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Inter- and Intraspecific Hybrids among Japanese, European and American Toads

By

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(With 24 Text-figures and 22 Plates)

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INTRODUCTION

The earliest studies on the interspecific hybridizations in the genus *Bufo* were made in Europe about one hundred years ago. The first researcher was DE L'ISLE (1872) who made reciprocal crosses between *Bufo vulgaris* (=*B. bufo*) and *Bufo calamita* by artificial fertilization. The eggs fertilized with foreign sperm mostly died during the embryonic stage. Although only a small number of them hatched, they could not reach the metamorphosing stage. HÉRON-ROYER (1883) also made reciprocal crosses between the same two species by natural fertilization following the amplexus. According to him, five of the hybrids produced from a female *Bufo vulgaris* by mating with a male *Bufo calamita* completed metamorphosis, while all the others usually died of deformities at the embryonic and tadpole stages. HÉRON-ROYER (1891) afterwards obtained fertilized eggs by pairing a female *Bufo vulgaris* (=*B. bufo*) with a male *Bufo arabicus* (=*B. viridis*). Only one of these eggs developed normally through metamorphosis and died at the age of 15 months and the others died of abnormalities at various developmental stages. BORN (1883, 1886) made reciprocal crossings between *Bufo cinereus* (=B. *bufo*) and *Bufo variabilis* (=*B. viridis*) and found that the two kinds of crossings differed from each other in the results. While the hybrids between female *Bufo cinereus* and male *Bufo variabilis* could metamorphose, the reciprocal hybrids all died at the embryonic or early tadpole stage. PFLÜGER and SMITH (1883) presented the result of a crossing, *Bufo variabilis* (=*B. viridis*) ♀ × *Bufo calamita* ♂, in a table attached to their report, together with those of many other kinds of crossings. The eggs of *Bufo variabilis* inseminated with sperm of *Bufo calamita* cleaved either normally or abnormally and all of them ceased their development. G. HERTWIG (1918, 1930) confirmed BORN's results by repeating reciprocal crosses between *Bufo communis* (=*B. bufo*) and *Bufo viridis*. The cross, *Bufo communis* ♀ × *Bufo viridis* ♂, produced many normally metamorphosing hybrids besides some inviable ones. In contrast, the hybrids produced from the cross, *Bufo viridis* ♀ × *Bufo communis* ♂, were inviable, although a few of them could survive for 12 days. Most of them died of abnormalities that were attributable to retention of the yolk plug at the embryonic stage.

MONTALENTI (1933) also confirmed that reciprocal hybrids between *Bufo vulgaris* (=*B. bufo*) and *Bufo viridis* were very different in viability. While the great majority of the embryos produced from the cross, *Bufo vulgaris*♀ × *Bufo viridis*♂, reached metamorphosis earlier than the controls and showed a low mortality, the reciprocal hybrids began to show abnormalities at the blastula or gastrula stage and gradually died. Even the best developed hybrids perished with symptoms of hydropsy before the hind limbs were formed. The development and external characteristics of the hybrids between a female *Bufo vulgaris* and a male *Bufo viridis* were described in detail by MONTALENTI (1938a). However, the reproductive capacity of these hybrids as well as their sex ratio were not clarified (MONLALENTI, 1938b). Recently, W. F. BLAIR (1972) obtained a small number of metamorphosed hybrids by one of three crossings, *Bufo viridis*♀ × *Bufo bufo bufo*♂.

In the United States of America, MILLER and CHAPIN (1910) and DECKERT (1917) reported that there was some possibility of natural hybridization between *Bufo americanus* and *Bufo fowleri*. PICKENS (1927) found two intermediate specimens, one between *Bufo americanus* and *Bufo fowleri* and the other between *Bufo fowleri* and *Bufo terrestris*, together with a fairly typical *Bufo fowleri*. SMITH (1934) described the area of intergradation between *Bufo woodhousei* and *Bufo fowleri*. Natural hybridization in the genus *Bufo* was also suggested by HARPER (1935) and BURT (1938) between *Bufo americanus* and *Bufo terrestris*, by BRAGG (1939) between *Bufo americanus* and *Bufo cognatus* and by the same author (1940) between *Bufo americanus* and *Bufo woodhousei*. A. P. BLAIR (1941, 1942, 1946) made extensive studies on the isolating mechanisms among five allied *Bufo* species, *americanus*, *fowleri*, *terrestris*, *valliceps* and *woodhousei*, by field work and laboratory experiments. In addition to the above five kinds of intermediate individuals, he found another kind between *fowleri* and *valliceps* in the area where the distribution regions of these two species overlapped. By experimental crossings following the amplexus, he obtained six kinds of metamorphosed hybrids between *americanus* and *fowleri*, *americanus* and *terrestris*, *fowleri* and *terrestris*, *woodhousei* and *fowleri*, *americanus* and *woodhousei*, and *terrestris* and *valliceps*. Of these kinds of hybrids, three obtained from crosses between *americanus* and *fowleri*, *woodhousei* and *fowleri*, and *americanus* and *woodhousei* attained sexual maturity. Male hybrids between *americanus* and *fowleri*, and *americanus* and *woodhousei* were found to be fertile by backcrossing to female *americanus*. VOLPE (1952 a, b) suggested from his field observations and laboratory experiments that there had been introgression of *Bufo americanus* into *Bufo fowleri*. THORNTON (1955) confirmed that some males of the hybrids between female *Bufo woodhousei* and male *Bufo valliceps* reached sexual maturity and produced mostly inviable zygotes when backcrossed to females of the parental species, while the reciprocal hybrids were inviable in the late neurula stage. A. P. BLAIR (1955) found natural hybrids between *Bufo microscaphus* and *Bufo woodhousei* at all the localities where the two species coexisted. Experimental crossings were made by him between *Bufo microscaphus* and *Bufo woodhousei*, *Bufo punctatus*, *Bufo compactilis*, or *Bufo boreas*. It was found that reciprocal

hybrids between *microscaphus* and *woodhousei* and the hybrids between female *microscaphus* and male *punctatus, compactilis* or *boreas* could reach metamorphosis, and that the hybrids between female *punctatus* and male *microscaphus* were difficult in gastrulation and could not reach metamorphosis in contrast with the reciprocal hybrids. CORY and MANION (1955) found *Bufo americanus* in wooded areas, *Bufo fowleri* in open areas and hybrid populations in ecologically mixed areas.

Natural hybridizations of *Bufo* species in Europe were reported by FLINDT and HEMMER (1967a, b) for the first time. The hybrids produced in the laboratory from a cross, *Bufo viridis*♀ × *Bufo calamita*♂, became mostly adult toads, while the reciprocal hybrids were abnormal tadpoles and died before becoming 31 days old. Cross-pairing of these two species as well as individuals resembling the laboratory hybrids between them were observed by these authors in nature. W. F. BLAIR (1972) performed hundreds of hybridization experiments among numerous *Bufo* species collected from North, Central and South America, Europe and Africa, and developed an evolutionary history of this genus on the basis of the results of experiments. He reared various kinds of viable hybrids passing over metamorphosis until sexual maturity and examined their reproductive capacity. BOGART (1972) examined the chromosomes of 50 *Bufo* species of the four continents and 175 hybrid combinations among these species.

No hybridization experiments were performed among toads distributed in the East except for those by CHEN (1940). Thus, Professor CEI, Faculty of Sciences, University of Lisbon, Portugal, recommended in 1975 that we make a study on artificial hybridization between Japanese and European toads. The present authors accepted Professor CEI's proposal and decided to conduct hybridization experiments in order to elucidate the existence of reproductive isolation between Japanese and European toads as well as between different kinds of Japanese toads. In addition to Japanese and European species and subspecies, an American species and a Chinese subspecies were used as complementary materials in the present study. The main results of the experiments performed hitherto will be reported here.

MATERIALS AND METHODS

During the four years from 1976 to 1979, forty kinds of crosses were made by using the following nine species and subspecies of *Bufo*.

1. *Bufo bufo japonicus* SCHLEGEL from the plateau of Kammuri, Hiroshima Prefecture. A total of eleven females and seven males were collected by one of the present authors; two females (Nos. 1 and 2) and three males (Nos. 1～3) in 1976, nine females (Nos. 3～11) and four males (Nos. 4～7) in 1977.

2. *Bufo bufo miyakonis* OKADA from Miyakojima Island. Three females (Nos. 1～3) were collected by Dr. M. KURAMOTO in April, 1977 and two females (Nos. 4 and 5) and two males (Nos. 1 and 2) were collected by Mr. M. MATSUI in February, 1979.

3. *Bufo bufo yakushimensis* OKADA from Yakushima Island. One male (No. 1)

was collected by Mr. S. OHTA in July, 1978. As this toad was immature at that time, it was used after rearing for about one year.

4. *Bufo torrenticola* M. MATSUI from the plateau of Odaigahara, Nara Prefecture. Four females (Nos. 1～4) and four males (Nos. 1～4) were collected by H. UEDA, one of the present authors in May, 1977.

5. *Bufo bufo bufo* (L.) from the province of Minho, Portugal. Five females (Nos. 1～5) and five males (Nos. 1～5) were sent here by Dr. E. CRESPO in January, 1976.

6. *Bufo bufo* subspecies from the forest of Chize, France. One male (No. 1) was collected by Drs. H. HOTZ and S. BRUNO on April 18, 1976. This toad was sent here in October, 1976 by Professor B. LANZA.

7. *Bufo bufo* subspecies from the Pindo Mountains, Metsovon, Greece. One male (No. 1) was collected by Drs. H. HOTZ and S. BRUNO on August 12, 1976, and sent here in October, 1976 by Professor B. LANZA.

8. *Bufo viridis* LAURENTI from Turkey. One female (No. 1) and one male (No. 1) were given us by Dr. M. KURAMOTO in April, 1977.

9. *Bufo americanus* HOLBROOK from Ann Arbor, Michigan, U.S.A. Three females (Nos. 1～3) and three males (Nos. 1～3) were collected by M. NISHIOKA, one of the present authors, in August, 1976 and reared from about one year in the Laboratory.

In addition to these species and subspecies, the following subspecies from Peking (Beiging), China was used in mating with male hybrids between two Japanese species or subspecies.

10. *Bufo bufo gargarizans* CANTOR from Peking (Beiging). Males and females were collected by Professor CHIH-YE CHANG, Institute of Zoology, Academia Sinica, China.

Ovulation was accelerated by injecting *Rana catesbeiana* pituitaries. Sperm suspension was made by crushing a piece of a testis in a small quantity of distilled water. This piece was removed from the testis of a male after laparotomy. All the matings were made by the artificial fertilization method. Fertilized eggs were developed at shade temperature (17～24°C) until the stage shortly after hatching. The feeding tadpoles produced in 1976 and 1977 were all kept at about 18°C, while those obtained in 1978, 1979 and 1980 were not always kept at this constant temperature. Tadpoles fed on boiled spinach. After metamorphosis, nymphs at various developmental stages and adults of the two-spotted cricket, *Gryllus bimaculatus* DE GEER were given to the toads (NISHIOKA and MATSUURA, 1977).

In order to observe the inner structure of gonads, the gonads were removed from the toads and fixed in NAVASHIN's fluid for 24 hours. Serial sections were made at 12 μ after imbedded in paraffin and stained with HEIDENHAIN's iron hematoxylin. The chromosomes of tadpoles were observed in squash preparations of tail-tips which were made in accordance with MAKINO and NISHIMURA (1952) as follows. Without keeping the tadpoles in a colchicine solution before preparations were made, their tail-tips were cut off and immersed in distilled water

for 60~120 minutes. They were stained with 1% orcein (Chroma) dissolved in 45% acetic acid for 30~60 minutes on a slide glass, and then squashed under a cover glass after heated for 20~30 seconds. They were lastly mounted with PVLB*. The chromosomes of adult toads were observed in bone marrow cells, whose preparations were made by OMURA's method (1967). A small amount of colchicine (Merk) dissolved in RINGER's solution** was previously injected into abdominal cavity. After 15~18 hours, the bone marrow cells were pushed out from long bones of the hind legs with sodium citrate solution. They were first fixed with a ethanol vaper and then with a mixture of ethanol and glacial acetic acid. After air-dried, they were stained with GIEMSA stain.

The following abbreviations are used for designation of each kind of toads.

jap. — *Bufo bufo japonicus* SCHLEGEL
miy. — *Bufo bufo miyakonis* OKADA
yak. — *Bufo bufo yakushimensis* OKADA
tor. — *Bufo torrenticola* M. MATSUI
bufo P. — *Bufo bufo bufo* (L.) from Portugal
bufo F. — *Bufo bufo* subspecies from France
bufo G. — *Bufo bufo* subspecies from Greece
vir. — *Bufo viridis* LAURENTI from Turkey
ame. — *Bufo americanus* HOLBROOK
gar. — *Bufo bufo gargarizans* CANTOR from China
W — Field-caught toad

OBSERVATION

I. Developmental capacity of hybrids

1. Crosses between female *Bufo bufo japonicus* and eight kinds of males

a. Control matings of *Bufo bufo japonicus*

In the breeding seasons of the years 1976~1979, matings were made between eight females (Nos. 1~8) and five males (Nos. 1, 2, 4, 6 and 7). The results showed that 85.0~99.4% of the respecitve number of eggs, 3578 (90.8%) of 3941 eggs in total, cleaved normally. While 807 eggs died of various abnormalities during the embryonic stage, 55.1~94.9%, 70.3% on the average, hatched normally. However, 47.5~80.5%, 59.6% on the average ate normally. Eventually, 33.8~71.2%, 47.5% on the average, that is, 39.8~71.6%, 52.3% on the average, of normally cleaved eggs completed metamorphosis. There were 1873 normally metamorphosed toads in total (Table 1).

b. Matings with males of three kinds of Japanese toads

Three female *Bufo bufo japonicus* (Nos. 3~5) were mated with a male *Bufo torrenticola* (No. 1) in 1977, and another female *Bufo bufo japonicus* (No. 8) was

* Paraffin, vaseline, lanolin and Canada balsam=2: 1: 1: 1

** 50 mg of colchicine in 1,000 ml of RINGER's solution

TABLE 1

Developmental capacity of hybrids between female *Bufo bufo japonicus* and eight kinds of male *Bufo* and their controls

Years	Parents*		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally meta-morphosed toads
	Female	Male					
1976	*jap.* W, Nos. 1, 2	*jap.* W, Nos. 1, 2	2047	1740 (85.0%)	1127 (55.1%)	973 (47.5%)	692 (33.8%)
		bufo P. W, Nos. 1, 2	3758	2291 (61.0%)	1269 (33.8%)	1156 (30.8%)	897 (23.9%)
1977	*jap.* W, Nos. 3~5	*jap.* W, No. 4	874	846 (96.8%)	718 (82.2%)	570 (65.2%)	520 (59.5%)
		tor. W, No. 1	650	482 (74.2%)	441 (67.8%)	396 (60.9%)	373 (57.4%)
		bufo F. W, No. 1	771	484 (62.8%)	290 (37.6%)	239 (31.0%)	141 (18.3%)
		bufo G. W, No. 1	1197	906 (75.7%)	661 (55.2%)	405 (33.8%)	382 (31.9%)
		ame. W, No. 1	1321	332 (25.1%)	0	0	0
1978	*jap.* W, Nos. 6, 7	*jap.* W, No. 6	708	682 (96.3%)	630 (89.0%)	570 (80.5%)	439 (62.0%)
		vir. W, No. 1	952	669 (70.3%)	411 (43.2%)	366 (38.4%)	276 (29.0%)
		ane. W, No. 2	1057	828 (78.3%)	0	0	0
1979	*jap.* W, No. 8	*jap.* W, No. 7	312	310 (99.4%)	296 (94.9%)	237 (76.0%)	222 (71.2%)
		yak. W, No. 1	447	403 (90.2%)	307 (68.7%)	289 (64.7%)	187 (41.8%)
		miy. W, No. 1	348	340 (97.7%)	337 (96.8%)	308 (88.5%)	291 (83.6%)
Total	*jap.* (8)	*jap.* (5)	3941	3578 (90.8%)	2771 (70.3%)	2350 (59.6%)	1873 (47.5%)
	jap. (1)	*yak.* (1)	447	403 (90.2%)	307 (68.7%)	289 (64.7%)	187 (41.8%)
	jap. (1)	*miy.* (1)	348	340 (97.7%)	337 (96.8%)	308 (88.5%)	291 (83.6%)
	jap. (3)	*tor.* (1)	650	482 (74.2%)	441 (67.8%)	396 (60.9%)	373 (57.4%)
	jap. (2)	*bufo* P. (2)	3758	2291 (61.0%)	1269 (33.8%)	1156 (30.8%)	897 (23.9%)
	jap. (3)	*bufo* F. (1)	771	484 (62.8%)	290 (37.6%)	239 (31.0%)	141 (18.3%)
	jap. (3)	*bufo* G. (1)	1197	906 (75.7%)	661 (55.2%)	405 (33.8%)	382 (31.9%)
	jap. (2)	*vir.* (1)	952	669 (70.3%)	411 (43.2%)	366 (38.4%)	276 (29.0%)
	jap. (5)	*ame.* (2)	2378	1160 (48.8%)	0	0	0

* Parentheses show the number of toads.

mated with a male *Bufo bufo yakushimensis* (No. 1) and a male *Bufo bufo miyakonis* (No. 1) in 1979. By these three kinds of matings, 74.2%, 90.2% and 97.7% of the respective total number of eggs cleaved normally. While 41, 96 and 3 eggs died of abnormalities during the embryonic stage, 67.8%, 68.7% and 96.8% hatched normally. These percentages were not inferior to those in the control matings. After the hatching stage embryos produced from the three kinds of crosses grew normally, 60.9%, 64.7% and 88.5% became feeding tadpoles and 57.4%, 41.8% and 83.6%, that is, 77.4%, 46.4% and 85.6% of normally cleaved eggs metamorphosed normally (Table 1).

