150 rads of X-rays were mated with three field-caught females Nos. 4~6 (Table 9). The survival curve of the second-generation offspring produced from each of the three males is shown in Fig. 27c. The proportions of normally cleaved eggs in the three matings Nos. 1~3 were 91.8%, 44.2% and 60.2%, 67.3% on the average, of the respective total number of eggs. The eggs in No. 1 scarcely differed in developmental capacity from the control until the stage immediately before metamorphosis. However, 249 of 522 tadpoles had no normal forelegs at the metamorphosis stage, while the other 273, 41.3% of the total number of eggs, became normally metamorphosed frogs. A small number of individuals in No. 2 died of various kind of abnormalities at various developmental stages; normally metamorphosed frogs corresponded to 29.1% of the total number of eggs. In No. 3, about two-thirds of the normally cleaved eggs died of microcephaly or edema during the embryonic stage. After the hatching stage a few tadpoles became edematous and died, while nearly one half of the others had underdeveloped forelegs at the metamorphosis stage. Normally metamorphosed frogs corresponded to 23.0% of the total number of eggs.

ii) J♀×(EX–250♀×J♂)♂, Nos. 1∼3

Three males produced from eggs which had been exposed to 250 rads of X-rays were mated with the same three females as in the above matings. The survival curve of the second-generation offspring produced from each of the three males is shown in Fig. 27d. The proportions of normally cleaved eggs were 81.5%, 89.6% and 30.4%, 70.5% on the average, in the three matings Nos. 1∼3. In Nos. 1 and 2, edematous embryos increased with development and became most numerous at the hatching stage. The proportions of normally hatched tadpoles were 50.3% and 75.9% of the respective total number of eggs. In the tadpole stage, numerous individuals in No. 1 and a few in No. 2 also became edematous; the proportions of normally metamorphosed frogs were 28.5% and 67.8% of the respective total number of eggs. A small number of the normally cleaved eggs in No. 3 gradually died of various kinds of abnormalities during their development. The proportion of normally metamorphosed frogs was eventually 17.2% of the total number of eggs.

d. Experimental series derived from neutron-irradiated grandparental sperm

i) J♀×(J♀×SN–100♂)♂, Nos. 1∼3

Three males produced from spermatozoa which had been exposed to 100 rads of neutrons were mated with three field-caught females Nos. 7∼9 (Table 9). The survival curve of the second-generation offspring produced from each of the three males is shown in Fig. 28a. The proportions of normally cleaved eggs in the three matings Nos. 1∼3 were 57.0%, 88.0% and 90.0%, 74.6% on the average. The eggs in No. 1 were distinctly inferior to the control in the later development, too. Many embryos and tadpoles died of edema, and 41.8% and 35.6% of the total number of eggs became normally hatched and metamorphosed frogs, respectively. In No. 2, 74.1% of the total number of eggs became normally metamorphosed frogs, while a small number of embryos and tadpoles died of various kinds of abnormalities at various stages. In No. 3, 74.3% of the total number of eggs hatched normally, while the other embryos mostly died of edema. After the hatching stage, edematous tadpoles gradually increased in number and eventually 59.3% became normally metamorphosed frogs.

ii) J♀×(J♀×SN–150♂)♂, Nos. 1∼4

Four males produced from spermatozoa which had been exposed to 150 rads of neutrons were mated with three field-caught females Nos. 7∼9. The survival curve of the second-generation offspring produced from each of the four males is shown in Fig. 28b. The proportions of normally cleaved eggs in the four matings Nos. 1∼4 were 73.0%, 79.6%, 87.5% and 81.1%, 80.2% on the average. In No. 1, 156 out of 563 embryos were microcephalous and edematous at the neurula stage. Only a few of the remaining embryos became edematous afterwards, and 392, 50.0% of the total number of eggs, hatched normally. However, 200 of the tadpoles gradually became edematous by the metamorphosis

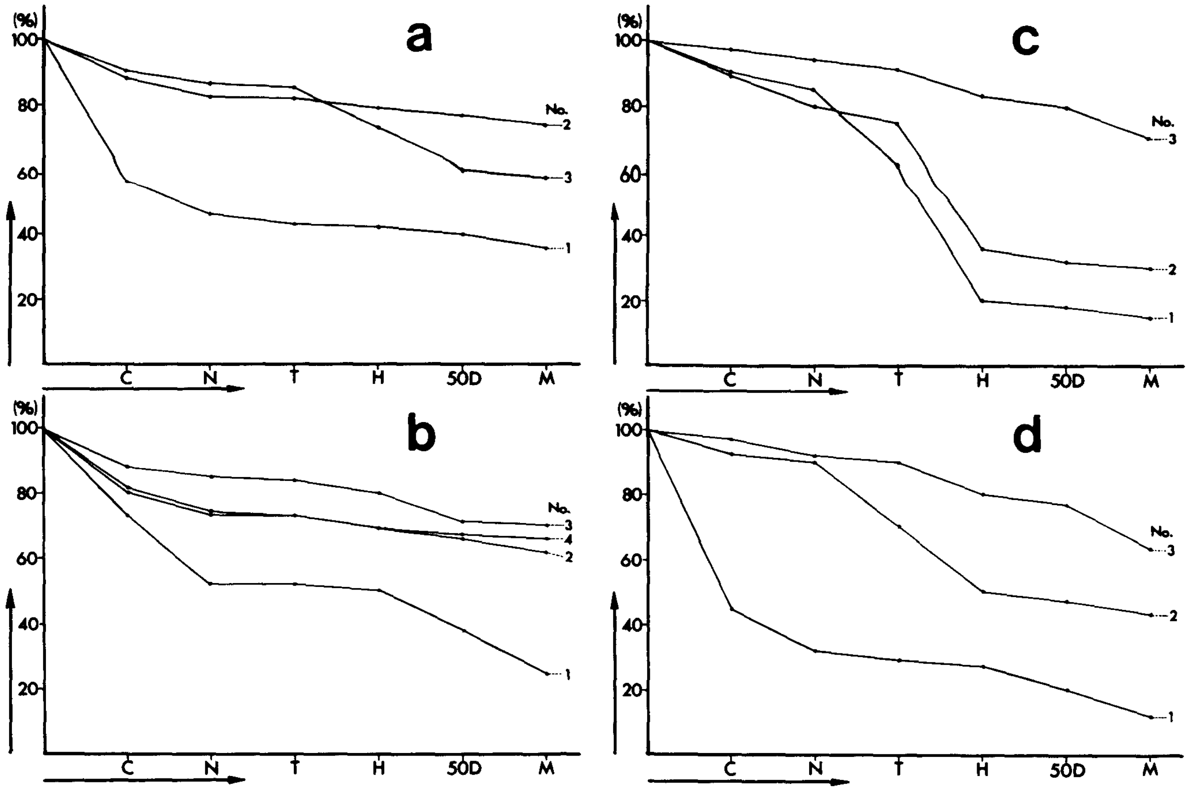


Fig. 28. Survival curves of second-generation offspring derived from neutron-irradiated grandparental gametes by passing over male first-generation offspring.

a. J♀ × (J♀ × SN–100♂) ♀, Nos. 1~3
b. J♀ × (J♀ × SN–150♂) ♂, Nos. 1~4
c. J♀ × (EN–150♀ × J♂) ♀, Nos. 1~3
d. J♀ × (EN–250♀ × J♂) ♂, Nos. 1~3

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

stage, and eventually 192 (24.5%) completed metamorphosis. In the other three matings Nos. 2, 3 and 4, many individuals revealed various kinds of abnormalities, such as microcephaly, bicephaly, curvature of the body and shrinkage of the skin, and gradually died at various stages. The numbers of normally metamorphosed frogs in these three matings were eventually 62.4%, 70.1% and 66.4% of the respective total number of eggs.

iii) J♀ × (J♀ × SN–250♂) ♂, Nos. 1 and 2

Two males produced from spermatozoa which had been exposed to 250 rads of neutrons were mated with one field-caught female No. 13. The proportions of normally cleaved eggs in the two matings were 87.6% and 85.5%, 86.5% on the average, of the respective total number of eggs. Except a few which died of microcephaly and edema at the hatching stage, they developed normally into swimming tadpoles. Although 66.9% and 76.0%, 71.6% on the average, became normally metamorphosed frogs, 42.3% and 53.0% from the two matings were temporarily edematous early in the tadpole stage.

e. Experimental series from neutron-irradiated grandparental eggs

i) J♀ × (EN–150♀ × J♂)♂, Nos. 1 ~ 3

Three males produced from eggs which were exposed to 150 rads of neutrons were mated with three field-caught females Nos. 11 ~ 13 (Table 9). The survival curve of the second-generation offspring produced from each of the three males is shown in Fig. 28c. The proportions of normal cleavages in the three matings Nos. 1 ~ 3 were 89.5%, 88.6% and 97.1%, 91.7% on the average. In No. 1, 121 and 230 of the normally cleaved eggs died of various kinds of abnormalities at the tail-bud and the hatching stage, respectively, while the others, 20.1% of the total number of eggs, hatched normally and became swimming tadpoles. A small number of the latter gradually died by the metamorphosis stage, and eventually 83 (15.2%) became normally metamorphosed frogs. In No. 2, more than 20 embryos gradually died of edema by the stage immediately before hatching. At the hatching stage 200 embryos which were microcephalous became promptly edematous and died, while 185 (36.1%) hatched normally. A small number of swimming tadpoles gradually became edematous, and 154 (30.1%) completed their metamorphosis. In No. 3, 83.1% of the total number of eggs hatched normally and 70.4% became normally metamorphosed frogs, while a small number of normally cleaved eggs died at various embryonic and tadpole stages.

ii) J♀ × (EN–250♀ × J♂)♂, Nos. 1 ~ 3

Three males produced from eggs which had been exposed to 250 rads of neutrons were mated with the same females as in the above matings. The survival curve of the second-generation offspring produced from each of the three males is shown in Fig. 28d. The proportions of normal cleavages were 44.9%, 91.5% and 97.0%, 79.1% on the average, in the three matings Nos. 1 ~ 3. Many of the normally cleaved eggs in No. 1 died of incomplete gastrulation or some other abnormalities during the embryonic stage, while 27.1% of the total number of eggs hatched normally. The majority of the tadpoles also died of ill-development or edema; 12.4% eventually became normally metamorphosed frogs. In No. 2, 107 and 103 embryos promptly died of edema at the tail-bud and the hatching stage, respectively, and 264 embryos, 50.2% of the total number of eggs, hatched normally. A small number of tadpoles gradually died of edema afterwards, and eventually 224 (42.5%) became normally metamorphosed frogs. In No. 3, 79.9% of the total number of eggs hatched normally, while a small number of normally cleaved eggs died of various kinds of abnormalities by the hatching stage. At the metamorphosis stage there were 65 tadpoles which formed no forelegs; 298 (63.3%) were normally metamorphosed.

2. Chromosome aberrations in normally shaped tadpoles

Chromosomes were observed in normally shaped 50-day-old tadpoles (V ~ X stages) of the second generation of males raised from X- or neutron-irradiated gametes and in the controls. The second-generation offspring were produced from male first-generation offspring by mating with normal females collected from the field. The tadpoles were those produced from nine matings of the four experimental series (Table 10).

TABLE 10

Chromosomal analysis of normally shaped 50-day-old tadpoles produced from males 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	
J.W69, Nos. 1~3	J·J, No. 1	20	19 (95.0%)	0	0	1 (5.0%)
J.W69, Nos. 1~3	J·SX–150, No. 3	24	18 (75.0%)	3 (12.5%)	1 (4.2%)	2 (8.3%)
J.W69, Nos. 1~3	J·SX–250, No. 2	27	18 (66.7%)	4 (14.8%)	2 (7.4%)	3 (11.1%)
J.W69, Nos. 4~6	EX–150·J, No. 1	25	18 (72.0%)	3 (12.0%)	1 (4.0%)	3 (12.0%)
J.W69, Nos. 4~6	EX–250·J, No. 2	24	17 (70.8%)	2 (8.3%)	1 (4.2%)	4 (16.7%)
J.W69, Nos. 7~9	J·SN–100, No. 2	32	23 (71.9%)	5 (15.6%)	2 (6.3%)	2 (6.3%)
J.W69, Nos. 7~9	J·SN–150, No. 3	25	20 (80.0%)	3 (12.0%)	1 (4.0%)	1 (4.0%)
J.W69, No. 13	J·SN–250, No. 2	27	20 (74.1%)	4 (14.8%)	1 (3.7%)	2 (7.4%)
J.W69, Nos. 10~12	EN–150·J, No. 3	23	15 (65.2%)	3 (13.0%)	2 (8.7%)	3 (13.0%)
J.W69, Nos. 10~12	EN–250·J, No. 3	22	14 (63.6%)	4 (18.2%)	2 (9.1%)	2 (9.1%)

a. Control series, J♀ × (J♀ × J♂)♂, No. 1

Squash preparations were made from the tail-tips of 30 normally shaped tadpoles produced from one (No. 1) of the three matings of the control series. As a result of examination, 20 of these tadpoles were analyzable. While a tadpole was a mosaic of normally diploid (2n=26) and abnormal cells which had a deletion in a chromosome, the other 19 were solely constructed of normally diploid cells.

b. Experimental series derived from X-irradiated grandparental sperm

i) J♀ × (J♀ × SX–150♂)♂, No. 3

From 30 normally shaped tadpoles produced from one (No. 3) of the three matings between three male first-generation offspring derived from spermatozoa which had been exposed to 150 rads of X-rays and normal females, the tail-tips were cut off, squashed and stained. Twenty-four of these tadpoles were analyzable (Table 10). Eighteen consisted solely of normally diploid cells, while the other six were composed of abnormal cells alone or a mixture of normally diploid and abnormal cells. Three of the six tadpoles had 26 chromosomes, among which there was a chromosome with a deletion or translocation; all the metaphase spreads of each tadpole were the same in karyotype. Another tadpole consisted of a mixture of mitoses containing a chromosome with a deletion and those containing both chromosome with a deletion and that with a translocation, although all the mitoses of this tadpole were 26 in chromosome number. Still another tadpole was a mosaic of normally diploid cells and abnormally diploid ones containing a ring chromosome. The remaining one was a mosaic of normally diploid cells and hyperdiploid (2n+1) cells.

ii) J♀×(J♀×SX–250♂)♂, No. 2

Twenty-seven of 36 normally shaped tadpoles produced from one (No. 2) of the three matings between three male first-generation offspring derived from spermatozoa which had been exposed to 250 rads of X-rays and normal females were analyzable in karyotype. Eighteen of them were normal diploids. Four others had solely abnormal mitoses, although each tadpole had a definite karyotype; a tadpole was a hyperdiploid (2n+1) having an additional small chromosome, two others were abnormal diploids having a chromosome with a deletion, and the remaining one was an abnormal diploid having a ring chromosome. Two other tadpoles were mosaics consisting of two kinds of abnormal chromosome complements; a tadpole was constructed of hyperdiploid cells having an additional small chromosome and hypodiploid cells losing the latter, and the other was constructed of abnormally diploid cells having a ring chromosome and those having a chromosome with a deletion. The remaining three tadpoles were mosaics of normally diploid and abnormal cells; a tadpole consisted of diploid and triploid cells, and the other two consisted of normally diploid cells and abnormally diploid cells having chromosomes with a deletion or translocation.

c. Experimental series derived from X-irradiated grandparental eggs

i) J♀×(EX–150♀×J♂)♂, No. 1

Preparations were made from the tail-tips of 30 normally shaped tadpoles produced from one (No. 1) of the three matings between three male first-generation offspring derived from eggs which had been exposed to 150 rads of X-rays and normal females. Twenty-five of them were analyzable in karyotype (Table 10). Of these tadpoles, 18 were normal diploids, while three others had nothing but abnormally diploid mitoses, although each tadpole had a definite karyotype. One of these three tadpoles had a deletion in a chromosome, another had a deletion in a chromosome and a translocation in another chromosome, and the other had a chromosome (No. 5) having a translocation in the long arm. One tadpole was a mosaic of two kinds of abnormally diploid cells; one kind had a deletion, while the other had a translocation in the long arm of one of the No. 4 chromosomes. The remaining three tadpoles were mosaics of normally diploid and abnormal cells. These abnormal cells were triploid cells, hyperdiploid (2n+1) cells having an additional small chromosome, or abnormally diploid cells having a ring chromosome.

ii) J♀×(EX–250♀×J♂)♂, No. 2

The tail-tips of 30 normally shaped tadpoles produced from one (No. 2) of the three matings between three male first-generation offspring derived from eggs which had been exposed to 250 rads of X-rays and normal females were squashed and stained. Of these tadpoles, 24 were analyzable in karyotype. Seventeen of them were normal diploids. Two others had no other than abnormal mitoses, although each tadpole was definite in karyotype. One of these two was a hyperdiploid (2n+1) having an additional small chromosome, while the other was an abnormal diploid in which one of the No. 10 chromosomes had a deletion in the short arm. Another tadpole was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) cells with an addition or a loss of a small chromosome. The remaining four tadpoles were mosaics of normally diploid and abnormal cells; one contained triploid cells, one contained hyperdiploid (2n+1) cells with an additional small chromosome, one contained abnormally diploid cells with a dicentric chromosome and a fragment, and one contained hypodiploid cells in which one of the No. 9 chromosomes was translocated to the long arm of one of the No. 5 chromosomes.

d. Experimental series derived from neutron-irradiated grandparental sperm

i) J♀×(J♀×SN-100♂)♂, No. 2

Preparations were made from the tail-tips of 40 normally shaped tadpoles produced by one (No. 2) of the three matings between three male first-generation offspring derived from spermatozoa which had been exposed to 100 rads of neutrons and normal females. Of these tadpoles, 32 were analyzable in karyotype (Table 10). Twenty-three of them contained solely normally diploid mitoses, while five others did not contain these, although each tadpole had a definite karyotype. One of the five was a hyperdiploid (2n+1) having an additional small chromosome, while the others were abnormal diploids having chromosomes with a deletion or translocation. Two other tadpoles were mosaics of two or more kinds of abnormal cells; a tadpole consisted of a mixture of abnormally diploid cells having a chromosome with a deletion and hypodiploid (2n−1) cells having a dicentric chromosome, while the other consisted of abnormally diploid cells having chromosomes with a deletion or translocation. The remaining two tadpoles were mosaics of normally and abnormally diploid cells; a tadpole consisted of a mixture of normally diploid cells and abnormally diploid cells having a ring chromosome, while the other consisted of a mixture of diploid and hyperdiploid (2n+1) cells having an additional small chromosome.

ii) J♀×(J♀×SN-150♂)♂, No. 3

Twenty-five of 30 normally shaped tadpoles produced from one (No. 3) of the four matings between four male first-generation offsprings derived from spermatozoa which had been exposed to 150 rads of neutrons and normal females were analyzable in karyotype. Of these tadpoles, 20 were normal diploids, while three others had solely abnormal mitoses, although all the mitoses of each tadpole were the same in karyotype. Two of these three were hyperdiploids (2n+1) having an additional small chromosome, and the other was an abnormal diploid having a deletion in a chromosome and a translocation in another one. Another tadpole was a mosaic of hyperdiploid (2n+1) cells having an additional small chromosome and hypodiploid (2n−1) cells losing the same chromosome. The remaining one tadpole was a mosaic of normally diploid cells and abnormally diploid cells having a chromosome with a deletion.

iii) J♀×(J♀×SN-250♂)♂, No. 2

Of 36 normally shaped tadpoles produced from one (No. 2) of the two matings between two male first-generation offspring derived from spermatozoa which had been exposed to 250 rads of neutrons and normal females, 27 were analyzable in karyotype. Twenty of them had nothing other than normally diploid mitoses. Four others did not contain such mitoses, although each of them had a definite karyotype; one tadpole was a hyperdiploid (2n+1) having an additional small chromosome, while the other four were abnormal diploids having chromosomes with a deletion, a translocation or a ring chromosome. Still another tadpole was a mosaic of hyperdiploid (2n+1) cells having an additional small chromosome and hypodiploid (2n−1) cells losing the same chromosome. The remaining two tadpoles were mosaics of normally and abnormally diploid cells; a tadpole contained mitoses having a dicentric chromosome and a fragment, while the other contained those having a ring chromosome.

e. Experimental series derived from neutron-irradiated grandparental eggs

i) J♀×(EN–150♀×J♂)♂, No. 3

Preparations were made from the tails of 30 normally shaped tadpoles produced from one (No. 3) of the three matings between three male first-generation offspring derived from eggs which had been exposed to 150 rads of neutrons and normal females. Their karyotypes were analyzable in 23 tadpoles (Table 10). Fifteen of the latter had solely normally diploid mitoses, while three others had no other than abnormal ones, although each tadpole had a definite karyotype. One of these three was a hyperdiploid (2n+1) having an additional small chromosome, while the others were abnormal diploids having a deletion in a chromosome and a translocation in another one. Two other tadpoles were mosaics of two kinds of abnormal cells; one consisted of a mixture of hyperdiploid (2n+1) and hypodiploid (2n−1) cells in which an addition or a loss of a small chromosome, while the other consisted of a mixture of abnormally diploid cells having a dicentric chromosome and a fragment and those having a deletion in a chromosome. The remaining three tadpoles were mosaics of normally diploid and abnormal cells; the latter in a tadpole were triploid, those in another were tetraploid, and those in the other were abnormally diploid and had a ring chromosome.

ii) J♀×(EN–250♀×J♂)♂, No. 3

Twenty-two of 30 normally shaped tadpoles produced from one (No. 3) of the three matings between three male first-generation offsprings derived from eggs which had been exposed to 250 rads of neutrons and normal females were analyzable in karyotype. Fourteen of them were normal diploids. Four others had no other than abnormal mitoses, although each tadpole had a definite karyotype; a tadpole was a triploid, another was a hyperdiploid (2n+1) having an additional small chromosome, still another was an abnormal diploid having a deletion in a chromosome, and the remainder was an abnormal diploid in which a segment of a chromosome was transferred to another chromosome. Two other tadpoles were mosaics of two kinds of abnormal mitoses; one consisted of a mixture of diploid mitoses having a ring chromosome and diploid ones having a deletion in a chromosome, while the other consisted of a mixture of diploid mitoses having a translocation in a chromosome and hypodiploid (2n—1) mitoses. The remaining two tadpoles were mosaics of normally diploid and abnormal cells; the latter in a tadpole were triploid, while those in the other were hypodiploid (2n—1) and had a dicentric chromosome.

3. Viability and sex of metamorphosed frogs

a. Viability

The days from fertilization to metamorphosis and the body length immediately after metamorphosis in the individuals of the experimental series and the controls are presented in Table 11. As shown in the table, there were no remarkable differences in these respects between the experimental and the control series, and between X-rays and neutrons to which sperm or eggs were exposed. No remarkable differences were also found between different amounts of X-rays or neutrons.

About one month after metamorphosis, 100 living frogs were removed from each experimental series and continuously reared in order to make them sexually mature, except three series, from which 31～99 frogs were left (Table 11). The remaining frogs were killed and preserved to examine the structure of gonads. The number of mature frogs in each experimental series is presented in Table 8, together with that in the controls. In the experimental series, many frogs died before the breeding season of the following year, and 0～71% of the young frogs attained sexual maturity, while 92% of the controls did. It was also clear that the series derived from gametes which had been exposed to a larger amount of irradiation was smaller in the number of mature frogs than that exposed to a smaller dosage. While the difference in the number of mature frogs between X-rays and neutrons to which the gametes were exposed was scarce, there was a remarkable difference in this respect between the series derived from irradiated eggs and those from irradiated sperm. The offspring of the males raised from spermatozoa which had been exposed to 250 rads of neutrons could not attain sexual maturity.

b. Sex

The sex of juvenile frogs which died or were killed within one month after metamorphosis is presented in Table 11. In the control series, there were males and females of nearly the same number among 454 frogs, that is, 224 males with normal testes and 227 females with normal ovaries, besides two hermaphrodites and a female with underdeveloped ovaries. When the two hermaphrodites were added to males, as they were considered to become males before long, the number of males was 49.8% of the total number of frogs.

The experimental series remarkably differed from the control series in that there were considerably many hermaphrodites as well as females with underdeveloped ovaries. Moreover, there was a distinct inequality of number between males and females in most experimental series. When hermaphrodites were

TABLE 11

Number, size and sex of metamorphosed frogs produced

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed frogs	Mean body length of 100 frogs immediately after metamorphosis (mm)	
Female	Male				No. of frogs
J.W69, Nos. 1~12	J·J, Nos. 1~3	77~94 (85.1)	601	17.5±0.2	454
J.W69, Nos. 1~3	J·SX-150, Nos. 1~3	77~109 (89.6)	868	17.2±0.2	697
	J·SX-250, Nos. 1~3	77~101 (87.5)	703	16.9±0.2	587
J.W69, Nos. 4~6	EX-150·J, Nos. 1~3	77~98 (86.6)	552	17.2±0.2	436
	EX-250·J, Nos. 1~3	77~110 (88.0)	578	17.3±0.2	445
J.W69, Nos. 7~9	J·SN-100, Nos. 1~3	77~98 (85.1)	794	17.6±0.2	685
	J·SN-150, Nos. 1~4	77~98 (86.6)	1588	16.7±0.2	1147
J.W69, No. 13	J·SN-250, Nos. 1,2	77~100 (87.9)	683	17.0±0.2	647
J.W69, Nos. 10~12	EN-150·J, Nos. 1~3	77~98 (87.6)	586	16.8±0.2	443
	EN-250·J, Nos. 1~3	77~99 (87.6)	576	16.7±0.2	323

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

added to males, the number of the latter was more than 55% of a total number of frogs in three experimental series, Nos. J♀×(EX-150♀×J♂)♂, J♀×(EX-250♀×J♂)♂ and J♀×(EN-150♀×J♂)♂, and less than 45% in three experimental series, Nos. J♀×(J♀×SN-150♂)♂, J♀×(J♀×SN-250♂)♂ and J♀×(EN-250♀×J♂)♂. In the remaining three experimental series, Nos. J♀×(J♀×SX-150♂)♂, J♀×(J♀×SX-250♂)♂ and J♀×(J♀×SN-100♂)♂, the rates of males were 50.8%, 50.4% and 46.6%.

The percentages of males including hermaphrodites stated above were those of the offspring produced by two to four male parents in each series. Accordingly, the inequality of males and females was more remarkable in the offspring of each of the male parent. Such offspring were generally feeble and many of them died within one month after metamorphosis. The sex ratio of the offspring of each male parent in each of the experimental and control series was as follows:

In each of the three control matings Nos. 1~3, the total number of males and hermaphrodites was 48.1~52.9% of all the frogs.

i) Experimental series, J♀×(J♀×SX-150♂)♂, Nos. 1~3

The total number of males and hermaphrodites in each of the three matings was 45.5~51.0% of all the frogs.

from males raised from irradiated sperm or eggs

Sex of frogs dead or killed about one month after metamorphosis					No. of frogs removed and reared	Sex of mature frogs			Sex of all frogs examined		
♀N	♀U	⚥	♂N	♂ (%)*		No. of frogs	♀	♂	Total	♀	♂ (%)*
227	1	2	224	(49.8)	100	92	44	48	546	272	274 (50.2)
323	20	17	337	(50.8)	80	57	25	32	754	368	386 (51.2)
278	13	5	291	(50.4)	100	32	17	15	619	308	311 (50.2)
171	14	21	230	(57.6)	100	26	14	12	462	199	263 (56.9)
154	5	9	277	(64.3)	100	22	11	11	467	170	297 (63.6)
354	12	11	308	(46.6)	100	69	33	36	754	399	355 (47.1)
731	32	31	353	(33.5)	100	42	21	21	1189	784	405 (34.1)
385	7	12	243	(39.4)	31	0	0	0	647	392	255 (39.4)
180	18	11	234	(55.3)	100	27	7	20	470	205	265 (56.4)
178	8	14	123	(42.4)	99	16	6	10	339	192	147 (43.4)

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

ii) Experimental series, J♀ × (J♀ × SX–250♂) ♂, Nos. 1~3

There was no distinct difference in number between males and females in two of the three matings, although 58.4% of the frogs were males in one of the matings, No. 2. In mating No. 3, only 11.8% of 34 frogs were males and hermaphrodites. The individuals produced by this mating were very feeble in the tadpole stage, and these 34 frogs were all that completed their metamorphosis.

iii) Experimental series, J♀ × (EX–150♀ × J♂) ♂, Nos. 1~3

While there was no distinct difference in number between males and females in matings Nos. 1 and 2, males were far more numerous than females in No. 3. Of 118 frogs, 74 (62.7%) and 4 (3.4%) were males and hermaphrodites, respectively.

iv) Experimental series, J♀ × (EX–250♀ × J♂) ♂, Nos. 1~3

In mating No. 2, 236 (64.0%) and 4 (1.1%) of 369 frogs were males and hermaphrodites, respectively. In the other two matings, the number of frogs was too small to determine the preponderance of the males and hermaphrodites over females with normal or underdeveloped ovaries.

v) Experimental series, J♀ × (J♀ × SN–100♂) ♂, Nos. 1~3

In each of the three matings, there was no distinct difference in number between males and females, that is, the total number of males and hermaphrodites was 45.5∼47.7% of all the frogs.

vi) Experimental series, J♀ × (J♀ × SN–150♂) ♂, Nos. 1∼4

While there were males and females of nearly the same number in two of the four matings, both sexes were extremely different from each other in number in the other two matings. In mating No. 3, 18 (6.6%) and 12 (4.4%) of 272 were males and hermaphrodites, respectively, while 242 (89.0%) were females with normal or underdeveloped ovaries. In No. 4, 121 (28.4%) and 11 (2.6%) of 426 were males and hermaphrodites, respectively.

vii) Experimental series, J♀ × (J♀ × SN–250♂) ♂, Nos. 1 and 2

There was no distinct difference in number between males and females in mating No. 1. In contrast, males were very few in No. 2, that is, there were 86 (24.9%) males and 10 (2.9%) hermaphrodites among 345 frogs.

viii) Experimental series, J♀ × (EN–150♀ × J♂) ♂, Nos. 1∼3

While 152 (48.1%) of 316 frogs were males and hermaphrodites in the mating No. 3, 78 (75.7%) of 103 were those in No. 2. The number of frogs was very small in No. 1, although 62.5% of them were males and hermaphrodites.

ix) Experimental series, J♀ × (EN–250♀ × J♂) ♂, Nos. 1∼3

There were males and females of nearly the same number among 206 frogs produced by mating No. 2, when hermaphrodites were added to males. In contrast with this, there were 28 (25.0%) males and 4 (3.6%) hermaphrodites among 112 frogs in mating No. 3. There were three males and two females among five frogs of No. 1, although the latter were very small in number.

x) Mature frogs

The sex ratio of mature frogs is presented in Table 11. In the control series, there were 48 (52.2%) males and 44 females among 92 frogs. In six of the nine experimental series, 46.2∼56.1% of the respective total number of frogs were males, while in two others, 20 (74.1%) of 27 and 10 (62.5%) of 16 frogs were males. There were no mature frogs in the remaining one experimental series. The two experimental series which showed a distinct male preponderance contained a total of 11 and 16 frogs produced by two matings, J♀ × (EN–150♀ × J♂) ♂, Nos. 1 and 2. While the 11 frogs of No. 1 consisted of six males and five females, the 16 frogs of No. 2 consisted of 14 males and two females. This ratio corresponded on the whole with the male preponderance in the juvenile frogs produced by this mating. It was noteworthy that mating No. 2 was the only one that produced many mature frogs among the matings from which remarkably unequal number of male and female juvenile frogs were produced.

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

1. Developmental capacity

In the breeding season of 1970, 35 females in eight experimental series as well as five females in the control were mated with five males collected from the field (Table 12). All these females were two years old. The females of the experimental series were those raised from spermatozoa or eggs irradiated with

TABLE 12

Testes of field-caught males used for mating experiments in 1970

Kind	Individual no.	Body length (mm)	Size of the testes		Inner structure (Type)
			Left (mm)	Right (mm)	
J.W70	1	38.0	3.0×2.5	3.0×2.5	1
	2	39.0	3.0×2.5	3.0×3.0	1
	3	40.0	3.0×2.5	3.0×2.5	1
	4	38.5	3.0×3.0	3.0×2.0	1
	5	40.0	3.5×2.5	4.0×2.5	1

TABLE 13

Eggs of female parents used for mating experiments in 1970

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs Total	Mean diameter of 100 eggs (mm)
(J·J), No. 1	1	2	43.0	914	1.72±0.02
	2	2	41.0	926	1.71±0.02
	3	2	42.5	1003	1.53±0.02
	4	2	40.0	814	1.57±0.02
	5	2	42.5	865	1.64±0.02
(J·SX–150), No. 1	1	2	42.0	615	1.68±0.02
	2	2	41.5	792	1.52±0.02
	3	2	40.0	710	1.50±0.02
	4	2	41.0	811	1.68±0.02
	5	2	42.5	693	1.64±0.02
(J·SX–250), No. 1	1	2	41.5	729	1.63±0.02
	2	2	41.0	837	1.54±0.05
	3	2	42.0	704	1.73±0.05
	4	2	41.5	118 — 61	1.3 ~1.6
				57	2.5 ~3.0
	5	2	40.5	134 — 40	1.01±0.05
				50	1.63±0.05
				44	3.02±0.05
(EX–150·J), No. 1	1	2	43.5	713	1.43±0.02
	2	2	41.5	725	1.51±0.02
	3	2	42.0	637	1.54±0.02
	4	2	42.0	899	1.59±0.02
	5	2	41.5	231 — 85	1.33±0.02
				55	1.60±0.02
				91	1.91±0.02
(EX–250·J), No. 1	1	2	42.0	740	1.52±0.02
	2	2	41.0	634	1.62±0.02
(J·SN–100), No. 1	1	2	40.5	514	1.67±0.02
	2	2	43.5	627 — 311	1.81±0.02
				316	1.35±0.02
	3	2	42.0	591 — 288	1.74±0.02
				303	1.38±0.02
	4	2	41.0	714	1.73±0.02
	5	2	42.0	785	1.54±0.02

Continued

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs Total	Mean diameter of 100 eggs (mm)
(J·SN–150), No. 1	1	2	41.5	964	1.55±0.02
	2	2	43.0	659	1.63±0.02
	3	2	40.0	72 — 37	1.51±0.02
				╲ 35	2.22±0.06
	4	2	40.0	810	1.53±0.02
	5	2	40.0	0	—
(EN–150·J), No. 1	1	2	39.0	875	1.63±0.02
	2	2	40.0	717	1.74±0.02
	3	2	41.0	694	1.54±0.02
	4	2	43.5	958	1.52±0.02
	5	2	41.0	673	1.63±0.02
(EN–250·J), No. 1	1	2	42.0	921	1.53±0.02
	2	2	42.5	805	1.56±0.02
	3	2	40.0	756	1.72±0.02
	4	2	42.0	625 — 310	1.57±0.02
				╲ 315	2.01±0.02
	5	2	37.5	0	—

(J·J), No. 1: Females obtained by J.W68♀, No. 1×J.W68♂, No. 1
(J·SX–150 or –250), No. 1: Females obtained by J.W68♀, No. 1×SX–150 or –250♂, No. 1
(EX–150 or –250·J), No. 1: Females obtained by EX–150 or –250♀, No. 1×J.W68♂, No. 1
(J·SN–100 or –150), No. 1: Females obtained by J.W68♀, No. 1×SN–100 or –150♂, No. 1
(EN–150 or –250·J), No. 1: Females obtained by EN–150 or –250♀, No. 1×J.W68♂, No. 1

TABLE 14

Developmental capacity of the offspring of females

Parents		No. of eggs	No. of cleaved eggs		No. of gastrulae	
Female	Male		Normal	Abnormal	Normal	Abnormal
J·J, Nos. 1∼5	J.W70, Nos. 1∼5	1106	1089 (98.5%)	0	1050 (94.9%)	39 (3.5%)
J·SX–150, Nos. 1∼5	J.W70, Nos. 1∼5	1766	1338 (75.8%)	58 (3.3%)	1170 (66.3%)	168 (9.5%)
J·SX–250, Nos. 1∼5		1253	865 (69.0%)	133 (10.6%)	324 (25.9%)	535 (42.7%)
EX–150·J, Nos. 1∼5	J.W70, Nos. 1∼5	1465	1271 (86.8%)	43 (2.9%)	1171 (79.9%)	100 (6.8%)
EX–250·J, Nos. 1,2	J.W70, Nos. 1,2	580	533 (91.9%)	16 (2.8%)	435 (75.0%)	98 (16.9%)
J·SN–100, Nos. 1∼5	J.W70, Nos. 1∼5	1619	1077 (66.5%)	41 (2.5%)	883 (54.5%)	194 (12.0%)
J·SN–150, Nos. 1∼4	J.W70, Nos. 1∼4	583	431 (73.9%)	32 (5.5%)	330 (56.6%)	101 (17.3%)
EN–150·J, Nos. 1∼5	J.W70, Nos. 1∼5	2041	1370 (67.1%)	19 (0.9%)	1175 (57.6%)	195 (9.6%)
EN–250·J, Nos. 1∼4	J.W70, Nos. 1∼4	1521	1122 (73.8%)	15 (1.0%)	916 (60.2%)	206 (13.5%)

X-rays or neutrons by fertilization with normal gametes in the breeding season of 1968. The control females were those which developed from artificially fertilized eggs at the same time as those of the experimental series. In all the experimental and control series, the same five males were used for mating with the females. The developmental capacity of the second-generation offspring in each of the eight experimental series and the control is presented in Table 14 and shown in Figs. 29, 30, 33 and 57∼59.

a. Control series, (J♀ ×J♂)♀ ×J♂, Nos. 1∼5

After pituitary transplantation normal ovulation occurred in all the five females; each one produced about 900 normal eggs. The eggs of each of the five females Nos. 1∼5 were uniform in size, that is, 1.7, 1.7, 1.5, 1.6 and 1.6 mm in diameter, respectively (Table 13). The five females were mated with five males. The mean rate of normal cleavages and the developmental capacity of normally cleaved eggs are presented in Table 14 and shown in Fig. 57. The survival curve of the second-generation offspring produced from each of the five females is shown in Fig. 29.

The proportions of normal cleavages in five matings were very high, being 96.6∼100%, 98.5% on the average. While a small number of normally cleaved eggs died of various abnormalities, 84.3∼94.5%, 90.1% on the average, of the respective total number of eggs hatched normally (Fig. 29). Some tadpoles gradually died of ill-development or edema. A few tadpoles died suddenly in spite of their normal appearance. Eventually, 66.0∼93.4%, 79.1% on the

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		
1036	14	1015	21	997	18	926	875
(93.7%)	(1.3%)	(91.8%)	(1.9%)	(90.1%)	(1.6%)	(83.7%)	(79.1%)
1098	72	954	144	858	96	739	679
(62.2%)	(4.1%)	(54.0%)	(8.2%)	(48.6%)	(5.4%)	(41.8%)	(38.4%)
241	83	220	21	205	15	181	159
(19.2%)	(6.6%)	(17.6%)	(1.7%)	(16.4%)	(1.2%)	(14.4%)	(12.7%)
1061	110	919	142	750	169	675	624
(72.4%)	(7.5%)	(62.7%)	(9.7%)	(51.2%)	(11.5%)	(46.1%)	(42.6%)
388	47	339	49	183	156	161	157
(66.9%)	(8.1%)	(58.4%)	(8.4%)	(31.6%)	(26.9%)	(27.8%)	(27.1%)
848	35	779	69	526	253	485	434
(52.4%)	(2.2%)	(48.1%)	(4.3%)	(32.5%)	(15.6%)	(30.0%)	(26.8%)
320	10	281	39	266	15	222	191
(54.9%)	(1.7%)	(48.2%)	(6.7%)	(45.6%)	(2.6%)	(38.1%)	(32.8%)
1126	49	1081	45	877	204	802	691
(55.2%)	(2.4%)	(53.0%)	(2.2%)	(43.0%)	(10.0%)	(39.3%)	(33.9%)
847	69	710	137	358	352	274	242
(55.7%)	(4.5%)	(46.7%)	(9.0%)	(23.5%)	(23.1%)	(18.0%)	(15.9%)

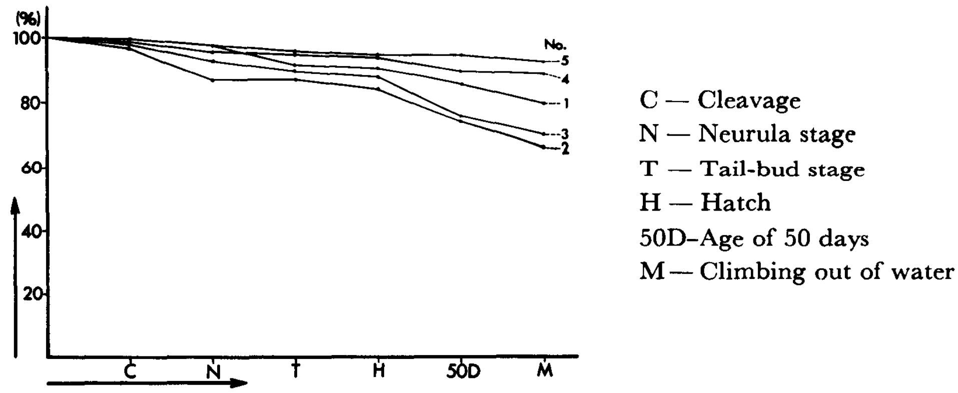


Fig. 29. Survival curves of control second-generation offspring derived from untreated grandparental gametes by matings, (J♀ ×J♂) ♀ ×J♂, Nos. 1~5.

average, of the respective total number of eggs completed their metamorphosis and became normal frogs.

b. Experimental series derived from X-irradiated grandparental sperm

i) (J♀ ×SX–150♂)♀ ×J♂, Nos. 1~5

Five female parents were raised from normal eggs fertilized with spermatozoa exposed to 150 rads of X-rays. By pituitary transplantation all these females discharged normal eggs which were quite similar in size and number to those of the control females, that is, 1.5~1.7 mm in diameter and about 700 in number (Table 13).

The offspring of the five females derived from the X-irradiated spermatozoa were produced by mating with the same five males as those used in the control series. The mean rate of normal cleavages and the developmental capacity of normally cleaved eggs are presented in Table 14. The survival curve of the second-generation offspring produced from each of the five females is shown in Fig. 30a. Normal cleavages in each of five matings occurred in 36.0~97.2%, 75.8% on the average, of the respective total number of eggs. In contrast with the control series, there was a great diversity among the rates of normal cleavages in the five matings. Some of the normally cleaved eggs died of various types of abnormalities at various embryonic stages. These types and stages as well as the viability differed with the female parents. In matings Nos. 1 and 5, embryos died of various types of abnormalities at various stages, while most eggs in No. 2 died of incomplete invagination at the gastrula stage after revealing rough surfaces. In Nos. 3 and 4, some embryos were microcephalous and became edematous (Fig. 31b). In the five matings, 2.7~78.9%, 48.6% on the average, of the respective total number of eggs hatched normally and became swimming tadpoles. However, some tadpoles died afterwards of edema, ill-development or some other abnormalities such as those of legs. Eventually, 2.0~64.5%, 38.4% on the average, completed metamorphosis.

ii) (J♀ ×SX–250♂)♀ ×J♂, Nos. 1~5

Female parents were raised from normal eggs fertilized with spermatozoa

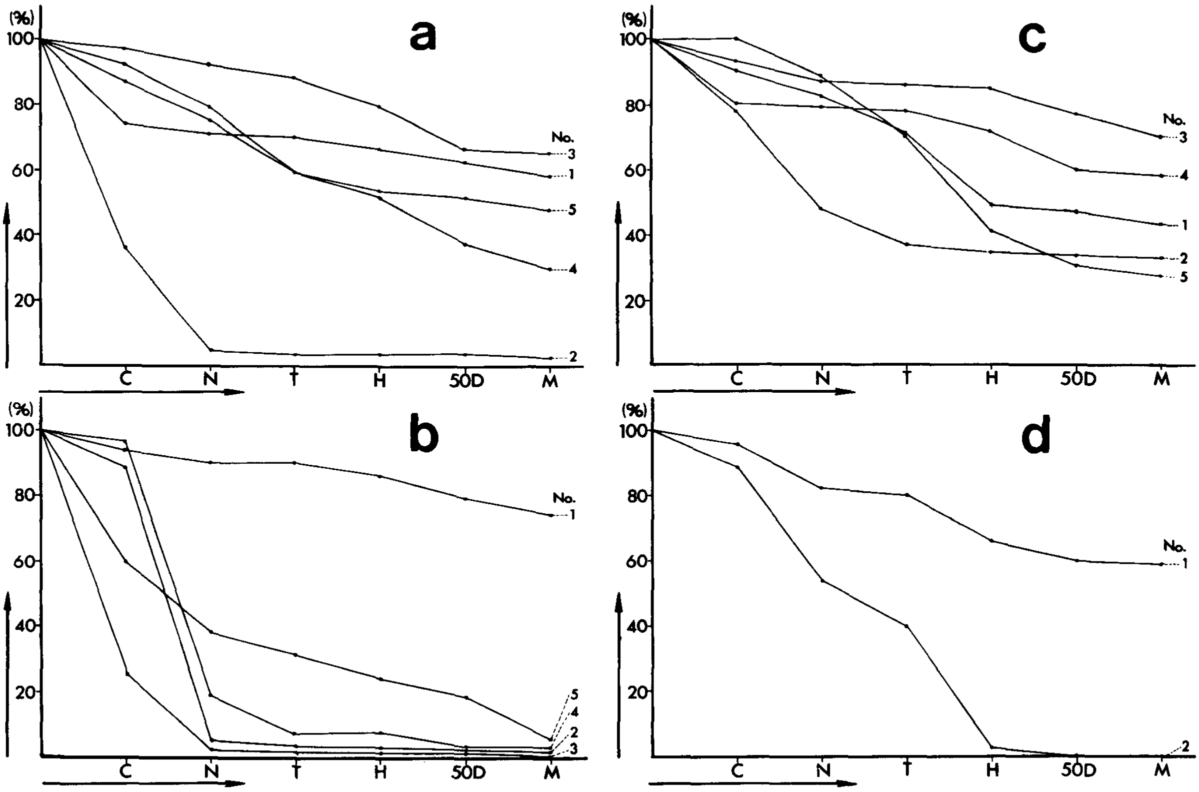


Fig. 30. Survival curves of second-generation offspring derived from X-irradiated grandparental gametes by passing over female first-generation offspring.

a. (J♀ × SX-150♂)♀ × J♂, Nos. 1∼5
b. (J♀ × SX-250♂)♀ × J♂, Nos. 1∼5
c. (EX-150♀ × J♂)♀ × J♂, Nos. 1∼5
d. (EX-250♀ × J♂)♀ × J♂, Nos. 1 and 2

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

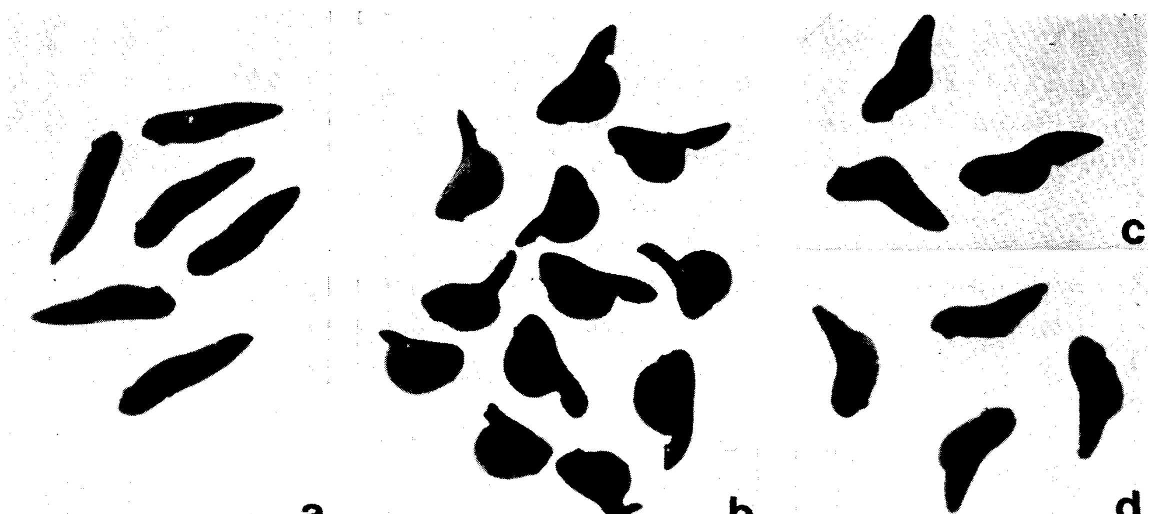


Fig. 31. Abnormalities at the late embryonic stage in the second-generation offspring derived from irradiated sperm. × 2.0

a. Control *Rana japonica* embryos at the hatching stage.
b. Abnormal (ascitic) embryos produced from a mating, (J♀ × SX-150♂)♀ × J♂, No. 3
c. Abnormal (curved) embryos produced from a mating, (J♀ × SX-250♂)♀ × J♂, No. 3
d. Abnormal (curved) embryos produced from a mating, (J♀ × SN-150♂)♀ × J♂, No. 2

which had been exposed to 250 rads of X-rays. Of five females whose ovulation was accelerated by pituitary transplantation, two laid a small number of eggs which were extremely unequal in size, while the other three laid eggs which were nearly normal in number and size. The eggs of females Nos. 1, 2 and 3 were 1.6 mm, 1.5 mm and 1.7 mm in mean diameter, respectively. Unlike these eggs, there were nearly an equal number of large and small eggs in No. 4, that is, 1.3∼1.6 mm and 2.5∼3.0 mm in diameter. In the remaining female No. 5, there were nearly an equal number of three kinds of eggs, being about 1.0 mm, 1.6 mm and 3.0 mm in diameter (Table 13). The eggs obtained from each of the two females Nos. 4 and 5 were less than one-fifth of those of the other females in number. The eggs utilized for insemination were always less than 1.6 mm in diameter, as larger eggs were apt to be injured when taken out of the cloaca.

Second-generation offspring were obtained from the five females by mating with the same five males as those of the control series (Table 14, Fig. 29). The survival curve of the second-generation offspring produced from each of the five females is shown in Fig. 30b. Normal cleavages occurred in 68.6% of the total number of eggs obtained from the five females. Mating No. 1 scarcely differed from the control in the rate of normal cleavages and the viability of embryos; 94.0% of the total number of eggs cleaved normally and 74.1% became normally metamorphosed frogs. However, some embryos produced from mating No. 1 revealed a uniform abnormality which was not found in the control embryos at the hatching stage. Such embryos had ill-developed gills, a wrinkled body, rough surfaces and a curved tail (Fig. 32a). Although 499 (88.9%) of 561 eggs cleaved normally in mating No. 2, 414 made an incomplete invagination at the gastrula stage, 42 died of edema at the neurula stage, and only 14 (2.5%) embryos hatched normally. Moreover, some of the latter became abnormal tadpoles having three or four hind legs, while some others became edematous and died. Eventually, six tadpoles, 1.1% of the total number of eggs, completed metamorphosis. Mating No. 3 was very low in fertilization rate; 99 (25.1%) of 395 eggs cleaved normally. Nearly all of them became abnormal in the embryonic (Fig. 31c) and tadpole stages and died. Incomplete invagination at the gastrula stage and malformation at the neurula stage occurred most frequently. Many embryos were bicephalous or microcephalous and could not live longer. As a result, only one tadpole could complete metamorphosis. In mating No. 4, 53 (98.1%) of 54 eggs cleaved normally. However, most of them became abnormal at the gastrula and neurula stages. The others gradually died of edema; a single tadpole eventually completed metamorphosis. In mating No. 5, 25 (59.5%) of 42 eggs cleaved normally. Only two (4.8%) attained the completely metamorphosed stage, while the others died of edema at various embryonic and tadpole stages.

c. Experimental series from X-irradiated grandparental eggs

i) (EX–150♀ ×J♂)♀ ×J♂, Nos. 1∼5

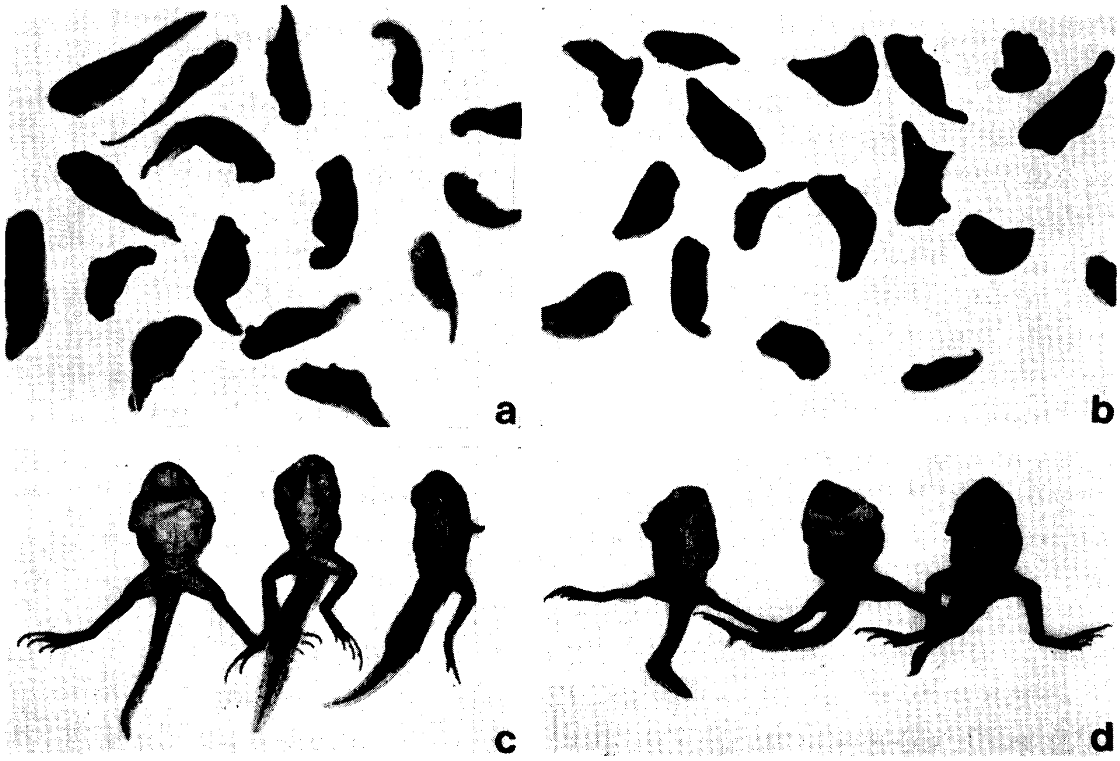


Fig. 32. Abnormalities at the hatching and metamorphosis stages in the second-generation offspring derived from irradiated sperm or oviducal eggs.

a. Extremely deformed embryos produced from a mating, (J♀ ×SX-250♂)♀ ×J♂, No. 1 ×2.0

b. Extremely deformed embryos produced from a mating, (J♀ ×SN-100♂)♀ ×J♂, No. 5 ×2.0

c. Metamorphosing frogs with ill-developed forelegs produced from a mating, (EX-150♀ ×J♂)♀ ×J♂, No. 3 ×0.8

d. Metamorphosing frogs with ill-developed forelegs produced from a mating, (EN-150♀ ×J♂)♀ ×J♂, No. 2 ×0.8

Five female parents were produced from eggs exposed to 150 rads of X-rays by insemination with sperm of a normal male. All these females ovulated normally after pituitary transplantation. One (No. 5) of them deposited three kinds of eggs, that is, large, medium and small ones. Specifically, they were about 1.9 mm, 1.6 mm and 1.3 mm in diameter, and there were 91 large, 55 medium and 85 small eggs. Each of the other females (Nos. 1, 2, 3 and 4) laid eggs which were almost uniform in size; the eggs of these four females were about 1.4 mm, 1.5 mm, 1.5 mm and 1.6 mm in diameter, respectively (Table 13).

Second-generation offspring were produced by mating these five females with the same five males as those of the control series (Table 14, Fig. 29). The survival curve of the second-generation offspring produced from each of the five females is shown in Fig. 30c. In five matings Nos. 1～5, 77.7～100%, 86.8% on the average, of the respective total number of eggs cleaved normally. In matings Nos. 1 and 5, a small number of normally cleaved eggs died of incomplete invagination at the gastrula stage and of edema at the neurula stage. More numerous individuals died of ascites during the stages from the tail-bud to the hatching, while 49.1% and 41.1% hatched normally, respectively. After some of the hatched tadpoles gradually died of edema, 42.9% and 26.8% became normally metamorphosed frogs, respectively. In mating No. 2, 35.1% of the total number of eggs hatched normally, after most of the normally cleaved eggs died of incomplete invagination at the gastrula stage and of various abnormalities, such as malformation of the neural tube, bicephaly, microcephaly, acephaly and cauda bifida at the tail-bud stage. At the metamorphosis stage, there were more than ten individuals with one or more excessive fore- or hind legs; 32.5% of the total number of eggs became normal frogs. In matings Nos. 3 and 4, many of the normally cleaved eggs died of various abnormalities, such as incomplete invagination, microcephaly, edema, ascites, ill-development of the forelegs in the embryonic and tadpole stages (Fig. 32c), while 69.5% and 57.7% of the respective total number of eggs attained completion of metamorphosis, respectively.

ii) (EX–250♀ ×J♂)♀ ×J♂, Nos. 1 and 2

Two females were raised from eggs exposed to 250 rads of X-rays by insemination with sperm of a normal male. By pituitary transplantation, ovulation occurred normally in them. The eggs of female No. 1 were 1.5 mm in diameter, while those of female No. 2 were 1.6 mm (Table 13).

