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Color Variants Induced by Radiation and their Inheritance in *Rana nigromaculata*

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(With 2 Plates)

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INTRODUCTION

It is strange that reports on visible mutants induced by irradiation are extremely scarce in amphibians, differing from that in many other forms of life. RUGH (1939) first suggested that both dominant and recessive (lethal) mutations may be produced by X-ray bombardment (15~50,000 r) of sperm chromosomes in *Rana pipiens*. According to his report, 68% of fertilized eggs hatched and many died after hatching when the X-ray exposure of sperm was given at 250 r, while only 1.6% hatched and the majority died as gastrulae or neurulae at 10,000 r.

Soon afterwards, SAUNDERS and RUGH (1943) reported that eggs seemed to be more resistant to damage than did sperm at all irradiation levels. While formed chromosomes in the spermatogenesis of *Cynops pyrrhogaster* showed immediate and prolonged physical damage by irradiation with such a large dose of X-rays as 5,000～50,000 r (RUGH, 1950), chromosomal aberrations such as deletion, translocation, fragmentation and formation of dicentric chromosomes were found in somatic cells of larvae or adults of *Pleurodeles waltlii* when the fertilized eggs had been irradiated with a small dose of γ-rays such as 70～280 r (GALLIEN, LABROUSSE and LACROIX, 1963, 1966; GALLIEN, LABROUSSE, PICHERAL and LACROIX, 1965; LABROUSSE, 1967; GALLIEN, 1969). A female adult produced from an egg exposed to 70 r of γ-rays revealed an abnormally dense keratinization in the epidermis surrounding the pores of dermal glands. Besides, this female was abnormal in karyotype, having a deletion and a translocation. The first- and second-generation offspring had also a translocation analogous to that of the female in their karyotypes (LABROUSSE, 1969). However, they neither had a deletion such as found in the karyotype of the female nor revealed any abnormality in the keratinization of the skin. Translocations and deletions were also found in the karyotypes of the offspring of irradiated male *Pleurodeles waltlii* mated with an unirradiated female (JAYLET and BACQUIER, 1967). KAWAMURA and NISHIOKA (1967, 1977) and NISHIOKA (1969, 1977) have reported on chromosomal aberrations found abundantly in the karyotypes of the offspring of three generations derived from X- or neutron-irradiated eggs or spermatozoa in two Japanese ranid species, *Rana nigromaculata* and *Rana japonica*. These aberrations were always accompanied with physical abnormalities. As no individuals with chromosomal or physical abnormalities attained sexual maturity, the inheritance of such abnormalities could not be pursued.

It was surprising to the author that various kinds of color mutations occurred in more than one-third of X- or neutron-irradiated eggs or spermatozoa of *Rana nigromaculata* during the author's recent experiments. All these mutations were well-defined in morphological expression as well as in inheritance, differing from almost all the chromosomal aberrations stated above. In this paper, the origin, character and inheritance of each of nine color variants obtained by the author will be reported.

MATERIALS AND METHODS

Mature males and females of *Rana nigromaculata* HALLOWELL in the breeding season are mostly yellow-ocher and blackish-brown on the dorsal surface of the body, respectively. However, there is no difference in color between immature males and females. They are green or brown all over the dorsal surface, or green in the fore part and brown in the hind part. In these three color types, a gene for green color is always dominant over a gene for brown, although the inheritance of the size of the green area in the last color type is so complicated that it has not yet been fully clarified (NISHIOKA, 1971).

In 1967, three males and three females collected from Hiro near Hiroshima were used as materials. One of the three females was green and the other two were brown all over the dorsal surface at the immature frog stage. Ovulation of the female was accelerated by injecting suspension of frog pituitaries into coelom. Irradiation of gametes with X-rays or neutrons was performed at the Research Institute for Nuclear Medicine and Biology of Hiroshima University.

The X-ray equipment was of Toshiba KXC-18-2 Type and operated at 180 KVp, 25 mA, the conversion coefficient and exposure dose rate being 0.95 rad/r and ca. 20 rads/min, respectively. Unfertilized eggs were exposed to 90, 145 or 200 rads, while spermatozoa were to 90, 170 or 240 rads. These three dosages in each case were determined by a preliminary test as those which would allow about 60%, 40% or 25% of fertilized eggs to hatch normally. Eggs differ slightly from spermatozoa in sensitivity to X-rays.

The neutron generator was of Toshiba (d. n) He Reaction Type, producing fast neutrons 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. It is generally

TABLE 1

Viability of frogs raised from irradiated eggs or spermatozoa in 1967

Parents: Female	Parents: Male	No. of eggs	No. of normal cleavages (%)	No. of normal neurulae (%)	No. of normal tail-bud embryos (%)	No. of normal hatched tadpoles (%)	No. of 50-day-old tadpoles (%)	No. of metamorphosed frogs (%)
W67, Nos. 1∼3	W67, Nos. 1∼3	495	423 (85.5)	415 (83.8)	412 (83.2)	410 (82.8)	405 (81.8)	402 (81.2)
W67, Nos. 1∼3	SX–90, Nos. 1∼3	714	625 (87.5)	573 (80.3)	501 (70.2)	475 (66.5)	357 (50.0)	301 (42.2)
	SX–170, Nos. 1∼3	865	767 (88.7)	684 (79.1)	559 (64.6)	431 (49.8)	280 (32.4)	266 (30.8)
	SX–240, Nos. 1∼3	783	679 (86.7)	580 (74.1)	438 (55.9)	303 (38.7)	192 (24.5)	154 (19.7)
EX–90, Nos. 1∼3	W67, Nos. 1∼3	786	678 (86.3)	654 (83.2)	612 (77.9)	534 (67.9)	341 (43.4)	300 (38.2)
EX–145, Nos. 1∼3		754	679 (90.1)	632 (83.8)	576 (76.4)	435 (57.7)	246 (32.6)	215 (28.5)
EX–200, Nos. 1∼3		851	732 (86.0)	694 (81.6)	540 (63.5)	315 (37.0)	141 (16.6)	103 (12.1)
W67, Nos. 1∼3	SN–50, Nos. 1∼3	805	741 (92.0)	705 (87.6)	605 (75.2)	579 (71.9)	434 (53.9)	306 (38.0)
	SN–90, Nos. 1∼3	793	690 (87.0)	635 (80.1)	589 (74.3)	428 (54.0)	333 (42.0)	242 (30.5)
	SN–130, Nos. 1∼3	716	635 (88.7)	550 (76.8)	431 (60.2)	275 (38.4)	179 (25.0)	135 (18.9)
EN–50, Nos. 1∼3	W67, Nos. 1∼3	912	820 (89.9)	811 (88.9)	729 (79.9)	634 (69.5)	473 (51.9)	346 (37.9)
EN–90, Nos. 1∼3		860	739 (85.9)	687 (79.9)	535 (62.2)	473 (55.0)	291 (33.8)	241 (28.0)
EN–130, Nos. 1∼3		992	846 (85.3)	694 (70.0)	516 (52.0)	295 (29.7)	152 (15.3)	102 (10.3)

W67: A male or female whose gametes were not irradiated.

SX–90, 170 or 240: Male *Rana nigromaculata* whose spermatozoa were exposed to 90, 170 or 240 rads of X-rays.

EX–90, 145 or 200: Female *Rana nigromaculata* whose eggs were exposed to 90, 145 or 200 rads of X-rays.

SN–50, 90 or 130: Male *Rana nigromaculata* whose spermatozoa were exposed to 50, 90 or 130 rads of neutrons.

EN–50, 90 or 130: Female *Rana nigromaculata* whose eggs were exposed to 50, 90 or 130 rads of neutrons.

believed that the neutrons produced by this apparatus contain no trace of γ-rays. Unfertilized eggs or spermatozoa were exposed to 50, 90 or 130 rads. As in the case of X-rays, these dosages were determined by a preliminary test as those which would allow about 60%, 40% or 25% of fertilized eggs to hatch normally. Differing from X-rays, eggs do not differ from spermatozoa in sensitivity to neutrons.

Artificial insemination was made between X- or neutron-irradiated eggs or spermatozoa and normal gametes. The developmental capacity of fertilized eggs in twelve experimental series was observed until the stage of metamorphosis (Table 1). One month after metamorphosis, 120 or 100 frogs from each of the twelve experimental series were left and continuously reared in order to mature them, while the other frogs were killed at once. In the control series, 60 frogs were reared after metamorphosis.

In the breeding season of 1970 only a small number of the frogs alive in each experimental series were survivors, while about half of the frogs alive were living in the control series (Table 2). They were three years old and quite matured. There was nearly an equal number of males and females in each experimental

TABLE 2

Sex of mature frogs raised from eggs or sperm irradiated in 1967

Kinds	No. of frogs	No. of frogs dead or killed within 1 month after metamorphosis	No. of living frogs 1 month after metamorphosis	No. of 3-year-old mature frogs		
				Total	♀	♂
W·W, Nos. 1∼3	402	342	60	31	15	16
W·SX–90, Nos. 1∼3	301	181	120	32	14	18
W·SX–170, Nos. 1∼3	266	146	120	25	12	13
W·SX–240, Nos. 1∼3	154	34	120	7	3	4
EX–90·W, Nos. 1∼3	300	180	120	25	11	14
EX–145·W, Nos. 1∼3	215	95	120	32	15	17
EX–200·W, Nos. 1∼3	103	3	100	10	4	6
W·SN–50, Nos. 1∼3	306	186	120	16	7	9
W·SN–90, Nos. 1∼3	242	122	120	11	5	6
W·SN–130, Nos. 1∼3	135	15	120	27	13	14
EN–50·W, Nos. 1∼3	346	226	120	17	8	9
EN–90·W, Nos. 1∼3	241	121	120	14	7	7
EN–130·W, Nos. 1∼3	102	2	100	21	10	11

W·W: Frogs raised from untreated eggs by fertilization with untreated spermatozoa.

W·SX–90, 170 or 240: Frogs raised from untreated eggs by fertilization with spermatozoa exposed to 90, 170 or 240 rads of X-rays.

EX–90, 145 or 200·W: Frogs raised from eggs exposed to 90, 145 or 200 rads of X-rays by fertilization with untreated spermatozoa.

W·SN–50, 90 or 130: Frogs raised from untreated eggs by fertilization with spermatozoa exposed to 50, 90 or 130 rads of neutrons.

EN–50, 90 or 130·W: Frogs raised from eggs exposed to 50, 90 or 130 rads of neutrons by fertilization with untreated spermatozoa.

series as well as in the control, although males were slightly more numerous than females in total. In the experimental series derived from X-irradiated gametes, the larger the dosage, the fewer were the survivors as a whole, while there was no such tendency in those derived from neutron-irradiated gametes. All the mature females were injected with suspension of frog pituitaries in order to accelerate their ovulation. When ovulation occurred, eggs were taken out of the females and initiated to develop into gynogenetic diploids by the following method.

Sperm suspension obtained from a male *Rana brevipoda* is exposed for 3 minutes to UV emitted from a mercury lamp, GUL-5·J Type of Toshiba Co., 2537 Å in main wave length at a distance of 20 cm. By such an exposure the nuclei of spermatozoa become incompetent. However, the spermatozoa are motile and enter into eggs; pseudofertilization occurs there. After the inseminated eggs are left as they are for 20 minutes at the room temperature (17～25°C), they are exposed to low temperature (0.2～0.7°C) for about 3 hours. By this treatment, the nucleus of the second polar body is retained in the egg and then united with the egg pronucleus into a diploid nucleus; the egg develops as a gynogenetic diploid. As homologous chromosomes are separated from each other at the first meiotic division of oocytes, half the number of diploid gynogenetic eggs usually become homozygous for one member of each pair of alleles, while the other half become also homozygous for the other member. Accordingly, every recessive mutation produced by irradiation of gametes can be easily determined by making use of the method of diploid gynogenesis.

The diploidy and homozygosity of the individuals produced by the above method are warranted at the tadpole stage by their viability and external characters. Almost all the haploid embryos reveal a haploid syndrome and die of edema. Even if some of them hatch normally, they become feeble and dwarf tadpoles and can not develop into mature frogs. When diploid or triploid hybrids are produced as a result of a failure in the experimental procedure, they are easily identified by their appearance. It is possible that autotriploids are gynogenetically produced by chance. However, they are detectable at the stage of sexual maturity, as they are sterile to a large extent. It has always confirmed that the nuclei of spermatozoa irradiated with UV were completely incompetent to participate in the development of eggs, as all the embryos were haploids when they were not refrigerated shortly after fertilization.

A part of eggs obtained from each female was fertilized with non-treated spermatozoa of males collected from the field. These normally fertilized eggs were utilized to compare with gynogenetic diploids in developmental capacity, as well as to obtain frogs heterozygous for an allelic pair of each color mutation produced by irradiation.

OBSERVATION

I. Developmental capacity and color variation of gynogenetic diploids

1. Control series
(W♀ × W♂)♀GD, Nos. 1∼15

Of 15 mature females which had been produced from matings between normal males and females in the control series, ten were injected with frog pituitary suspension. As ovulation occurred in all these females, a part of eggs obtained from each female was inseminated with spermatozoa of a normal male (W70,

TABLE 3
Viability of second-generation offspring produced from first-generation females

Year	Parents: Female	Parents: Male	No. of eggs	No. of normal cleavages (%)	No. of normal neurulae (%)
1970	W·W, No. 1	W70, No. 1	215	183 (85.1)	183 (85.1)
		GD, No. 1	165	132 (80.0)	124 (75.2)
	W·W, No. 2	W70, No. 1	263	254 (96.6)	252 (95.8)
		GD, No. 2	192	132 (68.8)	108 (56.3)
	W·W, No. 3	W70, No. 1	194	189 (97.4)	186 (95.9)
		GD, No. 3	134	102 (76.1)	84 (62.7)
	W·W, No. 4	W70, No. 1	152	138 (90.8)	137 (90.1)
		GD, No. 4	155	136 (87.7)	124 (80.0)
	W·W, No. 5	W70, No. 1	81	76 (93.8)	75 (92.6)
		GD, No. 5	168	124 (73.8)	116 (69.0)
	W·W, No. 6	W70, No. 1	132	121 (91.7)	120 (90.9)
		GD, No. 6	353	275 (77.9)	251 (71.1)
	W·W, No. 7	W70, No. 1	124	112 (90.3)	112 (90.3)
		GD, No. 7	397	253 (63.7)	240 (60.5)
	W·W, No. 8	W70, No. 1	121	107 (88.4)	107 (88.4)
		GD, No. 8	474	315 (66.5)	293 (61.8)
	W·W, No. 9	W70, No. 1	156	136 (87.2)	132 (84.6)
		GD, No. 9	426	276 (64.8)	242 (56.8)
	W·W, No. 10	W70, No. 1	140	132 (94.3)	130 (92.9)
		GD, No. 10	511	344 (67.3)	316 (61.8)
	W·W, Nos. 1∼10	W70, No. 1	1578	1448 (91.8)	1434 (90.9)
		GD, Nos. 1∼10	2975	2089 (70.2)	1898 (63.8)
1971	W·W, No. 11	GD, No. 11	754	531 (70.4)	462 (61.3)
	W·W, No. 12	GD, No. 12	560	413 (73.8)	374 (66.8)
	W·W, No. 13	GD, No. 13	427	216 (50.6)	201 (47.1)
	W·W, No. 14	GD, No. 14	610	465 (76.2)	417 (68.4)
	W·W, No. 15	GD, No. 15	564	314 (55.7)	304 (53.9)
	W·W, Nos. 11∼15	GD, Nos. 11∼15	2915	1939 (66.5)	1758 (60.3)

W·W: A female raised from an untreated egg fertilized with an untreated spermatozoon.
W70: A male collected from the field in the breeding season of 1970.
GD: Diploid gynogenesis.

No. 1) collected from the field, while the other part was induced to develop by the method of diploid gynogenesis (Table 3). As presented in the table, gynogenetic eggs were remarkably inferior to eggs inseminated with normal spermatozoa in developmental capacity. In the group of 10 matings performed by artificial insemination, 85.1 ~ 97.4%, 91.8% on the average, of the respective total number of eggs cleaved normally, while 63.7 ~ 87.7%, 70.2% on the average, did so in the group of 10 experiments of diploid gynogenesis. In the former group, 80.8 ~ 95.8%, 88.3% on the average, hatched normally after normal development in the embryonic stage, while 48.1 ~ 74.8%, 58.0% on the average, did so after many embryos died of curvature of the body or edema. In the group of artificial insemination, almost all the tadpoles continued their normal development; 78.1 ~ 94.3%, 84.3% on the average, metamorphosed normally. In the

by mating with a field-caught male or by diploid gynogenesis in the control series

No. of normal tail-bud embryos (%)	No. of normal hatched tadpoles (%)	No. of 50-day-old tadpoles (%)	No. of metamorphosed frogs (%)	No. of frogs 1 month after metamorph. (%)	No. of frogs 2 months after metamorph. (%)
183 (85.1)	179 (83.3)	171 (79.5)	168 (78.1)	167 (77.7)	167 (77.7)
122 (73.9)	117 (70.9)	58 (35.2)	40 (24.2)	38 (23.0)	31 (18.8)
252 (95.8)	252 (95.8)	250 (95.1)	248 (94.3)	245 (93.2)	243 (92.4)
108 (56.3)	102 (53.1)	86 (44.8)	61 (31.8)	60 (31.3)	60 (31.3)
180 (92.8)	178 (91.8)	167 (86.1)	162 (83.5)	162 (83.5)	162 (83.5)
82 (61.2)	78 (58.2)	75 (56.0)	75 (56.0)	74 (55.2)	72 (53.7)
135 (88.8)	132 (86.8)	124 (81.6)	122 (80.3)	121 (79.6)	121 (79.6)
122 (78.7)	116 (74.8)	110 (71.0)	109 (70.3)	95 (61.3)	82 (52.9)
74 (91.4)	72 (88.9)	72 (88.9)	72 (88.9)	72 (88.9)	72 (88.9)
111 (66.1)	109 (64.9)	82 (48.8)	71 (42.3)	69 (41.1)	69 (41.1)
117 (88.6)	116 (87.9)	114 (86.4)	113 (85.6)	110 (83.3)	105 (79.5)
244 (69.1)	221 (62.6)	173 (49.0)	149 (42.2)	122 (34.6)	114 (32.3)
110 (88.7)	110 (88.7)	107 (86.3)	105 (84.7)	103 (83.1)	100 (80.6)
237 (59.7)	232 (58.4)	185 (46.6)	134 (33.8)	115 (29.0)	112 (28.2)
105 (86.8)	101 (83.5)	101 (83.5)	95 (78.5)	93 (76.9)	93 (76.9)
288 (60.8)	247 (52.1)	202 (42.6)	175 (36.9)	146 (30.8)	123 (25.9)
130 (83.3)	126 (80.8)	125 (80.1)	122 (78.2)	120 (76.9)	120 (76.9)
237 (55.6)	205 (48.1)	144 (33.8)	112 (26.3)	103 (24.2)	94 (22.1)
129 (92.1)	127 (90.7)	125 (89.3)	123 (87.9)	120 (85.7)	119 (85.0)
306 (59.9)	298 (58.3)	216 (42.3)	167 (32.7)	124 (24.3)	124 (24.3)
1415 (89.7)	1393 (88.3)	1356 (85.9)	1330 (84.3)	1313 (83.2)	1302 (82.5)
1857 (62.4)	1725 (58.0)	1331 (44.7)	1093 (36.7)	946 (31.8)	881 (29.6)
424 (56.2)	391 (51.9)	353 (46.8)	346 (45.9)	346 (45.9)	343 (45.5)
323 (57.7)	320 (57.1)	299 (53.4)	281 (50.2)	280 (50.0)	276 (49.3)
193 (45.2)	175 (41.0)	156 (36.5)	150 (35.1)	147 (34.4)	142 (33.3)
405 (66.4)	376 (61.6)	354 (58.0)	352 (57.7)	352 (57.7)	342 (56.1)
277 (49.1)	228 (40.4)	206 (36.5)	200 (35.5)	199 (35.3)	197 (34.9)
1622 (55.6)	1490 (51.1)	1368 (46.9)	1329 (45.6)	1324 (45.4)	1300 (44.6)

group of diploid gynogenesis, many tadpoles died of edema or underdevelopment; 24.2 ~ 70.3%, 36.7% on the average, became normal, metamorphosed frogs.

All the frogs that developed gynogenetically or by normal fertilization were quite normal in color; their dorsal surfaces were brown or green. No color variants were found among them.

While ten of the 15 mature females were used as materials in 1970, the remaining five (Nos. 11 ~ 15) were used for producing gynogenetic diploids in 1971. In the 5 experiments, 50.6 ~ 76.2%, 66.5% on the average, of the respective total number of eggs cleaved normally. Many of the normally cleaved eggs died during embryonic and tadpole stages; 40.4 ~ 61.6%, 51.1% on the average, hatched normally, and 35.1 ~ 57.7%, 45.6% on the average, became normal, metamorphosed frogs. All these frogs were quite normal in color like those produced in the previous year; they were brown or green, and there were no color variations.

2. Experimental series using X-irradiated spermatozoa

a. (W♀ × SX–90♂) ♀GD, Nos. 1 ~ 9

Fourteen females that had been derived from spermatozoa exposed to 90 rads of X-rays matured in 1970. Ten of them were injected with frog pituitary suspension. Ovulation occurred normally in 9 females. The eggs removed from each female were divided into two parts. One part was induced to develop by the method of diploid gynogenesis, while the other part was inseminated with spermatozoa of the same male (W70, No. 1) as that used in the control series (Table 4).

While 64.2 ~ 97.6%, 85.6% on the average, of the respective total number of eggs cleaved normally in 9 matings Nos. 1 ~ 9, 31.5 ~ 81.9%, 59.0% on the average, did so in nine experiments of diploid gynogenesis. Many of the normally cleaved eggs became abnormal and died before the hatching stage. While 1.1 ~ 73.3%, 54.4% on the average, hatched normally in the nine matings, 2.5 ~ 66.2%, 17.4% on the average, did so in the nine experiments of diploid gynogenesis. Many tadpoles died afterwards of various abnormalities before metamorphosis; 0 ~ 65.3%, 37.6% on the average, and 0 ~ 17.2%, 7.3% on the average, became normal, metamorphosed frogs in the nine matings and the experiments of diploid gynogenesis, respectively.

The frogs that developed from eggs fertilized with normal spermatozoa were quite normal in color. In contrast with these frogs, color variants appeared in three (Nos. 1, 2 and 5) of the 9 experiments of diploid gynogenesis (Table 8). Of 42 gynogenetic frogs of experiment No. 1 examined 3 months after metamorphosis, 20 were green, 19 brown and three blue, while of 33 frogs produced by insemination with normal spermatozoa, 17 were brown and 16 green. The blue frogs were the same in the electron-microscopic structure of the skin as the green ones, except that the xanthophores contained no carotenoid vesicles.

In experiment No. 2, 36 of 86 fifty-day-old gynogenetic tadpoles were gray-eyed variants, while the other 50 had normal eyes. The gray-eyed variants were characterized by a semi-transparent body, very small quantity of melanine in the skin and ash color of the eyes. All of them died without making metamorphosis. Although they could develop almost normally until the stage of tiny hind-limb buds (stage V in TAYLOR and KOLLROS' table), they died sooner or later of edema or ill-development. In experiment No. 5, 25 of 52 fifty-day-old gynogenetic tadpoles were black-eyed variants, while the other 27 were quite normal. The black-eyed variants were characterized by a semi-transparent body, a large quantity of melanin in the skin and black eyes. Although these mutants were very inferior to the other wild-type tadpoles in viability, a few of them could normally metamorphose. In experiments Nos. 6 and 7, all the tadpoles died before the metamorphosing stage. Accordingly, no mutants were detected among them. From the remaining 4 experiments Nos. 3, 4, 8 and 9, no color variants appeared among tadpoles or frogs produced by diploid gynogenesis.

b. (W♀×SX–170♂) ♀GD, Nos. 1～9

Twelve females that had been derived from spermatozoa exposed to 170 rads of X-rays were injected with frog pituitary suspension. Ovulation occurred in nine of them. A part of the eggs taken out of each female was fertilized with sperm of the same male as that used in the control series, while the other part was induced to develop by the method of diploid gynogenesis (Table 4).

In 9 matings Nos. 1～9, 22.0～99.2%, 86.7% on the average, of the respective total number of eggs cleaved normally, while 7.5～87.2%, 49.8% on the average, did so in 9 experiments of diploid gynogenesis. If mating No. 1 as well as experiment No. 1 of diploid gynogenesis was excluded, more than 76.7% and 29.1% cleaved normally in the other 8 matings and 8 gynogenetic experiments, respectively. At the embryonic stage, many individuals died of various abnormalities; 11.3～84.5%, 60.6% on the average, hatched normally in the 9 matings, while 0.4～24.9%, 12.9% on the average, did so in the 9 gynogenetic experiments. During the tadpole stage, many individuals became abnormal and died; 8.6～70.4%, 48.9% on the average, metamorphosed normally in the 9 matings, while 0.4～20.9%, 10.0% on the average, did so in the 9 gynogenetic experiments.

Color variants were found among the frogs produced from 3 experiments (Nos. 2, 3 and 9) of diploid gynogenesis (Table 8). In experiment No. 2, two of 12 gynogenetic frogs examined 3 months after metamorphosis were blue mutants, while four others were green and the remaining six brown. In mating No. 2, 35 of 67 frogs produced from fertilization with normal spermatozoa were green and the other 32 brown. In experiment No. 3, three of 11 gynogenetic frogs examined 3 months after metamorphosis were blue mutants, while the other eight were green. In mating No. 3, all the 26 frogs produced by fertilization with normal spermatozoa were green when observed at the same stage. In experiment No. 9, 14 of 29 gynogenetic tadpoles had a semi-transparent bluish belly. After metamorphosis, such tadpoles became frogs that were olive in dorsal ground color. The other tadpoles were normal in external characters and became frogs that were brown in dorsal ground color.