When the present authors were rearing the hybrid tadpoles obtained from reciprocal crosses between *japonicus* and *torrenticola*, they found that they were intermediate in behavior between the two kinds of controls. When water in the glass basin was eddied, the *torrenticola* tadpoles, torrent dwellers, stuck to the wall of the basin at once, while the *japonicus* tadpoles, still-water dwellers, were drawn into the eddy without sticking to the wall. The hybrid tadpoles were intermediate in sticking ability between the two species.

c. Matings with males of four kinds of European toads

Two female *Bufo bufo japonicus* (Nos. 1 and 2) were mated with two *Bufo bufo bufo* from Portugal (Nos. 1 and 2) in 1976, while three other female *Bufo bufo japonicus* (Nos. 3~5) were mated with a male *Bufo bufo* from France (No. 1) and a male *Bufo bufo* from Greece (No. 1). By these three kinds of matings, 61.0%, 62.8%

and 75.7% of the respective total number of eggs cleaved normally. While 44.6%, 40.1% and 27.0% of the normally cleaved eggs died of various abnormalities at the embryonic stage, 16.2%, 30.8% and 30.8% died of ill-development or some other abnormalities during the tadpole stage. Eventually, 33.8%, 37.6% and 55.2% of the respective total number of eggs hatched normally and 23.9%, 18.3% and 31.9%, that is, 39.2%, 29.1% and 42.2% of normally cleaved eggs metamorphosed normally. These percentages were nearly equal to or somewhat lower than those in the control matings.

Two other female *Bufo bufo japonicus* (Nos. 6 and 7) were mated with a male *Bufo viridis* in 1978. It was found that 70.3% of the total number of eggs cleaved normally and 43.2% hatched normally. While 29.0%, that is, 41.3% of normally cleaved eggs completed metamorphosis, about one-third of normally hatched individuals died during the tadpole stage. These percentages indicated that this kind of hybrids scarcely differed in viability from those obtained from the matings between the females of Japanese *bufo* and the males of the three kinds of European *bufo*.

d. Matings with male American toads

Three female *Bufo bufo japonicus* (Nos. 3~5) were mated with a male *Bufo americanus* (No. 1) in 1977. The results showed that only 25.1% of eggs cleaved normally. Although the normally cleaved eggs were somewhat delayed in development, 21.7% became normal neurulae. The other were incomplete in gastrulation and died of severe abnormality at the neurula stage. All the normally

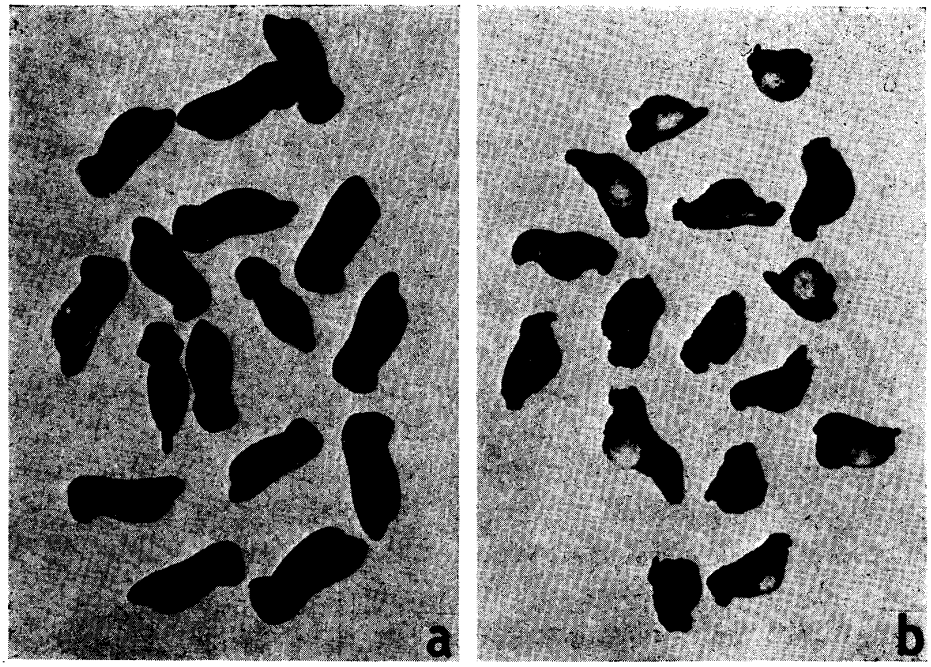

Fig. 1. Abnormalities at the embryonic stage in the hybrids between a female *Bufo bufo japonicus* and a male *Bufo americanus*. ×2.5

a. Controls, *japonicus* ♀ No. 3 × *japonicus* ♂ No. 4

b. Hybrids, *japonicus* ♀ No. 3 × *americanus* ♂ No. 1

shaped neurulae became abnormal at the tail-bud stage and most of them died before the hatching stage (Fig. 1).

Two other female *Bufo bufo japonicus* (Nos. 6 and 7) were mated with another male *Bufo americanus* (No. 2). While 78.3% of eggs cleaved normally, all the cleaved eggs ceased their development at the neurula stage and shortly died.

2. Crosses between female *Bufo bufo miyakonis* and six kinds of males

a. Control matings of *Bufo bufo miyakonis*

Matings were made between two females (Nos. 2 and 3) and one male (No. 1) in 1979 (Table 2). The results indicated that 97.7% of eggs cleaved normally, and 96.5% hatched normally. After 91.4% became feeding tadpoles, 82.8%, that is 84.8% of normally cleaved eggs, metamorphosed normally.

b. Matings with males of three kinds of Japanese toads

Three female *Bufo bufo miyakonis* (Nos. 1~3) were mated with two male *Bufo bufo japonicus* (Nos. 6 and 7), one male *Bufo bufo yakushimensis* (No. 1) and two male *Bufo torrenticola* (Nos. 3 and 4) in 1978 and 1979. By these three kinds of matings, 97.2%, 99.2% and 97.2% of the respective total number of eggs cleaved normally. While a few of these normally cleaved eggs died at the embryonic stage, 95.7%, 97.4% and 94.2% hatched normally, respectively, and 89.2%, 96.1% and 86.9% began to eat, respectively. The other hatched embryos became abnormal and died without taking food. After some tadpoles died of ill-development, 79.1%, 51.5% and 86.1%, that is, 81.4%, 51.9% and 88.6% of normally cleaved eggs attained the completion of metamorphosis (Table 2).

TABLE 2

Developmental capacity of hybrids between female *Bufo bufo miyakonis* and six kinds of male *Bufo* and their controls

Years	Parents*		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally meta-morphosed toads
	Female	Male					
1978	*miy.* W, No. 1	*jap.* W, No. 6	648	637 (98.3%)	637 (98.3%)	596 (92.0%)	530 (81.8%)
		tor. W, No. 3	517	517 (100 %)	500 (96.7%)	463 (89.6%)	463 (89.6%)
		bufo P. W, No. 5	405	278 (68.6%)	277 (68.4%)	268 (66.2%)	234 (57.8%)
		vir. W, No. 1	678	512 (75.5%)	492 (72.6%)	464 (68.4%)	410 (60.5%)
		ame. W, No. 2	1044	1015 (97.2%)	0	0	0
1979	*miy.* W, Nos. 2, 3	*jap.* W, No. 7	165	153 (92.7%)	141 (85.5%)	129 (78.2%)	113 (68.5%)
		yak. W, No. 1	388	385 (99.2%)	378 (97.4%)	373 (96.1%)	200 (51.5%)
		miy. W, No. 1	256	250 (97.7%)	247 (96.5%)	234 (91.4%)	212 (82.8%)
		tor. W, No. 4	161	142 (88.2%)	139 (86.3%)	126 (78.3%)	121 (75.2%)
		bufo P. W, No. 5	159	130 (81.8%)	112 (70.4%)	77 (48.4%)	56 (35.2%)
		vir. W, No. 1	204	150 (73.5%)	124 (60.8%)	92 (45.1%)	91 (44.6%)
		ame. W, No. 3	265	96 (36.2%)	0	0	0
Total	*miy.* (3)	*jap.* (2)	813	790 (97.2%)	778 (95.7%)	725 (89.2%)	643 (79.1%)
	miy. (2)	*yak.* (1)	388	385 (99.2%)	378 (97.4%)	373 (96.1%)	200 (51.5%)
	miy. (2)	*miy.* (1)	256	250 (97.7%)	247 (96.5%)	234 (91.4%)	212 (82.8%)
	miy. (3)	*tor.* (2)	678	659 (97.2%)	639 (94.2%)	589 (86.9%)	584 (86.1%)
	miy. (3)	*bufo* P. (1)	564	408 (72.3%)	389 (69.0%)	345 (61.2%)	290 (51.4%)
	miy. (3)	*vir.* (1)	882	662 (75.1%)	616 (69.8%)	556 (63.0%)	501 (56.8%)
	miy. (3)	*ame.* (2)	1300	1111 (85.5%)	0	0	0

* Parentheses show the number of toads.

c. Matings with males of two kinds of European toads

The same three female *Bufo miyakonis* (Nos. 1～3) as used in the above matings were mated with one male *Bufo bufo bufo* from Portugal (No. 5) and one male *Bufo viridis* (No. 1) in 1978 and 1979. By these two kinds of matings, 72.3% and 75.1% of the respective total number of eggs cleaved normally. These percentages were somewhat lower than those in the matings with male *miyakonis*, *japonicus*, *yakushimensis* and *torrenticola*. While some of the normally cleaved eggs died of various abnormalities or ill-development at the embryonic and tadpole stages, 69.0% and 69.8% of the respective total number of eggs hatched normally, and eventually, 51.4% and 56.8%, that is, 71.1% and 75.7% of normally cleaved eggs metamorphosed normally. These percentages were somewhat lower than those in the control matings (Table 2).

When the *miyakonis* tadpoles were compared with the Portuguese tadpoles at the feeding tadpole stage (stage 25), the two kinds of tadpoles resembled closely each other in appearance, although they differed from each other in size in accordance with the difference in egg size. The hybrid tadpoles produced from the cross, *miyakonis* ♀ × Portuguese *bufo* ♂, differed from the two kinds of control

24.9% and 3.0% of normally cleaved eggs attained the completion of metamorphosis. The others of the normally cleaved eggs died of various abnormalities or ill-development at the embryonic and tadpole stages. It was evident that the hybrids were remarkably inferior in viability to the controls as well as to the hybrids derived from males of the other Japanese toad species (Table 3).

d. Mating with a male American toad

A female *Bufo torrenticola* (No. 3) was mated with a male *Bufo americanus* (No. 3) in 1979. While 73.0% of eggs cleaved normally, they began to delay in development at the gastrula stage. All the embryos became abnormal at the neurula stage and died sooner or later (Fig. 3).

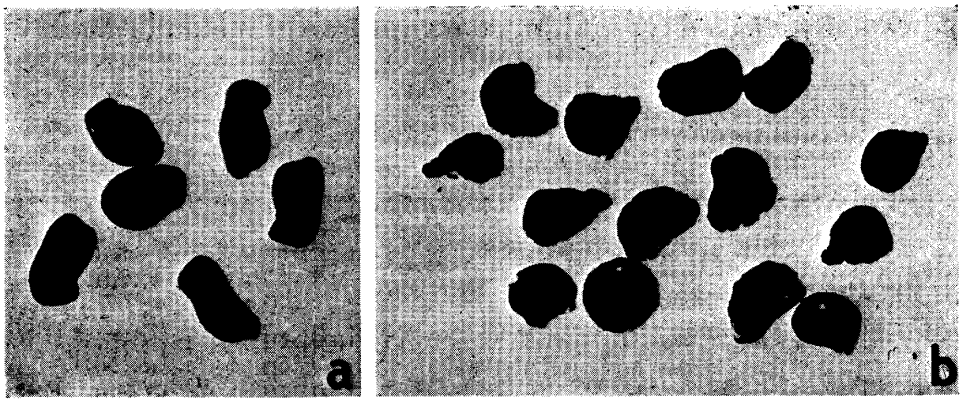

Fig. 3. Abnormalities at the embryonic stage in the hybrids between a female *bufo torrenticola* and a *Bufo americanus*. ×2.5

a. Controls, *torrenticola* ♀ No. 3 × *torrenticola* ♂ No. 4

b. Hybrids, *torrenticola* ♀ No. 3 × *americanus* ♂ No. 3

found that 97.2% and 36.2% of the respective number of eggs cleaved normally in 1978 and 1979, respectively. The normally cleaved eggs began to be delayed in development at the gastrula stage and became abnormal at the neurula stage. Although some individuals became abnormal tail-bud embryos, no embryos hatched normally (Table 2).

3. Crosses between female *Bufo torrenticola* and six kinds of males

a. Control matings of *Bufo torrenticola*

Three females (Nos. 1~3) were mated with two males (Nos. 2 and 4) in 1977 and 1979. It was found that 93.1% and 97.5% of the total number of eggs cleaved normally, and 82.3% and 94.9% hatched normally in 1977 and 1979, respectively. While some tadpoles died of ill-development, 60.4% and 88.9%, that is, 65.0% and 91.2% of normally cleaved eggs, attained completion of metamorphosis (Table 3).

TABLE 3

Developmental capacity of hybrids between female *Bufo torrenticola* and six kinds of male *Bufo* and their controls

Years	Parents		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally meta-morphosed toads
	Female	Male					
1977	*tor.* W, Nos. 1, 2	*tor.* W, No. 2	2228	2074 (93.1%)	1834 (82.3%)	1684 (75.6%)	1346 (60.4%)
		jap. W, No. 5	2180	1984 (91.0%)	1815 (83.3%)	1556 (71.4%)	1142 (52.4%)
1979	*tor.* W, No. 3	*tor.* W, No. 4	314	306 (97.5%)	298 (94.9%)	283 (90.1%)	279 (88.9%)
		yak. W, No. 1	236	215 (91.1%)	203 (86.0%)	197 (83.5%)	152 (64.4%)
		miy. W, No. 1	328	316 (96.3%)	303 (92.4%)	297 (90.5%)	296 (90.2%)
		bufo P. W, No. 5	401	374 (93.3%)	225 (56.1%)	202 (50.4%)	93 (23.2%)
		vir. W, No. 1	283	165 (58.3%)	88 (31.1%)	73 (25.8%)	5 (1.8%)
		ame. W, No. 3	400	292 (73.0%)	0	0	0

b. Matings with males of three kinds of Japanese toads

Two female *Bufo torrenticola* (Nos. 1 and 2) were mated with a male *Bufo bufo japonicus* (No. 5) in 1977. Another female *Bufo torrenticola* (No. 3) was mated with a male *Bufo bufo yakushimensis* (No. 1) and a male *Bufo bufo miyakonis* (No. 1) in 1979. By these three kinds of matings, 91.0%, 91.1% and 96.3% of the respective number of eggs cleaved normally. While some eggs died at the embryonic and tadpole stages, 83.3%, 86.0% and 92.4% hatched normally, and eventually 52.4%, 64.4% and 90.2%, that is, 57.6%, 70.7% and 93.7% of normally cleaved eggs attained the completion of metamorphosis. These percentages indicated that the hybrids, *torrenticola*♀ × *japonicus*♂ and *torrenticola*♀ × *miyakonis*♂, were nearly the same as the controls in viability, while the hybrids, *torrenticola*♀ × *yakushimensis*♂, were slightly inferior in this respect (Table 3).

c. Matings with males of two kinds of European toads

A female *Bufo torrenticola* (No. 3) was mated with a male *Bufo bufo bufo* from Portugal (No. 5) and a male *Bufo viridis* (No. 1). By these two kinds of matings, 93.3% and 58.3% of the respective number of eggs cleaved normally. However, 56.1% and 31.1% could normally hatched, and only 23.2% and 1.8%, that is,

24.9% and 3.0% of normally cleaved eggs attained the completion of metamorphosis. The others of the normally cleaved eggs died of various abnormalities or ill-development at the embryonic and tadpole stages. It was evident that the hybrids were remarkably inferior in viability to the controls as well as to the hybrids derived from males of the other Japanese toad species (Table 3).

d. Mating with a male American toad

A female *Bufo torrenticola* (No. 3) was mated with a male *Bufo americanus* (No. 3) in 1979. While 73.0% of eggs cleaved normally, they began to delay in development at the gastrula stage. All the embryos became abnormal at the neurula stage and died sooner or later (Fig. 3).

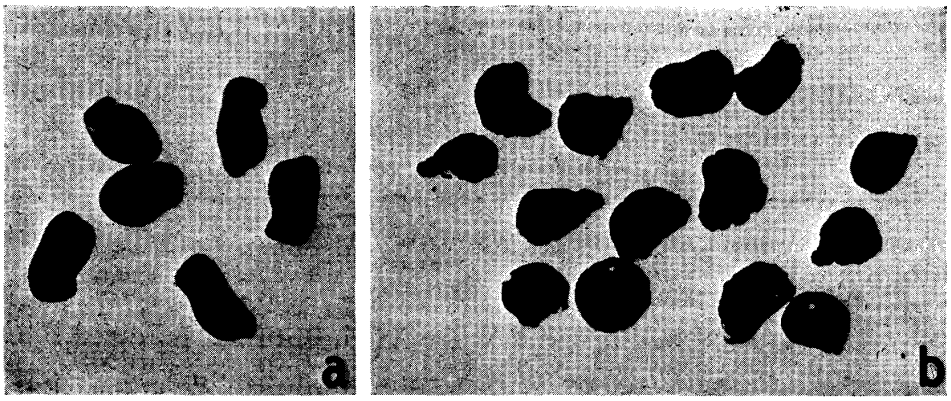

Fig. 3. Abnormalities at the embryonic stage in the hybrids between a female *bufo torrenticola* and a *Bufo americanus*. ×2.5

a. Controls, *torrenticola* ♀ No. 3 × *torrenticola* ♂ No. 4
b. Hybrids, *torrenticola* ♀ No. 3 × *americanus* ♂ No. 3

4. Crosses between female *Bufo bufo bufo* from Portugal and seven kinds of males

a. Control matings of *Bufo bufo bufo* from Portugal

Matings were made between five females (Nos. 1～5) and three males (Nos. 3～5) in 1976～1979. By these matings, 40.6～90.7%, 80.5% on the average, of the respective number of eggs cleaved normally. While about half of the normally cleaved eggs died of various abnormalities at the embryonic and feeding tadpole stages and of ill-development or edema at the later tadpole stage, the other grew normally. Of the respective number of eggs, 29.2～86.1%, 72.1% on the average, hatched normally, 27.1～85.6%, 62.3% on the average, became normally feeding tadpoles, and 18.8～60.5%, 40.0% on the average, that is, 39.8～66.7%, 49.7% on the average, of normally cleaved eggs attained completion of metamorphosis (Table 4).

b. Matings with males of three kinds of Japanese toads

A female *Bufo bufo bufo* from Portugal (No. 1) was mated with a male *Bufo bufo japonicus* (No. 3) in 1976. Two other female *Bufo bufo bufo* from Portugal

TABLE 4
Developmental capacity of hybrids between *Bufo bufo bufo* from Portugal and seven kinds of male *Bufo* and their controls