Second-generation offspring were produced by mating these females with two of the five males used in the control series (Table 14, Fig. 29). The survival curve of the second-generation offspring produced from each of the two females is shown in Fig. 30d. In mating No. 1, 95.5% of the total number of eggs cleaved normally. About one-third of the normally cleaved eggs died of various abnormalities, such as incomplete invagination at the gastrula stage, malformation of the neural tube at the neurula stage, edema, bicephaly, microcephaly, acephaly or cauda bifida at the tail-bud stage and edema, microcephaly or curvature of the body at the hatching stage. A small number of the hatched tadpoles died afterwards of ill-development or edema. Eventually, 65.5% of the total number of eggs hatched normally, and 58.8% became normally metamorphosed frogs. In mating No. 2, 278 (88.8%) of 313 eggs cleaved normally. However, 69 died of incomplete invagination at the gastrula stage, and 36 and 44 died at the neurula and the tail-bud stages, respectively. At the hatching stage 117 embryos died of edema, while only eight hatched normally. Moreover, all the hatched tadpoles became edematous and died shortly after they began to feed.

d. Experimental series from neutron-irradiated grandparental sperm

i) (J♀ ×SN–100♂)♀ ×J♂, Nos. 1∼5

Female parents were raised from eggs inseminated with spermatozoa which had been exposed to 100 rads of neutrons. Ovulation was accelerated in five females by pituitary transplantation. The eggs of three females Nos. 1, 4 and 5 were 1.7 mm, 1.7 mm and 1.5 mm in diameter, respectively, while the other two females Nos. 2 and 3 deposited nearly an equal number of large and small eggs, that is, 1.8 mm and 1.4 mm, and 1.7 mm and 1.4 mm in diameter, respectively (Table 13).

Second-generation offspring were produced by mating these five females with the same five males as those of the control series (Table 14, Fig. 29). The survival curve of the second-generation offspring produced from each of the five females is shown in Fig. 33a. While more than 82% of the respective total number of eggs cleaved normally in three matings Nos. 1, 4 and 5, 43.4% in No. 2 and only 4.6% in No. 3 did so. Normal cleavages occurred, on the average, in 66.5% of the eggs obtained from the five females. Most of the normally cleaved eggs in mating No. 1 died of edema at the neurula and tail-bud stages; only 12.9% of the total number of eggs hatched normally, and 9.4% attained the completion of metamorphosis. All the normally cleaved eggs in mating No. 2 became abnormal at the gastrula stage and did not develop further. In No. 3, a single egg became a normal, metamorphosed frog, while the others which were only 13 in number became abnormal at various developmental stages and died. In No. 4, 99.4% of the total number of eggs cleaved normally, and

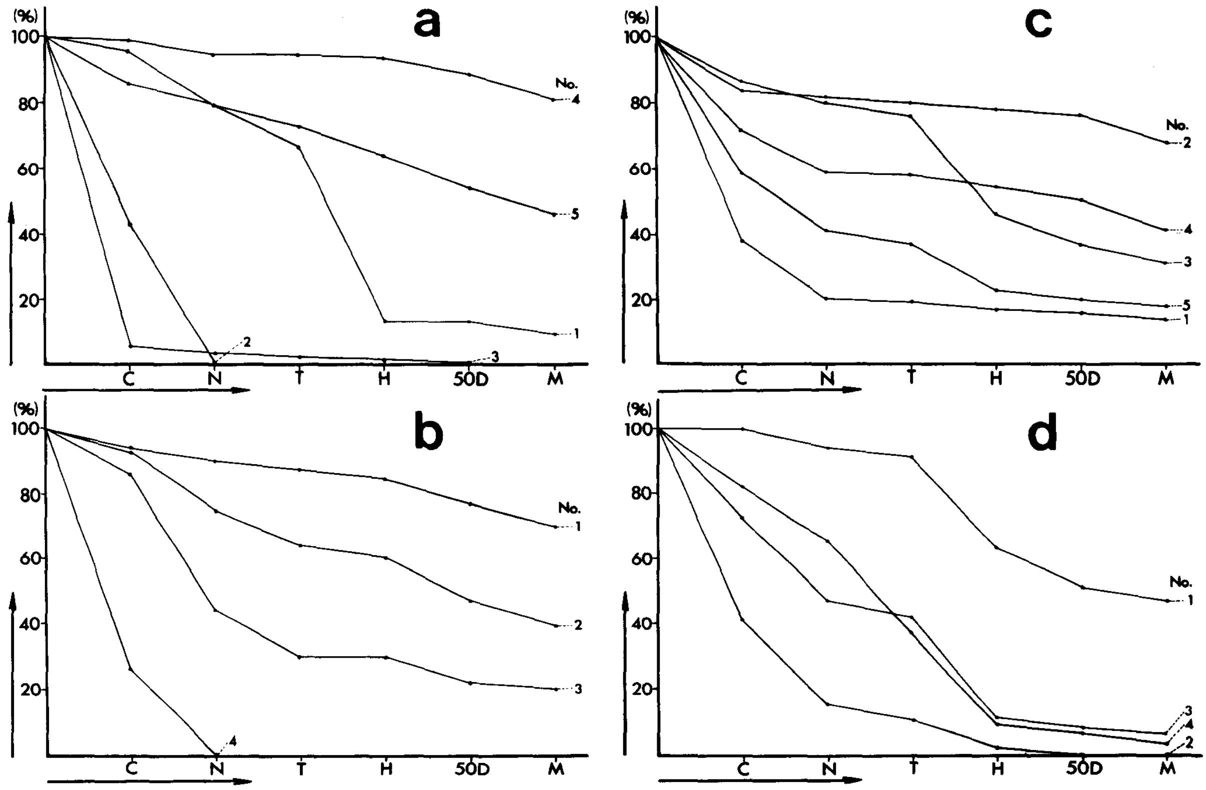


Fig. 33. Survival curves of second-generation offspring derived from neutron-irradiated grand-parental gametes by passing over female first-generation offspring.

a. (J♀ × SN–100♂)♀ × J♂, Nos. 1∼5 C—Cleavage N—Neurula stage
b. (J♀ × SN–150♂)♀ × J♂, Nos. 1∼4 T—Tail-bud stage H—Hatch
c. (EN–150♀ × J♂)♀ × J♂, Nos. 1∼5 50D—Age of 50 days M—Climbing out of water
d. (EN–250♀ × J♂)♀ × J♂, Nos. 1∼4

81.4% became normally metamorphosed frogs. Although 95.5% of the total number of eggs cleaved normally in mating No. 5, many of them died of various abnormalities during the stages from the tail-bud to the hatching (Fig. 32b). Eventually, 45.7% of the total number of eggs reached the completion of metamorphosis.

ii) (J♀ × SN–150♂)♀ × J♂, Nos. 1∼4

Five females were raised from eggs fertilized with spermatozoa which had been exposed to 150 rads of neutrons. They were transplanted with pituitary bodies; as a result, ovulation occurred in four of them. The eggs of three (Nos. 1, 2 and 4) of the four females were about 1.6 mm, 1.6 mm and 1.5 mm in diameter, respectively. The other female (No. 3) deposited less than 100 eggs consisting of nearly an equal number of large and small eggs which were about 2.2 mm and 1.5 mm in diameter, respectively (Table 13).

Second-generation offspring were produced by mating these four females with four of the five males used in the control series (Table 14). The survival curve of the second-generation offspring produced from each of the four females is shown in Fig. 33b. As shown in this figure, there were large differences among four survival curves, while the second-generation offspring produced by mating No. 1 scarcely differed in viability from those of the control matings. In mating No. 1, 93.9% of the total number of eggs cleaved normally and 70.4% attained completion of metamorphosis. Although 92.6% cleaved normally in mating No. 2, many of them became abnormal in the embryonic stage. Various abnormalities such as incomplete invagination at the gastrula stage, malformation at the neurula stage, shrinkage of the body or tail, bicephaly, microcephaly, acephaly, curvature of the body, ascites and some other abnormalities at the tail-bud stage, and ill-development, rough surfaces and curvature of the body at the hatching stage (Fig. 31d) occurred frequently, and then 59.7% hatched normally. Many of the hatched tadpoles died of ill-development of the body or tail, and eventually 38.8% attained the completion of metamorphosis. In mating No. 3, 86.0% of the total number of eggs cleaved normally. However, most of the large eggs died of incomplete invagination at the gastrula stage and of shrinkage of the body during the stages from the neurula to the tail-bud. A small number of small eggs also died of edema and curvature of the body. As a result, 20.0% became normally metamorphosed frogs. In spite of the existence of many eggs making an abnormal cleavage, 25.6% of the total number of eggs cleaved normally in mating No. 4. However, all the normally cleaved eggs performed incomplete invagination at the gastrula stage and died after becoming abnormal embryos with rough surfaces.

e. Experimental series derived from neutron-irradiated grandparental eggs

i) (EN–150♀ × J♂)♀ × J♂, Nos. 1∼5

Five females were raised from eggs exposed to 150 rads of neutrons by insemination with sperm of a normal male. By pituitary transplantation these females (Nos. 1∼5) laid normally their eggs, which were 1.6 mm, 1.7 mm, 1.5 mm, 1.5 mm and 1.6 mm in diameter, respectively (Table 13).

Second-generation offspring were produced from the five females by mating with the same five males as those of the control series (Table 14). The survival curve of the second-generation offspring produced from each of the five females is shown in Fig. 33c. In five matings Nos. 1~5, 36.6%, 84.0%, 87.2%, 72.3% and 59.3% of the respective total number of eggs cleaved normally, respectively. The normally cleaved eggs in mating No. 2 were nearly the same in developmental capacity as those in the control series, although there were about 20 metamorphosing tadpoles with ill-developed forelegs (Fig. 32d). The number of normally metamorphosed frogs was 269, being 68.3% of the total number of eggs. In matings Nos. 3 and 4, 46.2% and 53.6% of a total number of eggs hatched normally, respectively, while the remainders of the normally cleaved eggs died of various abnormalities, especially of edema at various embryonic stages. Many of the hatched tadpoles in the two matings became edematous and died by the metamorphosis stage; 30.5% and 40.8% eventually became normal, metamorphosed frogs, respectively. The normally cleaved eggs obtained by matings Nos. 1 and 5 mostly died of incomplete invagination, edema, curvature of the body, bicephaly, microcephaly or some other abnormalities in the embryonic stage, while 17.3% and 22.7% hatched normally, respectively. A small number of the hatched tadpoles also died of ill-development or edema; as a result, 14.3% and 17.8% reached the completion of metamorphosis, respectively.

ii) (EN-250♀ ×J♂)♀ ×J♂, Nos. 1~4

Five female parents were raised from eggs exposed to 250 rads of neutrons by insemination with sperm of a normal male. After pituitary transplantation, three of the five females laid eggs which were about 1.5 mm, 1.6 mm and 1.7 mm in diameter, respectively. Another female deposited nearly an equal number of large and small eggs, which were about 2.0 mm and 1.6 mm in diameter, respectively. The remainder did not ovulate (Table 13).

Second-generation offspring were produced by mating the former four females with four of the five males used in the control series. The mean rate of normal cleavages and the viability of embryos and tadpoles are presented in Table 14 and shown in Fig. 59. The survival curve of the second-generation offspring produced from each of the four females is shown in Fig. 33d.

Normal cleavages occurred in 99.8%, 40.5%, 73.2% and 82.2% of the respective total number of eggs in four matings Nos. 1~4. In mating No. 1, only a few offspring died of edema at the gastrula, neurula and tail-bud stages, while about one-third of embryos died of edema suddenly and simultaneously at the hatching stage. As a result, 288 embryos, 63.0% of the total number of eggs, hatched normally and became swimming tadpoles. In the tadpole stage, a small number of individuals became edematous and died, and eventually 213 tadpoles, 46.6% of the total number of eggs, completed metamorphosis. In mating No. 2, the rate of normal cleavages was very low. At the gastrula stage, nearly half the number of normally cleaved eggs became rough on their surfaces and died of incomplete invagination. Most of the remainders also died of edema or some other abnormalities at the neurula and tail-bud stages. Although only nine embryos eventually hatched normally, all of them died of ill-development, without taking food. In matings Nos. 3 and 4, about 80% of the respective total number of eggs cleaved normally. However, most of the normally cleaved eggs gradually became edematous and died in the embryonic stage, while 10.5% and 8.7% of the respective total number of eggs hatched normally and became swimming tadpoles. Many of the latter died of edema in the tadpole stage, and then only 6.2% and 2.9% became normal, metamorphosed frogs.

2. Chromosome aberrations in normally shaped tadpoles

Chromosomes were observed in normally shaped 50-day-old tadpoles (V~X stages) produced from eight matings of the experimental series and one mating of the control series (Table 15). These matings were selected as those which produced approximately the most numerous healthy tadpoles among those of each experimental series or the control matings.

TABLE 15

Chromosomal analysis of normally shaped 50-day-old tadpoles produced from females 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	
J·J, No. 2	J.W70, No. 2	45	45 (100 %)	0	0	0
J·SX–150, No. 3	J.W70, No. 3	32	22 (68.8%)	3 (9.4%)	3 (9.4%)	4 (12.5%)
J·SX–250, No. 1	J.W70, No. 1	30	20 (66.7%)	4 (13.3%)	2 (6.7%)	4 (13.3%)
EX–150·J, No. 3	J.W70, No. 3	27	18 (66.7%)	4 (14.8%)	2 (7.4%)	3 (11.1%)
EX–250·J, No. 1	J.W70, No. 1	31	22 (71.0%)	5 (16.1%)	2 (6.5%)	2 (6.5%)
J·SN–100, No. 4	J.W70, No. 4	27	19 (70.4%)	3 (11.1%)	2 (7.4%)	3 (11.1%)
J·SN–150, No. 2	J.W70, No. 2	30	21 (70.0%)	5 (16.7%)	1 (3.3%)	3 (10.0%)
EN–150·J, No. 2	J.W70, No. 2	30	20 (66.7%)	6 (20.0%)	1 (3.3%)	3 (10.0%)
EN–250·J, No. 1	J.W70, No. 1	26	17 (65.4%)	3 (11.5%)	3 (11.5%)	3 (11.5%)

a. Control series, (J♀ ×J♂)♀ ×J♂, No. 2

Squash preparations were made from the tail tips of 50 normally shaped tadpoles produced from one (No. 2) of the five matings of the control series. Of these tadpoles, 45 were analyzable in karyotype, as they had more than ten good metaphase spreads.

All the 45 tadpoles were pure diploids, having no other than normally diploid mitoses.

b. Experimental series derived from X-irradiated grandparental sperm

i) (J♀ ×SX–150♂)♀ ×J♂, No. 3

From 40 normally shaped tadpoles produced from one (No. 3) of the five matings between five females raised from normal eggs fertilized with spermatozoa which had been exposed to 150 rads of X-rays and normal males, the tail-tips were removed, squashed and stained. As a result, 32 tadpoles were analyzable in karyotype. Of these tadpoles, 22 were normal diploids. Three tadpoles had no normally diploid mitoses, although the metaphase spreads of each of them were the same in karyotype; one was a triploid, another was a hyperdiploid (2n+1) and the remainder was an abnormal diploid in which a segment of one of the No. 2 chromosomes was translocated to one of the No. 5 chromosomes. Three other tadpoles consisted of a mixture of two kinds of abnormal cells; one was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) cells, and the other two were mosaics of two kinds of abnormally diploid cells, which contained a chromosome with a deletion and another with a translocation. The remaining four tadpoles were mosaics of normally diploid and abnormal cells; one contained triploid cells, another contained hyperdiploid (2n+1) cells, still another contained abnormally diploid cells having a ring chromosome, and the remainder contained abnormally diploid cells having a deletion in the short arm of one of the No. 4 chromosomes, in addition to normally diploid cells.

ii) (J♀×SX-250♂)♀×J♂, No. 1

Thirty of 40 normally shaped tadpoles produced from one (No. 1) of the five matings between five females raised from normal eggs fertilized with spermatozoa which had been exposed to 250 rads of X-rays and normal males, were analyzable in karyotype. Twenty tadpoles were normal diploids. Four tadpoles consisted solely of abnormal cells, although the metaphase spreads of each of them were the same in karyotype; one was a triploid, another was a hyperdiploid (2n+1), still another was an abnormal diploid having a ring chromosome, and the remainder was an abnormal diploid having a translocation in one of the No. 5 chromosomes. Two tadpoles were mosaics of abnormal cells; one consisted of a mixture of abnormally diploid cells having a chromosome with a deletion and those having a ring chromosome, and the other consisted of a mixture of abnormally diploid cells having a chromosome with a translocation and hyperdiploid (2n+1) cells having an additional small chromosome. The remaining four tadpoles were mosaics of normally diploid and abnormal cells; one contained triploid cells, another contained hyperdiploid (2n+1) and hypodiploid (2n−1) cells, and the other two contained abnormally diploid cells having a chromosome with a deletion or a translocation, in addition to normally diploid cells.

c. Experimental series derived from X-irradiated grandparental eggs

i) (EX-150♀×J♂)♀×J♂, No. 3

Squash preparations were made from the tail-tips of 36 normally shaped tadpoles produced from one (No. 3) of the five matings between five females raised from eggs which had been exposed to 150 rads of X-rays and normal males. Of these tadpoles, 27 were analyzable in karyotype. Eighteen tadpoles were normal diploids. Four tadpoles had solely abnormal cells, although the metaphase spreads of each of them were of the same karyotype; one was a triploid, another was a hyperdiploid (2n+1), still another was an abnormal diploid having a chromosome with a deletion and another chromosome with a translocation, and the remainder was an abnormal diploid having a chromosome with a deletion. Two other tadpoles were mosaics of two kinds of abnormal cells; one consisted of a mixture of hyperdiploid (2n+1) and hypodiploid (2n−1) cells and the other consisted of a mixture of hyperdiploid (2n+1) cells and abnormally diploid ones having a chromosome with a deletion. The remaining three tadpoles were mosaics of normally diploid and abnormal cells; one contained triploid cells, and the other two contained abnormally diploid cells which have a chromosome with a deletion and another chromosome with a translocation, in addition to normally diploid cells.

ii) (EX-250♀×J♂)♀×J♂, No. 1

Thirty-one of 36 normally shaped tadpoles produced from one (No. 1) of the two matings between two females raised from eggs which had been exposed to 250 rads of X-rays and normal males were analyzable in karyotype. Twenty-two of these tadpoles were normal diploids, while the others were not normal. Five tadpoles had solely abnormal cells, although the metaphase spreads of each of them were of the same karyotype; one was a triploid, two others were hyperdiploids (2n+1), still another was an abnormal diploid having a chromosome with a translocation, and the remainder was an abnormal diploid having a ring chromosome. Two other tadpoles were mosaics of two kinds of abnormal cells; one consisted of a mixture of hypertriploid (3n+1) and hypotriploid (3n−1) cells, and the other consisted of abnormally diploid cells having a dicentric and a fragment chromosome and those having a chromosome with a deletion. The remaining two tadpoles were mosaics of normally diploid and abnormal cells; one contained abnormally diploid cells having a chromosome with a deletion and another chromosome with a translocation, and the other contained hypodiploid (2n−1) cells losing a small chromosome, in addition to normally diploid cells.

d. Experimental series derived from neutron-irradiated grandparental sperm

i) (J♀×SN-100♂)♀×J♂, No. 4

Preparations were made from the tail-tips of 36 normally shaped tadpoles produced from one (No. 4) of the five matings between five females raised from normal eggs fertilized with spermatozoa which had been exposed to 100 rads of neutrons and normal males. Twenty-seven of these tadpoles were analyzable in karyotype. While 19 tadpoles were normal diploids, the others were not. Three tadpoles had solely abnormal cells, although the metaphase spreads of each of them had the same karyotype; one was a hyperdiploid (2n+1), another was an abnormal diploid, having a chromosome with a translocation, and the remainder was an abnormal diploid having a chromosome with a deletion and another chromosome with a translocation. Two tadpoles were mosaics of two kinds of abnormal cells; one consisted of a mixture of abnormally diploid cells having a chromosome with a deletion and hypodiploid (2n−1) cells with a dicentric chromosome, and the other consisted of a mixture of abnormally diploid cells which varied in the site of a deletion or translocation. The remaining three tadpoles were mosaics of normally diploid and abnormal cells; one contained triploid cells, another contained hyperdiploid (2n+1) and hypodiploid (2n−1) cells and the other contained hypodiploid (2n−1) cells having a dicentric chromosomes, in addition to normally diploid cells.

ii) (J♀×SN-150♂)♀×J♂, No. 2

Thirty of 40 normally shaped tadpoles produced by one (No. 2) of the four matings between four females raised from normal eggs fertilized with spermatozoa which had been exposed to 150 rads of neutrons and normal males, were analyzable in karyotype. Twenty-one tadpoles were normal diploids. Five others had solely abnormal cells, although the metaphase spreads of each of them were the same in karyotype; one was a triploid, one was a hypertriploid (3n+1), one was a hyperdiploid (2n+1), one was an abnormal diploid, having a ring chromosome, and the remainder was an abnormal diploid in which a segment of a chromosome was translocated to another chromosome. Another tadpole was a mosaic of abnormally diploid cells having a chromosome with a deletion and hypodiploid (2n−1) cells having a dicentric chromosome. The remaining three tadpoles were mosaics of normally diploid and abnormal cells; one contained abnormally diploid cells having a ring chromosome, another contained those having a chromosome with a deletion and the other contained those having a chromosome with a translocation, in addition to normally diploid cells.

e. Experimental series derived from neutron-irradiated grandparental eggs

i) (EN-150♀×J♂)♀×J♂, No. 2

Squash preparations were made from 40 normally shaped tadpoles produced from one (No. 2) of the five matings between five females raised from eggs exposed to 150 rads of neutrons and normal males. Of these tadpoles, 30 were analyzable in karyotype. While 20 tadpoles were normal diploids, the others were abnormal. Six tadpoles had solely abnormal mitoses, although the metaphase spreads of each of them were of the same karyotype; one was a hypertriploid (3n+1), one was a triploid, one was a hyperdiploid (2n+1), one was an abnormal diploid having a chromosome with a translocation, and the other two were abnormal diploids having a chromosome with a deletion and another chromosome with a translocation. Another tadpole was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) cells. The remaining three were mosaics of normally diploid and abnormal cells; one contained hyperdiploid (2n+1) cells having a ring and a fragment chromosome, and the other two contained abnormally diploid cells having a chromosome with a deletion or a translocation, in addition to normally diploid cells.

ii) (EN-250♀×J♂)♀×J♂, No. 1

Twenty-six of 36 normally shaped tadpoles produced by one (No. 1) of the four matings between four females raised from eggs which had been exposed to 250 rads of neutrons and normal males were analyzable in karyotype. While

17 of them were normal diploids, the other nine were abnormal. Three tadpoles had solely abnormal cells, although the metaphase spreads of each of them were of the same karyotype; one was a hypertriploid (3n+1), another was a hyperdiploid (2n+1) and the remainder was an abnormal diploid in which a segment of the long arm of one of the No. 4 chromosomes was translocated to the long arm of one of the No. 5 chromosomes. Three other tadpoles were mosaics of two kinds of abnormal cells; one consisted of a mixture of hypertriploid (3n+1) and hypotriploid (3n−1) cells, another consisted of a mixture of abnormally diploid cells having a ring chromosome and those having a chromosome with a deletion and a chromosome with a translocation, and the other consisted of a mixture of abnormally diploid cells having a chromosome with a deletion and those having a chromosome with a translocation. The remaining three tadpoles were mosaics of normally diploid and abnormal cells; one contained abnormally diploid cells having a dicentric and a fragment chromosome, another contained abnormally diploid cells having a chromosome with a deletion, and the other contained abnormally diploid cells having a chromosome with a deletion and another chromosome with a translocation, in addition to normally diploid cells.

3. Viability and sex of metamorphosed frogs

a. Viability

The period of time from fertilization to metamorphosis and the body length

TABLE 16

Number, size and sex of metamorphosed frogs produced

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed frogs	Mean body length of 100 frogs immediately after metamorphosis (mm)	
Female	Male				No. of frogs
J·J, Nos. 1∼5	J.W70, Nos. 1∼5	74∼120 (94.4)	875	18.2±0.2	624
J·SX–150, Nos. 1∼5	J.W70, Nos. 1∼5	74∼122 (91.7)	679	17.6±0.2	372
J·SX–250, Nos. 1∼5		80∼113 (96.8)	159	18.0±0.2	68
EX–150·J, Nos. 1∼5	J.W70, Nos. 1∼5	74∼118 (94.1)	624	17.7±0.2	450
EX–250·J, Nos. 1,2	J.W70, Nos. 1,2	70∼109 (88.4)	157	17.8±0.2	64
J·SN–100, Nos. 1∼5	J.W70, Nos. 1∼5	74∼111 (90.8)	434	18.1±0.2	296
J·SN–150, Nos. 1∼4	J.W70, Nos. 1∼4	74∼104 (91.8)	191	18.2±0.2	141
EN–150·J, Nos. 1∼5	J.W70, Nos. 1∼5	83∼118 (94.3)	691	17.6±0.2	400
EN–250·J, Nos. 1∼4	J.W70, Nos. 1∼4	83∼116 (98.7)	242	17.9±0.2	129

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

of the individuals of the experimental series and the controls immediately after metamorphosis are presented in Table 16. There were no distinct differences in these respects between the individuals of the experimental series and the controls. There were also no distinct differences between the individuals of the series from X-irradiated sperm or eggs and those from neutron-irradiated sperm or eggs, or between the individuals from sperm or eggs exposed to different dosages of X-rays or neutrons.

About one month after metamorphosis, 50 frogs were removed from each group of offspring in which the karyotype analysis of normally shaped tadpoles had been performed and continuously reared until the next year in order to make them sexually mature. In a series, (J♀ × SX–150♂)♀ × J♂, No. 3, however, 100 frogs were exceptionally removed and reared.

There were 39 mature frogs in the control series. In contrast, there were 8～22 mature frogs in seven experimental series, and 65 in the exceptional series where 100 young frogs had been removed. Mature frogs were usually fewer in the series derived from gametes exposed to higher dosages of irradiation.

b. Sex

The sex of immature frogs killed within one month after metamorphosis is presented in Table 16. In the control series, there were 310 females and 314 males. Although a single female had underdeveloped ovaries, there were

from females raised from irradiated sperm or eggs

Sex of frogs dead or killed about one month after metamorphosis					No. of frogs removed and reared	Sex of mature frogs			Sex of all frogs examined		
♀N	♀U	⚥	♂N	♂ (%)*		No. of frogs	♀	♂	Total	♀	♂ (%)*
309	1	0	314	(50.3)	50	39	20	19	663	330	333 (50.2)
185	3	4	180	(49.5)	100	65	13	52	437	201	236 (54.0)
31	1	2	34	(52.9)	50	15	3	12	83	35	48 (57.8)
225	7	2	216	(48.4)	50	14	8	6	464	240	224 (48.3)
30	2	0	32	(50.0)	50	10	5	5	74	37	37 (50.0)
132	2	3	159	(54.7)	50	22	7	15	318	141	177 (55.7)
41	3	4	93	(68.8)	50	8	1	7	149	45	104 (69.8)
165	9	11	215	(56.5)	50	14	7	7	414	181	233 (56.3)
61	3	1	64	(50.4)	50	13	7	6	142	71	71 (50.0)

⚥—Hermaphrodites ♂N—Males with normal testes * Including hermaphrodites no hermaphrodites. In each of the five control matings, there was also no distinct difference in number between males and females.

While there was no distinct difference in number between males and females in six of the eight experimental series, as in the control, there was a male preponderance in the other two series. In a series, (J♀×SN–150♂)♀×J♂, there were 44 females and 97 males, if hermaphrodites were counted as males. This male preponderance was attributable to that among the second-generation offspring produced from two (Nos. 1 and 2) of the four matings of this series. In another series, (EN–150♀×J♂)♀×J♂, there were 174 females and 226 males, and this male preponderance was attributable to that among the second-generation offspring produced from one (No. 1) of the five matings of this experimental series, that is, there were 11 females and 50 males. Among the second-generation offspring produced from other than the above three matings of the two experimental series, there was no distinct difference in number between males and females.

It was also remarkable that there were a few juvenile hermaphrodites in seven of the eight experimental series. While there was no hermaphrodite among 624 control frogs, there were 1～11 hermaphrodites among 68～450 frogs in each of the experimental series. In the experimental series which had no hermaphrodite, the number of frogs was very small, being only 64.

Concerning the sex ratio of mature frogs, there was a distinct difference between the experimental series from irradiated sperm and those from irradiated eggs as well as the control series. There were 20 females and 19 males among the control frogs, and also nearly the same number of males and females in the four experimental series from X- or neutron-irradiated eggs. In contrast with this, there was a distinct male preponderance among mature frogs in each of the four experimental series from X- or neutron-irradiated sperm (Table 16).

Of the mature male second-generation offspring derived from X- or neutron-irradiated sperm, many had somewhat abnormal testes. In some of them, there was an extreme difference in size between the right and left testes. Some other males had no or ill-developed testis on one side, while there was a normal or extraordinarily large one on the other side. In still some others, the testes were lobular in shape and appeared to have transformed from ovaries by sex-reversal. It was evident that the deficiency or ill-development of a testis always occurred on the right side.

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

1. Developmental capacity

Third-generation offspring derived from X- or neutron-irradiated sperm or eggs were produced in the breeding season of 1970 by making use of male second-generation offspring. The second-generation offspring produced in 1969 matured in the next year (Table 17). They were divided into eight series in

TABLE 17

Testes of male parents used for mating experiments in 1970

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure (Type)
				Left (mm)	Right (mm)	
J(J·J), No. 2	1	1	30.5	2.0×1.5	2.0×1.5	1
	2	1	31.0	2.5×1.5	2.5×1.5	1
	3	1	34.5	3.0×2.0	3.0×2.0	1
J(J·SX–150), No. 3	1	1	31.0	2.0×1.0	2.0×2.0	1
	2	1	32.0	3.0×2.0	2.5×2.0	1
	3	1	38.0	4.0×2.0	4.0×2.0	1
	4	1	30.0	2.5×2.0	2.5×2.0	1
	5	1	28.5	2.0×1.5	2.0×1.5	2
J(J·SX–250), No. 1	1	1	30.0	2.0×1.0	2.0×1.0	1
	2	1	33.0	3.5×2.5	3.5×2.5	1
	3	1	28.0	2.5×2.0	2.5×2.0	1
	4	1	28.5	2.0×2.0	2.0×2.0	1
J(EX–150·J), No. 1	1	1	37.5	1.0×0.5	3.5×2.5	1
	2	1	34.5	2.5×1.5	2.5×1.5	1
	3	1	35.5	3.0×2.5	4.5×2.5	1
	4	1	35.0	3.0×2.0	3.0×1.5	1
J(EX–250·J), No. 2	1	1	30.0	1.5×1.5	2.0×1.5	3
	2	1	33.0	2.5×2.0	2.5×2.0	1
	3	1	33.5	3.0×2.0	3.0×2.0	1
	4	1	34.5	2.5×2.0	2.5×2.0	1
J(J·SN–100), No. 2	1	1	32.0	2.0×1.5	2.0×1.5	1
	2	1	33.5	3.5×2.5	3.5×2.5	1
	3	1	36.0	3.0×2.5	3.0×2.5	1
	4	1	36.5	4.0×3.5	0.3×0.2	1
J(J·SN–150), No. 3	1	1	33.0	1.5×1.0	1.5×1.0	1
	2	1	34.0	2.0×1.5	2.0×1.5	1
	3	1	33.0	2.5×2.0	2.5×2.0	1
	4	1	32.0	2.0×1.5	2.5×2.0	1
J(EN–150·J), No. 3	1	1	33.0	1.5×1.0	2.5×2.0	1
	2	1	33.5	2.5×2.0	3.0×2.5	2
	3	1	32.0	1.5×1.0	1.5×1.0	1
	4	1	32.0	2.0×1.0	3.0×1.0	1
J(EN–250·J), No. 3	1	1	31.0	2.0×1.5	2.0×1.5	1
	2	1	33.5	2.5×2.0	2.5×2.0	1
	3	1	34.5	2.5×2.5	3.0×2.5	3

J(J·J), No. 2: Males obtained by J.W69♀, Nos. 1∼12×(J·J)♂, No. 2

J(J·SX–150 or –250), No. 3 or 1: Males obtained by J.W69♀, Nos. 1∼3×(J·SX–150 or –250)♂, No. 3 or 1

J(EX–150 or –250·J), No. 1 or 2: Males obtained by J.W69♀, Nos. 4∼6×(EX–150 or –250·J)♂, No. 1 or 2

J(J·SN–100 or –150), No. 2 or 3: Males obtained by J.W69♀, Nos. 7∼10×(J·SN–100 or –150)♂, No. 2 or 3

J(EN–150 or –250·J), No. 3: Males obtained by J.W69♀, Nos. 11∼13×(EN–150 or –250·J)♂, No. 3

accordance with their origin, and mated with normal females collected from the field (Table 18). In each series, three to five male second-generation offspring

TABLE 18

Eggs of field-caught female frogs used for mating experiments in 1970

Kind	Individual no.	Body length (mm)	No. of eggs	Mean diameter of 50 eggs (mm)
J.W70	1	42.5	1213	1.50±0.01
	2	42.0	1115	1.51±0.01
	3	43.0	979	1.52±0.01
	4	45.0	1047	1.42±0.01
	5	43.5	1253	1.55±0.01
	6	41.0	1184	1.59±0.01
	7	45.0	1270	1.49±0.01
	8	44.0	1132	1.58±0.01
	9	43.0	985	1.63±0.01
	10	42.5	1057	1.67±0.01
	11	41.0	1079	1.54±0.01
	12	40.0	991	1.50±0.01

were mated with each of three females by artificial fertilization. The developmental capacity of the third-generation offspring as well as the reproductive ability of the male second-generation offspring in each of the experimental series and the control is presented in Table 19 and shown in Figs. 34∼36 and 57∼59.

a. Control series, J♀×{J♀×(J♀×J♂)♂}♂, Nos. 1∼3

Three mature males were mated with each of 12 females collected from the

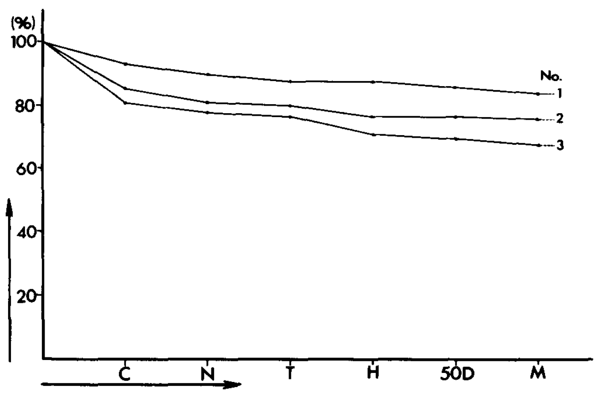
TABLE 19

Developmental capacity of the offspring of males derived

Parents		No. of eggs	No. of cleaved eggs		No. of gastrulae	
Female	Male		Normal	Abnormal	Normal	Abnormal
J.W70, Nos. 1∼12	J(J·J), Nos. 1∼3	399	347 (87.0%)	0	340 (85.2%)	7 (1.8%)
J.W70, Nos. 1∼3	J(J·SX–150), Nos. 1∼5	664	555 (83.6%)	18 (2.7%)	519 (78.2%)	36 (5.4%)
	J(J·SX–250), Nos. 1∼4	581	533 (91.7%)	8 (1.4%)	498 (85.7%)	35 (6.0%)
J.W70, Nos. 4∼6	J(EX–150·J), Nos. 1∼4	553	482 (87.2%)	0	452 (81.7%)	30 (5.4%)
	J(EX–250·J), Nos. 1∼4	532	387 (72.7%)	3 (0.6%)	380 (71.4%)	7 (1.3%)
J.W70, Nos. 7∼9	J(J·SN–100), Nos. 1∼4	561	507 (90.4%)	0	483 (86.1%)	24 (4.3%)
	J(J·SN–150), Nos. 1∼4	555	475 (85.6%)	0	468 (84.3%)	7 (1.3%)
J.W70, Nos. 10∼12	J(EN–150·J), Nos. 1∼4	535	438 (81.9%)	5 (0.9%)	429 (80.2%)	9 (1.7%)
	J(EN–250·J), Nos. 1∼3	428	329 (76.9%)	0	326 (76.2%)	3 (0.7%)

field. These males were second-generation offspring produced from a male offspring between a male and a female collected from the field, by mating with a female collected from the field. In other words, the male first- and second-generation offspring were frogs raised in our laboratory as controls, while all the females were those collected from the field (Table 19). The survival curve of the third-generation offspring produced by each of the three males is shown in Fig. 34.

By three matings with these three males, 93.3%, 85.0% and 80.6%, 87.0% on the average, of the respective total number of eggs cleaved normally. However, as a small number of normally cleaved eggs died one after another of ascites,



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

Fig. 34. Survival curves of control third-generation offspring derived from untreated great-grandparental gametes by matings, J♀ × {J♀ × (J♀ × J♂) ♂} ♂, Nos. 1∼3.

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		
335	5	328	7	317	11	312	306
(84.0%)	(1.3%)	(82.2%)	(1.8%)	(79.4%)	(2.8%)	(78.2%)	(76.7%)
512	7	501	11	444	57	429	417
(77.1%)	(1.1%)	(75.5%)	(1.7%)	(66.9%)	(8.6%)	(64.6%)	(62.8%)
474	24	461	13	409	52	336	315
(81.6%)	(4.1%)	(79.3%)	(2.2%)	(70.4%)	(9.0%)	(57.8%)	(54.2%)
439	13	410	29	344	66	319	307
(79.4%)	(2.4%)	(74.1%)	(5.2%)	(62.2%)	(11.9%)	(57.7%)	(55.5%)
365	15	357	8	322	35	301	283
(68.6%)	(2.8%)	(67.1%)	(1.5%)	(60.5%)	(6.6%)	(56.6%)	(53.2%)
446	37	427	19	384	43	360	347
(79.5%)	(6.6%)	(76.1%)	(3.4%)	(68.4%)	(7.7%)	(64.2%)	(61.9%)
460	8	438	22	378	60	337	307
(82.9%)	(1.4%)	(78.9%)	(4.0%)	(68.1%)	(10.8%)	(60.7%)	(55.3%)
417	12	402	15	383	19	361	346
(77.9%)	(2.2%)	(75.1%)	(2.8%)	(71.6%)	(3.6%)	(67.5%)	(64.7%)
321	5	304	17	258	46	252	229
(75.0%)	(1.2%)	(71.0%)	(4.0%)	(60.3%)	(10.7%)	(58.9%)	(53.5%)

blisters, curvature of the body, microcephaly, ill-development and some other abnormalities by the stage of metamorphosis. Eventually, 84.0%, 75.7% and 68.2%, 76.7% on the average, of the respective total number of eggs became normally metamorphosed eggs.

b. Experimental series derived from X-irradiated great-grandparental sperm

i) J♀ × {J♀ × (J♀ × SX-150♂)♂}♂, Nos. 1~5

Five male second-generation offspring derived from spermatozoa exposed to 150 rads of X-rays were mated with three normal females Nos. 1~3 collected from the field (Table 19). The survival curve of the third-generation offspring obtained from each of the five males is shown in Fig. 35a.

In three (Nos. 1, 2 and 3) of five matings, the proportions of normal cleavages were very high, being 92.3%, 97.8% and 93.7% of the respective total number of eggs, while 83.6% on the average cleaved normally in the five matings. Of the normally cleaved eggs of No. 1, many died of incomplete invagination at the gastrula stage, and afterwards of edema. Eventually, 69.8% and 64.8% became normally hatched tadpoles and normally metamorphosed frogs, respec-

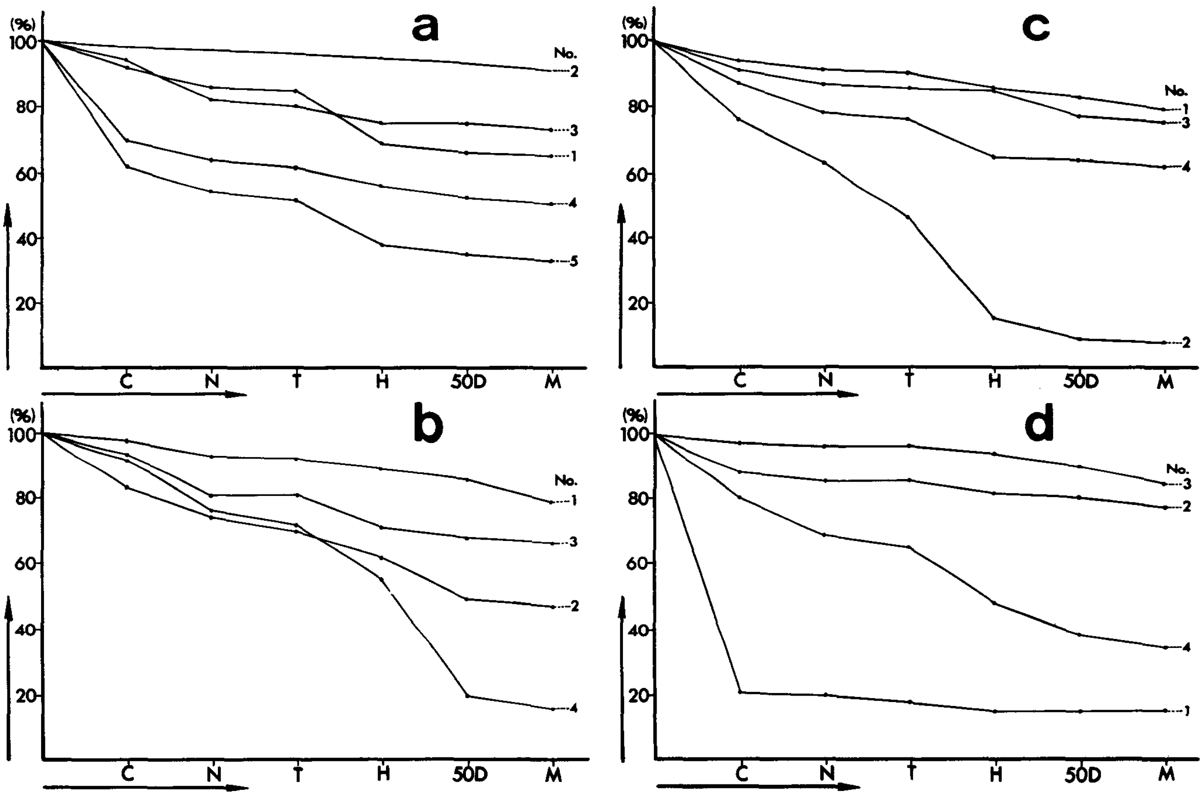


Fig. 35. Survival curves of third-generation offspring derived from X-irradiated great-grandparental gametes by passing over male first- and second-generation offspring.

a. J♀ × {J♀ × (J♀ × SX-150♂)♂}♂, Nos. 1~5
b. J♀ × {J♀ × (J♀ × SX-250♂)♂}♂, Nos. 1~4
c. J♀ × {J♀ × (EX-150♀ × J♂)♂}♂, Nos. 1~4
d. J♀ × {J♀ × (EX-250♀ × J♂)♂}♂, Nos. 1~4

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

tively. In contrast, the normally cleaved eggs of matings Nos. 2 and 3 were nearly the same in developmental capacity as the controls, although a small number of eggs died of various kinds of abnormalities. In these two matings, 91.2% and 73.2% of the respective total number of eggs became normally metamorphosed frogs. The rate of normal cleavages was slightly low in Nos. 4 and 5, that is, 70.1% and 62.4%. The normally cleaved eggs in these two matings were also inferior in developmental capacity to the controls; only 50.0% and 32.8% developed into normally metamorphosed frogs, respectively.

ii) J♀ × {J♀ × (J♀ × SX–250♂)♂}♂, Nos. 1∼4

Four male second-generation offspring derived from spermatozoa exposed to 250 rads of X-rays were mated with the same three females as those in the above experimental series. The survival curve of the third-generation offspring produced from each of the four males is shown in Fig. 35b.

The percentages of normal cleavages in four matings Nos. 1∼4 were very high, that is, 98.1%, 82.8%, 93.8% and 91.8%, 91.7% on the average, respectively. The normally cleaved eggs obtained by two matings Nos. 1 and 3 were nearly the same as those of the control series in developmental capacity at the embryonic and tadpole stages. While a small number of them died of various kinds of abnormalities, 79.0% and 65.8% of the respective total number of eggs developed into normally metamorphosed frogs. In the other two matings Nos. 2 and 4, many of the normally cleaved eggs died of incomplete invagination at the gastrula stage or of blisters at the embryonic stage. Besides, a small number of them died of microcephaly, curvature of the body or edema, while 62.3% and 54.9% hatched normally. In the tadpole stage, many also died of ill-development of teeth or ascites, and eventually 47.0% and 16.4% attained the completion of metamorphosis.

c. Experimental series derived from X-irradiated great-grandparental eggs

i) J♀ × {J♀ × (EX–150♀ × J♂)♂}♂, Nos. 1∼4

Four male second-generation offspring derived from eggs exposed to 150 rads of X-rays were mated with three normal females Nos. 4∼6 collected from the field (Table 19). The survival curve of the third-generation offspring produced from each of the four males is shown in Fig. 35c.

In four matings Nos. 1∼4, 94.3%, 75.9%, 91.2% and 87.9%, 87.2% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs obtained from three matings Nos. 1, 3 and 4 did not differ in developmental capacity from the controls; 79.3%, 75.0% and 62.1% became normally metamorphosed frogs. However, most of the normally cleaved eggs obtained from mating No. 2 died of various kinds of abnormalities, such as incomplete invagination at the gastrula stage, abnormal neurulation, acephaly, microcephaly, shrivelling of the tail and edema. Especially at the hatching stage, about four-fifths of embryos died of ascites, formation of blisters or curvature of the body. Eventually, 22 embryos, 15.2% of the total number of eggs, hatched normally, and only 12 (8.3%) completed metamorphosis after the other tadpoles died of ill-development.

ii) J♀×{J♀×(EX–250♀×J♂)♂}♂, Nos. 1~4

Four male second-generation offspring derived from eggs exposed to 250 rads of X-rays were mated with the same three females as those used in the above experimental series. The survival curve of the third-generation offspring obtained from each of the four males is shown in Fig. 35d.

While the rate of normal cleavages in mating No. 1 was very low (20.8%), those in the other matings Nos. 2, 3 and 4 were normal, that is, 88.4%, 97.2% and 80.3%, respectively. It was 72.7% on the average in these four matings. Although a small number of eggs in mating No. 1 died at the stages from the tail-bud to the hatching, nearly all the others developed normally, that is, 15.2% became normally metamorphosed frogs. The normally cleaved eggs in Nos. 2 and 3 were very similar to the controls in developmental capacity; 76.7% and 83.7% of the respective total number of eggs became normal frogs. In contrast with these eggs, a large number of the normally cleaved eggs in mating No. 4 died of edema at the stages from the neurula to the hatching, and 48.2% hatched normally. During the tadpole stage, a small number of individuals gradually died of edema, and eventually 34.3% became normally metamorphosed frogs.

d. Experimental series derived from neutron-irradiated great-grandparental sperm

i) J♀×{J♀×(J♀×SN–100♂)♂}♂, Nos. 1~4

Four male second-generation offspring derived from spermatozoa exposed to 100 rads of neutrons were mated with three normal females Nos. 7~9 (Table 19). The survival curve of the third-generation offspring obtained from each of the four males is shown in Fig. 36a.

In four matings Nos. 1~4, 94.8%, 88.3%, 99.3% and 76.0%, 90.4% on the average, of the respective total number of eggs cleaved normally. While the normally cleaved eggs obtained from three matings Nos. 2~4 did not remarkably differ from the controls in developmental capacity during the period from gastrulation to metamorphosis, 70.1%, 85.1% and 61.2% became normally metamorphosed frogs. More than half of the normally cleaved eggs obtained from mating No. 1 died of various kinds of abnormalities at various developmental stages, such as incomplete invagination at the gastrula stage, ill-formation of the neural tube at the neurula stage, acephaly, microcephaly and edema at the tail-bud stage, and microcephaly, formation of blisters, curvature of the body and ascites at the later embryonic stage. After 45.8% of the total number of eggs hatched normally, many tadpoles also died of ill-development of teeth or ascites, and eventually 32.9% completed metamorphosis.

ii) J♀×{J♀×(J♀×SN–150♂)♂}♂, Nos. 1~4

Four male second-generation offspring derived from spermatozoa exposed to 150 rads of neutrons were mated with the same three normal females as those used in the above experimental series. The survival curve of the third-generation offspring obtained from each of the four males is shown in Fig. 36b.

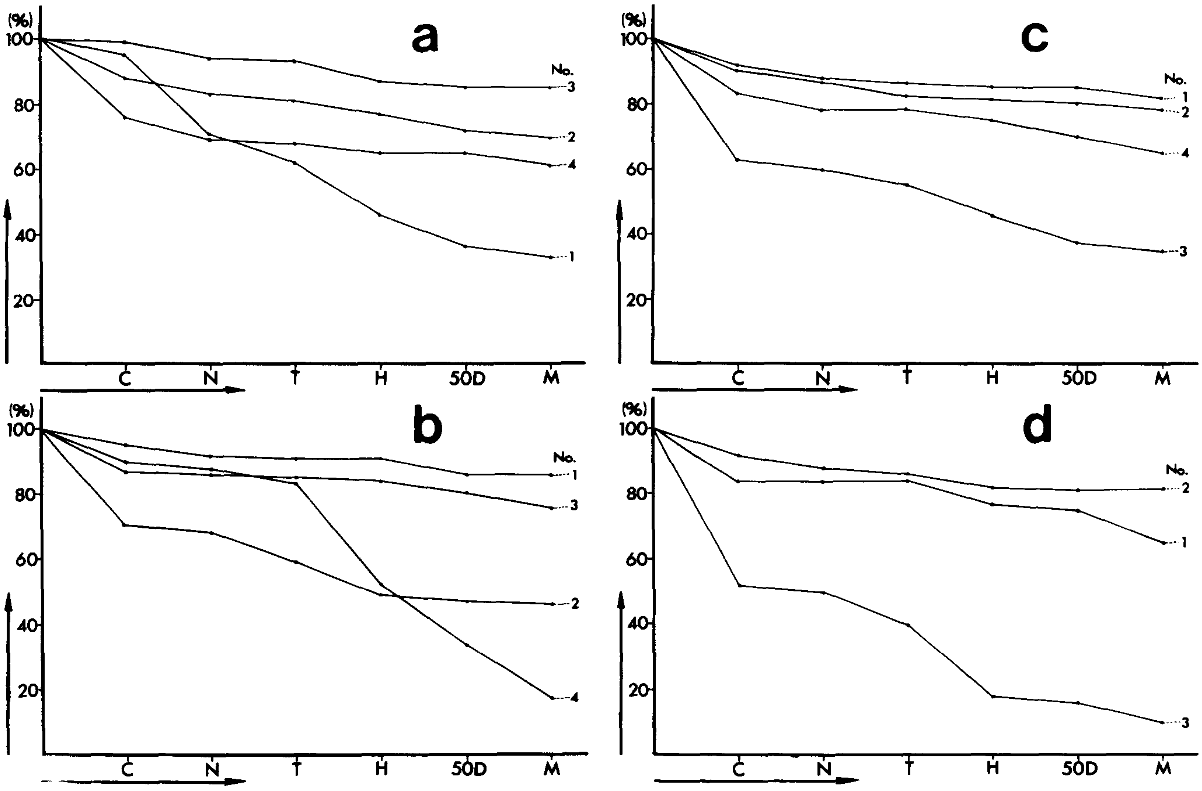


Fig. 36. Survival curves of third-generation offspring derived from neutron-irradiated great-grandparental gametes by passing over male first- and second-generation offspring.

a. J♀ × {J♀ × (J♀ × SN–100♂) ♂} ♂, Nos. 1∼4 C—Cleavage N—Neurula stage
b. J♀ × {J♀ × (J♀ × SN–150♂) ♂} ♂, Nos. 1∼4 T—Tail-bud stage H—Hatch
c. J♀ × {J♀ × (EN–150♀ × J♂) ♂} ♂, Nos. 1∼4 50D—Age of 50 days
d. J♀ × {J♀ × (EN–250♀ × J♂) ♂} ♂, Nos. 1∼3 M—Climbing out of water

In four matings Nos. 1∼4, 71.1∼95.4%, 85.6% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs obtained from matings Nos. 1 and 3 were the same as the controls in developmental capacity, that is, 86.2% and 75.8% became normally metamorphosed frogs. In mating No. 2, which was the lowest (71.1%) in the rate of normal cleavages among the four matings, many of the normally cleaved eggs died of edema from the tail-bud stage to the hatching stage, and 49.0% hatched normally. While only a few individuals died afterwards, 46.3% became normally metamorphosed frogs. In mating No. 4, the rate of normal cleavages was high (90.3%) and the normally cleaved eggs did not differ from the controls in developmental capacity during the embryonic stage. However, at the hatching stage many embryos simultaneously died of edema, and 52.8% hatched normally. In the tadpole stage, most individuals also died of edema, and only 18.1% completed metamorphosis.

e. Experimental series derived from neutron-irradiated great-grandparental eggs

i) J♀ × {J♀ × (EN–150♀ × J♂) ♂} ♂, Nos. 1∼4

Four male second-generation offspring derived from eggs exposed to 150 rads of neutrons were mated with three normal females Nos. 10 ~ 12 collected from the field (Table 19). The survival curve of the third-generation offspring produced from each of the four males is shown in Fig. 36c.

The rates of cleavages in matings Nos. 1 ~ 4 were 92.0%, 90.2%, 63.2% and 83.1%, 81.9% on the average, of the respective total number of eggs. Although a small number of normally cleaved eggs in three matings Nos. 1, 2 and 4 died of various kinds of abnormalities at various stages, the survival curves in these matings were similar to those of the controls. Eventually, 82.4%, 78.0% and 64.8% became normally metamorphosed frogs. In mating No. 3, 46.3% of the total number of eggs hatched normally after numerous eggs became edematous and died during the embryonic stage. At the tadpole stage a small number of individuals also died of edema; 36.8% were normal 50 days after the insemination, and eventually, 35.3% became normally metamorphosed frogs.

ii) J♀ × {J♀ × (EN–250♀ × J♂) ♂} ♂, Nos. 1 ~ 3

Three male second-generation offspring derived from eggs exposed to 250 rads of neutrons were mated with the same three females as those used in the above experimental series. The survival curve of the third-generation offspring produced from each of the three males is shown in Fig. 36d.

Normal cleavages occurred in 84.2%, 91.9% and 51.9%, 76.9% on the average, of the respective total number of eggs in three matings Nos. 1 ~ 3. The normally cleaved eggs in Nos. 1 and 2 scarcely differ from the controls in development during the embryonic and tadpole stages, although a small number of them died of various kinds of abnormalities at various stages; 65.1% and 81.2% eventually became normally metamorphosed frogs. In mating No. 3, most of the normally cleaved eggs died of edema at the stages from the tail-bud to the hatching, while only 18.0% of the total number of eggs hatched normally. At the tadpole stage numerous individuals died of edema, and 9.8% became normally metamorphosed frogs.