TABLE 4

Viability of second-generation offspring produced from females raised from X-irradiated

Parents		No. of eggs	No. of normal cleavages (%)	No. of normal neurulae (%)
Female	Male			
W·SX–90, No. 1	W70, No. 1	271	174 (64.2)	165 (60.9)
	GD, No. 1	421	230 (54.6)	88 (20.9)
W·SX–90, No. 2	W70, No. 1	285	250 (88.0)	212 (74.4)
	GD, No. 2	334	200 (59.9)	123 (36.8)
W·SX–90, No. 3	W70, No. 1	316	294 (93.0)	244 (77.2)
	GD, No. 3	327	116 (35.5)	51 (15.6)
W·SX–90, No. 4	W70, No. 1	205	200 (97.6)	155 (75.6)
	GD, No. 4	174	138 (79.3)	115 (66.1)
W·SX–90, No. 5	W70, No. 1	167	146 (87.4)	94 (56.3)
	GD, No. 5	151	115 (76.2)	111 (73.5)
W·SX–90, No. 6	W70, No. 1	112	99 (88.4)	93 (83.0)
	GD, No. 6	175	128 (73.1)	108 (61.7)
W·SX–90, No. 7	W70, No. 1	87	78 (89.7)	3 (3.4)
	GD, No. 7	159	110 (69.2)	94 (59.1)
W·SX–90, No. 8	W70, No. 1	124	103 (83.1)	96 (77.4)
	GD, No. 8	155	127 (81.9)	105 (67.7)
W·SX–90, No. 9	W70, No. 1	101	83 (82.2)	51 (50.5)
	GD, No. 9	162	51 (31.5)	32 (19.8)
W·SX–90, Nos. 1∼9	W70, No. 1	1668	1427 (85.6)	1113 (66.7)
	GD, Nos. 1∼9	2058	1215 (59.0)	827 (40.2)
W·SX–170, No. 1	W70, No. 1	186	41 (22.0)	29 (15.6)
	GD, No. 1	266	20 (7.5)	2 (0.8)
W·SX–170, No. 2	W70, No. 1	245	230 (93.9)	151 (61.6)
	GD, No. 2	203	59 (29.1)	24 (11.8)
W·SX–170, No. 3	W70, No. 1	262	260 (99.2)	224 (85.5)
	GD, No. 3	213	69 (32.4)	41 (19.2)
W·SX–170, No. 4	W70, No. 1	204	195 (95.6)	151 (74.1)
	GD, No. 4	314	169 (53.8)	70 (22.3)
W·SX–170, No. 5	W70, No. 1	272	269 (98.9)	253 (93.0)
	GD, No. 5	201	70 (34.8)	33 (16.4)
W·SX–170, No. 6	W70, No. 1	233	224 (96.1)	209 (89.7)
	GD, No. 6	249	210 (84.3)	79 (31.7)
W·SX–170, No. 7	W70, No. 1	73	56 (76.7)	46 (63.0)
	GD, No. 7	109	52 (47.7)	42 (38.5)
W·SX–170, No. 8	W70, No. 1	133	112 (84.2)	100 (75.2)
	GD, No. 8	215	149 (69.3)	130 (60.5)
W·SX–170, No. 9	W70, No. 1	145	132 (91.0)	123 (84.8)
	GD, No. 9	226	197 (87.2)	170 (75.2)
W·SX–170, Nos. 1∼9	W70, No. 1	1753	1519 (86.7)	1286 (73.4)
	GD, Nos. 1∼9	1996	995 (49.8)	591 (29.6)
W·SX–240, No. 1	W70, No. 1	211	182 (86.3)	174 (82.5)
	GD, No. 1	152	71 (46.7)	70 (46.1)
W·SX–240, No. 2	W70, No. 1	194	112 (57.7)	109 (56.2)
	GD, No. 2	241	28 (11.6)	25 (10.4)
W·SX–240, Nos. 1, 2	W70, No. 1	405	294 (72.6)	283 (69.9)
	GD, Nos. 1, 2	393	99 (25.2)	95 (24.2)

W·SX–90, 170 or 240: A female raised from an untreated egg fertilized with a spermatozoon
GD: Diploid gynogenesis.

spermatozoa by mating with a field-caught male or by diploid gynogenesis

No. of normal tail-bud embryos (%)	No. of normal hatched tadpoles (%)	No. of 50-day-old tadpoles (%)	No. of metamor-phosed frogs (%)	No. of frogs 1 month after metamorph. (%)	No. of frogs 2 months after metamorph. (%)
154 (56.4)	135 (49.8)	130 (48.0)	115 (42.4)	97 (35.8)	85 (31.4)
76 (18.1)	52 (12.4)	48 (11.4)	45 (10.7)	45 (10.7)	42 (10.0)
209 (73.3)	209 (73.3)	201 (70.5)	186 (65.3)	178 (62.5)	54 (18.9)
118 (35.3)	95 (28.4)	86 (25.7)	41 (12.3)	32 (9.6)	22 (6.6)
225 (71.2)	212 (67.1)	170 (53.8)	142 (44.9)	103 (32.6)	82 (25.9)
46 (14.1)	29 (8.9)	10 (3.1)	9 (2.8)	4 (1.2)	2 (0.6)
134 (65.4)	118 (57.6)	69 (33.7)	67 (32.7)	65 (31.7)	65 (31.7)
101 (58.0)	36 (20.7)	30 (17.2)	30 (17.2)	25 (14.4)	23 (13.2)
77 (46.1)	42 (25.1)	24 (14.4)	16 (9.6)	16 (9.6)	11 (6.6)
105 (69.5)	100 (66.2)	52 (34.4)	3 (2.0)	3 (2.0)	2 (1.3)
85 (75.9)	81 (72.3)	32 (28.6)	5 (4.5)	3 (2.7)	3 (2.7)
45 (25.7)	8 (4.6)	3 (1.7)	0	0	0
3 (3.4)	1 (1.1)	1 (1.1)	0	0	0
23 (14.5)	5 (3.1)	0	0	0	0
87 (70.2)	72 (58.1)	66 (53.2)	61 (49.2)	60 (48.4)	58 (46.8)
80 (51.6)	29 (18.7)	25 (16.1)	21 (13.5)	16 (10.3)	15 (9.7)
39 (38.6)	37 (36.6)	36 (35.6)	35 (34.7)	24 (23.8)	17 (16.8)
29 (17.9)	4 (2.5)	3 (1.9)	2 (1.2)	2 (1.2)	2 (1.2)
1013 (60.7)	907 (54.4)	729 (43.7)	627 (37.6)	546 (32.7)	375 (22.5)
623 (30.3)	358 (17.4)	257 (12.5)	151 (7.3)	127 (6.2)	108 (5.2)
29 (15.6)	21 (11.3)	16 (8.6)	16 (8.6)	16 (8.6)	13 (7.0)
2 (0.8)	1 (0.4)	1 (0.4)	1 (0.4)	0	0
141 (57.6)	126 (51.4)	120 (49.0)	108 (44.1)	103 (42.0)	95 (38.9)
19 (9.4)	16 (7.9)	16 (7.9)	13 (6.4)	13 (6.4)	12 (5.9)
207 (79.0)	195 (74.4)	183 (69.8)	126 (48.1)	114 (43.5)	93 (35.5)
28 (13.1)	27 (12.7)	15 (7.0)	13 (6.1)	13 (6.1)	11 (5.2)
126 (61.8)	118 (57.8)	118 (57.8)	104 (51.0)	102 (50.0)	33 (16.2)
68 (21.7)	61 (19.4)	56 (17.8)	52 (16.6)	50 (15.9)	45 (14.3)
238 (87.5)	211 (77.6)	202 (74.3)	179 (65.8)	153 (56.3)	79 (29.0)
31 (15.4)	30 (14.9)	24 (11.9)	19 (9.5)	18 (9.0)	15 (7.5)
203 (87.1)	197 (84.5)	197 (84.5)	164 (70.4)	116 (49.8)	59 (25.3)
74 (29.7)	62 (24.9)	56 (22.5)	52 (20.9)	52 (20.9)	48 (19.3)
42 (57.5)	41 (56.2)	37 (50.7)	35 (47.9)	34 (46.6)	34 (46.6)
31 (28.4)	7 (6.4)	5 (4.6)	5 (4.6)	5 (4.6)	4 (3.7)
92 (69.2)	74 (55.6)	70 (52.6)	64 (48.1)	64 (48.1)	64 (48.1)
78 (36.3)	16 (7.4)	16 (7.4)	16 (7.4)	16 (7.4)	16 (7.4)
120 (82.8)	80 (55.2)	76 (52.4)	62 (42.8)	61 (42.1)	43 (29.7)
129 (57.1)	38 (16.8)	32 (14.2)	29 (12.8)	29 (12.8)	27 (11.9)
1198 (68.3)	1063 (60.6)	1019 (58.1)	858 (48.9)	763 (43.5)	513 (29.3)
460 (23.0)	258 (12.9)	221 (11.1)	200 (10.0)	196 (9.8)	178 (8.9)
171 (81.0)	119 (56.4)	115 (54.5)	102 (48.3)	80 (37.9)	80 (37.9)
70 (46.1)	40 (26.3)	32 (21.1)	14 (9.2)	13 (8.6)	10 (6.6)
105 (54.1)	69 (35.6)	50 (25.8)	44 (22.7)	44 (22.7)	42 (21.6)
25 (10.4)	20 (8.3)	17 (7.1)	5 (2.1)	2 (0.8)	2 (0.8)
276 (68.1)	188 (46.4)	165 (40.7)	146 (36.0)	124 (30.6)	122 (30.1)
95 (24.2)	60 (15.3)	49 (12.5)	19 (4.8)	15 (3.8)	12 (3.1)

exposed to 90, 170 or 240 rads of X-rays.

c. (W♀ × SX–240♂) ♀GD, Nos. 1 and 2

Three mature females that had been derived from spermatozoa exposed to 240 rads of X-rays were injected with frog pituitary suspension. As ovulation occurred in two of them, a part of the eggs removed from each female was induced to develop by the method of diploid gynogenesis, while the other part was inseminated with spermatozoa of the same male as that used in the control series (Table 4). While 86.3% and 57.7%, 72.6% on the average, of the respective total number of eggs cleaved normally in matings Nos. 1 and 2, 46.7% and 11.6%, 25.2% on the average, did so in experiments Nos. 1 and 2, respectively. Many of the normally cleaved eggs died of various abnormalities at the embryonic stage; 26.3% and 8.3%, 15.3% on the average, hatched normally in gynogenetic experiments Nos. 1 and 2, while 56.4% and 35.6%, 46.4% on the average, did so in matings Nos. 1 and 2. After the hatching stage, many tadpoles died of various abnormalities; only 9.2% and 2.1%, 4.8% on the average, metamorphosed normally in gynogenetic experiments Nos. 1 and 2, while 48.3% and 22.7%, 36.0% on the average, did so in matings Nos. 1 and 2. No color mutants were produced from the gynogenetic experiments as well as from the matings.

3. Experimental series using X-irradiated eggs

a. (EX–90♀ × W♂) ♀GD, Nos. 1∼6

Of mature females that had been derived from eggs exposed to 90 rads of X-rays, six ovulated normally after injection of frog pituitary suspension. A part of the eggs taken out of each female was induced to develop by the method of diploid gynogenesis, while the other part was inseminated with spermatozoa of a normal male (W70, No. 2) collected from the field (Table 5). In 6 gynogenetic experiments Nos. 1∼6, 2.2∼89.8%, 53.6% on the average, of the respective total number of eggs cleaved normally, while 67.8∼98.6%, 83.4% on the average, did so in 6 matings. Many of the normally cleaved eggs died of various abnormalities at various embryonic stages; 1.7∼41.8%, 22.4% on the average, hatched normally in the gynogenetic experiments, while 50.3∼90.9%, 67.9% on the average, did so in the matings. Afterwards, 0∼25.8%, 12.6% on the average, metamorphosed normally in the gynogenetic experiments, while 40.0∼66.0%, 56.2% on the average, did so in the matings.

Color variants were found among the frogs produced from 2 experiments (Nos. 5 and 6) of diploid gynogenesis (Table 8). In experiment No. 5, eleven of 54 fifty-day-old tadpoles were gray-eyed variants. They had a semi-transparent body and gray eyes. Their skin contained an extremely small quantity of melanin. All these mutants died without making metamorphosis. Although they developed almost normally until the stage of small limb buds (stage V in Taylor and Kollros' table), they died sooner or later without making further development. In experiment No. 6, nine of 21 fifty-day-old tadpoles were black-eyed variants. They had a semi-transparent body and black eyes. Their skin contained a very large quantity of melanin. Only four of them completed metamorphosis and became frogs. No color variants were produced from the remaining gynogenetic experiments as well as from the matings.

b. (EX–145♀ × W♂)♀GD, Nos. 1 ~ 12

Fifteen mature females which had been derived from eggs exposed to 145 rads of X-rays by inseminating with untreated spermatozoa of a normal male were injected with frog pituitary suspension. As a result, ovulation occurred in twelve of them. A part of the eggs taken out of each female was made to develop by the method of diploid gynogenesis, while the other part was inseminated with spermatozoa of the male W70, No. 2 (Table 5). In the twelve gynogenetic experiments (Nos. 1 ~ 12), 9.8 ~ 49.6%, 35.9% on the average, of the respective total number of eggs cleaved normally, while 3.1 ~ 79.2%, 45.3% on the average, did so in the twelve matings. During the embryonic stage, many of the normally cleaved eggs died of various abnormalities; 0 ~ 21.3%, 10.6% on the average, hatched normally in the gynogenetic experiments, while 1.3 ~ 50.4%, 28.0% on the average, did so in the matings. Many tadpoles died afterwards of edema or underdevelopment; 0 ~ 17.9%, 5.9% on the average, metamorphosed normally in the gynogenetic experiments, while 0 ~ 42.4%, 21.3% on the average, did so in the matings.

Color variants appeared in 3 experiments (Nos. 1, 4 and 11) of diploid gynogenesis (Table 8). When examined one month after metamorphosis, 15 of 30 and 24 of 49 frogs were olive variants in experiments Nos. 1 and 4, respectively. In experiment No. 11, one of 16 individuals at the hatching stage was an albino. In 4 experiments (Nos. 5, 8, 10 and 12) of diploid gynogenesis, all the tadpoles died before the metamorphosing stage. Accordingly, it was almost impossible to ascertain the existence of color variants. In the remaining 5 experiments, no color mutants were detected. The frogs produced from eggs fertilized with non-treated spermatozoa were green or brown in ground color.

c. (EX–200♀ × W♂)♀GD, Nos. 1 and 2

Four mature females that had been derived from eggs exposed to 200 rads of X-rays were injected with frog pituitary suspension. As ovulation occurred in two of them, a part of the eggs taken out of each female was induced to develop by the method of diploid gynogenesis, while the other part was inseminated with non-treated spermatozoa of male W70, No. 2 (Table 5). In the gynogenetic experiments Nos. 1 and 2, 21.5% and 16.2%, 18.2% on the average, of the respective total number of eggs cleaved normally, while 80.9% and 73.1%, 77.2% on the average, did so in matings Nos. 1 and 2. Many of the normally cleaved eggs died of various abnormalities at various embryonic stages; 0 and 1.9%, 1.2% on the average, hatched normally in experiments Nos. 1 and 2, while 59.5% and 23.3%, 42.4% on the average, did so in matings Nos. 1 and 2. All the gynogenetic tadpoles died before the metamorphosing stage. Accordingly, it was almost impossible to ascertain the existence of color variants. In matings Nos. 1 and 2, 32.1% and 14.5%, 23.8% on the average, metamorphosed normally.

TABLE 5

Viability of second-generation offspring produced from females raised from

Parents		No. of eggs	No. of normal cleavages (%)	No. of normal neurulae (%)
Female	Male			
EX–90·W, No. 1	W70, No. 2	208	190 (91.3)	181 (87.0)
	GD, No. 1	179	4 (2.2)	3 (1.7)
EX–90·W, No. 2	W70, No. 2	290	220 (75.9)	215 (74.1)
	GD, No. 2	256	17 (6.6)	17 (6.6)
EX–90·W, No. 3	W70, No. 2	209	206 (98.6)	201 (96.2)
	GD, No. 3	194	163 (84.0)	140 (72.2)
EX–90·W, No. 4	W70, No. 2	174	118 (67.8)	114 (65.5)
	GD, No. 4	209	163 (78.0)	125 (59.8)
EX–90·W, No. 5	W70, No. 2	240	207 (86.3)	193 (80.4)
	GD, No. 5	221	182 (82.4)	167 (75.6)
EX–90·W, No. 6	W70, No. 2	145	115 (79.3)	101 (69.7)
	GD, No. 6	108	97 (89.8)	80 (74.1)
EX–90·W, Nos. 1~6	W70, No. 2	1266	1056 (83.4)	1005 (79.4)
	GD, Nos. 1~6	1167	626 (53.6)	532 (45.6)
EX–145·W, No. 1	W70, No. 2	294	60 (20.4)	56 (19.0)
	GD, No. 1	521	212 (40.7)	128 (24.6)
EX–145·W, Nos. 2	W70, No. 2	257	142 (55.3)	135 (52.5)
	GD, No. 2	519	172 (33.1)	74 (14.3)
EX–145·W, No. 3	W70, No. 2	237	76 (32.1)	62 (26.2)
	GD, No. 3	260	117 (45.0)	91 (35.0)
EX–145·W, No. 4	W70, No. 2	275	133 (48.4)	121 (44.0)
	GD, No. 4	357	177 (49.6)	99 (27.7)
EX–145·W, No. 5	W70, No. 2	177	49 (27.7)	37 (20.9)
	GD, No. 5	125	21 (16.8)	7 (5.6)
EX–145·W, No. 6	W70, No. 2	244	152 (62.3)	130 (53.3)
	GD, No. 6	67	29 (43.3)	17 (25.4)
EX–145·W, No. 7	W70, No. 2	246	95 (38.6)	77 (31.3)
	GD, No. 7	121	52 (43.0)	28 (23.1)
EX–145·W, No. 8	W70, No. 2	160	5 (3.1)	4 (2.5)
	GD, No. 8	193	19 (9.8)	3 (1.6)
EX–145·W, No. 9	W70, No. 2	279	221 (79.2)	199 (71.3)
	GD, No. 9	127	30 (23.6)	19 (15.0)
EX–145·W, No. 10	W70, No. 2	104	64 (61.5)	51 (49.0)
	GD, No. 10	111	27 (24.3)	20 (18.0)
EX–145·W, No. 11	W70, No. 2	125	89 (71.2)	81 (64.8)
	GD, No. 11	112	53 (47.3)	50 (44.6)
EX–145·W, No. 12	W70, No. 2	92	43 (46.7)	33 (35.9)
	GD, No. 12	76	20 (26.3)	15 (19.7)
EX–145·W, Nos. 1~12	W70, No. 2	2490	1129 (45.3)	986 (39.6)
	GD, Nos. 1~12	2589	929 (35.9)	551 (21.3)
EX–200·W, No. 1	W70, No. 2	215	174 (80.9)	149 (69.3)
	GD, No. 1	130	28 (21.5)	14 (10.8)
EX–200·W, No. 2	W70, No. 2	193	141 (73.1)	80 (41.5)
	GD, No. 2	216	35 (16.2)	13 (6.0)
EX–200·W, Nos. 1, 2	W70, No. 2	408	315 (77.2)	229 (56.1)
	GD, Nos. 1, 2	346	63 (18.2)	27 (7.8)

EX–90, 145 or 200·W: A female raised from an egg exposed to 90, 145 or 200 rads of X-rays by

GD: Diploid gynogenesis.

X-irradiated eggs by mating with a field-caught male or by diploid gynogenesis

No. of normal tail-bud embryos (%)	No. of normal hatched tadpoles (%)	No. of 50-day-old tadpoles (%)	No. of metamorphosed frogs (%)	No. of frogs 1 month after metamorph. (%)	No. of frogs 2 months after metamorph. (%)
180 (86.5)	137 (65.9)	127 (61.1)	121 (58.2)	121 (58.2)	118 (56.7)
3 (1.7)	3 (1.7)	3 (1.7)	0	0	0
210 (72.4)	198 (68.3)	189 (65.2)	163 (56.2)	128 (44.1)	88 (30.3)
17 (6.6)	11 (4.3)	10 (3.9)	7 (1.7)	3 (1.2)	3 (1.2)
197 (94.3)	190 (90.9)	182 (87.1)	138 (66.0)	116 (55.5)	98 (46.9)
130 (67.0)	81 (41.8)	68 (35.1)	50 (25.8)	49 (25.2)	49 (25.2)
110 (63.2)	100 (57.5)	93 (53.4)	87 (50.0)	80 (46.0)	73 (42.0)
123 (58.9)	71 (34.0)	44 (21.1)	38 (18.2)	38 (18.2)	38 (18.2)
189 (78.8)	161 (67.1)	151 (62.9)	144 (60.0)	141 (58.8)	139 (57.9)
115 (52.0)	72 (32.6)	54 (24.4)	36 (16.3)	31 (14.0)	31 (14.0)
93 (64.1)	73 (50.3)	70 (48.3)	58 (40.0)	54 (37.2)	50 (34.5)
68 (63.0)	23 (21.3)	21 (19.4)	16 (14.8)	10 (9.3)	10 (9.3)
979 (77.3)	859 (67.9)	812 (64.1)	711 (56.2)	640 (50.6)	566 (44.7)
456 (39.1)	261 (22.4)	200 (17.1)	147 (12.6)	131 (11.2)	131 (11.2)
51 (17.3)	50 (17.0)	49 (16.7)	32 (10.9)	29 (9.9)	27 (9.2)
83 (15.9)	58 (11.1)	40 (7.6)	30 (5.8)	30 (5.8)	30 (5.8)
112 (43.6)	101 (39.3)	77 (30.0)	67 (26.1)	64 (24.9)	53 (20.6)
65 (12.5)	31 (6.0)	24 (4.6)	17 (3.3)	15 (2.9)	3 (0.6)
51 (21.5)	48 (20.3)	45 (19.0)	42 (17.7)	41 (17.3)	33 (13.9)
37 (14.2)	35 (13.5)	31 (11.9)	19 (7.3)	14 (5.4)	7 (2.7)
108 (39.3)	92 (33.5)	83 (30.2)	77 (28.0)	77 (28.0)	75 (27.3)
80 (22.4)	76 (21.3)	52 (14.6)	50 (14.0)	49 (13.7)	44 (12.3)
32 (18.1)	27 (15.3)	20 (11.3)	14 (7.9)	14 (7.9)	8 (4.5)
7 (5.6)	0	0	0	0	0
119 (48.8)	112 (45.9)	104 (42.6)	98 (40.2)	98 (40.2)	92 (37.7)
14 (20.9)	14 (20.9)	14 (20.9)	12 (17.9)	11 (16.4)	10 (14.9)
71 (28.9)	41 (16.7)	40 (16.3)	31 (12.6)	30 (12.2)	4 (1.6)
22 (18.2)	20 (16.5)	16 (13.2)	2 (1.7)	2 (1.7)	0
2 (1.3)	2 (1.3)	2 (1.3)	0	0	0
0	0	0	0	0	0
197 (70.6)	103 (36.9)	82 (29.4)	74 (26.5)	74 (26.5)	70 (25.1)
17 (13.4)	15 (11.8)	14 (11.0)	12 (9.4)	9 (7.1)	4 (3.1)
46 (44.2)	32 (30.8)	30 (28.8)	22 (21.2)	20 (19.2)	20 (19.2)
17 (15.3)	6 (5.4)	3 (2.7)	0	0	0
77 (61.6)	63 (50.4)	59 (47.2)	53 (42.4)	53 (42.4)	45 (36.0)
17 (15.2)	16 (14.3)	13 (11.6)	11 (9.8)	5 (4.5)	5 (4.5)
30 (32.6)	27 (29.3)	20 (21.7)	20 (21.7)	17 (18.5)	15 (16.3)
10 (13.2)	3 (3.9)	0	0	0	0
896 (36.0)	698 (28.0)	611 (24.5)	530 (21.3)	517 (20.8)	442 (17.8)
369 (14.3)	274 (10.6)	207 (8.0)	153 (5.9)	135 (5.2)	103 (4.0)
149 (69.3)	128 (59.5)	100 (46.5)	69 (32.1)	49 (22.8)	41 (19.1)
7 (5.4)	0	0	0	0	0
71 (36.8)	45 (23.3)	37 (19.1)	28 (14.5)	28 (14.5)	4 (2.0)
6 (2.8)	4 (1.9)	2 (0.9)	0	0	0
220 (53.9)	173 (42.4)	137 (33.6)	97 (23.8)	77 (18.9)	45 (11.0)
13 (3.8)	4 (1.2)	2 (0.6)	0	0	0

fertilization with an untreated spermatozoon.