Years	Parents*		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally metamorphosed toads
	Female	Male					
1976	*bufo* P. W, No. 1	*jap.* W, No. 3	576	509 (88.4%)	495 (85.9%)	483 (83.9%)	399 (69.3%)
		bufo P. W, No. 3	410	372 (90.7%)	353 (86.1%)	351 (85.6%)	248 (60.5%)
1977	*bufo* P. W, No. 2	*bufo* P. W, No. 4	96	39 (40.6%)	28 (29.2%)	26 (27.1%)	18 (18.8%)
		bufo F. W, No. 1	330	154 (46.7%)	95 (28.8%)	82 (24.8%)	78 (23.6%)
		bufo G. W, No. 1	380	237 (62.4%)	150 (39.5%)	134 (35.3%)	121 (31.8%)
		ame. W, No. 1	512	246 (48.0%)	0	0	0
1978	*bufo* P. W, No. 3	*tor.* W, No. 3	372	291 (78.2%)	255 (68.5%)	198 (53.2%)	134 (36.0%)
		bufo P. W, No. 5	223	192 (86.1%)	189 (84.8%)	142 (63.7%)	80 (35.9%)
		vir. W, No. 1	376	158 (42.0%)	113 (30.1%)	20 (5.3%)	2 (0.5%)
		ame. W, No. 2	514	206 (40.1%)	0	0	0
1979	*bufo* P. W, Nos. 4, 5	*tor.* W, No. 4	383	290 (75.7%)	260 (67.9%)	231 (60.3%)	201 (52.5%)
		miy. W, No. 1	534	480 (89.9%)	455 (85.2%)	366 (68.5%)	324 (60.7%)
		bufo P. W, No. 5	597	465 (77.9%)	386 (64.7%)	307 (51.4%)	185 (31.0%)
		vir. W, No. 1	544	394 (72.4%)	244 (44.9%)	211 (38.8%)	97 (17.8%)
		ame. W, No. 3	699	439 (62.8%)	0	0	0
Total	*bufo* P. (1)	*jap.* (1)	576	509 (88.4%)	495 (85.9%)	483 (83.9%)	399 (69.3%)
	bufo P. (2)	*miy.* (1)	534	480 (89.9%)	455 (85.2%)	366 (68.5%)	324 (60.7%)
	bufo P. (3)	*tor.* (2)	755	581 (77.0%)	515 (68.2%)	429 (56.8%)	335 (44.4%)
	bufo P. (5)	*bufo* P. (3)	1326	1068 (80.5%)	956 (72.1%)	826 (62.3%)	531 (40.0%)
	bufo P. (1)	*bufo* F. (1)	330	154 (46.7%)	95 (28.8%)	82 (24.8%)	78 (23.6%)
	bufo P. (1)	*bufo* G. (1)	380	237 (62.4%)	150 (39.5%)	134 (35.3%)	121 (31.8%)
	bufo P. (3)	*vir.* (1)	920	552 (60.0%)	357 (38.8%)	231 (25.1%)	99 (10.8%)
	bufo P. (4)	*ame.* (3)	1725	891 (51.7%)	0	0	0

* Parentheses show the number of toads.

(Nos. 4 and 5) were mated with a male *Bufo bufo miyakonis* (No. 1) in 1979. In 1978 and 1979, three female *Bufo bufo bufo* from Portugal (Nos. 3~5) were mated with two male *Bufo torrenticola* (Nos. 3 and 4). By these three kinds of matings, 88.4%, 89.9% and 77.0% of the respective number of eggs cleaved normally and then 85.9%, 85.2% and 68.2% hatched normally. While a small number of tadpoles died of ill-development or various abnormalities, 69.3%, 60.7%, and 44.4%, that is, 78.4%, 67.5% and 57.7% of normally cleaved eggs metamorphosed normally. These percentages indicated that these hybrids were not inferior in viability to the controls (Table 4).

c. Matings with males of three kinds of European toads

A female *Bufo bufo bufo* from Portugal (No. 2) was mated with a male *Bufo bufo* from France (No. 1) and a male *Bufo bufo* from Greece (No. 1) in 1977. The results showed that 46.7% and 62.4% of the respective number of eggs cleaved normally. These percentages were not inferior to that of the control mating between the same female (No. 2) and a male *bufo* from Portugal (No. 4); 40.6% of eggs cleaved normally in this mating. The normally cleaved eggs were not inferior to the controls in the subsequent development, too. While 29.2% of eggs hatched normally and 18.8% metamorphosed normally in the control mating, 28.8% and 39.5% of the respective number of eggs hatched normally, and eventually, 23.6% and 31.8%, that is, 50.6% and 51.1% of normally cleaved eggs metamor-

phosed normally in the crosses. In other words, nearly half of the normally cleaved eggs attained completion of metamorphosis in each of the crosses as well as in the control mating (Table 4).

In 1978 and 1979, three female *Bufo bufo bufo* from Portugal (Nos. 3~5) were mated with a male *Bufo viridis* (No. 1). In contrast with the above crosses, the hybrids were remarkably inferior to the controls in viability, although 60.0% of eggs cleaved normally and 38.8% hatched normally. Most of the tadpoles died of ill-development or various abnormalities, and eventually 10.8%, that is, 17.9% of normally cleaved eggs attained completion of metamorphosis.

d. Matings with male American toads

Four female *Bufo bufo bufo* from Portugal (Nos. 2~5) were mated with three male *Bufo americanus* (Nos. 1~3) in 1977~1979. The results showed that 40.1~62.8%, 51.7% on the average, of the respective number of eggs cleaved normally. All the normally cleaved eggs became abnormal at the gastrula or neurula stage; there were no normal embryos at the tail-bud stage (Table 4).

5. Crosses between a female *Bufo viridis* and six kinds of males

a. Control mating of *Bufo viridis*

In 1978, only 12 of 2581 eggs cleaved normally when a female *Bufo viridis* (No. 1) was mated with a male *Bufo viridis* (No. 1). Of these fertilized eggs, ten hatched normally and six could complete metamorphosis. The same male and female were mated again in 1979. It was found that 32.2% of eggs cleaved normally and 13.1% hatched normally. While most of the normally cleaved eggs died of various abnormalities at the embryonic stage, the normally hatched embryos mostly developed normally during the tadpole stage. Eventually, 11.1%, that is, 34.6% of normally cleaved eggs attained completion of metamorphosis (Table 5).

TABLE 5

Developmental capacity of hybrids between a female *Bufo viridis* and six kinds of male *Bufo* and their controls

Years	Parents		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally metamorphosed toads
	Female	Male					
1979	*vir.* W, No. 1	*jap.* W, No. 7	660	242 (36.7%)	81 (12.3%)	70 (10.6%)	23 (3.5%)
		yak. W, No. 1	1060	154 (14.5%)	66 (6.2%)	56 (5.3%)	40 (3.8%)
		miy. W, No. 1	876	200 (22.8%)	88 (10.0%)	79 (9.0%)	48 (5.5%)
		tor. W, No. 4	962	413 (42.9%)	288 (29.9%)	201 (20.9%)	74 (7.7%)
		bufo P. W, No. 5	979	118 (12.1%)	47 (4.8%)	20 (2.0%)	9 (0.9%)
		vir. W, No. 1	1480	477 (32.2%)	194 (13.1%)	176 (11.9%)	165 (11.1%)
		ame. W, No. 3	1126	335 (29.8%)	102 (9.1%)	0	0

b. Matings with males of four kinds of Japanese toads

The above female (No. 1) was mated with a male *Bufo bufo japonicus* (No. 7), a male *Bufo bufo yakushimensis* (No. 1), a male *Bufo bufo miyakonis* (No. 1) and a male *Bufo torrenticola* (No. 4) in 1979. By these four kinds of matings, 36.7%,

14.5%, 22.8% and 42.9% of the respective number of eggs cleaved normally and then 12.3%, 6.2%, 10.0% and 29.9% hatched normally. Although the four kinds of hybrids were not remarkably inferior in viability to the controls until the hatching stage, they were much more feeble than the controls during the tadpole stage. After many tadpoles died of ill-development or various abnormalities, 3.5%, 3.8%, 5.5% and 7.7%, that is, 9.5%, 26.0%, 24.0% and 17.9% of normally cleaved eggs attained completion of metamorphosis (Table 5).

c. Mating with a male European toad

The above female (No. 1) was mated with a male *Bufo bufo bufo* from Portugal (No. 5) in 1979. Only 12.1% of eggs cleaved normally and then 4.8% hatched normally. More than half of the normally hatched embryos died before they began to eat, and eventually only 0.9%, that is, 7.6% of normally cleaved eggs metamorphosed normally.

d. Mating with a male American toad

The above female (No. 1) was mated with a male *Bufo americanus* (No. 3) in 1979. It was observed that 29.8% of eggs cleaved normally, and most of them died of various abnormalities at the neurula or tail-bud stage. The remaining embryos, 9.1% of the total number of eggs, could hatch, although all of them were abnormal in shape. There were no individuals which began to eat (Table 5).

6. Crosses between female *Bufo americanus* and seven kinds of males

a. Control matings of *Bufo americanus*

Matings were made between three females (Nos. 1~3) and three males (Nos. 1~3). By these matings, 86.0~88.7%, 87.2% on the average, of the respective number of eggs cleaved normally. While a small number of embryos died of various abnormalities, 77.3~86.1%, 79.7% on the average, hatched normally. During the tadpole stage, numerous individuals died of ill-development; eventually 15.4~45.2%, 35.1% on the average, that is, 17.5~52.6%, 40.3% on the average, of normally cleaved eggs metamorphosed normally (Table 6).

b. Matings with males of three kinds of Japanese toads

The same three females (Nos. 1~3) as used in the control matings were mated with three male *Bufo bufo japonicus* (Nos. 4, 6 and 7) in 1977~1979. Two (Nos. 2 and 3) of the three females were mated with two male *Bufo torrenticola* (Nos. 3 and 4) in 1978 and 1979, while two females (Nos. 2 and 3) were mated with a male *Bufo bufo miyakonis* (No. 1). By these three kinds of matings, 85.4%, 86.8% and 89.8% of the respective number of eggs cleaved normally. After some embryos died of various abnormalities, 59.4%, 46.4% and 81.2% hatched normally, respectively (Fig. 4b). All the three kinds of hybrids were remarkably inferior to the controls in viability during the tadpole stage. More than half of the hybrids derived from male *japonicus* died without taking food. The remaining hybrids mostly died of various abnormalities at the tadpole stage, and eventually

TABLE 6

Developmental capacity of hybrids between female *Bufo americanus* and seven kinds of male *Bufo* and their controls

Years	Parents*		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally metamorphosed toads
	Female	Male					
1977	*ame.* W, Nos. 1, 2	*jap.* W, No. 4	1264	994 (78.6%)	641 (50.7%)	214 (16.9%)	181 (14.3%)
		bufo P. W, No. 4	1036	422 (40.7%)	3 (0.3%)	0	0
		bufo F. W, No. 1	1378	514 (37.3%)	28 (2.0%)	7 (0.5%)	0
		bufo G. W, No. 1	621	359 (57.8%)	0	0	0
		ame. W, No. 1	1704	1465 (86.0%)	1318 (77.3%)	993 (58.3%)	770 (45.2%)
1978	*ame.* W, No. 3	*jap.* W, No. 6	826	767 (92.9%)	492 (59.6%)	121 (14.6%)	78 (9.4%)
		tor. W, No. 3	935	814 (87.1%)	224 (24.0%)	0	0
		bufo P. W, No. 5	1213	1006 (82.9%)	140 (11.5%)	1 (0.1%)	1 (0.1%)
		vir. W, No. 1	1062	990 (93.2%)	514 (48.4%)	309 (29.1%)	264 (24.9%)
		ame. W, No. 2	788	699 (88.7%)	615 (78.0%)	428 (54.3%)	272 (34.5%)
1979	*ame.* W, Nos. 2, 3	*jap.* W, No. 7	772	683 (88.5%)	568 (73.6%)	422 (54.7%)	63 (8.2%)
		miy. W, No. 1	767	689 (89.8%)	623 (81.2%)	531 (69.2%)	114 (14.9%)
		tor. W, No. 4	677	585 (86.4%)	524 (77.4%)	182 (26.9%)	0
		bufo P. W, No. 5	838	728 (86.9%)	51 (6.1%)	38 (4.5%)	0
		vir. W, No. 1	819	712 (86.9%)	633 (77.3%)	591 (72.2%)	109 (13.3%)
		ame. W, No. 3	850	749 (88.1%)	732 (86.1%)	647 (76.1%)	131 (15.4%)
Total	*ame.* (3)	*jap.* (3)	2862	2444 (85.4%)	1701 (59.4%)	757 (26.5%)	322 (11.3%)
	ame. (2)	*miy.* (1)	767	689 (89.8%)	623 (81.2%)	531 (69.2%)	114 (14.9%)
	ame. (2)	*tor.* (2)	1612	1399 (86.8%)	748 (46.4%)	182 (11.3%)	0
	ame. (3)	*bufo* P. (2)	3087	2156 (69.8%)	194 (6.3%)	39 (1.3%)	1 (0.03%)
	ame. (2)	*bufo* F. (1)	1378	514 (37.3%)	28 (2.0%)	7 (0.5%)	0
	ame. (2)	*bufo* G. (1)	621	359 (57.8%)	0	0	0
	ame. (2)	*vir.* (1)	1881	1702 (90.5%)	1147 (61.0%)	900 (47.8%)	373 (19.8%)
	ame. (3)	*ame.* (3)	3342	2913 (87.2%)	2665 (79.7%)	2068 (61.9%)	1173 (35.1%)

* Parentheses show the number of toads.

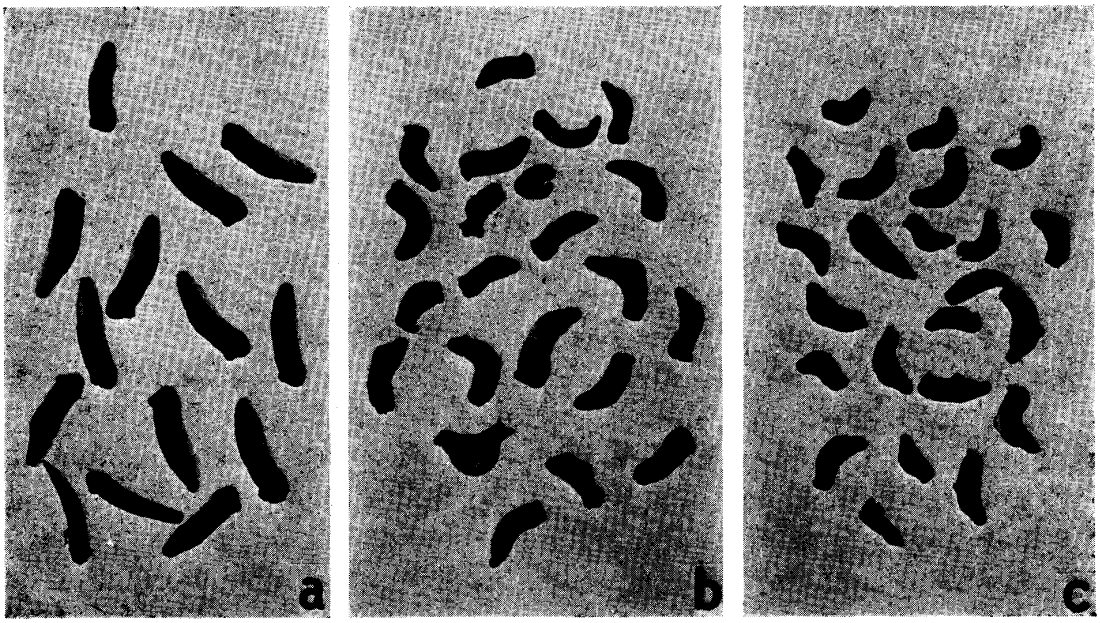

Fig. 4. Abnormalities at the post-hatching stage in the hybrids between a female *Bufo americanus* and a male *Bufo bufo japonicus* or *Bufo bufo bufo* from Portugal. ×2.5

a. Controls, *americanus* ♀ No. 1 × *americanus* ♂ No. 1

b. Hybrids, *americanus* ♀ No. 1 × *japonicus* ♂ No. 4

c. Hybrids, *americanus* ♀ No. 1 × *bufo* from Portugal ♂ No. 4

11.3%, that is, 13.2% of normally cleaved eggs attained completion of metamorphosis. Although the hybrids derived from a male *miyakonis* mostly began to eat after hatching, about four-fifths of them died of various abnormalities during the tadpole stage. Eventually, 14.9%, that is, 16.8% of normally cleaved eggs metamorphosed normally. In contrast with these two kinds of hybrids, those derived from male *torrenticola* all died during the tadpole stage, although 11.3% of the total number of eggs became feeding tadpoles (Table 6).

c. Matings with males of four kinds of European toads

The above three females (Nos. 1~3) were mated with two male *Bufo bufo bufo* from Portugal (Nos. 4 and 5) in 1977~1979. The results showed that 40.7~86.9%, 69.8% on the average, of the respective number of eggs cleaved normally. However, they showed delay in development at the gastrula stage and mostly died of various abnormalities before the hatching stage (Fig. 4c); 6.3% of the total number of eggs hatched normally, and 1.3% became feeding tadpoles. All of the latter died of ill-development, edema or various abnormalities during the tadpole stage, except for one tadpole which could complete metamorphosis. This single individual died without taking food after metamorphosis (Table 6).

Two (Nos. 1 and 2) of the three females were mated with a male *Bufo bufo* from France and a male *Bufo bufo* from Greece in 1977. By these two kinds of matings, 37.3% and 57.8% of the respective number of eggs cleaved normally. These normally cleaved eggs showed delay in development at the gastrula stage. Although 34.1% and 45.2% became nearly normal neurulae, only 10.6% and 10.6% could attain the tail-bud stage, respectively. While 2.0% hatched normally in the matings with a male *bufo* from France, no embryos could normally be hatched in those with a male *bufo* from Greece. One-fourth of the former hybrids began to eat after hatching, but died of ill-development before metamorphosis (Table 6).

Two (Nos. 2 and 3) of the three females were mated with a male *Bufo viridis* (No. 1) in 1978 and 1979. It was found that 90.5% of the total number of eggs cleaved normally, and 61.0% hatched normally. Most tadpoles died of ill-development, edema or some other abnormalities before metamorphosis, and eventually 19.8%, that is, 21.9% of normally cleaved eggs attained the completion of metamorphosis (Table 6).