2. Chromosome aberrations in normally shaped tadpoles

Chromosomal analysis was made in normally shaped 50-day-old tadpoles (V ~ X stages) produced from eight matings of the experimental series and one of the control matings (Table 20).

a. Control series, J♀ × {J♀ × (J♀ × J♂) ♂} ♂, No. 1

The tail-tips of 16 normally shaped tadpoles produced from one (No. 1) of the three control matings were examined by the squash method. Fifteen of these tadpoles were analyzable in karyotype; all of them had nothing but diploid mitoses whose chromosomes were normal in number and shape.

b. Experimental series derived from X-irradiated great-grandparental sperm

i) J♀ × {J♀ × (J♀ × SX–150♂) ♂} ♂, No. 2

Eighteen of 20 normally shaped tadpoles produced from one (No. 2) of the

TABLE 20

Chromosomal analysis of normally shaped 50-day-old tadpoles produced from males 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	Mosaics	
J.W70, Nos. 1∼3	J(J·J), No. 1	15	15 (100 %)	0	0	0
J.W70, Nos. 1∼3	J(J·SX–150), No. 2	18	14 (77.8%)	1 (5.6%)	1 (5.6%)	2 (11.1%)
J.W70, Nos. 1∼3	J(J·SX–250), No. 1	20	16 (80.0%)	2 (10.0%)	0	2 (10.0%)
J.W70, Nos. 4∼6	J(EX–150·J), No. 1	19	15 (78.9%)	1 (5.3%)	1 (5.3%)	2 (10.5%)
J.W70, Nos. 4∼6	J(EX–250·J), No. 3	20	16 (80.0%)	2 (10.0%)	1 (5.0%)	1 (5.0%)
J.W70, Nos. 7∼9	J(J·SN–100), No. 3	21	16 (76.2%)	2 (9.5%)	1 (4.8%)	2 (9.5%)
J.W70, Nos. 7∼9	J(J·SN–150), No. 1	20	17 (85.0%)	2 (10.0%)	0	1 (5.0%)
J.W70, Nos. 10∼12	J(EN–150·J), No. 1	17	13 (76.5%)	1 (5.9%)	1 (5.9%)	2 (11.8%)
J.W70, Nos. 10∼12	J(EN–250·J), No. 2	21	16 (76.2%)	2 (9.5%)	1 (4.8%)	2 (9.5%)

five matings between five male second-generation offspring derived from spermatozoa exposed to 150 rads of X-rays and normal females were analyzable in karyotype. Fourteen of them were normal diploids, while the others had abnormal mitoses solely or together with normally diploid ones. One of the latter was a hyperdiploid (2n+1), having an additional No. 2 chromosome, another consisted of a mixture of abnormally diploid mitoses having a ring chromosome and those having a chromosome with a deletion. The remaining two tadpoles were mosaics of normally diploid and abnormal cells; one had triploid mitoses, while the others had abnormally diploid mitoses having a chromosome with a deletion.

ii) J♀ × {J♀ × (J♀ × SX–250♂) ♂} ♂, No. 1

Twenty of 22 normally shaped tadpoles produced from one (No. 1) of the four matings between four male second-generation offspring derived from spermatozoa exposed to 250 rads of X-rays and normal females were analyzable in karyotype. Of these tadpoles 16 were normal diploids, while the other four had abnormal mitoses. Two of the latter tadpoles had nothing but abnormally diploid mitoses; in one tadpole the long arm of a No. 4 chromosome was lost, and in the other a part of the long arm of a No. 4 chromosome was translocated to the long arm of a No. 5 chromosome. The remaining two tadpoles were mosaics consisting of normally diploid and abnormal cells. One of them had hypodiploid (2n−1) mitoses in which a small chromosome was lost, while the other had abnormally diploid mitoses containing a dicentric chromosome and a fragment in place of normal Nos. 2 and 12 chromosomes.

c. Experimental series derived from X-irradiated great-grandparental eggs

i) J♀ × {J♀ × (EX–150♀ × J♂) ♂} ♂, No. 1

Of 22 normally shaped tadpoles produced from one (No. 1) of the four matings between four male second-generation offspring derived from eggs exposed to 150 rads of X-rays and normal females, 19 were analyzable in karyotype. While 15 tadpoles were normal diploids, the other four were abnormal. One of the latter was an abnormal diploid; in all the metaphase spreads a part of a No. 7 chromosome was translocated to the long arm of a No. 5 chromosome. Another was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, in which there were three or only one No. 12 chromosome. The remaining two tadpoles were mosaics of normally diploid and abnormal mitoses; one of them had triploid mitoses, while the other had abnormally diploid mitoses containing a dicentric chromosome and a fragment.

ii) J♀×{J♀×(EX-250♀×J♂)♂}♂, No. 3

Twenty of 22 normally shaped tadpoles produced from one (No. 3) of the four matings between four male second-generation offspring derived from eggs exposed to 250 rads of X-rays and normal females were analyzable in karyotype. Sixteen of them were normal diploids. Two others consisted solely of abnormal cells; one was a hypertriploid (3n+1) and the other was an abnormal diploid, having a chromosome with a translocation. Another tadpole was a mosaic of hypodiploid (2n−1) mitoses with a dicentric chromosome and abnormally diploid mitoses containing a chromosome with a translocation. The remaining tadpole was a mosaic of normally diploid and hyperdiploid (2n+1) mitoses.

d. Experimental series derived from neutron-irradiated great-grandparental sperm

i) J♀×{J♀×(J♀×SN-100♂)♂}♂, No. 3

Of 22 normally shaped tadpoles produced from one (No. 3) of the four matings between four male second-generation offspring derived from spermatozoa exposed to 100 rads of neutrons and normal females, 21 were analyzable in karyotype. Sixteen of them were normal diploids. Two of the other five tadpoles had solely abnormal mitoses; a tadpole was an abnormal diploid having a chromosome with a deletion, while the other tadpole was a hyperdiploid (2n+1) having an additional small chromosome. Another tadpole was a mosaic of two different kinds of abnormally diploid cells, that is, mitoses having a chromosome with a deletion and those having a chromosome with a translocation. The remaining two tadpoles were mosaics of normally and abnormally diploid cells. One of them had mitoses containing a ring chromosome, while the other had those containing a minute chromosome which was derived from a small chromosome by loss of a part.

ii) J♀×{J♀×(J♀×SN-150♂)♂}♂, No. 1

Twenty of 22 normally shaped tadpoles produced from one (No. 1) of the four matings between four male second-generation offspring derived from spermatozoa exposed to 150 rads of neutrons and normal females were analyzable in karyotype. Seventeen of them were normal diploids. Two others had no normally diploid mitoses; one was a triploid, and the other was an abnormally diploid tadpole having a No. 4 chromosome with a deletion. The remaining tadpole was a mosaic consisting of normally diploid mitoses and hyperdiploid (2n+1) ones with an additional small chromosome.

e. Experimental series derived from neutron-irradiated great-grandparental eggs

i) J♀×{J♀×(EN–150♀×J♂)♂}♂, No. 1

Seventeen of 20 normally shaped tadpoles produced from one (No. 1) of the four matings between four male second-generation offspring derived from eggs exposed to 150 rads of neutrons and normal females were analyzable in karyotype. Thirteen of them were normal diploids, while the other four had abnormal mitoses. One of these four tadpoles was an abnormal diploid, having a ring chromosome. Another was a mosaic consisting of hyperdiploid (2n+1) and hypodiploid (2n−1) mitoses, in which there was an addition or a loss of a small chromosome. The remaining two were mosaics of normally diploid and abnormal mitoses; one had triploid mitoses and the other had abnormally diploid mitoses, in which there was a chromosome with a deletion or a translocation.

ii) J♀×{J♀×(EN–250♀×J♂)♂}♂, No. 2

Twenty-one of 22 normally shaped tadpoles produced from one (No. 2) of the three matings between three male second-generation offspring produced from eggs exposed to 250 rads of neutrons and normal females were analyzable in karyotype. Sixteen of them were normal diploids, while two others had no normally diploid mitoses. One of these two tadpoles was a hypotriploid (3n−1), in which a small chromosome was lost from the triploid complement, while the other was a hyperdiploid (2n+1), having an additional small chromosome. Another was a mosaic of two kinds of abnormally diploid mitoses, in which there was a chromosome with a deletion or a translocation. The remaining two were mosaics of normally and abnormally diploid mitoses; one had a minute chromosome, while the other had a ring.

3. Viability and sex of metamorphosed frogs

a. Viability

The age of tadpoles at the time of climbing out of water and the body length of frogs immediately after metamorphosis in the experimental and the control series are presented in Table 21. As found in the second-generation offspring, there were no distinct differences in these respects between the individuals of the experimental series and the controls. There were also no distinct differences between the individuals derived from X-irradiated sperm or eggs and those derived from neutron-irradiated sperm or eggs, or between the individuals derived from sperm or eggs exposed to different dosages of X-rays or neutrons.

About one month after metamorphosis, 50 frogs were removed from each mating group of third-generation offspring in which the karyotype analysis of normally shaped tadpoles had been made, and reared until sexual maturity. However, from each of two matings, J♀×{J♀×(EX–250♀×J♂)♂}♂ and J♀×{J♀×(EN–250♀×J♂)♂}♂, 40 frogs were removed and reared. As presented in Table 21, less than half the number of the young frogs in the experimental series attained sexual maturity in the next breeding season, while about two-thirds of the control frogs did so. It was moreover clear that the frogs derived from gametes exposed to larger dosages of X-rays or neutrons were lower in viability than those from gametes exposed to smaller dosages of the same irradiation. In four series including the former frogs, only 10~18% of young frogs attained sexual maturity, while 32~48% did so in four series including the latter frogs. There were no differences in viability between the irradiation with X-rays and that with neutrons and between the irradiation of sperm and that of eggs.

b. Sex

The sex of immature frogs examined within one month after metamorphosis is presented in Table 21. In the control series, there were nearly an equal number of males and females, that is, 131 were females and 124 males, although two females had underdeveloped ovaries. There were no hermaphrodites. No distinct difference was found in number between males and females produced from each of three matings of the control series. In contrast with the control frogs, there were much more females than males in three of the eight experimental series, while there were more numerous males than females in two other experimental series.

TABLE 21

Number, size and sex of metamorphosed frogs produced from males

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed frogs	Mean body length of all frogs immediately after metamorphosis (mm)	
Female	Male				No. of frogs
J.W70, Nos. 1~12	J(J·J), Nos. 1~3	76~121 (92.3)	306	18.6±0.2	255
J.W70, Nos. 1~3	J(J·SX–150), Nos. 1~5	75~120 (94.1)	417	18.3±0.2	365
	J(J·SX–250), Nos. 1~4	77~121 (95.1)	315	18.1±0.2	264
J.W70, Nos. 4~6	J(EX–150·J), Nos. 1~4	74~119 (92.7)	307	17.7±0.2	257
	J(EX–250·J), Nos. 1~4	75~120 (93.6)	283	18.1±0.2	243
J.W70, Nos. 7~9	J(J·SN–100), Nos. 1~4	74~120 (92.6)	347	17.9±0.2	296
	J(J·SN–150), Nos. 1~4	76~121 (96.3)	307	17.7±0.2	252
J.W70, Nos. 10~12	J(EN–150·J), Nos. 1~4	74~115 (92.7)	346	18.0±0.2	296
	J(EN–250·J), Nos. 1~3	75~120 (94.4)	229	17.9±0.2	186

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

i) Experimental series, J♀ × {J♀ × (J♀ × SX–150♂)♂}♂

Of 365 frogs produced from five matings Nos. 1∼5, 220 were females, 16 hermaphrodites and 129 males, although ten females had underdeveloped ovaries. The female preponderance in this series was attributable to that in the third-generation offspring produced from two (Nos. 2 and 4) of five matings Nos. 1∼5; 50 were females, 5 hermaphrodites and 19 males among 74 frogs produced from mating No. 2; 64 were females with normal ovaries, one a female with underdeveloped ovaries and 2 hermaphrodites among 67 frogs from mating No. 4. In each of two other matings (Nos. 1 and 5), nearly an equal number of males and females were produced; of 90 frogs produced from mating No. 1, 45 were females with normal ovaries, one a female with underdeveloped ovaries, 2 hermaphrodites and 42 males; of 41 frogs produced from No. 5, 20 were females with normal ovaries, one a female with underdeveloped ovaries, one a hermaphrodite and 19 males. There was a male preponderance in mating No. 3; 31 were females with normal ovaries, 7 females with underdeveloped ovaries, 6 hermaphrodites and 49 males among 93 frogs.

ii) Experimental series, J♀ × {J♀ × (J♀ × SX–250♂)♂}♂

Of 264 frogs produced from four matings Nos. 1∼4, 157 were females, 11 hermaphrodites and 96 males. All the 47 frogs produced from mating No. 2 were females, while there were nearly an equal number of males and females

derived from irradiated grandparental sperm or eggs, I

Sex of frogs dead or killed about one month after metamorphosis					No. of frogs removed and reared	Sex of mature frogs			Sex of all frogs examined		
♀$_N$	♀$_U$	⚥	♂$_N$	♂ (%)*		No. of frogs	♀	♂	Total	♀	♂ (%)*
129	2	0	124	(48.6)	50	32	16	16	287	147	140 (48.8)
210	10	16	129	(39.7)	50	21	7	14	386	227	159 (41.2)
154	3	11	96	(40.5)	50	5	2	3	269	159	110 (40.9)
133	2	1	121	(47.5)	50	24	9	15	281	144	137 (48.8)
97	1	3	142	(59.7)	40	4	1	3	247	99	148 (59.9)
141	6	10	139	(50.3)	50	16	5	11	312	152	160 (51.3)
166	7	7	72	(31.3)	50	9	2	7	261	175	86 (33.0)
116	1	4	175	(60.5)	50	17	7	10	313	124	189 (60.4)
85	2	2	97	(53.2)	40	6	2	4	192	89	103 (53.6)

⚥—Hermaphrodites ♂$_N$—Males with normal testes * Including hermaphrodites

among the frogs produced from each of matings Nos. 1, 3 and 4.

iii) Experimental series, J♀ × {J♀ × (EX–150♀ × J♂) ♂} ♂

There were nearly an equal number of males and females among the frogs produced from each of four matings Nos. 1 ~ 4.

iv) Experimental series, J♀ × {J♀ × (EX–250♀ × J♂) ♂} ♂

Of 243 frogs produced from four matings Nos. 1 ~ 4, 98 were females, 3 hermaphrodites and 142 males. Such a male preponderance was attributable to that in the third-generation offspring produced from one (No. 3) of the four matings, while there were nearly an equal number of males and females among the frogs produced from each of the other matings.

v) Experimental series, J♀ × {J♀ × (J♀ × SN–100♂) ♂} ♂

There were nearly an equal number of males and females among the frogs produced from each of four matings, Nos. 1 ~ 4.

vi) Experimental series, J♀ × {J♀ × (J♀ × SN–150♂) ♂} ♂

Of 252 frogs produced from four matings, Nos. 1 ~ 4, 166 were females with normal ovaries, 7 females with underdeveloped ovaries, 7 hermaphrodites and 72 males. Of 95 frogs produced from one (No. 3) of these matings, 92 were females with normal ovaries and 3 hermaphrodites, while there were nearly an equal number of males and females among the frogs produced from each of the other matings.

vii) Experimental series, J♀ × {J♀ × (EN–150♀ × J♂) ♂} ♂

Of 296 frogs produced from four matings Nos. 1 ~ 4, 117 were females, 4 hermaphrodites and 175 males. This male preponderance was because 28 were females with normal ovaries, one a female with underdeveloped ovaries, 3 hermaphrodites and 71 males among 103 frogs produced by one (No. 2) of these matings, while there were nearly an equal number of males and females among the frogs produced from each of the other three matings.

viii) Experimental series, J♀ × {J♀ × (EN–250♀ × J♂) ♂} ♂

There was no distinct difference in number between males and females produced from each of three matings Nos. 1 ~ 3. Of 186 frogs, 87 were females, 2 hermaphrodites and 97 males.

Of 32 sexually mature frogs in the control series, 16 were females and 16 males. On the other hand, of 102 sexually mature frogs in the eight experimental series, 35 were females and 67 males. Such a male preponderance was always found in each of the experimental series and in contrast with the female preponderance among the immature frogs produced from three experimental series derived from X- or neutron-irradiated sperm (Table 21).

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

1. Developmental capacity

In the breeding season of 1970, female second-generation offspring (Table 22) derived from X- or neutron-irradiated sperm or eggs were mated with normal

TABLE 22

Eggs of female parents used for mating experiments in 1970

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 100 eggs (mm)
J(J·J), No. 2	1	1	35.0	439	1.64±0.02
	2	1	36.5	899	1.70±0.02
	3	1	36.5	1074	1.71±0.02
	4	1	36.0	991	1.67±0.02
J(J·SX–150), No. 3	1	1	37.0	524	1.74±0.02
	2	1	38.0	463	1.64±0.02
	3	1	39.5	615	1.58±0.02
	4	1	36.0	386	1.69±0.02
J(J·SX–250), No. 1	1	1	35.5	520	1.68±0.02
	2	1	39.5	672	1.63±0.02
	3	1	38.0	921	1.72±0.02
	4	1	40.0	0	—
J(EX–150·J), No. 1	1	1	39.5	768	1.62±0.02
	2	1	35.0	912	1.53±0.02
	3	1	34.0	674	1.51±0.02
	4	1	35.5	0	—
J(EX–250·J), No. 2	1	1	35.0	469	1.64±0.02
	2	1	38.0	695	1.68±0.02
	3	1	36.5	0	—
	4	1	35.5	0	—
J(J·SN–100), No. 2	1	1	40.0	736	1.63±0.02
	2	1	34.5	438	1.64±0.02
	3	1	32.5	521	1.52±0.02
	4	1	33.5	637	1.50±0.02
J(J·SN–150), No. 3	1	1	36.0	694	1.62±0.02
	2	1	35.0	648	1.67±0.02
	3	1	36.5	474	1.69±0.02
	4	1	34.5	551	1.63±0.02
J(EN–150·J), No. 3	1	1	36.5	563	1.64±0.02
	2	1	36.0	647	1.53±0.02
	3	1	35.0	492	1.47±0.02
	4	1	35.5	0	—
J(EN–250·J), No. 3	1	1	37.0	749	1.71±0.02
	2	1	36.0	648	1.60±0.02
	3	1	35.0	0	—
	4	1	37.0	0	—

J(J·J), No. 2: Females obtained by J.W69♀, Nos. 1～12×(J·J)♂, No. 2

J(J·SX–150 or –250), No. 3 or 1: Females obtained by J.W69♀, Nos. 1～3×(J·SX–150 or –250)♂, No. 3 or 1

J(EX–150 or –250·J), No. 1 or 2: Females obtained by J.W69♀, Nos. 4～6×(EX–150 or –250·J)♂, No. 1 or 2

J(J·SN–100 or –150), No. 2 or 3: Females obtained by J.W69♀, Nos. 7～10×(J·SN–100 or –150)♂, No. 2 or 3

J(EN–150 or –250·J), No. 3: Females obtained by J.W69♀, Nos. 11～13×(EN–150 or –250·J)♂, No. 3

males (Table 23) collected from the field in order to produce third-generation offspring. The female second-generation offspring were those which were produced in 1969 from male first-generation offspring by mating with normal females collected from the field. They were divided into eight experimental series in accordance with their origin. In each series, two to four female second-generation offspring were mated with each of four normal males by artificial fertilization. The developmental capacity of the third-generation offspring in each experimental series and the controls is presented in Table 24 and shown in Figs. 37～39 and 57～59.

TABLE 23

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

Kind	Individual no.	Body length (mm)	Size of the testes		Inner structure (Type)
			Left (mm)	Right (mm)	
J.W70	6	45.0	4.0×2.5	4.0×2.5	1
	7	40.0	3.5×2.0	3.5×2.0	1
	8	41.5	3.5×2.0	3.5×2.0	1
	9	42.0	4.0×2.0	4.0×2.0	1

a. Control series, {J♀×(J♀×J♂)♂}♀×J♂, Nos. 1～4

Four mature females (Nos. 1～4) were transplanted with frog pituitaries. As normal ovulation occurred in all of them, they were mated with four males (Nos. 6～9) collected from the field. The females were second-generation

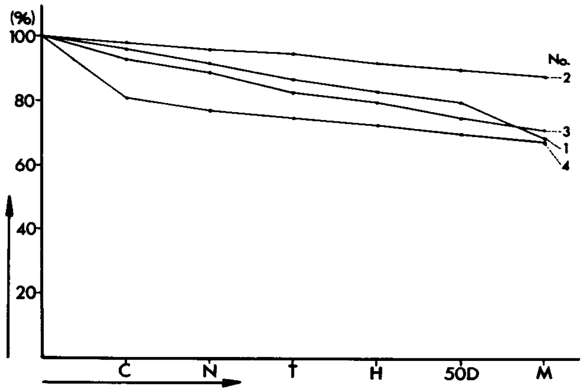
TABLE 24

Developmental capacity of the offspring of females derived from

Parents		No. of eggs	No. of cleaved eggs		No. of gastrulae	
Female	Male		Normal	Abnormal	Normal	Abnormal
J(J·J), Nos. 1～4	J.W70, Nos. 6～9	539	497 (92.2%)	2 (0.4%)	484 (89.8%)	13 (2.4%)
J(J·SX–150), Nos. 1～4	J.W70, Nos. 6～9	623	415 (66.6%)	22 (3.5%)	389 (62.4%)	26 (4.2%)
J(J·SX–250), Nos. 1～3		397	284 (71.5%)	13 (3.3%)	264 (66.5%)	20 (5.0%)
J(EX–150·J), Nos. 1～3	J.W70, Nos. 6～9	388	299 (77.1%)	11 (2.8%)	271 (69.8%)	28 (7.2%)
J(EX–250·J), Nos. 1,2		279	257 (92.1%)	2 (0.7%)	245 (87.8%)	12 (4.3%)
J(J·SN–100), Nos. 1～4	J.W70, Nos. 6～9	594	445 (74.9%)	23 (3.9%)	398 (67.0%)	47 (7.9%)
J(J·SN–150), Nos. 1～4		560	428 (76.4%)	20 (3.6%)	401 (71.6%)	27 (4.8%)
J(EN–150·J), Nos. 1～3	J.W70, Nos. 6～9	364	320 (87.9%)	16 (4.4%)	314 (86.3%)	6 (1.6%)
J(EN–250·J), Nos. 1,2		227	205 (90.3%)	1 (0.4%)	196 (86.3%)	9 (4.0%)

offspring of a male first-generation offspring mated with a female collected from the field. The male first-generation offspring was one of those which were obtained from field-caught males and females as the control of the frogs raised from X- or neutron-irradiated spermatozoa or eggs. The survival curve of the third-generation offspring produced from each of the four females is shown in Fig. 37.

In four matings, 96.1%, 98.1%, 93.2% and 80.6%, 92.2% on the average, of the respective total number of eggs cleaved normally. A small number of them died of various kinds of abnormalities, such as ascites, microcephaly, curvature of the body, or ill-development at various developmental stages, and



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

Fig. 37. Survival curves of control third-generation offspring derived from untreated great-grandparental gametes by matings, {J♀ × (J♀ × J♂) ♂} ♀ × J♂, Nos. 1~4.

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		
478	6	460	18	443	17	427	402
(88.7%)	(1.1%)	(85.3%)	(3.3%)	(82.2%)	(3.2%)	(79.2%)	(74.6%)
374	15	340	34	326	14	245	232
(60.0%)	(2.4%)	(54.6%)	(5.5%)	(52.3%)	(2.2%)	(39.3%)	(37.2%)
252	12	225	27	188	37	180	173
(63.5%)	(3.0%)	(56.7%)	(6.8%)	(47.4%)	(9.3%)	(45.3%)	(43.6%)
248	23	236	12	220	16	209	193
(63.9%)	(5.9%)	(60.8%)	(3.1%)	(56.7%)	(4.1%)	(53.9%)	(49.7%)
222	23	205	17	147	58	130	114
(79.6%)	(8.2%)	(73.5%)	(6.1%)	(52.7%)	(20.8%)	(46.6%)	(40.9%)
359	39	296	63	277	19	245	229
(60.4%)	(6.6%)	(49.8%)	(10.6%)	(46.6%)	(3.2%)	(41.2%)	(38.6%)
367	34	326	41	311	15	274	225
(65.5%)	(6.1%)	(58.2%)	(7.3%)	(55.5%)	(2.7%)	(48.9%)	(40.2%)
299	15	283	16	270	13	258	232
(82.1%)	(4.1%)	(77.7%)	(4.4%)	(74.2%)	(3.6%)	(70.9%)	(63.7%)
189	7	187	2	181	6	170	150
(83.3%)	(3.1%)	(82.4%)	(0.9%)	(79.7%)	(2.6%)	(74.9%)	(66.1%)

eventually 69.3%, 88.0%, 70.7% and 68.2%, 74.6% on the average, became normally metamorphosed frogs.

b. Experimental series derived from X-irradiated great-grandparental sperm

i) {J♀ × (J♀ × SX–150♂)♂} ♀ × J♂, Nos. 1∼4

Four female second-generation offspring (Nos. 1∼4) derived from spermatozoa exposed to 150 rads of X-rays were mated with the same four normal males as those used in the control series, after their ovulation was accelerated by pituitary transplantation (Table 24, Fig. 37). The survival curve of the third-generation offspring produced from each of the four females is shown in Fig. 38a. There was no distinct difference in survival curve between the offspring produced from two (Nos. 1 and 3) of four matings (Nos. 1∼4) and the controls. In these two matings, 78.1% and 95.1% of the respective total number of eggs cleaved normally, and eventually 63.1% and 70.8% became normally metamorphosed frogs, while the remaining eggs died of various abnormalities at various embryonic and tadpole stages. The survival rates of the offspring produced from the other two matings (Nos. 2 and 4) were very low, as compared with those of the controls.

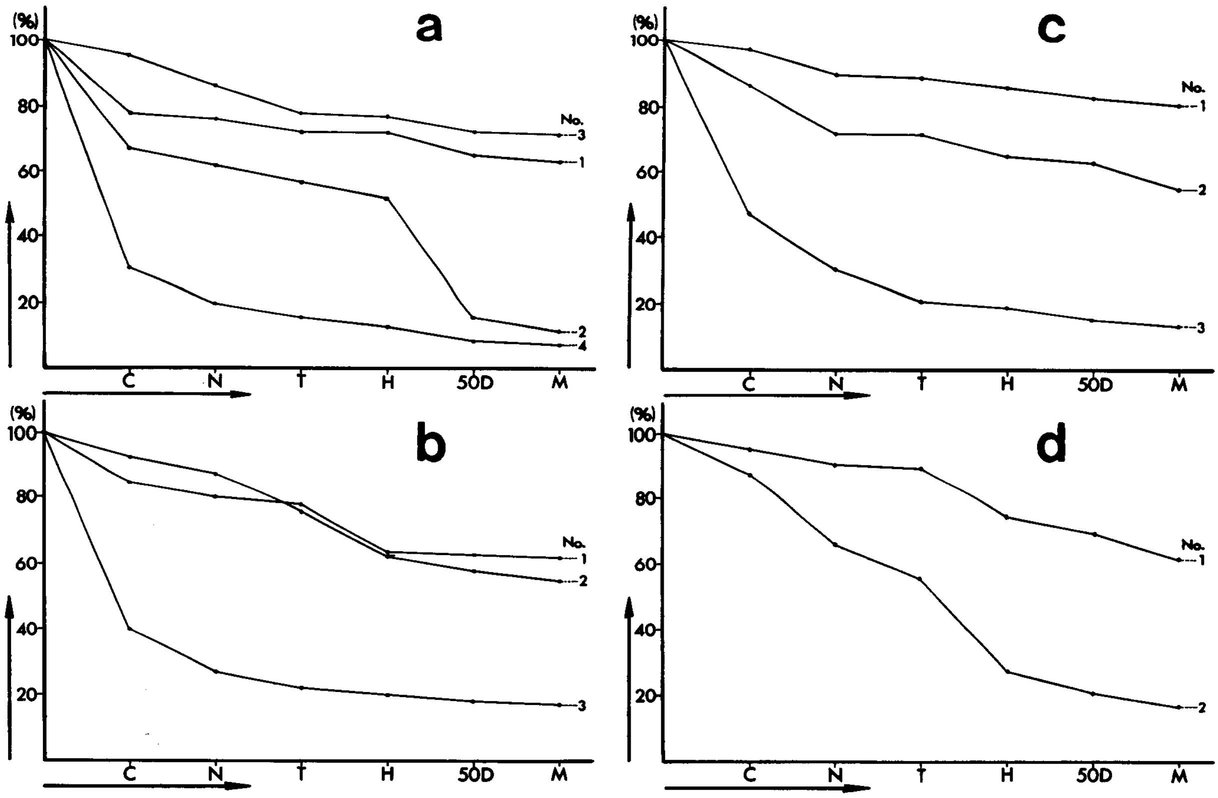


Fig. 38. Survival curves of third-generation offspring derived from X-irradiated great-grandparental gametes by passing over male first- and female second-generation offspring.

a. {J♀ × (J♀ × SX–150♂)♂} ♀ × J♂, Nos. 1∼4
b. {J♀ × (J♀ × SX–250♂)♂} ♀ × J♂, Nos. 1∼3
c. {J♀ × (EX–150♀ × J♂)♂} ♀ × J♂, Nos. 1∼3
d. {J♀ × (EX–250♀ × J♂)♂} ♀ × J♂, Nos. 1 and 2

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

In mating No. 2, 66.9% of the total eggs cleaved normally, and 51.9% hatched normally after a small number of embryos died of various abnormalities. However, most of the hatched tadpoles were ill-developed and became thin. At the age of 50 days, 24 of 80 tadpoles were appeared normal, while the other 56 were seriously ill. Seventeen of the healthy tadpoles, that is, 11.0% of the total number of eggs normally completed metamorphosis, while the other seven remained without making metamorphosis for several dozen days afterwards. In mating No. 4, only 50 (30.3%) eggs cleaved normally. While 15 of them died of incomplete invagination at the gastrula stage, and 15 others died of edema from the tail-bud to the hatching stage, the remaining 20 (12.1%) hatched normally. Of these hatched tadpoles, 12, that is, 7.3% of the total number of eggs completed metamorphosis, while the other 8 died of edema during the tadpole stage.

ii) {J♀ × (J♀ × SX–250♂)♂}♀ × J♂, Nos. 1 ~ 3

Four female second-generation offspring derived from spermatozoa exposed to 250 rads of X-rays were transplanted with frog pituitaries; ovulation occurred in three of them. These three females were mated with the same four males as those used in the control series. The survival curve of the third-generation offspring produced from each of the three females is shown in Fig. 38b. From three matings Nos. 1 ~ 3, 84.7%, 93.1% and 40.1% of the respective total number of eggs cleaved normally. A few of the normally cleaved eggs produced from mating No. 1 died of rough surfaces, blisters or microcephaly at the embryonic stage. At the hatching stage, about one-fifth of the remaining embryos died of edema at the same time, while 63.7% hatched normally, and afterwards 62.1% became normally metamorphosed frogs. Of the normally cleaved eggs produced from mating No. 2, only a few died of various abnormalities at the embryonic stage, as those from mating No. 1. However, many of the remaining embryos died of edema from the tail-bud to the hatching stage, while 61.8% of the total number of eggs hatched normally, and 55.0% became normally metamorphosed frogs. About half of the normally cleaved eggs obtained by the mating (No. 3) gradually died of edema and some other abnormalities at the embryonic stage, while 19.7% of the total number of eggs hatched normally and 16.9% became normally metamorphosed frogs.

c. Experimental series derived from X-irradiated great-grandparental eggs

i) {J♀ × (EX–150♀ × J♂)♂}♀ × J♂, Nos. 1 ~ 3

Four female second-generation offspring derived from eggs exposed to 150 rads of X-rays were transplanted with frog pituitaries. As a result, ovulation occurred in three of them. These three females were mated with the same four males as those used in the control series (Table 24, Fig. 37). The survival curve of the third-generation offspring produced from each of them is shown in Fig. 38c. From matings Nos. 1 and 2, 96.8% and 86.7% of the respective total number of eggs cleaved normally. While some of the normally cleaved eggs died of various abnormalities such as edema, blisters, curvature of the body or shrinkage of the tail at various developmental stages, 81.0%

and 54.8% became normally metamorphosed frogs. In other words, the eggs of mating No. 1 did not differ from the controls in developmental capacity, while those of No. 2 were somewhat inferior. In mating No. 3, the proportion of normally cleaved eggs was very low, that is, 47.2%. The great majority of them died of various abnormalities such as incomplete invagination at the gastrula stage, or acephaly, microcephaly, edema, and curvature or shrinkage of the body during the later embryonic stages, while only 13.4% became normally metamorphosed frogs.

ii) {J♀×(EX–250♀×J♂)♂}♀×J♂, Nos. 1 and 2

Four female second-generation offspring derived from eggs exposed to 250 rads of X-rays were transplanted with frog pituitaries; ovulation occurred in two of them. These two females were mated with the same four males as those used in the control series. The survival curve of the third-generation offspring produced from each of them is shown in Fig. 38d. From two matings Nos. 1 and 2, 95.9% and 88.0% of the respective total number of eggs cleaved normally. Although the normally cleaved eggs in mating No. 1 were nearly the same as the controls in developmental capacity until the late embryonic stage, many of them became edematous simultaneously at the hatching stage. After a small number of tadpoles gradually died of edema, 62.3% of the total number of eggs became normally metamorphosed frogs. In mating No. 2, most of the normally cleaved eggs died of ill-formation of the neural tube or edema at the neurula stage, and also of acephaly, microcephaly, dicephaly, curvature of the body or edema from the tail-bud to the hatching stage, while 27.8% of the total number of eggs hatched normally. Afterwards, only 17.3% became normally metamorphosed frogs, while the remaining tadpoles died of underdevelopment at the early feeding stage.

d. Experimental series derived from neutron-irradiated great-grandparental sperm

i) {J♀×(J♀×SN–100♂)♂}♀×J♂, Nos. 1∼4

Four female second-generation offspring derived from spermatozoa exposed to 100 rads of neutrons were mated with the same four males as those used in the control series, after their ovulation was accelerated by pituitary transplantation (Table 24, Fig. 37). The survival curve of the third-generation offspring produced from each of them is shown in Fig. 39a. From matings Nos. 2 and 4, 92.1% and 93.7% of the respective total number of eggs cleaved normally. The third-generation offspring produced from these matings did not differ in developmental capacity from the controls during the embryonic stage; 76.8% and 83.1% hatched normally. Except that a fairly large number of tadpoles produced from mating No. 2 died of malformation of the teeth before the age of 50 days, the tadpoles obtained from the two matings grew normally, and 60.9% and 78.9% became normally metamorphosed frogs.

In the other matings Nos. 1 and 3, the rates of normally cleaved eggs were low, that is, 62.8% and 53.0%. During the stages from the neurula to the tail-

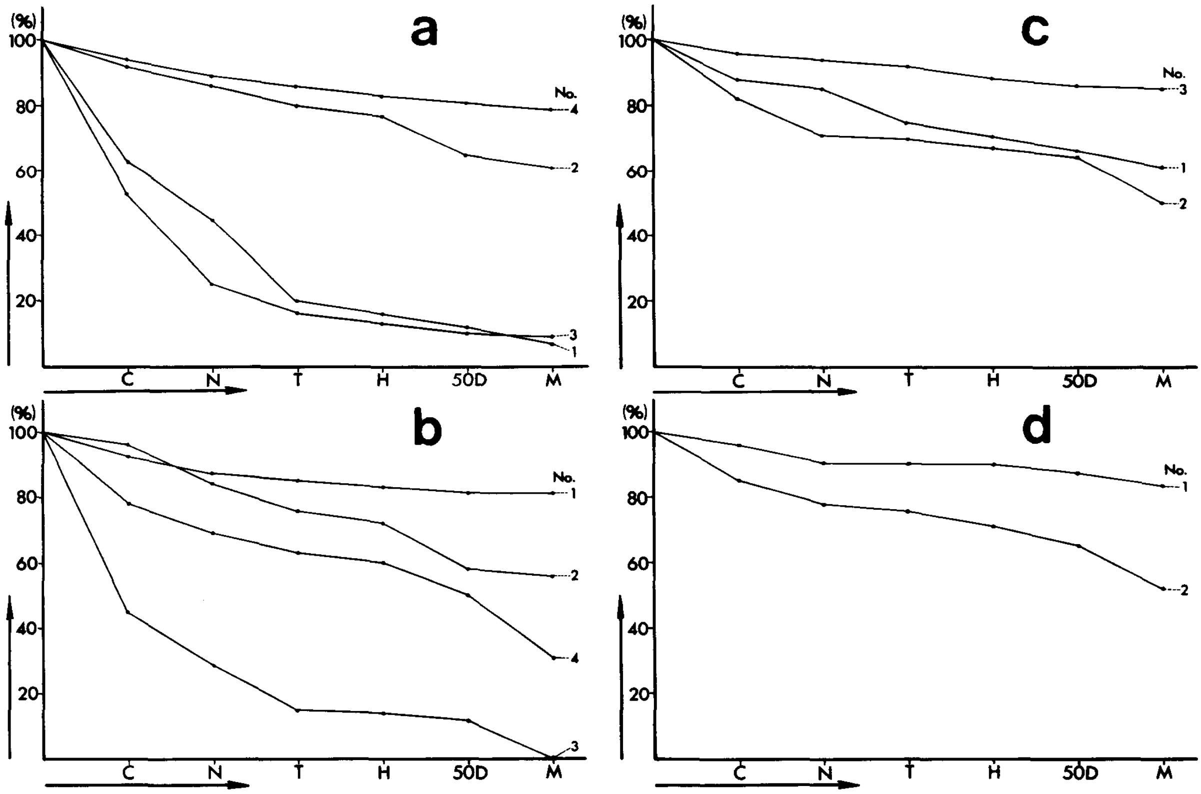


Fig. 39. Survival curves of third-generation offspring derived from neutron-irradiated great-grandparental gametes by passing over male first- and female second-generation offspring.

a. {J♀ × (J♀ × SN-100♂) ♂} ♀ × J♂, Nos. 1∼4 C—Cleavage N—Neurula stage
b. {J♀ × (J♀ × SN-150♂) ♂} ♀ × J♂, Nos. 1∼4 T—Tail-bud stage H—Hatch
c. {J♀ × (EN-150♀ × J♂) ♂} ♀ × J♂, Nos. 1∼3 50D—Age of 50 days
d. {J♀ × (EN-250♀ × J♂) ♂} ♀ × J♂, Nos. 1 and 2 M—Climbing out of water

bud, a large majority of the embryos obtained from mating No. 1 died of various abnormalities, such as malformation or shrinkage of the body, microcephaly or acephaly. Most of the embryos obtained from mating No. 3 died of various abnormalities such as malformation of the body accompanied with blisters on the surfaces, acephaly, microcephaly or dicephaly at the embryonic stage. Eventually, 16.1% and 12.8% hatched normally. Many of the hatched tadpoles afterwards became edematous or underdeveloped, while 7.3% and 9.1% became normally metamorphosed frogs.

ii) {J♀ × (J♀ × SN-150♂) ♂} ♀ × J♂, Nos. 1∼4

Four female second-generation offspring derived from spermatozoa exposed to 150 rads of neutrons were mated with the same four males as those used in the control series, after their ovulation was accelerated by pituitary transplantation. The survival curve of the third-generation offspring produced from each of them is shown in Fig. 39b. The eggs in one (No. 1) of four matings were similar to the controls in cleavage and developmental capacity, that is, 91.9% cleaved normally and 80.9% became normally metamorphosed frogs. Although 96.0% of the total number of eggs cleaved normally in mating No. 2, many of them gradually died of ascites since the neurula stage. In this mating, 55.6% became normally metamorphosed frogs. There were numerous abnormal cleavages in the other two matings Nos. 3 and 4, while 45.1% and 78.2% of the respective total number of eggs cleaved normally. Many of the normally cleaved eggs died of incomplete invagination at the gastrula stage and of edema at the tail-bud stage. Eventually, no normal frogs were produced from mating No. 3, while 31.3% of the total number of eggs became normally metamorphosed frogs in mating No. 4.

e. Experimental series derived from neutron-irradiated great-grandparental eggs

i) {J♀×(EN-150♀×J♂)♂}♀×J♂, Nos. 1~3

Four female second-generation offspring derived from eggs exposed to 150 rads of neutrons were transplanted with frog pituitaries; ovulation occurred in three of them. These three females were mated with the same three males as those used in the control series (Table 24, Fig. 37). The survival curve of the third-generation offspring produced from each of them is shown in Fig. 39c. From three matings Nos. 1~3, 87.9%, 82.0% and 96.0% of the respective total number of eggs cleaved normally. The normally cleaved eggs in mating No. 3 did not differ in developmental capacity from the controls; 85.1% of the total number of eggs became normally metamorphosed frogs. In mating No. 1, 12% of the total number of embryos at the tail-bud stage were dicephalous. Many other embryos afterwards were underdeveloped and died, while 61.3% of the total number of eggs became normally metamorphosed frogs. In mating No. 2, 11% of the total embryos died of ill-formation of the neural tube at the neurula stage, and a few others afterwards revealed various abnormalities and died. It was peculiar that 19 (21%) of 89 tadpoles had underdeveloped forelegs at the metamorphosis stage. Eventually, 50.4% of the total number of eggs became normally metamorphosed frogs.

ii) {J♀×(EN-250♀×J♂)♂}♀×J♂, Nos. 1 and 2

Four female second-generation offspring derived from eggs exposed to 250 rads of neutrons were transplanted with frog pituitaries, and ovulation was accelerated in two of them. These two females were mated with the same four males as those used in the control series. The survival curve of the third-generation offspring produced from each of them is shown in Fig. 39d. From two matings Nos. 1 and 2, 95.9% and 88.0% of the respective total number of eggs cleaved normally. In mating No. 1, the normally cleaved eggs did not differ from the controls in developmental capacity. In contrast, many edematous tadpoles began to appear shortly after the hatching stage in mating No. 2, and 16 (20%) of 79 tadpoles died of edema immediately before metamorphosis, while 51.6% of the total number of eggs became normally metamorphosed frogs.

2. Chromosome aberrations in normally shaped tadpoles

Chromosomal analysis was made in normally shaped 50-day-old tadpoles

TABLE 25

Chromosomal analysis of normally shaped 50-day-old tadpoles produced from females derived from irradiated grandparental sperm or eggs

Parents: Female	Parents: Male	No. of analyzed tadpoles	Number of tadpoles: With normal cells only	With abnormal cells only: Pure	With abnormal cells only: Mosaics	With normal and abnormal cells
J(J·J), No. 2	J.W70, Nos. 6∼9	21	21 (100 %)	0	0	0
J(J·SX–150), No. 3	J.W70, Nos. 6∼9	23	19 (82.6%)	1 (4.3%)	1 (4.3%)	2 (8.7%)
J(J·SX–250), No. 1	J.W70, Nos. 6∼9	20	15 (75.0%)	3 (15.0%)	1 (5.0%)	1 (5.0%)
J(EX–150·J), No. 1	J.W70, Nos. 6∼9	24	20 (83.3%)	2 (8.3%)	1 (4.2%)	1 (4.2%)
J(EX–250·J), No. 1	J.W70, Nos. 6∼9	22	16 (72.7%)	3 (13.6%)	2 (9.1%)	1 (4.5%)
J(J·SN–100), No. 4	J.W70, Nos. 6∼9	25	19 (76.0%)	4 (16.0%)	0	2 (8.0%)
J(J·SN–150), No. 1	J.W70, Nos. 6∼9	21	16 (76.2%)	3 (14.3%)	1 (4.8%)	1 (4.8%)
J(EN–150·J), No. 3	J.W70, Nos. 6∼9	23	17 (73.9%)	4 (17.4%)	1 (4.3%)	1 (4.3%)
J(EN–250·J), No. 1	J.W70, Nos. 6∼9	20	15 (75.0%)	2 (10.0%)	1 (5.0%)	2 (10.0%)

(V∼X stages) produced from eight matings of the experimental series and one mating of the control series (Table 25).

a. Control series, {J♀×(J♀×J♂)♂}♀×J♂, No. 2

Tail-tips of 26 normally shaped tadpoles produced from one (No. 2) of the four matings of the control series were examined by the squash method. Twenty-one of these tadpoles were analyzable in karyotype; all of them had no other than diploid mitoses which were normal in the number and shape of chromosomes.

b. Experimental series derived from X-irradiated great-grandparental sperm

i) {J♀×(J♀×SX–150♂)♂}♀×J♂, No. 3

The tail-tips of 26 normally shaped tadpoles produced from one (No. 3) of the four matings between four female second-generation offspring derived from spermatozoa exposed to 150 rads of X-rays and normal males were examined by the squash method. Of these tadpoles 23 were analyzable in karyotype. While 19 of them were normal diploids, the other four had abnormal mitoses solely or together with normally diploid mitoses. One consisted of abnormally diploid mitoses, in which a part of the long arm of a No. 4 chromosome was translocated to a No. 5 chromosome. Another tadpole was a mosaic of hyperdiploid ($2n+1$) and hypodiploid ($2n-1$) cells. While in the hyperdiploid mitoses there was an additional No. 11 chromosome, this chromosome was lost in the hypodiploid mitoses. The remaining two tadpoles were mosaics of normally diploid cells and abnormal ones; in addition to normally diploid mitoses, there were hypertriploid ($3n+2$) mitoses in one of them, and abnormally diploid mitoses with a ring chromosome in the other.

ii) {J♀×(J♀×SX–250♂)♂}♀×J♂, No. 1

Twenty of 26 normally shaped tadpoles produced from one (No. 1) of the three matings between three female second-generation offspring derived from spermatozoa exposed to 250 rads of X-rays and normal males were analyzable in karyotype. Fifteen of them were normal diploids. Three other tadpoles consisted solely of abnormal cells. One of them was a triploid, another was a hyperdiploid, having an additional small chromosome, and the remainder was an abnormal diploid having a chromosome with a deletion. Another tadpole was a mosaic constructed of two kinds of abnormally diploid cells, that is, diploid mitoses containing a ring chromosome and those containing a chromosome with a deletion. The remaining one tadpole was a mosaic of normally diploid cells and hyperdiploid (2n+1) cells, in which there was an additional small chromosome.

c. Experimental series derived from X-irradiated great-grandparental eggs

i) {J♀ × (EX–150♀ × J♂)♂}♀ × J♂, No. 1

Of 26 normally shaped tadpoles produced from one (No. 1) of the three matings between three female second-generation offspring derived from eggs exposed to 150 rads of X-rays and normal males, 24 were analyzable in karyotype. While 20 tadpoles were normal diploids, two others had nothing but abnormal mitoses. These two were an abnormal diploid and a hypodiploid (2n−1). In their mitoses, a part of a chromosome or the whole of a small chromosome was translocated to another chromosome. Another tadpole was a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) cells, in which there was an addition or a loss of a small chromosome. The remaining tadpole was a mosaic of normally diploid cells and triploid cells.

ii) {J♀ × (EX–250♀ × J♂)♂}♀ × J♂, No. 1

Twenty-two of 26 normally shaped tadpoles produced from one (No. 1) of the two matings between two female second-generation offspring derived from eggs exposed to 250 rads of X-rays and normal males were analyzable in karyotype. Sixteen of them were normal diploids. Three of the other six tadpoles had nothing but abnormal mitoses; one was a hyperdiploid (2n+1), whose mitoses had an additional No. 13 chromosome, and the other two were abnormal diploids, having a chromosome with a deletion. Two other tadpoles were mosaics of two kinds of abnormal cells; one consisted of a mixture of hyperdiploid (2n+1) and hypodiploid (2n−1) cells, in which there was an addition or a loss of a small chromosome, and the other consisted of a mixture of hypodiploid (2n−1) cells having a dicentric chromosome and abnormally diploid cells having a chromosome with a deletion. The remaining tadpole was a mosaic of normally diploid cells and hyperdiploid (2n+2) cells, in which there were additional No. 4 and No. 12 chromosomes.

d. Experimental series derived from neutron-irradiated great-grandparental sperm

i) {J♀ × (J♀ × SN–100♂)♂}♀ × J♂, No. 4

Of 26 normally shaped tadpoles produced from one (No. 4) of the four matings between four female second-generation offspring derived from spermatozoa exposed to 100 rads of neutrons and normal males, 25 were analyzable in karyotype. While 19 of them were normal diploids, four others had no other than abnormal mitoses. One of these four tadpoles was hypotriploid (3n−1) losing a No. 12 chromosome, two others were abnormal diploids having a chromosome with a deletion, and the remainder was a hypodiploid (2n−1), in which a No. 13 chromosome was translocated to a No. 3 chromosome. The other two tadpoles were mosaics of normally diploid cells and abnormal ones; one had triploid mitoses and the other had hypodiploid (2n−1) mitoses losing a No. 12 chromosome, in addition to normal diploid cells.

ii) {J♀ × (J♀ × SN–150♂)♂}♀ × J♂, No. 1

Twenty-one of 26 normally shaped tadpoles produced from one (No. 1) of the four matings between four female second-generation offspring derived from spermatozoa exposed to 150 rads of neutrons and normal males were analyzable in karyotype. Sixteen of them were normal diploids. Three others had nothing but abnormal mitoses; one was a hyperdiploid (2n+1) with an additional small chromosome, another was an abnormal diploid having a No. 5 chromosome with a deletion, and the other was an abnormal diploid having a ring chromosome. Another tadpole was a mosaic of hypodiploid (2n−1) cells having a dicentric chromosome and abnormally diploid cells having a chromosome with a deletion. The remaining tadpole was a mosaic of normally diploid cells and hypodiploid (2n−1) cells, in which a small chromosome was lost.

e. Experimental series derived from neutron-irradiated great-grandparental eggs

i) {J♀ × (EN–150♀ × J♂)♂}♀ × J♂, No. 3

Of 26 normally shaped tadpoles produced from one (No. 3) of the three matings between three female second-generation offspring derived from eggs exposed to 150 rads of neutrons and normal males, 23 were analyzable in karyotype. Seventeen of these tadpoles were normal diploids. Four of the other six had nothing but abnormal mitoses; one was a triploid, another a hypertriploid (3n+1), still another an abnormal diploid, in which a part of a chromosome was translocated to another chromosome, and the remainder an abnormal diploid, having a chromosome with a deletion. One of the remaining two tadpoles was a mosaic of hyperdiploid (2n+1) and hypodiploid cells, in which there was an addition or a loss of a No. 13 chromosome. The other tadpole was a mosaic of normally diploid cells and hypodiploid (2n−1) cells, in which a No. 7 chromosome was translocated to a No. 5 chromosome.

ii) {J♀ × (EN–250♀ × J♂)♂}♀ × J♂, No. 1

Twenty of 26 normally shaped tadpoles produced from one (No. 1) of the two matings between two female second-generation offspring derived from eggs exposed to 250 rads of neutrons and normal males. Fifteen of them were normal diploids. Three other tadpoles had nothing but abnormal mitoses; one of them was a triploid, another an abnormal diploid having a ring chromosome, and the remainder a mosaic of hyperdiploid (2n+1) and hypodiploid (2n−1) cells. The remaining two tadpoles were mosaics of normally diploid cells and abnormal ones; one had triploid mitoses and the other had abnormally diploid mitoses containing a dicentric chromosome and a fragment, in addition to normally diploid mitoses.

3. Viability and sex of metamorphosed frogs

a. Viability

The age of tadpoles at the time of climbing out of water and the body length of the frogs immediately after metamorphosis in the experimental and the control series are presented in Table 26. While the age of 402 control tadpoles was 83∼124 days, 96.4 days on the average, that of 114∼232 tadpoles in the eight experimental series was 82∼126 days, 95.1∼97.1 days on the average in each series. While the frogs immediately after metamorphosis in the control series were 19.3±0.2 mm in body length, those of the eight experimental series were 18.6±0.2∼19.5±0.2 mm. In other words, the frogs in the experimental series scarcely differed from the controls in age at the time of metamorphosis as well as in body length immediately after metamorphosis. As can be seen in the table, there were also no distinct differences in these two respects between the frogs derived from X-irradiated sperm or eggs and those derived from neutron-irradiated sperm or eggs, or between the frogs derived from sperm or eggs exposed to different

TABLE 26

Number, size and sex of metamorphosed frogs produced from

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed frogs	Mean body length of all frogs immediately after metamorphosis (mm)	
Female	Male				No. of frogs
J(J·J), Nos. 1∼4	J.W70, Nos. 6∼9	83∼124 (96.4)	402	19.3±0.2	323
J(J·SX−150), Nos. 1∼4	J.W70, Nos. 6∼9	83∼126 (97.1)	232	19.2±0.2	178
J(J·SX−250), Nos. 1∼3		84∼125 (96.3)	173	19.4±0.2	132
J(EX−150·J), Nos. 1∼3	J.W70, Nos. 6∼9	82∼123 (96.7)	193	19.2±0.2	146
J(EX−250·J), Nos. 1,2		82∼123 (95.1)	114	18.7±0.2	83
J(J·SN−100), Nos. 1∼4	J.W70, Nos. 6∼9	82∼123 (95.2)	229	19.1±0.2	175
J(J·SN−150), Nos. 1∼4		84∼124 (95.6)	225	18.9±0.2	174
J(EN−150·J), Nos. 1∼3	J.W70, Nos. 6∼9	83∼126 (96.6)	232	18.6±0.2	181
J(EN−250·J), Nos. 1,2		82∼121 (95.2)	150	19.5±0.2	110

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

dosages of X-rays or neutrons.

About one month after metamorphosis, 50 frogs were removed from each mating group of third-generation offspring in which the karyotype analysis of normally shaped tadpoles had been made, and continuously reared until sexual maturity. However, from each of four mating groups which had less than 50 frogs, 30 or 40 frogs were removed and reared to make them sexually mature, as presented in Table 26. In the control series, 35 (70%) of 50 frogs attained sexual maturity in the next breeding season, while the other 15 died at the immature stage. In contrast, more than half the number of frogs did not attain sexual maturity in the experimental series. The frogs derived from gametes exposed to larger dosages of X-rays or neutrons were far lower in viability during the young frog stage than those derived from gametes exposed to smaller dosages. While 48%, 40%, 35% and 22% of the frogs in the four series derived from gametes exposed to smaller dosages attained sexual maturity, only 30%, 30%, 23% and 13% of the frogs in the other four series derived from gametes exposed to larger dosages did so. The frogs derived from X- or neutron-irradiated eggs were somewhat lower in viability than those derived from X- or neutron-irradiated spermatozoa, as only 13～35% of the frogs attained sexual maturity in the former series, while 30～48% of the frogs did so in the latter series. On the other hand, the frogs derived from eggs or spermatozoa irradiated with neutrons were slightly lower in viability than those derived from eggs or sperma-

females derived from irradiated grandparental sperm or eggs, I

Sex of frogs dead or killed about one month after metamorphosis					No. of frogs removed and reared	Sex of mature frogs			Sex of all frogs examined		
♀$_N$	♀$_U$	⚥	♂$_N$	♂ (%)*		No. of frogs	♀	♂	Total	♀	♂ (%)*
159	0	0	164	(50.8)	50	35	18	17	358	177	181 (50.6)
47	2	3	126	(72.5)	50	24	6	18	202	55	147 (72.8)
44	3	4	81	(64.4)	40	12	4	8	144	51	93 (64.6)
73	1	2	70	(49.3)	40	14	4	10	160	78	82 (51.3)
39	1	2	41	(51.8)	30	7	2	5	90	42	48 (53.3)
66	1	12	96	(61.7)	50	20	8	12	195	75	120 (61.5)
64	2	7	101	(62.1)	50	15	4	11	189	70	119 (63.0)
80	1	1	99	(55.2)	50	11	5	6	192	86	106 (55.2)
57	1	0	52	(47.3)	40	5	2	3	115	60	55 (47.8)

⚥—Hermaphrodites ♂$_N$—Males with normal testes * Including hermaphrodites

tozoa irradiated with X-rays, as 13 ~ 40% of the frogs attained sexual maturity in the former series, while 23 ~ 48% of the frogs did so in the latter series.

b. Sex

The sex of immature frogs examined within one month after metamorphosis in the experimental and the control series is presented in Table 26. In the control series, there was nearly an equal number of males and females in each of the four matings Nos. 1 ~ 4, and moreover, there was neither female with underdeveloped ovaries nor hermaphrodite. The ovaries of all the females were filled with growing auxocytes. In contrast with the control series, there were remarkably more males than females in each of the four experimental series derived from X- or neutron-irradiated great-grandparental sperm. Moreover, a few juvenile hermaphrodites as well as a few females with underdeveloped ovaries were always found in these series.

i) Experimental series, {J♀ × (J♀ × SX-150♂)♂}♀ × J♂

Of 178 frogs produced from four matings Nos. 1 ~ 4, 49 were females, 3 hermaphrodites and 126 males. Two of the females had underdeveloped ovaries, which contained no growing auxocytes in spite of the existence of numerous germ cells. In mating No. 1, there were 31 females, 2 hermaphrodites and 64 males. Seventeen frogs from mating No. 2 were all males. In mating No. 3, there were 13 females, one hermaphrodite and 38 males. Fifty other frogs from this mating were continuously reared to make them sexually mature. Of 12 frogs from mating No. 4, 5 were females and 7 males. One female from each of Nos. 1 and 3 had underdeveloped ovaries.

ii) Experimental series, {J♀ × (J♀ × SX-250♂)♂}♀ × J♂

Of 132 frogs produced from three matings Nos. 1 ~ 3, 47 were females, 4 hermaphrodites and 81 males. Fifty frogs from mating No. 1 were continuously reared to make them sexually mature. Of 37 other frogs from this mating, 18 were females, one a hermaphrodite and 18 males, that is, there was no difference in number between males and females. Nearly the same results were found among the frogs from mating No. 3; there were 13 females, 2 hermaphrodites and 9 males. In contrast, in mating No. 2, there were 16 females, one hermaphrodite and 54 males. One female from each of these three matings had underdeveloped ovaries.

iii) Experimental series, {J♀ × (J♀ × SN-100♂)♂}♀ × J♂

Of 175 frogs produced from four matings Nos. 1 ~ 4, 67 were females, 12 hermaphrodites and 96 males. There were quite or nearly an equal number of males and females in two matings Nos. 1 and 3, while male preponderance was found in the other two Nos. 2 and 4. Among 10 frogs from mating No. 1, there were 5 females and 5 males. Among 15 frogs from No. 3, there were 7 females, 2 hermaphrodites and 6 males. In contrast with these matings, among 89 frogs from No. 2, there were 31 females, 7 hermaphrodites and 51 males. From mating No. 4, 50 frogs were removed and continuously reared to make them sexually mature. Of 61 other frogs, 24 were females, 3 hermaphrodites and

34 males. Only a female from mating No. 3 had underdeveloped ovaries.

iv) Experimental series, {J♀ × (J♀ × (SN–150♂) ♂} ♀ × J♂

No metamorphosed frogs were obtained from one (No. 3) of the four matings. Fifty of 111 frogs from mating No. 1 were continuously reared to make them sexually mature, while the sex of the other 60 was examined at once; 22 were females, 2 hermaphrodites and 36 males. Among 68 frogs from mating No. 2, there were 20 females, 5 hermaphrodites and 43 males. From mating No. 4, nearly an equal number of males and females were produced; 24 were females and 22 were males. Two females from No. 2 had underdeveloped ovaries.