TABLE 6

Viability of second-generation offspring produced from females raised from

Parents		No. of eggs	No. of normal cleavages (%)	No. of normal neurulae (%)
Female	Male			
W·SN–50, No. 1	W70, No. 3	240	181 (75.4)	98 (40.8)
	GD, No. 1	232	101 (43.5)	6 (2.6)
W·SN–50, No. 2	W70, No. 3	236	199 (84.3)	166 (70.3)
	GD, No. 2	314	274 (87.3)	111 (35.4)
W·SN–50, No. 3	W70, No. 3	205	181 (88.3)	167 (81.5)
	GD, No. 3	419	315 (75.2)	202 (48.2)
W·SN–50, No. 4	W70, No. 3	195	155 (79.5)	146 (74.9)
	GD, No. 4	564	453 (80.3)	244 (43.3)
W·SN–50, No. 5	W70, No. 3	264	212 (80.3)	188 (71.2)
	GD, No. 5	111	45 (40.5)	40 (36.0)
W·SN–50, Nos. 1∼5	W70, No. 3	1140	928 (81.4)	765 (67.1)
	GD, Nos. 1∼5	1640	1188 (72.4)	603 (36.8)
W·SN–90, No. 1	W70, No. 3	184	171 (92.9)	163 (88.6)
	GD, No. 1	253	194 (76.7)	185 (73.1)
W·SN–90, No. 2	W70, No. 3	310	258 (83.2)	248 (80.0)
	GD, No. 2	244	198 (81.1)	166 (68.0)
W·SN–90, No. 3	W70, No. 3	243	231 (95.1)	202 (83.1)
	GD, No. 3	310	236 (76.1)	226 (72.9)
W·SN–90, Nos. 1∼3	W70, No. 3	737	660 (89.6)	613 (83.2)
	GD, Nos. 1∼3	807	628 (77.8)	577 (71.5)
W·SN–130, No. 1	W70, No. 3	407	345 (84.8)	289 (71.0)
	GD, No. 1	348	190 (54.6)	73 (21.0)
W·SN–130, No. 2	W70, No. 3	147	122 (83.0)	112 (76.2)
	GD, No. 2	72	22 (30.6)	12 (16.7)
W·SN–130, No. 3	W70, No. 3	180	141 (78.3)	125 (69.4)
	GD, No. 3	207	119 (57.5)	55 (26.6)
W·SN–130, No. 4	W70, No. 3	206	157 (76.2)	146 (70.9)
	GD, No. 4	284	87 (30.6)	27 (9.5)
W·SN–130, No. 5	W70, No. 3	210	194 (92.4)	170 (81.0)
	GD, No. 5	245	135 (55.1)	67 (27.3)
W·SN–130, No. 6	W70, No. 3	241	217 (90.0)	199 (82.6)
	GD, No. 6	354	309 (87.3)	293 (82.8)
W·SN–130, No. 7	W70, No. 3	175	114 (65.1)	102 (58.3)
	GD, No. 7	216	159 (73.6)	107 (49.5)
W·SN–130, No. 8	W70, No. 3	115	95 (82.6)	88 (76.5)
	GD, No. 8	173	130 (75.1)	102 (59.0)
W·SN–130, No. 9	W70, No. 3	212	177 (83.5)	152 (71.7)
	GD, No. 9	305	156 (51.1)	104 (34.1)
W·SN–130, No. 10	W70, No. 3	295	261 (88.5)	261 (88.5)
	GD, No. 10	299	181 (60.5)	165 (55.2)
W·SN–130, Nos. 1∼10	W70, No. 3	2188	1823 (83.3)	1644 (75.1)
	GD, Nos. 1∼10	2503	1488 (59.4)	1005 (40.2)

W·SN–50, 90 or 130: A female raised from an untreated egg by fertilization with a spermatozoon

GD: Diploid gynogenesis.

neutron-irradiated spermatozoa by mating with a field-caught male or by diploid gynogenesis

No. of normal tail-bud embryos (%)	No. of normal hatched tadpoles (%)	No. of 50-day-old tadpoles (%)	No. of metamorphosed frogs (%)	No. of frogs 1 month after metamorph. (%)	No. of frogs 2 months after metamorph. (%)
94 (39.2)	92 (38.3)	87 (36.3)	69 (28.8)	64 (26.7)	55 (22.9)
5 (2.2)	5 (2.2)	2 (0.9)	1 (0.4)	0	0
146 (61.9)	142 (60.2)	123 (52.1)	120 (50.8)	99 (41.9)	97 (41.1)
104 (33.1)	95 (30.3)	80 (25.5)	8 (2.5)	6 (1.9)	6 (1.9)
164 (80.0)	154 (75.1)	143 (69.8)	136 (66.3)	121 (59.0)	115 (56.1)
147 (35.1)	143 (34.1)	124 (29.6)	77 (18.4)	65 (15.5)	63 (15.0)
141 (72.3)	131 (67.5)	126 (64.6)	124 (63.6)	100 (51.3)	75 (38.5)
223 (39.5)	201 (35.6)	199 (35.3)	178 (31.6)	169 (30.0)	152 (27.0)
165 (62.5)	164 (62.1)	163 (61.7)	157 (59.5)	140 (53.0)	130 (49.2)
34 (30.6)	32 (28.8)	15 (13.5)	10 (9.0)	5 (4.5)	5 (4.5)
710 (62.3)	683 (59.9)	642 (56.3)	606 (53.2)	524 (46.0)	472 (41.4)
513 (31.3)	476 (29.0)	420 (25.6)	274 (16.7)	245 (14.9)	226 (13.8)
163 (88.6)	159 (86.4)	157 (85.3)	140 (76.1)	125 (67.9)	120 (65.2)
165 (65.2)	139 (54.9)	81 (32.0)	72 (28.5)	72 (28.5)	68 (26.9)
204 (65.8)	174 (56.1)	148 (47.7)	140 (45.2)	109 (35.2)	50 (16.1)
149 (61.1)	115 (47.1)	44 (18.0)	33 (13.5)	32 (13.1)	20 (8.2)
202 (83.1)	196 (80.7)	196 (80.7)	192 (79.0)	170 (70.0)	148 (60.9)
202 (65.2)	171 (55.2)	99 (31.9)	90 (29.0)	90 (29.0)	84 (27.1)
569 (77.2)	529 (71.8)	501 (68.0)	472 (64.0)	404 (54.8)	318 (43.1)
516 (63.9)	425 (52.7)	224 (27.8)	195 (24.2)	194 (24.0)	172 (21.3)
278 (68.3)	272 (66.8)	242 (59.5)	212 (52.1)	212 (52.1)	186 (45.7)
67 (19.3)	52 (14.9)	52 (14.9)	38 (10.9)	30 (8.6)	15 (4.3)
107 (72.8)	100 (68.0)	60 (40.8)	56 (38.1)	56 (38.1)	37 (25.2)
9 (12.5)	9 (12.5)	6 (8.3)	6 (8.3)	5 (6.9)	4 (5.6)
123 (68.3)	112 (62.2)	105 (58.3)	90 (50.0)	79 (43.9)	30 (16.7)
32 (15.5)	23 (11.1)	21 (10.1)	15 (7.2)	15 (7.2)	12 (5.8)
136 (66.0)	117 (56.8)	114 (55.3)	95 (46.1)	82 (39.8)	79 (38.3)
20 (7.0)	19 (6.7)	6 (2.1)	5 (1.8)	5 (1.8)	3 (1.1)
165 (78.6)	140 (66.7)	133 (63.3)	79 (37.6)	49 (23.3)	44 (21.0)
62 (25.3)	49 (20.0)	26 (10.6)	21 (8.6)	20 (8.2)	17 (6.9)
194 (80.5)	187 (77.6)	185 (76.8)	171 (71.0)	161 (66.8)	108 (44.8)
285 (80.5)	116 (32.8)	85 (24.0)	61 (17.2)	52 (14.7)	52 (14.7)
95 (54.3)	82 (46.9)	75 (42.9)	70 (40.0)	69 (39.4)	69 (39.4)
77 (35.6)	25 (11.6)	23 (10.6)	20 (9.3)	15 (6.9)	14 (6.5)
77 (67.0)	62 (53.9)	58 (50.4)	49 (42.6)	48 (41.7)	42 (36.5)
91 (52.6)	23 (13.3)	21 (12.1)	17 (9.8)	17 (9.8)	17 (9.8)
140 (66.0)	137 (64.6)	131 (61.8)	125 (59.0)	124 (58.5)	124 (58.5)
85 (27.9)	45 (14.8)	45 (14.8)	42 (13.8)	40 (13.1)	36 (11.8)
259 (87.8)	254 (86.1)	234 (79.3)	209 (70.8)	181 (61.4)	137 (46.4)
123 (41.1)	87 (29.1)	80 (26.8)	41 (13.7)	40 (13.4)	39 (13.0)
1574 (71.9)	1463 (66.9)	1337 (61.1)	1156 (52.8)	1061 (48.5)	856 (39.1)
851 (34.0)	448 (17.9)	365 (14.6)	266 (10.6)	239 (9.5)	209 (8.3)

exposed to 50, 90 or 130 rads of neutrons.

4. Experimental series using neutron-irradiated spermatozoa

a. (W♀ × SN–50♂)♀GD, Nos. 1~5

Seven mature females that had been derived from spermatozoa exposed to 50 rads of neutrons were injected with frog pituitary suspension. As normal ovulation occurred in five of them, a part of the eggs taken out of each female was induced to develop by diploid gynogenesis, while the other part was inseminated with spermatozoa of a normal male (W70, No. 3) collected from the field (Table 6). In 5 gynogenetic experiments (Nos. 1~5), 40.5~87.3%, 72.4% on the average, of the respective total number of eggs cleaved normally, while 75.4~88.3%, 81.4% on the average, did so in 5 matings (Nos. 1~5). Many of the normally cleaved eggs died of various abnormalities during the embryonic stage; 2.2~35.6%, 29.0% on the average, hatched normally in the gynogenetic experiments, while 38.3~75.1%, 59.9% on the average, did so in the matings. During the tadpole stage, many individuals died of edema or underdevelopment; 0.4~31.6%, 16.7% on the average, metamorphosed normally in the gynogenetic experiments, while 28.8~66.3%, 53.2% on the average, did so in the matings.

Color variants appeared among the tadpoles produced from one of the five experiments of diploid gynogenesis (Table 8). Of 32 newly hatched tadpoles produced from experiment No. 5, six were albinos, while the others were of normal color. No color variants were produced from the other gynogenetic experiments as well as from all the matings.

b. (W♀ × SN–90♂)♀GD, Nos. 1~3

Five mature females that had been derived from spermatozoa exposed to 90 rads of neutrons were injected with frog pituitary suspension. As three of them ovulated normally, a part of the eggs taken out of each female was induced to develop by diploid gynogenesis, while the other part was inseminated with spermatozoa of male W70, No. 3 (Table 6). In 3 gynogenetic experiments (Nos. 1~3), 76.1~81.1%, 77.8% on the average, of the respective total number of eggs cleaved normally, while 83.2~95.1%, 89.6% on the average, did so in 3 matings. Many of the normally cleaved eggs died of various abnormalities during the embryonic stage; 47.1~55.2%, 52.7% on the average, hatched normally in the gynogenetic experiments, while 56.1~86.4%, 71.8% on the average, did so in the matings. Many individuals died afterwards of edema or underdevelopment during the tadpole stage; 13.5~29.0%, 24.2% on the average, metamorphosed normally in the gynogenetic experiments, while 45.2~79.0%, 64.0% on the average, did so in the matings.

No color variants were detected among the tadpoles or frogs produced by diploid gynogenesis as well as from matings with the normal male. All these tadpoles and frogs were of normal color.

c. (W♀ × SN–130♂)♀GD, Nos. 1~10

Thirteen mature females that had been derived from spermatozoa exposed to

130 rads of neutrons were injected with frog pituitary suspension. As normal ovulation occurred in ten of them, a part of the eggs taken out of each female was induced to develop by the method of diploid gynogenesis, while the other part was inseminated with spermatozoa of male W70, No. 3 (Table 6). In 10 gynogenetic experiments (Nos. 1 ~ 10), 30.6 ~ 87.3%, 59.4% on the average, of the respective total number of eggs cleaved normally, while 65.1 ~ 92.4%, 83.3% on the average, did so in 10 matings. Many of the normally cleaved eggs died of various abnormalities during the embryonic stage; 6.7 ~ 32.8%, 17.9% on the average, hatched normally in the gynogenetic experiments, while 46.9 ~ 86.1%, 66.9% on the average, did so in the matings. During the tadpole stage, many individuals also died of edema or underdevelopment; 1.8 ~ 17.2%, 10.6% on the average, metamorphosed normally in the gynogenetic experiments, while 37.6 ~ 71.0%, 52.8% on the average, did so in the matings.

Color variants appeared in six of the 10 gynogenetic experiments (Table 8). In experiment No. 1, 15 of 30 frogs examined one month after metamorphosis were olive mutants, which were olive in dorsal ground color. Their bodies were somewhat semi-transparent. In experiment No. 3, four of 23 tadpoles were albinos at the hatching stage. Moreover, six of the other 19 tadpoles were olive variants when examined at the age of 50 days. In experiment No. 4, two of 5 frogs were blue mutants when examined one month after metamorphosis, while the other three were normally green in dorsal ground color. In experiment No. 5, five of 49 newly hatched tadpoles were albinos. Of 17 frogs examined one month after metamorphosis in experiment No. 8, two were blue variants and four were olive variants whose body walls were semi-transparent, while the other eleven were normal in color, that is, four were green and seven brown. In experiment No. 9, 22 of 45 tadpoles examined at the age of 50 days were olive variants, while the other 23 were normally brown. All the tadpoles or frogs produced by normal fertilization were normal in color.

5. Experimental series using neutron-irradiated eggs

a. (EN–50♀ × W♂) ♀GD, Nos. 1 ~ 7

Eight mature females that had been derived from eggs exposed to 50 rads of neutrons were injected with frog pituitary suspension. As seven of them ovulated normally, a part of the eggs taken out of each female was induced to develop by the method of diploid gynogenesis, while the other part was inseminated with spermatozoa of a normal male (W70, No. 4) collected from the field (Table 7). In 7 gynogenetic experiments (Nos. 1 ~ 7), 30.9 ~ 83.5%, 58.2% on the average, of the respective total number of eggs cleaved normally, while 84.2 ~ 98.3%, 93.0% on the average, did so in 7 matings. Many of the normally cleaved eggs died of various abnormalities during the embryonic stage; 0.4 ~ 27.6%, 17.6% on the average, hatched normally in the gynogenetic experiments, while 67.1 ~ 86.8%, 80.1% on the average, did so in the matings. Many individuals died afterwards of edema or underdevelopment during the tadpole stage; 0.4 ~ 20.4%, 8.7% on the average, metamorphosed normally in the gynogenetic experiments,

TABLE 7

Viability of second-generation offspring produced from females raised from neutron-

Parents: Female	Parents: Male	No. of eggs	No. of normal cleavages (%)	No. of normal neurulae (%)
EN–50·W, No. 1	W70, No. 4	258	249 (96.5)	225 (87.2)
	GD, No. 1	314	97 (30.9)	63 (20.1)
EN–50·W, No. 2	W70, No. 4	301	289 (96.0)	276 (91.7)
	GD, No. 2	294	132 (44.9)	85 (28.9)
EN–50·W, No. 3	W70, No. 4	234	230 (98.3)	211 (90.2)
	GD, No. 3	256	139 (54.3)	70 (27.3)
EN–50·W, No. 4	W70, No. 4	279	237 (84.9)	224 (80.3)
	GD, No. 4	299	190 (63.5)	70 (23.4)
EN–50·W, No. 5	W70, No. 4	378	371 (98.1)	349 (92.3)
	GD, No. 5	269	134 (49.8)	26 (9.7)
EN–50·W, No. 6	W70, No. 4	146	123 (84.2)	119 (81.5)
	GD, No. 6	243	203 (83.5)	183 (75.3)
EN–50·W, No. 7	W70, No. 4	172	145 (84.3)	135 (78.5)
	GD, No. 7	342	279 (81.6)	258 (75.4)
EN–50, Nos. 1∼7	W70, No. 4	1768	1664 (93.0)	1539 (87.0)
	GD, Nos. 1∼7	2017	1174 (58.2)	755 (37.4)
EN–90·W, No. 1	W70, No. 4	282	136 (48.2)	131 (46.5)
	GD, No. 1	254	145 (57.1)	130 (51.2)
EN–90·W, No. 2	W70, No. 4	239	161 (67.4)	159 (66.5)
	GD, No. 2	231	88 (38.1)	11 (4.8)
EN–90·W, No. 3	W70, No. 4	216	159 (73.6)	151 (69.9)
	GD, No. 3	287	62 (21.6)	23 (8.0)
EN–90·W, No. 4	W70, No. 4	143	98 (68.5)	89 (62.2)
	GD, No. 4	391	145 (37.1)	128 (32.7)
EN–90, Nos. 1∼4	W70, No. 4	880	554 (63.0)	530 (60.2)
	GD, Nos. 1∼4	1163	440 (37.8)	292 (25.1)
EN–130·W, No. 1	W70, No. 4	164	145 (88.4)	140 (85.4)
	GD, No. 1	215	46 (21.4)	41 (19.1)
EN–130·W, No. 2	W70, No. 4	243	169 (69.5)	161 (66.3)
	GD, No. 2	260	75 (28.8)	67 (25.8)
EN–130·W, No. 3	W70, No. 4	126	122 (96.8)	95 (75.4)
	GD, No. 3	293	232 (79.2)	135 (46.1)
EN–130·W, No. 4	W70, No. 4	125	87 (69.6)	86 (68.8)
	GD, No. 4	412	205 (49.8)	163 (39.6)
EN–130·W, No. 5	W70, No. 4	103	64 (62.1)	63 (61.2)
	GD, No. 5	375	139 (37.1)	113 (30.1)
EN–130·W, No. 6	W70, No. 4	104	78 (75.0)	76 (73.1)
	GD, No. 6	434	211 (48.6)	198 (45.6)
EN–130·W, Nos. 1∼6	W70, No. 4	865	665 (76.9)	621 (71.8)
	GD, Nos. 1∼6	1989	908 (45.7)	717 (36.0)

EN–50, 90 or 130·W: A female raised from an egg exposed to 50, 90 or 130 rads of neutrons by
GD: Diploid gynogenesis.

while 55.5∼81.2%, 69.8% on the average, did so in the matings.

Color variants appeared from four of the seven experiments of diploid gynogenesis (Table 8). In experiment No. 1, seven of 17 frogs examined one month irradiated eggs by mating with a field-caught male or by diploid gynogenesis

No. of normal tail-bud embryos (%)	No. of normal hatched tadpoles (%)	No. of 50-day-old tadpoles (%)	No. of metamorphosed frogs (%)	No. of frogs 1 month after metamorph. (%)	No. of frogs 2 months after metamorph. (%)
225 (87.2)	204 (79.1)	203 (78.7)	173 (67.1)	168 (65.1)	94 (36.4)
59 (18.8)	55 (17.5)	28 (8.9)	21 (6.7)	17 (5.4)	13 (4.1)
273 (90.7)	253 (84.1)	247 (82.1)	216 (71.8)	166 (55.1)	155 (51.5)
80 (27.2)	68 (23.1)	63 (21.4)	60 (20.4)	57 (19.4)	50 (17.0)
208 (88.9)	193 (82.5)	187 (79.9)	162 (69.2)	143 (61.1)	100 (42.7)
58 (22.7)	51 (19.9)	25 (9.8)	17 (6.6)	15 (5.9)	11 (4.3)
223 (79.9)	212 (76.0)	197 (70.6)	190 (68.1)	165 (59.1)	149 (53.4)
58 (19.4)	49 (16.4)	48 (16.1)	43 (14.4)	43 (14.4)	41 (13.7)
335 (88.6)	328 (86.8)	323 (85.4)	307 (81.2)	196 (51.9)	146 (38.6)
26 (9.7)	1 (0.4)	1 (0.4)	1 (0.4)	0	0
100 (68.5)	98 (67.1)	91 (62.3)	81 (55.5)	63 (43.2)	46 (31.5)
163 (67.1)	67 (27.6)	31 (12.8)	19 (7.8)	19 (7.8)	19 (7.8)
131 (76.2)	128 (74.4)	118 (68.6)	105 (61.0)	88 (51.2)	81 (47.1)
255 (74.6)	65 (19.0)	32 (9.4)	14 (4.1)	7 (2.0)	7 (2.0)
1495 (84.6)	1416 (80.1)	1366 (77.3)	1234 (69.8)	989 (55.9)	771 (43.6)
699 (34.7)	356 (17.6)	228 (11.3)	175 (8.7)	158 (7.8)	141 (7.0)
124 (44.0)	119 (42.2)	111 (39.4)	98 (34.8)	90 (31.9)	89 (31.6)
107 (42.1)	91 (35.8)	53 (20.9)	30 (11.8)	25 (9.8)	23 (9.1)
152 (63.6)	147 (61.5)	141 (59.0)	120 (50.2)	86 (36.0)	55 (23.0)
10 (4.3)	10 (4.3)	10 (4.3)	6 (2.6)	6 (2.6)	6 (2.6)
144 (66.7)	138 (63.9)	125 (57.9)	115 (53.2)	110 (50.9)	95 (44.0)
22 (7.7)	20 (7.0)	15 (5.2)	10 (3.5)	10 (3.5)	10 (3.5)
86 (60.1)	84 (58.7)	80 (55.9)	76 (53.1)	75 (52.4)	46 (32.2)
123 (31.5)	87 (22.3)	80 (20.5)	21 (5.4)	21 (5.4)	20 (5.1)
506 (57.5)	488 (55.5)	457 (51.9)	409 (46.5)	361 (41.0)	285 (32.4)
262 (22.5)	208 (17.9)	158 (13.6)	67 (5.8)	62 (5.3)	59 (5.1)
137 (83.5)	92 (56.1)	84 (51.2)	64 (39.0)	59 (36.0)	49 (29.9)
33 (15.3)	28 (13.0)	27 (12.6)	27 (12.6)	20 (9.3)	15 (7.0)
100 (41.2)	88 (36.2)	88 (36.2)	72 (29.6)	70 (28.8)	63 (25.9)
28 (10.8)	25 (9.6)	25 (9.6)	2 (0.8)	2 (0.8)	0
71 (56.3)	51 (40.5)	48 (38.1)	33 (26.2)	32 (25.4)	32 (25.4)
126 (43.0)	90 (30.7)	73 (24.9)	50 (17.1)	14 (4.8)	9 (3.1)
85 (68.0)	83 (66.4)	72 (57.6)	72 (57.6)	71 (56.8)	67 (53.6)
152 (36.9)	128 (31.1)	102 (24.8)	53 (12.9)	53 (12.9)	53 (12.9)
62 (60.2)	60 (58.3)	59 (57.3)	45 (43.7)	44 (42.7)	41 (39.8)
106 (28.3)	73 (19.5)	59 (15.7)	7 (1.9)	7 (1.9)	7 (1.9)
76 (73.1)	73 (70.2)	65 (62.5)	45 (43.3)	42 (40.4)	38 (36.5)
172 (39.6)	51 (11.8)	26 (6.0)	11 (2.5)	11 (2.5)	11 (2.5)
531 (61.4)	447 (51.7)	416 (48.1)	331 (38.3)	318 (36.8)	290 (33.5)
617 (31.0)	395 (19.9)	312 (15.7)	150 (7.5)	107 (5.4)	95 (4.8)

fertilization with an untreated spermatozoon.

after metamorphosis were olive variants, while the others were normally brown or green in dorsal ground color. In experiment No. 2, five of 57 frogs examined immediately after metamorphosis were blue variants, while all the others were normally green. All the 41 frogs developed from the eggs of the same female by normal fertilization were also green. In experiment No. 4, 22 of 43 frogs examined immediately after metamorphosis were olive variants, while the others were normally brown. The frogs developed from the eggs of the same female by normal fertilization were also all normally brown. In experiment No. 6, nine of 19 frogs were blue variants when examined one month after metamorphosis, while the other ten were normally green.

b. (EN–90♀ ×W♂)♀GD, Nos. 1~4

Seven mature females that had been derived from eggs exposed to 90 rads of neutrons were injected with frog pituitary suspension. As normal ovulation occurred in four of them, a part of the eggs of each female was induced to develop by the method of diploid gynogenesis, while the other part was inseminated with spermatozoa of male W70, No. 4 (Table 7). In 4 gynogenetic experiments (Nos. 1~4), 21.6~57.1%, 37.8% on the average, of the respective total number of eggs cleaved normally, while 48.2~73.6%, 63.0% on the average, did so in 4 matings. Many of the normally cleaved eggs died of various abnormalities during the embryonic stage; 4.3~35.8%, 17.9% on the average, hatched normally in the gynogenetic experiments, while 42.2~63.9%, 55.5% on the average, did so in the matings. During the tadpole stage, many tadpoles died of edema or underdevelopment; 2.6~11.8%, 5.8% on the average, metamorphosed normally in the gynogenetic experiments, while 34.8~53.2%, 46.5% on the average, did so in the matings.

Color mutants appeared from one (No. 3) of the four gynogenetic experiments (Table 8). Five of 15 tadpoles were gray-eyed mutants at the age of 50 days, while the other ten were normal. All the 5 gray-eyed mutants died nearly at the stage of small hind-limb buds. No color mutants were detected among the tadpoles or frogs produced from the other gynogenetic experiments as well as from the matings with the normal male.

c. (EN–130♀ ×W♂)♀GD, Nos. 1~6

Ten mature females that had been derived from eggs exposed to 130 rads of neutrons were injected with frog pituitary suspension. As normal ovulation occurred in six of them, a part of the eggs taken out of each female was induced to develop by diploid gynogenesis, while the other part was inseminated with spermatozoa of male W70, No. 4 (Table 7). In 6 gynogenetic experiments (Nos. 1~6), 21.4~79.2%, 45.7% on the average, of the respective total number of eggs cleaved normally, while 62.1~96.8%, 76.9% on the average, did so in 6 matings (Nos. 1~6). Many of the normally cleaved eggs died of various abnormalities during the embryonic stage; 9.6~31.1%, 19.9% on the average, hatched normally in the gynogenetic experiments, while 36.2~70.2%, 51.7% on the average, did so in the matings. During the tadpole stage, many individuals also died of edema or underdevelopment; 0.8~17.1%, 7.5% on the average, metamorphosed normally, while 26.2~57.6%, 38.3% on the average, did

TABLE 8

Number and kind of color variants found in the offspring of females raised from irradiated gametes

Females			Stage of offspring analysed	No. of gynogenetic diploids			Kind of color variants
Individual no.	Phenotype	Genotype		Total	Wild	Variants	
W·SX–90, No. 1	Green	*XxEe*	3 months after metamorphosis	42	39	3	Blue
W·SX–90, No. 2	Brown	*Gg*	50-day-old tadpole	86	50	36	Gray-eyed
W·SX–90, No. 5	Brown	*Bb*	50-day-old tadpole	52	27	25	Black-eyed
W·SX–170, No. 2	Green	*XxEe*	3 months after metamorphosis	12	10	2	Blue
W·SX–170, No. 3	Green	*XxEE*	3 months after metamorphosis	11	8	3	Blue
W·SX–170, No. 9	Brown	*XxIiee*	50-day-old tadpole	29	15	14	Olive group
EX–90·W, No. 5	Brown	*Gg*	50-day-old tadpole	54	43	11	Gray-eyed
EX–90·W, No. 6	Brown	*Bb*	50-day-old tadpole	21	12	9	Black-eyed
EX–145·W, No. 1	Brown	*XXIiee*	1 month after metamorphosis	30	15	15	Olive group
EX–145·W, No. 4	Brown	*XXIiee*	1 month after metamorphosis	49	25	24	Olive group
EX–145·W, No. 11	Brown	*Mm*	Hatching tadpole	16	15	1	Albino
W·SN–50, No. 5	Brown	*Mm*	Hatching tadpole	32	26	6	Albino
W·SN–130, No. 1	Brown	*XXIiee*	1 month after metamorphosis	30	15	15	Olive group
W·SN–130, No. 3	Brown	*XXIieeMm*	Hatching tadpole	23	13	4	Albino
			50-day-old tadpole			6	Olive group
W·SN–130, No. 4	Green	*XxEE*	1 month after metamorphosis	5	3	2	Blue
W·SN–130, No. 5	Green	*Mm*	Hatching tadpole	49	44	5	Albino
W·SN–130, No. 8	Green	*XxIiEe*	1 month after metamorphosis	17	11	2	Blue
						4	Olive group
W·SN–130, No. 9	Brown	*XxIiee*	50-day-old tadpole	45	23	22	Olive group
EN–50·W, No. 1	Green	*XXIiEe*	1 month after metamorphosis	17	10	7	Olive group
EN–50·W, No. 2	Green	*XxEE*	Immediately after metamorphosis	57	52	5	Blue
EN–50·W, No. 4	Brown	*XXIiee*	Immediately after metamorphosis	43	21	22	Olive group
EN–50·W, No. 6	Green	*XxEE*	1 month after metamorphosis	19	10	9	Blue
EN–90·W, No. 3	Brown	*Gg*	50-day-old tadpole	15	10	5	Gray-eyed
EN–130·W, No. 1	Brown	*Bb*	50-day-old tadpole	27	14	13	Black-eyed

so in the matings.