II. Viability and sex

1. Hybrids produced in 1976 between Japanese and European toads

From crosses between two female *Bufo bufo japonicus* (Nos. 1 and 2) and two male *Bufo bufo bufo* from Portugal (Nos. 1 and 2), 897 normally metamorphosed hybrids were produced (Table 7). These toads climbed out of water at the age

TABLE 7

Number, size and sex of reciprocal hybrids produced in 1976 between *Bufo bufo japonicus* and *Bufo bufo bufo* from Portugal and their controls
The number in parentheses shows that of the toads measured

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed toad	Body lengths		Sex of toads, one or two years old		
				Juveniles taken at random immediately after metamorphosis mm	The largest toads, about one-year old mm	No. of toads	Female	Male
Female	Male							
jap. W, Nos. 1, 2	*jap.* W, Nos. 1, 2	37～46 m. 40.3	692	9.69±0.04 (40)	108.02±2.07 (50)	215	113	102 (47.4%)
	bufo P. W, Nos. 1, 2	39～52 m. 42.5	897	11.56±0.02 (40)	97.33±1.09 (50)	232	111	121 (52.2%)
bufo P. W, No. 1	*jap.* W, No. 3	42～49 m. 44.6	399	11.97±0.11 (40)	95.83±2.16 (50)	166	57	109 (65.7%)
	bufo P. W, No. 3	48～56 m. 52.4	248	12.60±0.05 (40)	90.15±0.75 (50)	146	76	70 (47.9%)

m., Mean

of 39～52 days, 42.5 days on the average. Forty of these toads, of which 20 came from female No. 1 and male No. 1 and the other 20 came from female No. 2 and male No. 2, were measured before taking food immediately after metamorphosis. It was found that they were 10.0～12.5 mm, 11.56 mm on the average, in body length. From the control matings between the two female *Bufo bufo japonicus* (Nos. 1 and 2) and two male *Bufo bufo japonicus* (Nos. 1 and 2), 692 normally metamorphosed toads were obtained. They climbed out of water at the age of 37～46 days, 40.3 days on the average. Forty toads, of which 20 came from female No. 1 and male No. 1 and the other 20 came from female No. 2 and male No. 2, were measured. The results indicated that the hybrids were larger than the controls; the latter were 9.0～10.5 mm, 9.69 mm on the average, in body length before taking food immediately after metamorphosis.

A total of 399 hybrids was produced from a cross between a female *Bufo bufo bufo* from Portugal (No. 1) and a male *Bufo bufo japonicus* (No. 3). They climbed out of water at the age of 42～49 days, 44.6 days on the average. When 40 of them were measured before taking food immediately after metamorphosis, they were 11.5～12.5 mm, 11.97 mm on the average, in body length. In contrast with this, 248 toads produced from the control mating between the female *Bufo bufo bufo* from Portugal (No. 1) and a male *Bufo bufo bufo* from Portugal (No. 3) climbed out of water at the age of 48～56 days, 52.4 days on the average. Forty of them were 12.0～13.0 mm, 12.60 mm on the average, in body length when measured before taking food immediately after metamorphosis. These figures showed that the hybrids were slightly smaller than the controls. Moreover, comparison of the toads produced from the four kinds of matings, indicated that *bufo* and *japonicus* were the largest and the smallest in body length, respectively, and reciprocal hybrids were intermediate between the two subspecies. The hybrids between a female *bufo* and a male *japonicus* were slightly larger than the reciprocal hybrids.

About one-fourth to three-fourths of the toads produced from each of the

above four kinds of matings were continuously reared after divided into three groups. While the toads produced from the mating between female *japonicus* No. 1 and male *japonicus* No. 1 or *Bufo bufo bufo* No. 1 were divided into the first and second group, those produced from the mating between female *japonicus* No. 2 and male *japonicus* No. 2 or *bufo* No. 2 were placed as the third group. The toads produced from the mating between female *bufo* No. 1 and male *japonicus* No. 3 or *bufo* No. 3 were divided into the first, second and third groups. The first group of toads produced from each kind of matings consisted of 50 toads which were reared under sufficient supply of food in order to accelerate their growth as much as possible. The second and third groups consisted of 50 to 60 toads and 50 to 100 toads, respectively. While the toads of the first and second groups were kept in a plastic 40 cm × 60 cm × 15 cm vessel which was put indoors, those of the third group were kept in an aquarium located outdoors. When the toads produced from each kind of matings were about one year old, it was found that only a few toads perished during the period from the completion of metamorphosis to this age, and that all the 50 toads belonging to the first group were alive. The body length of these toads was measured at the age of about one year. The result indicated that the control *japonicus* and *bufo* were the largest and the smallest, respectively, and the reciprocal hybrids were intermediate between the two controls (Table 7). It was noteworthy that the order of the four kinds of toads in size was inverted within one year, and that the two kinds of hybrids were slightly nearer to toads of the maternal species than those of the paternal in body length at the age of one year as well as at the stage immediately after metamorphosis.

Most of the male hybrids and controls reared indoors and outdroos matured sexually at the age of one year, while the remainders did one year later. In contrast, the female hybrids and controls sexually matured at the age of two or three years. The sex ratio was examined in all the toads produced from the four kinds of matings after they matured sexually at the age of one, two or three years (Table 7). While 102 (47.4%) of 215 control toads produced from matings between two female *japonicus* and two male *japonicus* were males, 121 (52.2%) of 232 hybrids produced from crosses between the same females and two male *bufo* were males. A remarkable male preponderance in sex ratio was found in the reciprocal hybrids. While 70 (47.9%) of 146 controls were males, 109 (65.7%) of 166 hybrids produced from the cross between a female *bufo* and a male *japonicus* were males.

2. Hybrids produced in 1977 among Japanese, European and American toads

Four kinds of female toads, *Bufo bufo japonicus*, *Bufo torrenticola*, *Bufo bufo bufo* from Portugal and *Bufo americanus*, were mated with males of these four kinds of toads and two others, *Bufo bufo* from France and *Bufo bufo* from Greece. The growth and sex ratio were observed in the metamorphosed toads produced from these matings (Table 8).

a. Hybrids from female *Bufo bufo japonicus*

From control matings between three female *Bufo bufo japonicus* (Nos. 3~5) and a male *Bufo bufo japonicus* (No. 4), 520 metamorphosed toads were produced (Table 8). They climbed out of water at the age of 36~40 days, 38.4 days on the average. A total of 50 toads, of which 25 were obtained from female No. 3 and the other 25 were from female No. 4, were measured before taking food immediately after metamotphosis. They were 9.0~10.0 mm, 9.65 mm on the average, in body length. From the same three female *japonicus* (Nos. 3~5), three kinds of hybrids were produced by mating with a male *torrenticola* (No. 1), a male *bufo* from France (No. 1) and a male *bufo* from Greece (No. 1). The number of metamorphosed toads, their age at the time of climbing out of water and the body length before taking food immediately after metamorphosis were as follows (Table 8).

TABLE 8

Number, size and sex of hybrids produced in 1977 among Japanese, European and American toads and their controls. The number in parentheses shows that of the toads measured

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed toads	Body lengths		Sex of toads, one or two years old		
Female	Male			Juveniles taken at random immediately after metamorphosis mm	The largest toads, about one year old mm	No. of toads	Female	Male
jap. W, Nos. 3~5	*jap.* W, No. 4	36~40 m. 38.4	520	9.65±0.04 (50)	97.46±1.54 (50)	159	80	79 (49.7%)
	tor. W, No. 1	36~38 m. 37.4	373	11.17±0.04 (50)	93.12±1.73 (50)	100	50	50 (50.0%)
	bufo F. W, No. 1	36~40 m. 38.1	141	11.37±0.03 (50)	93.00±1.95 (50)	98	10	88 (89.8%)
	bufo G. W, No. 1	36~41 m. 39.8	382	11.10±0.03 (50)	89.17±2.09 (50)	115	60	55 (47.8%)
tor. W, Nos. 1, 2	*jap.* W, No. 5	31~36 m. 32.2	1142	11.25±0.04 (50)	87.60±1.68 (50)	142	72	70 (49.3%)
	tor. W, No. 2	31~36 m. 32.1	1346	11.55±0.03 (50)	90.29±2.10 (50)	107	52	55 (51.4%)
bufo P. W, No. 2	*bufo* P. W, No. 4	43~45 m. 43.3	18	12.54±0.04 (18)*	75.71±1.90 (10)*	10	6	4 (40.0%)
	bufo F. W, No. 1	39~41 m. 39.3	78	12.31±0.05 (50)	75.34±1.76 (48)*	48	42	6 (12.5%)
	bufo G. W, No. 1	40~50 m. 41.3	121	12.14±0.05 (50)				
ame. W, Nos. 1, 2	*jap.* W, No. 4	42~58 m. 47.4	181	9.25±0.14 (100)	82.56±1.14 (50)	57	27	30 (52.6%)
	ame. W, No. 1	44~58 m. 50.0	770	11.07±0.03 (50)	73.83±1.30 (50)	94	46	48 (51.1%)

* All living toads were measured. m., Mean

From crosses *japonicus*♀×*torrenticola*♂, 373 hybrids were produced. They climbed out of water at the age of 36 to 38 days, 37.4 days on the average and were 10.5~12.0 mm, 11.17 mm on the average, in body length, which is somewhat larger than the controls. From crosses *japonicus*♀×*bufo* from France♂, 141 hybrids were produced. They climbed out of water at the age of 36~40 days, 38.1 days on the average, and were 11.0~11.5 mm, 11.37 mm on the average, in body length. From crosses *japonicus*♀×*bufo* from Greece♂, 382 hybrids

were produced. They climbed out of water at the age of 36～41 days, 39.8 days on the average, and were 10.5～11.5 mm, 11.10 mm on the average, in body length. It was found that the above three kinds of hybrids were nearly the same with each other but larger than the controls of the maternal species in body length, although there was no remarkable difference in the age at the time of climbing out of water between the hybrids and the controls.

While 160 of the metamorphosed toads produced from the control matings were continuously reared, 100 to 120 of the metamorphosed hybrids produced from three female *japonicus* (Nos. 3, 4 and 5) by mating with a male *torrenticola*, *bufo* from France or *bufo* from Greece were continuously reared in order to make them sexually mature. Although only a few of these hybrids and controls died or were lost, all the others were healthy at the age of about one year. Of the hybrids and controls, those produced from No. 3 had been reared under sufficient supply of food in order to accelerate their growth as much as possible. Fifty of the hybrids produced from each of the three kinds of crosses as well as fifty controls were measured at the age of about one year (Table 8). The results showed that the control *japonicus* were the largest in body length, the hybrids *japonicus*♀ × *bufo* from Greece♂ were the smallest and the other kinds of hybrids were intermediate. It was noteworthy that the *japonicus* were larger than the three kinds of hybrids, though they were evidently smaller at the stage immediately after metamorphosis.

When the hybrids and the controls produced from two female *japonicus* (Nos. 3 and 4) by mating with a male *Bufo torrenticola* (No. 1), a male *bufo* from France (No. 1), a male *bufo* from Greece (No. 1) and a male *japonicus* (No. 4) matured sexually at the age of one or two years, their sex ratio was examined (Table 8). It was found that 79 (49.7%) of 159 controls, 50 (50.0%) of 100 hybrids derived from the male *torrenticola* and 55 (47.8%) of 115 hybrids derived from the male *bufo* from Greece were males, while 88 (89.8%) of 98 hybrids derived from the male *bufo* from France were males.

b. Hybrids from female *Bufo torrenticola*

A total of 1142 metamorphosed hybrids was produced from crosses between two female *Bufo torrenticola* (Nos. 1 and 2) and a male *Bufo bufo japonicus* (No. 5). They climbed out of water at the age of 31～36 days, 32.2 days on the average. Fifty hybrids, of which 25 were obtained from female No. 1 and the other 25 were from female No. 2, were 10.5～12.0 mm, 11.25 mm on the average, in body length before taking food immediately after metamorphosis, while 50 controls were 11.0～12.0 mm, 11.55 mm on the average. A total of 150 hybrids and 110 controls produced from the two female *torrenticola* was continuously reared. Of these toads, 50 hybrids and 50 controls produced from female No. 1 were reared under sufficient supply of food in order to accelerate their growth as much as possible. Although a few toads were lost, all the other hybrids and controls produced from the two females were healthy at the age of one year. The 50 hybrids and the 50 controls produced from female No. 1 were measured at this stage. The results indicated that the hybrids were 87.60 mm on the average in body

length and the controls were 90.29 mm.

When the hybrids and the controls matured sexually at the age of one or two years, their sex ratio was examined. It was found that 70 (49.3%) of 142 hybrids and 55 (51.4%) of 107 controls were males.

c. Hybrids from a female *Bufo bufo bufo* from Portugal

Only 18 metamorphosed toads were produced from a control mating between a female *Bufo bufo bufo* from Portugal (No. 2) and a male *Bufo bufo bufo* from Portugal (No. 4). They climbed out of water at the age of 43~45 days, 43.3 days on the average, and were 11.5~13.0 mm, 12.54 mm on the average, in body length before taking food immediately after metamorphosis. From crosses of the same female with a male *Bufo bufo* from France (No. 1) and a male *Bufo bufo* from Greece (No. 1), 78 and 121 toads were produced, respectively. These two kinds of toads climbed out of water at the age of 39~41 days, 39.3 days on the average, and 40~50 days, 41.3 days on the average, respectively. When 50 hybrids produced by mating with each of the two males were measured before taking food immediately after metamorphosis, they were 11.5~13.0 mm, 12.31 mm on the average, or 11.5~12.5 mm, 12.14 mm on the average.

Ten of the control toads and 50 hybrids produced by mating with each of the two males from France and Greece were continuously reared. However, all the toads obtained by mating with the male from Greece were lost by an accident during hibernation, although they did not differ in viability and growth from the controls and the hybrids obtained by mating with the male from France. At the age of about one year, the ten controls and 48 of the 50 hybrids were alive; they were 75.71 mm and 75.34 mm in body length, respectively. Their sex was examined when they matured sexually at the age of one or two years. Of the ten controls, six were females and four males. In contrast with this, there were 42 females and six (12.5%) males among the 48 toads produced by mating with the male from France.

d. Hybrids from female *Bufo americanus*

From crosses between two female *Bufo americanus* (Nos. 1 and 2) and a male *Bufo bufo japonicus* (No. 4), 181 metamorphosed toads were produced, while 770 were obtained from control matings between the same females and a male *americanus* (No. 1). The hybrids climbed out of water at the age of 42~58 days, 47.4 days on the average, while the controls did at the age of 44~58 days, 50.0 days on the average. All the toads produced by mating with several kinds of males and females in 1977 were reared under nearly the same circumstances. It was found that the control *americanus* were the latest in metamorphosis and the hybrids, *americanus*♀×*japonicus*♂, were the next as a whole. When 100 of these hybrids and 50 of the controls produced by female *americanus* (No. 1) were measured before taking food immediately after metamorphosis, they were 6.5~11.0 mm, 9.25 mm on the average, and 10.0~12.0 mm, 11.07 mm on the average, respectively.

The 100 hybrids and 100 controls produced by female *americanus* No. 1 were continuously reared. The hybrids were remarkably small and feeble for a short period after metamorphosis as compared with the controls. At the age of about one year, 57 hybrids and 94 controls were alive; 50 hybrids and 50 controls of these toads were 82.56 mm and 73.83 mm on the average in body length, respectively (Table 8).

Sex ratio was examined in sexually mature toads at the age of one or two years. Of the control toads, 46 were females and 48 (51.1%) were males, while 27 of the hybrids were females and 30 (52.6%) were males.

3. Hybrids produced in 1978 among Japanese, European and American toads

Five kinds of female toads, *Bufo bufo japonicus, Bufo bufo miyakonis, Bufo bufo bufo* from Portugal, *Bufo viridis* and *Bufo americanus*, were mated with five kinds of male toads, *Bufo bufo japonicus, Bufo torrenticola, Bufo bufo bufo* from Portugal, *Bufo viridis* and *Bufo americanus*. The growth and sex ratio of the metamorphosed toads produced from these matings were examined (Table 9).

a. Hybrids from female *Bufo bufo japonicus*

From control matings between two female *Bufo bufo japonicus* (Nos. 6 and 7) and a male *Bufo bufo japonicus* (No. 6), 439 metamorphosed toads were produced, while 276 hybrids were obtained from crosses between the same females and a male *Bufo viridis* (No. 1). They climbed out of water at the age of 36~50 days, 37.6 days on the avergae, and 34~52 days, 38.4 days on the avergae, respectively. Forty toads, of which 20 came from female No. 6 and the other 20 came from female No. 7, were measured before taking food immediately after metamorphosis in each of the crossing and control series. It was found that the hybrids were 11.0~14.0 mm, 12.34 mm on the average, in body length, while the control were 9.0~9.5 mm, 9.38 mm on the average. The hybrids were distinctly larger than the controls and intermediate in size between their parental species, although they considerably varied in size.

One hundred of the hybrid toads and 25 of the controls produced by female *japonicus* No. 6 were continuously reared to make them sexually mature. At the age of about one year, the 25 controls and 50 of the hybrids were measured. It was found that they were 95.83 mm and 70.13 mm on the average in body length, respectively (Table 9).

The sex of mature toads was examined at the age of one or two years. The results showed that all 84 hybrids were males, while nine of 21 controls were females and the other twelve (57.1%) were males.

b. Hybrids from a female *Bufo bufo miyakonis*

From crosses of a female *Bufo bufo miyakonis* (No. 1) with a male *Bufo bufo japonicus* (No. 6), a male *Bufo torrenticola* (No. 3), a male *Bufo bufo bufo* from Portugal (No. 5) and a male *Bufo viridis* (No. 1), 530, 463, 234 and 410 metamorphosed

toads were produced, respectively (Table 9). They climbed out of water at the age of 43～73 days, 46.6 days on the average, 41～53 days, 45.4 days on the average, 41～53 days, 43.1 days on the average, and 45～87 days, 62.6 days on the average, respectively. Forty of the toads produced by mating with each of male *japonicus*, *torrenticola*, *bufo* from Portugal and *viridis* were measured before taking food immediately after metamorphosis; they were 10.60 mm, 10.74 mm, 9.92 mm and 9.97 mm, respectively. It was noteworthy that two kinds of hybrids, *miyakonis*♀×*bufo*♂ and *miyakonis*♀×*viridis*♂, were smaller than the other two, although their parental species were remarkably larger than *japonicus* at the time immediately after metamorphosis.

Of these four kinds of juvenile toads, 50 produced by mating with the male *japonicus*, 100 produced by mating with each of the male *torrenticola* and *bufo* from Portugal and 200 produced by mating with the male *viridis* were continuously reared. At the age of about one year, 50 of toads produced from each of the four kinds of matings were measured. It was found that the hybrids produced by mating with the male *japonicus*, *torrenticola*, *bufo* from Portugal and *viridis* were 77.95 mm, 68.97 mm, 57.77 mm and 68.24 mm on the average in body length, respectively. These values indicated that the hybrids, *miyakonis*♀× *bufo* from Portugal♂, were distinctly smaller than the other three kinds of hybrids.