In each of the four experimental series derived from X- or neutron-irradiated great-grandparental eggs, there were nearly an equal number of males and females. Moreover, juvenile hermaphrodites and females with underdeveloped ovaries were fewer than those among the offspring derived from irradiated great-grandparental sperm, although there were a few of them, in contrast with the state in the control series.

The sex of mature frogs in the experimental and the control series is presented in Table 26. While there were 18 females and 17 males in the control series, males were always more numerous than females in each of the eight experimental series. It was remarkable that such male preponderance was also found among the offspring derived from X- or neutron-irradiated eggs, differing from the state found among the young frogs within one month after metamorphosis in the same experimental series. While there was an equal number of males and females besides one hermaphrodite among the offspring obtained from mating No. 1 in the experimental series, {J♀ × (J♀ × SX–250♂) ♂} ♀ × J♂, 12 mature frogs obtained from the same mating consisted of 4 females and 8 males. The male preponderance among the mature frogs obtained from the other three matings between female second-generation offspring derived from X- or neutron-irradiated spermatozoa and normal males, was also found among the young frogs from the same matings, as stated above.

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

1. Developmental capacity

In the breeding season of 1971, male second-generation offspring derived from X- or neutron-irradiated sperm or eggs were mated with normal females collected from the field. The male second-generation offspring were those which had been produced in 1970 from male first-generation offspring by mating with normal field-caught males (Tables 27, 28). They were divided into eight experimental series in accordance with their origin. In each series, two to seven male second-generation offspring were mated with each of three or four normal females by artificial fertilization. The developmental capacity of the third-generation offspring in each of the eight experimental series and the control is presented in Table 29 and shown in Figs. 40～42.

TABLE 27

Eggs of normal 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)
J.W71	1	49.0	1417	1.62±0.02
	2	47.0	1564	1.58±0.02
	3	47.0	1215	1.52±0.02
	4	47.5	1702	1.41±0.01
	5	50.0	1345	1.67±0.02
	6	49.5	1636	1.53±0.01
	7	48.0	1329	1.54±0.02
	8	45.0	1578	1.42±0.01
	9	46.5	1054	1.48±0.02
	10	47.0	1137	1.56±0.02
	11	47.5	1206	1.47±0.02
	12	48.0	1456	1.41±0.02
	13	49.0	1382	1.54±0.02
	14	47.0	1451	1.49±0.02
	15	47.5	1362	1.52±0.02

TABLE 28

Testes of male parents used for mating experiments in 1971

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure (Type)
				Left (mm)	Right (mm)	
(J·J)J, No. 2	1	1	32.0	1.5×1.0	1.5×1.0	1
	2	1	30.0	2.0×1.5	2.0×1.5	1
	3	1	31.5	2.5×2.0	2.5×2.0	1
	4	1	28.5	2.0×1.5	2.0×1.5	1
	5	1	29.5	3.0×2.0	3.0×2.0	1
(J·SX–150)J, No. 3	1	1	32.5	2.5×1.5	2.5×1.5	1
	2	1	34.0	2.5×2.0	2.5×2.0	1
	3	1	30.5	2.0×1.0	2.0×1.0	1
	4	1	35.0	3.0×2.5	3.0×2.0	2
	5	1	34.0	3.0×2.0	3.5×2.5	4
	6	1	36.5	3.5×1.5	3.5×1.5	4
(J·SX–250)J, No. 1	1	1	30.5	2.0×1.5	2.0×1.5	1
	2	1	33.0	2.5×3.0	2.5×1.0	1
	3	1	30.0	3.0×1.5	3.0×1.5	1
	4	1	36.0	3.5×2.0	3.5×2.0	1
	5	1	37.0	3.0×2.0	3.0×2.0	1
	6	1	29.0	2.5×1.5	2.0×1.5	1
(EX–150·J)J, No. 3	1	1	29.0	2.0×1.5	2.0×1.5	1
	2	1	32.5	2.5×2.0	2.5×1.5	1
	3	1	34.5	3.5×2.5	3.5×2.5	1
	4	1	33.0	3.5×2.5	3.5×2.0	3
(EX–250·J)J, No. 1	1	1	36.0	3.5×2.5	3.5×2.0	1
	2	1	34.5	3.5×2.0	3.0×3.0	1
	3	1	30.0	3.0×2.0	3.0×1.5	3
(J·SN–100)J, No. 4	1	1	28.5	2.0×1.5	2.0×1.5	1
	2	1	30.0	2.0×1.5	2.0×1.5	1
	3	1	32.5	3.0×1.5	3.0×2.0	1
	4	1	29.0	3.0×1.5	3.0×1.5	1
	5	1	36.0	4.0×2.0	4.0×2.0	1

Continued

Kind	Individual no.	Age (year)	Body length (mm)	Size of testes Left (mm)	Size of testes Right (mm)	Inner structure (Type)
(J·SN–150)J, No. 1	1	1	35.0	3.0×2.0	3.0×1.5	1
	2	1	36.0	3.0×2.0	3.0×2.0	1
	3	1	29.0	2.5×2.0	2.5×2.0	1
	4	1	37.0	4.0×3.0	2.0×1.0	4
	5	1	35.0	3.0×2.0	3.0×2.0	3
	6	1	28.5	2.0×1.5	2.0×1.5	4
	7	1	32.5	3.5×3.0	3.5×2.5	5
(EN–150·J)J, No. 2	1	1	33.0	3.0×2.0	3.0×2.0	1
	2	1	30.5	2.5×2.0	3.0×2.0	3
(EN–250·J)J, No. 1	1	1	30.0	2.5×2.0	2.5×2.0	1
	2	1	29.0	2.5×1.5	2.0×1.5	2
	3	1	34.0	3.0×2.0	3.0×2.0	1
	4	1	37.0	4.0×3.5	4.0×3.0	5
	5	1	34.5	3.5×2.0	3.0×2.0	1
	6	1	32.0	3.0×1.5	3.0×1.5	2

(J·J)J, No. 2: Males obtained by (J·J) ♀, No. 2×J.W70♂, No. 2

(J·SX–150 or –250)J, No. 3 or 1: Males obtained by (J·SX–150 or –250) ♀, No. 3 or 1×J.W70♂, No. 3 or 1

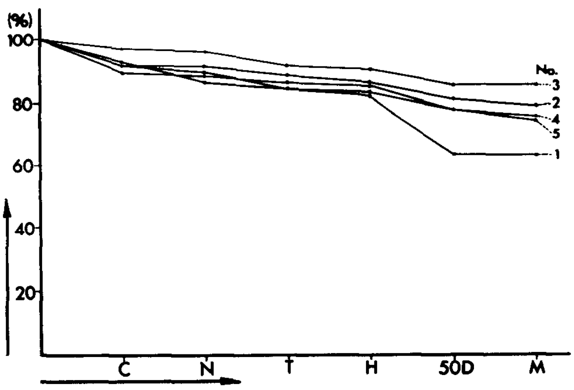
(EX–150 or –250·J)J, No. 3 or 1: Males obtained by (EX–150 or –250·J) ♀, No. 3 or 1×J.W70♂, No. 3 or 1

(J·SN–100 or –150)J, No. 4 or 1: Males obtained by (J·SN–100 or –150) ♀, No. 4 or 1×J.W70♂, No. 4 or 1

(EN–150 or –250·J)J, No. 2 or 1: Males obtained by (EN–150 or –250·J) ♀, No. 2 or 1×J.W70♂, No. 2 or 1

a. Control series, J♀×{(J♀×J♂)♀×J♂}♂, Nos. 1∼5

Fifteen normal females Nos. 1∼15 collected from the field were mated with five males Nos. 1∼5 which had been reared in our laboratory (Table 29, Fig. 57). These males were second-generation offspring produced from a female first-generation offspring by mating with a field-caught male. The female first-generation offspring was one of those which had been obtained by mating of field-caught males and females as the controls of the offspring raised from X- or neutron-irradiated spermatozoa or eggs. The survival curve of the offspring produced from each of the five males is shown in Fig. 40.



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

Fig. 40. Survival curves of control third-generation offspring derived from untreated great-grandparental gametes by matings, J♀×{(J♀×J♂)♀×J♂}♂, Nos. 1∼5.

TABLE 29

Developmental capacity of the offspring of males derived

Parents		No. of eggs	No. of cleaved eggs	
Female	Male		Normal	Abnormal
J.W71, Nos. 1～15	(J·J)J, Nos. 1～5	2303	2102 (91.3%)	12 (0.5%)
J.W71, Nos. 1～4	(J·SX–150)J, Nos. 1～6	2310	1376 (59.6%)	32 (1.4%)
	(J·SX–250)J, Nos. 1～6	2803	2330 (83.1%)	9 (0.3%)
J.W71, Nos. 5～7	(EX–150·J)J, Nos. 1～4	1541	957 (62.1%)	19 (1.2%)
	(EX–250·J)J, Nos. 1～3	1052	698 (66.3%)	12 (1.1%)
J.W71, Nos. 8～11	(J·SN–100)J, Nos. 1～5	2587	2213 (85.5%)	14 (0.5%)
	(J·SN–150)J, Nos. 1～7	2181	1408 (64.6%)	16 (0.7%)
J.W71, Nos. 12～15	(EN–150·J)J, Nos. 1,2	736	432 (58.7%)	0
	(EN–250·J)J, Nos. 1～6	2005	1468 (73.2%)	22 (1.1%)

In five matings Nos. 1～5, 89.1～97.1%, 91.3% on the average, of the respective total number of eggs cleaved normally. However, a small number of normally cleaved eggs died of edema, blisters, or curvature or shrinkage of the body at various embryonic stages, while 82.3～89.9%, 85.0% on the average, hatched normally. A few individuals gradually died of edema at the tadpole stage, although 99 of 423 tadpoles produced from mating No. 1 died mostly of ill-development at the age of about 50 days. Eventually, 63.0～84.8%, 75.2% on the average, of the respective total number of eggs became normally metamorphosed frogs.

b. Experimental series derived from X-irradiated great-grandparental sperm

i) J♀ × {(J♀ × SX–150♂)♀ × J♂}♂, Nos. 1～6

The same four normal females Nos. 1～4 as those used in the control series were mated with six male second-generation offspring Nos. 1～6, derived from spermatozoa exposed to 150 rads of X-rays (Table 29, Fig. 58). The survival curve of the offspring produced from each of the six males is shown in Fig. 41a.

There were great differences in the percentage of normally cleaved eggs among six matings; 89.1%, 93.8%, 90.8%, 59.2%, 5.4% and 0.9% of the respective total number of eggs cleaved normally in matings Nos. 1～6. While the eggs in mating No. 6 all died of abnormalities by the tail-bud stage, the normally cleaved eggs in the other five matings mostly developed normally, and only a small number of them died of various abnormalities as found in the controls. In matings Nos. 1～5, 80.3%, 90.0%, 83.4%, 51.0% and 2.3% hatched nor-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		
2066	33	1990	76	1957	33	1765	1733
(89.7%)	(1.4%)	(86.4%)	(3.3%)	(85.0%)	(1.4%)	(76.6%)	(75.2%)
1347	49	1305	42	1265	40	1200	960
(58.3%)	(2.1%)	(56.5%)	(1.8%)	(54.8%)	(1.7%)	(51.9%)	(41.6%)
2280	50	2200	80	2085	115	1944	1684
(81.3%)	(1.8%)	(78.5%)	(2.9%)	(74.4%)	(4.1%)	(69.4%)	(60.1%)
918	39	869	49	784	85	719	620
(59.6%)	(2.5%)	(56.4%)	(3.2%)	(50.9%)	(5.5%)	(46.7%)	(40.2%)
660	38	632	28	506	126	485	446
(62.7%)	(3.6%)	(60.1%)	(2.7%)	(48.1%)	(12.0%)	(46.1%)	(42.4%)
2071	92	1952	119	1895	57	1828	1613
(80.1%)	(3.6%)	(75.5%)	(4.6%)	(73.3%)	(2.2%)	(70.7%)	(62.4%)
1330	78	1299	31	1278	21	1026	920
(61.0%)	(3.6%)	(59.6%)	(1.4%)	(58.6%)	(1.0%)	(47.0%)	(42.2%)
417	15	404	13	398	6	378	331
(56.7%)	(2.0%)	(54.9%)	(1.8%)	(54.1%)	(0.8%)	(51.4%)	(45.0%)
1378	90	1329	49	1303	26	1249	1127
(68.7%)	(4.5%)	(66.3%)	(2.4%)	(65.0%)	(1.3%)	(62.3%)	(56.2%)

mally, respectively. Until the age of 50 days, the normally cleaved eggs from the five matings did not differ in developmental capacity from the controls. However, shortly before metamorphosis, comparatively numerous tadpoles, 94 of 422 from mating No. 1, 34 of 367 from mating No. 2 and 84 of 260 from mating No. 3, suffered from extremely swollen hind legs and an edematous abdomen and died soon afterwards. In mating No. 4, a small number of tadpoles died of edema during the tadpole stage. While 80.0% of the total number of eggs became normally metamorphosed frogs in mating No. 2, 59.1%, 52.2% and 40.4% did so in matings Nos. 1, 3 and 4, respectively. No frogs were produced from mating No. 5. On the average, 41.6% of the total number of eggs became normal frogs from the six matings.

ii) J♀ × {(J♀ × SX–250♂)♀ × J♂}♂, Nos. 1 ~ 6

The four normal females Nos. 1 ~ 4 were mated with six male second-generation offspring Nos. 1 ~ 6 derived from spermatozoa exposed to 250 rads of X-rays (Table 29, Fig. 58). The survival curve of the offspring produced from each of the six males is shown in Fig. 41b. In six matings Nos. 1 ~ 6, 69.1 ~ 99.2%, 83.1% on the average, of the respective total number of eggs cleaved normally. About one-fourth of the normally cleaved eggs in mating No. 1 became suddenly edematous at the tail-bud and hatching stages, and 70.9% hatched normally, although this mating was the highest in the percentage of normally cleaved eggs among the six matings. A small number of the hatched tadpoles became gradually edematous, and 66.7% of the total number of eggs attained the completion of metamorphosis. Mating No. 2 was the lowest in the percentage of

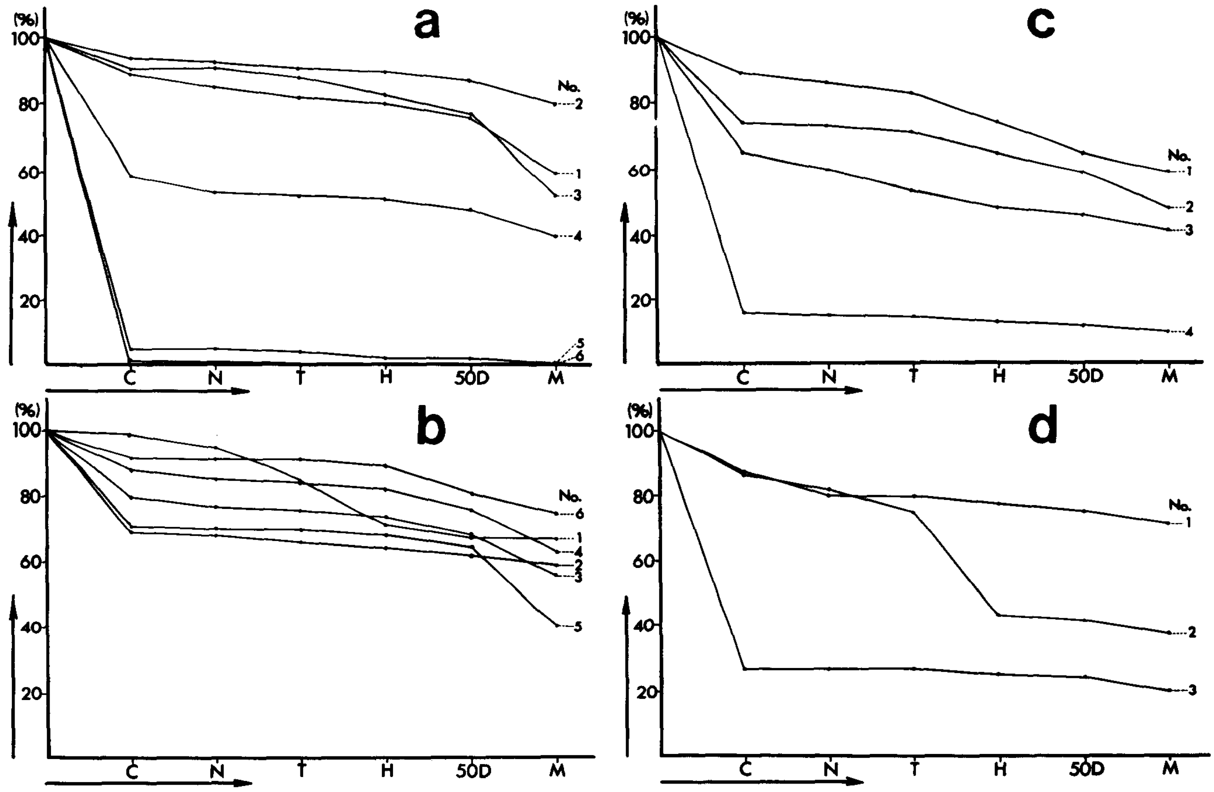


Fig. 41. Survival curves of third-generation offspring derived from X-irradiated great-grandparental gametes by passing over female first- and male second-generation offspring.

a. J♀ × {(J♀ × SX–150♂)♀ × J♂}♂, Nos. 1~6
b. J♀ × {(J♀ × SX–250♂)♀ × J♂}♂, Nos. 1~6
c. J♀ × {(EX–150♀ × J♂)♀ × J♂}♂, Nos. 1~4
d. J♀ × {(EX–250♀ × J♂)♀ × J♂}♂, Nos. 1~3

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

normally cleaved eggs. A small number of the normally cleaved eggs afterwards died of edema, blisters or curvature or shrinkage of the body, while 64.4% hatched normally. During the tadpole stage, some of them died of ill-development or edema, and 59.3% of the total number of eggs became normally metamorphosed frogs. The normally cleaved eggs from the other matings Nos. 3, 4, 5 and 6 developed as normally as the controls did until the hatching stage. However, a small number of the hatched tadpoles became gradually edematous and died since they began to eat, and 56.5%, 62.6%, 40.3% and 75.2%, 60.1% on the average, of the respective total number of eggs became normally metamorphosed frogs in matings No. 3, 4, 5 and 6. It was remarkable that 13.0% and 23.7% became normal, metamorphosing frogs with underdeveloped forelegs in matings Nos. 4 and 5, respectively.

c. Experimental series derived from X-irradiated great-grandparental eggs

i) J♀ × {(EX–150♀ × J♂)♀ × J♂}♂, Nos. 1~4

The same three normal females Nos. 5~7 as those used in the control series were mated with four male second-generation offspring derived from eggs exposed to 150 rads of X-rays (Table 29, Fig. 58). The survival curve of the offspring produced from each of the four males is shown in Fig. 41c. In four matings Nos. 1∼4, 89.4%, 73.8%, 64.7% and 15.8%, 62.1% on the average, of the respective total number of eggs cleaved normally. In these matings, about one-third of the normally cleaved eggs died of edema, blisters, curvature, underdevelopment of the body or some other abnormalities at various embryonic and tadpole stages. Especially in mating No. 2, 49 of 252 tadpoles did not protrude their forelegs. In these four matings, 59.1%, 47.9%, 40.9% and 10.2%, 40.2% on the average, of the respective total number of eggs attained the completion of metamorphosis.

ii) J♀ × {(EX–250♀ × J♂)♀ × J♂}♂, Nos. 1∼3

The three normal females Nos. 5∼7 were mated with three male second-generation offspring Nos. 1∼3 derived from eggs exposed to 250 rads of X-rays (Table 29, Fig. 58). The survival curve of the third-generation offspring produced from each of the three males is shown in Fig. 41d. In three matings Nos. 1∼3, 86.6%, 85.6% and 27.4%, 66.3% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs in matings Nos. 1 and 3 did not differ in developmental capacity from the controls; 71.4% and 20.3% became normally metamorphosed frogs. In mating No. 2, about half of the normally cleaved eggs became edematous and died from the neurula to hatching stage. Especially at the later embryonic stage, 115 of 271 embryos suffered from edema and died at the same time. Although the other embryos became normal tadpoles, a small number of them also died of edema afterwards; 134 tadpoles, 37.0% of the total number of eggs, attained completion of metamorphosis. In the three matings (Nos. 1∼3), 42.4% of the total number of eggs became normal frogs.

d. Experimental series derived from neutron-irradiated great-grandparental sperm

i) J♀ × {(J♀ × SN–100♂)♀ × J♂}♂, Nos. 1∼5

The same four normal females Nos. 8∼11 as those used in the control series were mated with five male second-generation offspring derived from spermatozoa exposed to 100 rads of neutrons (Table 29, Fig. 59). The survival curve of the offspring produced from each of the five males is shown in Fig. 42a. In five matings Nos. 1∼5, 77.9∼91.4%, 85.5% on the average, of the respective total number of eggs cleaved normally. However, a small number of the normally cleaved eggs in mating No. 1 died of incomplete invagination at the gastrula stage and some others died afterwards of various abnormalities, while 66.7% of the total number of eggs became normally metamorphosed frogs. Although more than 90% of eggs cleaved normally in matings Nos. 2 and 5, a comparatively large number of normally cleaved eggs died of edema at various developmental stages, mainly at the embryonic stage from the neurula to the tail-bud as well as immediately before metamorphosis; eventually, 57.1% and 60.8% became normally metamorphosed frogs, respectively. In mating No. 3 which was the

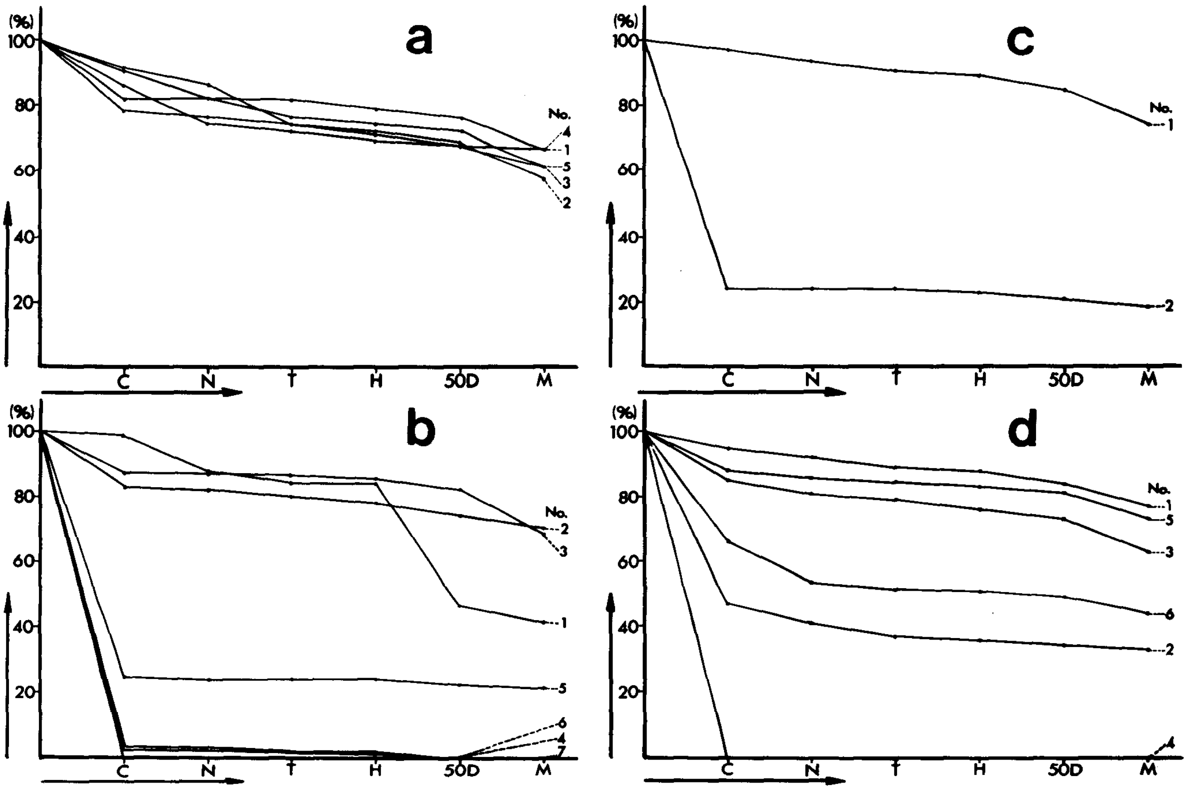


Fig. 42. Survival curves of third-generation offspring derived from neutron-irradiated great-grandparental gametes by passing over female first- and male second-generation offspring.

a. J♀ × {(J♀ × SN-100♂)♀ × J♂}♂, Nos. 1∼5
b. J♀ × {(J♀ × SN-150♂)♀ × J♂}♂, Nos. 1∼7
c. J♀ × {(EN-150♀ × J♂)♀ × J♂}♂, Nos. 1 and 2
d. J♀ × {(EN-250♀ × J♂)♀ × J♂}♂, Nos. 1∼6

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

lowest in the percentage of normal cleavages, the eggs did not differ in developmental capacity from the controls. After a small number of eggs died of various abnormalities at various developmental stages, 60.9% became normally metamorphosed frogs. In mating No. 4, all the normally cleaved eggs, that is, 81.8% of the total number of eggs hatched normally. However, about one-fifth of the tadpoles gradually died of edema, while 60.8% of the total number of eggs attained completion of metamorphosis. In the five matings (Nos. 1∼5) 62.4% of the total number of eggs became normal frogs.

ii) J♀ × {(J♀ × SN-150♂)♀ × J♂}♂, Nos. 1∼7

The normal females Nos. 8∼11 were mated with seven male second-generation offspring Nos. 1∼7 derived from spermatozoa exposed to 150 rads of neutrons (Table 29, Fig. 59). The survival curve of the third-generation offspring produced from each of the seven males is shown in Fig. 42b. While in matings Nos. 1∼3, 99.3%, 83.2% and 87.4%, and in mating No. 5, 25.0% of the respective total number of eggs cleaved normally, only a few eggs did so at the most in the other matings. In the seven matings, 64.6% of the total number of eggs cleaved normally. In mating No. 1, a small number of normally cleaved eggs died of edema or blisters; 67 of 565 died at the neurula, 19 at the tail-bud and 4 at the hatching stage, while 475, that is, 83.5% of the total number of eggs hatched normally and became tadpoles. However, 214 of the latter did not normally grow and gradually died of ill-development by the age of 50 days. While 234 tadpoles, 41.1% of the total number of eggs, became normally metamorphosed frogs, the other 27 did not complete metamorphosis for several months. In mating No. 2, normally cleaved eggs did not differ from the controls in developmental capacity; 70.8% became normally metamorphosed frogs. In mating No. 3, the normally cleaved eggs did not differ from the control in developmental capacity, except that 59 of 366 tadpoles had underdeveloped forelegs at the metamorphosis stage. Eventually, 307 tadpoles, 69.1% of the total number of eggs, became normally metamorphosed frogs. In matings Nos. 4, 6 and 7, all the cleaved eggs died before metamorphosis, while in mating No. 5, 21.4% became normally metamorphosed frogs, although the percentage of normal cleavages was very low. In the seven matings (Nos. 1~7), 42.2% of the total number of eggs became normal frogs.

e. Experimental series derived from neutron-irradiated great-grandparental eggs

i) J♀×{(EN-150♀×J♂)♀×J♂}♂, Nos. 1 and 2

The same four normal females Nos. 12~15 as those used in the control series were mated with two male second-generation offspring Nos. 1 and 2 derived from eggs exposed to 150 rads of neutrons (Table 29, Fig. 59). The survival curve of the offspring produced from each of the two males is shown in Fig. 42c. In two matings Nos. 1 and 2, 97.4% and 24.0%, 58.7% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs scarcely differed in developmental capacity from the controls; 73.9% and 19.1%, 45.0% on the average, of the respective total number of eggs became normal, metamorphosed frogs. Besides, 39 of 296 tadpoles produced from mating No. 1 became abnormal frogs with underdeveloped forelegs.

ii) J♀×{(EN-250♀×J♂)♀×J♂}♂, Nos. 1~6

The four normal females Nos. 12~15 were mated with six male second-generation offspring Nos. 1~6 derived from eggs exposed to 250 rads of neutrons (Table 29, Fig. 59). The survival curve of the third-generation offspring produced from each of the six males is shown in Fig. 42d. In six matings Nos. 1~6, 94.9%, 46.5%, 85.0%, 0%, 88.0% and 65.6%, 73.2% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs from matings Nos. 1, 3 and 5 scarcely differed in developmental capacity from the controls, except that 36 of 458 in mating No. 1, 35 of 257 in No. 2 and 27 of 257 in No. 5 suffered from ascites and swollen hind legs immediately before metamorphosis. In these three matings, 77.4%, 62.9% and 72.6% of the respective total number of eggs became normally metamorphosed frogs. In matings Nos. 2 and 6, about one-third of the normally cleaved eggs died gradually of edema since the neurula stage, while 32.5% and 43.5% of the respective total number of eggs attained completion of metamorphosis. In six matings Nos. 1~6, 56.2% of the total number of eggs became normal frogs.

2. Viability and sex of metamorphosed frogs

a. Viability

The age of tadpoles at the time of climbing out of water and the body length of frogs immediately after metamorphosis in eight experimental and the control series are presented in Table 30. While the age of 1733 control tadpoles at the time of climbing out of water was 68~104 days, 92.9 days on the average, that of 331~1684 tadpoles was 66~126 days in the eight experimental series, 89.2~98.9 days on the average in each of the latter. While about 100 frogs which were removed at random from the control series immediately after metamorphosis were 17.5±0.2 mm in body length, those from each of the experimental series were 16.7±0.2~17.3±0.2 mm. Thus, there were no significant differences between the frogs of the experimental series and the controls in the age of metamorphosis as well as in the body length immediately after metamorphosis. There were also no remarkable differences in these respects among the frogs of the eight experimental series which differed from one another in the kind and dosage of irradiation, as presented in Table 30. About one month after metamorphosis, 57~100 frogs obtained from two or exceptionally one mating were removed from each of the experimental and the control series and reared continuously in order to make them sexually mature (Table 30). By the breeding

TABLE 30
Number, size and sex of metamorphosed frogs produced

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed frogs	Mean body length of 100 frogs immediately after metamorphosis (mm)	
Female	Male				No. of frogs
J.W71, Nos. 1~15	(J·J)J, Nos. 1~5	68~104 (92.9)	1733	17.5±0.2	424
J.W71, Nos. 1~4	(J·SX–150)J, Nos. 1~6	71~116 (98.1)	960	17.1±0.2	473
	(J·SX–250)J, Nos. 1~6	66~126 (91.6)	1684	17.0±0.2	702
J.W71, Nos. 5~7	(EX–150·J)J, Nos. 1~4	80~113 (93.9)	620	17.3±0.2	475
	(EX–250·J)J, Nos. 1~3	80~117 (94.8)	446	17.3±0.2	389
J.W71, Nos. 8~11	(J·SN–100)J, Nos. 1~5	69~111 (89.2)	1613	16.7±0.2	805
	(J·SN–150)J, Nos. 1~7	68~118 (95.1)	920	17.1±0.2	410
J.W71, Nos. 12~15	(EN–150·J)J, Nos. 1,2	80~120 (93.2)	331	17.0±0.2	254
	(EN–250·J)J, Nos. 1~6	81~118 (98.9)	1127	17.2±0.2	711

♀$_N$—Females with normal ovaries ♀$_U$—Females with underdeveloped ovaries season of the next year, 71 (89%) of 80 frogs attained sexual maturity in the control series, while the remaining frogs died. In four of the eight experimental series, 88～100% of the frogs attained sexual maturity, while 43～56% did so in the remaining. In the two experimental series derived from X-irradiated great-grandparental sperm, 88% and 95% of the respective number of frogs matured. In contrast with this, 43% and 47% of the respective number of frogs attained sexual maturity in the two experimental series derived from X-irradiated great-grandparenal eggs. In these experimental series derived from X-irradiated sperm or eggs, there was no distinct difference in the percentage of mature frogs between small and large dosages of irradiation. While 100% of the frogs derived from spermatozoa exposed to 100 rads of neutrons became sexually mature, only 45% of those derived from spermatozoa exposed to 150 rads of neutrons did so. Contrarily, 95% of the frogs derived from eggs exposed to 250 rads of neutrons became sexually mature, while 56% of those derived from eggs exposed to 150 rads of neutrons did so.

b. Sex of immature frogs

In the control series, 210 of 424 immature frogs which were killed or died within one month after metamorphosis were females, while the other 214 (50.5%) were males. One of the females had underdeveloped ovaries. There were nearly an equal number of males and females and no juvenile hermaphrodites

from males derived from irradiated grandparental sperm or eggs, II

Sex of frogs dead or killed within one month after metamorphosis					No. of frogs removed and reared	Sex of mature frogs			Sex of all frogs examined		
♀N	♀U	⚥	♂N	♂ (%)*		No. of frogs	♀	♂	Total	♀	♂ (%)*
209	1	0	214	(50.5)	80	71	39	32	495	249	246 (49.7)
196	6	9	262	(57.3)	60	53	21	32	526	223	303 (57.6)
276	42	33	351	(54.7)	60	57	22	35	759	340	419 (55.2)
241	20	11	203	(45.1)	60	26	13	13	501	274	227 (45.3)
190	10	14	175	(48.6)	57	27	10	17	416	210	206 (49.5)
385	2	4	414	(51.9)	100	100	48	52	905	435	470 (51.9)
132	26	27	225	(61.5)	60	27	13	14	437	171	266 (60.9)
116	1	2	135	(53.9)	75	42	21	21	296	138	158 (53.4)
311	11	15	374	(54.7)	60	56	25	31	767	347	420 (54.8)

⚥—Hermaphrodites ♂N—Males with normal testes * Including hermaphrodites among the frogs obtained from each of the five matings of the control series. In contrast with the control series, comparatively numerous hermaphrodites and females with underdeveloped ovaries were found in most of the experimental series (Table 30). Moreover, there was nearly the same number of males and females in only two of the eight experimental series when hermaphrodites were counted as males.

i) Experimental series, J♀ × {(J♀ × SX–150♂)♀ × J♂}♂

Of 473 frogs produced from four matings Nos. 1∼4, 202 were females, 9 hermaphrodites and 262 males. Six of the females had underdeveloped ovaries. When the hermaphrodites were counted as males, 57.3% of the total number of frogs were males. However, this male preponderance was almost attributable to that among the frogs obtained from mating No. 4; among 118 frogs, there were 30 females with normal ovaries, 3 females with underdeveloped ovaries, 4 hermaphrodites and 81 males. Among the frogs produced from the other three matings Nos. 1∼3, there were nearly an equal number of males and females.

ii) Experimental series, J♀ × {(J♀ × SX–250♂)♀ × J♂}♂

Of 702 frogs produced from six matings Nos. 1∼6, 318 were females, 33 hermaphrodites and 351 males. Of the females, 42 had underdeveloped ovaries. When hermaphrodites were counted as males, 54.7% of the total number of frogs were males. This male preponderance was attributable to that among the frogs produced from one of the six matings. Among 195 frogs produced from mating No. 5, there were 37 females with normal ovaries, 20 females with underdeveloped ovaries, 23 hermaphrodites and 115 males. Thirty other frogs obtained from this mating were removed and continuously reared. There was no remarkable difference in number between males and females produced from each of the other five matings.

iii) Experimental series, J♀ × {(EX–150♀ × J♂)♀ × J♂}♂

Of 475 frogs produced from four matings Nos. 1∼4, 241 were females with normal ovaries, 20 females with underdeveloped ovaries, 11 hermaphrodites and 203 males. When the hermaphrodites were counted as males, 45.1% of the total number of frogs were males. The female preponderance in this series was attributable to that among the frogs produced from the mating No. 2; there were 128 females with normal ovaries, 19 females with underdeveloped ovaries, 6 hermaphrodites and 50 males among 203 frogs. However, all the 35 frogs from mating No. 4 were males. In the other two matings Nos. 1 and 3, there was no difference in number between males and females.

iv) Experimental series, J♀ × {(EX–250♀ × J♂)♀ × J♂}♂

There was nearly an equal number of males and females among the frogs produced from each of three matings Nos. 1∼3. When hermaphrodites were counted as males, 48.6% of the total number of frogs were males.

v) Experimental series, J♀ × {(J♀ × SN–100♂)♀ × J♂}♂

There was nearly an equal number of males and females among the frogs produced from five matings Nos. 1∼5. When 4 hermaphrodites were counted as males, 418 (51.9%) of 805 frogs were males.

vi) Experimental series, J♀ × {(J♀ × SN–150♂)♀ × J♂}♂

Of 410 frogs produced from four matings Nos. 1, 2, 3 and 5, 132 were females with normal ovaries, 26 females with underdeveloped ovaries, 27 hermaphrodites and 225 males. When the hermaphrodites were counted as males, 61.5% of the total number of frogs were males. This male preponderance was attributable to that among the frogs produced from matings Nos. 1 and 5. Among 232 frogs produced from mating No. 1, there were 72 females with normal ovaries, 11 females with underdeveloped ovaries, 13 hermaphrodites and 136 males, while among 70 frogs from mating No. 5, there were 15 females with normal ovaries, 5 females with underdeveloped ovaries, 9 hermaphrodites and 41 males. In the other two matings Nos. 2 and 3, there were nearly an equal number of males and females.

vii) Experimental series, J♀ × {(EN–150♀ × J♂)♀ × J♂}♂

Of 254 frogs produced from two matings Nos. 1 and 2, 117 were females, 2 hermaphrodites and 135 males. Only one of the females had underdeveloped ovaries. When the hermaphrodites were counted as males, 53.9% of the total number of frogs were males. This male preponderance was mainly attributable to that among the frogs produced from mating No. 1. Among 180 frogs produced from this mating, there were 81 females with normal ovaries, a female with underdeveloped ovaries, 2 hermaphrodites and 96 males, while among 74 frogs from the other mating, there were 35 females and 39 males.

viii) Experimental series, J♀ × {(EN–250♀ × J♂)♀ × J♂}♂

Of 711 frogs produced from six matings Nos. 1～6, 311 were females with normal ovaries, 11 females with underdeveloped ovaries, 15 hermaphrodites and 374 males. When the hermaphrodites were counted as males, 54.7% of the total number of frogs were males. This male preponderance was attributable to that among the frogs produced from mating No. 2; among 125 frogs from the latter, there were 26 females with normal ovaries, 5 females with underdeveloped ovaries, 7 hermaphrodites and 87 males. Among the frogs obtained from the other five matings, there was no remarkable difference in number between males and females.

c. Sex of mature frogs

The sex of mature frogs in the experimental and control series is presented in Table 30. In the control series, 32 (45%) of 71 frogs were males. While there was just or nearly an equal number of males and females among the frogs in four of the eight experimental series, 55～63% of the respective total number of frogs were males in the other experimental series.

Of 53 mature frogs in experimental series, J♀ × {(J♀ × SX–150♂)♀ × J♂}♂, 21 were females and 32 (60%) were males. These frogs were survivors from among 60 young frogs obtained from two matings Nos. 1 and 3, in which there was nearly an equal number of males and females at the stage immediately after metamorphosis.

In experimental series, J♀ × {(J♀ × SX–250♂)♀ × J♂}♂, there were 22 females and 35 (61%) males. These frogs were survivors from among 60 young frogs obtained from two matings Nos. 1 and 5. While there were 15 males and 15 females among 30 mature frogs obtained from mating No. 1, there were 7 females and 20 males among 27 mature frogs from mating No. 5. Such a male preponderance among the mature frogs obtained from the mating No. 5 was already found among the young frogs immediately after metamorphosis. Four of the mature males obtained from this mating had a right underdeveloped hind leg which was abnormal in skeleton. In experimental series, J♀ × {(J♀ × SN–100♂)♀ × J♂}♂, 48 of 100 mature frogs produced from matings Nos. 3 and 4 were females and the other 52 were males. Such a ratio of males and females also existed among the young frogs obtained from the same matings. A similar situation was found in three other experimental series, J♀ × {(J♀ × SN–150♂)♀ × J♂}♂, J♀ × {(EX–150♀ × J♂)♀ × J♂}♂ and J♀ × {(EN–150♀ × J♂)♀ × J♂}♂.

Of 27 mature frogs in experimental series, J♀ × {(EX–250♀ × J♂)♀ × J♂}♂, 10 were females and 17 (63%) males. These frogs were survivors from among 57 immature frogs produced from matings Nos. 1 and 3. Of these mature frogs, 4 females and 8 males were obtained from mating No. 1 and 6 females and 9 males were from mating No. 3, while there was nearly an equal number of males and females among the immature frogs produced by the same matings. In experimental series, J♀ × {(EN–250♀ × J♂)♀ × J♂}♂, 25 of 56 mature frogs obtained from matings Nos. 1 and 3 were females and the other 31 were males. Of these frogs, 5 females and 11 males were obtained from mating No. 1, while 20 females and 20 males were from mating No. 3. At the stage within one month after metamorphosis, there was nearly an equal number of males and females among the immature frogs obtained from mating No. 1. Four of the 11 mature males obtained from this mating had abnormal hind legs whose skeleton was also abnormal.

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

1. Developmental capacity

In the breeding season of 1971, female second-generation offspring derived from X- or neutron-irradiated great-grandparenal sperm or eggs were mated with normal, field-caught males (Tables 31, 32). The female second-generation offspring were produced in 1970 from female first-generation offspring by mating with normal, field-caught males. They were divided into eight experimental series in accordance with their origin. In each series, one to six females were mated with three normal, field-caught males Nos. 1 ∼ 3 by artificial fertilization. The developmental capacity of the third-generation offspring in each experimental series and the control is presented in Table 33 and shown in Figs. 43, 44 and 46.

TABLE 31

Eggs of female parents used for mating experiments in 1971, II

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 100 eggs (mm)
(J·J)J, No. 2	1	1	35.0	739	1.63±0.02
	2	1	37.0	894	1.57±0.02
	3	1	34.0	715	1.52±0.02
	4	1	37.0	967	1.66±0.02
	5	1	33.5	542	1.54±0.02
(J·SX–150)J, No. 3	1	1	35.0	806	1.42±0.02
	2	1	32.0	471	1.74±0.02
	3	1	39.0	798	1.57±0.02
	4	1	38.0	652	1.61±0.02
	5	1	36.0	684	1.53±0.02
(J·SX–250)J, No. 1	1	1	35.5	569	1.51±0.02
	2	1	36.0	770	1.50±0.02
	3	1	39.0	834	1.72±0.02
(EX–150·J)J, No. 3	1	1	36.0	531	1.47±0.01
	2	1	38.5	667	1.64±0.02
	3	1	33.5	633	1.41±0.02
	4	1	37.0	459	1.65±0.02
	5	1	36.5	574	1.67±0.02
	6	1	37.0	472	1.73±0.02
	7	1	35.0	0	—
	8	1	36.0	0	—
(EX–250·J)J, No. 1	1	1	36.5	498	1.56±0.02
	2	1	37.5	802	1.40±0.02
	3	1	34.0	637	1.48±0.02
	4	1	33.0	425	1.52±0.02
	5	1	41.0	0	—
(J·SN–100)J, No. 4	1	1	34.5	316	1.73±0.02
	2	1	36.0	579	1.64±0.02
	3	1	36.0	730	1.51±0.02
	4	1	37.0	941	1.48±0.02
	5	1	34.5	598	1.57±0.02
	6	1	35.0	422	1.63±0.02
	7	1	39.5	0	—
(J·SN–150)J, No. 1	1	1	37.5	338	1.71±0.02
(EN–150·J)J, No. 2	1	1	36.0	411	1.52±0.02
	2	1	34.5	635	1.54±0.02
	3	1	33.5	573	1.53±0.02
	4	1	37.0	792	1.62±0.02
	5	1	36.0	676	1.63±0.02
	6	1	36.0	0	—
	7	1	32.0	0	—
(EN–250·J)J, No. 1	1	1	33.5	559	1.54±0.02
	2	1	34.0	732	1.41±0.02
	3	1	34.5	796	1.52±0.02
	4	1	35.0	684	1.60±0.02
	5	1	36.0	835	1.61±0.02
	6	1	36.5	0	—
	7	1	33.0	0	—

(J·J)J, No. 2: Females obtained by (J·J) ♀, No. 2 × J.W70 ♂, No. 2

(J·SX–150 or –250)J, No. 3 or 1: Females obtained by (J·SX–150 or –250) ♀, No. 3 or 1 × J.W70 ♂, No. 3 or 1

(EX–150 or –250·J)J, No. 3 or 1: Females obtained by (EX–150 or –250·J) ♀, No. 3 or 1 × J.W70 ♂, No. 3 or 1

(J·SN–100 or –150)J, No. 4 or 1: Females obtained by (J·SN–100 or –150) ♀, No. 4 or 1 × J.W70 ♂, No. 4 or 1

(EN–150 or –250·J)J, No. 2 or 1: Females obtained by (EN–150 or –250·J) ♀, No. 2 or 1 × J.W70 ♂, No. 2 or 1

TABLE 32

Testes of normal field-caught male frogs used for mating experiments in 1971, II

Kind	Individual no.	Body length (mm)	Size of the testes		Inner structure (Type)
			Left (mm)	Right (mm)	
J.W71	1	37.5	3.0×2.0	3.0×2.0	1
	2	40.0	3.5×2.5	3.5×2.5	1
	3	39.0	3.0×2.0	3.0×2.0	1

a. Control series, {(J♀×J♂)♀×J♂}♀×J♂, Nos. 1∼5

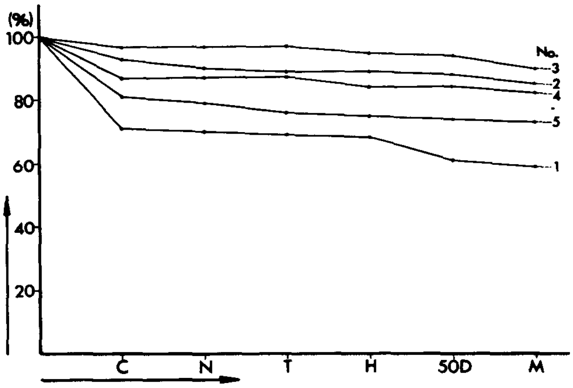
Five female second-generation offspring Nos. 1∼5 produced from a control female first-generation offspring in 1970 by mating with a field-caught male were transplanted with frog pituitaries. As normal ovulation occurred in all these females, they were mated with normal, field-caught males Nos. 1∼3. The female first-generation offspring was one of those which had been obtained from males and females collected from the field as the control of the frogs raised from X- or neutron-irradiated spermatozoa or eggs. The survival curve of the offspring produced from each of the five females is shown in Fig. 43.

By five matings Nos. 1∼5, 71.0%, 93.0%, 97.4%, 87.2% and 80.9%, 84.4% on the average, of the respective total number of eggs cleaved normally, and later 67.8%, 89.1%, 94.8%, 84.2% and 74.8%, 80.5% on the average, hatched normally. At the tadpole stage, a small number of individuals died of ill-development or edema, and eventually 58.9%, 84.5%, 90.3%, 81.6% and 73.0%, 76.1% on the average, became normally metamorphosed frogs.

TABLE 33

Developmental capacity of the offspring of females derived

Parents		No. of eggs	No. of cleaved eggs	
Female	Male		Normal	Abnormal
(J·J)J, Nos. 1∼5	J.W71, Nos. 1∼3	924	780 (84.4%)	5 (0.5%)
(J·SX–150)J, Nos. 1∼5	J.W71, Nos. 1∼3	1026	653 (63.6%)	26 (2.5%)
(J·SX–250)J, Nos. 1∼3		530	331 (62.5%)	43 (8.1%)
(EX–150·J)J, Nos. 1∼6	J.W71, Nos. 1∼3	1158	775 (66.9%)	31 (2.7%)
(EX–250·J)J, Nos. 1∼4		739	477 (64.5%)	34 (4.6%)
(J·SN–100)J, Nos. 1∼6	J.W71, Nos. 1∼3	1217	838 (68.9%)	28 (2.3%)
(J·SN–150)J, No. 1		265	170 (64.2%)	15 (5.7%)
(EN–150·J)J, Nos. 1∼5	J.W71, Nos. 1∼3	1001	607 (60.6%)	67 (6.7%)
(EN–250·J)J, Nos. 1∼5		908	666 (73.3%)	20 (2.2%)



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

Fig. 43. Survival curves of control third-generation offspring derived from untreated great-grandparental gametes by matings, {(J♀ × J♂) ♀ × J♂} ♀ × J♂, Nos. 1∼5 .

b. Experimental series derived from X-irradiated great-grandparental sperm

i) {(J♀ × SX–150♂) ♀ × J♂} ♀ × J♂, Nos. 1∼5

Five female second-generation offspring Nos. 1∼5 derived from spermatozoa exposed to 150 rads of X-rays were transplanted with frog pituitaries. As normal ovulation occurred in all of them, they were mated with the same three males as those used in the control series. The survival curve of the third-generation offspring produced from each of the five females is shown in Fig. 44a.

In five matings Nos. 1∼5, 80.7%, 92.6%, 36.2%, 82.1% and 16.7%, 63.6% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs from matings Nos. 2, 3, 4 and 5 did not differ from the

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		
765	11	757	8	744	13	723	703
(82.8%)	(1.2%)	(81.9%)	(0.9%)	(80.5%)	(1.4%)	(78.2%)	(76.1%)
617	36	577	40	548	29	527	484
(60.1%)	(3.5%)	(56.2%)	(3.9%)	(53.4%)	(2.8%)	(51.4%)	(47.2%)
321	8	295	26	267	28	252	224
(60.6%)	(1.5%)	(55.7%)	(4.9%)	(50.4%)	(5.3%)	(47.5%)	(42.3%)
739	20	702	37	652	50	610	576
(63.8%)	(1.7%)	(60.6%)	(3.2%)	(56.3%)	(4.3%)	(52.7%)	(49.7%)
440	27	396	44	356	40	334	317
(59.5%)	(3.7%)	(53.6%)	(6.0%)	(48.2%)	(5.4%)	(45.2%)	(42.9%)
679	128	623	56	582	41	560	551
(55.8%)	(10.5%)	(51.2%)	(4.6%)	(47.8%)	(3.4%)	(46.0%)	(45.3%)
164	6	161	3	160	1	160	159
(61.9%)	(2.3%)	(60.8%)	(1.1%)	(60.4%)	(0.4%)	(60.4%)	(60.0%)
579	20	556	23	510	46	488	476
(57.8%)	(2.0%)	(55.5%)	(2.3%)	(50.9%)	(4.6%)	(48.8%)	(47.6%)
630	27	600	30	554	46	527	500
(69.4%)	(3.0%)	(66.1%)	(3.3%)	(61.0%)	(5.1%)	(58.0%)	(55.1%)

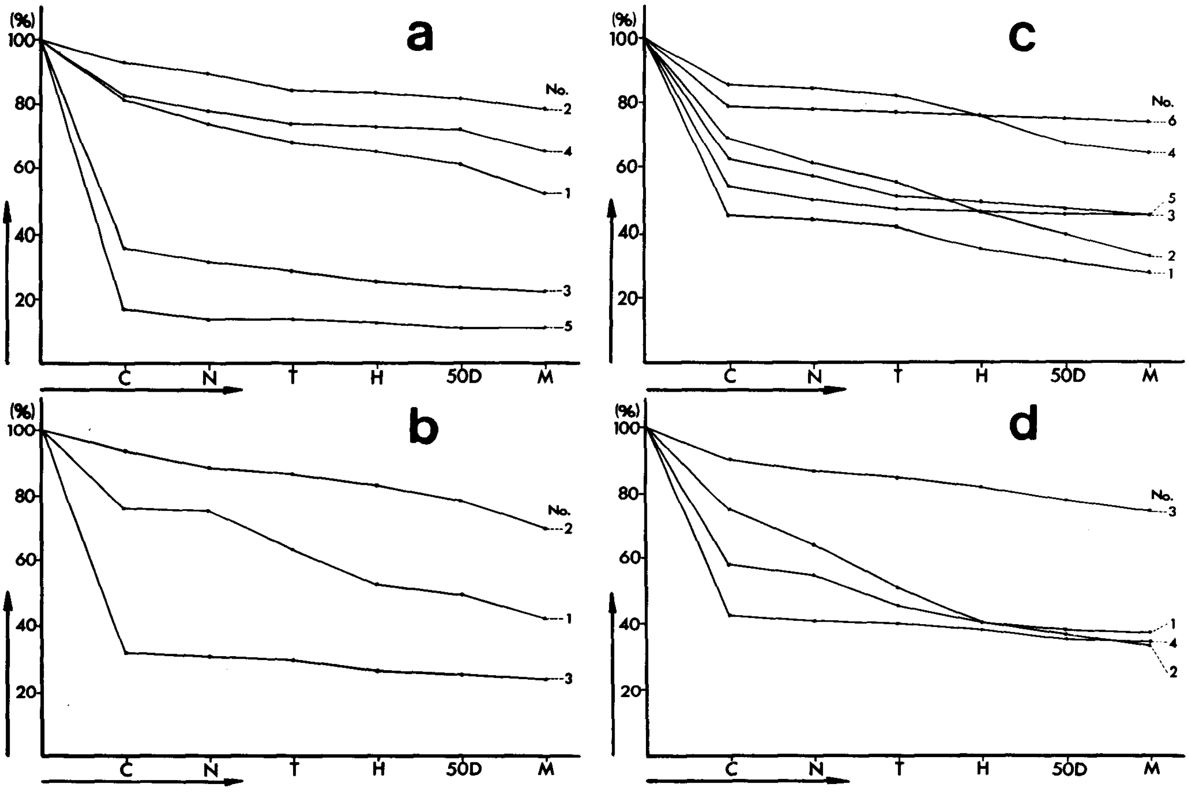


Fig. 44. Survival curves of third-generation offspring derived from X-irradiated great-grandparental gametes by passing over female first- and second-generation offspring.

a. {(J♀ × SX–150♂)♀ × J♂} ♀ × J♂, Nos. 1∼5
b. {(J♀ × SX–250♂)♀ × J♂} ♀ × J♂, Nos. 1∼3
c. {(EX–150♀ × J♂)♀ × J♂} ♀ × J♂, Nos. 1∼6
d. {(EX–250♀ × J♂)♀ × J♂} ♀ × J♂, Nos. 1∼4

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

controls in developmental capacity, while those from No. 1 were somewhat inferior. After a small number of normally cleaved eggs died of edema, blisters, curvature of the body or some other abnormalities at various embryonic stages, 65.0%, 82.5%, 24.6%, 73.4% and 12.8%, 53.4% on the average, hatched normally. At the metamorphosis stage, comparatively numerous individuals produced from mating No. 1 died of ill-development of forelegs (Fig. 45c). In the five matings, 52.0%, 78.3%, 22.1%, 65.2% and 10.6%, 47.2% on the average, became normally metamorphosed frogs.

ii) {(J♀ × SX–250♂)♀ × J♂}♀ × J♂, Nos. 1∼3

Three female second-generation offspring Nos. 1∼3 derived from spermatozoa exposed to 250 rads of X-rays were transplanted with frog pituitaries. As normal ovulation occurred in all of them, they were mated with the same three males as those used in the control series. The survival curve of the third-generation offspring produced from each of the three females is shown in Fig. 44b.