Color mutants appeared from only one of the six gynogenetic experiments (Table 8). Of 27, fifty-day-old tadpoles produced from experiment No. 1, 13 were black-eyed mutants, while the other 14 were normally brown. The body walls of the mutants were semi-transparent and had a large quantity of melanine. Their eyes looked black. No color mutants were detected among the tadpoles or frogs produced by the other gynogenetic experiments (Nos. 2～6). All the individuals produced from matings with the normal male were normal in color.

6. Summary of the four experimental series

In summary, a total of 75 females derived from X- or neutron-irradiated spermatozoa or eggs were examined to detect color-mutation genes by applying the method of diploid gynogenesis to their eggs. While the offspring of 11 females died at the embryonic stage probably by doubling of a recessive lethal gene, the other 64 females produced tadpoles and frogs which were analyzable. Color variants were detected among the offspring of 24 females (Table 8). All the offspring of the other 40 females were quite normal in color. Of the former females, one that had been derived from a spermatozoon exposed to 130 rads of neutrons produced two kinds of color variants, albino and olive, and another also produced two kinds, blue and olive variants. Accordingly, a total of 26 stocks of color variants including 4 albino (*mm*), 3 gray-eyed (*gg*), 3 black-eyed (*bb*), 9 blue (*xx*) and 9 olive (*ii*) were induced by irradiation of 64 gametes (Table 9). By the experiments of diploid gynogenesis on the eggs of 15 females in the control series, it was ascertained that the three father and three mother frogs collected from the field in 1967 had no color-mutation genes.

TABLE 9

Numbers of recessive color mutations detected by the diploid-gynogenetic method

Kind of females	Number of females			Number of color mutations					
	Total	Wild-type	Color variants	Total	*mm*	*gg*	*bb*	*xx*	*ii*
Cont. W·W	15	15	0	0	0	0	0	0	0
W·SX–90	9	4	3	3	0	1	1	1	0
W·SX–170	9	5	3	4	0	0	0	3	1
W·SX–240	2	2	0	0	0	0	0	0	0
EX–90·W	6	3	2	2	0	1	1	0	0
EX–145·W	12	5	3	3	1	0	0	0	2
EX–200·W	2	—	—	—	—	—	—	—	—
W·SN–50	5	3	1	1	1	0	0	0	0
W·SN–90	3	3	0	0	0	0	0	0	0
W·SN–130	10	4	6	9	2	0	0	3	4
EN–50·W	7	3	4	4	0	0	0	2	2
EN–90·W	4	3	1	1	0	1	0	0	0
EN–130·W	6	5	1	1	0	0	1	0	0
Total	75	40	24	28	4	3	3	9	9

mm, *gg*, *bb*, *xx* and *ii*: cf. texts in p. 49, p. 54, p. 58, p. 61 and p. 68, respectively.

II. Inheritance of color variants

1. Albino

Rana nigromaculata have three kinds of dermal chromatophores, xanthophores, iridophores and melanophores. In albinos, the melanophores are abnormal, while the other two kinds of chromatophores are quite normal. Their melanophores contain colorless premelanosomes in place of melanosomes; the synthesis of melanin stops halfway. Accordingly, albino tadpoles or frogs of this species are yellow or yellowish-white in dorsal ground color and have red or pink eyes. Albinos are distinguishable from normal individuals at stage 19 or 20 (SHUMWAY, 1940), where black pigment appears around the eyes of normal individuals; no black pigment is produced in albinos. Accordingly, all the albinos could be accurately identified at stage 20, that is, at the hatching stage in the following experiments.

Albinos were detected among gynogenetic diploids produced from each of 4 females, No. 11 from EX–145♀×W♂, No. 5 from W♀×SN–50♂, and Nos. 3 and 5 from W♀×SN–130♂ (Table 8). This indicates that these females were heterozygous (*Mm*) for the albino gene (*m*). Although about half the eggs deposited by each female should have *mm*, albinos were always remarkably few. Such scarcity of albinos was clearly attributable to their weakness that had probably been increased by refrigeration of the eggs at 0.2∼0.7°C for about 3 hours. In addition their tolerance to intense light and low temperature was low.

Half the number of the frogs produced from females which are presumed to be heterozygous for the albino gene by mating with normal males collected from the field should be heterozygous for this mutation gene. When brother and sister matings are made at random among these second-generation offspring derived from irradiated gametes, about one-fourth of the third-generation offspring should become albinos in accordance with MENDEL's law.

a. Experiments using offspring of female No. 11 from EX–145♀×W♂

A single albino was produced in 1970 from female No. 11 obtained from EX–145♀×W♂, by the method of diploid gynogenesis (Table 8). Accordingly, this female was presumed to be heterozygous for the albino gene. The offspring of this female and a normal male (W70, No. 2) collected from the field were all of the wild type, although half of them should be heterozygous for the albino gene. Twelve matings between 4 female (Nos. 1∼4) and 3 male (Nos. 1∼3) offspring as well as 4 experiments of diploid gynogenesis were made in 1972 (Table 10). The result was that two (Nos. 2 and 3) of the 4 females and one (No. 3) of the 3 males were heterozygous for the albino gene. In short, three of the 7 offspring were heterozygotes.

From the mating between female No. 2 and male No. 3, 120 tadpoles at the

TABLE 10

Production of albinos from female second-generation offspring derived from irradiated gametes by brother and sister mating or by diploid gynogenesis, I

Parents		No. of eggs	No. of normal cleavages	No. of tadpoles (St. 20)		
Female	Male			Total	Wild	Albino
$(EX\text{–}145\cdot W)_{11}W$, No. 1	$(EX\text{–}145\cdot W)_{11}W$, No. 1	107	35 (32.7%)	20 (18.7%)	20	0
	$(EX\text{–}145\cdot W)_{11}W$, No. 2	89	21 (23.6%)	13 (14.6%)	13	0
	$(EX\text{–}145\cdot W)_{11}W$, No. 3	93	26 (28.0%)	15 (16.1%)	15	0
$(EX\text{–}145\cdot W)_{11}W$, No. 2	$(EX\text{–}145\cdot W)_{11}W$, No. 1	125	108 (86.4%)	93 (74.4%)	93	0
	$(EX\text{–}145\cdot W)_{11}W$, No. 2	146	132 (90.4%)	126 (86.3%)	126	0
	$(EX\text{–}145\cdot W)_{11}W$, No. 3	135	132 (97.8%)	120 (88.9%)	90	30
	GD, No. 2	230	65 (28.3%)	19 (8.3%)	14	5
$(EX\text{–}145\cdot W)_{11}W$, No. 3	$(EX\text{–}145\cdot W)_{11}W$, No. 1	102	66 (64.7%)	60 (58.8%)	60	0
	$(EX\text{–}145\cdot W)_{11}W$, No. 2	81	51 (63.0%)	46 (56.8%)	46	0
	$(EX\text{–}145\cdot W)_{11}W$, No. 3	193	63 (32.6%)	47 (24.4%)	36	11
	GD, No. 3	269	73 (27.1%)	34 (12.6%)	17	17
$(EX\text{–}145\cdot W)_{11}W$, No. 4	$(EX\text{–}145\cdot W)_{11}W$, No. 1	72	54 (75.0%)	31 (43.1%)	31	0
	$(EX\text{–}145\cdot W)_{11}W$, No. 2	72	29 (40.3%)	20 (27.8%)	20	0
	$(EX\text{–}145\cdot W)_{11}W$, No. 3	52	11 (21.2%)	10 (19.2%)	10	0

$(EX\text{–}145\cdot W)_{11}W$: A female or male produced from mating between female EX–145·W, No. 11 and a field-caught male.

GD: Diploid gynogenesis.

hatching stage were produced; 90 were of the wild type and 30 albinos. From the mating between female No. 3 and male No. 3, 47 tadpoles at the hatching stage were produced; 36 were of the wild type, and eleven albinos. These figures show that about one-fourth of the whole tadpoles were albinos. After the hatching stage, about half the albino tadpoles died before metamorphosis. Of 41 albinos in total, 21 metamorphosed normally at the age of 65~87 days, 73.6 days on the average, while 92 of 126 wild-type tadpoles in total did so at the age of 63~77 days, 69.7 days on the average.

Two females Nos. 2 and 3 produced 53 tadpoles by the method of diploid gynogenesis; 31 were of the wild type and 22 albinos. The smaller number of albinos is probably attributable to their weakness increased by refrigeration of the eggs at 0.2~0.7°C. Of these 22 albino tadpoles, eleven metamorphosed normally at the age of 66~75 days, 70.6 days on the average, while 29 of 31 wild-type tadpoles did so at the age of 66~77 days, 70.2 days on the average.

b. Experiments using offspring of female No. 5 from W♀ × SN–50♂

Six albinos were produced from female No. 5 obtained from W♀ × SN–50♂ by diploid gynogenesis in 1970 (Table 8). Of these albinos, only one metamorphosed normally and afterwards grew into a mature frog. On the other hand, this female was mated with a normal male (W70, No. 3) to produce her offspring. As the latter matured in 1972, 9 matings between 3 female (Nos. 1~3) and 3 male (Nos. 1~3) offspring as well as 3 experiments of diploid gynogenesis were made to obtain albinos (Table 11). The result was that two (Nos. 1 and 2) of the 3 females and one (No. 1) of the 3 males were heterozygous for the albino gene.

From the mating between female No. 1 and male No. 1, 46 tadpoles of the

TABLE 11
Production of albinos from female second-generation offspring derived from irradiated gametes by brother and sister mating or by diploid gynogenesis, II

Parents		No. of eggs	No. of normal cleavages	No. of tadpoles (St. 20)		
Female	Male			Total	Wild	Albino
$(W·SN-50)_5W$, No. 1	$(W·SN-50)_5W$, No. 1	114	75 (65.8%)	66 (57.9%)	46	20
	$(W·SN-50)_5W$, No. 2	146	110 (75.3%)	102 (69.9%)	102	0
	$(W·SN-50)_5W$, No. 3	98	90 (91.8%)	85 (86.7%)	85	0
	GD, No. 1	233	94 (40.3%)	61 (26.2%)	46	15
$(W·SN-50)_5W$, No. 2	$(W·SN-50)_5W$, No. 1	63	27 (42.9%)	24 (38.1%)	19	5
	$(W·SN-50)_5W$, No. 2	78	36 (46.2%)	34 (43.6%)	34	0
	$(W·SN-50)_5W$, No. 3	67	53 (79.1%)	48 (71.6%)	48	0
	GD, No. 2	301	135 (44.9%)	119 (39.5%)	89	30
$(W·SN-50)_5W$, No. 3	$(W·SN-50)_5W$, No. 1	103	63 (61.2%)	36 (35.0%)	36	0
	$(W·SN-50)_5W$, No. 2	97	52 (53.6%)	31 (32.0%)	31	0
	$(W·SN-50)_5W$, No. 3	115	54 (47.0%)	30 (26.1%)	30	0

$(W·SN-50)_5W$: A female or male produced from mating between female W·SN–50, No. 5 and a field-caught male.
GD: Diploid gynogenesis.

wild type and 20 albino tadpoles were produced, while 19 tadpoles of the wild type and 5 albinos were from the mating between female No. 2 and male No. 1. Of 90 tadpoles in total, 65 were of the wild type and 25 albinos. Twelve of the latter afterwards metamorphosed normally at the ages of 67∼86 days, 78.9 days on the average, while 56 of the wild-type tadpoles did so at the ages of 65∼77 days, 73.7 days on the average. All the other albino and wild-type tadpoles died before the metamorphosing stage.

Two females Nos. 1 and 2 produced 180 tadpoles at the hatching stage by the method of diploid gynogenesis; 135 were of the wild type and 45 albinos. The smaller number of albinos is probably attributable to their weakness increased by refrigeration of the eggs. After the hatching stage, 37 of the wild-type tadpoles died, while the other 98 metamorphosed normally at the age of 61∼77 days, 72.5 days on the average. Of the 45 albinos at the hatching stage, 24 metamorphosed normally at the age of 67∼87 days, 79.1 days on the average, while the other 21 died before long after hatching.

c. Experiments using offspring of female No. 3 from W♀ × SN–130♂

Four albinos were produced from female No. 3 obtained from W♀ × SN–130♂ by the method of diploid gynogenesis in 1970 (Table 8). These albinos were detected at the hatching stage, although they all died before long. However, 9 wild-type tadpoles produced by diploid gynogenesis all metamorphosed normally. Six of them were olive variants. Accordingly, it was presumed that the female was heterozygous for both albino and olive mutation genes. Of the offspring produced from a mating between this female and a normal male (W70, No. 3), half the number should be heterozygous for the albino gene, as well as for the olive mutation gene. As many of these offspring matured in 1972, 24 brother and sister matings between 8 females (Nos. 1∼8) and 6 males (Nos. 1∼6) as well as 8 experiments of diploid gynogenesis were made to obtain albinos

TABLE 12

Production of albinos from female second-generation offspring derived from irradiated gametes by brother and sister mating or by diploid gynogenesis, III

Parents		No. of eggs	No. of normal cleavages	No. of tadpoles (St. 20)		
Female	Male			Total	Wild	Albino
$(W\cdot SN–130)_3W$, No. 1	$(W\cdot SN–130)_3W$, No. 1	96	36 (37.5%)	26 (27.1%)	26	0
	$(W\cdot SN–130)_3W$, No. 2	82	36 (43.9%)	25 (30.5%)	25	0
	$(W\cdot SN–130)_3W$, No. 3	113	53 (46.9%)	44 (38.9%)	44	0
$(W\cdot SN–130)_3W$, No. 2	$(W\cdot SN–130)_3W$, No. 1	104	65 (62.5%)	51 (49.0%)	51	0
	$(W\cdot SN–130)_3W$, No. 2	84	43 (51.2%)	26 (31.0%)	26	0
	$(W\cdot SN–130)_3W$, No. 3	118	72 (61.0%)	47 (39.8%)	47	0
$(W\cdot SN–130)_3W$, No. 3	$(W\cdot SN–130)_3W$, No. 1	125	75 (60.0%)	75 (60.0%)	75	0
	$(W\cdot SN–130)_3W$, No. 2	115	63 (54.8%)	63 (54.8%)	63	0
	$(W\cdot SN–130)_3W$, No. 3	113	62 (54.9%)	62 (54.9%)	62	0
$(W\cdot SN–130)_3W$, No. 4	$(W\cdot SN–130)_3W$, No. 1	65	49 (75.4%)	35 (53.8%)	24	11
	$(W\cdot SN–130)_3W$, No. 2	102	65 (63.7%)	43 (42.2%)	32	11
	$(W\cdot SN–130)_3W$, No. 3	93	40 (43.0%)	23 (24.7%)	23	0
	GD, No. 4	259	62 (23.9%)	28 (10.8%)	22	6
$(W\cdot SN–130)_3W$, No. 5	$(W\cdot SN–130)_3W$, No. 1	81	29 (35.8%)	27 (33.3%)	18	9
	$(W\cdot SN–130)_3W$, No. 2	89	31 (34.8%)	29 (32.6%)	24	5
	$(W\cdot SN–130)_3W$, No. 3	82	27 (32.9%)	25 (30.5%)	25	0
	GD, No. 5	356	172 (48.3%)	90 (25.3%)	61	29
$(W\cdot SN–130)_3W$, No. 6	$(W\cdot SN–130)_3W$, No. 4	118	103 (87.3%)	96 (81.4%)	72	24
	$(W\cdot SN–130)_3W$, No. 5	95	87 (91.6%)	81 (85.3%)	81	0
	$(W\cdot SN–130)_3W$, No. 6	124	99 (79.8%)	89 (71.8%)	89	0
	GD, No. 6	112	31 (27.7%)	5 (4.5%)	4	1
$(W\cdot SN–130)_3W$, No. 7	$(W\cdot SN–130)_3W$, No. 4	106	105 (99.1%)	102 (96.2%)	102	0
	$(W\cdot SN–130)_3W$, No. 5	71	71 (100 %)	63 (88.7%)	63	0
	$(W\cdot SN–130)_3W$, No. 6	70	66 (94.3%)	58 (82.9%)	58	0
$(W\cdot SN–130)_3W$, No. 8	$(W\cdot SN–130)_3W$, No. 4	103	95 (92.2%)	89 (86.4%)	67	22
	$(W\cdot SN–130)_3W$, No. 5	227	179 (78.9%)	153 (67.4%)	153	0
	$(W\cdot SN–130)_3W$, No. 6	117	79 (67.5%)	70 (59.8%)	70	0
	GD, No. 8	252	84 (33.3%)	16 (6.3%)	13	3

$(W\cdot SN–130)_3W$: A female or male produced from mating between female W·SN–130, No. 3 and a field-caught male.

GD: Diploid gynogenesis.

(Table 12). The result was that 4 (Nos. 4, 5, 6 and 8) females and 3 (Nos. 1, 2 and 4) males, seven of the 14 frogs in total, were heterozygous for the albino gene. Olive variants are here counted as wild-type tadpoles for the convenience sake, as they are indistinguishable from the latter at the hatching stage.

From the mating between female No. 4 and male No. 1, 24 wild-type and 11 albino tadpoles at the hatching stage were produced, while 32 wild-type and 11 albino tadpoles were from the mating between the same female and male No. 2. From the mating between female No. 5 and male No. 1, 18 wild-type and 9 albino tadpoles were produced, while 24 wild-type and 5 albino tadpoles were from that between the same female and male No. 2. From the mating between female No. 6 and male No. 4, 72 wild-type and 24 albino tadpoles were produced, while 67 wild-type and 22 albino tadpoles were from the mating between female No.8 and the same male. In summing up these figures, 82 tadpoles were albinos and 237 of the wild type. More than half the number of these tadpoles died of edema before the metamorphosing stage; only 26 albinos metamorphosed normally at the age of 67∼86 days, 75.8 days on the average, while 113 wild-type tadpoles did so at the age of 67∼77 days, 70.6 days on the average.

TABLE 13

Production of albinos from female second-generation offspring derived from irradiated gametes by brother and sister mating or by diploid gynogenesis, IV

Parents		No. of eggs	No. of normal cleavages	No. of tadpoles (St. 20)		
Female	Male			Total	Wild	Albino
(W·SN–130)$_5$W, No. 1	(W·SN–130)$_5$W, No. 1	164	75 (45.7%)	63 (38.4%)	63	0
	(W·SN–130)$_5$W, No. 2	167	56 (33.5%)	42 (25.1%)	32	10
	(W·SN–130)$_5$W, No. 3	180	64 (35.6%)	54 (30.0%)	41	13
	(W·SN–130)$_5$W, No. 4	91	35 (38.5%)	29 (31.9%)	29	0
	GD, No. 1	416	186 (44.7%)	92 (22.1%)	71	21
(W·SN–130)$_5$W, No. 2	(W·SN–130)$_5$W, No. 1	113	57 (50.4%)	45 (39.8%)	45	0
	(W·SN–130)$_5$W, No. 2	151	82 (54.3%)	78 (51.7%)	59	19
	(W·SN–130)$_5$W, No. 3	84	35 (41.7%)	28 (33.3%)	20	8
	(W·SN–130)$_5$W, No. 4	105	78 (74.3%)	65 (61.9%)	65	0
	GD, No. 2	558	320 (57.3%)	110 (19.7%)	81	29
(W·SN–130)$_5$W, No. 3	(W·SN–130)$_5$W, No. 1	72	35 (48.6%)	33 (45.8%)	33	0
	(W·SN–130)$_5$W, No. 2	106	81 (76.4%)	72 (67.9%)	72	0
	(W·SN–130)$_5$W, No. 3	110	63 (57.3%)	55 (50.0%)	55	0
	(W·SN–130)$_5$W, No. 4	103	60 (58.3%)	46 (44.7%)	46	0
(W·SN–130)$_5$W, No. 4	(W·SN–130)$_5$W, No. 1	75	36 (48.0%)	31 (41.3%)	31	0
	(W·SN–130)$_5$W, No. 2	104	76 (73.1%)	70 (67.3%)	70	0
	(W·SN–130)$_5$W, No. 3	115	78 (67.8%)	66 (57.4%)	66	0
	(W·SN–130)$_5$W, No. 4	86	42 (48.8%)	35 (40.7%)	35	0

(W·SN–130)$_5$W: A female or male produced from mating between female W·SN–130, No. 5 and a field-caught male.

GD: Diploid gynogenesis.

By the method of diploid gynogenesis, the 4 females Nos. 4, 5, 6 and 8 produced albinos together with wild-type tadpoles. Female No. 4 produced 22 wild-type and 6 albino tadpoles, No. 5 produced 61 wild-type and 29 albino tadpoles, No. 6 produced 4 wild-type and an albino tadpoles, and No. 8 produced 13 wild-type and 3 albino tadpoles. In summing up these figures, 39 of 139 tadpoles at the hatching stage were albinos. The smaller number of albinos is probably attributable to their weakness increased by refrigeration of the eggs. All the albino tadpoles died during the tadpole stage, except that only one completed normally its metamorphosis. While 32 of the wild-type tadpoles died before the metamorphosing stage, the other 68 metamorphosed normally at the ages of 65～79 days, 73.7 days on the average.

d. Experiments using offspring of female No. 5 from W♀ × SN–130♂

Five albinos were produced from female No. 5 obtained from W♀ × SN–130♂, by the method of diploid gynogenesis (Table 8). Accordingly, it was clear that this female was heterozygous for the albino gene. Of the offspring produced from a mating between this female and a normal male (W70, No. 3), half the number should be heterozygous for the albino gene. As the offspring of female No. 5 matured sexually in 1972, 16 brother and sister matings between 4 females (Nos. 1～4) and 4 males (Nos. 1～4) as well as 4 experiments of diploid gynogenesis were made (Table 13). The result was that two (Nos. 1 and 2) of the females and two (Nos. 2 and 3) of the males were heterozygous for the albino gene.

From the mating between female No. 1 and male No. 2, 32 wild-type and 10 albino tadpoles at the hatching stage were produced, while 41 wild-type and 13 albino tadpoles were from that between the same female and male No. 3. From the mating between female No. 2 and male No. 2, 59 wild-type and 19 albino tadpoles were produced, while 20 wild-type and 8 albino tadpoles were from that between the same female and male No. 3. In summing up these figures, 50 of 202 tadpoles at the hatching stage were albinos. The half of the albinos died of edema or underdevelopment at the early tadpole stage, while the other 25 metamorphosed normally at the age of 68～87 days, 77.1 days on the average. Of the 152 wild-type tadpoles, 32 died also of edema or underdevelopment at the early tadpole stage, while the other 120 metamorphosed normally at the age of 65～76 days, 71.5 days on the average.

Two females Nos. 1 and 2 produced many albinos by the method of diploid gynogenesis; No. 1 produced 71 wild-type and 21 albino tadpoles, and No. 2 produced 81 wild-type and 29 albino ones at the hatching stage. The 2 females produced 152 wild-type and 50 albino tadpoles in total. After hatching, 38 wild-type and 29 albino tadpoles died of edema or underdevelopment before long; 21 albinos metamorphosed normally at the age of 67～85 days, 75.4 days on the average, while 114 wild-type tadpoles did so at the ages of 65～75 days, 71.3 days on the average.

e. Matings between different stocks of albinos

Albino mutants were derived from 4 sources, that is, females No. 11 from EX–145♀×W♂, No. 5 from W♀×SN–50♂, No. 3 from W♀×SN–130♂ and No. 5 from W♀×SN–130♂. Matings were made between different stocks of albinos by various combinations. The result was that all the 4 stocks of albinos had the same albino gene, since the matings always produced albinos and no wild-type tadpoles.

2. Gray-eyed variant

The gray-eyed variant was named from the character that the iris is gray, while the pupil is whitish. The body is yellowish and semi-transparent. In this mutation, the 3 kinds of dermal chromatophores, xanthophores, iridophores and melanophores are abnormal and indistinguishable from one another. Some portions of the skin have no chromatophores, while the other have a single layer of abnormal chromatophores which contain a small amount of abnormal reflecting platelets, melanosomes and pterinosomes. In accordance with such abnormalities, the skin is distinctly thin and semi-transparent, so that the visceral organs can be seen from the outside. Gray-eyed variants develop almost normally for about ten days after hatching. However, the development slows down rapidly, when tadpoles are about 30 mm in total length. These tadpoles are abnormal in the development of teeth. Although they barely live with a nearly normal appearance for more than one month after hatching, all of them gradually die, and no individual attains the metamorphosing stage. The individuals heterozygous for the gray-eyed mutation gene are remarkably superior to the control diploids in developmental capacity and grow into frogs that are almost twice as large as the latter at the age of one year.