Sex ratio was examined when the toads matured sexually at the age of one or two years. The results indicated that 128 hybrids produced by the male *viridis* were all males, while the sex ratio was nearly 1: 1 among the hybrids produced from each of the other three kinds of crosses (Table 9).

c. Hybrids from a female *Bufo bufo bufo* from Portugal

From matings between a female *Bufo bufo bufo* from Portugal (No. 3) and a male *Bufo bufo bufo* from Portugal (No. 5), a male *Bufo torrenticola* (No. 3) and a male *Bufo viridis* (No. 1), 80, 134 and 2 metamorphosed toads were produced, respectively (Table 9). They climbed out of water at the age of 49～78 days, 61.3 days on the average, 46～85 days, 60.4 days on the average, and 56 days and 67 days, 61.5 days on the average, respectively. Forty of the toads produced by mating with each of the male *bufo* and *torrenticola* were measured before taking food immediately after metamorphosis. They were 11.5～13.5 mm, 12.17 mm on the average, and 11.0～13.0 mm, 11.21 mm on the average, respectively. The two hybrids produced by mating with the male *viridis* died of ill-development before the completion of metamorphosis.

One hundred of the hybrids produced by mating with the male *torrenticola* and all the controls were continuously reared to make them sexually mature. Fifty of the hybrids and 50 of the controls were measured at the age of about one year; they were 70.38 mm and 72.80 mm on the average in body length, respectively.

Sex ratio was examined in 36 hybrids and 34 controls which matured sexually at the age of one or two years. Of the hybrids, 15 were females and 21 (58.3%) were males, while 18 of the controls were females and 16 (47.1%) were males.

d. Controls from a female *Bufo viridis*

Only six metamorphosed toads were produced from a mating between a female *Bufo viridis* (No. 1) and a male *Bufo viridis* (No. 1). These toads climbed out of water at the age of 46～52 days, 50.2 days on the average, and were 16.25 mm on the average in body length before taking food immediately after metamorphosis (Table 9). At the age of about one year, they were 69.74 mm on the average in body length. All of them were males.

e. Hybrids from a female *Bufo americanus*

From matings between a female *Bufo americanus* (No. 3) and a male *Bufo bufo japonicus* (No. 6), a male *Bufo viridis* (No. 1) and a male *Bufo americanus* (No. 2), 78, 264 and 272 metamorphosed toads were produced, respectively (Table 9). They climbed out of water at the age of 41～50 days, 42.6 days on the average, 42～129 days, 50.0 days on the average, and 42～85 days, 50.7 days on the average, respectively. Forty of the toads produced by each of the male *japonicus*, *viridis* and *americanus* were measured before taking food immediately after metamorphosis; they were 9.78 mm, 11.66 mm and 10.74 mm on the average in body length, respectively.

All the toads produced by mating with the male *japonicus* and 100 of those

TABLE 9

Number, size and sex of the hybrids produced in 1978 among Japanese, European and American toads and their controls. The number in parentheses shows that of the toads measured

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed toads	Body length		Sex of toads, one or two years old		
Female	Male			Juveniles taken at random immediately after metamorphosis mm	The largest toads, about one year old mm	No. of toads	Female	Male
jap. W, Nos. 6, 7	*jap.* W, No. 6	36～50 m. 37.6	439	9.38±0.03 (40)	95.83±1.30 (25)	21	9	12 (57.1%)
	vir. W, No. 1	34～52 m. 38.4	276	12.34±0.11 (40)	70.13±3.10 (50)	84	0	84 (100 %)
miy. W, No. 1	*jap.* W, No. 6	43～73 m. 46.6	530	10.60±0.03 (40)	77.95±2.11 (50)	50	24	26 (52.0%)
	tor. W, No. 3	41～53 m. 45.4	463	10.74±0.11 (40)	68.97±1.35 (50)	96	45	51 (53.1%)
	bufo P. W, No. 5	41～53 m. 43.1	234	9.92±0.20 (40)	57.77±1.48 (50)	63	31	32 (50.9%)
	vir. W, No. 1	45～87 m. 62.6	410	9.97±0.04 (40)	68.24±0.75 (50)	128	0	128 (100 %)
bufo P. W, No. 3	*tor.* W, No. 3	46～85 m. 60.4	134	11.21±0.14 (40)	70.38±1.06 (50)	36	15	21 (58.3%)
	bufo P. W, No. 5	49～78 m. 61.3	80	12.17±0.05 (40)	72.80±1.21 (50)	34	18	16 (47.1%)
	vir. W, No. 1	56, 67 m. 61.5	2					
vir. W, No. 1	*vir.* W, No. 1	46～52 m. 50.2	6	16.25±0.07 (6)*	69.74±1.89 (6)*	6	0	6 (100 %)
ame. W, No. 3	*jap.* W, No. 6	41～50 m. 42.6	78	9.78±0.15 (40)	71.18±2.95 (50)	27	13	14 (51.9%)
	vir. W, No. 1	42～129 m. 50.0	264	11.66±0.04 (40)				
	ame. W, No. 2	42～85 m. 50.7	272	10.74±0.02 (40)	60.88±1.10 (50)	40	18	22 (55.0%)

* All living toads were measured. m., Mean

produced by mating with each of the male *viridis* and *americanus* were continuously reared after metamorphosis. However, the hybrids produced by mating with the male *viridis* all died by an accident during their hibernation, and, moreover, many of the controls died of underdevelopment within three months after metamorphosis. At the age of about one year, 50 of the remaining hybrids and 50 of the controls were measured; they were 71.18 mm and 60.88 mm on the average in body length, respectively.

Sex ratio was examined in 27 hybrids and 40 controls which matured sexually at the age of one or two years. Of these hybrids, 13 were females and 14 (51.9%) were males, while of the controls 18 were females and 22 (55.0%) were males.

4. Hybrids produced in 1979 among Japanese, European and American toads

In the breeding season of 1979, six kinds of female toads, *Bufo bufo japonicus*, *Bufo bufo miyakonis*, *Bufo torrenticola*, *Bufo bufo bufo* from Portugal, *Bufo viridis* and *Bufo americanus*, were mated with seven kinds of male toads, *Bufo bufo japonicus*, *Bufo bufo yakushimensis*, *Bufo bufo miyakonis*, *Bufo torrenticola*, *Bufo bufo bufo* from Portugal, *Bufo viridis* and *Bufo americanus*. The growth of the metamorphosed hybrids and controls produced from these matings was observed (Table 10). The sex of some of these toads was also examined.

a. Hybrids from a female *Bufo bufo japonicus*

From matings between a female *Bufo bufo japonicus* (No. 8) and a male *Bufo bufo japonicus* (No. 7), a male *Bufo bufo yakushimensis* (No. 1) and a male *Bufo bufo miyakonis* (No. 1), 222, 187 and 291 metamorphosed toads were produced, respectively (Table 10). They climbed out of water at the age of 42~63 days, 49.3 days on the average, 50~56 days, 50.6 days on the average, and 41~59 days, 42.6 days on the average, respectively. Thirty of the toads produced by mating with each of the male *japonicus*, *yakushimensis* and *miyakonis* were measured before taking food immediately after metamorphosis; they were 9.0~10.0 mm, 9.50 mm on the average, 9.0~10.5 mm, 9.54 mm on the average, and 9.5~11.5 mm, 10.86 mm on the average, in body length, respectively. These values indicated that the hybrids produced by mating with the male *miyakonis* were intermediate in growth between the two kinds of controls, *japonicus* and *miyakonis* (Table 10).

b. Hybrids from female *Bufo bufo miyakonis*

From matings between two female *Bufo bufo miyakonis* (Nos. 2 and 3) and a male *Bufo bufo japonicus* (No. 7), a male *Bufo bufo yakushimensis* (No. 1), a male *Bufo bufo miyakonis* (No. 1), a male *Bufo torrenticola* (No. 4), a male *Bufo bufo bufo* from Portugal (No. 5) and a male *Bufo viridis* (No. 1), 113, 200, 212, 121, 56 and 91 metamorphosed toads were produced, respectively. The hybrids produced by mating with the male *yakushimensis* climbed out of water at the age of 62~83 days, 70.4 days on the average, while the other four kinds of hybrids and the

TABLE 10
Number and size of the hybrids produced in 1979 among Japanese, European and American toads and their controls. The number in parentheses shows that of the toads measured

Parents		Age at the time of climbing out of water (days)	No. of metamorphosed toads	Body length of juveniles taken at random immediately after metamorphosis mm
Female	Male			
jap. W, No. 8	*jap.* W, No. 7	42～63 m. 49.3	222	9.50±0.02 (30)
	yak. W, No. 1	50～56 m. 50.6	187	9.54±0.02 (30)
	miy. W, No. 1	41～59 m. 42.6	291	10.86±0.07 (30)
miy. W, Nos. 2, 3	*jap.* W, No. 7	54～76 m. 56.1	113	10.00±0.10 (30)
	yak. W, No. 1	62～83 m. 70.4	200	9.32±0.26 (30)
	miy. W, No. 1	53～77 m. 59.6	212	11.68±0.06 (30)
	tor. W, No. 4	47～72 m. 52.0	121	11.24±0.05 (30)
	bufo P. W, No. 5	51～74 m. 56.8	56	10.86±0.12 (10)
	vir. W, No. 1	43～80 m. 55.3	91	12.73±0.16 (30)
tor. W, No. 3	*yak.* W, No. 1	50～63 m. 53.5	152	11.77±0.09 (30)
	miy. W, No. 1	41～55 m. 42.7	296	11.51±0.09 (30)
	tor. W, No. 4	43～67 m. 45.6	279	11.66±0.06 (30)
	bufo P. W, No. 5	49～77 m. 51.6	93	12.12±0.12 (30)
	vir. W, No. 1	69～85 m. 75.8	5	
bufo P. W, Nos. 4, 5	*tor.* W, No. 4	55～79 m. 57.7	201	11.38±0.07 (40)
	miy. W, No. 1	48～78 m. 58.9	324	11.62±0.13 (40)
	bufo P. W, No. 5	55～85 m. 64.8	185	12.31±0.07 (40)
	vir. W, No. 1	53～85 m. 70.3	97	12.68±0.27 (40)
vir. W, No. 1	*jap.* W, No. 7	56～65 m. 60.0	23	11.85±0.32 (20)
	yak. W, No. 1	65～92 m. 73.8	40	11.68±0.08 (30)
	miy. W, No. 1	56～89 m. 71.9	48	13.34±0.11 (30)
	tor. W, No. 4	55～95 m. 68.7	74	13.17±0.20 (30)
	bufo P. W, No. 5	58～81 m. 74.7	9	12.00±0.20 (6)
	vir. W, No. 1	56～85 m. 60.2	165	14.50±0.06 (30)
ame. W, Nos. 2, 3	*jap.* W, No. 7	55～94 m. 67.6	63	9.88±0.14 (30)
	miy. W, No. 1	56～101 m. 82.6	114	
	vir. W, No. 1	65～92 m. 73.2	109	11.83±0.08 (30)
	ame. W, No. 3	71～94 m. 80.5	131	11.50±0.05 (30)

m., Mean

controls did at the age of 43～80 days, 52.0～59.6 days on the average. Thirty toads, of which 15 came from female No. 2 and the other 15 came from female No. 3 were measured before taking food immediately after metamorphosis in each of four kinds of hybrids and the controls. The results showed that the hybrids produced by mating with the male *yakushimensis* were 9.32 mm on the average and remarkably varied in body length, while the hybrids produced by mating with the male *japonicus*, *torrenticola* and *viridis* were 10.00 mm, 11.24 mm and 12.73 mm on the average, respectively. The thirty controls and ten of the hybrids produced from female No. 2 by mating with the male *bufo* from Portugal were 11.68 mm and 10.86 mm on the average in body length, respectively.

Some of the hybrids produced from each of the matings, *miyakonis*♀ × *japonicus*♂, *yakushimensis*♂, *miyakonis*♂, *torrenticola*♂ and *bufo* from Portugal♂,

matured at the age of one year. In these toads, there were both males and females. Of the 91 metamorphosed toads produced from the cross, *miyakonis*♀ × *viridis*♂, 34 were alive at the age of one year. Twenty-three of them were mature males that were 63.0~89.0 mm in body length. The sex of the other eleven toads, 40.0~56.0 mm in body length, was unknown, as they were immature and still alive.

c. Hybrids from a female *Bufo torrenticola*

From matings between a female *Bufo torrenticola* (No. 3) and a male *Bufo bufo yakushimensis* (No. 1), a male *Bufo bufo miyakonis* (No. 1), a male *Bufo torrenticola* (No. 4), a male *Bufo bufo bufo* from Portugal (No. 5) and a male *Bufo viridis* (No. 1), 152, 296, 279, 93 and 5 metamorphosed toads were produced, respectively (Table 10). The five hybrids produced by mating with the male *Bufo viridis* climbed out of water at the age of 69~85 days, 75.8 days on the average, and died before completion of metamorphosis. The other three kinds of hybrids and the controls climbed out of water at the age of 41~77 days, 42.7~53.5 days on the average. Thirty of the toads produced by mating with each of male *yakushimensis miyakonis*, *torrenticola* and *bufo* were measured before taking food immediately after metamorphosis; they were 11.51~12.12 mm on the average.

d. Hybrids from female *Bufo bufo bufo* from Portugal

From matings between two female *Bufo bufo bufo* from Portugal (Nos. 4 and 5) and a male *Bufo torrenticola* (No. 4), a male *Bufo bufo miyakonis* (No. 1), a male *Bufo bufo bufo* from Portugal (No. 5) and a male *Bufo viridis* (No. 1), 201, 324, 185 and 97 metamorphosed toads were produced, respectively (Table 10). The hybrids produced by mating with the male *viridis* climbed out of water at the age of 53~85 days, 70.3 days on the average, which is somewhat later than the controls of the two parental species. The other two kinds of hybrids produced by mating with the male *torrenticola* and *miyakonis* climbed out of water at the age of 55~79 days, 57.7 days on the average, and 48~78 days, 58.9 days on the average, respectively, while the controls did at the age of 55~85 days, 64.8 days on the average. Forty toads, of which 20 came from female No. 4 and the other 20 came from female No. 5, were measured before taking food immediately after metamorphosis in each kinds of hybrids and the controls. While the controls were 12.31 mm on the average in body length, the hybrids produced by mating with the male *torrenticola* and *miyakonis* were 11.38 mm and 11.62 mm, respectively, and those produced by mating with the male *viridis* were 12.68 mm.

At the age of one year, there were many mature males and females in the toads produced from each of the matings, *bufo*♀ × *torrenticola*♂, *miyakonis*♂ and *bufo*♂. In contrast, there were no females in the hybrids, *bufo*♀ × *viridis*♂. Of these hybrids, 20 were mature males that were 62.0~81.0 mm in body length, while the other 12 were immature and 34.5~56.5 mm in body length. It was found that all these immature hybrids were males by examining their gonads after preservation.

e. Hybrids from a female *Bufo viridis*

From matings between a female *Bufo viridis* (No. 1) and a male *Bufo bufo japonicus* (No. 7), a male *Bufo bufo yakushimensis* (No. 1), a male *Bufo bufo miyakonis* (No. 1), a male *Bufo torrenticola* (No. 4), a male *Bufo bufo bufo* from Portugal (No. 5) and a male *Bufo viridis* (No. 1), 23, 40, 48, 74, 9 and 165 metamorphosed toads were produced, respectively (Table 10). Of these kinds of hybrids, those obtained by mating with the male *miyakonis, torrenticola* and *bufo* climbed out of water at the age of 56~89 days, 71.9 days on the average, 55~95 days, 68.7 days on the average, and 58~81 days, 74.7 days on the average, respectively. It was found from these figures that the three kinds of hybrids were distinctly delayed in metamorphosis as compared with their parental species. The hybrids produced by mating with a male *yakushimensis* climbed out of water at the age of 65~92 days, 73.8 days on the average. Although there were no controls obtained by mating this male with a female of the same species, it was assumed that this kind of hybrids was also remarkably delayed in metamorphosis. The hybrids produced by mating with the male *japonicus* climbed out of water at the age of 56~65 days, 60.0 days on the average, like the control *viridis* which did at the age of 56~85 days, 60.2 days on the average.

When body length was measured before taking food immediately after metamorphosis in 20 hybrids produced by mating with the male *japonicus*, 30 hybrids produced by mating with each of the male *yakushimensis, miyakonis* and *torrenticola*, 6 hybrids produced by matings with the male *bufo* and 30 controls, it was 11.85 mm, 11.68 mm, 13.34 mm, 13.17 mm, 12.00 mm and 14.50 mm on the average, respectively (Table 10). These figures indicate that the hybrids were intermediate in body length between the parental species, except that the hybrids obtained by matings with the male *bufo* were similar to the paternal species.

There were some mature males and females in one-year-old hybrids produced from each of the crosses, *viridis*♀×*japonicus*♂, *yakushimensis*♂, *miyakonis*♂, *torrenticola*♂ and *bufo*♂. In contrast, there were no mature females in 129 control toads produced from the same female by mating with a male *viridis*. Of these control toads, 75 were mature males that were 64.0~85.0 mm in body length, while the others were immature and 34.5~56.5 mm in body length. Their sex was unknown, as they were still living.

f. Hybrids from female *Bufo americanus*

From matings between two female *Bufo americanus* (Nos. 2 and 3) and a male *Bufo bufo japonicus* (No. 7), a male *Bufo bufo miyakonis* (No. 1), a male *Bufo viridis* (No. 1) and a male *Bufo americanus* (No. 3), 63, 114, 109 and 131 metamorphosed toads were obtained, respectively (Table 10). While the controls climbed out of water at the age of 71~94 days, 80.5 days on the average, the three kinds of hybrids did at the age of 55~94 days, 67.6 days on the average, 56~101 days, 82.6 days on the average, and 65~92 days, 73.2 days on the average, respectively. It was found that the hybrids obtained by mating with the male *miyakonis* were somewhat delayed in metamorphosis as compared with the parental species,

while the other two kinds of hybrids were intermediate. Thirty toads, of which 15 came from female No. 2 and the other 15 came from female No. 3, were measured before taking food immediately after metamorphosis. The results showed that the hybrids obtained by mating with the male *japonicus* and *viridis* were 9.88 mm and 11.83 mm on the average, respectively, while the controls were 11.50 mm on the average. These figures indicated that the two kinds of hybrids were similar to one parental species which was slower than the other in growth during the tadpole stage. The hybrids produced by mating with the male *miyakonis* were not measured.