In three matings Nos. 1∼3, 75.9%, 92.5% and 30.5%, 62.5% on the average, of the respective total number of eggs cleaved normally. In mating No. 1, 91 (52.3%) normally cleaved eggs hatched normally, while 21 and 18 died of

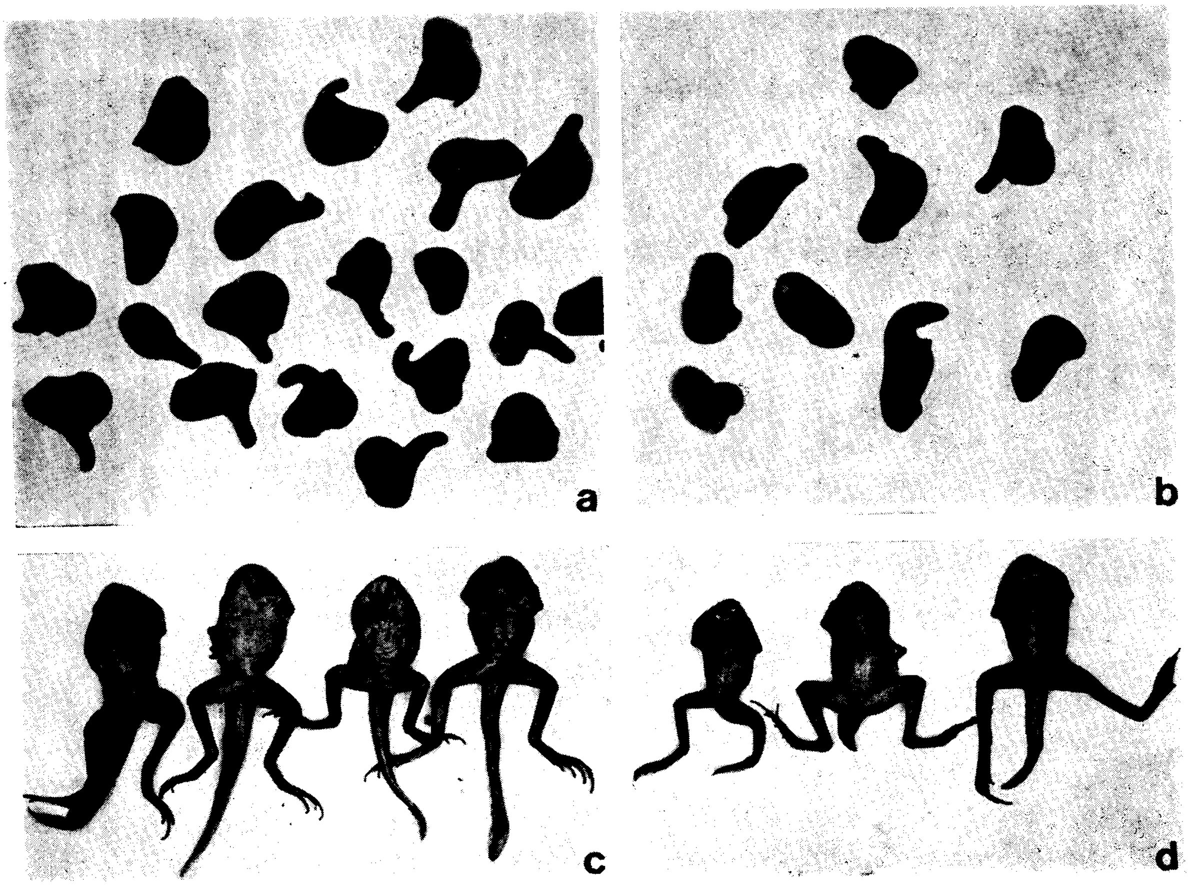


Fig. 45. Abnormalities at the embryonic and metamorphosis stages in the third-generation offspring derived from X-irradiated sperm or oviducal eggs.

a. Extremely deformed and ascitic embryos produced from a mating, {(J♀ ×SX–250♂)♀ ×J♂} ♀ ×J♂, No. 1 ×2.0

b. Extremely deformed embryos produced from a mating, {(EX–250♀ ×J♂)♀ ×J♂} ♀ ×J♂, No. 2 ×2.0

c. Metamorphosing frogs with ill-developed forelegs produced from a mating, {(J♀ ×SX–150♂)♀ ×J♂} ♀ ×J♂, No. 1 ×0.8

d. Metamorphosing frogs with ill-developed forelegs produced from a mating, {(EX–250♀ ×J♂)♀ ×J♂} ♀ ×J♂, No. 2 ×0.8

edema at the tail-bud and hatching stages, respectively (Fig. 45a). In mating No. 2, the normally cleaved eggs did not remarkably differ from the controls in development; 82.9% of the total number of eggs hatched normally. In mating No. 3, the normally cleaved eggs did not also differ from the controls in developmental capacity, although the percentage of normal cleavages was very low. While a few of them died of various abnormalities, 26.2% of the total number of eggs hatched normally. On the average, 50.4% of the total number of eggs in this experimental series hatched normally, and afterwards 42.0%, 68.5% and 24.3%, 42.3% on the average, became normally metamorphosed frogs, while a few died of edema or ill-development at the tadpole stage.

c. Experimental series derived from X-irradiated great-grandparental eggs

i) {(EX–150♀ ×J♂)♀ ×J♂}♀ ×J♂, Nos. 1∼6

Eight female second-generation offspring derived from eggs exposed to 150 rads of X-rays were transplanted with frog pituitaries. As six of them ovulated normally, these females (Nos. 1∼6) were mated with the same three males as those used in the control series. The survival curve of the third-generation offspring produced from each of the six females is shown in Fig. 44c.

In six matings Nos. 1∼6, 45.2%, 69.1%, 53.6%, 85.4%, 61.7% and 79.2%, 66.9% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs in matings Nos. 3 and 6 were nearly the same in developmental capacity as the controls, while those in the other matings were more or less inferior. After some embryos died of various abnormalities, 35.1%, 46.3%, 45.5%, 75.5%, 48.7% and 75.8%, 56.3% on the average, of the respective total number of eggs in the six matings hatched normally, and afterwards 26.8%, 32.0%, 45.0%, 63.7%, 44.8% and 73.8%, 49.7% on the average, became normally metamorphosed frogs. The other tadpoles obtained from each of the matings died of ill-development or edema.

ii) {(EX–250♀ ×J♂)♀ ×J♂}♀ ×J♂, Nos. 1∼4

Five female second-generation offspring derived from eggs exposed to 250 rads of X-rays were transplanted with frog pituitaries. As four of them ovulated normally, they (Nos. 1∼4) were mated with the same three males as those used in the control series. The survival curve of the third-generation offspring produced from each of the four females is shown in Fig. 44d.

In four matings Nos. 1∼4, 58.4%, 75.0%, 90.3% and 43.4%, 64.5% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs obtained from matings Nos. 3 and 4 did not differ from the controls in developmental capacity. After a few of them died of ill-development or the curvature of the body at the embryonic stage in these matings, 81.8% and 38.4% hatched normally. In matings Nos. 1 and 2, a comparatively large number of normally cleaved eggs died of edema or curvature of the body by the hatching stage (Fig. 45b); 39.5% and 40.3% of the respective total number of eggs hatched normally. While some tadpoles produced from matings Nos. 1 and 4 died of edema and some produced from Nos. 2 and 3 died of ill-development of the forelegs by the completion of metamorphosis (Fig. 45d), 36.8%, 33.0%, 74.0% and 34.2%, 42.9% on the average, became normally metamorphosed frogs in matings Nos. 1∼4.

d. Experimental series derived from neutron-irradiated great-grandparental sperm

i) {(J♀ ×SN–100♂)♀ ×J♂}♀ ×J♂, Nos. 1∼6

Seven female second-generation offspring derived from spermatozoa exposed to 100 rads of neutrons were transplanted with frog pituitaries. As normal ovulation occurred in six of them, these six females (Nos. 1∼6) were mated with the same three males as those used in the control series. The survival curve of the third-generation offspring produced from each of the six females is shown in Fig. 46a.

In six matings Nos. 1∼6, 75.6%, 72.9%, 89.5%, 91.9%, 31.4% and 46.7%, 68.9% on the average, of the respective total number of eggs cleaved normally. About one-third of the normally cleaved eggs obtained from mating No. 1 died of edema at the tail-bud and hatching stages, and 49.8% of the total number of eggs hatched normally. More than two-thirds of the normally cleaved eggs obtained from mating No. 4 became abnormal and died at the gastrula and neurula stages. A few of the remaining embryos died of edema at the stages from the tail-bud to the hatching; only 21.8% of the total number of eggs hatched normally. While a few of the normally cleaved eggs obtained from each of four matings Nos. 2, 3, 5 and 6 became abnormal, 65.3%, 83.0%, 25.4% and 41.0% of the respective total number of eggs in these matings hatched normally. In all the six matings, 47.8% hatched normally. Only a few of the tadpoles produced from each of six matings Nos. 1∼6 died of underdevelopment or edema; 46.2%, 62.8%, 83.0%, 20.4%, 23.2% and 35.5%, 45.3% on the average, of the respective total number of eggs became normally metamorphosed frogs.

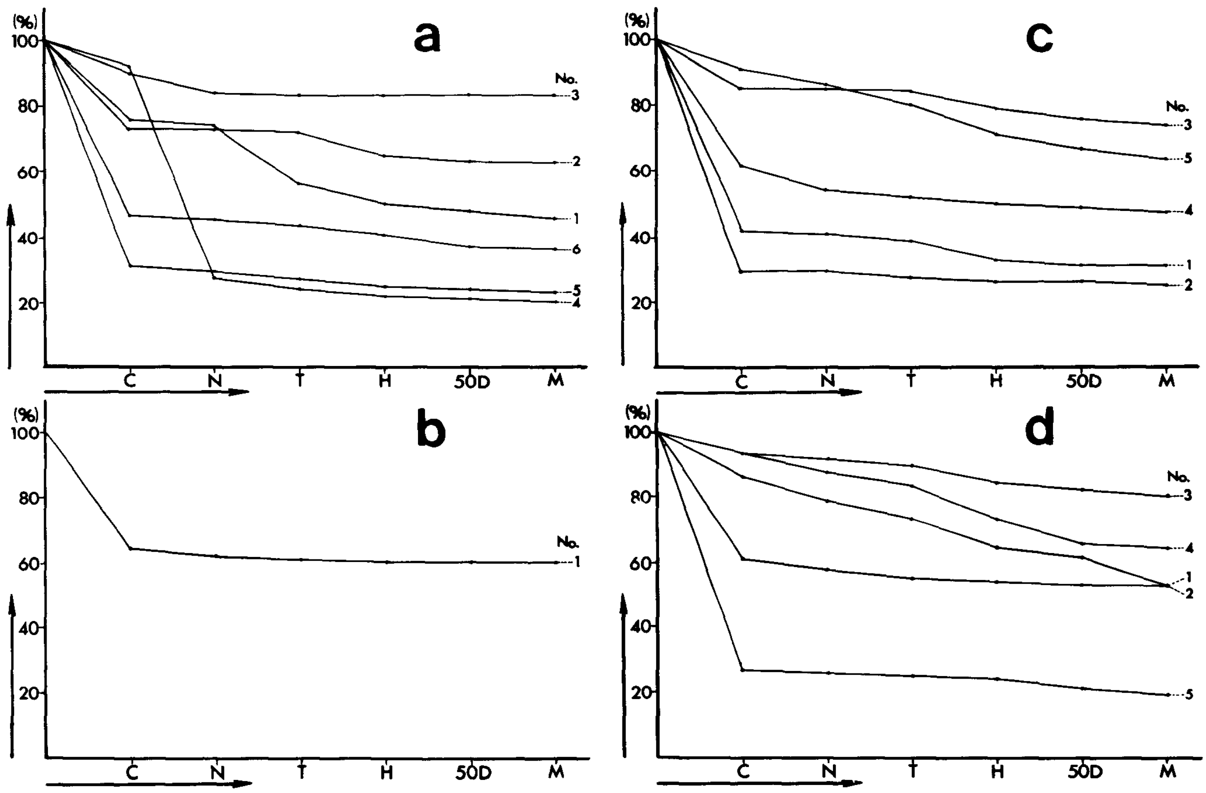


Fig. 46. Survival curves of third-generation offspring derived from neutron-irradiated great-grandparental gametes by passing over female first- and second-generation offspring.

a. {(J♀ × SN–100♂)♀ × J♂}♀ × J♂, Nos. 1∼6 C—Cleavage N—Neurula stage
b. {(J♀ × SN–150♂)♀ × J♂}♀ × J♂, No. 1 T—Tail-bud stage H—Hatch
c. {(EN–150♀ × J♂)♀ × J♂}♀ × J♂, Nos. 1∼5 50D—Age of 50 days
d. {(EN–250♀ × J♂)♀ × J♂}♀ × J♂, Nos. 1∼5 M—Climbing out of water

ii) {(J♀ × SN–150♂)♀ × J♂}♀ × J♂, No. 1

A female second-generation offspring derived from a spermatozoon exposed to 150 rads of neutrons was transplanted with frog pituitaries. As normal ovulation occurred, this female was mated with the same three males as used in the control series. The survival curve of the third-generation offspring produced from her is shown in Fig. 46b. In this mating (No. 1), 64.2% of the total number of eggs cleaved normally. While several eggs died of various kinds of abnormalities in the embryonic stage, 60.4% hatched normally, and 60.0% became normally metamorphosed frogs.

e. Experimental series derived from neutron-irradiated great-grandparental eggs

i) {(EN–150♀ × J♂)♀ × J♂}♀ × J♂, Nos. 1～5

Seven female second-generation offspring derived from eggs exposed to 150 rads of neutrons were transplanted with frog pituitaries. As normal ovulation occurred in five of them, these females (Nos. 1～5) were mated with the same three males as used in the control series. The survival curve of the third-generation offspring produced from each of the five females is shown in Fig. 46c.

In five matings Nos. 1～5, 42.1%, 29.0%, 85.4%, 61.8% and 91.3%, 60.6% on the average, of the respective total number of eggs cleaved normally. While matings Nos. 3 and 5 did not differ in the percentage of normal cleavages from the controls, the other three were remarkably inferior. After a small number of the normally cleaved eggs died of underdevelopment, edema, blisters or curvature of the body (Fig. 47b), 32.7%, 26.2%, 79.0%, 50.3% and 71.1%, 50.9%

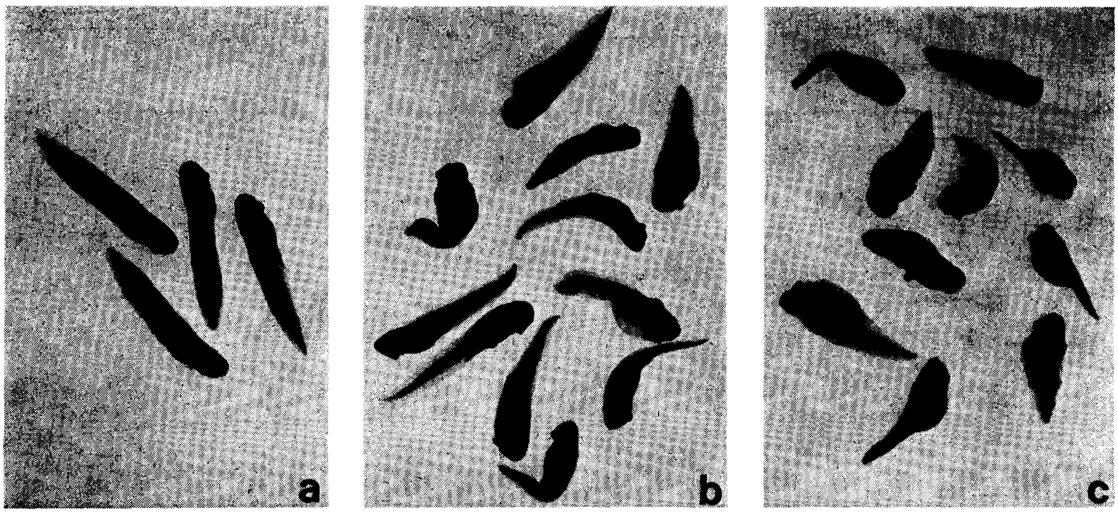


Fig. 47. Abnormalities at the hatching stage in the third-generation offspring derived from neutron-irradiated oviducal eggs. ×2.0

a. Control *Rana japonica* immediately after hatch, produced from a mating, {(J♀ × J♂)♀ × J♂}♀ × J♂, No. 1

b. Edematous, curved or emaciated individuals produced from a mating, {(EN–150♀ × J♂)♀ × J♂}♀ × J♂ No. 5

c. Edematous embryos produced from a mating, {(EN–250♀ × J♂)♀ × J♂}♀ × J♂, No. 4

on the average, of the respective total number of eggs hatched normally in five matings Nos. 1 ~ 5. In the tadpole stage, a small number of individuals died of underdevelopment or edema, and then 30.8%, 24.8%, 73.7%, 48.2% and 64.2%, 47.6% on the average, became normally metamorphosed frogs. Accordingly, the normally cleaved eggs obtained from the five matings were nearly the same as the controls in developmental capacity, except those from mating No. 5.

ii) {(EN-250♀ ×J♂)♀ ×J♂}♀ ×J♂, Nos. 1 ~ 5

Seven female second-generation offspring derived from eggs exposed to 250 rads of neutrons were transplanted with frog pituitaries. As normal ovulation occurred in five of them, these females (Nos. 1 ~ 5) were mated with the same three males as used in the control series. The survival curve of the third-generation offspring produced from each of the five females is shown in Fig. 46d.

In five matings Nos. 1 ~ 5, 85.8%, 61.0%, 93.3%, 92.7% and 27.4%, 73.3% on the average, of the respective total number of eggs cleaved normally. A comparatively large number of the normally cleaved eggs obtained from matings Nos. 1 and 4 died of edema by the hatching stage, while a small number of those obtained from matings Nos. 2, 3 and 5 died of underdevelopment or curvature of the body. In the five matings, 64.2%, 54.0%, 83.6%, 73.3% and 24.0%, 61.0% on the average, of the respective total number of eggs hatched normally. A small number of the tadpoles obtained from matings Nos. 1 and 4 died of edema (Fig. 47c), and 52.9% and 64.0% of the respective total number of eggs became normally metamorphosed frogs. In contrast with matings Nos. 1 and 4, a few tadpoles obtained from matings Nos. 2, 3 and 5 died of underdevelopment, and 53.1%, 80.0% and 18.5% became normally metamorphosed frogs. In all the five matings, 55.1% of the total number of eggs developed into normally metamorphosed frogs.

2. Viability and sex of metamorphosed frogs

a. Viability

The age of tadpoles at the time of climbing out of water and the body length of the frogs immediately after metamorphosis in eight experimental and the control series are presented in Table 34. While 703 tadpoles in the control series were 114 ~ 146 days old, 130.7 days old on the average, in the age at the time of climbing out of water, 159 ~ 576 tadpoles were 112 ~ 148 days old in the eight experimental series, or 127.0 ~ 135.4 days old on the average in each of these series. While about 100 frogs removed at random from the control series immediately after metamorphosis were 17.2 ± 0.3 mm in body length, those from each of the experimental series were $16.9 \pm 0.5 \sim 17.6 \pm 0.2$ mm. Thus, it was clear that there were no remarkable differences in the age at the time of metamorphosis as well as in body length between the frogs of the experimental series and the controls. There were also no distinct differences between the frogs of two kinds of experimental series which were different from each other in the kind or the dosage of irradiation, as presented in Table 34.

TABLE 34

Number, size and sex of metamorphosed frogs produced

Parents: Female	Parents: Male	Age at the time of climbing out of water (days)	No. of metamorphosed frogs	Mean body length of 100 frogs immediately after metamorphosis (mm)	No. of frogs
(J·J)J, Nos. 1∼5	J.W71, Nos. 1∼3	114∼146 (130.7)	703	17.2±0.3	641
(J·SX–150)J, Nos. 1∼5	J.W71, Nos. 1∼3	118∼144 (132.8)	484	17.0±0.4	402
(J·SX–250)J, Nos. 1∼3		118∼144 (135.4)	224	16.9±0.5	147
(EX–150·J)J, Nos. 1∼6	J.W71, Nos. 1∼3	122∼146 (134.4)	576	17.3±0.3	490
(EX–250·J)J, Nos. 1∼4		120∼141 (131.6)	317	17.5±0.2	235
(J·SN–100)J, Nos. 1∼6	J.W71, Nos. 1∼3	114∼139 (127.0)	551	17.3±0.3	476
(J·SN–150)J, No. 1		115∼146 (133.0)	159	17.6±0.2	93
(EN–150·J)J, Nos. 1∼5	J.W71, Nos. 1∼3	116∼147 (133.6)	476	17.1±0.2	371
(EN–250·J)J, Nos. 1∼5		112∼148 (134.0)	500	17.2±0.3	400

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

About two months after metamorphosis, 50 frogs obtained from a mating in each of the eight experimental and the control series were removed and continuously reared in order to make them sexually mature (Table 34). In the control series, 43 (86%) of the 50 frogs attained sexual maturity by the breeding season of the next year, while the others died. In contrast, less than 40 frogs survived and sexually matured in the experimental series. The fourth-generation offspring derived from irradiated eggs were inferior in viability to those from irradiated spermatozoa. The fourth-generation offspring derived from eggs or spermatozoa exposed to a large dosage of X-rays or neutrons were generally inferior in viability to those derived from eggs or spermatozoa exposed to a small dosage, although there was an exceptional case of the experimental series derived from neutron-irradiated spermatozoa, as presented in Table 34. In this series, 37 of 50 immature frogs derived from spermatozoa exposed to 150 rads of neutrons became mature frogs, while 31 of 50 derived from spermatozoa exposed to 100 rads of neutrons did so.

b. Sex

The sex of both immature and mature frogs in the experimental and the control series is presented in Table 34. While there was nearly an equal number of males and females in the control series, males were more or less numerous than from females derived from irradiated grandparental sperm or eggs, II

Sex of frogs killed within one or two months after metamorphosis					No. of frogs removed and reared	Sex of mature frogs			Sex of all frogs examined		
♀N	♀U	⚥	♂N	♂ (%)*		No. of frogs	♀	♂	Total	♀	♂ (%) *
317	3	2	319	(50.1)	50	43	21	22	684	341	343 (50.1)
181	3	6	212	(54.2)	50	39	14	25	441	198	243 (55.1)
54	10	11	72	(56.5)	50	20	7	13	167	71	96 (57.5)
213	9	13	255	(54.7)	50	24	9	15	514	231	283 (55.1)
86	17	15	117	(56.2)	50	16	5	11	251	108	143 (57.0)
203	21	17	235	(52.9)	50	31	14	17	507	238	269 (53.1)
40	5	6	42	(51.6)	50	37	17	20	130	62	68 (52.3)
139	40	32	160	(51.8)	50	21	10	11	392	189	203 (51.8)
162	23	20	195	(53.8)	50	14	7	7	414	192	222 (53.6)

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

females in each experimental series. Moreover, there were comparatively numerous females with underdeveloped ovaries as well as hermaphrodites among immature frogs in the experimental series.

In the control series, 12 of 703 normally metamorphosed frogs produced from five matings Nos. 1 ~ 5 died within one or two months after metamorphosis in spite of their normal appearance. Fifty of 126 frogs from mating No. 1 were removed and continuously reared. The remaining 641 from the five matings were killed to examine their sex. As presented in Table 34, 317 of them were females with normal ovaries, 3 females with underdeveloped ovaries, 2 hermaphrodites and 319 males. When the two hermaphrodites were counted as males, 50% of the total number of immature frogs examined were males. Of the 50 immature frogs removed, 43 attained sexual maturity by the breeding season of the next year. Twenty-one of them were females and 22 males (51%), while 37 (49%) of the 76 immature frogs obtained from the same mating No. 1 and killed shortly after metamorphosis were males including a hermaphrodite.

i) Experimental series, {(J♀ × SX–150♂)♀ × J♂}♀ × J♂

Thirty-two of 484 normally metamorphosed frogs produced from five matings Nos. 1 ~ 5 died within one or two months after metamorphosis. Among the 452 survivors, 50 of 164 frogs obtained from mating No. 2 were removed, while the remaining 402 from the five matings were killed to examine their sex. As a result, 181 were females with normal ovaries, 3 females with underdeveloped ovaries, 6 hermaphrodites and 212 males. When the hermaphrodites were counted as males, 54.2% of the immature frogs were males. Of 39 mature frogs which survived from among the 50 immature frogs removed, 14 were females and 25 (64%) were males, while 64 (53%) of the 120 immature frogs which were obtained from the same mating No. 2 and killed shortly after metamorphosis were males including 2 hermaphrodites.

ii) Experimental series, {(J♀ × SX–250♂)♀ × J♂}♀ × J♂

Twenty-seven of 224 frogs produced from three matings Nos. 1 ~ 3 died within one or two months after metamorphosis. While 50 of 100 immature frogs obtained from mating No. 2 were removed and continuously reared, the remaining 147 from the three matings were killed to examine their sex. In this series, there were comparatively numerous hermaphrodites as well as females with underdeveloped ovaries; 54 were females with normal ovaries, 10 females with underdeveloped ovaries, 11 hermaphrodites and 72 males. When the hermaphrodites were counted as males, 56.5% of the immature frogs were males. After a year, 20 of the 50 frogs removed matured sexually. Among these 20 frogs, there were 7 females and 13 (65%) males, while 28 (56%) were males including 5 hermaphrodites among 50 immature frogs which were produced from the same mating No. 2 and killed shortly after metamorphosis.

iii) Experimental series, {(EX–150♀ × J♂)♀ × J♂}♀ × J♂

Thirty-six of 576 frogs produced from six matings Nos. 1 ~ 6 died within one or two months after metamorphosis. The survivors were killed to examine their sex, except 50 frogs produced by mating No. 6. These were continuously reared. Of 490 frogs examined, 213 were females with normal ovaries, 9 females with underdeveloped ovaries, 13 hermaphrodites and 255 males. When the hermaphrodites were counted as males, 54.7% of the immature frogs were males. Of 24 mature frogs which survived from among the 50 immature frogs, 9 were females and 15 (63%) were males, while 67 (53%) of 127 immature frogs which were produced from the same mating No. 6 and killed shortly after metamorphosis were males including 2 hermaphrodites.

iv) Experimental series, {(EX–250♀ × J♂)♀ × J♂}♀ × J♂

Thirty-two of 317 frogs produced from four matings Nos. 1 ~ 4 died within one or two months after metamorphosis. Of the remaining 285 frogs, 50 of 114 frogs produced from mating No. 3 were removed and continuously reared. The other 235 frogs from the four matings were killed to examine their sex. As a result, it was found that 86 were females with normal ovaries, 17 females with underdeveloped ovaries, 15 hermaphrodites and 117 males. When the hermaphrodites were counted as males, 56.2% of the immature frogs examined were males. Of the 50 frogs removed, only 16 became sexually mature frogs. Five of them were females and the other 11 (69%) were males, while 33 (52%) of 64 immature frogs which were produced from the same mating No. 3 and killed shortly after metamorphosis were males including 4 hermaphrodites.

v) Experimental series, {(J♀ × SN–100♂)♀ × J♂}♀ × J♂

Twenty-five of 551 frogs produced from six matings Nos. 1~6 died within one or two months after metamorphosis. Fifty frogs were removed and continuously reared, and the remaining 476 were killed to examine their sex. Of these immature frogs, 203 were females with normal ovaries, 21 females with underdeveloped ovaries, 17 hermaphrodites and 235 males. When the hermaphrodites were counted as males, 52.9% of the immature frogs were males. Of 31 mature frogs which survived from among the 50 immature frogs, 14 were females and 17 (55%) were males. These 50 immature frogs were those removed from among 166 frogs produced from mating No. 3. Of the remaining 116, 65 (56%) were males including 4 hermaphrodites.

vi) Experimental series, {(J♀ ×SN–150♂)♀ ×J♂}♀ ×J♂

Sixteen of 159 frogs produced from a mating died within one or two months after metamorphosis. Of the other 143 frogs, 50 were removed and continuously reared, while 93 were killed to examine their sex. As a result, it was found that 40 were females with normal ovaries, 5 females with underdeveloped ovaries, six hermaphrodites and 42 males. When the hermaphrodites were counted as males, 51.6% of the immature frogs examined were males. Of the 50 frogs removed, 37 matured sexually. Seventeen of the latter were females and 20 (54%) were males.

vii) Experimental series, {(EN–150♀ ×J♂)♀ ×J♂}♀ ×J♂

Fifty-five of 476 frogs produced from five matings Nos. 1~5 died within one or two months after metamorphosis. Of the remaining 421 immature frogs, 50 obtained from mating No. 1 were removed and continuously reared, while the other 371 were killed to examine their sex. Of these frogs, 139 were females with normal ovaries, 40 females with underdeveloped ovaries, 32 hermaphrodites and 160 males. When the hermaphrodites were counted as males, 51.8% of the immature frogs were males. Twenty-one of the 50 immature frogs removed survived and attained sexual maturity by the breeding season of the next year; 10 were females and 11 were (52%) males. Of 16 immature frogs which were produced from the same mating No. 1 and killed shortly after metamorphosis, 9 (56%) were males including a hermaphrodite.

viii) Experimental series, {(EN–250♀ ×J♂)♀ ×J♂}♀ ×J♂

Fifty of 500 frogs produced from five matings Nos. 1~5, 50 died within one or two months after metamorphosis. Fifty of the remaining 450 immature frogs were removed and continuously reared, while the others were killed to examine their sex. As a result, 162 were females with normal ovaries, 23 females with underdeveloped ovaries, 20 hermaphrodites and 195 males. When the hermaphrodites were counted as males, 53.8% of the immature frogs were males. Only 14 of the 50 immature frogs removed survived and attained sexual maturity. Seven of them were females and 7 (50%) were males. These 50 immature frogs were those removed from among 156 frogs produced from mating No. 3. Of the remaining 106 frogs which were killed shortly after metamorphosis, 53% were males including 3 hermaphrodites.

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

1. Developmental capacity

In the breeding season of 1972, male third-generation offspring derived from X- or neutron-irradiated sperm or eggs were mated with normal, field-caught females (Tables 35, 36). The male third-generation offspring were those which were produced from male second-generation offspring in 1970 by mating with normal, field-caught females. The male second-generation offspring were in turn produced from male first-generation offspring in 1969 by mating with normal, field-caught females. The male third-generation offspring were divided into eight experimental series in accordance with their origin. In each series, three to five males were mated with four females by artificial fertilization. The developmental capacity of the fourth-generation offspring in each experimental series and the control is presented in Table 37 and Figs. 48～50.

TABLE 35

Testes of male parents used for mating experiments in 1972, I

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure (Type)
				Left (mm)	Right (mm)	
J{J(J·J)}, No. 1	1	2	43.0	3.0×2.5	3.0×2.5	1
	2	2	39.0	3.5×2.0	3.5×2.0	1
	3	2	42.5	4.0×2.5	4.0×2.0	1
	4	2	40.5	3.0×1.5	3.0×1.5	1
	5	2	38.0	3.0×1.5	3.0×1.5	1
J{J(J·SX–150)}, No. 2	1	2	38.5	3.0×1.5	3.0×1.5	1
	2	2	40.0	2.5×1.0	2.5×1.0	1
	3	2	41.5	3.0×2.0	3.5×1.5	1
	4	2	41.0	3.5×1.5	2.0×1.0	1
	5	2	39.0	2.5×1.0	2.5×1.5	1
J{J(J·SX–250)}, No. 1	1	2	39.0	2.5×1.5	3.0×2.0	1
	2	2	40.5	3.0×2.0	3.0×2.0	1
	3	2	43.0	5.5×3.5	2.0×1.0	1
J{J(EX–150·J)}, No. 1	1	2	39.0	3.5×1.5	3.5×1.5	1
	2	2	42.5	4.0×2.0	4.0×2.0	1
	3	2	45.5	4.0×2.0	4.0×2.0	1
	4	2	41.5	3.5×1.5	3.5×1.5	1
	5	2	41.0	3.5×2.0	3.5×2.0	1
J{J(EX–250·J)}, No. 3	1	2	42.0	3.5×2.5	3.5×2.0	1
	2	2	42.0	3.5×2.0	3.5×2.0	1
	3	2	40.0	3.5×1.5	3.5×1.5	1
J{J(J·SN–100)}, No. 3	1	2	38.5	3.0×1.5	3.0×1.5	1
	2	2	45.0	4.0×2.0	3.5×2.0	1
	3	2	43.5	3.5×2.0	3.5×2.0	1
	4	2	44.0	4.0×2.5	4.0×2.5	1
	5	2	39.0	3.0×1.5	3.0×1.5	1

Continued

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure (Type)
				Left (mm)	Right (mm)	
J{J(J·SN–150)}, No. 1	1	2	37.5	3.0×2.0	3.0×1.5	1
	2	2	39.0	3.0×1.5	3.0×1.5	1
	3	2	38.5	3.5×2.0	3.5×2.0	1
	4	2	41.5	3.5×2.0	3.5×2.0	1
	5	2	40.5	3.5×1.5	3.5×2.0	1
J{J(EN–150·J)}, No. 1	1	2	43.0	4.0×2.0	3.0×2.0	1
	2	2	41.0	4.0×2.0	4.0×2.5	1
	3	2	37.5	3.5×1.5	3.5×1.5	1
	4	2	38.0	3.0×1.5	3.0×1.5	1
	5	2	43.0	3.5×2.0	4.0×2.0	1
J{J(EN–250·J)}, No. 2	1	2	41.5	3.5×2.0	3.5×2.0	1
	2	2	42.5	4.0×2.5	4.0×2.5	1
	3	2	40.0	4.0×2.0	4.0×2.0	1
	4	2	39.5	3.5×2.0	3.5×2.0	1

J{J(J·J)}, No. 1: Males obtained by J.W70♀, Nos. 1~12×J(J·J)♂, No. 1
J{J(J·SX–150 or –250)}, No. 2 or 1: Males obtained by J.W70♀, Nos. 1~3×J(J·SX–150 or –250)♂, No. 2 or 1
J{J(EX–150 or –250·J)}, No. 1 or 3: Males obtained by J.W70♀, Nos. 4~6×J(EX–150 or –250·J)♂, No. 1 or 3
J{J(J·SN–100 or –150)}, No. 3 or 1: Males obtained by J.W70♀, Nos. 7~9×J(J·SN–100 or –150)♂, No. 3 or 1
J{J(EN–150 or –250·J)}, No. 1 or 2: Males obtained by J.W70♀, Nos. 10~12×J(EN–150 or –250·J)♂, No. 1 or 2

TABLE 36

Eggs of normal 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)
J.W72	1	53.0	1821	1.63±0.02
	2	49.0	1356	1.54±0.02
	3	55.0	1720	1.49±0.02
	4	48.5	1497	1.51±0.02
	5	47.0	1532	1.43±0.02
	6	47.0	1098	1.57±0.02
	7	50.0	1274	1.60±0.02
	8	51.5	1228	1.62±0.02
	9	51.5	1349	1.48±0.02
	10	50.5	1421	1.57±0.02
	11	48.5	1093	1.63±0.02
	12	52.0	986	1.72±0.02
	13	47.5	1004	1.54±0.02
	14	49.0	1575	1.42±0.02
	15	49.0	1178	1.61±0.02
	16	47.5	993	1.59±0.02

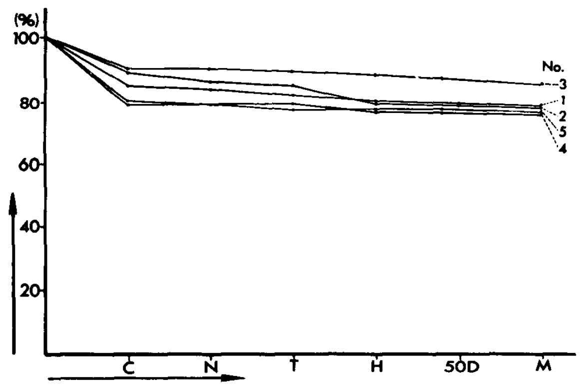
a. Control series, J♀×[J♀×{J♀×(J♀×J♂)♂}♂]♂, Nos. 1~5

Five males Nos. 1~5 were mated with 16 normal, field-caught females Nos. 1~16 (Table 37, Fig. 48). These males were third-generation offspring which

TABLE 37

Developmental capacity of the offspring of males derived

Parents		No. of eggs	No. of cleaved eggs	
Female	Male		Normal	Abnormal
J.W72, Nos. 1∼16	J{J(J·J)}, Nos. 1∼5	1030	873 (84.8%)	13 (1.3%)
J.W72, Nos. 1∼4	J{J(J·SX–150)}, Nos. 1∼5	1053	872 (82.8%)	24 (2.3%)
	J{J(J·SX–250)}, Nos. 1∼3	701	537 (76.6%)	14 (2.0%)
J.W72, Nos. 5∼8	J{J(EX–150·J)}, Nos. 1∼5	1033	843 (81.6%)	15 (1.5%)
	J{J(EX–250·J)}, Nos. 1∼3	693	570 (82.3%)	10 (1.4%)
J.W72, Nos. 9∼12	J{J(J·SN–100)}, Nos. 1∼5	1057	864 (81.7%)	20 (1.9%)
	J{J(J·SN–150)}, Nos. 1∼5	1020	843 (82.6%)	4 (0.4%)
J.W72, Nos. 13∼16	J{J(EN–150·J)}, Nos. 1∼5	1039	836 (80.5%)	13 (1.3%)
	J{J(EN–250·J)}, Nos. 1∼4	841	673 (80.0%)	20 (2.4%)



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

Fig. 48. Survival curves of control fourth-generation offspring derived from untreated great-great-grandparental gametes by matings, J♀ × [J♀ × {J♀ × (J♀ × J♂) ♂} ♂] ♂, Nos. 1∼5.

were produced from a male second-generation offspring in 1970 by mating with a normal, field-caught female. The male second-generation offspring was in turn obtained from a male first-generation offspring in 1969 by mating with a normal female from the field; the male first-generation was one of those which were produced from males and females from the field as the controls of the frogs raised from X- or neutron-irradiated spermatozoa or eggs. The survival curve of the fourth-generation offspring produced from each of the five males is shown in Fig. 48.

In five matings Nos. 1∼5, 84.7%, 89.4%, 89.7%, 79.3% and 80.0%, 84.8% on the average, of the respective total number of eggs cleaved normally. While about 10% of the normally cleaved eggs produced from the mating No. 2 died by the hatching stage, less than 5% of those from the other matings died by this from irradiated great-grandparental sperm or eggs

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		
861 (83.6%)	10 (1.0%)	847 (82.2%)	14 (1.4%)	822 (79.8%)	25 (2.4%)	815 (79.1%)	804 (78.1%)
851 (80.8%)	16 (1.5%)	828 (78.6%)	23 (2.2%)	792 (75.2%)	36 (3.4%)	764 (72.6%)	755 (71.7%)
531 (75.7%)	5 (0.7%)	523 (74.6%)	8 (1.1%)	500 (71.3%)	23 (3.3%)	500 (71.3%)	495 (70.6%)
836 (80.9%)	7 (0.7%)	823 (79.7%)	13 (1.3%)	783 (75.8%)	40 (3.9%)	769 (74.4%)	765 (74.1%)
562 (81.1%)	7 (1.0%)	553 (79.8%)	9 (1.3%)	526 (75.9%)	27 (3.9%)	518 (74.7%)	513 (74.0%)
855 (80.9%)	9 (0.9%)	841 (79.6%)	14 (1.3%)	798 (75.5%)	43 (4.1%)	793 (75.0%)	771 (72.9%)
838 (82.2%)	9 (0.9%)	823 (80.7%)	15 (1.5%)	783 (76.8%)	40 (3.9%)	775 (76.0%)	767 (75.2%)
821 (79.0%)	8 (0.8%)	802 (77.2%)	19 (1.8%)	777 (74.8%)	25 (2.4%)	774 (74.5%)	766 (73.7%)
663 (78.8%)	8 (1.0%)	654 (77.8%)	9 (1.1%)	630 (74.9%)	24 (2.9%)	626 (74.4%)	621 (73.8%)

stage. Eventually, 79.6%, 79.3%, 87.6%, 75.9% and 76.8%, 79.8% on the average, hatched normally and became tadpoles, in the five matings. After only a few tadpoles died, 77.8%, 76.7%, 85.1%, 74.9% and 76.3%, 78.1% on the average, of the respective total number of eggs became normally metamorphosed frogs.

b. Experimental series derived from X-irradiated great-great-grandparental sperm

i) J♀ × [J♀ × {J♀ × (J♀ × SX-150♂)♂}♂]♂, Nos. 1 ~ 5

Five male third-generation offspring Nos. 1 ~ 5 derived from spermatozoa exposed to 150 rads of X-rays were mated with the same four females Nos. 1 ~ 4 as those used in the control series (Table 37, Fig. 58). The survival curve of the fourth-generation offspring produced from each of the five males is shown in Fig. 49a.

In matings Nos. 1 ~ 5, 81.0%, 81.8%, 80.6%, 81.5% and 89.8%, 82.8% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were scarcely inferior to the control eggs in developmental capacity until the hatching stage; 73.5%, 70.1%, 71.1%, 79.1% and 83.2%, 75.2% on the average, hatched and became normal tadpoles. While comparatively many tadpoles produced from mating No. 5 died before the completion of metamorphosis, the tadpoles from the other matings Nos. 1 ~ 4 were nearly the same in viability as the controls; 71.5%, 64.5%, 70.3%, 78.7% and 74.0%,

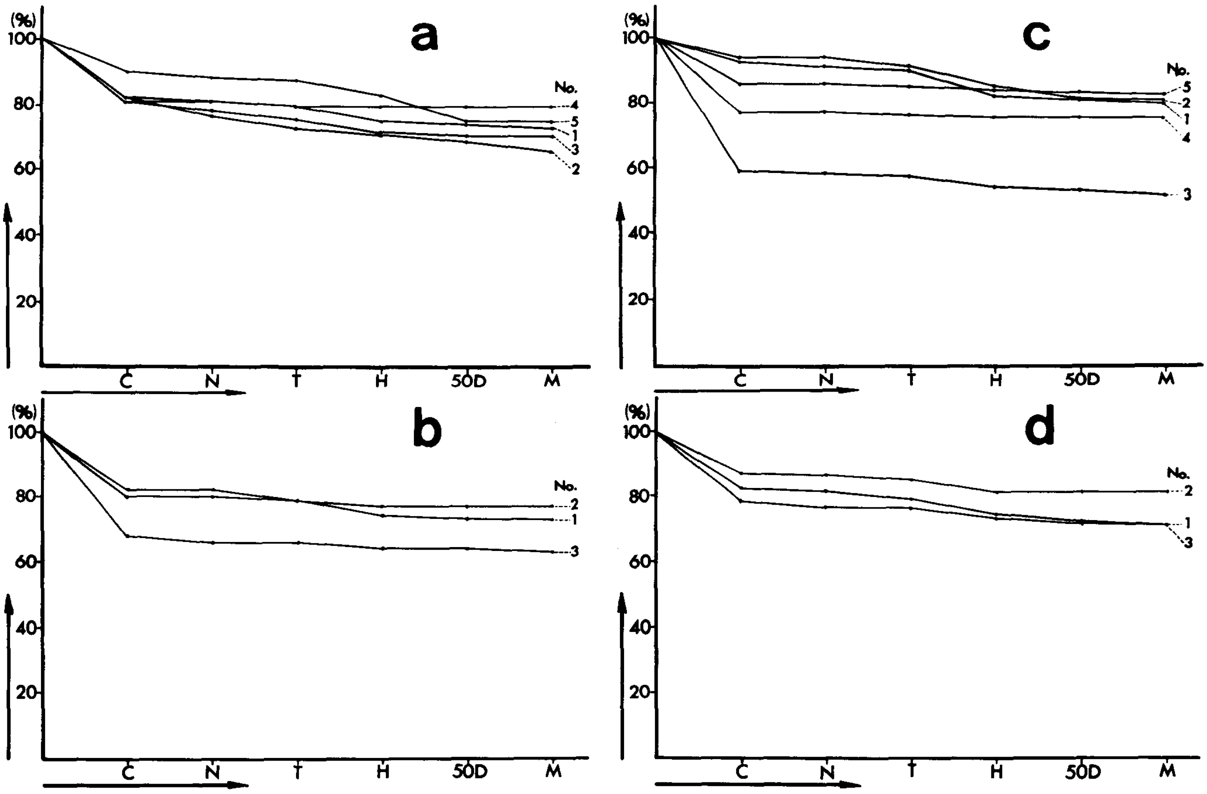


Fig. 49. Survival curves of fourth-generation offspring derived from X-irradiated great-great-grandparental gametes by passing over male first-, second- and third-generation offspring.

a. J♀×[J♀×{J♀×(J♀×SX-150♂)♂}♂]♂, Nos. 1∼5
b. J♀×[J♀×{J♀×(J♀×SX-250♂)♂}♂]♂, Nos. 1∼3
c. J♀×[J♀×{J♀×(EX-150♀×J♂)♂}♂]♂, Nos. 1∼5
d. J♀×[J♀×{J♀×(EX-250♀×J♂)♂}♂]♂, Nos. 1∼3

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

71.7% on the average, of the respective total number of eggs became normally metamorphosed frogs.

ii) J♀×[J♀×{J♀×(J♀×SX-250♂)♂}♂]♂, Nos. 1∼3

Three male third-generation offspring Nos. 1∼3 derived from spermatozoa exposed to 250 rads of X-rays were mated with the same four females Nos. 1∼4 as those used in the control series (Table 37, Fig. 58). The survival curve of the fourth-generation offspring produced from each of the three males is shown in Fig. 49b.

In matings Nos. 1∼3, 82.4%, 79.7% and 68.0%, 76.6% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to the controls; 73.4%, 77.1% and 63.9%, 71.3% on the average, hatched and became normal tadpoles. The latter grew almost normally, and 72.5%, 77.1% and 62.7%, 70.6% on the average, became normally metamorphosed frogs.

c. Experimental series derived from X-irradiated great-great-grandparental eggs

i) J♀×[J♀×{J♀×(EX-150♀×J♂)♂}♂]♂, Nos. 1∼5

Five male third-generation offspring derived from eggs exposed to 150 rads of X-rays were mated with the same four females Nos. 5∼8 as those used in the control series (Table 37, Fig. 58). The survival curve of the fourth-generation offspring produced from each of the five males is shown in Fig. 49c.

In matings Nos. 1∼5, 93.2%, 93.6%, 59.0%, 77.3% and 86.3%, 81.6% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to the controls; 82.2%, 84.8%, 54.3%, 75.0% and 83.5%, 75.8% on the average, hatched normally, and 80.1%, 80.9%, 52.4%, 75.0% and 82.5%, 74.1% on the average, became normally metamorphosed frogs.

ii) J♀×[J♀×{J♀×(EX–250♀×J♂)♂}♂]♂, Nos. 1∼3

Three male third-generation offspring Nos. 1∼3 derived from eggs exposed to 250 rads of X-rays were mated with the same four females Nos. 5∼8 as those used in the control series (Table 37, Fig. 58). The survival curve of the fourth-generation offspring produced from each of the three males is shown in Fig. 49d.

In three matings Nos. 1∼3, 82.3%, 86.7% and 78.1%, 82.3% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to the controls; 73.6%, 80.9% and 73.4%, 75.9% on the average, hatched normally, and 71.0%, 80.9% and 70.5%, 74.0% on the average, became normally metamorphosed frogs.

d. Experimental series derived from neutron-irradiated great-great-grandparental sperm

i) J♀×[J♀×{J♀×(J♀×SN–100♂)♂}♂]♂, Nos. 1∼5

Five male third-generation offspring Nos. 1∼5 derived from spermatozoa exposed to 100 rads of neutrons were mated with the same four females Nos. 9∼12 as those used in the control series (Table 37, Fig. 59). The survival curve of the fourth-generation offspring produced from each of the five males is shown in Fig. 50a.

In five matings Nos. 1∼5, 85.4%, 76.1%, 86.1%, 84.8% and 76.0%, 81.7% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were scarcely inferior in developmental capacity to the controls; 76.9%, 71.1%, 81.1%, 80.4% and 67.7%, 75.5% on the average, hatched normally and 73.1%, 69.0%, 76.6%, 78.7% and 66.8%, 72.9% on the average, became normally metamorphosed frogs.

ii) J♀×[J♀×{J♀×(J♀×SN–150♂)♂}♂]♂, Nos. 1∼5

Five male third-generation offspring Nos. 1∼5 derived from spermatozoa exposed to 150 rads of neutrons were mated with the same four females Nos. 9∼12 as those used in the control series (Table 37, Fig. 59). The survival curve of the fourth-generation offspring produced from each of the five males is shown in Fig. 50b.

In five matings Nos. 1∼5, 77.8%, 97.6%, 68.1%, 89.2% and 82.1%, 82.6% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to the con-

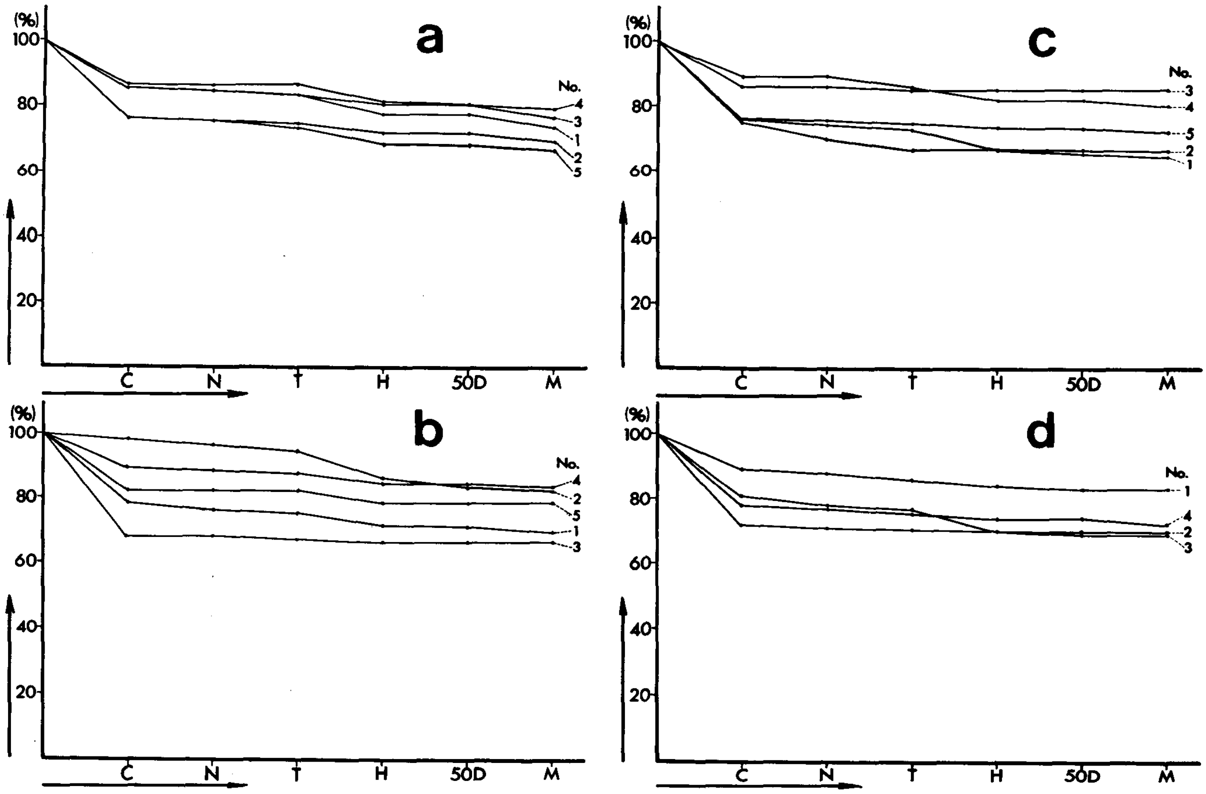


Fig. 50. Survival curves of fourth-generation offpsring derived from neutron-irradiated great-great-grandparental gametes by passing over male first-, second- and third-generation offspring.

a. J♀×[J♀×{J♀×(J♀×SN–100♂)♂}♂]♂, Nos. 1∼5
b. J♀×[J♀×{J♀×(J♀×SN–150♂)♂}♂]♂, Nos. 1∼5
c. J♀×[J♀×{J♀×(EN–150♀×J♂)♂}♂]♂, Nos. 1∼5
d. J♀×[J♀×{J♀×(EN–250♀×J♂)♂}♂]♂, Nos. 1∼4

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

trols; 70.8%, 86.3%, 65.7%, 84.3% and 78.1%, 76.8% on the average, hatched normally, and 69.4%, 82.0%, 65.7%, 82.7% and 77.6%, 75.2% on the average, became normally metamorphosed frogs.

e. Experimental series derived from neutron-irradiated great-great-grand-parental eggs

i) J♀×[J♀×{J♀×(EN–150♀×J♂)♂}♂]♂, Nos. 1∼5

Five male third-generation offspring derived from eggs exposed to 150 rads of neutrons were mated with the same four females Nos. 13∼16 as those used in the control series (Table 37, Fig. 59). The survival curve of the fourth-generation offspring produced from each of the five males is shown in Fig. 50c.

In matings Nos. 1∼5, 75.5%, 75.2%, 86.4%, 89.2% and 76.4%, 80.5% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to the controls; 67.0%, 66.7%, 85.0%, 82.1% and 73.1%, 74.8% on the average, hatched normally, and 65.1%, 66.7%, 85.0%, 80.0% and 72.2%, 73.7% on the average, became normally metamorphosed frogs.

ii) J♀×[J♀×{J♀×(EN–250♀×J♂)♂}♂]♂, Nos. 1～4

Four male third-generation offspring Nos. 1～4 derived from eggs exposed to 250 rads of neutrons were mated with the same four females Nos. 1～4 as those used in the control series (Table 37, Fig. 59). The survival curve of the fourth-generation offspring produced from each of the four males is shown in Fig. 50d.

In matings Nos. 1～4, 88.5%, 80.7%, 71.6% and 78.2%, 80.0% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior in developmental capacity to the controls; 84.2%, 70.3%, 69.6% and 73.9%, 74.9% on the average, hatched normally, and 82.9%, 70.3%, 68.6% and 72.0%, 73.8% on the average, developed into normally metamorphosed frogs.

2. Viability and sex of metamorphosed frogs

a. Viability

The age of tadpoles at the time of climbing out of water and the body length of frogs immediately after metamorphosis in eight experimental and control series are presented in Table 38. While 804 tadpoles in the control series were 111～137 days old, 129.2 days old on the average, when they climbed out of water, 513～771 tadpoles in the eight experimental series were 111～150 days old, 122.4～132.2 days old on the average in each of these experimental series. While about 100 frogs removed at random from the control series immediately after metamorphosis were 16.7±0.2 mm in body length, those from each of the experimental series were 16.4±0.2～17.0±0.2 mm. There were no remarkable differences in the age at the time of climbing out of water as well as in the body length immediately after metamorphosis between the frogs of the experimental series and the controls. There were also no distinct differences in these respects between the frogs of two kinds of experimental series which were different from each other in the kind or the dosage of irradiation, and between the frogs derived from spermatozoa and eggs irradiated, as presented in Table 38.

While about 3% of the total number of normally metamorphosed frogs died within one or two months after metamorphosis, 1～4% did in each of the experimental series within the same period of time. In other words, there was no remarkable difference in viability within this period of time between the frogs of the experimental series and the controls. There were also no remarkable differences among the eight experimental series. All the living frogs were killed one or two months after metamorphosis in order to examine their sex.

b. Sex

The sex of immature frogs preserved during the period from about one to two months after metamorphosis is presented in Table 38. While there were nearly an equal number of males and females in the control series, males were more or less numerous than females in most of the experimental series. Moreover, there were comparatively numerous females with underdeveloped ovaries

TABLE 38

Number, size and sex of metamorphosed frogs produced from males

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed frogs
Female	Male		
J.W72, Nos. 1~16	J{J(J·J)}, Nos. 1~5	111~137 (129.2)	804
J.W72, Nos. 1~4	J{J(J·SX–150)}, Nos. 1~5	114~143 (125.8)	755
	J{J(J·SX–250)}, Nos. 1~3	111~140 (122.4)	495
J.W72, Nos. 5~8	J{J(EX–150·J)}, Nos. 1~5	111~139 (123.6)	765
	J{J(EX–250·J)}, Nos. 1~3	116~136 (125.3)	513
J.W72, Nos. 9~12	J{J(J·SN–100)}, Nos. 1~5	111~144 (128.8)	771
	J{J(J·SN–150)}, Nos. 1~5	120~146 (130.2)	767
J.W72, Nos. 13~16	J{J(EN–150·J)}, Nos. 1~5	116~150 (130.9)	766
	J{J(EN–250·J)}, Nos. 1~4	116~147 (132.2)	621

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

as well as hermaphrodites in each experimental series, while they were very scarce in the control series.