Gray-eyed variants are easily distinguished from wild-type individuals at the early tadpole stage (stage 25 in SHUMWAY's table) by the lack of glittering iridophores on the body surface. They were detected in 3 experiments of diploid gynogenesis using 3 females, No. 2 from W♀×SX–90♂, No. 5 from EX–90♀×W♂ and No. 3 from EN–90♀×W♂ (Tables 8 and 9). Accordingly, these 3 females were presumed to be heterozygous (*Gg*) for the gray-eyed mutation gene (*g*). Although nearly the same number of gray-eyed variants and wild-type individuals should be produced from these heterozygous females by diploid gynogenesis, the former were far fewer than the latter as the matter of fact. This was attributable to death of many gray-eyed variants. As the offspring between the heterozygous females and normal males collected from the field matured in 1972, the next generations were produced from brother and sister matings as well as by diploid gynogenesis to obtain gray-eyed variants.

a. Experiments using offspring of female No. 2 from W♀×SX–90♂

In 1970, 36 gray-eyed variants were produced from female No. 2 obtained from W♀×SX–90♂, by the method of diploid gynogenesis (Table 8). These variants all died during the tadpole stage. However, as the offspring between this female and a normal male (W70, No. 1) matured sexually in 1972, 52 brother and sister matings between 12 female (Nos. 1～12) and 18 male (Nos. 1～18) offspring as well as 12 experiments of diploid gynogenesis were made (Table 14). The result was that six (Nos. 1, 4, 5, 8, 11 and 12) of the females and nine (Nos. 5, 8, 9, 10, 11, 12, 16, 17 and 18) of the males were heterozygous for the gray-eyed mutation gene.

From 14 matings between the 6 heterozygous females and the 9 heterozygous males, 896 wild-type and 327 gray-eyed tadpoles were produced: 72 wild-type and 35 gray-eyed between female No. 1 and male No. 5, 144 wild-type and 48 gray-eyed between female No. 4 and males Nos. 8～10, 127 wild-type and 41 gray-eyed between female No. 5 and male Nos. 11 and 12, 171 wild-type and 50 gray-eyed between female No. 8 and males Nos. 11 and 12, 220 wild-type and 91 gray-eyed tadpoles between female No. 11 and males Nos. 16～18, and 162 wild-type and 62 gray-eyed tadpoles between female No. 12 and males Nos. 16～18. All the gray-eyed variants died during the tadpole stage, while almost all the wild-type tadpoles metamorphosed normally and afterwards grew more rapidly than the control frogs did.

From 6 females Nos. 1, 4, 5, 8, 11 and 12, 223 wild-type and 173 gray-eyed tadpoles were produced by diploid gynogenesis. These variants all died before attaining the metamorphosing stage, like those produced from the matings between heterozygous males and females.

b. Experiments using offspring of female No. 5 from EX–90♀×W♂

Eleven gray-eyed variants were produced from female No. 5 obtained from

TABLE 14

Production of gray-eyed variants from female second-generation offspring derived from irradiated gametes by brother and sister mating or by diploid gynogenesis, I

Parents		No. of eggs	No. of normal cleavages	No. of tadpoles (St. 25)		
Female	Male			Total	Wild	Gray-eyed
$(W·SX–90)_2W$, No. 1	$(W·SX–90)_2W$, No. 1	105	97 (92.4%)	94 (89.5%)	94	0
	$(W·SX–90)_2W$, No. 2	124	110 (88.7%)	107 (86.3%)	107	0
	$(W·SX–90)_2W$, No. 3	159	61 (38.4%)	53 (33.3%)	53	0
	$(W·SX–90)_2W$, No. 4	183	165 (90.2%)	114 (62.3%)	114	0
	$(W·SX–90)_2W$, No. 5	163	119 (73.0%)	107 (65.6%)	72	35
	GD, No. 1	236	96 (40.7%)	43 (18.2%)	25	18
$(W·SX–90)_2W$, No. 2	$(W·SX–90)_2W$, No. 1	130	103 (79.2%)	101 (77.7%)	101	0
	$(W·SX–90)_2W$, No. 2	130	103 (79.2%)	100 (76.9%)	100	0
	$(W·SX–90)_2W$, No. 3	167	134 (80.2%)	121 (72.5%)	121	0
	$(W·SX–90)_2W$, No. 4	192	84 (43.8%)	72 (37.5%)	72	0
	$(W·SX–90)_2W$, No. 5	185	65 (35.1%)	54 (29.2%)	54	0
$(W·SX–90)_2W$, No. 3	$(W·SX–90)_2W$, No. 6	136	82 (60.3%)	56 (41.2%)	56	0
	$(W·SX–90)_2W$, No. 7	154	126 (81.8%)	101 (65.6%)	101	0
	$(W·SX–90)_2W$, No. 8	81	9 (11.1%)	5 (6.2%)	5	0
	$(W·SX–90)_2W$, No. 9	57	34 (59.6%)	20 (35.1%)	20	0
	$(W·SX–90)_2W$, No. 10	103	95 (92.2%)	66 (64.1%)	66	0
$(W·SX–90)_2W$, No. 4	$(W·SX–90)_2W$, No. 6	116	89 (76.7%)	53 (45.7%)	53	0
	$(W·SX–90)_2W$, No. 7	114	31 (27.2%)	30 (26.3%)	30	0
	$(W·SX–90)_2W$, No. 8	160	120 (75.0%)	73 (45.6%)	59	14
	$(W·SX–90)_2W$, No. 9	152	108 (71.1%)	65 (42.8%)	50	15
	$(W·SX–90)_2W$, No. 10	95	83 (87.4%)	54 (56.8%)	35	19
	GD, No. 4	211	89 (42.2%)	52 (24.6%)	28	24
$(W·SX–90)_2W$, No. 5	$(W·SX–90)_2W$, No. 11	100	89 (89.0%)	77 (77.0%)	61	16
	$(W·SX–90)_2W$, No. 12	167	100 (59.9%)	91 (54.5%)	66	25
	$(W·SX–90)_2W$, No. 13	179	67 (37.4%)	64 (35.8%)	64	0
	$(W·SX–90)_2W$, No. 14	72	54 (75.0%)	49 (68.1%)	49	0
	GD, No. 5	364	132 (36.3%)	94 (25.8%)	50	44
$(W·SX–90)_2W$, No. 6	$(W·SX–90)_2W$, No. 11	101	98 (97.0%)	93 (92.1%)	93	0
	$(W·SX–90)_2W$, No. 12	104	101 (97.1%)	99 (95.2%)	99	0
	$(W·SX–90)_2W$, No. 13	67	58 (86.6%)	53 (79.1%)	53	0
	$(W·SX–90)_2W$, No. 14	82	66 (80.5%)	63 (76.8%)	63	0
$(W·SX–90)_2W$, No. 7	$(W·SX–90)_2W$, No. 11	130	122 (93.8%)	70 (53.8%)	70	0
	$(W·SX–90)_2W$, No. 12	125	116 (92.8%)	56 (44.8%)	56	0
	$(W·SX–90)_2W$, No. 13	126	119 (94.4%)	97 (77.0%)	97	0
	$(W·SX–90)_2W$, No. 14	139	113 (81.3%)	109 (78.4%)	109	0
$(W·SX–90)_2W$, No. 8	$(W·SX–90)_2W$, No. 11	105	104 (99.0%)	104 (99.0%)	86	18
	$(W·SX–90)_2W$, No. 12	124	122 (98.4%)	117 (94.4%)	85	32
	$(W·SX–90)_2W$, No. 13	137	134 (97.8%)	122 (89.1%)	122	0
	$(W·SX–90)_2W$, No. 14	129	118 (91.5%)	111 (86.0%)	111	0
	GD, No. 8	214	95 (44.4%)	56 (26.2%)	30	26
$(W·SX–90)_2W$, No. 9	$(W·SX–90)_2W$, No. 15	80	79 (98.8%)	69 (86.3%)	69	0
	$(W·SX–90)_2W$, No. 16	63	63 (100 %)	40 (63.5%)	40	0
	$(W·SX–90)_2W$, No. 17	40	38 (95.0%)	28 (70.0%)	28	0
	$(W·SX–90)_2W$, No. 18	50	47 (94.0%)	40 (80.0%)	40	0
$(W·SX–90)_2W$, No. 10	$(W·SX–90)_2W$, No. 15	50	44 (88.0%)	42 (84.0%)	42	0
	$(W·SX–90)_2W$, No. 16	31	30 (96.8%)	17 (54.8%)	17	0
	$(W·SX–90)_2W$, No. 17	46	36 (78.3%)	28 (60.9%)	28	0
	$(W·SX–90)_2W$, No. 18	63	45 (71.4%)	40 (63.5%)	40	0
$(W·SX–90)_2W$, No. 11	$(W·SX–90)_2W$, No. 15	141	116 (82.3%)	88 (62.4%)	88	0
	$(W·SX–90)_2W$, No. 16	140	118 (84.3%)	113 (80.7%)	79	34
	$(W·SX–90)_2W$, No. 17	108	104 (96.3%)	101 (93.5%)	78	23
	$(W·SX–90)_2W$, No. 18	106	103 (97.2%)	97 (91.5%)	63	34
	GD, No. 11	302	119 (39.4%)	83 (27.5%)	48	35
$(W·SX–90)_2W$, No. 12	$(W·SX–90)_2W$, No. 15	135	131 (97.0%)	125 (92.6%)	125	0
	$(W·SX–90)_2W$, No. 16	128	39 (30.5%)	38 (29.7%)	22	16
	$(W·SX–90)_2W$, No. 17	142	123 (86.6%)	115 (81.0%)	83	32
	$(W·SX–90)_2W$, No. 18	147	75 (51.0%)	71 (48.3%)	57	14
	GD, No. 12	226	111 (49.1%)	68 (30.0%)	42	26

$(W·SX–90)_2W$: A female or male produced from mating between female W·SX–90, No. 2 and a field-caught male.

GD: Diploid gynogenesis.

EX–90♀×W♂ by diploid gynogenesis in 1970 (Table 8). As the offspring between this female and a normal male (W70, No. 2) matured sexually in 1972, 10 brother and sister matings between 2 female (Nos. 1 and 2) and 5 male (Nos. 1∼5) offspring were made, together with 2 experiments of diploid gynogenesis by making use of the 2 females (Table 15). The result was that one (No. 1) of the females and two (Nos. 1 and 2) of the males were heterozygous for the gray-eyed mutation gene.

TABLE 15

Production of gray-eyed variants from female second-generation offspring derived from irradiated gametes by brother and sister mating or by diploid gynogenesis, II

Parents		No. of eggs	No. of normal cleavages	No. of tadpoles (St. 25)		
Female	Male			Total	Wild	Gray-eyed
$(EX–90·W)_5W$, No. 1	$(EX–90·W)_5W$, No. 1	230	133 (57.8%)	86 (37.4%)	65	21
	$(EX–90·W)_5W$, No. 2	211	154 (73.0%)	103 (48.8%)	75	28
	$(EX–90·W)_5W$, No. 3	195	125 (64.1%)	84 (43.1%)	84	0
	$(EX–90·W)_5W$, No. 4	202	112 (55.4%)	70 (34.7%)	70	0
	$(EX–90·W)_5W$, No. 5	183	96 (52.5%)	54 (29.5%)	54	0
	GD, No. 1	196	52 (26.5%)	19 (9.7%)	12	7
$(EX–90·W)_5W$, No. 2	$(EX–90·W)_5W$, No. 1	163	94 (57.7%)	71 (43.6%)	71	0
	$(EX–90·W)_5W$, No. 2	172	105 (61.0%)	56 (32.6%)	56	0
	$(EX–90·W)_5W$, No. 3	156	67 (42.9%)	29 (18.6%)	29	0
	$(EX–90·W)_5W$, No. 4	189	83 (43.9%)	43 (22.8%)	43	0
	$(EX–90·W)_5W$, No. 5	175	71 (40.6%)	41 (23.4%)	41	0

$(EX–90·W)_5W$: A female or male produced from mating between female EX–90·W, No. 5 and a field-caught male.

GD: Diploid gynogenesis.

From the mating between female No. 1 and male No. 1, 65 wild-type and 21 gray-eyed tadpoles were produced, while 75 wild-type and 28 gray-eyed tadpoles were from that between the same female and male No. 2. In summing up these figures, 49 of 189 tadpoles were gray-eyed variants. All these variants died during the tadpole stage, while almost all the 140 wild-type tadpoles metamorphosed normally. By diploid gynogenesis, 12 wild-type and 7 gray-eyed tadpoles were produced from female No. 1.

c. Experiments using offspring of female No. 3 from EN–90♀×W♂

Five gray-eyed variants were produced from female No. 3 obtained from EN–90♀×W♂, by diploid gynogenesis in 1970 (Table 8). As the offspring between this female and a normal male (W70, No. 4) matured sexually in 1972, 27 brother and sister matings between 6 females (Nos. 1∼6) and 9 males (Nos. 1∼9) were made (Table 16). The result was that three (Nos. 1, 5 and 6) of the females and five (Nos. 1, 3, 5, 7 and 8) of the males were heterozygous for the gray-eyed mutation gene.

From 8 matings between 3 heterozygous females Nos. 1, 5 and 6 and 5 heterozygous males Nos. 1, 3, 5, 7 and 8, 1113 wild-type and 396 gray-eyed tadpoles were produced: 124 wild-type and 47 gray-eyed between female No. 1 and males Nos. 1 and 3, 673 wild-type and 246 gray-eyed between female No. 5 and males

TABLE 16

Production of gray-eyed variants from female second-generation offspring derived from irradiated gametes by brother and sister mating or by diploid gynogenesis, III

Parents: Female	Parents: Male	No. of eggs	No. of normal cleavages	No. of tadpoles (St. 25): Total	Wild	Gray-eyed
$(EN\text{-}90{\cdot}W)_3W$, No. 1	$(EN\text{-}90{\cdot}W)_3W$, No. 1	204	130 (63.7%)	85 (41.7%)	61	24
	$(EN\text{-}90{\cdot}W)_3W$, No. 2	203	178 (87.7%)	42 (20.7%)	42	0
	$(EN\text{-}90{\cdot}W)_3W$, No. 3	398	284 (71.4%)	86 (21.6%)	63	23
	$(EN\text{-}90{\cdot}W)_3W$, No. 4	156	131 (84.0%)	38 (24.4%)	38	0
$(EN\text{-}90{\cdot}W)_3W$, No. 2	$(EN\text{-}90{\cdot}W)_3W$, No. 1	198	125 (63.1%)	79 (39.9%)	79	0
	$(EN\text{-}90{\cdot}W)_3W$, No. 2	153	132 (86.3%)	93 (60.8%)	93	0
	$(EN\text{-}90{\cdot}W)_3W$, No. 3	214	156 (72.9%)	102 (47.7%)	102	0
	$(EN\text{-}90{\cdot}W)_3W$, No. 4	303	232 (76.6%)	164 (54.1%)	164	0
$(EN\text{-}90{\cdot}W)_3W$, No. 3	$(EN\text{-}90{\cdot}W)_3W$, No. 1	172	86 (50.0%)	52 (30.2%)	52	0
	$(EN\text{-}90{\cdot}W)_3W$, No. 2	154	94 (61.0%)	65 (42.2%)	65	0
	$(EN\text{-}90{\cdot}W)_3W$, No. 3	230	139 (60.4%)	94 (40.9%)	94	0
	$(EN\text{-}90{\cdot}W)_3W$, No. 4	226	160 (70.8%)	105 (46.5%)	105	0
$(EN\text{-}90{\cdot}W)_3W$, No. 4	$(EN\text{-}90{\cdot}W)_3W$, No. 5	492	314 (63.8%)	149 (30.3%)	149	0
	$(EN\text{-}90{\cdot}W)_3W$, No. 6	376	119 (31.6%)	95 (25.3%)	95	0
	$(EN\text{-}90{\cdot}W)_3W$, No. 7	421	293 (69.6%)	102 (24.2%)	102	0
	$(EN\text{-}90{\cdot}W)_3W$, No. 8	480	356 (74.2%)	211 (44.0%)	211	0
	$(EN\text{-}90{\cdot}W)_3W$, No. 9	445	307 (69.0%)	174 (39.1%)	174	0
$(EN\text{-}90{\cdot}W)_3W$, No. 5	$(EN\text{-}90{\cdot}W)_3W$, No. 5	530	491 (92.6%)	378 (71.3%)	274	104
	$(EN\text{-}90{\cdot}W)_3W$, No. 6	365	351 (96.2%)	197 (54.0%)	197	0
	$(EN\text{-}90{\cdot}W)_3W$, No. 7	431	400 (92.8%)	289 (67.1%)	219	70
	$(EN\text{-}90{\cdot}W)_3W$, No. 8	415	405 (97.6%)	252 (60.7%)	180	72
	$(EN\text{-}90{\cdot}W)_3W$, No. 9	474	464 (97.9%)	196 (41.4%)	196	0
$(EN\text{-}90{\cdot}W)_3W$, No. 6	$(EN\text{-}90{\cdot}W)_3W$, No. 5	352	324 (92.0%)	188 (53.4%)	143	45
	$(EN\text{-}90{\cdot}W)_3W$, No. 6	375	297 (79.2%)	145 (38.7%)	145	0
	$(EN\text{-}90{\cdot}W)_3W$, No. 7	341	269 (78.9%)	157 (46.0%)	117	40
	$(EN\text{-}90{\cdot}W)_3W$, No. 8	284	254 (89.4%)	74 (26.1%)	56	18
	$(EN\text{-}90{\cdot}W)_3W$, No. 9	308	265 (86.0%)	121 (39.3%)	121	0

$(EN\text{-}90{\cdot}W)_3W$: A female or male produced from mating between female EN–90·W, No. 3 and a field-caught male.

Nos. 5, 7 and 8, and 316 wild-type and 103 gray-eyed between female No. 6 and males Nos. 5, 7 and 8. All the gray-eyed variants died during the tadpole stage, while almost all the wild-type tadpoles metamorphosed normally. Two-thirds of the wild-type frogs were males, regardless of their genotype being homozygous *GG* or heterozygous *Gg*.

d. Matings between different stocks of gray-eyed variants

Gray-eyed variants were derived from 3 sources, that is, females No. 2 from W♀ × SX–90♂, No. 5 from EX–90♀ × W♂ and No. 3 from EN–90♀ × W♂. As these variants all died during the tadpole stage, matings were made by making use of heterozygous frogs among the 3 stocks of gray-eyed mutation. From each mating, wild-type tadpoles and gray-eyed variants were produced nearly in the rate of 3: 1. Accordingly, it was clear that the three stocks had the same gray-eyed mutation gene.

3. Black-eyed variant

This variant is apparently similar to the "melanoid" reported in *Rana pipiens* by Richards, Tartof and Nace (1969). The whole body of the black-eyed variant is distinctly blackish and semi-transparent. The iridophores contain small, abnormally shaped reflecting platelets. The melanophores are distinctly expanded and filled with very large melanosomes. The xanthophores are usually normal. The name of black-eyed variant was given by the author from the character that the irises of a tadpole as well as of a frog are black. Black-eyed variants were identified in tadpoles at the age of 50 days. They were detected in 3 experiments of diploid gynogenesis using 3 females, No. 5 from W♀×SX–90♂, No. 6 from EX–90♀×W♂, and No. 1 from EN–130♀×W♂. Accordingly, these females were presumed to be heterozygous (*Bb*) for the black-eyed mutation gene (*b*). In these experiments, the variants were nearly the same in number as the wild-type tadpoles or slightly fewer than the latter, that is, they did not distinctly differ from the latter in viability. However, the variants were somewhat smaller than the wild-type siblings at the tadpole stage. Shortly before metamorphosis, many of the variants became edematous and died. Even if black-eyed variants began to metamorphose, many of them also died of edema. Of the black-eyed variants produced by diploid gynogenesis, a small number could normally metamorphose.

Half the number of the offspring between females heterozygous for the black-eyed mutation gene and normal males collected from the field should also be heterozygous. Brother and sister matings as well as experiments of diploid gynogenesis were performed by making use of these offspring to produce black-eyed variants.

a. Experiments using offspring of female No. 5 from W♀×SX–90♂

Twenty-five black-eyed variants were produced from female No. 5 obtained from W♀×SX–90♂ by diploid gynogenesis in 1970 (Table 8). Most of them died of edema shortly before metamorphosis, although only 5 tadpoles completed metamorphosis. These five were all sex-reversed, genetic females. One of them lived until sexual maturity. As four of the offspring between female No. 5 and a normal male matured sexually in 1972, and they consisted of 2 males (Nos. 1 and 2) and 2 females (Nos. 1 and 2), 4 brother and sister matings were made (Table 17).

From the mating between female No. 1 and male No. 1, 5 wild-type and one black-eyed tadpoles were produced. No variants were produced from the other 3 matings, between female No. 1 and male No. 2 and between female No. 2 and males Nos. 1 and 2. Accordingly, it was found that one of the 2 females as well as one of the 2 males was heterozygous for the black-eyed mutation gene.

b. Experiments using offspring of female No. 6 from EX–90♀×W♂

Nine black-eyed variants were produced from female No. 6 obtained from EX–90♀×W♂ by diploid gynogenesis in 1970 (Table 8). Only one of them metamorphosed normally, while the others died of edema before metamorphosis. Of the offspring between female No. 6 and a normal male, 3 males and 2 females attained sexual maturity in 1972. Brother and sister matings were made between

TABLE 17

Production of black-eyed variants from female second-generation offspring derived from irradiated gametes by brother and sister matings or by diploid gynogenesis

Parents		No. of eggs	No. of normal cleavages	No. of 50-day-old tadpoles		
Female	Male			Total	Wild	Black-eyed
$(W \cdot SX\text{–}90)_5W$, No. 1	$(W \cdot SX\text{–}90)_5W$, No. 1	152	85 (55.9%)	6 (3.9%)	5	1
	$(W \cdot SX\text{–}90)_5W$, No. 2	146	51 (34.9%)	30 (20.5%)	30	0
$(W \cdot SX\text{–}90)_5W$, No. 2	$(W \cdot SX\text{–}90)_5W$, No. 1	134	92 (68.7%)	53 (39.6%)	53	0
	$(W \cdot SX\text{–}90)_5W$, No. 2	160	92 (57.5%)	46 (28.8%)	46	0
$(EX\text{–}90 \cdot W)_6W$, No. 1	$(EX\text{–}90 \cdot W)_6W$, No. 1	127	54 (42.5%)	15 (11.8%)	15	0
	$(EX\text{–}90 \cdot W)_6W$, No. 2	151	79 (52.3%)	18 (11.9%)	18	0
	$(EX\text{–}90 \cdot W)_6W$, No. 3	133	80 (60.2%)	34 (25.6%)	34	0
$(EX\text{–}90 \cdot W)_6W$, No. 2	$(EX\text{–}90 \cdot W)_6W$, No. 1	125	63 (50.4%)	32 (25.6%)	32	0
	$(EX\text{–}90 \cdot W)_6W$, No. 2	110	58 (52.7%)	35 (31.8%)	35	0
	$(EX\text{–}90 \cdot W)_6W$, No. 3	119	73 (61.3%)	49 (41.2%)	36	13
	GD, No. 2	231	57 (24.7%)	11 (4.8%)	5	6
$(EN\text{–}130 \cdot W)_1W$, No. 1	$(EN\text{–}130 \cdot W)_1W$, No. 1	118	64 (54.2%)	23 (19.5%)	17	6
	$(EN\text{–}130 \cdot W)_1W$, No. 2	97	56 (57.7%)	33 (34.0%)	33	0
	$(EN\text{–}130 \cdot W)_1W$, No. 3	101	67 (66.3%)	45 (44.6%)	45	0
	GD, No. 1	202	55 (27.2%)	7 (3.5%)	4	3
$(EN\text{–}130 \cdot W)_1W$, No. 2	$(EN\text{–}130 \cdot W)_1W$, No. 1	104	71 (68.3%)	50 (48.1%)	50	0
	$(EN\text{–}130 \cdot W)_1W$, No. 2	132	86 (65.2%)	49 (37.1%)	49	0
	$(EN\text{–}130 \cdot W)_1W$, No. 3	156	104 (66.7%)	73 (46.8%)	73	0

$(W \cdot SX\text{–}90)_5W$: A female or male produced from mating between female W·SX–90, No. 5 and a field-caught male.

$(EX\text{–}90 \cdot W)_6W$: A female or male produced from mating between female EX–90·W, No. 6 and a field-caught male.

$(EN\text{–}130 \cdot W)_1W$: A female or male produced from mating between female EN–130·W, No. 1 and a field-caught male.

GD: Diploid gynogenesis.

them, together with an experiment of diploid gynogenesis (Table 17).

From the mating between female No. 2 and male No. 3, 36 wild-type and 13 black-eyed tadpoles were produced, while only wild-type tadpoles were from the other 5 matings. By diploid gynogenesis, 5 wild-type and 6 black-eyed tadpoles were produced from female No. 2. Accordingly, it was found that one (No. 2) of the 2 females as well as one (No. 3) of the 3 males was heterozygous (*Bb*) for the gene (*b*) of black-eyed mutation. Of the tadpoles produced from the brother and sister matings as well as by the experiment of diploid gynogenesis, only eight, 6 wild-type and 2 black-eyed, metamorphosed normally, although none attained sexual maturity. The 2 variants were males, while the 6 wild-type frogs consisted of 3 males and 3 females.

c. Experiments using offspring of female No. 1 from EN–130♀ × W♂

Thirteen black-eyed tadpoles were produced from female No. 1 obtained from EN–130♀ × W♂, by diploid gynogenesis in 1970, together with 14 wild-type siblings (Table 8). Two of the variants and 8 of the wild-type tadpoles metamorphosed normally, and eventually one variant and 5 wild-type frogs matured sexually. The single variant and two of the latter were sex-reversed, genetic females. In 1972, 3 male and 2 female offspring produced from a heterozygous female No. 1 by mating with a normal male attained sexual maturity. Between the male and female offspring, 6 matings were made together with an experiment of diploid gynogenesis to obtain black-eyed variants (Table 17). As a result, one (No. 1) of the 2 females as well as one (No. 1) of the 3 males was heterozygous (*Bb*) for the black-eyed mutation gene (*b*). From the mating between female No. 1 and male No. 1, 17 wild-type and 6 black-eyed tadpoles were produced. From the same female, 4 wild-type and 3 black-eyed tadpoles were produced by diploid gynogenesis. While 12 of 21 wild-type tadpoles in total produced from the brother and sister mating as well as by the diploid gynogenesis metamorphosed normally, no black-eyed tadpoles did so. All the variants became edematous shortly before metamorphosis and died. No variants were produced from the remaining five of the 6 brother and sister matings.

d. Matings between different stocks of black-eyed variants

Black-eyed variants were derived from 3 sources, that is, females No. 5 from W♀×SX–90♂, No. 6 from EX–90♀×W♂ and No. 1 from EN–130♀×W♂ (Table 8). In 1972, matings were made between heterozygous females produced from females, Nos. 6 and 1, by mating with normal males and a black-eyed male produced from female No. 5 by diploid gynogenesis. As a result, about half the number of the offspring obtained were black-eyed variants. Accordingly, it was clear that the 3 stocks of black-eyed variants had the same gene.