In one-year-old control *americanus*, there were many mature males and females. It was also found that there were both males and females in the hybrids, *americanus*♀ × *japonicus*♂ or *miyakonis*♂, when the sex of these hybrids was examined after preservation at the age of one year. However, all of 35 hybrids produced from the cross, *americanus*♀ × *viridis*♂, were males when their sex was examined after preservation at the age of one year. They were 40.0～72.0 mm in body length.

III. External characters of mature toads

1. Hybrids produced in 1976 between Japanese and European toads and the controls

Three females and three males of each of four kinds of mature toads, *Bufo bufo japonicus*♀ × *Bufo bufo bufo*♂ hybrids, the reciprocal hybrids, and the control *Bufo bufo japonicus* and *Bufo bufo bufo*, were observed in terms of external characters. Their parental *Bufo bufo bufo* were those collected from Portugal. Relative sizes of various body sites are presented in Table 11. All these toads were two years old.

a. Controls, *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo japonicus*♂ No. 1

Three females and three males were 144.3 mm and 116.3 mm in mean body length, respectively. The canthus rostralis was sharply edged. The iris was bright yellowish-orange. The tympanum was round in contrast with that of *Bufo bufo bufo* which was oval. The parotoid gland was spindle-shaped and situated closely to the posterior end of the upper eyelid.

The skin of the back was comparatively smooth. The main tubercles on the back were generally larger and fewer than those of *bufo*. Some of them had a strong tendency of arrangement in two rows along the median line of the back. Each tubercle had one or more black spots on its top. There was a row of comparatively large tubercles along each side of the body between the parotoid gland and the groin. The back was dark reddish-brown (♀) or olive-brown (♂) with or without a pale incomplete median stripe. The upper half of the parotoid gland was white, while the lower half was black. These white and black marks extended backward to the groin as a white narrow band and a black wide

TABLE 11

Relative sizes of various body sites of reciprocal hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo* from Portugal and their controls. All the toads were produced in 1976 and measured in 1978

Kind	*jap.*♀ × *jap.*♂	*jap.*♀ × *bufo* P.♂	*bufo* P.♀ × *jap.*♂	*bufo* P.♀ × *bufo* P.♂
No. of toads	♀3, ♂3	♀3, ♂3	♀3, ♂3	♀3, ♂3
Head length / Body length	0.25	0.25	0.26	0.24
Head width / Head length	1.29	1.40	1.35	1.36
Interorbital space / Head length	0.24	0.32	0.26	0.23
Diameter of tympanum / Head length	0.26	0.21	0.15	0.12
Length of parotoid / Head length	0.95	0.88	0.74	0.88
Width of parotoid gland / Length of parotoid gland	0.34	0.40	0.42	0.48
Arm length / Body length	0.49	0.54	0.59	0.60
Hind-leg length / Body length	1.02	1.07	1.15	1.15
Length of inner-metatarsal tubercle / Tibia length	0.16	0.17	0.16	0.17

jap., *Bufo bufo japonicus* *bufo* P., *Bufo bufo bufo* from Portugal

band. The underside of the body was whitish or yellowish with or without black or dark-gray flecks or vermiculations. The margin of the submaxilla was almost completely black or dark gray (Plate I, 1 and 2).

b. Controls, *Bufo bufo bufo*♀ No. 1 × *Bufo bufo bufo*♂ No. 3

Three females and three males were 100.3 mm and 94.7 mm in mean body length, respectively. The canthus rostralis was gently elevated. The iris was deep reddish-orange. The tympanum was somewhat elliptical and remarkably smaller in largest diameter than that of *japonicus* (Table 11). The parotoid gland was elliptical and broader than that of *japonicus*. It was situated 3~6 mm from the upper eyelid. The arms were longer than those of *japonicus*.

The dorsal skin was rough, that is, seamed with innumerable wrinkles. The main tubercles on the back were generally smaller and more numerable than those of *japonicus*. Some of them had a tendency of arrangement in two rows along the median line of the back. Each tubercle had no black spot on its top. There was no row of large tubercles along each side of the body between the parotoid gland and the groin. The back was light yellowish-brown with several pale large marks. There was no white band on the parotoid gland and the flank. Although the lower part of the parotoid gland was dark brown, there was no band of the same color between the parotoid gland and the groin. The underside of the body was whitish with or without light-gray flecks or vermicu-

lations. The margin of the submaxilla was completely whitish (Plate I, 7 and 8).

c. Hybrids, *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo bufo*♂ No. 1

Three females and three males were 109.3 mm and 99.8 mm in mean body length, respectively. The shape of the canthus rostralis was intermediate between the two subspecies. The color of the iris was similar to that of *japonicus* in 12 and to that of *bufo* in 11 of 50 toads observed. In the remaining 27 toads, the iris was intermediate in color between those of the two species. The tympanum was oval and slightly smaller in the largest diameter than that of *japonicus* (Table 11). The parotoid gland was much more similar in shape to that of *japonicus* than that of *bufo*. Its location was of *japonicus* type in 46 and of intermediate type in 4 of 50 toads. The arms were intermediate in length between those of the two subspecies.

The appearance of the dorsal skin and the number and size of the main tubercles on the back resembled more closely those of *japonicus*, although they were intermediate between those of the two subspecies. The color and pattern of the dorsal body surface were intermediate between those of the two subspecies. The parotoid glands and the flanks resembled more closely those of *japonicus* in color and pattern, although they were intermediate between those of the two subspecies. The ventral surface of the body was whitish or pale yellow with or without gray flecks or vermiculations. The margin of the submaxilla was partly gray or completely whitish (Plate I, 3 and 4).

d. Hybrids, *Bufo bufo bufo*♀ No. 1 × *Bufo bufo japonicus*♂ No. 3

Three females and three males were 110.2 mm and 103.2 mm in mean body length, respectively. The canthus rostralis was intermediate in shape between the two subspecies. The color of the iris was similar to that of *japonicus* in three, to that of *bufo* in seven and intermediate between the two subspecies in 40 of 50 toads observed. The tympanum was oval and somewhat smaller than that of the reciprocal hybrids (Table 11). The parotoid gland was very similar to that of *bufo* in shape and location. The arms were nearly the same in relative length as those of *bufo*.

The dorsal skin and its main tubercles resembled more closely those of *bufo* in appearance, although they were intermediate between the two subspecies. While the dorsal body surface was very similar in color and pattern to that of the reciprocal hybrids, the parotoid gland and the flank resembled more closely those of *bufo*, although they were intermediate between those of two subspecies. The ventral surface of the body was whitish with or without gray flecks or vermiculations. The margin of the submaxilla was almost completely or partly black or gray, or completely whitish (Plate I, 5 and 6).

2. Hybrids produced in 1977 between two Japanese toad species and the controls

Three females and three males of each of four kinds of mature toads, *Bufo bufo*

japonicus♀ × *Bufo torrenticola*♂, the reciprocal hybrids, and the control *Bufo bufo japonicus* and *Bufo torrenticola*, were observed in terms of external characters. Relative sizes of various body sites are presented in Table 12. All these toads were two years old.

TABLE 12

Relative sizes of various body sites of reciprocal hybrids between *Bufo bufo japonicus* and *Bufo torrenticola* and their controls. All the toads were produced in 1977 and measured in 1979

Kind	*jap.*♀ × *jap.*♂	*jap.*♀ × *tor.*♂	*tor.*♀ × *jap.*♂	*tor.*♀ × *tor.*♂
No. of toads	♀3, ♂3	♀3, ♂3	♀3, ♂3	♀3, ♂3
Head length / Body length	0.26	0.27	0.28	0.27
Head width / Head length	1.34	1.30	1.27	1.32
Interorbital space / Head length	0.26	0.22	0.22	0.26
Diameter of tympanum / Head length	0.20	0.15	0.14	0.10
Length of parotoid / Head length	0.85	0.78	0.70	0.58
Width of parotoid gland / Length of parotoid gland	0.30	0.29	0.32	0.35
Arm length / Body length	0.51	0.67	0.65	0.70
Hind-leg length / Body length	1.12	1.26	1.24	1.26
Length of inner-metatarsal tubercle / Tibia length	0.16	0.16	0.17	0.16

jap., *Bufo bufo japonicus* *tor.*, *Bufo torrenticola*

a. Controls, *Bufo bufo japonicus*♀ No. 3 × *Bufo bufo japonicus*♂ No. 4

Three females and three males were 122.8 mm and 118.2 mm in mean body length, respectively. The canthus rostralis was sharply edged. The iris was bright yellowish-orange. The tympanum was round. The parotoid gland was spindle-shaped and situated closely to the posterior end of the upper eyelid.

The dorsal skin of the body was comparatively smooth. The main tubercles on the back were round or elliptic and usually fewer than those of *bufo*. Some of them showed a strong tendency of arrangement in two rows along the median line of the back. There was a row of comparatively large tubercles between the parotoid gland and the groin. The back was dark reddish-brown (♀) or olive-brown (♂) with or without a vague reticular mark. The upper half of the parotoid gland was white, while the lower half was black. These white and black marks extended to the groin as a white narrow band and a black wide band, respectively. The underside of the body was whitish with black vermiculations. The margin of the submaxilla was almost completely black or dark gray (Plate II, 9 and 10).

b. Controls, *Bufo torrenticola*♀ No. 1 × *Bufo torrenticola*♂ No. 2

Three females and three males were 100.5 mm and 89.0 mm in mean body length, respectively. The shape of the canthus rostralis and the color of the iris were the same as those of *japonicus*. The tympanum was almost indistinct, although its position and outline were barely traceable. It was oval and remarkably smaller than that of *japonicus* (Table 12). The parotoid gland was a long ellipsoid in shape and shorter than that of *japonicus* and situated 1～2 mm from the posterior end of the eyelid. The arms were longer than those of *japonicus*.

The skin of the back was seamed with numerous wrinkles. The main tubercles on the back were round and usually more numerous than those of *japonicus*. Some of them showed a weak tendency of arrangement in two rows along the median line of the back. There was a row of comparatively large tubercles between the parotoid gland and the groin. The back was dark or light olive-brown, usually tinged with orange or equipped with wide or narrow orange areas. The upper half of the parotoid gland was white, while the lower half was black. This white mark extended toward the groin as a pale yellow band or a row of white or pale yellow dots. However, such a band or a row of dots usually disappeared on the way of extension. The black mark on the parotoid gland extended to the groin as a wide black band or disappeared on the way of extension after becoming a group of black or gray flecks. The underside of the body was pale yellow or orange and often had some black flecks. The margin of the submaxilla was almost completely or partly black or dark gray (Plate II, 15 and 16).

c. Hybrids, *Bufo bufo japonicus*♀ No. 3 × *Bufo torrenticola*♂ No. 1

Three females and three males were 105.3 mm and 100.5 mm in mean body length, respectively. The shape of the canthus rostralis and the color of the iris were the same as those of the two species. The tympanum was oval and intermediate between those of the two species in appearance and diameter (Table 12). The parotoid gland was long ellipsoid in shape and situated closely to or 1～2 mm from the posterior end of the upper eyelid. It was intermediate in relative length between those of the parental species, although it seemed to resemble more closely that of *Bufo bufo japonicus*. The arms were almost similar to those of *torrenticola* in relative length.

The skin of the back and its main tubercles resembled more closely those of *japonicus* in appearance, although they were intermediate between the two species. The back was similar in color and pattern to that of *japonicus*, except that the color of some toads was somewhat tinged with orange. The white and black bands between the parotoid gland and the groin resembled more closely those of *japonicus* in appearance, although they were intermediate between the two species. The underside of the body was pale yellow with black or gray flecks. The margin of the submaxilla was almost completely black or dark gray (Plate II, 11 and 12).

d. Hybrids, *Bufo torrenticola*♀ No. 1 × *Bufo bufo japonicus*♂ No. 5

Three females and three males were 103.7 mm and 102.3 mm in mean body length, respectively. The shape of the canthus rostralis and the color of the iris were the same as those of the two species. The tympanum was similar in shape and diameter to that of the reciprocal hybrids (Table 12). While the parotoid gland was similar to that of the reciprocal hybrids in position, it was intermediate between those of the two parental species in relative length. The arms were similar to those of the reciprocal hybrids in relative length.

The dorsal skin of the body and its main tubercles resembled more closely those of *torrenticola* in appearance, although they were intermediate between the two species. The back was dark olive-brown with or without narrow orange areas. The white and black bands between the parotoid gland and the groin resembled more closely those of *torrenticola*, although they were intermediate between the two species. The underside of the body was similar to that of the reciprocal hybrids in color and pattern. The margin of the submaxilla was almost completely or partly black or dark gray (Plate II, 13 and 14).

3. Hybrids produced in 1977 among Japanese, European and American toads and the controls

Three females and three males of each of five kinds of mature toads, *Bufo americanus*, *Bufo americanus*♀ × *Bufo bufo japonicus*♂, *Bufo bufo japonicus*♀ × *Bufo bufo* from France♂, *Bufo bufo japonicus*♀ × *Bufo bufo* from Greece♂ and *Bufo bufo bufo* from Portugal♀ × *Bufo bufo* from France♂, were observed in terms of external characters. Relative sizes of various body sites are presented in Table 13. All these toads were two years old. Besides, two male *Bufo bufo* collected from France and Greece were observed.

a. Controls, *Bufo americanus*♀ No. 1 × *Bufo americanus*♂ No. 1

Three females and three males were 78.5 mm and 70.0 mm in mean body length, respectively. The canthus rostralis was gently elevated. The iris was light gray or light greenish-gray. The tympanum was round or oval and nearly the same as that of *japonicus* in diameter (Table 13). The parotoid gland was of broad-bean shape. It was distinctively broader than that of *japonicus* and situated 3～4 mm from the posterior end of the eyelid. The arms were slightly longer than those of *japonicus*.

The skin of the back was rough. While the dorsal skin of females was remarkably granulous owing to the presence of many minute tubercles, that of males was seamed with innumerable wrinkles. The main tubercles on the back were more highly protruded than those of *japonicus*. There was no row of large tubercles between the parotoid gland and the groin. The back was grayish-brown with pale bilateral patches (♀) or dull greenish-yellow (♂). There was a pale median stripe. The main tubercles on the back were mostly bordered with a black mark. The large and small dermal tubercles on the back of females were reddish-brown, while those of males were greenish-yellow. The females had

TABLE 13

Relative sizes of various body sites of hybrids among *Bufo americanus*, *Bufo bufo japonicus* and *Bufo bufo* from France, Greece and Portugal, and of male *Bufo bufo* collected from France and Greece. All the toads were produced in 1977 except the field-caught and measured in 1979

Kind	*ame.* ♀ × *ame.* ♂	*ame.* ♀ × *jap.* ♂	*bufo* F.	*jap.* ♀ × *bufo* F. ♂	*bufo* G.	*jap.* ♀ × *bufo* G. ♂	*bufo* P. ♀ × *bufo* F. ♂
No. of toads	♀3, ♂3	♀3, ♂3	♂1	♀3, ♂3	♂1	♀3, ♂3	♀3, ♂3
Head length / Body length	0.23	0.24	0.25	0.24	0.24	0.25	0.26
Head width / Head length	1.48	1.46	1.29	1.42	1.40	1.33	1.33
Interorbital space / Head length	0.29	0.26	0.27	0.27	0.32	0.25	0.25
Diameter of tympanum / Head length	0.24	0.26	0.13	0.19	0.14	0.14	0.15
Length of parotoid / Head length	0.83	0.68	0.57	0.94	1.00	0.87	0.86
Width of parotoid gland / Length of parotoid gland	0.55	0.54	0.43	0.34	0.42	0.39	0.57
Arm length / Body length	0.62	0.63		0.67	0.73	0.65	0.70
Hind-leg length / Body length	1.17	1.18	1.19	1.23	1.29	1.23	1.37
Length of inner-metatarsal tubercle / Tibia length	0.20	0.20	0.21	0.18	0.21	0.18	0.15

ame., *Bufo americanus* *jap.*, *Bufo bufo japonicus* *bufo* F., *Bufo bufo* from France *bufo* G., *Bufo bufo* from Greece *bufo* P., *Bufo bufo bufo* from Portugal

many dark gray flecks dispersed on the whole back. The parotoid gland was light or dark brown. There was neither white band nor black one between the parotoid gland and the groin, while there were numerous dark gray flecks which tended to form an irregular reticulation. The underside of the body was light gray tinged very faintly with yellow. Some toads had many or a few gray flecks. The throat of males was dark gray. The margin of the submaxilla was almost whitish (Plate III, 21 and 22).

b. Hybrids, *Bufo americanus* ♀ No. 1 × *Bufo bufo japonicus* ♂ No. 4

Three females and three males were 93.0 mm and 89.7 mm in mean body length, respectively. The canthus rostralis was intermediate in shape between the two species. The iris was also intermediate in color, that is, light yellowish-orange. The tympanum was oval and nearly the same in diameter as that of *americanus* (Table 13). The parotoid gland closely resembled that of *americanus* in shape. It was situated 0.5～3 mm from the posterior end of the eyelid. The arms were nearly the same in relative length as those of *americanus*.

The dorsal skin of the body and its tubercles were intermediate between the two species. There was a row of large tubercles between the parotoid gland and the groin, as found in *japonicus*. The back is dull greenish-yellow, mostly tinged with light or dark brown. There was often a pale median stripe. The main

tubercles were not usually bordered with a black mark. The color of the large and small tubercles as well as the pattern of the back was intermediate between those of the two species. The parotoid gland and the flank were also intermediate in color and pattern between those of the two species. The underside of the body was pale yellow with many light or dark gray flecks dispersed over the entire surface. The margin of the submaxilla was almost whitish (Plate III, 19 and 20).

c. A field-caught *Bufo bufo* from France

A single male was 89.6 mm in body length. The canthus rostralis was gently elevated and the iris was deep reddish-orange, as in *bufo* from Portugal. The tympanum was nearly round and small in diameter, although its outline was not well defined (Table 13). The parotoid gland was ellipsoidal and similar in shape to that of *bufo* from Portugal, although it was remarkably shorter than the latter. It was located 3.0 mm from the posterior end of the eyelid.