In the control series, 22 (3%) of 804 normally metamorphosed frogs produced from five matings died within one or two months after metamorphosis. All the remaining 782 frogs were killed to examine their sex. As a result, 391 were females with normal ovaries, 2 females with underdeveloped ovaries, one hermaphrodite and 388 males. When the hermaphrodite was counted as a male, 49.7% of the total number of frogs examined were males.

i) Experimental series, J♀ × [J♀ × {J♀ × (J♀ × SX–150♂) ♂} ♂] ♂

Fifteen of 755 normally metamorphosed frogs produced from five matings died within one or two months after metamorphosis. All the remaining 740 were killed to examine their sex. As a result of examination, 345 were females with normal ovaries, 12 females with underdeveloped ovaries, 16 hermaphrodites and 367 males. When the hermaphrodites were counted as males, 51.8% of the total number of frogs examined were males.

ii) Experimental series, J♀ × [J♀ × {J♀ × (J♀ × SX–250♂) ♂} ♂] ♂

Four of 495 frogs produced from three matings died within one or two months after metamorphosis. Of the remaining 491, 203 were females with normal ovaries, 16 females with underdeveloped ovaries, 20 hermaphrodites and 252 males. When the hermaphrodites were counted as males, 55.4% of the total number of frogs examined were males.

derived from irradiated great-grandparental sperm or eggs

Mean body length of 100 frogs immediately after metamorphosis (mm)	Sex of frogs killed about one or two months after metamorphosis					
	No. of frogs	♀N	♀U	⚥	♂N	♂ (%)*
16.7±0.2	782	391	2	1	388	(49.7)
16.8±0.2	740	345	12	16	367	(51.8)
17.0±0.2	491	203	16	20	252	(55.4)
16.5±0.3	755	340	21	24	370	(52.2)
16.9±0.2	500	212	18	21	249	(54.0)
16.7±0.2	749	346	24	27	352	(50.6)
16.4±0.2	743	333	23	19	368	(52.1)
16.6±0.3	734	341	15	15	363	(51.5)
16.5±0.3	613	268	17	22	306	(53.5)

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

iii) Experimental series, J♀[J♀ × {J♀ × (EX–150♀ ×J♂)♂}♂]♂

Ten of 765 frogs produced from five matings died within one or two months after metamorphosis. Of the remaining 755, 340 were females with normal ovaries, 21 females with underdeveloped ovaries, 24 hermaphrodites and 370 males. When the hermaphrodites were counted as males, 52.2% of the total number of frogs examined were males.

iv) Experimental series, J♀ × [J♀ × {J♀ × (EX–250♀ ×J♂)♂}♂]♂

Thirteen of 513 frogs produced from three matings died within one or two months after metamorphosis. Of the remaining 500, 212 were females with normal ovaries, 18 females with underdeveloped ovaries, 21 hermaphrodites and 249 males. When the hermaphrodites were counted as males, 54.0% of the total number of frogs examined were males.

v) Experimental series, J♀ × [J♀ × {J♀ × (J♀ ×SN–100♂)♂}♂]♂

Twenty-two of 771 frogs produced from five matings died within one or two months after metamorphosis. Of the remaining 749, 346 were females with normal ovaries, 24 females with underdeveloped ovaries, 27 hermaphrodites and 352 males. When the hermaphrodites were counted as males, 50.6% of the total number of frogs examined were males.

vi) Experimental series, J♀ × [J♀ × {J♀ × (J♀ ×SN–150♂)♂}♂]♂

Twenty-four of 767 frogs produced from five matings died within one or two months after metamorphosis. Of the remaining 743, 333 were females with normal ovaries, 23 females with underdeveloped ovaries, 19 hermaphrodites and 368 males. When the hermaphrodites were counted as males, 52.1% of the total number of frogs examined were males.

vii) Experimental series, J♀ × [J♀ × {J♀ × (EN–150♀ × J♂) ♂} ♂] ♂

Thirty-two of 766 frogs produced from five matings died within one or two months after metamorphosis. Of the remaining 734, 341 were females with normal ovaries, 15 females with underdeveloped ovaries, 15 hermaphrodites and 363 males. When the hermaphrodites were counted as males, 51.5% of the total number of frogs examined were males.

viii) Experimental series, J♀ × [J♀ × {J♀ × (EN–250♀ × J♂) ♂} ♂] ♂

Eight of 621 frogs produced from four matings died within one or two months after metamorphosis. Of the remaining 613, 268 were females with normal ovaries, 17 females with underdeveloped ovaries, 22 hermaphrodites and 306 males. When the hermaphrodites were counted as males, 53.5% of the total number of frogs examined were males.

IX. *Fourth-generation offspring derived from irradiated gametes by passing over female first-, second- and third-generation offspring*

1. Developmental capacity

In the breeding season of 1972, female third-generation offspring derived from X- or neutron-irradiated sperm or eggs were mated with normal, field-caught males (Tables 39, 40). The female third-generation offspring were

TABLE 39

Eggs of female parents used for mating experiments in 1972, I

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 50 eggs (mm)
{(J·J)J}J, No. 3	1	1	35.0	752	1.64±0.02
	2	1	33.5	719	1.64±0.02
	3	1	35.0	892	1.68±0.02
	4	1	32.5	996	1.64±0.02
	5	1	35.5	1011	1.56±0.02
{(J·SX–150)J}J, No. 2	1	1	34.0	658	1.71±0.02
	2	1	36.0	713	1.63±0.02
	3	1	33.0	450	1.82±0.02
	4	1	37.5	795	1.67±0.02
	5	1	40.5	892	1.65±0.02
	6	1	35.0	0	—
{(J·SX–250)J}J, No. 2	1	1	41.0	841	1.61±0.02
	2	1	37.5	910	1.51±0.02
	3	1	39.0	631	1.68±0.02
	4	1	39.5	885	1.74±0.02
	5	1	39.5	647	1.63±0.02
	6	1	35.0	0	—
	7	1	38.5	0	—

Continued

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 50 eggs (mm)
{(EX–150·J)J} J, No. 6	1	1	34.5	582	1.57±0.02
	2	1	36.5	830	1.65±0.02
	3	1	35.0	765	1.72±0.02
	4	1	37.5	1027	1.64±0.02
	5	1	34.0	619	1.61±0.02
	6	1	34.0	0	—
	7	1	37.0	0	—
{(EX–250·J)J} J, No. 3	1	1	37.5	379	1.82±0.02
	2	1	36.0	621	1.69±0.02
	3	1	34.0	0	—
	4	1	34.5	0	—
	5	1	33.0	0	—
{(J·SN–100)J} J, No. 3	1	1	38.0	842	1.67±0.02
	2	1	36.5	736	1.68±0.02
	3	1	38.0	781	1.73±0.02
	4	1	37.5	590	1.62±0.02
	5	1	37.0	403	1.54±0.02
	6	1	33.0	0	—
	7	1	42.0	0	—
	8	1	40.0	0	—
{(J·SN–150)J} J, No. 1	1	1	37.0	753	1.56±0.02
	2	1	35.5	628	1.71±0.02
	3	1	39.0	864	1.69±0.02
	4	1	39.0	703	1.70±0.02
	5	1	37.5	0	—
{(EN–150·J)J} J, No. 3	1	1	37.5	647	1.62±0.02
	2	1	38.0	531	1.67±0.02
	3	1	36.0	772	1.69±0.02
	4	1	35.5	927	1.64±0.02
	5	1	33.0	0	—
{(EN–250·J)J} J, No. 3	1	1	34.0	725	1.61±0.02
	2	1	37.5	683	1.69±0.02
	3	1	36.5	716	1.72±0.02
	4	1	31.5	0	—
	5	1	35.0	0	—

{(J·J)J} J, No. 3: Females obtained by (J·J)J♀, No. 3×J.W71♂, Nos. 1∼3
{(J·SX–150 or –250)J} J, No. 2: Females obtained by (J·SX–150 or–250)J♀, No. 2×J.W71♂, Nos. 1∼3
{(EX–150 or–250·J)J} J, No. 6 or 3: Females obtained by (EX–150 or–250·J)J♀, No. 6 or 3×J. W71♂, Nos. 1∼3
{(J·SN–100 or–150)J} J, No. 3 or 1: Females obtained by (J·SN–100 or–150)J♀, No. 3 or 1×J. W71♂, Nos. 1∼3
{(EN–150 or–250·J)J} J, No. 3: Females obtained by (EN–150 or–250·J)J♀, No. 3×J.W71♂. Nos. 1∼3

TABLE 40

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

Kind	Individual no.	Body length (mm)	Size of the testes		Inner structure (Type)
			Left (mm)	Right (mm)	
J.W72	1	48.5	4.5×2.0	4.5×2.0	1
	2	50.0	5.0×2.5	5.0×2.5	1
	3	52.0	5.0×2.5	5.0×2.5	1

produced from female second-generation offspring in 1971 by mating with normal, field-caught males. The female second-generation offspring were those produced from female first-generation offspring in 1969 by mating with normal, field-caught males. The female third-generation offspring were divided into eight experimental series in accordance with their origin. In each series, two to five females were mated with three males by artificial fertilization. The developmental capacity of the fourth-generation offspring in each of the experimental series and the control is presented in Table 41 and shown in Figs. 51～53.

a. Control series, [{(J♀×J♂)♀×J♂}♀×J♂]♀×J♂, Nos. 1～4

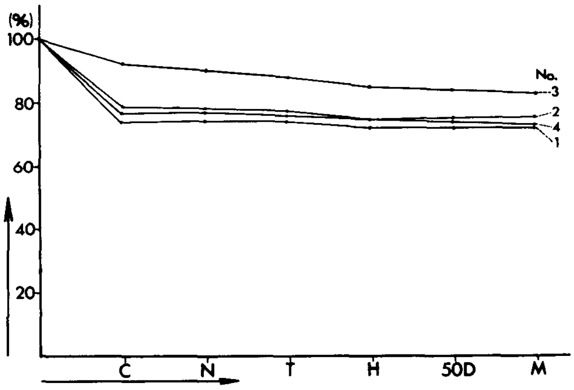
Four females Nos. 1～4 were mated with three normal, field-caught males Nos. 1～3. These females were third-generation offspring which were produced from a female second-generation offspring in 1971 by mating with a normal, field-caught male. The female second-generation offspring was in turn produced from a female first-generation offspring by mating with a normal, field-caught male. The female first-generation offspring was one of those which were produced from males and females from the field as the control of the frogs raised from X- or neutron-irradiated spermatozoa or eggs. The survival curve of the fourth-generation offspring produced from each of the four females is shown in Fig. 51.

In four matings Nos. 1～4, 74.4%, 77.4%, 92.0% and 79.1%, 80.5% on the average, of the respective total number of eggs cleaved normally. After a few of the normally cleaved eggs died of various abnormalities, 72.1%, 75.2%, 84.6% and 75.2%, 76.6% on the average, hatched normally and became

TABLE 41

Developmental capacity of the offspring of females derived

Parents: Female	Parents: Male	No. of eggs	No. of cleaved eggs: Normal	No. of cleaved eggs: Abnormal
{(J·J)J}J, Nos. 1～4	J.W72, Nos. 1～3	896	721 (80.5%)	2 (0.2%)
{(J·SX–150)J}J, Nos. 1～5	J.W72, Nos. 1～3	1077	847 (78.6%)	7 (0.6%)
{(J·SX–250)J}J, Nos. 1～5		1068	855 (80.1%)	7 (0.7%)
{(EX–150·J)J}J, Nos. 1～5	J.W72, Nos. 1～3	1116	857 (76.8%)	13 (1.2%)
{(EX–250·J)J}J, Nos. 1,2		484	369 (76.2%)	5 (1.0%)
{(J·SN–100)J}J, Nos. 1～5	J.W72, Nos. 1～3	1092	845 (77.4%)	35 (3.2%)
{(J·SN–150)J}J, Nos. 1～4		865	699 (80.8%)	6 (0.7%)
{(EN–150·J)J}J, Nos. 1～4	J.W72, Nos. 1～3	877	715 (81.5%)	33 (3.8%)
{(EN–250·J)J}J, Nos. 1～3		731	555 (75.9%)	14 (1.9%)



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

Fig. 51. Survival curves of control fourth-generation offspring derived from untreated great-great-grandparental gametes by matings, [{(J♀ ×J♂)♀ ×J♂} ♀ ×J♂]♀ ×J♂, Nos. 1~4.

swimming tadpoles. While a few tadpoles died before the completion of metamorphosis, 71.6%, 75.2%, 82.6% and 72.8%, 75.3% on the average, became normally metamorphosed frogs.

b. Experimental series derived from X-irradiated great-great-grandparental sperm

i) [{(J♀ ×SX–150♂)♀ ×J♂}♀ ×J♂]♀ ×J♂, Nos. 1~5

Six female third-generation offspring derived from spermatozoa exposed to 150 rads of X-rays were transplanted with frog pituitaries. As normal ovulation occurred in five of them, these five females (Nos. 1~5) were mated with the

from irradiated great-grandparental 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		
712 (79.5%)	6 (0.7%)	700 (78.1%)	12 (1.3%)	686 (76.6%)	14 (1.6%)	680 (75.9%)	675 (75.3%)
842 (78.2%)	5 (0.5%)	828 (76.9%)	14 (1.3%)	806 (74.8%)	22 (2.0%)	789 (73.3%)	780 (72.4%)
841 (78.7%)	7 (0.7%)	810 (75.8%)	31 (2.9%)	783 (73.3%)	27 (2.5%)	774 (72.5%)	765 (71.6%)
838 (75.1%)	13 (1.2%)	816 (73.1%)	22 (2.0%)	782 (70.1%)	34 (3.0%)	765 (68.5%)	754 (67.6%)
358 (74.0%)	8 (1.7%)	349 (72.1%)	9 (1.9%)	344 (71.1%)	5 (1.0%)	342 (70.7%)	338 (69.8%)
802 (73.4%)	22 (2.0%)	776 (71.1%)	26 (2.4%)	761 (69.7%)	15 (1.4%)	755 (69.1%)	745 (68.2%)
693 (80.1%)	6 (0.7%)	663 (76.6%)	30 (3.5%)	643 (74.3%)	20 (2.3%)	640 (74.0%)	633 (73.2%)
663 (75.6%)	38 (4.3%)	646 (73.7%)	17 (1.9%)	624 (71.2%)	22 (2.5%)	617 (70.4%)	612 (69.8%)
544 (74.4%)	6 (0.8%)	537 (73.5%)	7 (1.0%)	532 (72.8%)	5 (0.7%)	522 (71.4%)	511 (69.9%)

same three males Nos. 1∼3 as those used in the control series. The survival curve of the fourth-generation offspring produced from each of the five females is shown in Fig. 52a.

In five matings Nos. 1∼5, 83.9%, 85.2%, 90.0%, 60.8% and 75.2%, 78.6% on the average, of the respective total number of eggs cleaved normally. These normally cleaved eggs were not inferior in developmental capacity to the controls; 79.0%, 82.9%, 88.0%, 55.4% and 70.9%, 74.8% on the average, hatched normally, and 74.1%, 81.5%, 84.5%, 55.0% and 68.8%, 72.4% on the average, became normally metamorphosed frogs.

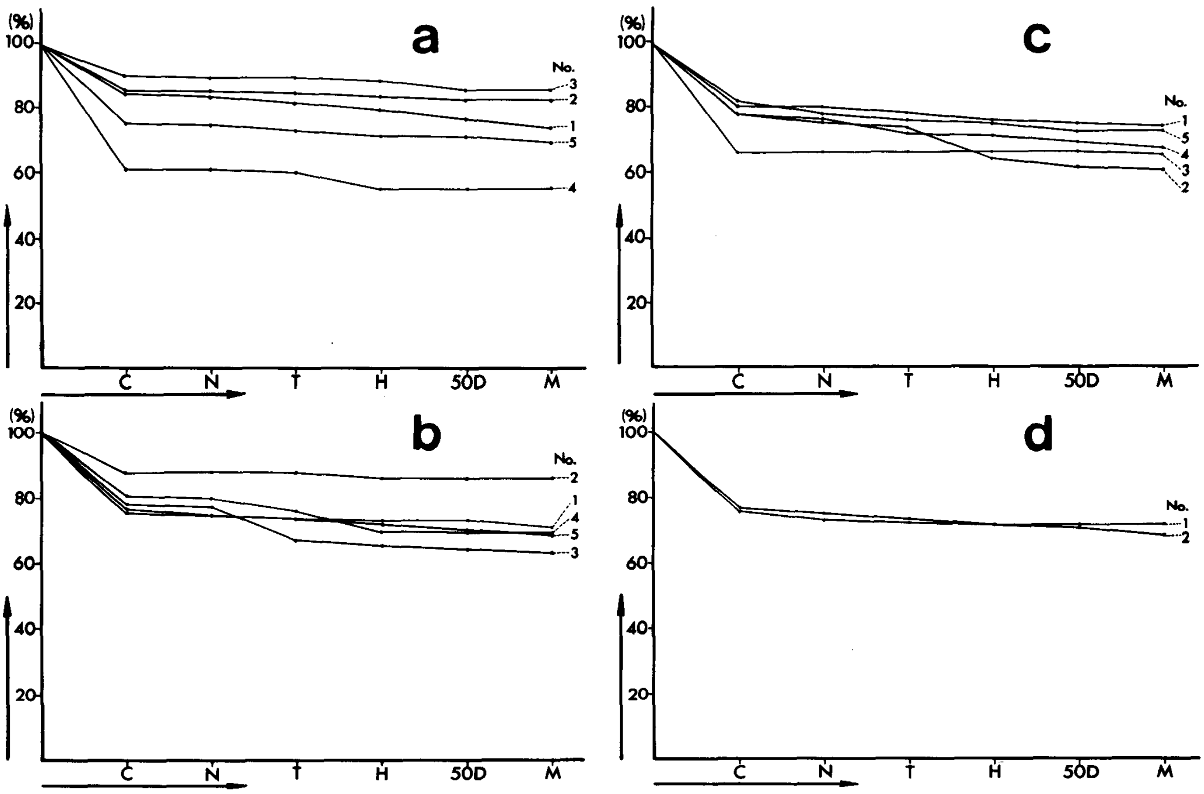


Fig. 52. Survival curves of fourth-generation offspring derived from X-irradiated great-great-grandparental gametes by passing over female first-, second- and third-generation offspring.

a. [{(J♀×SX-150♂)♀×J♂}♀×J♂]♀×J♂, Nos. 1∼5
b. [{(J♀×SX-250♂)♀×J♂}♀×J♂]♀×J♂, Nos. 1∼5
c. [{(EX-150♀×J♂)♀×J♂}♀×J♂]♀×J♂, Nos. 1∼5
d. [{(EX-250♀×J♂)♀×J♂}♀×J♂]♀×J♂, Nos. 1 and 2

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

ii) [{(J♀×SX-250♂)♀×J♂}♀×J♂]♀×J♂, Nos. 1∼5

Seven female third-generation offspring derived from spermatozoa exposed to 250 rads of X-rays were transplanted with frog pituitaries. As normal ovulation occurred in five of them, these five females (Nos. 1∼5) were mated with the same three males Nos. 1∼3 as those used in the control series. The survival curve of the fourth-generation offspring produced from each of the five females is shown in Fig. 52b.

In five matings Nos. 1~5, 76.5%, 88.1%, 78.0%, 81.0% and 76.1%, 80.1% on the average, of the respective total number of eggs cleaved normally. Some of the normally cleaved eggs died of various abnormalities by the hatching stage; 22 of 165 normally cleaved eggs produced from mating No. 3 died of edema at the tail-bud stage, while 13 of 160 from mating No. 4 died of edema at the hatching stage. Eventually, 82.8%, 86.3%, 64.5%, 70.0% and 72.2%, 73.3% on the average, hatched normally, and afterwards 71.4%, 85.8%, 62.6%, 69.0% and 68.3%, 71.6% on the average, became normally metamorphosed frogs.

c. Experimental series derived from X-irradiated great-great-grandparental eggs

i) [{(EX–150♀ ×J♂)♀ ×J♂}♀ ×J♂]♀ ×J♂, Nos. 1~5

Seven female third-generation offspring derived from eggs exposed to 150 rads of X-rays were transplanted with frog pituitaries. As normal ovulation occurred in five of them, these five females (Nos. 1~5) were mated with the same three males as those used in the control series. The survival curve of the fourth-generation offspring produced from each of the five females is shown in Fig. 52c.

In five matings Nos. 1~5, 80.4%, 77.5%, 66.4%, 77.8% and 81.9%, 76.8% on the average, of the respective total number of eggs cleaved normally. While 170 normally cleaved eggs produced from mating No. 2, 23 (14%) died of edema at the hatching stage, only a few from the other matings died of various abnormalities at various embryonic stages. Eventually, 75.9%, 63.6%, 65.5%, 71.1% and 74.5%, 70.1% on the average, in the five matings hatched normally. All the hatched tadpoles were not inferior to the controls in developmental capacity afterwards; 74.1%, 59.7%, 64.5%, 67.1% and 72.7%, 67.6% on the average, became normally metamorphosed frogs.

ii) [{(EX–250♀ ×J♂)♀ ×J♂}♀ ×J♂]♀ ×J♂, Nos. 1 and 2

Five female third-generation offspring derived from eggs exposed to 250 rads of X-rays were transplanted with frog pituitaries. As two of them ovulated normally, these two females (Nos. 1 and 2) were mated with the same three males as those used in the control series. The survival curve of the fourth-generation offspring produced from each of the females is shown in Fig. 52d.

In two matings Nos. 1 and 2, 76.9% and 75.6%, 76.2% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior to the controls in developmental capacity; 71.4% and 70.8%, 71.1% on the average, hatched normally, and 71.4% and 68.4%, 69.8% on the average, became normally metamorphosed frogs.

d. Experimental series derived from neutron-irradiated great-great-grandparental sperm

i) [{(J♀ ×SN–100♂)♀ ×J♂}♀ ×J♂]♀ ×J♂, Nos. 1~5

Eight female third-generation offspring derived from spermatozoa exposed to 100 rads of neutrons were transplanted with frog pituitaries. As normal ovulation occurred in five of them, these five females (Nos. 1~5) were mated with the same three males as those used in the control series. The survival curve of the fourth-generation offspring produced from each of these females is shown in Fig. 53a.

In five matings Nos. 1~5, 82.2%, 89.1%, 69.8%, 73.4% and 72.5%, 77.4% on the average, of the respective total number of eggs cleaved normally. While 46 (24%) of 189 normally cleaved eggs produced from mating No. 1 and 27 (16%) of 166 from mating No. 5 died of various abnormalities by the hatching stage, the normally cleaved eggs from the other matings were not inferior to the controls in developmental capacity. In the five matings, 62.2%, 87.7%, 67.0%, 72.5% and 60.7%, 69.7% on the average, hatched normally. The tadpoles produced from all these matings did not differ from the controls in developmental capacity; 58.7%, 87.2%, 65.1%, 72.0% and 59.8%, 68.2% on the average, became normally metamorphosed frogs.

ii) [{(J♀ ×SN-150♂)♀ ×J♂}♀ ×J♂]♀ ×J♂, Nos. 1~4

Five female third-generation offspring derived from spermatozoa exposed to 150 rads of neutrons were transplanted with frog pituitaries. As normal ovulation occurred in four of them, these four females (Nos. 1~4) were mated

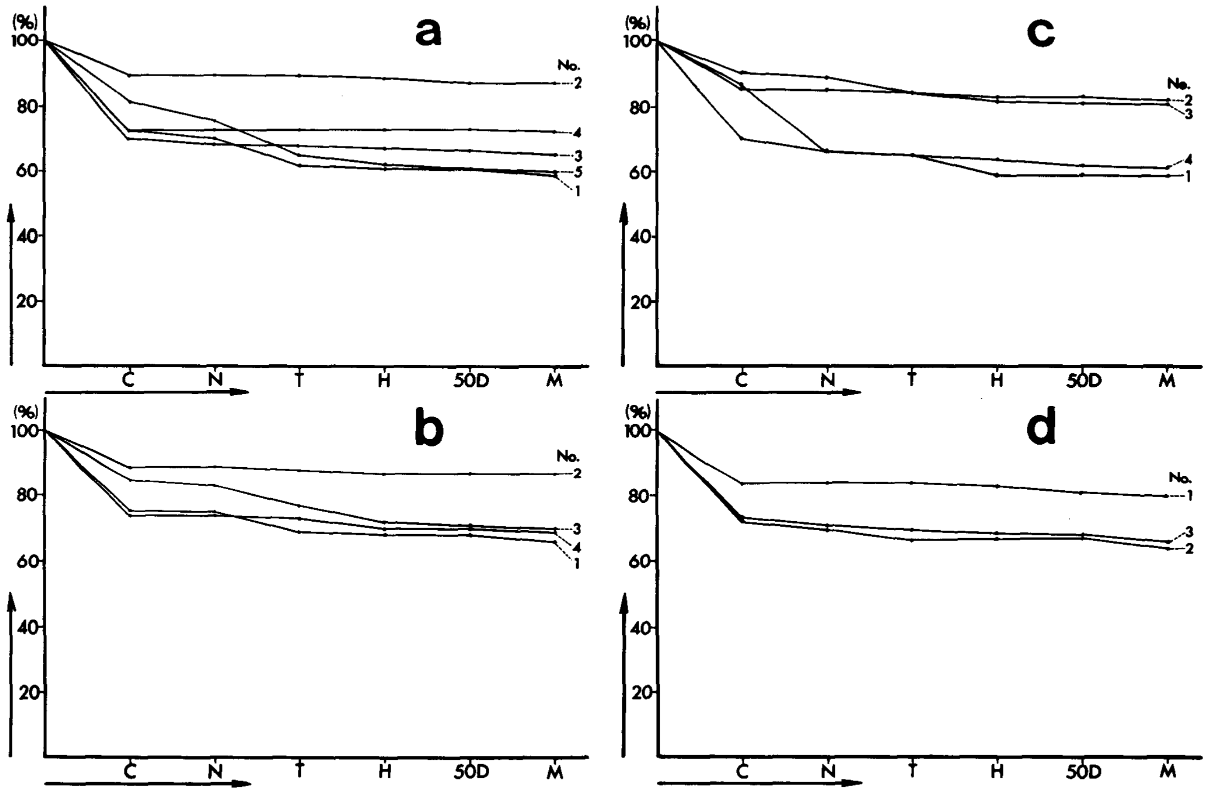


Fig. 53. Survival curves of fourth-generation offspring derived from neutron-irradiated great-great-grandparental gametes by passing over female first-, second- and third-generation offspring.

a. [{(J♀ ×SN-100♂)♀ ×J♂}♀ ×J♂]♀ ×J♂, Nos. 1~5
b. [{(J♀ ×SN-150♂)♀ ×J♂}♀ ×J♂]♀ ×J♂, Nos. 1~4
c. [{(EN-150♀ ×J♂)♀ ×J♂}♀ ×J♂]♀ ×J♂, Nos. 1~4
d. [{(EN-250♀ ×J♂)♀ ×J♂}♀ ×J♂]♀ ×J♂, Nos. 1~3

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

with the same three males as those used in the control series. The survival curve of the fourth-generation offspring produced from each of these females is shown in Fig. 53b.

In four matings Nos. 1∼4, 75.2%, 88.6%, 84.7% and 74.4%, 80.8% on the average, of the respective total number of eggs cleaved normally. While 15 (9%) of 161 normally cleaved eggs from mating No. 1 and 25 (14%) of 183 from mating No. 3 died of edema at the tail-bud and the hatching stage, respectively, the normally cleaved eggs from the other matings Nos. 2 and 4 developed normally except a few. In the four matings, 67.8%, 87.3%, 71.8% and 70.2%, 74.3% on the average, hatched normally, and afterwards 65.9%, 87.3%, 70.4% and 68.8%, 73.2% on the average, became normally metamorphosed frogs.

e. Experimental series derived from neutron-irradiated great-great-grandparental eggs

i) [{(EN–150♀ ×J♂)♀ ×J♂}♀ ×J♂]♀ ×J♂, Nos. 1∼4

Five female third-generation offspring derived from eggs exposed to 150 rads of neutrons were transplanted with frog pituitaries. As normal ovulation occurred in four of them, these four females (Nos. 1∼4) were mated with the same three males as those used in the control series. The survival curve of the fourth-generation offspring produced from each of these females is shown in Fig. 53c.

In four matings Nos. 1∼4, 69.5%, 84.8%, 89.7% and 85.6%, 81.5% on the average, of the respective total number of eggs cleaved normally. In mating No. 1, three, seven, two and fifteen of 182 normally cleaved eggs died of abnormalities at the blastula, neurula, tail-bud and hatching stages, respectively, while the remainders, that is, 59.2% of the total number of eggs hatched normally. In mating No. 4, 46 of 179 normally cleaved eggs died of abnormalities by the hatching stage. Most of the abnormalities appeared at the neurula stage. In this mating, 63.6% of the total number of eggs hatched normally. In matings Nos. 2 and 3, 83.4% and 82.1% of the respective total number of eggs hatched normally. On the average, 71.2% of the total number of eggs in the four matings hatched normally and became normal tadpoles. Only a few of the tadpoles produced from the four matings died of edema or underdevelopment afterwards, and 58.8%, 82.0%, 80.5% and 61.2%, 69.8% on the average, became normally metamorphosed frogs.

ii) [{(EN–250♀ ×J♂)♀ ×J♂}♀ ×J♂]♀ ×J♂, Nos. 1∼3

Five female third-generation offspring derived from eggs exposed to 250 rads of neutrons were transplanted with frog pituitaries. As normal ovulation occurred in three of them, these three females (Nos. 1∼3) were mated with the same three males as those used in the control series. The survival curve of the fourth-generation offspring produced from each of the three females is shown in Fig. 53d.

In three matings Nos. 1∼3, 83.5%, 72.1% and 72.5%, 75.9% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were not inferior to the controls in developmental capacity; 82.6%, 67.1%

and 69.8%, 72.8% on the average, hatched normally, and 80.1%, 64.2% and 65.9%, 69.9% on the average, became normally metamorphosed frogs.

2. Viability and sex of metamorphosed frogs

a. Viability

The age of tadpoles at the time of climbing out of water and the body length of frogs immediately after metamorphosis in eight experimental and the control series are presented in Table 42. While 675 tadpoles in the control series were 111 ~ 140 days old, 126.4 days old on the average, in the age at the time of climbing out of water, 338 ~ 780 tadpoles were 111 ~ 149 days old in the eight experimental series, 120.2 ~ 134.3 days old on the average in each of these experimental series. While about 100 frogs removed at random from the control series immediately after metamorphosis were 17.3±0.2 mm in body length, those from each of the experimental series were 16.9±0.3 ~ 17.3±0.2 mm. There were no remarkable differences in the age at the time of climbing out of water as well as in the body length immediately after metamorphosis between the frogs of the experimental series and the controls. There were also no distinct differences in these respects between the frogs of two kinds of matings which were different from each other in the kind or dosage of irradiation, and between the frogs derived from irradiated spermatozoa and those from irradiated eggs.

In each of the experimental series, 2 ~ 7% of the total number of normally metamorphosed frogs died within one or two months after metamorphosis, while

TABLE 42

Number, size and sex of metamorphosed frogs produced from

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed frogs
Female	Male		
{(J·J)J}J, Nos. 1~4	J.W72, Nos. 1~3	111~140 (126.4)	675
{(J·SX-150)J}J, Nos. 1~5	J.W72, Nos. 1~3	114~139 (126.8)	780
{(J·SX-250)J}J, Nos. 1~5		111~135 (120.2)	765
{(EX-150·J)J}J, Nos. 1~5	J.W72, Nos. 1~3	121~146 (126.4)	754
{(EX-250·J)J}J, Nos. 1,2		120~143 (130.5)	338
{(J·SN-100)J}J, Nos. 1~5	J.W72, Nos. 1~3	111~146 (129.3)	745
{(J·SN-150)J}J, Nos. 1~4		115~149 (134.3)	633
{(EN-150·J)J}J, Nos. 1~4	J.W72, Nos. 1~3	119~140 (129.6)	612
{(EN-250·J)J}J, Nos. 1~3		111~144 (126.0)	511

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

6% of the controls died within the same period of time. There were also no remarkable differences in the viability of metamorphosed frogs among the experimental series, except those derived from X-irradiated great-great-grandparental eggs; the fourth-generation offspring derived from eggs irradiated with 250 rads were slightly larger in the rate of inviable frogs than those derived from eggs with 150 rads. All the living frogs were killed one or two months after metamorphosis to examine their sex.

b. Sex

The sex of immature frogs is presented in Table 42. While there was nearly an equal number of males and females in the control series, males were somewhat more numerous than females among the frogs obtained from each of the experimental series.

In the control series, 41 of 675 normally metamorphosed frogs produced from four matings died within one or two months after metamorphosis. The other 634 were killed to examine their sex. Of these immature frogs, 314 were females with normal ovaries, 3 females with underdeveloped ovaries, one hermaphrodite and 316 males. When the hermaphrodite was counted as a male, just 50% of the total number of frogs examined were males.

i) Experimental series, [{(J♀ ×SX–150♂)♀ ×J♂}♀ ×J♂]♀ ×J♂

Fourteen of 780 normally metamorphosed frogs produced from five matings died within one or two months after metamorphosis. Of the remaining 766

females derived from irradiated great-grandparental sperm or eggs

Mean body length of 100 frogs immediately after metamorphosis (mm)	Sex of frogs killed about one or two months after metamorphosis					
	No. of frogs	♀$_N$	♀$_U$	⚥	♂$_N$	♂ (%)*
17.3±0.2	634	314	3	1	316	(50.0)
17.2±0.2	766	343	14	17	392	(53.4)
17.1±0.2	742	326	6	11	399	(55.3)
16.9±0.3	739	321	10	13	395	(55.2)
17.0±0.2	316	130	5	9	172	(57.3)
17.1±0.2	715	332	7	8	368	(52.6)
17.3±0.2	611	285	6	5	315	(52.4)
17.0±0.2	594	276	13	15	290	(51.3)
17.1±0.2	497	220	16	20	241	(52.5)

⚥—Hermaphrodites ♂$_N$—Males with normal testes * Including hermaphrodites

frogs, 343 were females with normal ovaries, 14 females with underdeveloped ovaries, 17 hermaphrodites and 392 males. When the hermaphrodites were counted as males, 53.4% of the total number of frogs examined were males.

ii) Experimental series, [{(J♀ × SX–250♂)♀ × J♂}♀ × J♂]♀ × J♂

Twenty-three of 765 normally metamorphosed frogs from five matings died within one or two months after metamorphosis. Of the remaining 742 immature frogs, 326 were females with normal ovaries, 6 females with underdeveloped ovaries, 11 hermaphrodites and 399 males. When the hermaphrodites were counted as males, 55.3% of the total number of frogs examined were males.

iii) Experimental series, [{(EX–150♀ × J♂)♀ × J♂}♀ × J♂]♀ × J♂

Fifteen of 754 frogs from five matings died within one or two months after metamorphosis. Of the remaining 739, 321 were females with normal ovaries, 10 females with underdeveloped ovaries, 13 hermaphrodites and 395 males. When the hermaphrodites were counted as males, 55.2% of the total number of frogs examined were males.

iv) Experimental series, [{(EX–250♀ × J♂)♀ × J♂}♀ × J♂]♀ × J♂

Twenty-two of 338 frogs from two matings died within one or two months after metamorphosis. Of the remaining 316 frogs, 130 were females with normal ovaries, 5 females with underdeveloped ovaries, 9 hermaphrodites and 172 males. When the hermaphrodites were counted as males, 57.3% of the total number of frogs examined were males.

v) Experimental series, [{(J♀ × SN–100♂)♀ × J♂}♀ × J♂]♀ × J♂

Thirty of 745 frogs from five matings died within one or two months after metamorphosis. Of the remaining 715, 332 were females with normal ovaries, 7 females with underdeveloped ovaries, 8 hermaphrodites and 368 males. When the hermaphrodites were counted as males, 52.6% of the total number of frogs examined were males.

vi) Experimental series, [{(J♀ × SN–150♂)♀ × J♂}♀ × J♂]♀ × J♂

Twenty-two of 633 frogs from four matings died within one or two months after metamorphosis. Of the remaining 611, 285 were females with normal ovaries, 6 females with underdeveloped ovaries, 5 hermaphrodites and 315 males. When the hermaphrodites were counted as males, 52.4% of the total number of frogs examined were males.

vii) Experimental series, [{(EN–150♀ × J♂)♀ × J♂}♀ × J♂]♀ × J♂

Eighteen of 612 frogs from four matings died within one or two months after metamorphosis. Of the remaining 594 frogs, 276 were males with normal ovaries, 13 females with underdeveloped ovaries, 15 hermaphrodites and 290 males. When the hermaphrodites were counted as males, 51.3% of the total number of frogs examined were males.

viii) Experimental series, [{(EN–250♀ × J♂)♀ × J♂}♀ × J♂]♀ × J♂

Fourteen of 511 frogs from three matings died within one or two months after metamorphosis. Of the remaining 497 frogs, 220 were females with normal ovaries, 16 females with underdeveloped ovaries, 20 hermaphrodites and 241 males. When the hermaphrodites were counted as males, 52.5% of the total number of frogs examined were males.

X. Fourth-generation offspring derived from irradiated gametes by matings between male and female third-generation offspring

1. Developmental capacity

In the breeding season of 1972, male and female third-generation offspring derived from X- or neutron-irradiated spermatozoa or eggs were mated. The female third-generation offspring were produced in 1970 from male second-generation offspring by mating with normal, field-caught females (Table 43). These male second-generation offspring were those produced in 1969 from male first-generation offspring by mating with normal, field-caught females. On the other hand, the third-generation males were produced in 1971 from female second-generation offspring by mating with normal, field-caught males (Table 44). These female second-generation offspring were those produced in 1969 from female first-generation offspring by mating with normal, field-caught males. The female third-generation offspring divided into eight series in accordance with their origin. In each series, one to five females were mated with the same number of males as that of the females by artificial fertilization. The developmental capacity of the fourth-generation offspring produced from each of the experimental and the control series is presented in Table 45 and shown in Figs. 54～56.

a. Control series, [J♀×{J♀×(J♀×J♂)♂}♂]♀×[{(J♀×J♂)♀×J♂}♀×J♂]♂, Nos. 1～5

Six females were transplanted with frog pituitaries. As five of them ovulated normally, these five females (Nos. 1～5) were mated with five males. Both males and females were third-generation offspring derived from normal, field-caught males and females as the controls of the frogs derived from X- or neutron-irradiated great-great-grandparental spermatozoa or eggs. The survival curve of the fourth-generation offspring produced from each of the five females is shown in Fig. 54.

In five matings Nos. 1～5, 77.6%, 75.2%, 73.1%, 83.1% and 80.5%, 77.9% on the average, of the respective total number of eggs cleaved normally. Except a few eggs which died of various abnormalities or edema at various developmental stages, the normally cleaved eggs developed normally; 75.6%, 73.4%, 70.5%, 80.9% and 76.2%, 75.3% on the average, hatched normally, and 75.2%, 72.4%, 68.7%, 80.4% and 74.9%, 74.4% on the average, became normally metamorphosed frogs.

TABLE 43

Eggs of female parents used for mating experiments in 1972, II

Kind	Individual no.	Age (year)	Body length (mm)	No. of eggs	Mean diameter of 50 eggs (mm)
J{J(J·J)}, No. 1	1	2	38.5	937	1.62±0.02
	2	2	43.5	1027	1.67±0.02
	3	2	44.0	1075	1.69±0.02
	4	2	41.5	982	1.72±0.02
	5	2	45.0	1146	1.75±0.02
J{J(J·SX–150)}, No. 2	1	2	42.5	794	1.73±0.02
	2	2	42.5	887	1.82±0.02
	3	2	43.0	632	1.69±0.02
	4	2	40.0	1033	1.64±0.02
	5	2	40.0	486	1.68±0.02
	6	2	38.0	0	—
J{J(J·SX–250)}, No. 1	1	2	43.0	791	1.64±0.02
	2	2	41.5	766	1.72±0.02
J{J(EX–150·J)}, No. 1	1	2	39.0	569	1.64±0.02
	2	2	43.5	864	1.69±0.02
	3	2	42.5	738	1.72±0.02
	4	2	45.0	1092	1.72±0.02
	5	2	43.0	1022	1.70±0.02
J{J(EX–250·J)}, No. 3	1	2	40.0	937	1.64±0.02
J{J(J·SN–100)}, No. 3	1	2	43.0	1012	1.59±0.02
	2	2	38.5	642	1.67±0.02
	3	2	41.0	944	1.63±0.02
	4	2	44.0	1130	1.58±0.02
	5	2	39.0	0	—
J{J(J·SN–150)}, No. 1	1	2	40.0	967	1.71±0.02
	2	2	45.0	1532	1.73±0.02
J{J(EN–150·J)}, No. 1	1	2	41.0	783	1.69±0.02
	2	2	39.0	569	1.62±0.02
	3	2	37.5	627	1.53±0.02
	4	2	44.0	991	1.82±0.02
	5	2	43.5	1033	1.69±0.02
	6	2	44.0	0	—
	7	2	41.0	0	—
J{J(EN–250·J)}, No. 2	1	2	42.5	962	1.67±0.02
	2	2	43.5	865	1.71±0.02

J{J(J·J)}, No. 1: Females obtained by J.W70 ♀, Nos. 1∼12×J(J·J) ♂, No. 1

J{J(J·SX–150 or –250)}, No. 2 or 1: Females obtained by J.W70 ♀, Nos. 1∼3×J(J·SX–150 or –250) ♂, No. 2 or 1

J{J(EX–150 or –250·J)}, No. 1 or 3: Females obtained by J.W70 ♀, Nos. 4∼6×J(EX–150 or –250·J) ♂, No. 1 or 3

J{J(J·SN–100 or –150)}, No. 3 or 1: Females obtained by J.W70 ♀, Nos. 7∼9×J(J·SN–100 or –150) ♂, No. 3 or 1

J{J(EN–150 or –250·J)}, No. 1 or 2: Females obtained by J.W70 ♀, Nos. 10∼12×J(EN–150 or –250·J) ♂, No. 1 or 2

TABLE 44

Testes of male parents used for mating experiments in 1972, II

Kind	Individual no.	Age (year)	Body length (mm)	Size of the testes		Inner structure (Type)
				Left (mm)	Right (mm)	
{(J·J)J} J, No. 3	1	1	34.0	3.0×2.0	3.0×2.0	1
	2	1	30.0	2.5×1.5	2.5×1.5	1
	3	1	29.5	2.5×2.0	2.5×1.5	1
	4	1	28.0	2.0×1.5	3.0×1.5	1
	5	1	32.5	3.0×2.0	3.0×1.5	1
{(J·SX–150)J} J, No. 2	1	1	30.5	2.5×1.5	2.5×1.5	1
	2	1	31.0	2.5×2.0	2.5×2.0	1
	3	1	33.0	3.0×1.5	3.0×1.5	1
	4	1	29.5	3.0×1.5	3.0×1.5	1
	5	1	34.5	3.5×2.0	3.0×2.5	1
{(J·SX–250)J} J, No. 2	1	1	32.5	3.0×2.0	3.5×2.0	1
	2	1	35.0	3.5×2.0	4.0×2.0	1
{(EX–150·J)J} J, No. 6	1	1	34.0	3.5×2.0	3.0×2.0	1
	2	1	31.0	3.0×1.5	3.0×1.0	1
	3	1	36.0	4.0×2.0	4.0×2.0	1
	4	1	30.0	3.0×1.5	3.0×1.5	1
	5	1	32.5	3.5×2.0	3.5×2.0	1
{(EX–250·J)J} J, No. 3	1	1	33.5	3.5×2.0	3.5×2.0	1
{(J·SN–100)J} J, No. 3	1	1	30.0	3.0×1.5	3.0×1.5	1
	2	1	31.0	3.0×1.5	3.0×1.5	1
	3	1	29.5	2.5×1.5	2.5×1.5	1
	4	1	32.0	3.0×2.0	3.5×2.0	1
{(J·SN–150)J} J, No. 1	1	1	31.5	3.5×2.0	3.5×2.0	1
	2	1	33.0	4.0×2.0	4.0×2.0	1
{(EN–150·J)J} J, No. 3	1	1	31.5	3.5×1.5	3.5×1.5	1
	2	1	31.0	3.0×1.5	3.0×1.5	1
	3	1	34.0	3.5×2.0	4.0×2.0	1
	4	1	36.0	4.0×2.0	4.0×2.0	1
	5	1	32.5	3.5×2.0	3.5×2.0	1
{(EN–250·J)J} J, No. 3	1	1	30.5	3.0×1.5	3.0×1.5	1
	2	1	32.5	3.5×2.0	3.5×2.0	1

{(J·J)J} J, No. 3: Males obtained by (J·J)J ♀, No. 3×J.W71 ♂, Nos. 1∼3

{(J·SX–150 or –250)J} J, No. 2: Males obtained by (J·SX–150 or –250)J ♀, No. 2×J.W71 ♂, Nos. 1∼3

{(EX–150 or –250·J)J} J, No. 6 or 3: Males obtained by (EX–150 or –250·J)J ♀, No. 6 or 3×J.W71 ♂, Nos. 1∼3

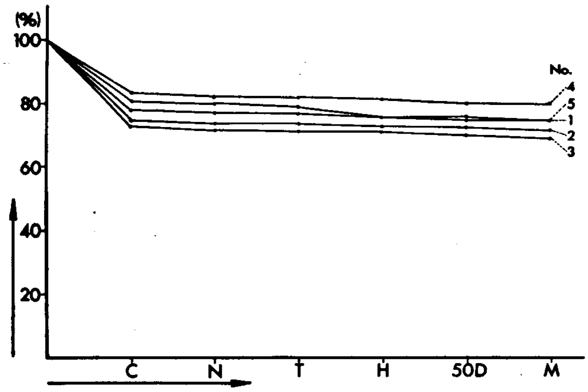
{(J·SN–100 or –150)J} J, No. 3 or 1: Males obtained by (J·SN–100 or –150)J ♀, No. 3 or 1× J.W71 ♂, Nos. 1∼3

{(EN–150 or –250·J)J} J, No. 3: Males obtained by (EN–150 or –250·J)J ♀, No. 3×J.W71 ♂, Nos. 1∼3

TABLE 45

Developmental capacity of the offspring of males and

Parents		No. of eggs	No. of cleaved eggs	
Female	Male		Normal	Abnormal
J{J(J·J)}, Nos. 1∼5	{(J·J)J}J, Nos. 1∼5	1151	897 (77.9%)	7 (0.6%)
J{J(J·SX–150)}, Nos. 1∼5	{(J·SX–150)J}J, Nos. 1∼5	1204	1006 (83.6%)	14 (1.2%)
J{J(J·SX–250)}, Nos. 1,2	{(J·SX–250)J}J, Nos. 1, 2	516	420 (81.4%)	3 (0.6%)
J{J(EX–150·J)}, Nos. 1∼5	{(EX–150·J)J}J, Nos. 1∼5	1139	904 (79.4%)	16 (1.4%)
J{J(EX–250·J)}, No. 1	{(EX–250·J)J}J, No. 1	260	210 (80.8%)	14 (5.4%)
J{J(J·SN–100)}, Nos. 1∼4	{(J·SN–100)J}J, Nos. 1∼4	874	710 (81.2%)	14 (1.6%)
J{J(J·SN–150)}, Nos. 1,2	{(J·SN–150)J}J, Nos. 1, 2	506	432 (85.4%)	5 (1.0%)
J{J(EN–150·J)}, Nos. 1∼5	{(EN–150·J)J}J, Nos. 1∼5	1113	910 (81.8%)	10 (0.9%)
J{J(EN–250·J)}, Nos. 1,2	{(EN–250·J)J}J, Nos. 1, 2	522	406 (77.8%)	3 (0.6%)



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

Fig. 54. Survival curves of control fourth-generation offspring derived from untreated great-great-grandparental gametes by matings, [J♀ × {J♀ × (J♀ × J♂) ♂} ♂] ♀ × [{(J♀ × J♂) ♀ × J♂} ♀ × J♂] ♂, Nos. 1∼5.

b. Experimental series derived from X-irradiated great-great-grandparental sperm

i) [J♀ × {J♀ × (J♀ × SX–150♂) ♂} ♂] ♀ × [{(J♀ × SX–150♂) ♀ × J♂} ♀ × J♂] ♂, Nos. 1∼5

Six female third-generation offspring derived from spermatozoa exposed to 150 rads of X-rays were transplanted with frog pituitaries. As normal ovulation occurred in five females (Nos. 1∼5), they were mated with five male third-generation offspring Nos. 1∼5 derived from spermatozoa exposed to the females derived from irradiated great-grandparental 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		
888	6	882	6	867	15	862	856
(77.2%)	(0.5%)	(76.6%)	(0.5%)	(75.3%)	(1.3%)	(74.9%)	(74.4%)
987	13	957	30	920	37	897	873
(82.0%)	(1.1%)	(79.5%)	(2.5%)	(76.4%)	(3.1%)	(74.5%)	(72.5%)
415	5	406	9	390	16	387	381
(80.4%)	(1.0%)	(78.7%)	(1.7%)	(75.6%)	(3.1%)	(75.0%)	(73.8%)
889	11	872	17	827	45	808	787
(78.1%)	(1.0%)	(76.6%)	(1.5%)	(72.6%)	(4.0%)	(70.9%)	(69.1%)
205	3	204	1	196	8	191	189
(78.8%)	(1.2%)	(78.5%)	(0.4%)	(75.4%)	(3.1%)	(73.5%)	(72.7%)
688	14	681	7	652	29	639	624
(78.7%)	(1.6%)	(77.9%)	(0.8%)	(74.6%)	(3.3%)	(73.1%)	(71.4%)
426	6	417	9	386	31	373	359
(84.2%)	(1.2%)	(82.4%)	(1.8%)	(76.3%)	(6.1%)	(73.7%)	(70.9%)
901	8	886	15	852	34	826	810
(81.0%)	(0.7%)	(79.6%)	(1.3%)	(76.5%)	(3.1%)	(74.2%)	(72.7%)
402	4	397	5	379	18	364	353
(77.0%)	(0.8%)	(76.1%)	(1.0%)	(72.6%)	(3.4%)	(69.7%)	(67.6%)

same dose. The survival curve of the fourth-generation offspring produced from each of the five females is shown in Fig. 55a.

In five matings Nos. 1∼5, 88.6%, 81.8%, 77.6%, 83.3% and 85.8%, 83.6% on the average, of the respective total number of eggs cleaved normally. A small number of the normally cleaved eggs died of various abnormalities by the hatching stage, and 82.9%, 75.2%, 68.5%, 75.1% and 80.0%, 76.4% on the average, hatched normally and became swimming tadpoles. Of a total of 920 tadpoles, 47 died of underdevelopment or edema afterwards. Eventually, 81.7%, 71.9%, 66.0%, 63.9% and 78.2%, 72.5% on the average, of the respective total number of eggs became normally metamorphosed frogs.

ii) [J♀×{J♀×(J♀×SX-250♂)♂}♂]♀×
[{(J♀×SX-250♂)♀×J♂}♀×J♂]♂, Nos. 1 and 2

Two female third-generation offspring Nos. 1 and 2 derived from spermatozoa exposed to 250 rads of X-rays were transplanted with frog pituitaries and then mated with two male third-generation offspring Nos. 1 and 2 derived from spermatozoa exposed to the same dose. The survival curve of the fourth-generation offspring produced from each of the two females is shown in Fig. 55b.

In two matings Nos. 1 and 2, 83.5% and 79.2%, 81.4% on the average, of the respective total number of eggs cleaved normally. About 90% of the normally cleaved eggs from mating No. 1 and about 95% of those from mating No. 2, that is, 75.6% of the total number of eggs in these two matings hatched normally, while the remaining eggs died of edema or abnormalities by the hatch-

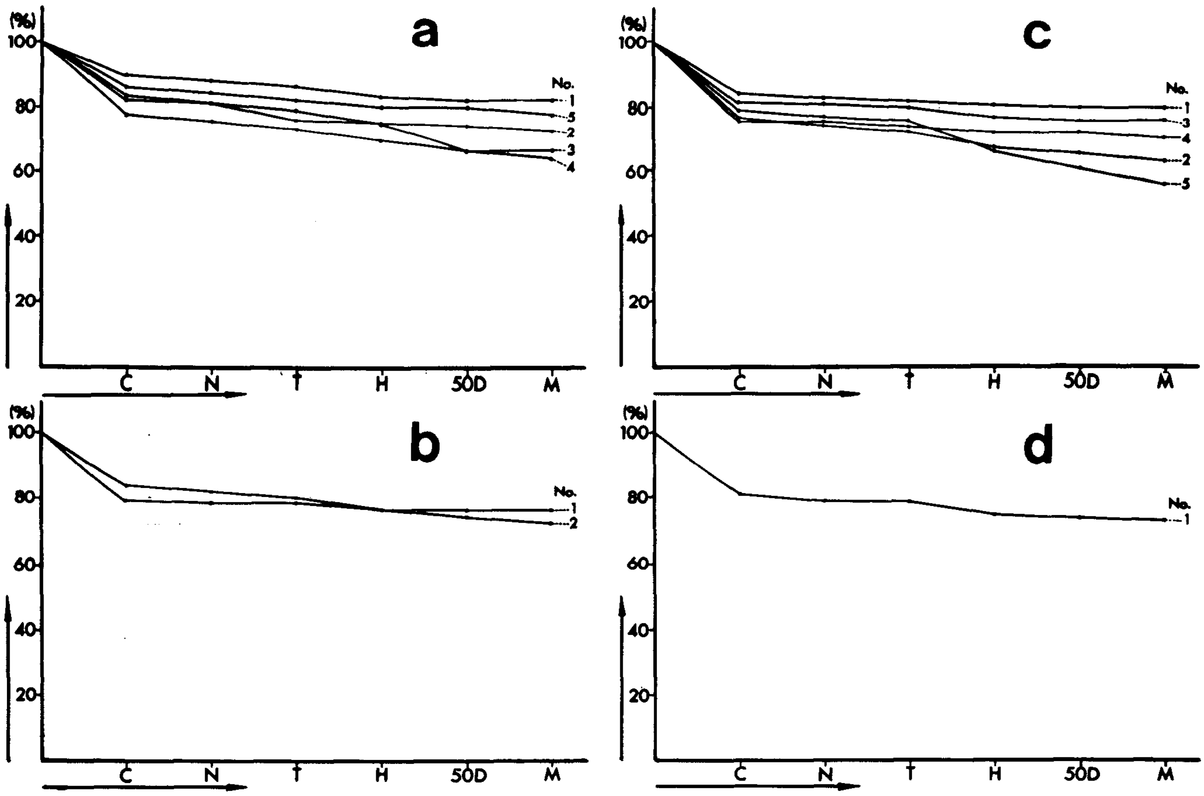


Fig. 55. Survival curves of fourth-generation offspring derived from X-irradiated great-great-grandparental gametes by matings between male and female third-generation offspring.

a. [J♀ × {J♀ × (J♀ × SX–150♂) ♂} ♂] ♀ × [{(J♀ × SX–150♂) ♀ × J♂} ♀ × J♂] ♂, Nos. 1∼5
b. [J♀ × {J♀ × (J♀ × SX–250♂) ♂} ♂] ♀ × [{(J♀ × SX–250♂) ♀ × J♂} ♀ × J♂] ♂, Nos. 1 and 2
c. [J♀ × {J♀ × (EX–150♀ × J♂) ♂} ♂] ♀ × [{(EX–150♀ × J♂) ♀ × J♂} ♀ × J♂] ♂, Nos. 1∼5
d. [J♀ × {J♀ × (EX–250♀ × J♂) ♂} ♂] ♀ × [{(EX–250♀ × J♂) ♀ × J♂} ♀ × J♂] ♂, No. 1

ing stage. All the tadpoles produced from mating No. 1 and about 90% of those from mating No. 2, that is, 73.8% of the total number of eggs in these two matings became normally metamorphosed frogs.

c. Experimental series derived from X-irradiated great-great-grandparental eggs

i) [J♀ × {J♀ × (EX–150♀ × J♂) ♂} ♂] ♀ × [{(EX–150♀ × J♂) ♀ × J♂} ♀ × J♂] ♂, Nos. 1∼5

Five female third-generation offspring Nos. 1∼5 derived from eggs exposed to 150 rads of X-rays were transplanted with frog pituitaries. As all of them ovulated normally, they were mated with five male third-generation offspring Nos. 1∼5 derived from eggs exposed to the same dose. The survival curve of the fourth-generation offspring produced from each of the five females is shown in Fig. 55c.

In five matings Nos. 1∼5, 83.9%, 76.5%, 81.0%, 76.4% and 78.7%, 79.4% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs obtained by matings Nos. 1, 3 and 4 were not inferior to the controls in developmental capacity, while those from matings Nos. 2 and 5 were slightly inferior; 80.9%, 66.8%, 76.8%, 72.2% and 66.0%, 72.6% on the average, hatched normally, and 80.4%, 63.3%, 75.5%, 70.4% and 55.7%, 69.1% on the average, became normally metamorphosed frogs.

ii) [J♀ × {J♀ × (EX–250♀ × J♂) ♂} ♂] ♀ ×
[{(EX–250♀ × J♂) ♀ × J♂} ♀ × J♂] ♂, No. 1

A female third-generation offspring derived from an egg exposed to 250 rads of X-rays was transplanted with frog pituitaries and then mated with a male third-generation offspring derived from an egg exposed to the same dose. The survival curve of the fourth-generation offspring produced from the female is shown in Fig. 55d.

In the mating, 80.8% of the total number of eggs cleaved normally. Of the normally cleaved eggs, two, three, one and eight died of abnormalities at the blastula, neurula, tail-bud and hatching stages, respectively, while 196, 75.4% of the total number of eggs hatched normally. After 7 tadpoles died of edema, the remaining 189, 72.7% became normally metamorphosed frogs.

d. Experimental series from neutron-irradiated great-great-grandparental sperm

i) [J♀ × {J♀ × (J♀ × SN–100♂) ♂} ♂] ♀ ×
[{(J♀ × SN–100♂) ♀ × J♂} ♀ × J♂] ♂, Nos. 1～4

Five female third-generation offspring derived from spermatozoa exposed to 100 rads of neutrons were transplanted with frog pituitaries. As four of them ovulated normally, these four females (Nos. 1～4) were mated with four male third-generation offspring Nos. 1～4 derived from spermatozoa exposed to the same dose. The survival curve of the fourth-generation offspring produced from each of the four females is shown in Fig. 56a.

In four matings Nos. 1～4, 75.8%, 80.2%, 75.7% and 92.3%, 81.2% on the average, of the respective total number of eggs cleaved normally. The normally cleaved eggs were scarcely inferior to the controls in developmental capacity, except those from mating No. 2 in which about one-fourth of them died of edema at the hatching or tadpole stage. Eventually, 71.1%, 70.0%, 70.7% and 85.5%, 74.6% on the average, of the respective total number of eggs hatched normally, and afterwards 71.1%, 58.9%, 69.4% and 84.6%, 71.4% on the average, became normally metamorphosed frogs.

ii) [J♀ × {J♀ × (J♀ × SN–150♂) ♂} ♂] ♀ ×
[{(J♀ × SN–150♂) ♀ × J♂} ♀ × J♂] ♂, Nos. 1 and 2

Two female third-generation offspring Nos. 1 and 2 derived from spermatozoa exposed to 150 rads of neutrons were transplanted with frog pituitaries and then mated with two male third-generation offspring Nos. 1 and 2 derived from spermatozoa exposed to the same dose. The survival curve of the fourth-generation offspring produced from each of the two females is shown in Fig. 56b.