4. Blue variant

Wild-type frogs are green or brown in dorsal ground color. The green is dominant and controlled by the expanding gene *E*. In the presence of *E*, all the 3 kinds of chromatophores expand and became flat, whereas *ee* results in brown, which is attributable to the contraction of all these three kinds of chromatophores (Nishioka and Ueda, 1977a).

The blue variant is blue in dorsal ground color. This color mutation occurs in frogs, *EE* or *Ee*, which are otherwise green in dorsal ground color, by the presence of *xx* (abnormal xanthophores) which gives rise to the absence of carotenoid vesicles in the xanthophores. The same abnormality of xanthophores occurs in frogs, *ee*, which are brown in dorsal ground color. In this case, the dorsal color is grayish-brown and hardly distinguishable from the wild-type brown in appearance, although the variants are easily distinguished from wild-type frogs by a microscopical examination of a piece of the dorsal skin. Such a grayish-brown variant is here included in the wild-type frogs for convenience sake.

Blue variants were detected in 7 experiments of diploid gynogenesis using 7 females, No. 1 from W♀×SX–90♂, Nos. 2 and 3 from W♀×SX–170♂, Nos. 4 and 8 from W♀×SN–130♂, and Nos. 2 and 6 from EN–50♀×W♂ (Table 8). Accordingly, these females were presumed to be heterozygous (*Xx*) for the gene *x* and, moreover, to be homozygous (*EE*) or heterozygous (*Ee*) for the gene *E* which expands all the three kinds of chromatophores. When both brown and green frogs are produced from a female by diploid gynogenesis, this female is presumed to be *Ee*. When no brown frogs are produced, she is presumed to be *EE*. The blue variant is indistinguishable from the green wild-type until the stage where the green makes its appearance on the backs of frogs. Accordingly, blue variants were separated from their normal siblings 3 months after metamorphosis. They were inferior to the latter in viability.

a. Experiments using offspring of female No. 1 from W♀ × SX–90♂

Three blue variants were produced together with 19 brown and 20 green wild-type frogs from female No. 1 obtained from W♀ × SX–90♂ by diploid gynogenesis in 1970 (Table 8). Of these frogs, one blue, 7 brown and 5 green frogs were living and matured sexually in the breeding season of 1972. Accordingly, it was presumed that the female was heterozygous (*XxEe*) for genes *x* and *e*. From the mating between this female and a normal brown-colored male collected from the field, 17 brown and 16 green offspring were produced. Half the number of these offspring should be heterozygous for gene *x*. The single blue variant produced gynogenetically was a female.

In 1972, offspring were produced from this blue variant by diploid gynogenesis as well as by mating with a green male (No. 1, *XxEe*) which had been obtained from the cross between the heterozygous female derived from an X-irradiated spermatozoon and a normal male collected from the field (Table 18). As a result, 35 gynogenetic diploids that were living 3 months after metamorphosis were all blue variants. From the mating between the blue female and the green male, 26 blue and 25 green frogs were produced. All these green frogs are heterozygous for the gene *x*.

b. Experiments using offspring of female No. 2 from W♀ × SX–170♂

Two blue variants were produced together with 4 green and 6 brown frogs from female No. 2 obtained from W♀ × SX–170♂ by diploid gynogenesis in 1970 (Table 8). From the mating between this female and a normal brown-colored male (*XXee*) collected from the field, 35 green and 32 brown frogs were produced. As one of the blue variants obtained gynogenetically was a female and matured sexually in the breeding season of 1972, this female was used for an experiment of diploid gynogenesis as well as matings with 3 males (Nos. 1∼3) of the above 35 green frogs.

Six frogs produced gynogenetically from the female blue variant were all blue 3 months after metamorphosis. From the matings between this female and males Nos. 1 and 2, 27 and 21 green frogs were produced, respectively. Differing from these matings, 10 blue and 12 green frogs were produced from the mating with male No. 3 (Table 18). Accordingly, it was presumed that males Nos. 1 and 2 were *XXEe*, while male No. 3 was *XxEe*, that is, heterozygous for the gene *x*.

c. Experiments using offspring of female No. 3 from W♀ × SX–170♂

In 1970, 3 blue and 8 green frogs were produced from female No. 3 obtained from W♀ × SX–170♂ by diploid gynogenesis (Table 8). All the 26 frogs produced from the mating between this female and a normal brown male collected from the field were green. The 3 blue frogs attained sexual maturity in the breeding season of 1972; two (Nos. 1 and 2) of them were males and the other (No. 1) a female. One (No. 1) of the 2 blue males was mated with a green female (No. 1) produced from the above mating, (W♀ × SX–170♂) ♀ No. 3 × W♂. From this mating, 8 blue and 9 green frogs were produced. The same green female (No. 1) produced 10 blue, 11 green and 23 brown frogs by diploid gynogenesis in the same year (Table 18). No ovulation occurred in the single blue female (No. 1) produced gynogenetically in 1972.

d. Experiments using offspring of female No. 2 from EN–50♀ × W♂

In 1970, 5 blue variants were produced together with 52 green frogs from female No. 2 obtained from EN–50♀ × W♂ by diploid gynogenesis. From the mating between this female and a normal brown male collected from the field, 41 green frogs were produced. As one (No. 1) of the blue variants produced gynogenetically was a female and matured sexually in 1972, offspring were produced from this female by diploid gynogenesis. All the six offspring were blue variants. From the mating between this female and a green male (No. 1) obtained from the above mating, (EN–50♀ × W♂) ♀ No. 2 × W♂, 9 blue and 10 green frogs were produced (Table 18).

e. Experiments using offspring of female No. 6 from EN–50♀ × W♂

Nine blue and 10 green frogs were produced from female No. 6 obtained from EN–50♀ × W♂ by diploid gynogenesis in 1970 (Table 8). From the mating between this female and a normal brown male collected from the field, 33 green frogs were produced. In 1972, one of the blue variants matured; this was a female. From this female, 13 blue variants were produced by diploid gynogenesis in this year. From the mating between this female and a green male (No. 1) obtained from the above mating, (EN–50♀ × W♂) ♀ No. 6 × W♂, 21 blue and 24 green frogs were produced (Table 18).

f. Experiments using offspring of female No. 4 from W♀ × SN–130♂

Two blue and 3 green frogs were produced from female No. 4 obtained from W♀ × SN–130♂ (Table 8). From the mating between this female and a normal brown male collected from the field, 27 green frogs were produced. In 1972, one of the blue variants matured. As this variant was a female, an experiment of diploid gynogenesis was made again; all the 11 offspring were blue. From the mating between this female and a green male (No. 1) obtained from the above mating, (W♀ × SN–130♂) ♀ No. 4 × W♂, 16 blue and 19 green frogs were produced (Table 18).

g. Experiments using offspring of females No. 1 from W♀ × SX–90♂ and No. 2 from W♀ × SX–170♂

A blue female (No. 2) obtained from a female offspring (No. 2) of W♀ × SX–170♂ by diploid gynogenesis in 1970 was mated in 1973 with 4 green males (Nos. 1 ~ 4) which were obtained in 1972 from a mating between a blue female

(No. 1) produced from W♀ × SX–90♂ by diploid gynogenesis and a green male (No. 1) produced from (W♀ × SX–90♂) ♀ × W♂ (Table 18). From each of the

TABLE 18

Production of blue variants from blue female second-generation offspring derived from irradiated gametes

Year	Parents: Female	Parents: Male	No. of eggs
1972	$(W{\cdot}SX{-}90)_1$GD, Blue, No. 1	$(W{\cdot}SX{-}90)_1$W, Green, No. 1	156
		GD^2, No. 1	247
	$(W{\cdot}SX{-}170)_2$GD, Blue, No. 1	$(W{\cdot}SX{-}170)_2$W, Green, No. 1	112
		$(W{\cdot}SX{-}170)_2$W, Green, No. 2	133
		$(W{\cdot}SX{-}170)_2$W, Green, No. 3	151
		GD^2, No. 1	193
	$(W{\cdot}SX{-}170)_3$W, Green, No. 1	$(W{\cdot}SX{-}170)_3$GD, Blue, No. 1	152
		GD^2, No. 1	189
	$(EN{-}50{\cdot}W)_2$GD, Blue, No. 1	$(EN{-}50{\cdot}W)_2$W, Green, No. 1	136
		GD^2, No. 1	155
	$(EN{-}50{\cdot}W)_6$GD, Blue, No. 1	$(EN{-}50{\cdot}W)_6$W, Green, No. 1	177
		GD^2, No. 1	139
	$(W{\cdot}SN{-}130)_4$GD, Blue, No. 1	$(W{\cdot}SN{-}130)_4$W, Green, No. 1	188
		GD^2, No. 1	130
1973	$(W{\cdot}SX{-}170)_2$GD, Blue, No. 2	{$(W{\cdot}SX{-}90)_1$GD, Blue, No. 1} × {$(W{\cdot}SX{-}90)_1$W, Green, No. 1}, Green, No. 1	142
		{$(W{\cdot}SX{-}90)_1$GD, Blue, No. 1} × {$(W{\cdot}SX{-}90)_1$W, Green, No. 1}, Green, No. 2	153
		{$(W{\cdot}SX{-}90)_1$GD, Blue, No. 1} × {$(W{\cdot}SX{-}90)_1$W, Green, No. 1}, Green, No. 3	144
		{$(W{\cdot}SX{-}90)_1$GD, Blue, No. 1} × {$(W{\cdot}SX{-}90)_1$W, Green, No. 1}, Green, No. 4	146
	$(W{\cdot}SX{-}170)_3$GD, Blue, No. 1	{$(W{\cdot}SX{-}170)_2$GD, Blue, No. 1} × {$(W{\cdot}SX{-}170)_2$W, Green, No. 1}, Green, No. 1	157
		{$(W{\cdot}SX{-}170)_2$GD, Blue, No. 1} × {$(W{\cdot}SX{-}170)_2$W, Green, No. 1}, Green, No. 2	174
	$(EN{-}50{\cdot}W)_2$GD, Blue, No. 1	{$(W{\cdot}SX{-}170)_3$W, Green, No. 1} × {$(W{\cdot}SX{-}170)_3$GD, Blue, No. 1}, Green, No. 1	145
		{$(W{\cdot}SX{-}170)_3$W, Green, No. 1} × {$(W{\cdot}SX{-}170)_3$GD, Blue, No. 1}, Green, No. 2	126
	$(EN{-}50{\cdot}W)_6$GD, Blue, No. 1	{$(EN{-}50{\cdot}W)_2$GD, Blue, No. 1} × {$(EN{-}50{\cdot}W)_2$W, Green, No. 1}, Green, No. 1	135
		{$(EN{-}50{\cdot}W)_2$GD, Blue, No. 1} × {$(EN{-}50{\cdot}W)_2$W, Green, No. 1}, Green, No. 2	146
		{$(EN{-}50{\cdot}W)_2$GD, Blue, No. 1} × {$(EN{-}50{\cdot}W)_2$W, Green, No. 1}, Green, No. 3	147

four matings, nearly equal number of blue and green frogs were produced: 51 of 108 frogs in total were blue and 57 were green. Accordingly, it was clear that

by mating with various male offspring derived from irradiated gametes or by diploid gynogenesis

No. of normal cleavages	No. of frogs 3 months after metamorphosis			
	Total	Brown	Green	Blue
93 (59.6%)	51 (32.7%)	0	25	26
76 (30.8%)	35 (14.2%)	0	0	35
54 (48.2%)	27 (24.1%)	0	27	0
80 (60.2%)	21 (15.8%)	0	21	0
92 (60.9%)	22 (14.6%)	0	12	10
87 (45.1%)	6 (3.1%)	0	0	6
54 (35.5%)	17 (11.2%)	0	9	8
93 (49.2%)	44 (23.3%)	23	11	10
67 (49.3%)	19 (14.0%)	0	10	9
53 (34.2%)	6 (3.9%)	0	0	6
76 (42.9%)	45 (25.4%)	0	24	21
95 (68.3%)	13 (9.4%)	0	0	13
59 (31.4%)	35 (18.6%)	0	19	16
57 (43.8%)	11 (8.5%)	0	0	11
74 (52.1%)	21 (14.8%)	0	12	9
65 (42.5%)	31 (20.3%)	0	16	15
79 (54.9%)	39 (27.1%)	0	19	20
87 (59.6%)	17 (11.6%)	0	10	7
55 (35.0%)	12 (7.6%)	0	6	6
41 (23.6%)	9 (5.2%)	0	4	5
36 (24.8%)	16 (11.0%)	0	9	7
57 (45.2%)	14 (11.1%)	0	7	7
66 (48.9%)	23 (17.0%)	0	12	11
65 (44.5%)	28 (19.2%)	0	15	13
91 (61.9%)	36 (24.5%)	0	19	17

TABLE 18. Continued

Year	Parents		No. of eggs
	Female	Male	
1973	(W·SN–130)$_4$GD, Blue, No. 1	{(EN–50·W)$_6$GD, Blue, No. 1} × {(EN–50·W)$_6$W, Green, No. 1}, Green, No. 1	146
		{(EN–50·W)$_6$GD, Blue, No. 1} × {(EN–50·W)$_6$W, Green, No. 1}, Green, No. 2	150
		{(EN–50·W)$_6$GD, Blue, No. 1} × {(EN–50·W)$_6$W, Green, No. 1}, Green, No. 3	121
		{(EN–50·W)$_6$GD, Blue, No. 1} × {(EN–50·W)$_6$W, Green, No. 1}, Green, No. 4	185

(W·SX–90 or 170)$_{1,2\text{or}3}$W: A female or male produced from mating between female W·SX–90
(W·SX–90 or 170)$_{1,2\text{or}3}$GD: A female or male produced gynogenetically from female W·SX–
(EN–50·W)$_{2\text{or}6}$W: A male produced from mating between female EN–50·W, No. 2 or 6 and a
(EN–50·W)$_{2\text{or}6}$GD: A female produced gynogenetically from female EN–50·W, No. 2 or 6.
(W·SN–130)$_4$W: A male produced from mating between female W·SN–130, No. 4 and a
(W·SN–130)$_4$GD: A female produced gynogenetically from female W·SN–130, No. 4.
GD^2: Diploid gynogenesis repeated twice.

the stock derived from a spermatozoon exposed to 90 rads of X-rays was the same in the gene of blue mutation as that derived from a spermatozoon exposed to 170 rads of X-rays.

h. Experiments using offspring of females Nos. 2 and 3 from W♀ × SX–170♂

A blue female (No. 1) obtained from a female offspring (No. 3) of W♀ × SX–170♂ by diploid gynogenesis in 1970 was mated in 1973 with two green males (Nos. 1 and 2) which were obtained in 1972 from a mating between a blue female (No. 1) produced from a female offspring of W♀ × SX–170♂ by diploid gynogenesis and a green male (No. 1) produced from the same female offspring by mating with a normal male from the field (Table 18). From each of the two matings, nearly an equal number of blue and green frogs were produced: 11 of 21 frogs in total were blue and 10 were green. Whether each of the 2 green males Nos. 1 and 2 used in 1973 was *XxEE* or *XxEe* in genotype was not determined.

i. Experiments using offspring of females No. 2 from EN–50♀ × W♂ and No. 3 from W♀ × SX–170♂

A blue female (No. 1) obtained from a female offspring (No. 2) of EN–50♀ × W♂ by diploid gynogenesis in 1970 was mated in 1973 with two green males (Nos. 1 and 2) which were obtained in 1972 from a mating between a green female offspring (No. 1) of (W♀ × SX–170♂)♀ No. 3 × W♂ and a blue male (No. 1), a sex-reversed genetic female produced from a female offspring (No. 3) of W♀ × SX–170♂ by diploid gynogenesis (Table 18). From each of the two matings

No. of normal cleavages	No. of frogs 3 months after metamorphosis			
	Total	Brown	Green	Blue
85 (58.2%)	26 (17.8%)	0	15	11
76 (50.7%)	24 (16.0%)	0	14	10
24 (19.8%)	7 (5.8%)	0	4	3
97 (52.4%)	67 (36.2%)	0	32	35

or 170, No. 1, 2 or 3 and a field-caught male.
90 or 170, No. 1, 2 or 3.
field-caught male.

field-caught male.

nearly equal number of blue and green frogs were produced: 14 of 30 frogs in total were blue and 16 were green. It was clear that two green males Nos. 1 and 2 used in 1973 were *XxEE* or *XxEe* in genotype and the same in the gene of blue mutation as blue female No. 1.

j. Experiments using offspring of females Nos. 2 and 6 from EN–50♀×W♂

A blue female (No. 1) obtained from a female offspring (No. 6) of EN–50♀× W♂ by diploid gynogenesis in 1970 was mated in 1973 with 3 green males (Nos. 1∼3) which were obtained in 1972 from a mating between a blue female (No. 1) produced from a female offspring (No. 2) of EN–50♀×W♂ by diploid gynogenesis and a green male offspring (No. 1) of (EN–50♀×W♂)♀ No. 2×W♂ (Table 18). Nearly an equal number of blue and green frogs were produced from each of the 3 matings: 41 of 87 frogs in total were blue and 46 were green. It was clear that each of the 3 green males Nos. 1∼3 used in 1973 was *XxEE* or *XxEe* in genotype.

k. Experiments using offspring of female No. 4 from W♀×SN–130♂ and female No. 6 from EN–50♀×W♂

A blue female (No. 1) obtained from a female offspring (No. 4) of W♀× SN–130♂ by diploid gynogenesis in 1970 was mated in 1973 with 4 green males (Nos. 1∼4) which were obtained in 1972 from a mating between a blue female (No. 1) produced from a female offspring (No. 6) of EN–50♀×W♂ by diploid gynogenesis and a green male (No. 1) produced from a mating, (EN–50♀×

W♂)♀ No. 6×W♂ (Table 18). From each of the four matings, nearly an equal number of blue and green frogs were produced: 59 of 124 frogs in total were blue and 65 were green. Accordingly, it was clear that the stock derived from a spermatozoon exposed to 130 rads of neutrons was the same in the gene of blue mutation as that from an egg exposed to 50 rads of neutrons. Each of the 4 green males used in 1973 was *XxEE* in genotype.

5. Olive variant

In this variant, the ventral surfaces of the body and legs are pale olive and semi-transparent in place of white. Their dorsal surfaces are somewhat olive in ground color. This variant occurs in the presence of abnormal iridophores which gives rise to decrease of reflecting platelets of iridophores in number and thickness. The melanophores are normal in structure. The olive variant is easily distinguishable from the wild type at the late tadpole stage, as the ventral surfaces are bluish-olive in place of white.

Olive variants (*ii*) were detected in 9 experiments of diploid gynogenesis using 9 females, No. 9 from W♀×SX–170♂, Nos. 1 and 4 from EX–145♀×W♂, Nos. 1, 3, 8 and 9 from W♀×SN–130♂ and Nos. 1 and 4 from EN–50♀×W♂. Albinos or blue frogs were produced together with olive variants from two of the 9 females, that is, Nos. 3 and 8 from W♀×SN–130♂. It was clear that the 9 females were heterozygous *Ii* in genotype.

TABLE 19

Production of olive variants from female second-generation offspring derived from

Parents	
Female	Male
$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 1	$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 1
	$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 2
	$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 3
	$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 4
	$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 5
	$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 6
	$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 7
$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 2	$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 1
	$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 2
	$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 3
	$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 4
	$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 5
	$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 6
	$(W{\cdot}SX\text{–}170)_9W$, Wild, No. 7
$(W{\cdot}SX\text{–}170)_9GD$, Olive, No. 1	$(W{\cdot}SX\text{–}170)_9GD$, Olive, No. 1
	$(W{\cdot}SX\text{–}170)_9GD$, Olive, No. 2
	$(W{\cdot}SX\text{–}170)_9GD$, Olive, No. 3
	$(W{\cdot}SX\text{–}170)_9GD$, Olive, No. 4
	$(W{\cdot}SX\text{–}170)_9GD$, Olive, No. 5

$(W{\cdot}SX\text{–}170)_9W$: A female or male produced from mating between female
$(W{\cdot}SX\text{–}170)_9GD$: A female or male produced gynogenetically from female

a. Experiments using offspring of female No. 9 from W♀ × SX–170♂

In 1970, 14 olive variants and 15 wild-type individuals were produced from a female offspring (No. 9) of W♀ × SX–170♂ by diploid gynogenesis. They were 50-day-old tadpoles, and afterwards all of them metamorphosed normally. At the age of two years, five of 6 olive variants as well as five of 10 brown-type frogs were sex-reversed into males.

As about half of the offspring of female No. 9 mated with a normal brown male collected from the field is presumed to be heterozygous for gene *i*, brother and sister matings were made among sexually mature offspring in 1972. In the same year, an olive female produced from female No. 9 by diploid gynogenesis was mated with 5 olive males, that is, sex-reversed genetic females produced simultaneously from the same female (Table 19). The brother and sister matings were made between 2 females (Nos. 1 and 2) and 7 males (Nos. 1∼7). As a result, it was found that a female (No. 1) and 4 males (Nos. 1, 2, 6 and 7) were heterozygous for gene *i*. Female No. 1 produced 12 olive and 34 wild-type, 10 olive and 31 wild-type, 5 olive and 14 wild-type and 7 olive and 20 wild-type tadpoles by mating with males Nos. 1, 2, 6 and 7, respectively. In total, 34 olive and 99 wild-type tadpoles were produced from the 4 matings. All the 146 tadpoles produced from 5 matings between the olive female No. 9 produced gynogenetically and the 5 olive sex-reversed phenotypic males were olive variants.

irradiated gametes by brother and sister mating, I

No. of eggs	No. of normal cleavages	No. of 50-day-old tadpoles		
		Total	Wild	Olive
93	59 (63.4%)	46 (49.5%)	34	12
102	72 (70.6%)	41 (40.2%)	31	10
106	52 (49.1%)	33 (31.1%)	33	0
98	66 (67.3%)	47 (48.0%)	47	0
112	91 (81.3%)	63 (56.3%)	63	0
114	46 (40.4%)	19 (16.7%)	14	5
99	54 (54.5%)	27 (27.3%)	20	7
97	42 (43.3%)	23 (23.7%)	23	0
74	36 (48.6%)	30 (40.5%)	30	0
85	51 (60.0%)	37 (43.5%)	37	0
113	55 (48.7%)	44 (38.9%)	44	0
109	68 (62.4%)	48 (44.0%)	48	0
101	70 (69.3%)	52 (51.5%)	52	0
100	63 (63.0%)	47 (47.0%)	47	0
80	41 (51.3%)	23 (28.8%)	0	23
129	60 (46.5%)	52 (40.3%)	0	52
93	53 (57.0%)	29 (31.2%)	0	29
77	44 (57.1%)	35 (45.5%)	0	35
91	20 (22.0%)	7 (7.7%)	0	7

W·SX–170, No. 9 and a field-caught male.

W·SX–170, No. 9.

TABLE 20

Production of olive variants from female second-generation offspring

Parents	
Female	Male
(EX–145·W)$_1$W, Wild, No. 1	(EX–145·W)$_1$W, Wild, No. 1 (EX–145·W)$_1$W, Wild, No. 2
(EX–145·W)$_1$W, Wild, No. 2	(EX–145·W)$_1$W, Wild, No. 1 (EX–145·W)$_1$W, Wild, No. 2
(EX–145·W)$_1$GD, Olive, No. 1	(EX–145·W)$_1$GD, Olive, No. 1 (EX–145·W)$_1$GD, Olive, No. 2
(EX–145·W)$_4$W, Wild, No. 1	(EX–145·W)$_4$W, Wild, No. 1 (EX–145·W)$_4$W, Wild, No. 2 (EX–145·W)$_4$W, Wild, No. 3
(EX–145·W)$_4$W, Wild, No. 2	(EX–145·W)$_4$W, Wild, No. 1 (EX–145·W)$_4$W, Wild, No. 2 (EX–145·W)$_4$W, Wild, No. 3
(EX–145·W)$_4$W, Wild, No. 3	(EX–145·W)$_4$W, Wild, No. 1 (EX–145·W)$_4$W, Wild, No. 2 (EX–145·W)$_4$W, Wild, No. 3
(EX–145·W)$_4$GD, Olive, No. 1	(EX–145·W)$_4$GD, Olive, No. 1 (EX–145·W)$_4$GD, Olive, No. 2 (EX–145·W)$_4$GD, Olive, No. 3

(EX–145·W)$_{1or4}$W: A female or male produced from mating between female
(EX–145·W)$_{1or4}$GD: A female or male produced gynogenetically from

b. Experiments using offspring of female No. 1 from EX–145♀ × W♂

Fifteen of 30 frogs produced from a female offspring (No. 1) of EX–145♀ × W♂ by diploid gynogenesis in 1970 were olive variants, while the other 15 were of brown type. Only 3 of these olive variants matured; one was a female and the other two were sex-reversed phenotypic males. Female offspring No. 1 of EX–145♀ × W♂ was mated with a normal brown male collected from the field. As 2 males (Nos. 1 and 2) and 2 females (Nos. 1 and 2) obtained from this mating matured sexually in 1972, brother and sister matings were made between these males and females (Table 20). As a result, it was found that female No. 1 and male No. 2 were heterozygous for gene *i*. Of 53 tadpoles produced from a mating between female No. 1 and male No. 2, 13 were olive and 40 of wild type. From 2 matings between the single olive female and the 2 olive, sex-reversed phenotypic males stated above, 76 olive tadpoles were produced.

c. Experiments using offspring of female No. 4 from EX–145♀ × W♂

Of 49 frogs produced from a female offspring (No. 4) of EX–145♀ × W♂ by diploid gynogenesis in 1970, 24 were olive and 25 brown. Seven of the olive variants matured sexually in 1972; two were females and five were sex-reversed phenotypic males. As normal ovulation occurred in one of the 2 females, 3 matings were made between this female and three (Nos. 1 ~ 3) of the 5 males (Table 20). From these matings, 70 olive tadpoles were produced.