The dorsal skin of the body was seamed with innumerable wrinkles. The main tubercles on the back were generally smaller and more numerous than those of *japonicus*. Large tubercles were extremely few. The back was dirty olive-brown and had no pattern, although the tubercles were generally dark brown. The lower part of the parotoid gland was blackish-brown, while the upper part was of nearly the same color as that of the back. There was no white band on the parotoid gland. The flank had neither white nor dark band or mark, as in *bufo* from Portugal. The underside of the body was pale yellowish-gray. The margin of the submaxilla was whitish (Plate IV, 27 and 28).

d. Hybrids, *Bufo bufo japonicus*♀ No. 3 × *Bufo bufo* from France♂ No. 1

Three females and three males were 89.7 mm and 105.8 mm in mean body length, respectively. The canthus rostralis and the parotoid gland were intermediate in shape between those of *Bufo bufo japonicus* and *Bufo bufo* from France. The iris was intermediate in color between those of two subspecies in most hybrids, while it was similar to that of one or the other subspecies in the remaining hybrids. The tympanum was oval and larger than that of *bufo* from France (Table 13). The parotoid gland was intermediate in shape between those of the two subspecies. It was located 1 ~ 3 mm from the posterior end of the eyelid.

The dorsal skin of the body was not so smooth as that of *japonicus*. The main tubercles on the back were somewhat smaller than those of *japonicus*. Some of them were arranged in two rows along the median line of the back. The color and pattern of the back were composed of an irregular, dirty-brown network and pale brownish-olive meshes. The upper half of the parotoid gland was dirty brown tinged with white, while the lower half was black or blackish brown. There was an irregular, dark-brown network between the parotoid gland and the groin. The underside of the body was whitish with gray or dark-brown flecks or vermiculations dispersed over the entire surface. The margin of the submaxilla was partly black or dark gray (Plate IV, 29 and 30).

e. A field-caught toads, *Bufo bufo* from Greece♂ No. 1

This male was 104.5 mm in body length. The canthus rostralis was gently elevated. The iris was deep reddish-orange. The tympanum was oval and distinctively smaller in diameter than that of *japonicus* (Table 13). The parotoid gland was of persimmon-stone shape and situated 2 mm from the posterior end of the eyelid. The arms were remarkably longer than those of *japonicus*.

The dorsal skin of the body was not so smooth as that of *japonicus*; it was seamed with innumerable wrinkles. The main tubercles on the back were smaller and more numerous than those of *japonicus*. Some of them had a very weak tendency of arrangement in two rows along the median-line of the back. There was a row of comparatively large tubercles between the parotoid gland and the groin. The back was greenish-gray with several orange flecks. The upper wide area of the parotoid gland was greenish gray, while the lower narrow area was orange with brown flecks. The flank between the parotoid gland and the groin was greenish gray with an irregular orange mark. The underside of the body was pale gray with an irregular greenish-gray network. The margin of the submaxilla was whitish (Plate IV, 23 and 24).

f. Hybrids, *Bufo bufo japonicus*♀ No. 3 × *Bufo bufo* from Greece♂ No. 1

Three females and three males were 110.7 mm and 96.5 mm in mean body length, respectively. The canthus rostralis was intermediate in shape between the two subspecies. The iris was intermediate in color between those of the two subspecies in most hybrids. In the remaining hybrids, it was similar to that of one or the other subspecies. The tympanum was oval and small; it was nearly the same in diameter as that of *bufo* from Greece (Table 13). The parotoid gland was intermediate in shape between those of the two subspecies and situated 1～3 mm from the posterior end of the eyelid.

The skin and its main tubercles of the back were intermediate in appearance between those of the two subspecies. Some of the tubercles were in two rows along the median line of the back. There was a row of comparatively large tubercles between the parotoid gland and the groin. The back was intermediate in color between the two subspecies; it was greenish-gray with dark brown blotches, flecks and spots. Almost all the large and small tubercles on the back were dark brown. In some toads, the anterior part of the back was tinged with orange. The upper half of the parotoid gland was greenish brown tinged with white or white tinged with greenish brown. The lower half was black or dark brown. A white or pale gray band and a reticular black or dark-brown mark were arranged above and below between the parotoid gland and the groin. The underside of the body was whitish with an irregular black or gray network. The margin of the submaxilla was partly black or gray (Plate IV, 25 and 26).

g. Hybrids, *Bufo bufo bufo* from Portugal♀ No. 2 × *Bufo bufo* from France♂ No. 1

Three females and three males were 92.2 mm and 70.0 mm in mean body

length, respectively. The canthus rostralis was gently elevated. The iris was deep reddish-orange. The tympanum was oval and remarkably small in diameter (Table 13). The parotoid gland was elliptical and distinctly broader than that of *japonicus*. It was situated 2~6 mm from the posterior end of the eyelid. The arms were remarkably longer than those of *japonicus*. In these respects, this kind of hybrids was very similar to *Bufo bufo bufo* from Portugal (Tables 11 and 13).

The dorsal skin of the body and its main tubercles were very similar in appearance to those of *bufo* from Portugal. Some of the tubercles have a weak tendency of arrangement in two rows along the median line of the back. The back was brown (♀), yellowish-brown (♀) or greenish-brown (♂), making an irregular network with pale-colored meshes. There was neither white band nor black one on the parotoid gland and flank. The lateral surface of the body showed a color and pattern similar to the dorsal surface. The underside of the body was whitish with or without pale gray (♀) or pale greenish-gray (♂) vermiculations. The margin of the submaxilla was whitish (Plate V, 33 and 34).

4. Hybrids produced in 1978 among Japanese, European and American toads and the controls

Three females and three males of each of four kinds of mature toads, *Bufo bufo miyakonis*♀ × *Bufo bufo japonicus*♂, *Bufo torrenticola*♂ and *Bufo bufo bufo* from Portugal♂, and *Bufo bufo bufo* from Portugal♀ × *Bufo torrenticola*♂, three male mature *Bufo viridis*, six male mature toads, *Bufo bufo miyakonis*♀ × *Bufo viridis*♂, and six male mature toads, *Bufo bufo japonicus*♀ × *Bufo viridis*♂, were observed in terms of external characters. All these toads were one year old. Besides, one female and three male field-caught *Bufo bufo miyakonis* and one female field-caught *Bufo viridis* were observed (Tables 14 and 15).

a. Field-caught *Bufo bufo miyakonis*

One female was 93.5 mm in body length, while three males were 90.8 mm in mean body length. The canthus rostralis was somewhat gentler in elevation than that of *japonicus*. The iris was deep brown. The tympanum was almost round. While the tympanum of the only female was nearly the same in diameter as that of *japonicus*, those of the males were smaller than the latter (Table 14). The parotoid gland was of spindle shape, being similar to that of *japonicus*. While the parotoid gland of the single female was nearly the same in length as that of *japonicus*, those of the males were remarkably shorter than the latter. The parotoid gland was situated closely to or 1 mm from the posterior end of the eyelid. The arms were longer than those of *japonicus*.

The dorsal skin of the body was seamed with innumerable wrinkles. Its main tubercles were spheres or short rods and nearly the same in size as those of *japonicus*. Some of them had a tendency of arrangement in two rows along the median line of the back. There was also a row of large tubercles between the parotoid gland and the groin. The back was grayish-brown and had a few pale yellowish-brown areas. The parotoid gland was light yellowish-brown or

TABLE 14

Relative sizes of various body sites of hybrids between *Bufo bufo miyakonis* and *Bufo bufo japonicus*, *Bufo torrenticola*, *Bufo bufo bufo* from Portugal and *Bufo viridis*, and of field-caught *Bufo bufo miyakonis*. All the toads were produced in 1978 except the field-caught and measured in 1979

Kind	*miy.* (field)	*miy.* ♀ × *jap.* ♂	*miy.* ♀ × *tor.* ♂	*miy.* ♀ × *bufo* P. ♂	*miy.* ♀ × *vir.* ♂
No. of toads	♀1, ♂3	♀3, ♂3	♀3, ♂3	♀3, ♂3	♂6
Head length / Body length	0.26	0.25	0.24	0.28	0.22
Head width / Head length	1.31	1.38	1.40	1.32	1.52
Interorbital space / Head length	0.31	0.27	0.33	0.30	0.29
Diameter of tympanum / Head length	0.16	0.18	0.18	0.18	0.19
Length of parotoid / Head length	0.63	0.83	0.71	0.83	0.85
Width of parotoid gland / Length of parotoid gland	0.35	0.34	0.42	0.36	0.43
Arm length / Body length	0.69	0.62	0.70	0.68	0.67
Hind-leg length / Body length	1.30	1.18	1.20	1.24	1.27
Length of inner-metatarsal tubercle / Tibia length	0.14	0.16	0.18	0.15	0.20

miy., *Bufo bufo miyakonis* *jap.*, *Bufo bufo japonicus* *tor.*, *Bufo torrenticola* *bufo* P., *Bufo bufo bufo* from Portugal *vir.*, *Bufo viridis*

grayish-brown. The row of large tubercles between the parotoid gland and groin was whitish. The underside of the body was whitish or pale gray with several black and orange flecks or blotches. The margin of the submaxilla was whitish (Plate V, 37 and 38).

b. Hybrids, *Bufo bufo miyakonis*♀ No. 1 × *Bufo bufo japonicus*♂ No. 6

Three females and three males were 106.3 mm and 83.8 mm in mean body length, respectively. The canthus rostralis was intermediate in shape between those of the two subspecies. The iris was light yellowish-brown. The tympanum was almost round and smaller in diameter than that of *japonicus* (Table 14). The parotoid gland was of spindle shape and situated closely to or 1～2 mm from the posterior end of the eyelid.

The dorsal skin of the body was intermediate in appearance between those of the two subspecies or similar to that of *miyakonis*. The size, number and arrangement of the main tubercles on the back were almost similar to those of the two subspecies. The back was dark reddish-brown, as in *japonicus*. The lateral sides of the body were similar in color and pattern to those of *japonicus*; there were an upper white band and a lower black band on the parotoid gland and the flank. The underside of the body was whitish with black or dark-gray vermiculations. The margin of the submaxilla was almost black (Plate VI, 41 and 42).

c. Hybrids, *Bufo bufo miyakonis*♀ No. 1 × *Bufo torrenticola*♂ No. 3

Three females and three males were 88.5 mm and 82.0 mm in mean body length, respectively. The canthus rostralis was intermediate in shape between those of the two species. The iris was light yellowish-brown. The tympanum was oval and larger in diameter than those of *torrenticola* and field-caught *miyakonis* (Tables 12 and 14). The parotoid gland was of spindle shape and situated 1～2 mm from the posterior end of the eyelid. The arms were nearly of the same length as those of the two species.

The dorsal skin of the body and its main tubercles were similar in appearance to those of the two species. The back was dirty greenish-brown (♂) or dirty yellowish- or reddish-brown (♀). The upper half of the parotoid gland was white or gray-brownish white, while the lower half was black. The row of large tubercles between the parotoid gland and the groin was white or pale yellow. The black mark of the parotoid gland extended toward the groin as a black or dark-colored band or a group of blackish flecks. The underside of the body was pale yellow with blackish vermiculations. The margin of the submaxilla was partly black (Plate VI, 43 and 44).

d. Hybrids, *Bufo bufo miyakonis*♀ No. 1 × *Bufo bufo bufo* from Portugal♂ No. 5

Three females and three males were 70.3 mm and 67.8 mm in mean body length, respectively. The canthus rostralis was gently elevated. The iris was deep orange. The tympanum was oval and somewhat larger in diameter than that of *bufo* from Portugal (Tables 11 and 14). The parotoid gland was intermediate in shape between those of *miyakonis* and *bufo*. It was situated close to or 1～2 mm from the posterior end of the eyelid.

The dorsal skin of the body was seamed with innumerable wrinkles. The main tubercles on the back were intermediate in number and size between the two subspecies. Some of them had a weak tendency of arrangement in two rows along the median line of the back. There was a row of large tubercles between the parotoid gland and the groin, although this row was not so distinct as that of *miyakonis*. The back was greenish-brown with some pale yellowish areas (♂) or reddish-brown with some paler areas (♀). While the upper wide part of the parotoid gland was reddish-brown, the lower narrow part was black or gray. The row of large tubercles between the parotoid gland and the groin was pale yellowish- or reddish-brown. There was no black or dark band on the flank. The underside of the body was whitish with gray flecks or vermiculations. The margin of the submaxilla was whitish (Plate V, 35 and 36).

e. Controls, *Bufo viridis*♀ No. 1 × *Bufo viridis*♂ No. 1 and a field-caught *Bufo viridis*

Three males produced by mating in the laboratory were 93.0 mm in mean body length, while a field-caught female was 93.5 mm. The canthus rostralis was gently elevated. The iris was bright bluish-green. The tympanum was round. The parotoid gland was of broad-bean shape and situated close to the

posterior end of the eyelid.

The dorsal skin of the body was seamed with innumerable wrinkles. The main tubercles on the back were spherical and somewhat smaller than those of *japonicus*, although they were more numerous than the latter. Some large tubercles were arranged in a row between the parotoid gland and groin. There were no tubercles which had a tendency of arrangement in two rows along the median line of the back. The back was yellow-green with a whitish or pale colored network. The parotoid gland was yellow-green and covered partly with pale colored network. The row of large tubercles between the parotoid gland and the groin was whitish. The underside of the body was whitish with several greenish-gray blotches. The margin of the submaxilla was whitish (Plate VII, 47 and 48).

f. Hybrids, *Bufo bufo miyakonis* ♀ No. 1 × *Bufo viridis* ♂ No. 1

Six males were 74.5 mm in mean body length. The canthus rostralis was gently elevated. The iris was light greenish-yellow. The tympanum was round. The parotoid gland was intermediate in shape between those of the two species or similar to that of *viridis*. They were situated close to the posterior end of the eyelid.

The dorsal skin of the body was seamed with innumerable wrinkles. All the main tubercles on the back were spherical. They were smaller and usually more numerous than those of *miyakonis*. Some of them had a weak tendency of arrangement in two rows along the median line of the back. Some large tubercles were arranged in a row between the parotoid gland and the groin. The back was dirty yellow-orange or green with a pale median stripe. A few toads had a vague, dull-green network on a paler ground. The upper wide part of the parotoid gland was white tinged with the same color as that of the back. The row of large tubercles between the parotoid gland and the groin was whitish. The underside of the body was whitish with several gray flecks. The margin of the submaxilla was whitish (Plate VII, 49 and 50).

g. Hybrids, *Bufo bufo japonicus* ♀ No. 7 × *Bufo viridis* ♂ No. 1

Six males were 89.2 mm in mean body length. The canthus rostralis was intermediate in shape between those of the two species. The iris was light greenish-yellow. The tympanum was round. The parotoid gland was intermediate in shape between those of the two species or similar to that of *viridis*. It was situated close to the posterior end of the eyelid. The arms were of the same length as those of *viridis*.

The dorsal skin of the body was intermediate in smoothness between those of the two species. All the main tubercles on the back were spherical. They were smaller and more numerous than those of *japonicus*. Some of them had a tendency of arrangement in two rows along the median line of the back. Some other large tubercles were arranged in a row between the parotoid gland and the groin. The ground of the back was pale yellow-green or pale brown. Several dirty yellow-green or dark brown areas formed a mark of somewhat bilateral symmetry.

There was a pale median stripe on the back. The parotoid gland was yellow-greenish white with dark-brown flecks or blotches. The row of large tubercles between the parotoid gland and the groin was whitish. Some toads had a wide black band beneath the row of whitish tubercles, while the others had not such a black band. The underside of the body was whitish or yellowish and had several black or gray flecks or blotches. The margin of the submaxilla was whitish or had a few gray flecks (Plate VII, 51 and 52).

h. Hybrids, *Bufo bufo bufo* from Portugal♀ No. 3 × *Bufo torrenticola*♂ No. 3

Three females and three males were 77.3 mm and 79.0 mm in mean body length, respectively. The canthus rostralis was intermediate in shape between those of the two species; it was somewhat sharply elevated. The iris was bright reddish-orange. The tympanum was oval and small in diameter (Table 15). The parotoid gland was ellipsoidal. Though it was similar in relative length to that of *torrenticola*, it was much wider than those of the two species.

The dorsal skin of the body was seamed with innumerable wrinkles. The main tubercles on the back were intermediate in shape, size and number between those of the two species. Some of them had a weak tendency of arrangement in two rows along the median line of the back. There was a row of large tubercles between the parotoid gland and the groin.

TABLE 15

Relative sizes of various body sites of hybrids among *Bufo bufo japonicus*, *Bufo viridis*, *Bufo bufo bufo* from Portugal and *Bufo torrenticola*, and of field-caught and the control *Bufo viridis*. All the toads were produced in 1978 except the field-caught and measured in 1979

Kind	*jap.*♀ × *vir.*♂	*vir.*♀ × *vir.*♂	*vir.* (field)	*bufo* P.♀ × *tor.*♂
No. of toads	♂6	♂3	♀1	♀3, ♂3
Head length / Body length	0.23	0.24	0.22	0.27
Head width / Head length	1.40	1.43	1.41	1.32
Interorbital space / Head length	0.26	0.21	0.19	0.23
Diameter of tympanum / Head length	0.21	0.19	0.20	0.15
Length of parotoid / Head length	0.93	1.02	1.02	0.58
Width of parotoid gland / Length of parotoid gland	0.49	0.52	0.57	0.66
Arm length / Body length	0.63	0.63	0.60	0.69
Hind-leg length / Body length	1.17	1.23	1.13	1.32
Length of inner-metatarsal tubercle / Body length	0.17	0.17	0.18	0.17

jap., *Bufo bufo japonicus* *vir.*, *Bufo viridis* *bufo* P., *Bufo bufo bufo* from Portugal
tor., *Bufo torrenticola*

The back was light or dark yellow-green (♂) or light or dark brown with pale-brown or reddish-orange areas (♀). The upper part of the parotoid gland was of the same color as that of the back. The lower part was black or dark brown. Some toads had a narrow whitish area between the upper and the lower part. The row of large tubercles between the parotoid gland and the groin was white tinged with reddish-orange or yellow-green. Under this row of whitish tubercles, there was a wide black or gray band which became a reticular mark or a group of blotches and sometimes disappeared on the way to the groin. The underside of the body was yellowish white with or without many gray blotches. The margin of the submaxilla was yellowish white (Plate VII, 53 and 54).