In two matings Nos. 1 and 2, 84.6% and 86.1%, 85.4% on the average, of the respective total number of eggs cleaved normally. Of the normally cleaved

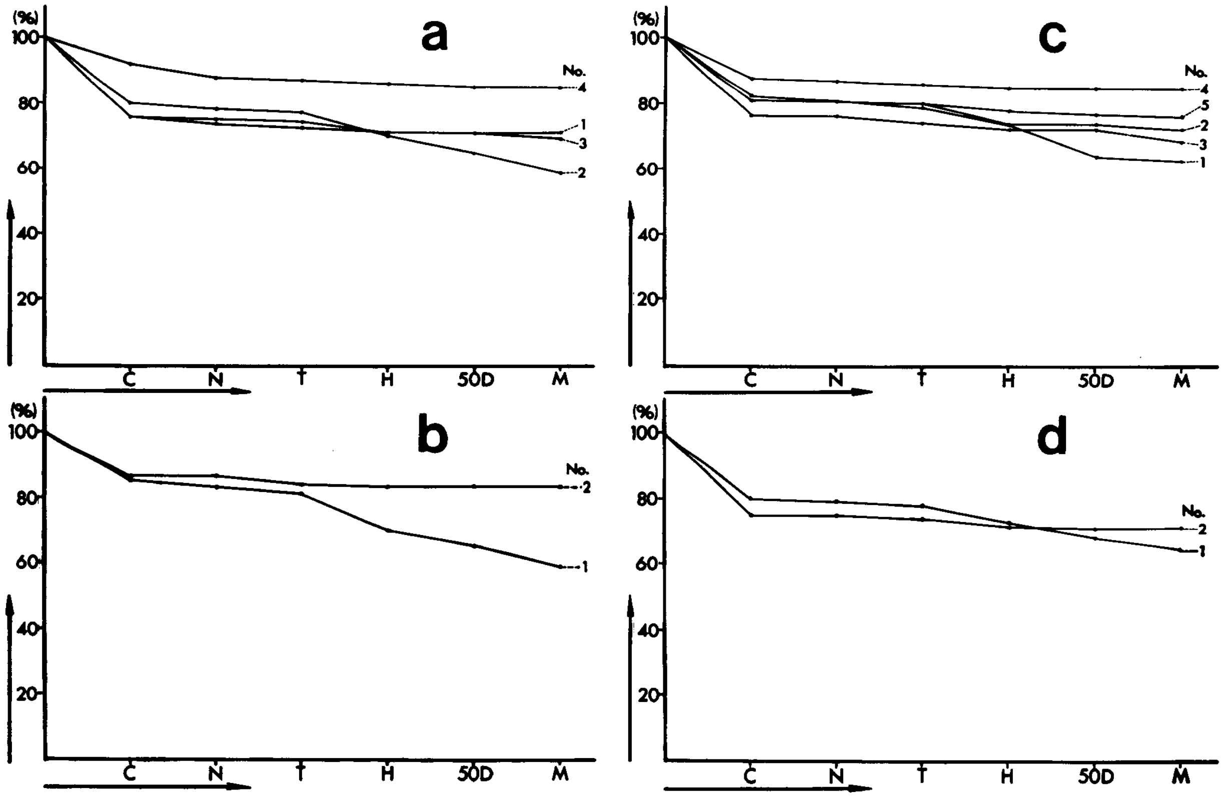


Fig. 56. Survival curves of fourth-generation offspring derived from neutron-irradiated great-great-grandparental gametes by matings between male and female third-generation offspring.

a. [J♀ × {J♀ × (J♀ × SN-100♂) ♂} ♂] ♀ × [{(J♀ × SN-100♂) ♀ × J♂} ♀ × J♂] ♂, Nos. 1~4

b. [J♀ × {J♀ × (J♀ × SN-150♂) ♂} ♂] ♀ × [{(J♀ × SN-150♂) ♀ × J♂} ♀ × J♂] ♂, Nos. 1 and 2

c. [J♀ × {J♀ × (EN-150♀ × J♂) ♂} ♂] ♀ × [{(EN-150♀ × J♂) ♀ × J♂} ♀ × J♂] ♂, Nos. 1~5

d. [J♀ × {J♀ × (EN-250♀ × J♂) ♂} ♂] ♀ × [{(EN-250♀ × J♂) ♀ × J♂} ♀ × J♂] ♂, Nos. 1 and 2

eggs obtained by mating No. 1, five, four, and 28 became edematous and died at the neurula, tail-bud and hatching stages, respectively, while the remaining 178, 70.1% of the total number of eggs hatched normally and became tadpoles. Of these tadpoles, 27 became edematous and died before the completion of metamorphosis. The remaining 151 tadpoles, 59.4% of the total number of eggs became normal frogs. The normally cleaved eggs from mating No. 2 were not inferior to the controls in developmental capacity; 82.5% hatched normally and afterwards became normally metamorphosed frogs. On the average, 76.3% and 70.9% of the total number of eggs in the two matings normally hatched and metamorphosed, respectively.

e. Experimental series derived from neutron-irradiated great-great-grand-parental eggs

i) [J♀ × {J♀ × (EN-150♀ × J♂) ♂} ♂] ♀ × [{(EN-150♀ × J♂) ♀ × J♂} ♀ × J♂] ♂, Nos. 1~5

Seven female third-generation offspring derived from eggs exposed to 150 rads of neutrons were transplanted with frog pituitaries. As normal ovulation occurred in five of them, these five females (Nos. 1∼5) were mated with five male third-generation offspring Nos. 1∼5 derived from eggs exposed to the same dose. The survival curve of the fourth-generation offspring produced from each of the five females is shown in Fig. 56c.

In five matings Nos. 1∼5, 82.4%, 80.8%, 76.0%, 88.2% and 81.9%, 81.8% on the average, of the respective total number of eggs cleaved normally. These normally cleaved eggs were not inferior to the controls in developmental capacity, except those obtained from mating No. 1. About 10% and 13% of the latter died of edema in the embryonic and tadpole stages, respectively. In the five matings, 73.9%, 74.1%, 72.7%, 84.8% and 78.0%, 76.5% on the average, hatched normally, and 63.0%, 71.9%, 69.4%, 84.7% and 76.3%, 72.8% on the average, became normally metamorphosed frogs.

ii) [J♀×{J♀×(EN–250♀×J♂)♂}♂]♀×
[{(EN–250♀×J♂)♀×J♂}♀×J♂]♂, Nos. 1 and 2

Two female third-generation offspring Nos. 1 and 2 derived from eggs exposed to 250 rads of neutrons were transplanted with frog pituitaries. As normal ovulation occurred in both of them, they were mated with two male third-generation offspring derived from eggs exposed to the same dose. The survival curve of the fourth-generation offspring produced from each of the two females is shown in Fig. 56d.

In two matings Nos. 1 and 2, 80.2% and 75.3%, 77.8% on the average, of the respective total number of eggs cleaved normally. While the normally cleaved eggs from mating No. 2 were not inferior to the controls in developmental capacity, about 9% and 11% of those from mating No. 1 died of edema in the embryonic and tadpole stages, respectively. In the two matings, 73.0% and 72.2%, 72.6% on the average, hatched normally, and 64.3% and 71.0%, 67.6% on the average, became normally metamorphosed frogs.

2. Viability and sex of metamorphosed frogs

a. Viability

The age of tadpoles at the time of climbing out of water and the body length of frogs immediately after metamorphosis in eight experimental and the control series are presented in Table 46. While 856 tadpoles in the control series were 111∼143 days old, 127.6 days old on the average, when they climbed out of water, 189∼873 tadpoles in the eight experimental series were 111∼151 days old, 125.7∼131.6 days old on the average in each of these experimental series. While about 100 frogs removed at random from the control series immediately after metamorphosis were 16.7±0.2 mm in body length, those from the experimental series were 16.5±0.2∼17.0±0.2 mm. There were no distinct differences in the age at the time of climbing out of water as well as in the body length immediately after metamorphosis between the frogs of the experimental series and the controls. There were also no distinct differences in these respects between the frogs of two kinds of experimental series which differed from each other in the kind or dosage of irradiation, and between the frogs derived from irradiated

TABLE 46

Number, size and sex of metamorphosed frogs produced from males and

Parents: Female	Parents: Male	Age at the time of climbing out of water (days)	No. of metamorphosed frogs
J{J(J·J)}, Nos. 1～5	{(J·J)J}J, Nos. 1～5	111～143 (127.6)	856
J{J(J·SX–150)}, Nos. 1～5	{(J·SX–150)J}J, Nos. 1～5	115～142 (130.8)	873
J{J(J·SX–250)}, Nos. 1,2	{(J·SX–250)J}J, Nos. 1,2	118～141 (129.8)	381
J{J(EX–150·J)}, Nos. 1～5	{(EX–150·J)J}J, Nos. 1～5	119～146 (130.5)	787
J{J(EX–250·J)}, No. 1	{(EX–250·J)J}J, No. 1	116～137 (131.5)	189
J{J(J·SN–100)}, Nos. 1～4	{(J·SN–100)J}J, Nos. 1～4	111～151 (128.5)	624
J{J(J·SN–150)}, Nos. 1,2	{(J·SN–150)J}J, Nos. 1,2	111～142 (125.7)	359
J{J(EN–150·J)}, Nos. 1～5	{(EN–150·J)J}J, Nos. 1～5	115～146 (127.1)	810
J{J(EN–250·J)}, Nos. 1,2	{(EN–250·J)J}J, Nos. 1,2	119～147 (131.6)	353

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

spermatozoa and those from irradiated eggs, as presented in Table 46.

While 5% of the total number of normally metamorphosed frogs died within one or two months after metamorphosis in the control series, 2～11% died in the eight experimental series. Among the latter, the offspring derived from gametes exposed to a large dose of X-rays or neutrons were slightly larger in the rate of inviable frogs than those from gametes exposed to a small dose. All the living frogs were killed one or two months after metamorphosis to examine their sex.

b. Sex

The sex of immature frogs is presented in Table 46. While there were nearly an equal number of males and females in the control series, males were somewhat more numerous than females in each of the experimental series. Moreover, comparatively numerous hermaphrodites as well as females with underdeveloped ovaries were always found in the experimental series, in contrast with the situation in the control.

In the control series, 43 of 856 normally metamorphosed frogs produced from five matings died within one or two months after metamorphosis. Of the remaining 813 frogs, 407 were females, one was a female with underdeveloped ovaries and 405 (49.8%) were males. There were no hermaphrodites.

i) Experimental series, [J♀×{J♀×(J♀×SX–150♂)♂}♂]♀× [{(J♀×SX–150♂)♀×J♂}♀×J♂]♂

Thirty-three of 873 normally metamorphosed frogs produced from five matfemales derived from irradiated great-grandparental sperm or eggs

Mean body length of 100 frogs immediately after metamorphosis (mm)	Sex of frogs killed about one to two months after metamorphosis					
	No. of frogs	♀$_{N}$	♀$_{U}$	⚥	♂$_{N}$	♂ (%)*
16.7±0.2	813	407	1	0	405	(49.8)
16.9±0.2	840	385	13	11	431	(52.6)
16.5±0.2	352	127	14	12	199	(59.9)
16.6±0.2	746	360	7	9	370	(50.8)
17.0±0.2	169	79	3	5	82	(51.5)
16.6±0.3	613	285	10	12	306	(51.9)
16.5±0.2	342	161	8	6	167	(50.6)
16.7±0.3	794	374	5	5	410	(52.3)
16.7±0.2	326	142	10	8	166	(53.4)

⚥—Hermaphrodites ♂$_{N}$—Males with normal testes * Including hermaphrodites

ings died within one or two months after metamorphosis. Of the remaining 840, 385 were females with normal ovaries, 13 females with underdeveloped ovaries, 11 hermaphrodites and 431 males. When the hermaphrodites were counted as males, 52.6% of the total number of frogs examined were males.

ii) Experimental series, [J♀ × {J♀ × (J♀ × SX–250♂) ♂} ♂] ♀ × [{(J♀ × SX–250♂) ♀ × J♂} ♀ × J♂] ♂

Twenty-nine of 381 normally metamorphosed frogs produced from two matings died within one or two months after metamorphosis. Of the remaining 352, 127 were females with normal ovaries, 14 females with underdeveloped ovaries, 12 hermaphrodites and 199 males. When the hermaphrodites were counted as males, 59.9% of the total number of frogs examined were males.

iii) Experimental series, [J♀ × {J♀ × (EX–150♀ × J♂) ♂} ♂] ♀ × [{(EX–150♀ × J♂) ♀ × J♂} ♀ × J♂] ♂

Forty-one of 787 normally metamorphosed frogs produced from five matings died within one or two months after metamorphosis. Of the remaining 746 frogs, 360 were females with normal ovaries, 7 females with underdeveloped ovaries, 9 hermaphrodites and 370 males. When the hermaphrodites were counted as males, 50.8% of the total number of frogs examined were males.

iv) Experimental series, [J♀ × {J♀ × (EX–250♀ × J♂) ♂} ♂] ♀ × [{(EX–250♀ × J♂) ♀ × J♂} ♀ × J♂] ♂

Twenty of 189 normally metamorphosed frogs produced from a mating died within one or two months after metamorphosis. Of the remaining 169 frogs, 79 were females with normal ovaries, 3 females with underdeveloped ovaries, 5 hermaphrodites and 82 males. When the hermaphrodites were counted as males, 51.5% of the total number of frogs examined were males.

v) Experimental series, [J♀ × {J♀ × (J♀ × SN–100♂)♂}♂]♀ × [{(J♀ × SN–100♂)♀ × J♂}♀ × J♂]♂

Eleven of 624 normally metamorphosed frogs produced from four matings died within one or two months after metamorphosis. Of the remaining 613 frogs, 285 were females with normal ovaries, 10 females with underdeveloped ovaries, 12 hermaphrodites and 306 males. When the hermaphrodites were counted as males, 51.9% of the total number of frogs examined were males.

vi) Experimental series, [J♀ × {J♀ × (J♀ × SN–150♂)♂}♂]♀ × [{(J♂ × SN–150♂)♀ × J♂}♀ × J♂]♂

Seventeen of 359 normally metamorphosed frogs produced from two matings died within one or two months after metamorphosis. Of the remaining 342 frogs, 161 were females with normal ovaries, 8 females with underdeveloped ovaries, 6 hermaphrodites and 167 males. When the hermaphrodites were counted as males, 50.6% of the total number of frogs examined were males.

vii) Experimental series, [J♀ × {J♀ × (EN–150♀ × J♂)♂}♂]♀ × [{(EN–150♀ × J♂)♀ × J♂}♀ × J♂]♂

Sixteen of 810 normally metamorphosed frogs produced from five matings died within one or two months after metamorphosis. Of the remaining 794 frogs, 374 were females with normal ovaries, 5 females with underdeveloped ovaries, 5 hermaphrodites and 410 males. When the hermaphrodites were counted as males, 52.3% of the total number of frogs examined were males.

viii) Experimental series, [J♀ × {J♀ × (EN–250♀ × J♂)♂}♂]♀ × [{(EN–250♀ × J♂)♀ × J♂}♀ × J♂]♂

Twenty-seven of 353 normally metamorphosed frogs produced from two matings died within one or two months after metamorphosis. Of the remaining 326 frogs, 142 were females with normal ovaries, 10 females with underdeveloped ovaries, 8 hermaphrodites and 166 males. When the hermaphrodites were counted as males, 53.4% of the total number of frogs examined were males.

XI. Summary of the developmental capacity and sex of individuals derived from four types of irradiated gametes

1. Developmental capacity

The developmental capacity of individuals in four generations derived from four types of irradiated gametes, X-irradiated sperm, X-irradiated oviducal eggs, neutron-irradiated sperm and neutron-irradiated oviducal eggs is presented in Tables 1, 9, 14, 19, 24, 29, 33, 37, 41 and 45. In order to facilitate the comparison between different generations as well as among the four types of irradiated gametes, the percentages of unfertilized or abnormally cleaved eggs, abnormal embryos, abnormal tadpoles and normally metamorphosed frogs in each series are shown in Figs. 58 and 59. While only 57% of untreated eggs of field-caught females became normally metamorphosed frogs by insemination with untreated sperm in the control series of the first generation, more than 74% of eggs always became such frogs in each of the control series of the second, third and fourth generations as shown in Fig. 57. The low percentage of normally metamorphosed frogs in the first generation was mainly attributable to abundance of unfertilized or abnormally cleaved eggs.

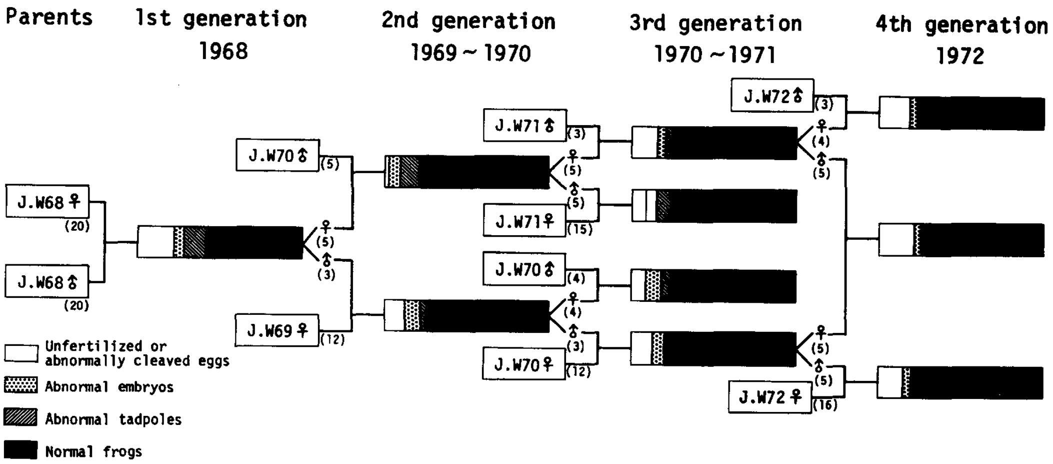


Fig. 57. Diagrammatic representation of the viabilities of first- to fourth-generation offspring derived from untreated gametes. The number of frogs used for each kind of matings is given in parentheses.

a. Descendants derived from X-irradiated sperm

The production and the developmental capacity of the first-, second-, third- and fourth-generation offspring derived from X-irradiated sperm by mating with field-caught males or females are graphically shown in Fig. 58. By sperm irradiated with 150 and 250 rads of X-rays, 26% and 13% of eggs obtained from field-caught females became normally metamorphosed frogs, respectively (Table 1, Fig. 1). This seems to show that the deleterious effects due to 250 rads of X-rays were two times larger than those by 150 rads. Such a decrease in the percentage of metamorphosed frogs with increase of dosage was scarcely found in the second- and third-generation offspring, although the normally metamorphosed frogs gradually increased as a whole with the advance of generations. However, they were remarkably lower in percentage than those of the controls. Chromosome aberrations were abundantly found in normally shaped tadpoles as well as abnormal embryos raised from X-irradiated sperm (Tables 2, 3). While 12 out of 20 abnormal embryos and all of 25 normally shaped tadpoles in the control series were normal diploids, only 12 out of 73 abnormal embryos and 18 out of 42 normally shaped raised tadpoles from 150 or 250 rad irradiated sperm were normal diploids. The remaining embryos and tadpoles had various kinds of chromosome aberrations. It was remarkable that normal diploids among the normally shaped tadpoles raised from 250 rad irradiated sperm were far fewer than those among such tadpoles raised from 150 rad irradiated sperm. This

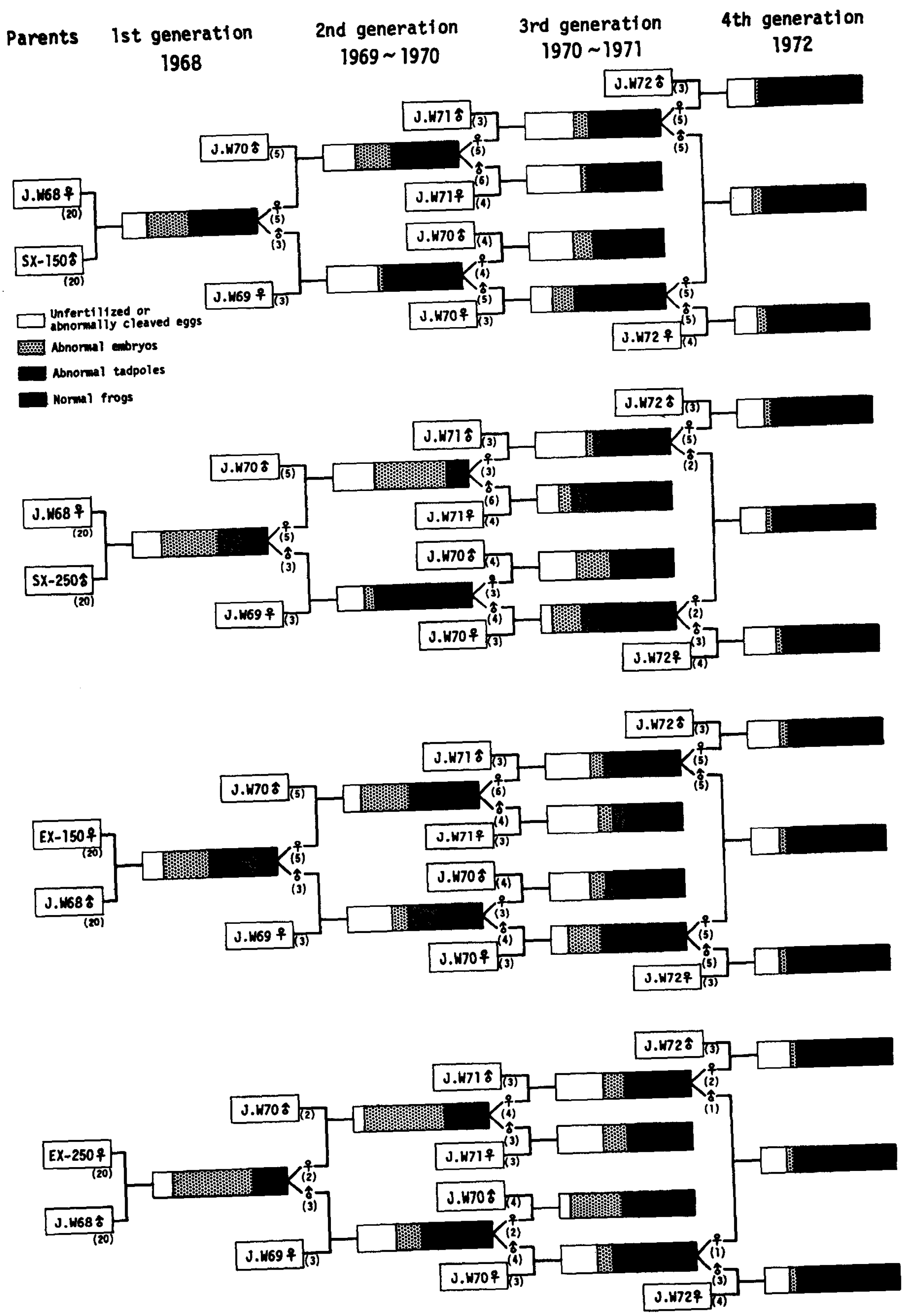


Fig. 58. Diagrammatic representation of the viabilities of first- to fourth-generation offspring derived from X-irradiated gametes. The number of frogs used for each kind of matings is given in parentheses.

corresponded to the difference in the percentage of normally metamorphosed frogs between the 150 and 250 rad series. In each of the 150 and 250 rad series, 100 normally metamorphosed frogs were continuously reared. As a result, 89 and 34 frogs attained the stage of sexual maturity, respectively, in contrast with 92 out of 100 controls (Table 4).

In order to produce second-generation offspring ten females and six males raised from 150 or 250 rad irradiated sperm were mated with field-caught frogs. These females laid 118~837 eggs, 614 eggs on the average, after pituitary injection (Table 13). This mean number was distinctly smaller than 904 eggs on the average of five control females. However, two females raised from 250 rad irradiated sperm were specific in that they laid a small number of eggs, that is, 118 and 134 eggs, respectively. Moreover, one of these two females laid two kinds of almost normal sized and remarkably large eggs in nearly an equal number, while the other laid three kinds of small, almost normal-sized and large eggs in nearly an equal number. The other eight females laid 736 eggs on the average. While two of the ten first-generation females were nearly equal to the controls in producing normally metamorphosed frogs, the others were inferior to various extents in this respect (Table 14, Figs. 29, 30a, b). Especially, five of them were extremely low in the percentage of normally metamorphosed frogs, regardless of the percentages of normally cleaved eggs obtained from them. Of 62 normally shaped tadpoles produced by two first-generation females, 20 had chromosome aberrations, while the others were normal diploids (Table 15). A total of 150 normally metamorphosed frogs produced by females raised from 150 or 250 rad irradiated eggs were continuously reared. The results showed that 65 out of 100 frogs in the 150 rad series and 15 out of 50 frogs in the 250 rad series attained the stage of sexual maturity, while 39 out of 50 controls did so (Table 16).

On the other hand, three, one and two of the six males used in producing second-generation offspring had testes of type 1, type 2 and type 3, respectively (Table 8). The intensity of abnormalities in the structure of testes corresponded roughly to the scarcity of normally metamorphosed frogs in the following generation. The three males with testes of type 1 were nearly equal or slightly inferior to the controls in producing normally metamorphosed frogs, while the only male with testes of type 2 was considerably inferior and the remaining two males with testes of type 3 were distinctly inferior to the controls in this respect (Table 8, Figs. 26, 27a, b). Of 51 normally shaped tadpoles produced by two first-generation males, 15 had chromosome aberrations, while the others were normal diploids (Table 10). A total of 180 normally metamorphosed frogs produced by first-generation males were continuously reared. As a result, 57 (71%) out of 80 frogs in the 150 rad series and 32 out of 100 frogs in the 250 rad series matured sexually, while 92 out of 100 controls did (Table 11).

Third-generation offspring were produced by mating 15 females and 21 males derived from sperm irradiated with 150 or 250 rads of X-rays with field-caught frogs. Eight of these females were those which had been produced by two first-generation females. They laid 471~834 eggs, 698 eggs on the average, after pituitary injection (Table 31). This mean number was somewhat smaller than 771 eggs on the average of five control females. Five of the eight females were slightly or remarkably inferior to the controls in producing normally metamorphosed frogs, while the others were nearly equal in this respect (Table 33, Figs. 43, 44a, b). A total of 100 normally metamorphosed frogs produced by second-generation females were continuously reared. It was found that 39 out of 50 frogs in the 150 rad series and 20 out of 50 frogs in the 250 rad series could sexually mature, while 43 out of 50 controls could so (Table 34).

Twelve of the 21 males used in producing third-generation offspring were brothers of these eight females. Nine, one and two had testes of type 1, type 2 and type 4, respectively (Table 28). Eight of the nine males with testes of type 1 were nearly equal or slightly inferior to the controls in producing normally metamorphosed frogs, while the remainder as well as the only male with testes of type 2 was considerably inferior in this respect (Table 29, Figs. 40, 41a, b). The two males with testes of type 4 were extremely low in the percentage of normally cleaved eggs and scarcely produced normally metamorphosed frogs. A total of 120 normally metamorphosed frogs produced by second-generation males were continuously reared. As a result, 53 (88%) out of 60 frogs in the 150 rad series and 57 (95%) out of 60 frogs in the 250 rad series attained sexual maturity, while 71 (89%) out of 80 controls did so (Table 30).

The remaining seven of the 15 females used in producing third-generation offspring were those which had been produced by two first-generation males raised from sperm irradiated with 150 or 250 rad of X-rays. They laid 386∼921 eggs, 586 eggs on the average, after pituitary injection. This mean number was far smaller than 851 eggs on the average of four control females (Table 22). There was another female which laid no eggs after pituitary injection. While four of the seven females were nearly equal or slightly inferior to the controls in producing normally metamorphosed frogs, the others were distinctly inferior in this respect (Table 24, Figs. 37, 38a, b). Of 43 normally shaped tadpoles produced by two second-generation females, nine had chromosome aberrations, while 34 were normal diploids (Table 25). A total of 90 normally metamorphosed frogs produced by second-generation females were continuously reared. As a result, 24 out of 50 frogs in the 150 rad series and 12 (30%) out of 40 frogs in the 250 rad series could sexually mature, while 35 out of 50 controls could so (Table 26).

Nine of the 21 males used in producing third-generation offspring were brothers of these second-generation females. Eight of them had testes of type 1, while the remainder had those of type 2 (Table 17). While five males with testes of type 1 were nearly equal to the controls in producing normally metamorphosed frogs, the other four with testes of type 1 or 2 were considerably inferior in this respect (Table 19, Figs. 34, 35a, b). Of 38 normally shaped tadpoles produced by two second-generation males which had been derived from 150 or 250 rad irradiated sperm, eight had chromosome aberrations, while the others were normal diploids (Table 20). A total of 100 normally metamorphosed frogs produced by second-generation males were continuously reared. It was found that 21 out of 50 frogs in the 150 rad series and five out of 50 frogs in the 250 rad series attained sexual maturity, while 32 out of 50 controls did so (Table 21).

Fourth-generation offspring were produced by 17 female and 15 male third-generation offspring derived from 150 or 250 rad irradiated sperm. While 21 third-generation females were injected with frog pituitaries, four did not ovulate. The eggs laid by the 17 females were 450～1033, 754 on the average, in number (Tables 39, 43). Ten control females laid 719～1146 eggs, 954 eggs on the average, although they were somewhat smaller as a whole in body length than the third-generation females. The testes of the above 15 males were all of type 1 in inner structure (Tables 35, 44). All these third-generation males and females were nearly equal or slightly inferior to the controls in producing normally metamorphosed frogs, although there were a male and a female third-generation offspring which were considerably lower than the controls in the percentage of normally cleaved eggs (Tables 37, 41, 45, Figs. 48, 49a, b, 51, 52a, b, 54, 55a, b).

b. Descendants derived from X-irradiated eggs

The production of descendants from X-irradiated oviducal eggs and their developmental capacity are shown in Fig. 58. By insemination with sperm of field-caught males, 14%, 8% and 2% of oviducal eggs irradiated with 150, 250 and 350 rads of X-rays became normally metamorphosed frogs, respectively (Table 1, Fig. 1). As found in the first-generation offspring raised from X-irradiated sperm, the deleterious effects given to oviducal eggs with 250 rads of X-rays seemed to be about two times larger than those with 150 rads, although they were remarkably larger than those given to sperm with the same doses. The deleterious effects given with 350 rads of X-rays seemed to be three or four times larger than those with 250 rads. Such a decrease in the percentage of normally metamorphosed frogs with an increase of the dosage was scarcely found in the second- and third-generation offspring between the 150 and 250 rad series, although the metamorphosed frogs gradually increased as a whole with the advance of generations. However, the percentages of normally metamorphosed frogs to the respective total number of eggs were distinctly lower than those of the controls.

Conspicuous chromosome aberrations were observed in normally shaped tadpoles as well as abnormal embryos raised from irradiated eggs. The percentage of normal diploids decreased remarkably with increase in irradiation dosage (Tables 2, 3). Of the normally metamorphosed frogs which were removed and reared, only a few could live until sexual maturity, although no mature frogs were obtained from among those raised from 350 rad irradiated eggs (Table 4). The testes of six mature first-generation males used for producing second-generation offspring were not always normal in inner structure; two, two, one and one of these males were of type 1, type 2, type 3 and type 4, respectively (Table 8). The two males having testes of type 3 or 4 and one of the two males having testes of type 2 were considerably defective in producing normally cleaved eggs, while the other three males having testes of type 1 or 2 were nearly normal in this respect. However, two of the latter as well as all the former three were remarkably inferior to the controls in producing normally metamorphosed frogs (Table 9, Figs. 26, 27c, d). The remaining one was nearly normal in this respect. One of the two males having testes of type 1 was not good in reproductive capacity in spite of a high percentage of normally cleaved eggs. Of 49 normally shaped tadpoles in the second-generation offspring derived from 150 or 250 rad irradiated grandparental eggs, 14 (29%) had chromosome aberrations, while the other 35 were normal diploids (Table 10). A total of 200 normally metamorphosed frogs produced by first-generation males were continuously reared. As a result, only 26 out of 100 frogs in the 150 rad series and only 22 out of 100 frogs in the 250 rad series could sexually mature, in contrast with 92 out of 100 controls (Table 11).

The other group of second-generation offspring was produced by seven females raised from X-irradiated eggs. These females laid 231～899 eggs, 654 eggs on the average, which were remarkably fewer than those of the controls (Table 13). Moreover, one of them laid three kinds of eggs that were different in size. While three females derived from X- irradiated eggs were not distinctly inferior to the controls in reproductive capacity, all the other females were remarkably inferior to the latter (Table 14, Figs. 29, 30c, d). However, the eggs of the seven females derived from X-irradiated eggs were nearly normal in the percentage of cleaved eggs. Of 58 normally shaped tadpoles produced by two first-generation females, 18 (31%) had chromosome aberrations, while the other 40 were normal diploids (Table 15). A total of 100 normally metamorphosed frogs produced by first-generation females were continuously reared. The results showed that only 14 out of 50 frogs in the 150 rad series and only 10 out of 50 frogs in the 250 rad series attained sexual maturity, in contrast with 39 out of 50 controls (Table 16).

Ten mature females and seven mature males produced by two first-generation females were mated with field-caught frogs in order to produce third-generation offspring. The ten females laid 425～802 eggs, 570 eggs on the average, after pituitary injection, while three other females laid no eggs (Table 31). This mean number of eggs was remarkably smaller than that of the controls. While three of them were nearly normal in reproductive capacity, the other seven were more or less inferior to the controls, regardless of the number of their eggs (Table 33, Figs. 43, 44c, d). However, normally cleaved eggs produced by five females derived from 150 or 250 rad irradiated eggs were not inferior to those of the controls in producing normally metamorphosed frogs, although they themselves were more or less inferior in percentage to those of the controls. A total of 100 normally metamorphosed frogs produced by second-generation females were continuously reared. As a result, 24 out of 50 frogs in the 150 rad series and 16 out of 50 frogs in the 250 rad series attained sexual maturity, whereas 43 out of 50 controls did so (Table 34).

On the other hand, five of the seven second-generation males had testes of type 1, while the other two had those of type 3 (Table 28). When they were mated with field-caught females, the latter two were extremely inferior to the controls in reproductive capacity (Table 29, Figs. 40, 41c, d). Only 16% and 27% of the respective number of eggs cleaved normally, and afterwards 10% and 20% became normally metamorphosed frogs, respectively. Two other males were nearly normal and the remaining three were more or less inferior to the controls in reproductive capacity. A total of 117 normally metamorphosed frogs produced by second-generation males were continuously reared. As a result, 53 of them could sexually mature, while 71 out of 80 controls could so (Table 30).

Five females and eight males of the second-generation offspring derived from 150 or 250 rad irradiated eggs by passing over first-generation males were mated with field-caught frogs in order to produce third-generation offspring. Although eight mature females were primarily injected with pituitaries, three of them laid no eggs. The other five laid 469～912 eggs, 704 eggs on the average (Table 22). This mean number was smaller than that of the controls which laid 851 eggs on an average. While two females were nearly normal in reproductive capacity, the other three were slightly or distinctly inferior (Table 24, Figs. 37, 38c, d). In the case of two of these three females, normally metamorphosed frogs were only raised from less than 20% of their eggs. Of 46 normally shaped tadpoles produced by two second-generation females, ten had chromosome aberrations, while the others were normal diploids (Table 25). A total of 70 normally metamorphosed frogs were continuously reared. It was found that only 14 out of 40 frogs in the 150 rad series and only seven out of 30 frogs in the 250 rad series could sexually mature, in contrast with 35 out of 50 controls (Table 26).

The eight second-generation males had testes of type 1, except one which had testes of type 3 (Table 17). As a result of mating experiments with field-caught females, this exceptional male was extremely low in the percentage of normally cleaved eggs. Two other males were distinctly inferior to the controls in reproductive capacity, while the remaining five were nearly normal in this respect (Table 19, Figs. 34, 35c, d). Of 39 normally shaped tadpoles produced by two second-generation males, eight had chromosome aberrations, while the others were normal diploids (Table 20). A total of 90 normally metamorphosed frogs were continuously reared. It was found that 24 out of 50 frogs in the 150 rad series and only four out of 40 frogs in the 250 rad series attained sexual maturity. In contrast 32 out of 50 controls did so (Table 21).

Fourth-generation offspring were produced by 13 females and 14 males of the third-generation offspring derived from X-irradiated oviducal eggs. Seven females mated with field-caught males were derived from 150 or 250 rad irradiated eggs by passing over first- and second-generation females. They laid 379～1027 eggs, 689 eggs on the average, after pituitary injection. This mean number was remarkably smaller than that of the control females (Table 39). Although twelve sisters were primarily injected with pituitaries, five of them laid no eggs. All the seven females were nearly equal or slightly inferior to the controls in reproductive capacity (Table 41, Figs. 51, 52c, d). Eight males derived from 150 or 250 rad irradiated eggs by passing over first- and second-generation males were mated with field-caught females in order to produce fourth-generation offspring. All of them had testes of type 1 (Table 35). They were nearly equal or slightly inferior to the controls in reproductive capacity, although one of them was considerably lower than the controls in the percentage of normally cleaved eggs (Table 37, Figs. 48, 49c, d). Six third-generation males derived from 150 or 250 rad irradiated eggs by passing over first- and second-generation females were mated with six third-generation females derived from 150 or 250 rad irradiated eggs by passing over first- and second-generation males. While all the third-generation males had testes of type 1 (Table 44), the third-generation females laid 569～1092 eggs, 870 eggs on the average (Table 43). This mean number is remarkably smaller than that of the five controls. While four matings were not inferior to the controls in the percentage of normally metamorphosed frogs, the other two were slightly inferior (Table 45, Figs. 54, 55c).

c. Descendants derived from neutron-irradiated sperm

The production and the developmental capacities of descendants from neutron-irradiated sperm are shown in Fig. 59. By sperm irradiated with 100, 150 and 250 rads of neutrons, 25%, 13% and 5% of eggs obtained from field-caught females became normally metamorphosed frogs, respectively (Table 1, Fig. 1). As in the case of X-irradiated sperm or oviducal eggs, the injurious effects given to sperm with 150 rads of neutrons seemed to be about two times larger than those with 100 rads. The effects given with 250 rads were nearly three times larger than those with 150 rads. Such a decrease in the percentage of normally metamorphosed frogs with an increase of the dosage was not found in the second- and third-generation offspring between the 100 and 150 rad series. The percentages of normally metamorphosed frogs gradually increased as a whole with the advance of generations, although they were distinctly smaller than those of the controls until the fourth generation.

Numerous chromosome aberrations were found in normally shaped tadpoles as well as abnormal embryos raised from irradiated sperm (Tables 2, 3). It was remarkable that normal diploids were less than half the number of analyzed tadpoles among normally shaped ones raised from 150 or 250 rad irradiated sperm. Most of the normally metamorphosed frogs which were removed and continuously reared died before they attained the stage of sexual maturity in contrast with the controls (Table 4). Nine mature males raised from 100, 150 or 250 rad irradiated sperm were used in producing offspring by mating with field-caught females. Seven of them had testes of type 1, while the other two had those of type 2 (Table 8). On the other hand, nine mature females raised from 100 or 150 rad irradiated sperm were mated with field-caught males. Although ten females were primarily injected with pituitaries, one of them laid no eggs. The nine females laid 72～964 eggs, 637 eggs on the average (Table 13). When the only female that laid 72 eggs was excluded, the other eight females laid more than 500 eggs, 708 eggs on the average. This mean number was distinctly smaller than that (904 eggs) of the five controls. More-

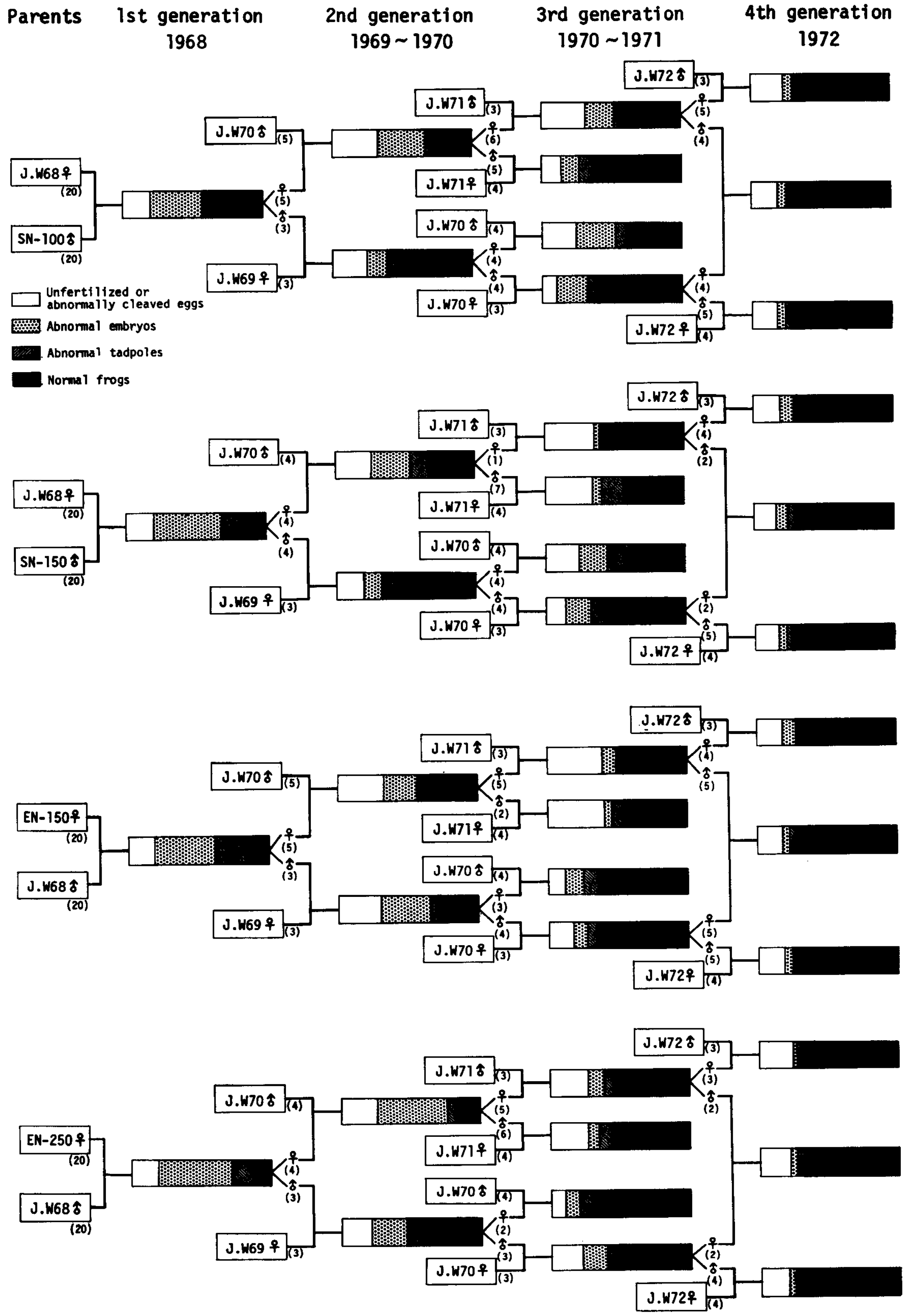


Fig. 59. Diagrammatic representation of the viabilities of first- to fourth-generation offspring derived from neutron-irradiated gametes. The number of frogs used for each kind of matings is given in parentheses.

over, each of three females laid large and small eggs in nearly an equal number. While two of the nine first-generation females were nearly normal in producing normally metamorphosed frogs, the other seven were inferior to various extents (Table 14, Figs. 29, 33a, b). The eggs of three females were very small in the percentage of normally cleaved eggs and produced no normally metamorphosed frogs. Of the seven first-generation males raised from 100 or 150 rad irradiated sperm, five were nearly equal or slightly inferior to the controls in producing normally metamorphosed frogs, while the remaining two were remarkably inferior to the controls in this respect (Table 8, Figs. 26, 28a, b).

Chromosomes were examined in 57 normally shaped tadpoles produced by two first-generation females raised from 100 or 150 rad irradiated sperm. As a result, 17 (30%) tadpoles had chromosome aberrations, while the others were normal diploids (Table 15). Of 100 normally metamorphosed frogs which were continuously reared, only 30 attained sexual maturity in contrast with 39 (78%) of 50 controls (Table 16). On the other hand, 84 normally shaped tadpoles produced by three first-generation males raised from 100, 150 or 250 rad irradiated sperm were examined in terms of chromosomes. Twenty-one (25%) of them had chromosome aberrations, while the others were normal diploids (Table 10). Of 231 normally metamorphosed frogs which were continuously reared, 111 (48%) attained sexual maturity, while 92 out of 100 controls did so (Table 11).

Seven females and 12 males of the second-generation offspring produced by four first-generation females which had been raised from 100 or 150 rad irradiated sperm were mated with field-caught frogs in order to produce third-generation offspring. Although eight second-generation females were primarily injected with pituitaries, one of them laid no eggs. The other seven laid 316~941 eggs, 561 eggs on the average. This mean number was remarkably smaller than 771 eggs on the average of the five controls (Table 31). While three of the seven females were nearly equal to the controls in producing normally metamorphosed frogs, the others were considerably inferior (Table 33, Figs. 43, 46a, b). There was no intimate correlation between the number of eggs laid by each female and the percentage of normally metamorphosed frogs raised from among these eggs. Of the 12 second-generation males used in producing third-generation offspring, eight had testes of type 1. One, two and one of the other males had testes of type 3, type 4 and type 5, respectively. All these four males were produced by a first-generation female which had been raised from a spermatozoon exposed to 150 rads of neutrons (Table 28). Normally cleaved eggs were scarcely obtained by three of them, while 25% of the eggs obtained from a field-caught female cleaved normally and 21% became normally metamorphosed frogs by sperm of the remaining male (Table 29, Figs. 40, 42b). Seven of the eight second-generation males which had testes of type 1 were nearly equal or slightly inferior to the controls in reproductive capacity, while the remainder was much inferior in this respect (Table 29, Figs. 40, 42a, b). A total of 160 normally metamorphosed frogs produced by second-generation males were continuously reared. It was found that all 100 frogs in the 100 rad series and 27 out of 60 frogs in the 150 rad series attained sexual maturity (Table 30).

Eight females and eight males of the second-generation offspring produced by four first-generation males which had been raised from 100 or 150 rad irradiated sperm were used in producing third-generation offspring by mating with field-caught frogs. These females laid 438~736 eggs, 587 eggs on the average, after pituitary injection. This mean number was remarkably smaller than that (851 eggs) of the controls (Table 22). While two of the eight females were nearly normal in producing normally metamorphosed frogs, two and three others were slightly and much inferior to the four controls in this respect. The only remainder produced no normally metamorphosed frogs, although 45% of the eggs cleaved normally (Table 24, Figs. 37, 39a, b). Of 46 normally shaped tadpoles which were produced by two females derived from 100 or 150 rad irradiated sperm, 11 had chromosome aberrations, while the other 35 were normal diploids (Table 25). Although 100 normally metamorphosed frogs were continuously reared, 35 of them attained sexual maturity, whereas 35 out of 50 control frogs did so (Table 26). On the other hand, all the eight males which were used in producing third-generation offspring had testes of type 1 (Table 17). Five of them were nearly equal or slightly inferior to the controls in producing normally metamorphosed frogs, while the other three were remarkably inferior in this respect (Table 19, Figs. 34, 36a, b). Of 41 normally shaped tadpoles which were produced by two males derived from 100 or 150 rad irradiated sperm, eight had chromosome aberrations, while the other 33 were normal diploids (Table 20). Although 100 normally metamorphosed frogs produced by second-generation males were continuously reared, only 25 attained the stage of sexual maturity, while 32 (64%) out of 50 control frogs did so (Table 21).

In order to produce fourth-generation offspring, 15 females and 16 males of third-generation offspring derived from 100 or 150 rad irradiated spermatozoa were used. Nine of the females were those that had been produced by passing over first- and second-generation females. Although 13 females were primarily injected with pituitaries, four laid no eggs. The other nine laid 403~864 eggs, 700 eggs on the average. This mean number was remarkably smaller than that (874 eggs) of the five controls (Table 39). However, these third-generation females were nearly equal or slightly inferior to the controls in producing normally metamorphosed frogs (Table 41, Figs. 51, 53a, b). Their six brothers were mated with six third-generation females derived from 100 or 150 rad irradiated sperm by passing over first- and second-generation males. All the third-generation males had testes of type 1 (Table 44), while the third-generation females laid 642~1532 eggs, 1038 eggs on the average (Table 43). This mean number of eggs was similar to that (1033 eggs) of the controls. Besides these females, there was a sister which laid no eggs after pituitary injection. Four of the six matings of the third-generation males and females were nearly the same as the controls in producing normally metamorphosed frogs, while the other two were slightly inferior in this respect (Table 45, Figs. 54, 56a, b). The remaining ten of the 16 males were brothers of the third-generation females derived by passing over first- and second-generation males. All of them had testes of type 1 (Table 35). As a result of mating with field-caught females, it was found that they were nearly equal or slightly inferior to the controls in producing normally metamorphosed frogs (Table 37, Figs. 48, 50a, b).

d. Descendants derived from neutron-irradiated eggs

The production of descendants from neutron-irradiated oviducal eggs and their developmental capacities are shown in Fig. 59. By insemination with sperm of field-caught males, 13% and 6% of oviducal eggs irradiated with 150 and 250 rads of neutrons became normally metamorphosed frogs, respectively (Table 1, Fig. 1). As in the other cases, the injurious effects given to oviducal eggs with 250 rads of neutrons seemed to be about two times larger than those with 150 rads. Such a decrease in the percentage of normally metamorphosed frogs with an increase of the dosage was not found in the second- and third-generation offspring. However, the percentages of normally metamorphosed frogs gradually increased as a whole with the advance of generations.

Of 77 abnormal embryos raised from 150 or 250 rad irradiated eggs, only seven were normal diploids, while the others had chromosome aberrations (Table 2). Such a high rate of chromosome aberrations was not found in 20 abnormal embryos obtained from untreated gametes; 12 of them were normal diploids. Chromosome aberrations were also found in 19 of 43 normally shaped tadpoles, while the others were normal diploids (Table 3). In contrast with this, 25 control tadpoles examined were all normal diploids. Of 200 normally metamorphosed frogs which were removed and continuously reared, only 26 (13%) attained sexual maturity, while 92 of 100 controls did so (Table 4). Nine females and six males raised from 150 or 250 rad irradiated eggs were used in producing second-generation offspring. These females laid 625～958 eggs, 780 eggs on the average, after pituitary injection. This mean number was considerably smaller than that (904 eggs) of the controls (Table 13). Moreover, one of them laid almost normal and somewhat larger eggs in nearly an equal number. There was another female that laid no eggs after pituitary injection. While one of the nine females was nearly equal to the controls in producing normally metamorphosed frogs, all the others were inferior to various extents (Table 14, Figs. 29, 33c, d). Especially by three females raised from 250 rad irradiated eggs, only a few frogs or none were produced. Of 56 normally shaped tadpoles produced by two females which had been raised from 150 or 250 rad irradiated eggs, 19 had chromosome aberrations, while none of 45 control tadpoles had these. Of 100 normally metamorphosed frogs which were continuously reared, only 27 attained sexual maturity (Table 16). On the other hand, three of the six males used in order to produce second-generation offspring had testes of type 1, while two and one of the others had those of type 2 and type 4, respectively (Table 8). While the five males having testes of type 1 or 2 were not inferior to the controls in producing normally cleaved eggs, only the male with testes of type 4 was remarkably inferior in this respect. However, the two males with testes of type 2 and two of the three males with testes of type 1 as well as the male with testes of type 4 were inferior to various extents in producing normally metamorphosed frogs (Table 9, Figs. 26, 28c, d). Only the remaining male with testes of type 1 was not inferior to the controls in this respect. Of 45 normally shaped tadpoles produced by two males which had been raised from 150 or 250 rad irradiated eggs, 16 had chromosome aberrations, while only one of 20 control tadpoles had them (Table 10). A total of 199 normally metamorphosed frogs produced by first-generation males were continuously reared. As a result, 27 out of 100 frogs in the 150 rad series and 16 out of 99 frogs in the 250 rad series attained sexual maturity (Table 11).

Third-generation offspring were produced by mating of 15 female or 15 male second-generation offspring derived from neutron-irradiated oviducal eggs with field-caught frogs. Ten of these females were those that had been produced by two first-generation females raised from 150 or 250 rad irradiated eggs. They laid 411~835 eggs, 669 eggs on the average, after pituitary injection (Table 31). This mean number of eggs was considerably smaller than that (771 eggs) of the controls. Four other females laid no eggs, although they were injected with pituitaries. While four of the ten females were nearly equal to the controls in producing normally metamorphosed frogs, the other six were slightly or distinctly inferior (Table 33, Figs. 43, 46c, d). Of 100 normally metamorphosed frogs which were continuously reared, only 35 attained sexual maturity, while 43 of 50 controls did so (Table 34). Eight brothers of the second-generation females used in producing offspring were mated with field-caught females (Table 29). While four of them had testes of type 1, two, one and one of the others had testes of type 2, type 3 and type 5, respectively (Table 28). The only male with testes of type 5 produced no normally cleaved eggs. The four males with testes of type 1 were nearly equal to the controls in producing normally metamorphosed frogs, while the remaining three males with testes of type 2 or 3 were distinctly inferior in this respect (Table 29, Figs. 40, 42c, d). Of 135 frogs which were continuously reared, 98 (73%) attained sexual maturity, while 71 (89%) of 80 controls did so (Table 30).

The remaining five female and seven male second-generation offspring used in producing third-generation offspring were obtained by first-generation males raised from 150 or 250 rad irradiated eggs. The females laid 492~749 eggs, 620 on the average, after pituitary injection. This mean number was remarkably smaller than that (851 eggs) of the controls. Besides these females, there were three that laid no eggs after pituitary injection (Table 22). Two of the five females were not inferior to the controls in producing normally metamorphosed frogs, while the other three were slightly inferior in this respect (Table 24, Figs. 37, 39c, d). Of 43 normally shaped tadpoles produced by two second-generation females derived from 150 or 250 rad irradiated eggs, eleven had chromosome aberrations, while the others were normal diploids (Table 25). A total of 90 normally metamorphosed frogs were continuously reared. It was found that only 11 out of 50 frogs in the 150 rad series and only five out of 40 frogs in the 250 rad series attained sexual maturity, whereas 35 out of 50 controls did so (Table 26).

Of the seven second-generation males, five had testes of type 1, while one and one of the other two had those of type 2 and type 3, respectively (Table 17). Four of the five males with testes of type 1 and one male with testes of type 2 were nearly equal to the controls in producing normally metamorphosed frogs, while the remaining male with testes of type 1 was considerably inferior and the only male with testes of type 3 was greatly inferior in this respect (Table 19, Figs. 34, 36c, d). Of 38 normally shaped tadpoles produced by two second-generation males derived from 150 or 250 rad irradiated eggs, nine had chromosome aberration, while the others were normal diploids (Table 20). A total of 90 normally metamorphosed frogs were continuously reared. It was found that 17 out of 50 frogs in the 150 rad series and only six out of 40 frogs in the 250 rad series attained sexual maturity, but 32 out of 50 controls did so (Table 21).

Fourth-generation offspring were produced by 14 female and 16 male third-generation offspring. Seven of the females were produced from 150 or 250 rad irradiated eggs by passing over first- and second-generation females. They laid 531～927 eggs, 714 eggs on the average, after pituitary injection. This mean number was remarkably smaller than that (874 eggs) of the controls (Table 39). There were three other females which did not lay eggs after pituitary injection. As a result of mating experiments, three of the seven females were rather superior to the controls in producing normally metamorphosed frogs, while the other four were slightly inferior in this respect (Table 39, Figs. 51, 53c, d).