Female No. 4 from EX–145♀ × W♂ was mated with a normal brown male derived from irradiated gametes by brother and sister mating, II

No. of eggs	No. of normal cleavages	No. of 50-day-old tadpoles		
		Total	Wild	Olive
97	46 (47.4%)	33 (34.0%)	33	0
91	68 (74.7%)	53 (58.2%)	40	13
89	53 (59.6%)	50 (56.2%)	50	0
104	74 (71.2%)	45 (43.3%)	45	0
102	26 (25.5%)	4 (3.9%)	0	4
263	114 (43.3%)	72 (27.4%)	0	72
152	133 (87.5%)	112 (73.7%)	83	29
115	72 (62.6%)	50 (43.5%)	50	0
105	81 (77.1%)	62 (59.0%)	48	14
112	86 (76.8%)	70 (62.5%)	70	0
93	69 (74.2%)	61 (65.6%)	61	0
96	65 (67.7%)	53 (55.2%)	53	0
125	85 (68.0%)	55 (44.0%)	55	0
104	72 (69.2%)	49 (47.1%)	49	0
117	66 (56.4%)	46 (39.3%)	46	0
173	37 (21.4%)	27 (15.6%)	0	27
166	30 (18.1%)	13 (7.8%)	0	13
189	51 (27.0%)	30 (15.9%)	0	30

EX–145·W, No. 1 or 4 and a field-caught male.
female EX–145·W, No. 1 or 4.

collected from the field in 1970. As the offspring matured sexually, brother and sister matings were made in 1972 between 3 females (Nos. 1∼3) and 3 males (Nos. 1∼3). As a result, it was found that female No. 1 and males Nos. 1 and 3 were heterozygous for the olive mutation gene. Of 174 tadpoles produced from 2 matings between the single female and the 2 males, 43 were olive and 131 of wild type.

d. Experiments using offspring of female No. 1 from W♀ × SN–130♂

Fifteen of 30 frogs produced from a female offspring (No. 1) of W♀ × SN–130♂ by diploid gynogenesis in 1970 were olive variants, while the other 15 were of brown type. Eight of the olive variants matured sexually in 1972; seven were females and one was a sex-reversed phenotypic male. As normal ovulation occurred in four (Nos. 1∼4) of the 7 females, they were mated with the single male (Table 21). From 4 matings, 124 tadpoles were produced; all of them were olive.

The offspring produced from the mating between female No. 1 from W♀ × SN–130♂ and a normal brown male collected from the field matured sexually in 1972. Brother and sister matings were made between 3 females (Nos. 1∼3) and 4 males (Nos. 1∼4). As a result, it was found that one (No. 1) of the females and two (Nos. 1 and 2) of the males were heterozygous for gene *i*. From 2 matings, 219 tadpoles were produced; 56 were olive and 163 of wild-type (Table 21).

TABLE 21

Production of olive variants from female second-generation offspring

Parents	
Female	Male
(W·SN–130)$_1$W, Wild, No. 1	(W·SN–130)$_1$W, Wild, No. 1 (W·SN–130)$_1$W, Wild, No. 2 (W·SN–130)$_1$W, Wild, No. 3 (W·SN–130)$_1$W, Wild, No. 4
(W·SN–130)$_1$W, Wild, No. 2	(W·SN–130)$_1$W, Wild, No. 1 (W·SN–130)$_1$W, Wild, No. 2 (W·SN–130)$_1$W, Wild, No. 3 (W·SN–130)$_1$W, Wild, No. 4
(W·SN–130)$_1$W, Wild, No. 3	(W·SN–130)$_1$W, Wild, No. 1 (W·SN–130)$_1$W, Wild, No. 2 (W·SN–130)$_1$W, Wild, No. 3 (W·SN–130)$_1$W, Wild, No. 4
(W·SN–130)$_1$GD, Olive, No. 1 (W·SN–130)$_1$GD, Olive, No. 2 (W·SN–130)$_1$GD, Olive, No. 3 (W·SN–130)$_1$GD, Olive, No. 4	(W·SN–130)$_1$GD, Olive, No. 1
(W·SN–130)$_3$GD, Olive, No. 1 (W·SN–130)$_3$GD, Olive, No. 2 (W·SN–130)$_3$GD, Olive, No. 3 (W·SN–130)$_3$GD, Olive, No. 4	(W·SN–130)$_3$GD, Olive, No. 1
(W·SN–130)$_8$GD, Olive, No. 1 (W·SN–130)$_8$GD, Olive, No. 2 (W·SN–130)$_8$GD, Olive, No. 3 (W·SN–130)$_8$GD, Olive, No. 4	(W·SN–130)$_8$GD, Olive, No. 1

(W·SN–130)$_1$W: A female or male produced from mating between female
(W·SN–130)$_{1,3or8}$GD: A female or male produced gynogenetically from

TABLE 22

Production of olive variants from female second-generation offspring

Parents	
Female	Male
(W·SN–130)$_9$W, Wild, No. 1	(W·SN–130)$_9$W, Wild, No. 1 (W·SN–130)$_9$W, Wild, No. 2 (W·SN–130)$_9$W, Wild, No. 3
(W·SN–130)$_9$W, Wild, No. 2	(W·SN–130)$_9$W, Wild, No. 1 (W·SN–130)$_9$W, Wild, No. 2 (W·SN–130)$_9$W, Wild, No. 3
(W·SN–130)$_9$W, Wild, No. 3	(W·SN–130)$_9$W, Wild, No. 1 (W·SN–130)$_9$W, Wild, No. 2 (W·SN–130)$_9$W, Wild, No. 3
(W·SN–130)$_9$W, Wild, No. 4	(W·SN–130)$_9$W, Wild, No. 1 (W·SN–130)$_9$W, Wild, No. 2 (W·SN–130)$_9$W, Wild, No. 3
(W·SN–130)$_9$GD, Olive, No. 1 (W·SN–130)$_9$GD, Olive, No. 2 (W·SN–130)$_9$GD, Olive, No. 3	(W·SN–130)$_9$GD, Olive, No. 1

(W·SN–130)$_9$W: A female or male produced from mating between female
(W·SN–130)$_9$GD: A female or male produced gynogenetically from female

derived from irradiated gametes by brother and sister mating, III

No. of eggs	No. of normal cleavages	No. of 50-day-old tadpoles		
		Total	Wild	Olive
112	87 (77.7%)	58 (51.8%)	44	14
296	182 (61.5%)	161 (54.4%)	119	42
116	94 (81.0%)	71 (61.2%)	71	0
125	85 (68.0%)	67 (53.6%)	67	0
103	77 (74.8%)	70 (68.0%)	70	0
120	82 (68.3%)	63 (52.5%)	63	0
115	56 (48.7%)	45 (39.1%)	45	0
98	70 (71.4%)	56 (57.1%)	56	0
76	48 (63.2%)	41 (53.9%)	41	0
85	73 (85.9%)	60 (70.6%)	60	0
101	87 (86.1%)	62 (61.4%)	62	0
110	71 (64.5%)	54 (49.1%)	54	0
83	37 (44.6%)	26 (31.3%)	0	26
112	65 (58.0%)	50 (44.6%)	0	50
97	31 (32.0%)	13 (13.4%)	0	13
71	44 (62.0%)	35 (49.3%)	0	35
123	84 (68.3%)	53 (43.1%)	0	53
141	72 (51.1%)	55 (39.0%)	0	55
115	54 (47.0%)	42 (36.5%)	0	42
126	43 (34.1%)	21 (16.4%)	0	21
70	32 (45.7%)	16 (22.9%)	0	16
125	84 (67.2%)	51 (40.8%)	0	51
53	26 (49.1%)	16 (30.2%)	0	16
57	18 (31.6%)	7 (12.3%)	0	7

W·SN–130, No. 1 and a field-caught male.
female W·SN–130, No. 1, 3 or 8.

derived from irradiated gametes by brother and sister mating, IV

No. of eggs	No. of normal cleavages	No. of 50-day-old tadpoles		
		Total	Wild	Olive
165	136 (82.4%)	56 (33.9%)	56	0
133	116 (87.2%)	90 (67.7%)	90	0
127	95 (74.8%)	61 (48.0%)	61	0
105	96 (91.4%)	32 (30.5%)	32	0
117	94 (80.3%)	54 (46.2%)	54	0
129	102 (79.1%)	58 (45.0%)	58	0
130	104 (80.0%)	62 (47.7%)	46	16
142	103 (72.5%)	92 (64.8%)	92	0
151	120 (79.5%)	70 (46.4%)	70	0
122	93 (76.2%)	46 (37.7%)	34	12
146	85 (58.2%)	44 (30.1%)	44	0
135	92 (68.1%)	60 (44.4%)	60	0
147	89 (60.5%)	35 (23.8%)	0	35
159	95 (59.7%)	43 (27.0%)	0	43
173	152 (87.9%)	61 (35.3%)	0	61

W·SN–130, No. 9 and a field-caught male.
W·SN–130, No. 9.

e. Experiments using offspring of females Nos. 3 and 8 from W♀ × SN–130♂

Immediately after hatching, four of 23 tadpoles produced from a female offspring (No. 3) of W♀ × SN–130♂ by diploid gynogenesis in 1970 were albinos, while the other 19 were of wild type in appearance. Six of the latter, however, were found to be olive variants at the late tadpole stage. These 6 variants all matured sexually in 1972; five were females and one was a sex-reversed phenotypic male. As normal ovulation occurred in four of the 5 females after pituitary injection, 4 matings were made between the 4 females and the single male (Table 21). All the 171 tadpoles produced from these matings were olive.

Two olive variants were produced from a female offspring (No. 8) of W♀ × SN–130♂ by diploid gynogenesis in 1970. As one of them was a sex-reversed phenotypic male and matured sexually in 1972, this male was mated with the above 4 olive females derived from female offspring No. 3 of W♀ × SN–130♂ (Table 21). All the 90 tadpoles produced from 4 matings were olive.

f. Experiments using offspring of female No. 9 from W♀ × SN–130♂

Of 45 tadpoles produced from a female offspring (No. 9) of W♀ × SN–130♂ by diploid gynogenesis, 22 were olive variants. Six of them metamorphosed normally and matured sexually in 1972; five were females and one was a sex-reversed phenotypic male. As normal ovulation occurred in 3 of the 5 females after pituitary injection, 3 matings were made between these females and the single male (Table 22). All the 139 tadpoles produced were olive.

Female No. 9 produced from W♀ × SN–130♂ was mated in 1970 with a normal brown male collected from the field. As the offspring matured sexually in 1972, brother and sister matings were made between 4 females (Nos. 1∼4) and 3 males (Nos. 1∼3). As a result, it was found that females Nos. 3 and 4 and male No. 1 were heterozygous for gene *i*. Of 108 tadpoles produced from

TABLE 23

Production of olive variants from female second-generation offspring

Parents	
Female	Male
(EN–50·W)$_1$W, Wild, No. 1	(EN–50·W)$_1$W, Wild, No. 1
	(EN–50·W)$_1$W, Wild, No. 2
	(EN–50·W)$_1$W, Wild, No. 3
(EN–50·W)$_1$W, Wild, No. 2	(EN–50·W)$_1$W, Wild, No. 1
	(EN–50·W)$_1$W, Wild, No. 2
	(EN–50·W)$_1$W, Wild, No. 3
(EN–50·W)$_1$W, Wild, No. 3	(EN–50·W)$_1$W, Wild, No. 1
	(EN–50·W)$_1$W, Wild, No. 2
	(EN–50·W)$_1$W, Wild, No. 3
(EN–50·W)$_1$GD, Olive, No. 1	(EN–50·W)$_1$GD, Olive, No. 1

(EN–50·W)$_1$W: A female or male produced from mating between female

(EN–50·W)$_1$GD: A female or male produced gynogenetically from female

2 matings between the 2 females and the single male, 28 were olive and 80 of wild type.

g. Experiments using offspring of female No. 1 from EN–50♀×W♂

Seven of 17 frogs produced from a female offspring (No. 1) of EN–50♀×W♂ by diploid gynogenesis in 1970 were olive variants, while five others were brown and the remaining five were green. Three of the 7 variants matured sexually in 1972; two were females and one was a sex-reversed phenotypic male. As normal ovulation occurred in one (No. 1) of the 2 females after pituitary injection, mating was made between this female and the single male (Table 23). All the 46 tadpoles produced were olive.

Brother and sister matings were made in 1972 between 3 females (Nos. 1∼3) and 3 males (Nos. 1∼3) produced in 1970 from a mating between female No. 1 from EN–50♀×W♂ and a normal male collected from the field (Table 23). As a result, it was found that females Nos. 2 and 3 and male No. 2 were heterozygous for gene *i*. Of 89 tadpoles produced from two matings between them, 21 were olive and 68 of wild type.

h. Experiments using offspring of female No. 4 from EN–50♀×W♂

Of 43 frogs produced from a female offspring (No. 4) of EN–50♀×W♂ by diploid gynogenesis in 1970, 22 were olive variants, while the other 21 were brown. Eight of the olive variants matured sexually in 1972; six were females and two were sex-reversed phenotypic males. As normal ovulation occurred in 4 (Nos. 1∼4) of the 6 females after pituitary injection, matings were made between these 4 females and one (No. 1) of the 2 males (Table 24). All the 185 tadpoles produced from the matings were olive.

The offspring between female No. 4 produced in 1970 from EN–50♀×W♂ and a normal brown male collected from the field matured sexually in 1972.

derived from irradiated gametes by brother and sister mating, V

No. of eggs	No. of normal cleavages	No. of 50-day-old tadpoles		
		Total	Wild	Olive
115	74 (64.3%)	50 (43.5%)	50	0
109	65 (59.6%)	36 (33.0%)	36	0
120	62 (51.7%)	45 (37.5%)	45	0
96	93 (96.9%)	42 (43.8%)	42	0
134	77 (57.5%)	30 (22.4%)	23	7
125	81 (64.8%)	56 (44.8%)	56	0
112	56 (50.0%)	31 (27.7%)	31	0
110	70 (63.6%)	59 (53.6%)	45	14
121	83 (68.6%)	52 (43.0%)	52	0
205	95 (46.3%)	46 (22.4%)	0	46

EN-50·W, No. 1 and a field-caught male.
EN-50·W, No. 1.

TABLE 24
Production of olive variants from female second-generation offspring

Parents	
Female	Male
(EN–50·W)$_4$W, Wild, No. 1	(EN–50·W)$_4$W, Wild, No. 1 (EN–50·W)$_4$W, Wild, No. 2 (EN–50·W)$_4$W, Wild, No. 3
(EN–50·W)$_4$W, Wild, No. 2	(EN–50·W)$_4$W, Wild, No. 1 (EN–50·W)$_4$W, Wild, No. 2 (EN–50·W)$_4$W, Wild, No. 3
(EN–50·W)$_4$W, Wild, No. 3	(EN–50·W)$_4$W, Wild, No. 1 (EN–50·W)$_4$W, Wild, No. 2 (EN–50·W)$_4$W, Wild, No. 3
(EN–50·W)$_4$W, Wild, No. 4	(EN–50·W)$_4$W, Wild, No. 1 (EN–50·W)$_4$W, Wild, No. 2 (EN–50·W)$_4$W, Wild, No. 3
(EN–50·W)$_4$GD, Olive, No. 1 (EN–50·W)$_4$GD, Olive, No. 2 (EN–50·W)$_4$GD, Olive, No. 3 (EN–50·W)$_4$GD, Olive, No. 4	(EN–50·W)$_4$GD, Olive, No. 1

(EN–50·W)$_4$W: A female or male produced from mating between female
(EN–50·W)$_4$GD: A female or male produced gynogenetically from female

Brother and sister matings were made between 4 female (Nos. 1∼4) and 3 male (Nos. 1∼3) offspring (Table 24). As a result, it was found that females Nos. 2 and 3 and males Nos. 1 and 2 were heterozygous for gene *i*. Of 414 tadpoles produced from four matings between these 2 females and 2 males, 109 were olive and 305 of wild type.

i. Experiments using heterozygous (*Ii*) females derived from different sources and a blue male

In 1972, matings were made between 9 females heterozygous for gene *i* and a blue male. Six of these females were No. 9 from W♀×SX–170♂, Nos. 1 and 4 from EX–145♀×W♂, No. 3 from W♀×SN–130♂, and Nos. 1 and 4 from EN–50♀×W♂, while the single male was No. 1 produced from a female offspring (No. 3) of W♀×SX–170♂ by diploid gynogenesis (Table 8). As a result of these matings, female No. 9 from W♀×SX–170♂ produced blue frogs together with green-type ones, while the other 5 females produced no blue frogs; all the offspring were green. On the other hand, no green-type frogs were produced from five females other than females No. 1 of EN–50♀×W♂ by diploid gynogenesis; the latter produced both green-type and brown-type frogs. As the blue male produced by diploid gynogenesis was *xxEE* in genotype, it was clear that female No. 9 from W♀×SX–170♂ was *XxIiee*, four other females Nos. 1 and 4 from EX–145♀×W♂, No. 3 from W♀×SN–130♂ and No. 4 from EN–50♀×W♂ were *XXIiee*, and female No. 1 from EN–50♀×W♂ was *XXIiEe* in genotype.

The remaining three of the 9 females used for mating experiments with the derived from irradiated gametes by brother and sister mating, VI

No. of eggs	No. of normal cleavages	No. of 50-day-old tadpoles		
		Total	Wild	Olive
120	86 (71.7%)	79 (65.8%)	79	0
180	85 (47.2%)	49 (27.2%)	49	0
103	45 (43.7%)	24 (23.3%)	24	0
207	89 (43.0%)	53 (25.6%)	40	13
175	156 (89.1%)	135 (77.1%)	101	34
198	101 (51.0%)	86 (43.4%)	86	0
185	94 (50.8%)	51 (27.6%)	36	15
226	203 (89.8%)	175 (77.4%)	128	47
178	115 (64.6%)	76 (42.7%)	76	0
106	75 (70.8%)	54 (50.9%)	54	0
124	82 (66.1%)	52 (41.9%)	52	0
155	79 (51.0%)	58 (37.4%)	58	0
171	16 (9.4%)	7 (4.1%)	0	7
192	171 (89.1%)	126 (65.6%)	0	126
113	46 (40.7%)	45 (39.8%)	0	45
123	8 (6.5%)	7 (5.7%)	0	7

EN–50·W, No. 4 and a field-caught male.
EN–50·W, No. 4.

blue male were Nos. 1, 8 and 9 from W♀×SN–130♂ (Table 8). All the offspring produced from the mating between female No. 1 and the blue male were of green type, while the matings between females Nos. 8 and 9 and the blue male produced both blue and green-type frogs. By diploid gynogenesis, both olive and brown-type frogs were produced from females Nos. 1 and 9, while olive and blue variants as well as brown- and green-type frogs were produced from female No. 8 (Table 8). On the basis of these results, it was clear that females Nos. 1, 8 and 9 from W♀×SN–130♂ were *XXIiee*, *XxIiEe* and *XxIiee* in genotype, respectively.

III. *Bluish-, greenish-, yellowish- and brownish-olive variants*

While mating experiments were repeated from generation to generation, it was found that olive variants (*ii*) could be divided into four kinds, bluish-, greenish-, yellowish- and brownish-olive variants, in accordance with the presence of *xx* or *ee*.

Bluish-olive variants seem to be derived from green-type frogs. Their dorsal surfaces are intermediate between blue and olive in ground color, while the ventral surfaces are pale bluish-olive and semi-transparent. Under an electron microscope, no carotenoid vesicles are found in the xanthophores and only a few thin reflecting platelets are in the iridophores. Accordingly, the bluish-olive variant occurs in coexistence of *xx* and *ii* in addition to *EE* or *Ee*. Greenish-olive variants seem to be similar to the bluish-olive ones, in that they derive from green-type frogs. Their dorsal surfaces are greenish-olive in ground color, while the ventral surfaces are pale bluish-olive and semi-transparent like those of bluish-olive variants. Under an electron microscope, the xanthophores are normal, while the iridophores are abnormal and contain a few thin reflecting platelets (NISHIOKA and UEDA, 1977a). Accordingly, the greenish-olive variant is *XiiE* in genotype. Yellowish-olive variants are very similar to brownish-olive ones in appearance, although there is a slight difference in ground color of dorsal surfaces. These two kinds of olive variants seem to derive from brown-type frogs, since all of them are homozygous for gene *e*. While the yellowish-olive variant is *Xiiee* in genotype, the brownish-olive one is *xxiiee*. Under an electron microscope, the xanthophores of the latter contain no carotenoid vesicles, differing from those of the former (NISHIOKA and UEDA, 1977a). The ventral surfaces of both the yellowish-olive and brownish-olive variants are pale bluish-olive and

TABLE 25

Production of four kinds of olive variants from male or female second-generation or

Year	Parents		No. of eggs
	Female	Male	
1972	(W·SN–130)$_8$GD, Bl-ol, No. 1	(W·SN–130)$_8$GD, Br-ol, No. 1	104
		GD^2, No. 1	195
		WL72, Br, No. 1	139
		(W·SN–130)$_9$GD, Ye-ol, No. 1	206
		(W·SN–130)$_8$W, Gr, No. 1	134
	(W·SN–130)$_9$GD, Br-ol, No. 1	(W·SN–130)$_9$GD, Ye-ol, No. 1	147
1973	WL73, Br, No. 1	{(W·SN–130)$_8$GD, Bl-ol, No. 1} × {(W·SN–130)$_8$GD, Br-ol, No. 1}, Bl-ol, No. 1	156
1974	{(W·SN–130)$_8$GD, Bl-ol, No. 1} × {(W·SN–130)$_8$GD, Br-ol, No. 1}, Bl-ol, No. 1	W·[{(W·SN–130)$_8$GD, Bl-ol, No. 1 × {(W·SN–130)$_8$GD, Br-ol, No. 1}], Br, No. 1	198
		{(W·SN–130)$_8$GD, Bl-ol, No. 1} × {(W·SN–130)$_9$GD, Ye-ol, No. 1}, Gr-ol, No. 1	246
	{(W·SN–130)$_9$GD, Br-ol, No. 1} × {(W·SN–130)$_9$GD, Ye-ol, No. 1}, Ye-ol, No. 1	{(W·SN–130)$_8$GD, Bl-ol, No. 1} × (WL72, Br, No. 1), Gr, No. 1	133
		{(W·SN–130)$_8$GD, Bl-ol, No. 1} × {(W·SN–130)$_9$GD, Ye-ol, No. 1}, Gr-ol, No. 1	151
	(W·SN–130)$_1$GD, Ye-ol, No. 1	{(W·SN–130)$_8$GD, Bl-ol, No. 1} × (WL72, Br, No. 1), Gr, No. 1	140
		{(W·SN–130)$_8$GD, Bl-ol, No. 1} × {(W·SN–130)$_9$GD, Ye-ol, No. 1}, Gr-ol, No. 1	163

Gr: Green. Br: Brown. Bl: Blue. Bl-ol: Bluish-olive. Gr-ol: Greenish-olive. Ye-ol:
(W·SN–130)$_{1,\,8\,or\,9}$GD: A female or male produced gynogenetically from female W·SN–130, No. 1,
(W·SN–130)$_8$W: A male produced from mating between female W·SN–130, No. 8 and a field-
WL72 or 73: Laboratory-raised frogs used in 1972 or 1973.
GD^2: Diploid gynogenesis repeated twice.

semi-transparent like those of the bluish-olive and greenish-olive variants. All the four kinds of olive variants were detected among the frogs which had derived from 3 spermatozoa exposed to 130 rads of neutrons.

1. Experiments performed in 1972

Of 17 frogs produced from female offspring No. 8 of W♀×SN–130♂ by diploid gynogenesis in 1970, four were green, seven brown, two blue, two brownish-olive and two bluish-olive when observed one month after metamorphosis. On the basis of this divergence, it was presumed that female No. 8 was *XxIiEe* in genotype. As a bluish-olive female (No. 1, *xxiiEE*) and a brownish-olive sex-reversed phenotypic male (No. 1, *xxiiee*) matured sexually in 1972, a mating was made between these two frogs (Table 25). As a result, 7 frogs were produced,

third-generation offspring derived from spermatozoa exposed to 130 rads of neutrons

No. of normal cleavages	No. of frogs	No. of frogs 3 months after metamorphosis						
		Gr	Br	Bl	Bl-ol	Gr-ol	Ye-ol or Br-ol	Total
37 (35.6%)	7 (6.7%)	0	0	0	2	0	0	2
86 (44.1%)	20 (10.3%)	0	0	0	16	0	0	16
82 (59.0%)	31 (22.3%)	24	0	0	0	0	0	24
115 (55.8%)	11 (5.3%)	0	0	0	0	7	0	7
74 (55.2%)	6 (4.5%)	2	0	0	1	1	0	4
89 (60.5%)	35 (23.8%)	0	0	0	0	0	20	20
104 (66.7%)	56 (35.9%)	20	23	0	0	0	0	43
184 (92.9%)	52 (26.3%)	8	13	6	6	7	10	50
139 (56.5%)	7 (2.8%)	0	0	0	2	2	1	5
117 (88.0%)	46 (34.6%)	9	8	4	4	8	9	42
71 (47.0%)	31 (20.5%)	0	0	0	0	15	10	25
105 (75.0%)	53 (37.9%)	11	9	0	0	11	16	47
29 (17.8%)	15 (9.2%)	0	0	0	0	5	6	11

Yellowish-olive. Br-ol: Brownish-olive.
8 or 9.
caught male.

although only two of them were living 3 months after metamorphosis. These two frogs were bluish-olive, being *xxiiEe* in genotype. From the same female No. 1, 16 offspring were produced by the second diploid gynogenesis. All these offspring were bluish-olive, being *xxiiEE* in genotype, too.

The same female was mated with a brown-type male WL72, No. 1 (*XXIIee* in genotype) in the control series and produced 24 green-type frogs at the stage of 3 months after metamorphosis. From a mating between the bluish-olive female No. 1 and a yellowish-olive, sex-reversed phenotypic male (*XXiiee* in genotype) obtained from a female offspring (No. 9) of W♀×SN–130♂ by diploid gynogenesis, 11 frogs were produced. Seven of them were living 3 months after metamorphosis. All the 7 frogs were greenish-olive, being *XxiiEe* in genotype. When the same female was mated with a green male (*XxIiEe* in genotype) which had been obtained from a mating between female No. 8 from W♀×SN–130♂ and a normal male collected from the field, 6 frogs were produced. Three months after metamorphosis, four of them were living; one was bluish-olive, one greenish-olive and the other two of green-type.

In 1970, half the number of tadpoles produced from female No. 9 obtained from W♀×SN–130♂ by diploid gynogenesis were olive variants. Two of them matured sexually in 1972; one was a brownish-olive female (No. 1), *xxiiee* in genotype, and the other was a yellowish-olive, sex-reversed phenotypic male (No. 1) which was used in the above mating. From a mating between them, 35 frogs were produced. Three months after metamorphosis, 20 of them were living; all of them were yellowish-olive variants, being *Xxiiee* in genotype.