TABLE 47

Sex of first-, second-, third- and fourth-generation

Generation (Group)	Series	Juveniles about one		
		Total no.	♀$_{N}$	♀$_{U}$
First	Control	106	55 (51.9%)	2 (1.9%)
	Experimental	1143	449 (39.3%)	218 (19.1%)
Second (from I♂)	Control	454	227 (50.0%)	1 (0.2%)
	Experimental	5410	2754 (50.9%)	129 (2.4%)
(from I♀)	Control	624	309 (49.5%)	1 (0.2%)
	Experimental	1920	870 (45.3%)	30 (1.6%)
Third (from I♂, II♂)	Control	255	129 (50.6%)	2 (0.8%)
	Experimental	2159	1102 (51.0%)	32 (1.5%)
(from I♂, II♀)	Control	323	159 (49.2%)	0
	Experimental	1179	470 (39.9%)	12 (1.0%)
(from I♀, II♂)	Control	424	209 (49.3%)	1 (0.2%)
	Experimental	4219	1847 (43.8%)	118 (2.8%)
(from I♀, II♀)	Control	641	317 (49.5%)	3 (0.5%)
	Experimental	2614	1078 (41.2%)	128 (4.9%)
Fourth (from I♂, II♂, III♂)	Control	782	391 (50.0%)	2 (0.3%)
	Experimental	5325	2388 (44.8%)	146 (2.7%)
(from I♀, II♀, III♀)	Control	634	314 (49.5%)	3 (0.5%)
	Experimental	4980	2233 (44.8%)	77 (1.5%)
(from I♂, II♂, III♀	Control	813	407 (50.1%)	1 (0.1%)
×I♀, II♀, III♂)	Experimental	4182	1913 (45.7%)	70 (1.7%)

♀$_{N}$—Females with normal ovaries ♀$_{U}$—Females with underdeveloped ovaries

Seven brothers of these third-generation females were mated with seven third-generation females which had been derived from 150 or 250 rad irradiated sperm by passing over first- and second-generation males (Fig. 56). While all the seven third-generation males had testes of type 1 (Table 44), the seven third-generation females laid 569～1033 eggs, 833 eggs on the average, after pituitary injection (Table 43). This mean number was remarkably smaller than that (1033 eggs) of the controls. There were two other females which laid no eggs after pituitary injection. The seven matings of the third-generation males and females were nearly equal or slightly inferior to the controls in producing normally metamorphosed frogs (Table 45, Figs. 54, 56c, d). Fourth-generation offspring were also produced by nine brothers of the third-generation females derived by passing over first- and second-generation males. All these third-generation males had testes of type 1 (Table 35). As the result of mating experiments with field-caught females, they were nearly equal or slightly inferior to the controls in producing normally metamorphosed frogs (Table 37, Figs. 48, 50c, d).

2. Sex ratio

The sex of juvenile and mature frogs in the first, second, third, and fourth-generation derived from X- or neutron-irradiated sperm or oviducal eggs is presented in Table 47. In each generation, the sex ratios of the descendants from irradiated gametes differed considerably from those of the controls, when examined at least in the mature frog stage. Males were always more numerous than females (Tables 4, 11, 16, 21, 26, 30, 34, 38, 42 and 46).

offspring derived from irradiated gametes

month after metamorphosis			Mature frogs		
⚥	♂$_N$	⚥ + ♂$_N$	Total no.	♀	♂
0	49 (46.2%)	49 (46.2%)	92	45 (48.9%)	47 (51.1%)
81 (7.1%)	395 (34.6%)	476 (41.6%)	248	103 (41.5%)	145 (58.5%)
2 (0.4%)	224 (49.3%)	226 (49.8%)	92	44 (47.8%)	48 (52.2%)
131 (2.4%)	2396 (44.3%)	2527 (46.7%)	291	134 (46.0%)	157 (54.0%)
0	314 (50.3%)	314 (50.3%)	39	20 (51.3%)	19 (48.7%)
27 (1.4%)	993 (51.7%)	1020 (53.1%)	161	51 (31.7%)	110 (68.3%)
0	124 (48.6%)	124 (48.6%)	32	16 (50.0%)	16 (50.0%)
54 (2.5%)	971 (45.0%)	1025 (47.5%)	102	35 (34.3%)	67 (65.7%)
0	164 (50.8%)	164 (50.8%)	35	18 (51.4%)	17 (48.6%)
31 (2.6%)	666 (56.5%)	697 (59.1%)	108	35 (32.4%)	73 (67.6%)
0	214 (50.5%)	214 (50.5%)	71	39 (54.9%)	32 (45.1%)
115 (2.7%)	2139 (50.7%)	2254 (53.4%)	388	173 (44.6%)	215 (55.4%)
2 (0.3%)	319 (49.8%)	321 (50.1%)	43	21 (48.8%)	22 (51.2%)
120 (4.6%)	1288 (49.3%)	1408 (53.9%)	202	83 (41.1%)	119 (58.9%)
1 (0.1%)	388 (49.6%)	389 (49.7%)			
164 (3.1%)	2627 (49.3%)	2791 (52.4%)			
1 (0.2%)	316 (49.8%)	317 (50.0%)			
98 (2.0%)	2572 (51.6%)	2670 (53.6%)			
0	405 (49.8%)	405 (49.8%)			
68 (1.6%)	2131 (51.0%)	2199 (52.6%)			

⚥—Hermaphrodites ♂$_N$—Males with normal testes

a. First-generation offspring derived from irradiated gametes

There were 449 (39%) females with normal ovaries, 218 (19%) females with underdeveloped ovaries, 81 (7%) hermaphrodites and 395 (35%) males with normal testes among a total of 1143 frogs dead or killed about one month after the completion of metamorphosis in ten experimental series (Table 4). When the hermaphrodites were counted as males, 42% were males. In contrast with this, there were 55 (52%) females with normal ovaries, two (2%) females with underdeveloped ovaries and 49 (46%) males with normal testes among 106 controls. If the control frogs were more numerous, it is probable that there was nearly an equal number of males and females. The existence of comparatively numerous females with underdeveloped ovaries as well as some hermaphrodites in the experimental series was in contrast with the controls. This showed that sex differentiation retarded in the tadpoles raised from irradiated gametes. Such retardation of sex differentiation was found in each of the ten experimental series (Table 4).

At the mature frog stage, there were 103 females and 145 (58%) males in the ten experimental series, while there were 45 females and 47 (51%) males among the controls. The predominance of males in number was found in each of eight experimental series. The testes of mature males raised from irradiated gametes were divided into five types from type 1 (normal) to type 5 (extremely abnormal) on the basis of their inner structure. As a result, 53 (37%), 35 (24%), 27 (19%), 18 (12%) and 12 (8%) out of 145 mature frogs in nine experimental series had testes of type 1, type 2, type 3, type 4 and type 5, respectively (Table 5). All of 47 controls had normal testes, that is, of type 1. It was noteworthy that most of the males raised from irradiated gametes had testes which were somewhat abnormal in inner structure.

On the other hand, the kidneys of the mature frogs stated above were divided into four types from type 1 (normal) to type 4 (very abnormal) on the basis of their inner structure. While 45 and two out of 47 mature control males had kidneys of type 1 and type 2, respectively, 69 (48%), 28 (19%), 25 (17%) and 23 (16%) of the 145 mature frogs had those of type 1, type 2, type 3 and type 4, respectively (Table 6). It was evident that the abnormalities in the inner structure of kidneys corresponded roughly to those in the inner structure of testes (Plates V, VI).

b. Second-generation offspring derived from irradiated gametes

The second-generation offspring derived from irradiated gametes were sorted into two groups. One group came from first-generation females, while the other came from first-generation males. Among a total of 7330 frogs dead or killed about one month after metamorphosis, there were 3624 (49%) females with normal ovaries, 159 (2%) females with underdeveloped ovaries, 158 (2%) hermaphrodites and 3389 (46%) males with normal testes (Tables 11, 16). When the hermaphrodites were counted as males, 48% were males. Among a total of 1078 controls, there were 536 (50%) females with normal ovaries, two (0.2%)

females with underdeveloped ovaries, two (0.2%) hermaphrodites and 538 (50%) males with normal testes. At the mature frog stage, there were 185 females and 267 (59%) males in nine experimental series, while there were 64 females and 67 (51%) males among the controls. As in the first-generation offspring, the second-generation offspring differed from the controls in abundance of hermaphrodites and females with underdeveloped ovaries, although these hermaphrodites and females were far fewer than those in the first-generation offspring.

It was remarkable that the two groups of second-generation offspring distinctly differed from each other in sex ratio. In the group derived from first-generation females, the rate of males was remarkably higher than that in the other group.

i) From first-generation males

A total of 5410 frogs dead or killed about one month after metamorphosis in nine experimental series were examined in terms of sex. As a result, 2754 (51%) were females with normal ovaries, 129 (2%) were females with underdeveloped ovaries, 131 (2%) were hermaphrodites and 2396 (44%) were males with normal testes (Table 11). When the hermaphrodites were counted as males, 47% were males. There were conspicuous differences in the percentage of males including hermaphrodites among the nine experimental series, as presented in Table 11. For example, 286 (64%) out of 445 frogs raised from 250 rad X-irradiated eggs were males, while 384 (33%) out of 1147 frogs raised from 150 rad neutron-irradiated sperm were males. Of a total of 454 controls, 227 (50%) were females with normal ovaries and 224 (49%) were males with normal testes. The remaining one (0.2%) and two (0.4%) were a female with underdeveloped ovaries and hermaphrodites, respectively. At the mature frog stage, there were 134 females and 157 (54%) males in nine experimental series, while 48 (52%) of 92 controls were males.

ii) From first-generation females

Of a total of 1920 frogs dead or killed about one month after metamorphosis in eight experimental series, 870 (45%) were females with normal ovaries, 30 (2%) females with underdeveloped ovaries, 27 (1%) were hermaphrodites and 993 (52%) were males with normal testes (Table 16). When the hermaphrodites were counted as males, 53% were males; while more than 53% of frogs were males in three of the eight experimental series, approximately 50% were males in the other experimental series. Of 624 controls, 309 (50%) were females with normal ovaries and 314 (50%) were males with normal testes. Besides, there was one female with underdeveloped ovaries. At the mature frog stage, 110 (68%) out of 161 frogs in the eight experimental series were males, while 19 (49%) out of 39 controls were males.

c. Third-generation offspring derived from irradiated gametes

The third-generation offspring derived from irradiated gametes in four groups of experimental series were not similar to the controls in sex ratio. A total of 10171 frogs dead or killed about one month after metamorphosis and a total of

800 sexually mature frogs were examined in terms of sex (Table 47). Of the juvenile frogs, 4497 (44%) were females with normal ovaries, 290 (3%) were females with underdeveloped ovaries, 320 (3%) were hermaphrodites and 5064 (50%) were males with normal testes. When the hermaphrodites were counted as males, 53% were males; in each of the four groups of experimental series, 47 ~ 59% of frogs were males. Of 1643 controls, 814 (50%) were females with normal ovaries and 821 (50%) were males with normal testes. Besides these males and females, there were only six (0.4%) females with underdeveloped ovaries and two (0.1%) hermaphrodites. At the mature frog stage, there were 326 females and 474 (59%) males in the four groups of experimental series, while 94 out of 181 mature control frogs were females and 87 (48%) were males.

i) From first- and second-generation males

Among a total of 2159 juvenile frogs in eight experimental series, there were 1102 (51%) females with normal ovaries, 32 (1%) females with underdeveloped ovaries, 54 (3%) hermaphrodites and 971 (45%) males with normal testes (Table 21). When the hermaphrodites were counted as males, 47% were males. However, more than 53% of frogs were males in three of the eight experimental series, while less than 41% were males in three other experimental series. In contrast with such diversity in sex ratio, there were 129 (51%) females with normal ovaries, two (0.8%) females with underdeveloped ovaries and 124 (49%) males with normal testes among 255 controls. No hermaphrodites were found among the controls. Of 102 mature frogs in the eight experimental series, 35 were females and 67 (66%) were males, while 16 (50%) out of 32 controls were males.

ii) From first-generation males and second-generation females

A total of 1179 juvenile and 108 mature frogs in eight experimental series were examined in terms of sex. Among the juveniles, there were 470 (40%) females with normal ovaries, 12 (1%) females with underdeveloped ovaries, 31 (3%) hermaphrodites and 666 (57%) males with normal testes. When the hermaphrodites were counted as males, 59% were males (Table 26). While more than 55% of frogs were males in five of the eight experimental series, 47 ~ 52% were males in the other experimental series. In contrast with this, there were 159 (49%) females with normal ovaries and 164 (51%) males with normal testes among 323 controls. Neither females with underdeveloped ovaries nor hermaphrodites were found among the controls. Of the mature frogs in the eight experimental series, 35 were females and 73 (68%) were males, while 18 were females and 17 (49%) were males among 35 controls.

iii) From first-generation females and second-generation males

The sex of 4219 juvenile and 388 mature frogs derived from irradiated gametes in eight experimental series were examined. Of the juveniles, 1847 (44%) were females with normal ovaries, 118 (3%) were females with underdeveloped ovaries, 115 (3%) were hermaphrodites and 2139 (51%) were males with normal testes (Table 30). When the hermaphrodites were counted as males, 53% were males. While more than 54% of frogs were males in four of the eight experimental series, 45～52% were males in the other experimental series. Of 424 controls, 209 (49%) were females with normal ovaries and 214 (50%) were males with normal testes. Besides, there was one female with underdeveloped ovaries. At the mature frog stage, there were a total of 173 females and 215 (55%) males in the eight experimental series, while there were 39 females and 32 (45%) males among 71 controls.

iv) From first- and second-generation females

Of a total of 2614 juvenile frogs in eight experimental series, 1078 (41%) were females with normal ovaries, 128 (5%) were females with underdeveloped ovaries, 120 (5%) were hermaphrodites and 1288 (49%) were males with normal testes (Table 34). When the hermaphrodites were counted as males, 54% were males. In each of the eight experimental series, 52～56% of frogs were males. Among 641 controls, there were 317 (49%) females with normal ovaries and 319 (50%) males with normal testes. Besides, there were three (0.5%) females with underdeveloped ovaries and two (0.3%) hermaphrodites. At the mature frog stage, 83 out of 202 frogs in the eight experimental series were females and the remaining 119 (59%) were males, while there were 21 females and 22 (51%) males among 43 controls.

d. Fourth-generation offspring derived from irradiated gametes

A total of 14487 juvenile fourth-generation offspring derived from irradiated gametes in three groups of experimental series were killed about one to two months after metamorphosis in order to examine their sex. As a result, 6534 (45%) were females with normal ovaries, 293 (2%) were females with underdeveloped ovaries, 330 (2%) were hermaphrodites and 7330 (51%) were males with normal testes (Table 47). When the hermaphrodites were counted as males, 53% were males; in each of the three groups, 52～54% were males. Of a total of 2229 controls, 1112 (50%) were females with normal ovaries and 1109 (50%) were males with normal testes. Besides these males and females, there were six (0.3%) females with underdeveloped ovaries and two (0.1%) hermaphrodites.

i) From first-, second- and third-generation males

Of a total of 5325 juvenile frogs in eight experimental series, 2388 (45%) were females with normal ovaries, 146 (3%) were females with underdeveloped ovaries, 164 (3%) were hermaphrodites and 2627 (49%) were males with normal testes (Table 38). When the hermaphrodites were counted as males, 52% were males; in each of the eight experimental series, 51～55% of frogs were males. Of a total of 782 controls, 391 (50%) were females with normal ovaries and 388 (50%) were males with normal testes. Besides, there were two (0.3%) females with underdeveloped ovaries and one (0.1%) hermaphrodite.

ii) From first-, second- and third-generation females

Among a total of 4980 juvenile frogs in eight experimental series, there were 2233 (45%) females with normal ovaries, 77 (2%) females with underdeveloped ovaries, 98 (2%) hermaphrodites and 2572 (52%) males with normal testes

(Table 24). When the hermaphrodites were counted as males, 54% were males; in each of the eight experimental series, 51 ~ 57% of frogs were males. Of 634 controls, 314 (50%) were females with normal ovaries and 316 (50%) were males with normal testes. Besides, only three (0.5%) females with underdeveloped ovaries and one (0.2%) hermaphrodite were found.

iii) From matings between third-generation females and males

Of a total of 4182 juvenile frogs in eight experimental series, 1913 (46%) were females with normal ovaries, 70 (2%) were females with underdeveloped ovaries, 68 (2%) were hermaphrodites and 2131 (51%) were males with normal testes (Table 46). When the hermaphrodites were counted as males, 53% were males; in each of the eight experimental series 51 ~ 60% were males. Among 813 controls, there were 407 (50%) females with normal ovaries and 405 (50%) males with normal testes. Besides these males and females, there was only one (0.1%) female with underdeveloped ovaries.

DISCUSSION

1. Radiosensitivity of *Rana japonica*

KAWAMURA and NISHIOKA (1978) have reported in an article of this volume on abnormalities in the descendants of *Rana nigromaculata* produced from oviducal eggs or sperm which were irradiated with X-rays or neutrons. While the oviducal eggs were exposed to 90, 145 or 200 rads of X-rays or to 50, 90 or 130 rads of neutrons, the sperm were exposed to 90, 170 or 240 rads of X-rays or to 50, 90 or 130 rads of neutrons in order to obtain similar degrees of effects. The three doses of neutrons were rather more deleterious to sperm than the three doses of X-rays, respectively, in the development of embryos and tadpoles raised from irradiated gametes. The irradiation of oviducal eggs with 50, 90 and 130 rads of neutrons had nearly the same degrees of deleterious effects as that with 90, 145 and 200 rads of X-rays, respectively, on the developmental capacity of embryos and tadpoles raised from these irradiated eggs (KAWAMURA and NISHIOKA, 1978). The fact that neutrons have more deleterious effects on frog gametes than X-rays was also confirmed in *Rana japonica*. Irradiation of sperm with 100 and 150 rads of neutrons was nearly the same in effect appearing in the embryonic and tadpole stages as that with 150 and 250 rads of X-rays, respectively (Fig. 1). Irradiation of oviducal eggs with 150 rads of neutrons was more deleterious than that with 150 rads of X-rays, while irradiation of oviducal eggs with 250 rads of neutrons was nearly the same in effect as that with 250 rads of X-rays. On the other hand, normal *Rana nigromaculata* eggs inseminated with X-irradiated sperm were somewhat lower than X-irradiated *Rana nigromaculata* eggs inseminated with normal sperm in percentage of normally cleaved eggs, and, afterwards, the former became somewhat higher than the latter in percentage of normally metamorphosed frogs. In contrast, normal *Rana nigromaculata* eggs inseminated with neutron-irradiated sperm were not always lower than neutron-irradiated eggs inseminated with normal sperm in percentage of normally cleaved eggs and not always higher than the latter in percentage of normally metamorphosed frogs (KAWAMURA and NISHIOKA, 1978). The same difference between X-rays and neutrons in the effects of irradiation on sperm and eggs in *Rana nigromaculata* were also found in *Rana japonica* (Fig. 1).

It is evident that the sperm and oviducal eggs of *Rana japonica* are more radio-resistant than those of *Rana nigromaculata*. When the degree of radioresistance was shown by comparing the percentage of normally metamorphosed frogs in each experimental series with that of the controls, the rates in the SX–240, SN–90, SN–130 and EN–130 series of *Rana nigromaculata* were 16.2%, 27.1%, 14.9% and 12.4%, respectively. In contrast with these, the rates in the SX–250, SN–100, SN–150 and EN–150 series of *Rana japonica* were 22.0%, 43.1%, 22.4% and 22.4%, respectively. A single doubtful case was found between the EX–145 series of *Rana nigromaculata* and the EX–150 series of *Rana japonica*. While the rate of normally metamorphosed frogs was 24.7% in the former, the rate was 24.8% in the latter. However, the rate in the EX–250 series of *Rana japonica* was 13.4%, while that in the EX–200 series of *Rana nigromaculata* was 10.4%. The other experimental series could not be utilized for comparison, since each of them in *Rana japonica* had no appropriate partner in *Rana nigromaculata*. Thus, the rates of normally metamorphosed frogs raised from X- or neutron-irradiated *Rana japonica* gametes were always higher than those from X- or neutron-irradiated *Rana nigromaculata* gametes, in spite of irradiation with slightly larger dose.

SPARROW, NAUMAN, DONNELLY, WILLIS and BAKER (1970) have reported that LD_{50} decreased with increasing nuclear and chromosome volume, when the LD_{50} values for *Rana pipiens, Desmognathus fuscus* and *Notophthalmus viridescens* were determined. According to GRIFFIN, SCOTT and PAPWORTH (1970), the higher chromosomal DNA content of *Bufo pardalis* as compared with those of both *B. marinus* and *B. calamita* resulted in higher frequency of deletions, just as an increase in radiation dose did. CONGER and CLINTON (1973) studied the relation of nuclear and chromosomal volumes and DNA contents to radiosensitivity in adult amphibians and confirmed that LD_{50} decreased with increasing nuclear parameters, volume and DNA contents per nucleus and per chromosome. Concerning radiation-induced mutations, ABRAHAMSON, BENDER, CONGER and WOLFF (1973) have insisted that the rate of radiation-induced mutations is proportional to the total genome size (DNA content) on the basis of the data obtained in various kinds of organisms, bacteria to mammals. Nuclear DNA amounts and relative nuclear sizes of some Palaearctic frogs and toads were determined by BACHMANN and NISHIOKA (1978). *Rana japonica* were 10.2 pg in diploid DNA amount and 23.4 in relative nuclear size, while *Rana nigromaculata* were 12.0 pg and 33.0, respectively. Thus, the higher radioresistance of *Rana japonica* gametes than that of *Rana nigromaculata* seems to be explicable by their smaller DNA amount and smaller relative nuclear size.

2. Developmental capacity of descendants

The descendants of four generations derived from irradiated gametes of *Rana japonica* were very similar to those of *Rana nigromaculata* in developmental capacity, as recognized by comparing Figs. 58 and 59 with the corresponding figures in KAWAMURA and NISHIOKA's paper (1978, cf. Figs. 29~32). When the four generations were compared with each other by the percentages of metamorphosed frogs, there was a distinct difference in developmental capacity between the first and the subsequent generations. In the first generation derived from irradiated *Rana japonica* gametes, 26% and 13% of eggs by sperm irradiated with 150 and 250 rads of X-rays, 14%, 8% and 2% of eggs irradiated with 150, 250 and 350 rads of X-rays, 25%, 13% and 5% of eggs by sperm irradiated with 100, 150 and 250 rads of neutrons and 13% and 6% of eggs irradiated with 150 and 250 rads of neutrons became normally metamorphosed frogs, respectively. From these figures, it was evident that the deleterious effects of irradiation increased approximately in proportion to the increase of dose as found in the first-generation offspring raised from irradiated *Rana nigromaculata* gametes. In contrast with the first-generation offspring, the second-, third- and fourth-generation offspring derived from the irradiated gametes did not show such an increase of deleterious effects proportional to the increase of dose. However, the number of normally metamorphosed frogs gradually increased as a whole with the advance of generations and became nearly the same as that of the controls in the fourth generation.

RUGH (1939) has reported that there is a typical experimental curve of hatching percentage dropping from 97.8% at 15 r to 1.6% at 10,000 r in *Rana pipiens*, when eggs were inseminated with sperm irradiated with various amounts of X-rays. He suggested that both dominant and recessive (lethal) mutations may be produced by X-ray bombardment of frog sperm chromosomes. RUGH (1950a, b) irradiated adult male *Cynops pyrrhogaster* with 5,000 r and 50,000 r of X-rays and found that X-irradiation made chromosomes at the spermatogenetic stage less viscous, more fluid and sticky. Thus, the chromosomes lost their identity and became clumped together. GALLIEN and his collaborators at the Faculty of Science, Paris, made a series of conspicuous studies on chromosome aberrations induced by irradiation in *Pleurodeles waltl*, after GALLIEN and LABROUSSE (1962) examined the change in radiation-susceptibility of neutron-irradiated, fertilized eggs during the period from second meiosis to first cleavage mitosis. At the beginning, GALLIEN, LABROUSSE and LACROIX (1963) exposed fertilized eggs of *Pleurodeles waltl* to γ-rays from radioactive cobalt and observed various kinds of chromosome aberrations, such as deletions, translocations and formation of dicentric chromosomes in abnormal embryos raised from irradiated eggs. LABROUSSE (1967) submitted fertilized, unsegmented eggs to the action of neutrons or γ-rays and assumed that the hypomorphogenesis like anencephalia, microcephalia, and various non-cephalic atrophies found in the tail-bud stage are related to aberrations of the karyotype. GALLIEN, LABROUSSE, PICHERAL and LACROIX (1965) observed various chromosome aberrations such as breakage, deletions, translocations and the presence of two centromeres per chromosome in abnormal embryos which had been produced by transplanting nuclei of embryos raised from γ-irradiated fertilized eggs into unfertilized eggs. GALLIEN, LABROUSSE and LACROIX (1966), LABROUSSE (1967, 1969, 1971), and GALLIEN (1969a, b) examined chromosomes in viable larvae which had been raised from fertilized eggs irradiated with weak γ-rays and confirmed that the larvae having chromosome aberrations were able to attain the adult stage and even to reproduce. Thus, the karyotypes of eight mature females in total raised from fertilized eggs exposed to 70 r of γ-rays were examined in detail. Four of these females had a deletion or a reciprocal translocation in mitotic or lampbrush chromosomes. Another female was a tetraploid. By mating with normal males, seven females laid a comparatively large or a small number of fertilized eggs. While all the eggs of two females died before the blastula stage, those of the other five females mostly or partly hatched normally. Approximately all or most of the normally hatched larvae seemed to be diploids, except that the larvae produced from the tetraploid seemed to be triploids (LABROUSSE, 1967). Of the two chromosome aberrations, reciprocal translocation and deletion, found in the karyotype of one female, the reciprocal translocation was disclosed in the karyotypes of her grandchildren, while the deletion was not found, that is, it had no effect on the germ cell line of the female (LABROUSSE, 1969; GALLIEN, 1969a). According to LABROUSSE (1971), among 30 larvae which were produced from a female having a reciprocal translocation and were morphologically normal at the hatching stage, there were 12 normal diploids, 12 abnormal diploids having a reciprocal translocation similar to that of their mother, three trisomics with a translocation and three individuals with secondary chromosome aberrations.

On the other hand, JAYLET and BACQUIER (1967) at the Faculty of Sciences, Toulouse, irradiated the hind parts of male *Pleurodeles waltl* with X-rays and mated them with unirradiated females. Of 80 one-year-old newts produced from these matings, 15 had chromosome aberrations. They studied in detail six of these newts which had chromosome aberrations. Three and one of the six newts had one and two translocations in karyotype, respectively, while the remaining two had one deletion. JAYLET (1971a, b) obtained a stock of fertile homozygotes for a reciprocal translocation as well as for a pericentric inversion in *Pleurodeles waltl*. These chromosome aberrations were first detected at the heterozygous state in a female produced from a cross between a normal female and an X-irradiated male. When a female heterozygous for the pericentric inversion was mated with a normal male, a little more than half of the fertilized eggs died during the embryonic stage. However, hatched larvae were not higher in mortality than the controls after they began to eat. Of the adults raised from the larvae that were phenotypically normal, approximately a half had the pericentric inversion in karyotype, while the other half were normal diploids. Several males and females heterozygous for the reciprocal translocation were produced by mating the heterozygous female obtained at the start with a normal male (JAYLET, 1971b). When these males and females were mated with normal females or normal males, four types of offspring were produced. One type had a deletion and was lethal, another had a translocation and was semi-lethal, still another was the same as the parents having a reciprocal translocation and phenotypically normal, and the remaining included normal diploids. CONTER and JAYLET (1974) established a stock of fertile heterozygotes for a triple translocation in *Pleurodeles waltl*. This translocation was first discovered in a female produced from a cross between a normal female and an X-irradiated male. When males and females heterozygous for the triple translocation were mated with normal females or normal males, ten kinds of phenotypes revealing different morphological characters were distinguished from one another in the offspring produced. About one-fourth of the offspring belonged to phenotype 1 and were morphologically normal. They were normal in karyotype or heterozygous for the triple translocation. In contrast, the other three-fourths belonging to phenotypes 1～9 were morphologically abnormal and lethal or semilethal, depending on their unbalanced karyotypes.

LACROIX and LOONES (1971) examined the lampbrush chromosomes of ten female *Pleurodeles waltl* produced from crosses between normal females and X-irradiated males. As a result, seven had one or two translocations, another had a triple translocation, and still another had a break occurring at the sphere organizer. They (1974) have also reported on chromosome mutations of *Pleurodeles poireti* which were disclosed in lampbrush chromosomes of seven female offspring of two X-irradiated males. These females were divided into two groups, one having simple abnormalities and the other having multiple abnormalities. The first group consisted of one female having a pericentric inversion and three females having a reciprocal translocation. While the offspring of the female with the inversion were normal in developmental capacity, those of the females with the translocation were defective to a large extent. Only a few of them attained sexual maturity, while the others mostly died during the embryonic stage. It was remarkable that the pericentric inversion of one female as well as the reciprocal translocation of one of the three females could not be disclosed in mitotic chromosomes. No offspring were produced by two of the three females having multiple abnormalities. In the other female, only 5% of eggs became feeding larvae and 0.6% grew into adults. When mitotic chromosomes were examined in larvae produced by the latter, nearly all of them were polyploids.

LACROIX (1967) and GUILLEMIN (1972, 1974) obtained fertile trisomic *Pleurodeles waltl* from triploid males or females by mating with diploid females or males. These trisomics produced many abnormal embryos with chromosome aberrations in addition to phenotypically normal trisomics and diploids.

It is very probable that many of the mature males and females which were derived from irradiated gametes and used in producing second- and third-generation offspring in the present study had almost balanced chromosome aberrations, such as reciprocal translocations, pericentric inversions or trisomy reported by GALLIEN, LABROUSSE and LACROIX (1966), LABROUSSE (1967, 1969, 1971), LACROIX (1967), GALLIEN (1969), JAYLET and BACQUIR (1967), JAYLET

(1971a, b), LACROIX and LOONES (1971), GUILLEMIN (1972, 1974) and CONTER and JAYLET (1974) in *Pleurodeles waltl* and by LACROIX and LOONES (1974) in *Pleurodeles poireti*. Although there is no doubt that irradiation gave rise to dominant and recessive lethal or semilethal genes as well as various kinds of aberrations in the chromosomes of irradiated gametes, the mature frogs raised from irradiated gametes does not seem to have had dominant lethal or semilethal genes or unbalanced chromosome aberrations. There was also no chance that recessive lethal genes had an effect on the development of their offspring, as the frogs raised from irradiated gametes were always mated with normal field-caught animals. Thus, the mutations that seriously injured the reproductive capacity of such frogs seem to be balanced, chromosome aberrations alone.

The karyotypes of mature frogs used in producing offspring were not examined in the present study. However, nearly half of the number of normally shaped tadpoles raised from irradiated gametes had various kinds of chromosome aberrations. Moreover, 92 (63%) out of 145 mature males raised from irradiated gametes were more or less abnormal in inner structure of the testes. Of 27 males used in producing second-generation offspring, 12 were also more or less abnormal in this respect. As assumed by JAYLET (1971a, b) and CONTER and JAYLET (1974), spermatozoa with an unbalanced set of chromosomes should have been produced in these abnormal testes, together with those with a normal as well as a balanced abnormal set of chromosomes. Gametogeneses similar to those in the testes should have occurred in the ovaries of many of the females which were raised from irradiated gametes and used in producing second-generation offspring. Of normally shaped tadpoles produced from first-generation males and females, 29% and 32% had chromosome aberrations, respectively (Tables 10, 15). About two-thirds of the first-generation males and females were more or less inferior to the controls in producing normally metamorphosed frogs (Figs. 26～30, 33). Four out of 32 mature second-generation males produced by first-generation males and 13 out of 39 mature second-generation males produced by first-generation females were more or less abnormal in inner structure of the testes (Tables 17, 28). Of normally shaped tadpoles produced by second-generation males and females, 21% and 23% had chromosome aberrations, respectively (Tables 20, 25). About half of the number of second-generation males and females were more or less inferior to the controls in producing normally metamorphosed frogs (Figs. 34～44, 46). In contrast to the first- and second-generation males, all the third-generation males used in producing fourth-generation offspring were normal in inner structure of the testes (Tables 35, 44). Mature third-generation males and females were equal or slightly inferior to the controls in producing normally metamorphosed frogs (Figs. 48～56). From these results, half of the number at least of the mature first-generation frogs as well as one-fourth at least of the mature second-generation frogs used in producing offspring seem to have had balanced chromosome aberrations like reciprocal or triple translocations or trisomy, while the mature third-generation frogs used in producing offspring scarcely seem to have had such chromosome aberrations.

It is remarkable that most of the normally shaped tadpoles with chromosome aberrations in first-, second- and third-generation offspring derived from irradiated gametes were chromosomal mosaics (Tables 3, 10, 15, 20, 25). They consisted of a mixture of two or more kinds of abnormal karyotypes or a mixture of normal and abnormal karyotypes. Such a lack of uniformity in karyotype of these tadpoles must have occurred at the first cleavage or later, as the result of abnormal mitoses like non-disjunction, elimination or fragmentation. It is evident that the mosaicism in normally shaped tadpoles was indirectly induced from balanced, chromosome aberrations of their parents on one side, and had an injurious effect on the viability of the tadpoles on the other side. In fact, the percentages of mature frogs to normally metamorphosed ones in the first, second and third generations derived from irradiated gametes were remarkably lower than those in the controls; of 829 normally metamorphosed frogs raised from irradiated gametes, 248 (29.9%) could mature, while 92 out of 100 controls could (Table 4). In the second generation, 291 (35.9%) out of 810 normally metamorphosed frogs produced by first-generation males and 161 (35.8%) out of 450 normally metamorphosed frogs produced by first-generation females could mature, while 92 out of 100 controls and 39 out of 50 controls could, respectively (Tables 11, 16). In the third generation derived from irradiated gametes by passing over first- and second-generation males, or by passing over first-generation males and second-generation females, 102 (26.8%) out of 380 or 108 (30.9%) out of 350 normally metamorphosed frogs could attain sexual maturity, while 32 (64%) out of 50 controls or 35 (70%) out of 50 controls could (Tables 21, 26). On the basis of these facts, it is very probable that the normally shaped tadpoles having chromosome aberrations died before they became mature, even if they could metamorphose. Thus, the mature frogs used in producing second- and third-generation offspring, seem to have had balanced chromosome aberrations which would not be disclosed in karyotype.

The mature frogs which were derived from irradiated great-grandparental sperm or eggs and used in producing fourth-generation offspring were not always completely normal in reproductive capacity. There were some males and females which were slightly inferior to the controls in this respect (Figs. 48~56). This seems to show that they had still some balanced chromosome aberrations. These chromosome aberrations as well as those stated above may be located in lampbrush chromosomes of oocytes.

3. Abnormality in sex differentiation

Male heterogamety of the genus *Rana* was postulated for the first time by WITSCHI (1914) in *R. temporaria* and *R. esculenta* on the basis of HERTWIG's crossing experiments between different local races in regard to sex differentiation. Thereafter, he (1923, 1929b) confirmed the male heterogamety of *Rana temporaria*, an European brown frog species, by his detailed investigations. The male heterogamety of *Rana japonica*, a Japanese brown frog species, was assumed by

KAWAMURA and YOKOTA (1959) on the basis of their result of research that about half of the number of males raised from androgen-injected tadpoles produced almost exclusively females. MORIWAKI (1959) also assumed the male heterogamety of *Rana japonica* from the sex of the offspring obtained by mating between a parthenogenetically produced male and a normal female. As the offspring were all females, it was evident that this male was a sex-reversed, genetic female which was homogametic. KAWAMURA and NISHIOKA (1977a) confirmed the male heterogamety of this species together with that of another Japanese brown frog species, *Rana tsushimensis*, and some other anuran species by examining the sex of diploid frogs produced gynogenetically.

The sex of first-, second-, third- and fourth-generation offspring derived from *Rana japonica* sperm or oviducal eggs irradiated with X-rays or neutrons was very similar to that of descendants derived from X- or neutron-irradiated *Rana nigromaculata* sperm or oviducal eggs, as clearly demonstrated by comparison between Table 47 in the present report and Table 63 in KAWAMURA and NISHIOKA's paper (1978). The descendants from irradiated gametes differed considerably in sex ratio from the controls. Males were usually more numerous than females in each generation. Sex differentiation in juveniles of each generation derived from irradiated gametes was more or less retarded as compared with the controls.

Rana japonica is a well-differentiated species in sex, as found in the control frogs of each generation (Table 47). There was always nearly an equal number of males and females at the juvenile and mature frog stages in each generation. Females with underdeveloped ovaries as well as hermaphrodites with gonads transforming from ovaries into testes were very few. In contrast, such females and hermaphrodites were comparatively numerous in the descendants of each generation derived from irradiated gametes. They were especially more numerous in the first generation than those in the second, third and fourth generation and did not decrease with the advance of generations. It was remarkable that 58% of mature frogs raised from irradiated gametes were males, in contrast with the finding that 42% of juveniles were males including hermaphrodites. This seems to show that about 8% of juvenile females might be genetic males and that about 8% of mature males might be sex-reversed genetic females. In the second-generation offspring produced by males raised from irradiated gametes, 47% of juveniles were males including hermaphrodites. In the third-generation offspring derived from irradiated great-grandparental gametes by passing over first- and second-generation males, 47% of juveniles were likewise males including hermaphrodites. These two cases may be explained as follows. A part of their fathers was sex-reversed genetic females and produced almost exclusively females, or some fathers had factors which delayed the sex differentiation of genetic males in their offspring. In either event, the cause for the smaller number of males seems to exist in the chromosomes of their fathers. Of the juveniles in each group of the second-, third- and fourth-generation offspring derived from irradiated gametes, 52～59% were males including hermaphrodites, except the two cases stated above. Of the mature frogs belonging to each group of the second- and third-generation offspring, 54~68% were males. Thus it is evident that sex reversal occurred in some genetic females.

Sex reversal of genetic females into phenotypic males in *Rana* arises from various causes, such as overripeness of oviducal eggs (KUSCHAKEWITSCH, 1910; R. HERTWIG, 1921; WITSCHI, 1924), rearing under high temperature (WITSCHI, 1929a, b), hybridization (DÜRKEN, 1935, 1938; KAWAMURA, 1943, 1950; KAWAMURA and KOBAYASHI, 1959, 1960) and triploidy (HUMPHREY, BRIGGS and FANKHAUSER, 1950; KAWAMURA and TOKUNAGA, 1952; KAWAMURA and NISHIOKA, 1967). Sex reversal has also occurred in descendants of nucleo-cytoplasmic hybrids in *Rana*. KAWAMURA and NISHIOKA (1972, 1977a, b) have reported a remarkable sex reversal in the descendants of seven consecutive generations derived from three male nucleo-cytoplasmic hybrids consisting of *Rana ornativentris* cytoplasm and *Rana japonica* nuclei. A similar phenomenon has been confirmed by the present author (1972) in the second- and third-generation offspring derived from a female nucleo-cytoplasmic hybrid consisting of *Rana brevipoda* cytoplasm and *Rana nigromaculata* nuclei. It is noteworthy that all these factors giving rise to sex reversal are closely related to chromosome aberrations or abnormal chromosome combinations as well as to anatomical abnormalities.

WITSCHI (1929a) has observed the process of sex reversal arising in female tadpoles of *Rana sylvatica* by high temperature. According to him, suppression of the normal development first occurs in the cortical portions of the ovaries. This seems to result in compensatory growth of the medullary portions, which then differentiate into the rete apparatus of testes. As stated in KAWAMURA and NISHIOKA's paper (1978), it is probable that chromosome aberrations bring about an abnormal metabolism of the body and in turn suppress the normal development of the ovarian cortices, as the latter is very sensitive to general metabolism. While embryos with severe chromosome aberrations die sooner or later of morphological and physiological deficiencies, those with slight, almost completely balanced, chromosome aberrations may be able to develop into mature frogs. However, the development of the cortical portions of the gonads in genetic males and females seems to be most sensitively affected in those viable individuals. In some cases, the suppression of the development of the cortical portions should be expressed as retardation of sex differentiation at the juvenile stage or as smallness in number of eggs produced by females at the mature stage. In some other cases, this seems to induce compensatory growth of the medullary portions in the ovaries of genetic females and to result in sex reversal before or after metamorphosis in *Rana* at least.

SUMMARY

1. In order to clarify the genetic effects of irradiation on the development and sex of amphibians, first- to fourth-generation offspring were produced from *Rana japonica* whose sperm or oviducal eggs were exposed to X-rays or neutrons.

2. While the number of normally metamorphosed frogs in the first-generation offspring decreased with increase of radiation dosage, such a decrease was not found in the second-, third- and fourth-generation offspring. However, the number of normally metamorphosed frogs gradually increased as a whole with the advance of generations and became nearly the same as that of the controls in the fourth generation.

3. When normally metamorphosed frogs were continuously reared in each of the first-, second- and third-generations derived from irradiated gametes, the frogs that could attain sexual maturity were distinctly fewer than the controls. In the third generations derived from first-generation males by passing over second-generation males or females as well as in the first and second generation, the percentage of mature frogs decreased more or less distinctly with increase of radiation dosage.

4. The descendants of each generation derived from X- or neutron-irradiated sperm or oviducal eggs differed considerably in sex ratio from the controls. Males were always more numerous than females at the mature stage at least. At the juvenile stage, moreover, there were always many females with underdeveloped ovaries and a considerable number of hermaphrodites, in contrast with the small number of such females and hermaphrodites in the controls.

5. Chromosome aberrations were abundantly found in normally shaped tadpoles as well as abnormal embryos raised from X- or neutron-irradiated sperm or oviducal eggs. Normal diploids found among normally shaped tadpoles decreased distinctly in number with an increase of radiation dosage. The other tadpoles had various kinds of chromosome aberrations in all or a part of the metaphase spreads prepared by the squash method.

6. Of 1143 juveniles raised from irradiated gametes, 476 (42%) were males including hermaphrodites. There were numerous females with underdeveloped ovaries and a considerable number of hermaphrodites, in contrast with the controls. At the mature stage, 145 (58%) out of 248 frogs were males, while 96 (48%) out of 198 controls were males.

7. When 145 mature males raised from irradiated gametes were classified into five types on the basis of inner structure of their testes, 53 (37%), 35 (24%), 27 (19%), 18 (12%) and 12 (8%) were of type 1 (normal), type 2 (slightly abnormal), type 3 (considerably reduced normal spermatozoa), type 4 (a few normal spermatozoa) and type 5 (no normal spermatozoa), respectively. All 47 controls were of type 1. When they were classified into four types mainly on the basis of inner structure of their kidneys, 69 (48%), 28 (19%), 25 (17%) and 23 (16%) of the above 145 males were of type 1 (normal), type 2 (partly dilated uriniferous tubules), type 3 (half very abnormal) and type 4 (mostly very abnormal), respectively. Of the 47 controls, 45 were of type 1, while the other two were of type 2. It was evident that the abnormalities in inner structure of the kidneys corresponded roughly to those in inner structure of the testes.

8. Second-generation offspring were produced by mating 35 females and 27 males raised from X- or neutron-irradiated sperm or oviducal eggs with field-

caught frogs. The females belonging to each kind of experimental series laid remarkably fewer eggs as a whole than the controls did, and, moreover, seven of them laid two or three kinds of eggs that differed in size. While eight of the 35 females were nearly equal to the controls in producing normally metamorphosed frogs, the others were more or less inferior in this respect. Six of them produced no normally metamorphosed frogs. All the seven females which laid two or three kinds of eggs were extremely inferior in producing normally metamorphosed frogs; two of them produced none of the latter. There were two other females which laid no eggs after pituitary injection.

9. Of the 27 males used in producing second-generation offspring, fifteen, seven, three and two had testes of type 1, type 2, type 3 and type 4, respectively, while three controls had testes of type 1. Of the males with testes of type 1 in the experimental series, 12 were nearly equal to the controls in producing normally metamorphosed frogs, while the others were somewhat inferior in this respect. Three and four of the seven males having testes of type 2 were nearly equal and somewhat inferior to the controls, respectively. The five males having testes of type 3 or 4 were extremely inferior to the controls.

10. In the second generation derived from X- or neutron-irradiated grandparental sperm or oviducal eggs, 322 (70%) out of 462 normally shaped tadpoles had various kinds of chromosome aberrations, while the others were normal diploids.

11. Of 5410 second-generation juveniles produced by mating first-generation males raised from irradiated gametes with field-caught females, 2527 (47%) were males including 131 hermaphrodites. At the mature stage, 157 (54%) out of 291 frogs were males. In contrast, 1020 (53%) out of 1920 second-generation juveniles produced by mating first-generation females raised from irradiated gametes with field-caught males were males including hermaphrodites. At the mature stage, 110 (68%) out of 161 frogs were males. Among the control frogs there was always nearly an equal number of males and females.

12. Third-generation offspring were produced by mating 60 second-generation females and 71 second-generation males derived from X- or neutron-irradiated grandparental sperm or oviducal eggs with field-caught frogs. The females belonging to each kind of experimental series laid remarkably fewer eggs as a whole after pituitary injection than the controls did, although the eggs were nearly equal to those of the controls in size. There were 15 other second-generation females which laid no eggs after pituitary injection.

13. Of 35 females produced by first-generation females, 13 were nearly equal to the controls in producing normally metamorphosed frogs, while the others were more or less inferior in this respect. Of 400 frogs reared continuously after metamorphosis, 202 (51%) attained sexual maturity, while 43 (86%) of 50 controls did so. On the other hand, 14 out of 25 females produced by first-generation males were nearly equal to the controls in producing normally metamorphosed frogs, while the others were more or less inferior. No normally metamorphosed frogs were produced by one female. Of 350 frogs reared continuously after meta-

morphosis, 108 (31%) matured sexually, while 35 (70%) out of 50 controls did. The females laying a comparatively small number of eggs were mostly inferior to the others in producing normally metamorphosed frogs.

14. Of the 71 males used in producing third-generation offspring, 54, 5, 6, 4 and 2 had testes of type 1, type 2, type 3, type 4 and type 5, respectively. No normally metamorphosed frogs were produced by six males with testes of types 4 and 5. While 39 out of the 54 males with testes of type 1 were nearly equal to the controls in producing normally metamorphosed frogs, the others were more or less inferior in this respect. While four of the five males with testes of type 2 were considerably inferior to the controls, the remainder was nearly equal to them. All the six males with testes of type 3 were extremely inferior to the controls. While 388 (73%) out of 532 frogs reared continuously after metamorphosis in the series derived from first-generation females attained sexual maturity, only 102 (27%) out of 380 frogs in the series derived from first-generation males did so.

15. In the third generation derived from X- or neutron-irradiated great-grandparental sperm or oviducal eggs by passing over first-generation males and second-generation females or males, 74 (22%) out of 334 normally shaped tadpoles whose chromosomes were examined had various kinds of chromosome aberrations, while the others were normal diploids.

16. In a group of frogs produced by passing over first- and second-generation males mated with field-caught females, 1025 (47%) out of 2159 juveniles were males including hermaphrodites, and 67 (66%) out of 102 matures frogs were males. In another group produced by passing over first-generation males and second-generation females mated with field-caught frogs, 697 (59%) out of 1179 juveniles were males including hermaphrodites, and 73 (68%) out of 108 mature frogs were males. In still another group of frogs produced by passing over first-generation females and second-generation males mated with field-caught frogs, 2254 (53%) out of 4219 juveniles were males including hermaphrodites, and 215 (55%) out of 388 mature frogs were males. In the remaining group of frogs produced by passing over first- and second-generation females mated with field-caught males, 1408 (54%) out of 2614 juveniles were males including hermaphrodites, and 119 (59%) of 202 mature frogs were males. The controls of these four groups always consisted of nearly an equal number of males and females.

17. Fourth-generation offspring were produced by 59 third-generation females and 61 third-generation males derived from X- or neutron-irradiated great-grandparental sperm or oviducal eggs. These third-generation females and males were those which had been produced by passing over first- and second-generation females or males. Mating experiments were performed between these males and females or between these and field-caught frogs. The females belonging to each kind of experimental series laid considerably fewer eggs than the controls did after pituitary injection, except only one kind of experimental series consisting of two females. There were 19 other females which laid no eggs

after pituitary injection. All the 61 third-generation males were of type 1 in inner structure of the testes.

All the third-generation males and females were nearly equal or slightly inferior to the controls in producing normally metamorphosed frogs.

18. In a group of frogs produced by passing over first-, second- and third-generation males mated with field-caught females, 2791 (52%) out of 5325 juveniles were males including hermaphrodites. In another group of frogs produced by passing over first-, second- and third-generation females mated with field-caught males, 2670 (54%) out of 4980 juveniles were males including hermaphrodites. In the remaining group of frogs produced by mating between third-generation females obtained by passing over first- and second-generation males and third-generation males obtained by passing over first- and second-generation females, 2199 (53%) out of 4182 juveniles were males including hermaphrodites. The controls of these three groups consisted of nearly an equal number of males and females.

19. The difference in radiosensitivity between *Rana japonica* and *R. nigromaculata* seems to be attributable to those in DNA amount and relative nuclear size. 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, one year old, raised from irradiated gametes of *Rana japonica*. 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 male produced from a mating, J♀ ×J♂, No. 1.
2. Type 1 testis of a male No. 2 produced from a mating, J♀ ×SX–150♂, No. 1.
3. Type 2 testis of a male No. 1 produced from a mating, J♀ ×SN–150♂, No. 1.
4. Type 3 testis of a male No. 3 produced from a mating, EX–250♀ ×J♂, No. 1.

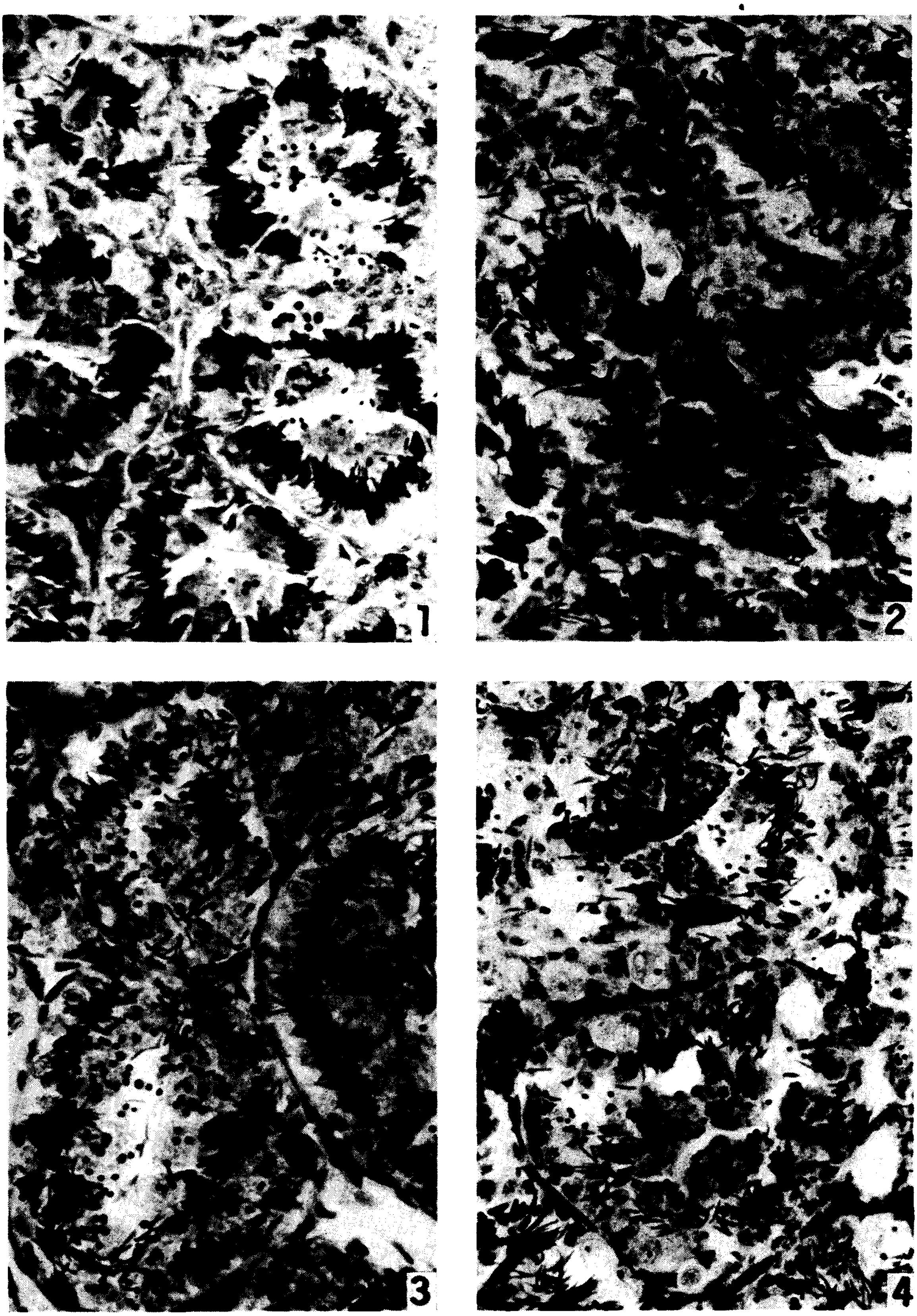

PLATE II

Cross-sections of seminal tubules of the testes of mature males, one year old, raised from irradiated gametes of *Rana japonica*. The testes of mature males were divided into five types on the basis of abnormality in inner structure. ×350

5. Type 4 testis of a male No. 2 produced from a mating, EX–150♀ ×J♂, No. 1.
6. Type 5 testis of a male No. 4 produced from a mating, J♀ ×SX–250♂, No. 1.
7. Type 4 testis of a male No. 1 produced from a mating, EN–250♀ ×J♂, No. 1.
8. Type 5 testis of a male No. 8 produced from a mating, J♀ ×SN–100♂, No. 1.

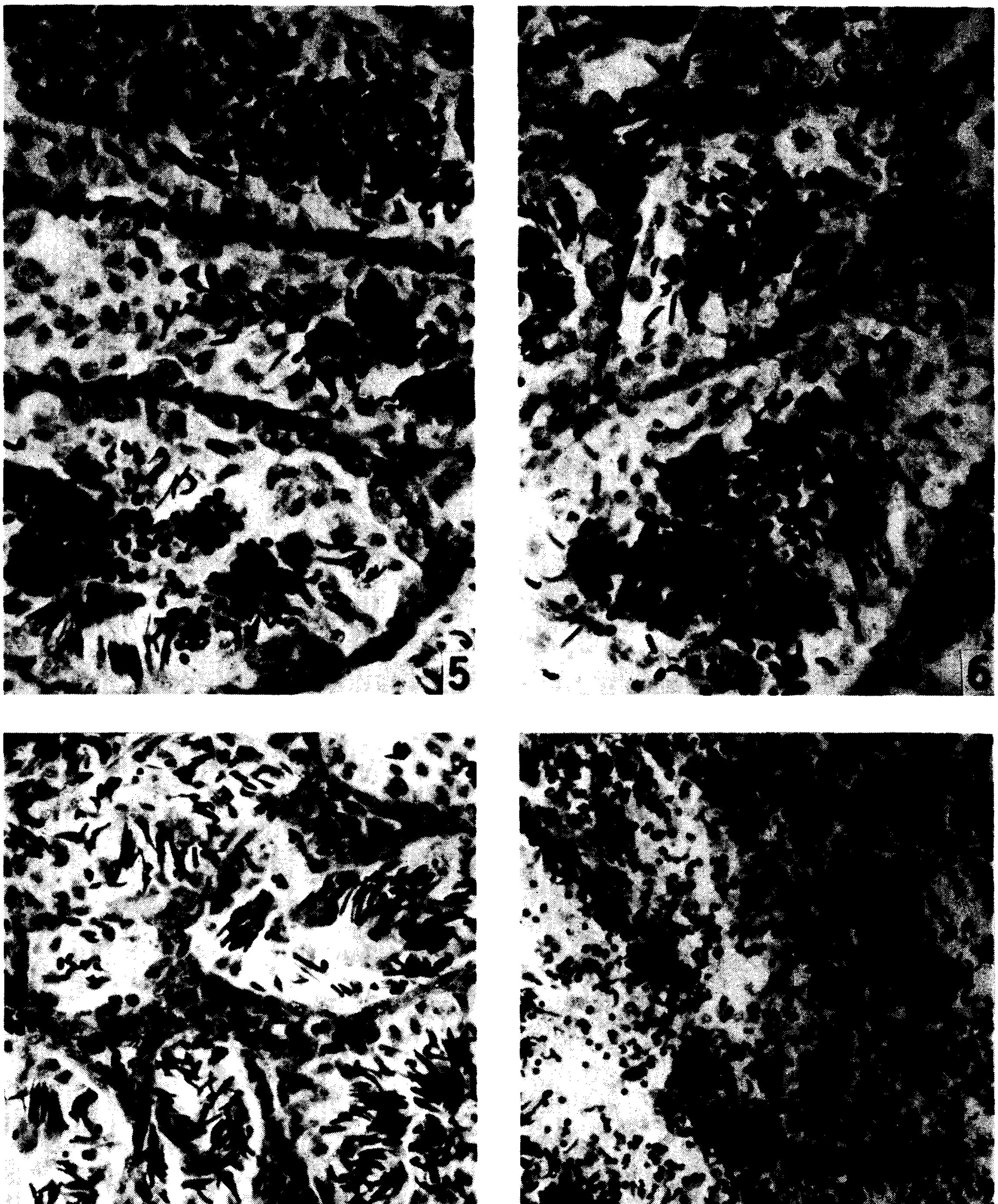

PLATE III

Cross-sections of the kidneys of mature males raised from irradiated sperm of *Rana japonica*. The kidneys of mature males were divided into four types on the basis of abnormality in inner structure. ×87

9. Type 1 kidney of a control male produced from a mating, J♀ ×J♂, No. 1.
10. Type 2 kidney of a male No. 2 produced from a mating, J♀ ×SX–150♂, No. 1.
11. Type 3 kidney of a male No. 8 produced from a mating, J♀ ×SN–100♂, No. 1.
12. Type 4 kidney of a male No. 4 produced from a mating, J♀ ×SX–250♂, No. 1.


9


10


11


12