2. Experiments performed in 1973 and 1974

A frog (No. 1) produced in 1972 from the mating between the bluish-olive female No. 1 and the brownish-olive, sex-reversed phenotypic male No. 1 was bluish-olive, being *xxiiEe* in genotype. As this frog was a sex-reversed phenotypic male and matured sexually in 1973, he was mated with a normal brown female WL73, No. 1 (*XXIIee* in genotype) in the control series. From this mating, 56 frogs were produced. Three months after metamorphosis 43 of them were living; 20 were green (*XxIiEe*) and 23 brown (*XxIiee*). As a male (No. 1) of these brown-colored frogs matured sexually in 1974, he was mated with a bluish-olive female (No. 1, *xxiiEe* in genotype) which was a sister of the bluish-olive, sex-reversed phenotypic male No. 1. Fifty-two frogs were produced from this mating, and 50 of them were living 3 months after metamorphosis; eight were green (*XxIiEe*), thirteen brown (*XxIiee*) or grayish-brown (*xxIiee*), six blue (*xxIiEe*), six bluish-olive (*xxiiEe*), seven greenish-olive (*XxiiEe*) and ten yellowish-olive (*Xxiiee*) or brownish-olive (*xxiiee*). The same bluish-olive female No. 1 (*xxiiEe*) was mated in 1974 with a greenish-olive male (No. 1, *XxiiEe*), which had been produced from a mating in 1972 between the other bluish-olive female No. 1 (*xxiiEE*) derived from female offspring No. 8 of W♀×SN–130♂ by diploid gynogenesis and the yellowish-olive, sex-reversed phenotypic male No. 1 (*XXiiee*) derived from the female offspring No. 9 of W♀×SN–130♂. From this mating 7 frogs were produced. Three months after metamorphosis, five of them were living; two were bluish-olive (*xxiiE*), two greenish-olive (*XxiiE*) and one yellowish-olive (*Xxiiee*) or brownish-olive (*xxiiee*).

A yellowish-olive female (No. 1, *Xxiiee*) produced in 1972 from a mating between the brownish-olive female No. 1 (*xxiiee*) and the yellowish-olive, sex-reversed phenotypic male No. 1 (*XXiiee*) derived from female offspring No. 9 of W♀ × SN–130♂ by diploid gynogenesis was mated in 1974 with a green male No. 1 (*XxIiEe*) which had been produced from a mating between bluish-olive female No. 1 (*xxiiEE*) derived from the female offspring No. 8 of W♀ × SN–130♂ by diploid gynogenesis and a normal brown male WL72, No. 1 (*XXIIee*) in the control series. From this mating 46 frogs were produced. Three months after metamorphosis, 42 of them were living; nine were green (*XIiEe*), eight brown (*XIiee*) or grayish-brown (*xxIiee*), four blue (*xxIiEe*), four bluish-olive (*xxiiEe*), eight greenish-olive (*XiiEe*) and nine yellowish-olive (*Xiiee*) or brownish-olive (*xxiiee*). The same yellowish-olive female No. 1 (*Xxiiee*) was mated in 1974 with a greenish-olive male (*XxiiEe*) which had been produced from a mating between the bluish-olive female No. 1 (*xxiiEE*) derived from the female offspring No. 8 of W♀ × SN–130♂ by diploid gynogenesis and the yellowish-olive male No. 1 (*XXiiee*) derived from the female offspring No. 9 of W♀ × SN–130♂ by diploid gynogenesis. From this mating 31 frogs were produced. Three months after metamorphosis, 25 of them were living; 15 were greenish-olive (*XiiEe*) and 10 yellowish-olive (*Xiiee*) or brownish-olive (*xxiiee*).

A yellowish-olive female (No. 1, *XXiiee*) produced in 1970 from a female offspring (No. 1) of W♀ × SN–130♂ by diploid gynogenesis was mated in 1974 with the green male No. 1 (*XxIiEe*) which had been produced in 1972 from the mating between the bluish-olive female No. 1 (*xxiiEE*) derived from the female offspring No. 8 of W♀ × SN–130♂ by diploid gynogenesis and the normal brown male WL72, No. 1 (*XXIIee*). From this mating 53 frogs were produced. Of these frogs, 47 were living 3 months after metamorphosis; eleven were green (*XIiEe*), nine brown (*XIiee*), eleven greenish-olive (*XiiEe*) and sixteen yellowish-olive (*Xiiee*). The same female No. 1 (*XXiiee*) was mated with the greenish-olive male No. 1 (*XxiiEe*) which had been produced from the mating between the bluish-olive female No. 1 (*xxiiEE*) derived from the female offspring No. 8 of W♀ × SN–130♂ and the yellowish-olive male No. 1 (*XXiiee*) derived from female No. 9 of W♀ × SN–130♂ by diploid gynogenesis. Fifteen frogs were produced from this mating. Eleven of them were living 3 months after metamorphosis; five were greenish-olive (*XiiEe*) and six yellowish-olive (*Xiiee*).

DISCUSSION

The inheritance of spontaneous color variants in the genus *Rana* has been studied by several authors. Moore (1942) clarified for the first time that the burnsi variant occurring occasionally in natural populations of *Rana pipiens* is controlled by a single dominant gene, although the existence of spotting and non-spotting modifiers is presumed by VOLPE (1961) and VOLPE and DASGUPTA (1962). Another variant of *Rana pipiens*, the kandiyohi, was genetically studied by VOLPE (1955). This variant is also controlled by a dominant gene, as is the burnsi variant. These two kinds of dominant genes are not allelic (VOLPE, 1960). They lack dominance with respect to each other and produce the color and pattern combining the characteristics of the two variants in their coexistence (ANDERSON and VOLPE, 1958; VOLPE, 1961). MORIYA (1952) reported on a variant characteristic of a black marbly pattern of the ventral surface, differing from the usual white color, in *Rana nigromaculata*. This variant is distributed in a limited area of Niigata Prefecture, Japan and seemed to be due to the presence of a recessive gene in the homozygous condition. BROWDER (1968) investigated the inheritance of the speckle and blue mutations in *Rana pipiens*. The speckle mutation is due to a dominant gene in the heterozygous condition. A typical heterozygote reduces numbers of guanophores and xanthophores in the dorsal and lateral skin, while homozygotes have none or a few of them. According to him, the blue mutation may be due to a recessive gene in the homozygous condition. A variant named "melanoid" was obtained by RICHARDS, TARTOF and NACE (1969) from two female *Rana pipiens* by the method of diploid gynogenesis. In the presence of the "melanoid" gene in the homozygous condition, neither xanthophores nor iridophores are produced in the skin. The first case of linkage between two mutant loci in anurans was reported by BROWDER (1972a). He postulated the existence of a dominant subvital gene linked to the allele of burnsi gene, in order to explain a large excess of burnsi progeny produced from a cross between a frog heterozygous for the dominant burnsi gene and a wild-type frog. The inheritance of albinism has been studied in *Rana temporaria* (SMALLCOMBE, 1949) and *Rana pipiens* (SMITH-GILL, RICHARDS and NACE, 1972; BROWDER, 1972b).

As anurans other than *Rana* species, the inheritance of albinism has been reported up to the present in *Xenopus laevis* and *Hyla arborea japonica*. Although albinism in *Xenopus laevis* is periodic in gene expression during the development of animals, it is due to a recessive gene in the homozygous condition (HOPERSKAYA, 1975). NISHIOKA and UEDA (1977b) report in this volume that albinism in *Hyla arborea japonica* is not so simple as reported hitherto in the other anuran species as well as in urodeles, birds and mammals. There are three recessive albino genes each of which has a locus differing from those of the other albino genes. Moreover, there are two dominant genes; one normalizes the color of the eyes and the other somewhat normalizes the color of the skin in one of the three kinds of albinos. Each of these two dominant genes is linked with a recessive albino gene.

In urodeles, one and four kinds of color variants that occurred spontaneously have been reported in *Pleurodeles waltlii* and *Ambystoma mexicanum*, respectively. The single color variant in *Pleurodeles waltlii* is characteristic of the absence of iridophores and an increase in number and distribution of melanophores in the skin of larvae. This variant is produced by a recessive gene in the homozygous condition (LACROIX and CAPURON, 1970). The four kinds of color variants in

Ambystoma mexicanum are the white, albinic, melanoid, and a new variant (HUMPHREY, 1971). White axolotls have been known since more than 100 years ago (DUMERIL, 1870). While the body is white, the eyes are pigmented. This phenotype occurs by the presence of a recessive gene affecting pigment cell distribution in the homozygous condition. The true albinic variant is due to a recessive gene which was introduced from an albino *Ambystoma tigrinum* into axolotls (HUMPHREY, 1967). The body of the melanoid variant is dark charcoal gray or dull black, because of increased numbers of melanophores, absence of iridophores and relatively small numbers of xanthophores. This variant is produced by a recessive gene in the homozygous condition (HUMPHREY and BAGNARA, 1967). The new variant listed by HUMPHREY (1971) lacks completely the xanthophores and guanophores, while the distribution of melanophores is normal. This variant is due to a recessive gene.

The wild specimens of *Rana nigromaculata* are green or brown in dorsal ground color at the immature frog stage. The green skin of this frog species is nearly the same as that of *Rana clamitans* described by BERNS and NARAYAN (1970) in the arrangement as well as in the electron-microscopic structures of the three kinds of dermal chromatophores. This green color is due to the presence of the three dominant genes, *X* for normal xanthophores, *I* for normal iridophores and *E* for expansion of the three kinds of dermal chromatophores. The brown color of wild frogs is due to the presence of *ee* in place of *E*; the recessive gene *e* contracts all the three kinds of dermal chromatophores.

In the present research, nine kinds of color variants were produced by irradiation of the eggs or spermatozoa with X-rays or neutrons. Three of them, the albino, gray-eyed and black-eyed, are each due to a recessive gene (*m*, *g*, and *b*) which is epistatic in the homozygous condition to the genes for various kinds of color and pattern. Each of the other six color variants, the blue, grayish-brown, greenish-olive, bluish-olive, yellowish-olive and brownish-olive, occurs by the presence of one or two kinds of recessive, mutation genes. The genotypes of nine color variants derived from irradiated eggs or spermatozoa are presented in Table 26.

Albinos occurred in four gametes, that is, an egg exposed to 145 rads of X-rays, a spermatozoon exposed to 50 rads of neutrons and two spermatozoa exposed to 130 rads of neutrons (Table 8). The albino genes which occurred in these gametes were the same in locus as well as in phenotypic expression. This is especially noteworthy, because more than three albino *Rana nigromaculata* strains which differ from one another in the locus of albino gene have been collected by the authors from the field (NISHIOKA and UEDA, unpublished), as in *Hyla arborea japonica* (NISHIOKA and UEDA, 1977b). The albino gene (*m*) produced by X- or neutron-irradiation was the same in locus as that of one of the three kinds of spontaneous albinos, although the former somewhat differs from the latter in phenotype. A detailed comparison in phenotype between the radiation-induced and spontaneous albinos will be reported in another paper (NISHIOKA and UEDA, unpublished).

TABLE 26

Genotypes of nine color variants raised from irradiated eggs or spermatozoa

	Phenotype	Genotype
Wild type	Green (Wild type)	*XXIIEE, XXIIEe*
	Green (Induced type)	*XxIIEE, XXIiEE, XxIIEe, XXIiEe*
		XxIiEE, XxIiEe
	Brown (Wild type)	*XXIIee*
	Brown (Induced type)	*XXIiee, XxIIee, XxIiee*
Color variant	Blue	*xxIIEE, xxIIEe, xxIiEE, xxIiEe*
	Grayish-brown	*xxIIee, xxIiee*
	Greenish-olive	*XXiiEE, XxiiEE, XXiiEe, XxiiEe*
	Bluish-olive	*xxiiEE, xxiiEe*
	Yellowish-olive	*XXiiee, Xxiiee*
	Brownish-olive	*xxiiee*
	Albino	*mm*
	Gray-eyed	*gg*
	Black-eyed	*bb*

The mutation genes of gray-eyed variants occurred in three irradiated gametes, a spermatozoon and an egg exposed to 90 rads of X-rays and an egg exposed to 90 rads of neutrons (Table 8) and were of the same locus (*g*). The gray-eyed variant is peculiar in that the three kinds of dermal chromatophores are so abnormal as they are indistinguishable from one another. As the abnormal chromatophores contain a small number of abnormal reflecting platelets, melanosomes and pterinosomes, this mutation seems to be a unique material for studying the mechanism of organella differentiation in each of the three kinds of chromatophores.

The black-eyed variant seems somewhat different in phenotype from the melanoid in *Ambystoma* (HUMPHREY and BAGNARA, 1967), the "melanoid" in *Rana pipiens* (RICHARDS, TARTOF and NACE, 1969) and the melanic in *Pleurodeles waltlii* (LACROIX and CAPURON, 1970), as the latter three kinds of variants have no iridophores; the black-eyed variant has iridophores which contain small, abnormally-shaped reflecting platelets. However, all these variants are the same in that they have black eyes without the glittering iris. The black-eyed mutation genes which occurred in three irradiated gametes, a spermatozoon and an egg exposed to 90 rads of X-rays and an egg exposed to 130 rads of neutrons (Table 8) were the same in locus (*b*). Moreover, the variants derived from these three different sources were similar to one another in phenotype as well as in viability. In these respects, they were strangely similar to the gray-eyed variant, although the latter could not attain the metamorphosing stage.

As the green and brown in the dorsal ground color of *Rana nigromaculata* are due to the presence of three kinds of genes, *XIE* and *XIee*, respectively, six kinds of color variants are produced by mutations of genes *X* and *I* in green and brown frogs. In the presence of *xx* and *ii*, the deficiency of carotenoid vesicles in xanthophores and the decrease of reflecting platelets in number and thickness in iridophores occur, respectively. Accordingly, the phenotypes of *xxIE*, *XiiE* and *xxiiE* are blue, greenish-olive and bluish-olive, respectively, while those of *xxIee*, *Xiiee* and *xxiiee* are grayish-brown, yellowish-olive and brownish-olive, respectively. While BERNS and NARAYAN (1970) could not determine whether xanthophores were greatly altered or quite absent from the blue integument of *Rana clamitans*, there were abnormal xanthophores containing abundant pterinosomes and no carotenoid vesicles in the dorsal skin of blue variants in *Rana nigromaculata* (NISHIOKA and UEDA, 1977a).

Abnormal-xanthophore and abnormal-iridophore mutations occurred in nine and nine gametes by X- or neutron-irradiation, respectively (Table 8). However, as these two kinds of mutations occurred together in each of three gametes, the gametes which gave rise to these mutations were 15 in the actual number. In one of these 15 gametes, the albino mutation (*m*) occurred together with *i* (Table 8). It was clarified that the abnormal-xanthophore and abnormal-iridophore mutations in the 15 gametes were the same in the locus, respectively.

In the present investigation, 75 females in total derived from X- or neutron-irradiated eggs or spermatozoa were used in order to obtain color variants by the method of diploid gynogenesis. However, the offspring of 11 of them died before the stage where color variants were detectable, probably from the presence of a lethal gene in the homozygous condition. Accordingly, a total of 28 mutations were found in 24 of the remaining 64 gametes. The radiation-induced mutation rates of the kinds of color genes *m*, *g*, *b*, *x* and *i* are presented in Table 27, although the gametes analyzed were so small as 64 in number. The five kinds of mutations occurred in 17 (50%) of 34 spermatozoa and in 11 (37%) of

TABLE 27
Mutation rates of color genes in 64 irradiated gametes

Kind of mutations	Number of mutations	Mutation rate in percentage
Albino (*m*)	4	6.3
Gray-eyed (*g*)	3	4.7
Black-eyed (*b*)	3	4.7
Abnormal xanthophores (*x*)	9	14.1
Abnormal iridophores (*i*)	9	14.1
Total	28	43.8

30 unfertilized eggs after irradiation with X-rays or neutrons. On the other hand, they occurred in 12 (40%) of 30 gametes irradiated with X-rays and in 16 (47%) of 34 gametes irradiated with neutrons. The mutations were induced in 11 (37%) of 30 gametes exposed to 50∼90 rads of X-rays or neutrons, while they were in 17 (50%) of 34 gametes exposed to 130∼170 rads. Although definite conclusions can not yet drawn from these data on account of fewness of analyzed gametes, there seems to be no large difference in mutation rate between spermatozoa and eggs, as well as between gametes irradiated with X-rays and neutrons.

SUMMARY

1. In order to produce color variants by irradiation in *Rana nigromaculata,* spermatozoa from 3 males and unfertilized eggs from 3 females were exposed to 90～240 rads of X-rays or 50～130 rads of neutrons. The wild-type frogs are green or brown in dorsal ground color at the immature stage. The green is dominant and controlled by gene E which expands all the 3 kinds of dermal chromatophores, xanthophores, iridophores and melanophores. In the presence of *ee*, the dorsal ground color becomes brown. Both green and brown frogs have at least gene X for normal xanthophores and gene I for normal iridophores, besides gene M for normal melanophores.

2. A total of 75 females derived from irradiated gametes were examined by the method of diploid gynogenesis in order to detect induced mutations. The offspring of 11 of them died at the tadpole stage probably by doubling of a recessive lethal gene.

3. Twenty-four of the remaining 64 females produced 9 kinds of color variants, albino, gray-eyed, black-eyed, blue, grayish-brown, greenish-olive, bluish-olive, yellowish-olive and brownish-olive, while the other 40 produced none of them.

4. The mutation gene for each of the albino, gray-eyed and black-eyed variants is recessive and epistatic in the homozygous condition to the genes for various kinds of color and pattern. Albino, gray-eyed and black-eyed mutations occurred in 4, 3 and 3 irradiated gametes, respectively. In each kind of color variants, the mutations which occurred by irradiation in the different gametes were the same in locus. Accordingly, the recessive albino, gray-eyed and black-eyed mutations are given signs *m*, *g* and *b*, respectively.

5. In the albino, the melanophores are abnormal, that is, they contain colorless premelanosomes in place of melanosomes, while the other two kinds of dermal chromatophores are quite normal. In the gray-eyed variant (*gg*) three kinds of dermal chromatophores are abnormal and indistinguishable from one another. There is a single layer of dermal chromatophores which contain a small amount of abnormal reflecting platelets, melanosomes and pterinosomes. In the black-eyed variant (*bb*), the iridophores are abnormal, that is, they contain small, abnormally-shaped reflecting platelets. The melanophores are filled with very large melanosomes. The xanthophores are usually normal.

6. The albino, gray-eyed and black-eyed variants are detectable at the tadpole stage. All of these variants are distinctly poor in viability. The gray-eyed variant can not attain the metamorphosing stage. The black-eyed variant scarcely attain sexual maturity after metamorphosis. Differing from these two kinds of variants, some albinos become mature frogs.

7. The blue variant and the olive group of variants were derived from 9 and 9 irradiated gametes, respectively. In each of these two kinds of color variants, the mutations in the different gametes were the same in locus. In the blue

variant, the xanthophores are abnormal, that is, they contained no carotenoid vesicles, while in the olive group of variants, the iridophores are abnormal, that is, the reflecting platelets contained in them decrease in number and thickness. The mutations giving rise to the abnormality of xanthophores and iridophores are given signs *x* and *i*, respectively.

8. Six kinds of color variants are produced by mutations of genes *X* and *I* in green and brown frogs. The phenotypes of *xxIE*, *XiiE* and *xxiiE* are blue, greenish-olive and bluish-olive, respectively, while those of *xxIee*, *Xiiee* and *xxiiee* are grayish-brown, yellowish-olive and brownish-olive, respectively. Of these variant, the grayish-brown is hardly distinguished from the brown wild-type in appearance, although the skins of these two are clearly different from each other under a microscope.

9. A total of 28 mutations consisting of five kinds of color genes *m*, *g*, *b*, *x* and *i* occurred in 17 (50%) of 34 spermatozoa and 11 (37%) of 30 unfertilized eggs after irradiation with X-rays or neutrons.

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EXPLANATION OF PLATES

PLATE I

Color variants induced by radiation in *Rana nigromaculata*, I.

1. Albino derived from an egg exposed to 145 rads of X-rays. ×0.7
2. Albino derived from a spermatozoon exposed to 130 rads of neutrons. ×0.7

3, 4. Black-eyed variant derived from a spermatozoon exposed to 90 rads of X-rays. ×1.2

5, 6. Gray-eyed variant derived from a spermatozoon exposed to 90 rads of X-rays. ×0.9

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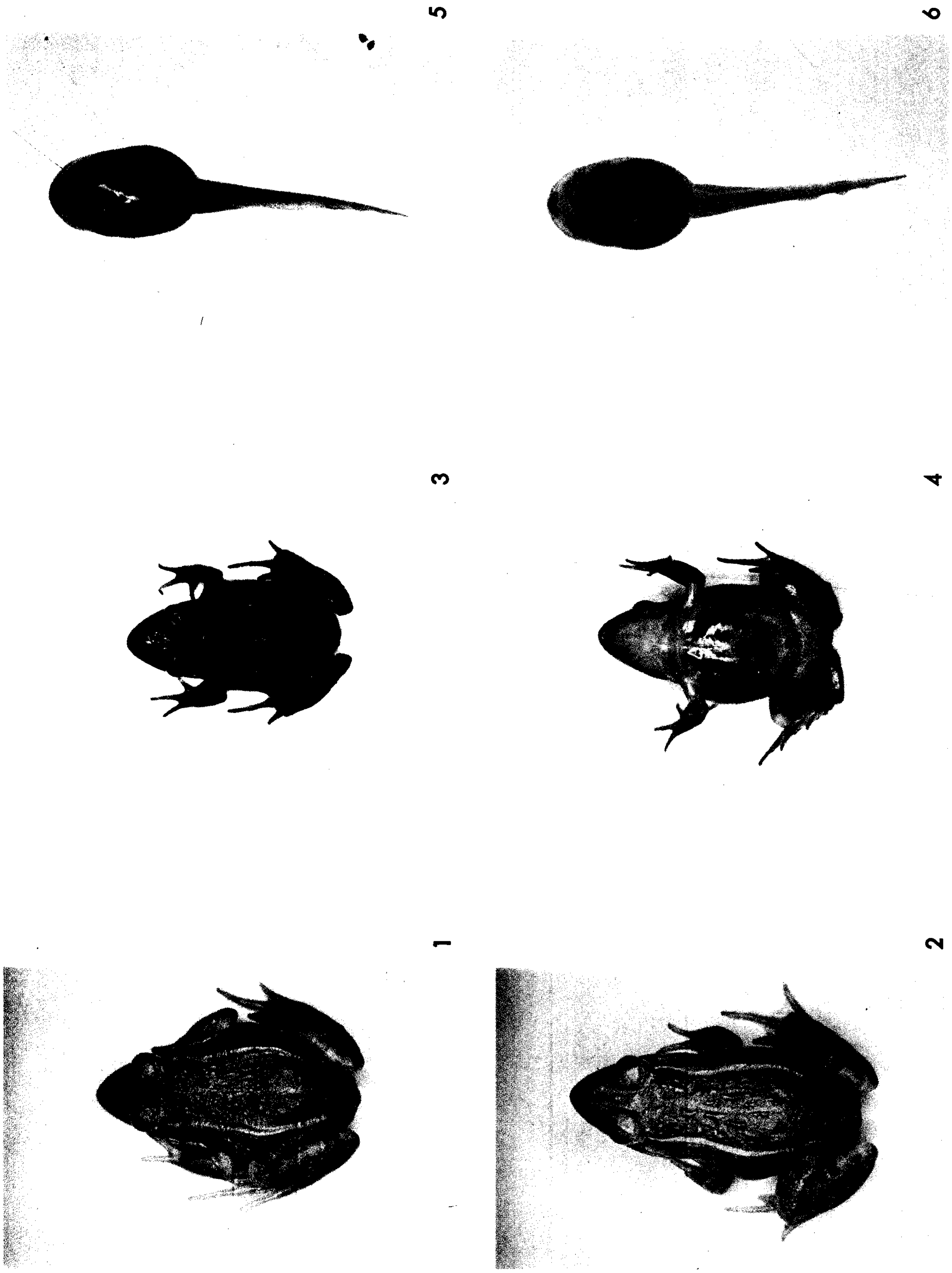

PLATE II

Color variants induced by radiation in *Rana nigromaculata*, II. ×0.9

7. Green-type control frog.
8. Brown-type control frog.
9. Blue variant derived from a spermatozoon exposed to 170 rads of X-rays.
10. Grayish-brown variant derived from a spermatozoon exposed to 170 rads of X-rays.
11. Greenish-olive variant derived from a spermatozoon exposed to 130 rads of neutrons.
12. Yellowish-olive variant derived from a spermatozoon exposed to 130 rads of neutrons.
13. Bluish-olive variant derived from a spermatozoon exposed to 130 rads of neutrons.
14. Brownish-olive variant derived from a spermatozoon exposed to 130 rads of neutrons.

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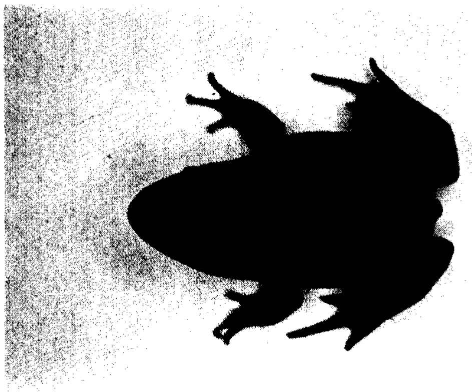
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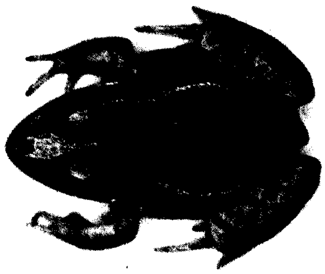
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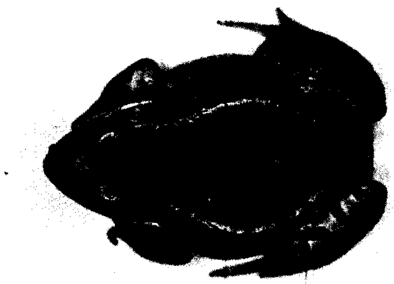
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