IV. Gonads of mature toads

1. Hybrids produced in 1976 between Japanese and European toads

a. Male

Almost all the male hybrids produced from reciprocal crosses between *Bufo bufo japonicus* and *Bufo bufo bufo* from Portugal and the control males revealed secondary sexual characters at the age of one year. The testes of one-, two-, three- and four-year-old hybrids and the controls were observed. In addition, BIDDER's organs of these hybrids and the controls were mostly measured.

i) Controls, *Bufo bufo japonicus* and *Bufo bufo bufo*

The testes of mature toads obtained from two matings, *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo japonicus*♂ No. 1 and *Bufo bufo bufo*♀ No. 1 × *Bufo bufo bufo*♂ No. 3, were measured (Table 16). Four one-year-old male *japonicus* were 89.0～103.5 mm, average of 95.5 mm, in body length and had testes which were 9.0～15.0 mm in length and 4.0～5.5 mm in width. In contrast, three one-year-old male *Bufo bufo bufo*, which were 70.5～74.0 mm, average of 72.7 mm, in body length, had somewhat smaller testes, being 6.0～9.0 mm in length and 3.5～4.5 mm in width. BIDDER's organs of the four one-year-old male *japonicus* were 3.0～11.5 mm in length and 2.5～4.0 mm in width, while those of the three one-year-old male *bufo* were 3.0～7.0 mm in length and 2.5～5.0 mm in width (Table 16). Four two-year-old male *japonicus*, 96.0～115.0 mm, average of 107.4 mm, in body length, had cylindrical testes, which were 17.0～21.5 mm in length and 4.5～7.0 mm in width. Two two-year-old male *bufo*, 82.5 mm and 84.0 mm in body length, had ellipsoidal testes, which were 8.5 and 10.5 mm in length and 4.5 and 5.0 mm in width and very similar to those of the one-year-old *japonicus* in size and shape.

When the testes of three-year-old male toads were observed, there was a greater difference in their size and shape between the two subspecies. A male *japonicus*, 149.0 mm in body length, had long cylindrical testes which were 32.0 mm × 6.0 mm and 32.5 mm × 5.5 mm in size, while the testes of a male *bufo*, 92.5 mm in

TABLE 16

Testes and BIDDER's organs of mature male hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo* from Portugal and the controls. All the toads were produced in 1976

Parents		Individual no.	Age	Body length	Size of testes		Size of BIDDER's organs	
Female	Male		year(s)	mm	Left mm	Right mm	Left mm	Right mm
jap. W, No. 1	*jap.* W, No. 1	1	1	89.0	12.0×4.5	10.0×4.0	11.5×3.0	6.0×3.0
		2	1	94.5	10.5×5.0	11.5×5.5	5.0×3.0	5.0×3.0
		3	1	95.0	9.0×4.0	9.0×4.0	6.0×4.0	5.5×3.0
		4	1	103.5	15.0×5.0	10.0×4.5	3.0×2.5	6.0×3.5
		5	2	96.0	20.5×6.0	17.0×4.5		
		6	2	108.5	20.5×6.0	21.5×6.0		
		7	2	110.0	18.0×7.0	20.0×6.5		
		8	2	115.0	20.5×6.0	18.5×6.0		
		9	4	118.5	21.0×7.5			
		10	4	124.0	22.5×7.0			
bufo P. W, No. 1	*bufo* P. W, No. 3	1	1	73.5	6.5×4.0	6.0×4.5	3.0×5.0	4.0×2.5
		2	1	74.0	9.0×3.5	6.0×4.0	6.5×4.0	5.5×4.5
		3	1	70.5	7.0×3.5	7.0×4.0	3.0×2.5	7.0×3.5
		4	2	82.5	10.0×4.5	8.5×5.0		
		5	2	84.0	10.5×4.5	10.0×5.0		
		6	4	90.5	12.0×6.0	10.5×6.0		
		7	4	91.0	13.0×6.0			
jap. W, No. 1	*bufo* P. W, No. 1	1	1	75.0	7.0×4.0	7.0×4.0	3.5×3.0	3.0×3.0
		2	1	82.0	5.5×3.5	6.5×4.0	2.5×4.0	3.0×3.0
		3	1	80.5	5.0×4.0	5.0×4.0	8.5×4.0	4.5×4.0
		4	1	80.0	3.0×2.0	10.0×4.5	5.0×3.5	3.5×3.0
		5	1	76.5	6.0×2.5	4.5×2.0	0	0
		6	2	105.0	13.0×6.5	12.5×6.0		
		7	2	97.0	12.0×6.0	9.5×5.5		
		8	2	96.0	10.5×5.5	9.0×5.5		
		9	2	94.0	9.5×6.5	7.5×5.0		
		10	2	85.0	8.0×5.5	6.5×5.0		
bufo P. W, No. 1	*jap.* W, No. 3	1	1	83.5	12.5×4.5	7.5×4.0	1.5×2.0	8.0×9.0
		2	1	85.5	2.0×2.0	7.0×4.5	9.5×6.5	6.0×5.5
		3	1	85.0	12.5×5.5	7.0×4.5	3.5×2.0	6.0×3.0
		4	1	82.5	5.0×3.0	10.5×6.5	0.5×0.5	0.5×0.5
		5	1	78.5	5.0×3.5	8.0×4.0	0.5×0.5	0.5×0.5
		6	2	101.0	8.5×4.5	8.5×4.0		
		7	2	92.0	6.5×4.0	6.5×4.0		
		8	2	98.0	7.0×4.5	7.0×4.5		
		9	2	96.0	7.5×5.5	7.5×5.0		
		10	2	94.0	4.5×3.0	4.5×3.0		

body length, were 11.0 mm × 7.5 mm and 10.5 mm × 6.0 mm in size (Fig. 5a, b).

Two four-year-old male *japonicus* (Nos. 9 and 10) were 118.5 mm and 124.0 mm in body length. Their left testes were 21.0 mm × 7.5 mm and 22.5 mm × 7.0 mm in size (Table 16). In contrast with these Japanese toads, two four-year-old male *bufo* from Portugal (Nos. 6 and 7) were 90.5 mm and 91.0 mm in body length. Their testes were 10.5 ~ 13.0 mm in length and 6.0 mm in width.

The testes of all the one- and two-year-old male *japonicus* and *bufo* were normal

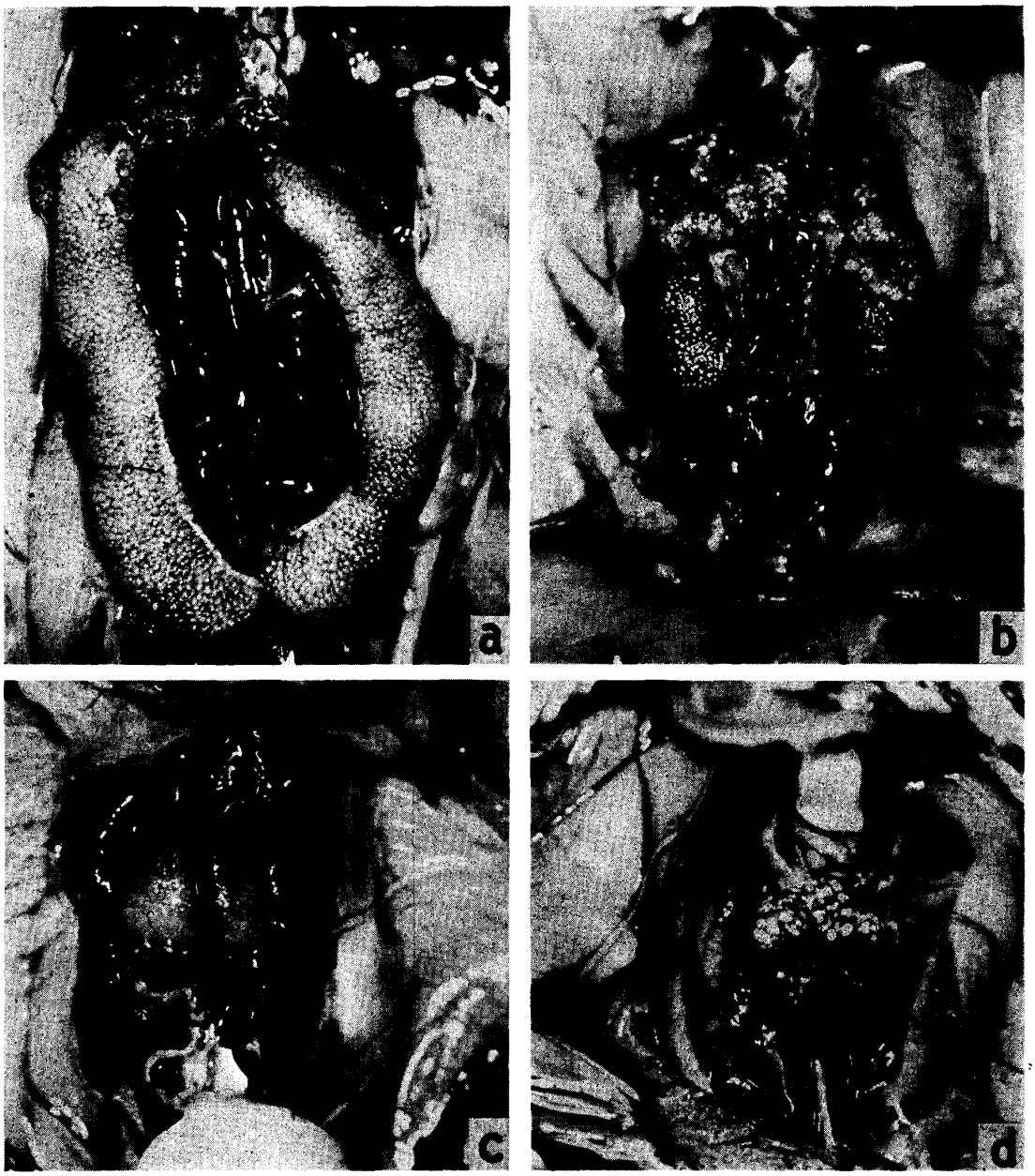

Fig. 5. Testes of three-year-old male hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo* from Portugal and the controls. ×2.0

a. Control, *japonicus* ♀ No. 1 × *japonicus* ♂ No. 1
b. Control, *bufo* from Portugal ♀ No. 1 × *bufo* Portugal ♂ No. 3
c. Hybrid, *japonicus* ♀ No. 1 × *bufo* from Portugal ♂ No. 1
d. Hybrid, *bufo* from Portugal ♀ No. 1 × *japonicus* ♂ No. 3

in inner structure. The cavities of seminiferous tubules were almost filled with compact bundles of normal spermatozoa (Plate VIII, 55 and 56). In each of the cross sections of seminiferous tubules, there were many first- and second spermatocytes and a few spermatogonia in addition to spermatozoa. BIDDER's organs of the two kinds of control toads were filled with oocytes which were about 100 μ in diameter (Plate XIX, 99 and 100).

ii) Reciprocal hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo*

The testes of reciprocal hybrids produced from two crosses, *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo bufo*♂ No. 1 and *Bufo bufo bufo*♀ No. 1 × *Bufo bufo japonicus*♂ No. 3, were measured (Table 16). They appeared generally to resemble those of *bufo* in shape. Five one-year-old male hybrids derived from female *japonicus* were 75.0～82.0 mm, average of 78.8 mm, in body length and had testes which were 3.0～10.0 mm in length and 2.0～4.5 mm in width. Five one-year-old male hybrids produced from female *bufo* No. 1 were 78.5～85.5 mm, average of 83.0 mm, in body length and had testes which were 2.0～12.5 mm in length and 2.0～6.5 mm in width. Although both kinds of hybrids were intermediate between the two kinds of controls in body length, their gonads were not always intermediate in size. Four of the five hybrids from female *japonicus* No. 1 and three of the five from female *bufo* No. 1 had a remarkably smaller testes than those of the control *bufo* on one side at least. While four of the five hybrids from female *japonicus* No. 1 and three of the five reciprocal hybrids from female *bufo* No. 1 had BIDDER's organs which were similar in size to those of the controls, the remaining three hybrids had no or extremely small BIDDER's organs (Table 16).

Five two-year-old hybrids derived from female *japonicus* No. 1 were 85.0～105.0 mm, average of 95.4 mm, in body length and had ellipsoidal testes which were very similar in size and shape to those of the two-year-old control *bufo*. Their testes were 6.5～13.0 mm in length and 5.0～6.5 mm in width. Five two-year-old hybrids derived from female *bufo* No. 1 were similar to the reciprocal hybrids in body length; they were 92.0～101.0 mm, average of 96.2 mm. While the testes of four of them were 6.5～8.5 mm in length and 3.0～5.5 mm in width, those of the remaining one were 4.5 mm in length and 3.0 mm in width. A three-year-old male hybrid derived from female *japonicus* No. 1 was 108.0 mm in body length and had testes which were 8.5 mm × 5.0 mm and 9.5 mm × 5.5 mm in size, while a three-year-old male hybrid derived from female *bufo* No. 1, 99.5 mm in body length, had testes which were 7.0 mm × 5.0 mm and 6.5 mm × 4.5 mm (Fig. 5c, d). It was found that the testes of the two male reciprocal hybrids at the age of three years were remarkably smaller than those of the male three-year-old *bufo*, which were in turn nearly one-third of those of the male three-year-old *japonicus* in size.

The testes of all the reciprocal hybrids between *japonicus* and *bufo* were distinctly abnormal in inner structure. There were no normal spermatozoa in the seminiferous tubules (Plate VIII, 57 and 58). In cross sections of the latter, there were a few abnormal spermatozoa and pycnotic nuclei besides primary and secondary spermatogonia and first spermatocytes. Germ cells seemed usually to degenerate at the metaphase or anaphase of the first reduction division. The basic structure of the testes except for germ cells was almost normal. BIDDER's organs of reciprocal hybrids somewhat differed from those of the control toads in inner structure (Plate XIX). The enlarged oocytes in BIDDER's organ of the hybrids were remarkably fewer and generally larger than those of the controls. Some of them were degenerating. Some hybrids had abundant oogonia in

BIDDER's organs (Plate XIX, 101 and 102).

b. Female

i) Controls, *Bufo bufo japonicus* and *Bufo bufo bufo*

All the female toads produced in 1976 from matings, *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo japonicus*♂ No. 1 and *Bufo bufo bufo*♀ No. 1 × *Bufo bufo bufo*♂ No. 3, were sexually immature at the age of one year. The female *japonicus*, 105.0～115.0 mm, average of 110.5 mm, in body length, had ovaries which were 32.5 mm × 9.5 mm～36.0 mm × 15.0 mm in size, while the female *bufo*, 75.0～90.5 mm, average of 83.4 mm, in body length, had ovaries which were 20.5 mm × 12.5 mm～30.5 mm × 13.0 mm (Fig. 6a, b). The ovaries of these one-year-old *japonicus* and *bufo* were filled with auxocytes which were mostly about 300 μ and 500 μ in diameter, respectively; the largest auxocytes were about 450 μ and 900 μ, respectively. The animal half of some auxocytes was dark to various degrees owing to deposition of melanin.

The control female *japonicus* and *bufo* matured sexually at the age of two years. Two female *japonicus* were 115.0 mm and 107.5 mm in body length and laid 6604 and 5927 eggs after pituitary injection (Table 17). Four female *bufo* were 84.0～95.5 mm, average of 88.8 mm, in body length and laid 1613～3785 eggs

TABLE 17

Eggs of mature female hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo* from Portugal and the controls. All the toads were produced in 1976

Parents		Individual no.	Age	Body length	No. of eggs	Mean diameter of eggs	
						Normal (50 eggs)	Large (10～30 eggs)
Female	Male		year(s)	mm		mm	mm
jap. W, No. 1	*jap.* W, No. 1	1	2	115.0	6604	2.46±0.02	
		2	2	107.5	5927		
bufo P. W, No. 1	*bufo* P. W, No. 3	1	2	88.5	3745	2.05±0.02	
		2	2	87.0	1613		
		3	2	95.5	3785		
		4	2	84.0	1720		
jap. W, No. 1	*bufo* P. W, No. 1	1	2	91.0	125		
		2	2	96.5	956		
		3	2	90.0	1467	1.88±0.01	
		4	2	87.5	511	2.00±0.07	2.43±0.02
		5	2	86.0	8		
		6	2	94.5	215		
		7	2	98.5	1402		
		8	4	90.0	1843	2.29±0.01	2.64±0.02
		9	4	73.5	1004	2.28±0.01	2.77±0.02
bufo P. W, No. 1	*jap.* W, No. 3	1	2	110.5	737		
		2	4	99.5	3867	2.18±0.01	2.80±0.00
		3	4	102.0	2274	2.42±0.03	2.84±0.01
		4	4	103.0	2259	2.20±0.01	2.64±0.01
		5	4	89.0	2905	2.19±0.01	2.57±0.03
		6	4	91.0	3326	2.11±0.02	2.66±0.02
		7	4	93.5	2663	2.17±0.01	2.73±0.03

after pituitary injection. The eggs of *japonicus* were distinctly larger than those of *bufo*. While 50 of the eggs of a female *japonicus* (No. 1) were 2.46±0.02 mm in diameter, those of a female *bufo* were 2.05±0.02 mm. Gelatinous strings enveloping eggs were measured about 5 hours after immersed in water. It was found that the gelatinous string of eggs obtained from female *japonicus* No. 1 was 8.5～10.0 mm in thickness, while that of eggs from female *bufo* No. 1 was 4.5～5.0 mm. The outer part of the gelatinous string of female *japonicus* formed a sheath composed of a single homogeneous layer which was somewhat solid. In contrast, the gelatinous sheath of female *bufo* appeared to be constructed of four homogeneous layers which were softer than those of *japonicus*. The wall of the sheath was about 1.0 mm in thickness. While the animal half of *japonicus*

Fig. 6. Ovaries of one-year-old female hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo* from Portugal and the controls. ×2.0

a. Control, *japonicus* ♀ No. 1 × *japonicus* ♂ No. 1
b. Control, *bufo* from Portugal ♀ No. 1 × *bufo* from Portugal ♂ No. 3
c. Hybrid, *japonicus* ♀ No. 1 × *bufo* from Portugal ♂ No. 1
d. Hybrid, *bufo* from Portugal ♀ No. 1 × *japonicus* ♂ No. 3

eggs was usually dark brown, that of *bufo* eggs was somewhat light grayish-brown.

ii) Hybrids, *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo bufo*♂ No. 1

Five one-year-old female hybrids, 88.0～120.5 mm, average of 109.7 mm, in body length, had ovaries which were 15.5 mm × 10.0 mm～32.0 mm × 15.0 mm in size (Fig. 6c); the largest ovaries were not inferior in size to the ovaries of the control *japonicus* and *bufo*. However, all the ovaries of these hybrids differed from those of the two kinds of controls in inner structure. In each ovary, there were some large auxocytes distributed separately or in small groups (Plate IX, 60). The auxocytes were mostly about 300 μ in diameter; the largest ones were about 700 μ. Some auxocytes were degenerating. Each ovary was mostly composed of oogonia, young oocytes and remnants of follicles whose auxocytes had degenerated.

Twenty-five two-year-old female hybrids, 86.5～120.0 mm, average of 98.5 mm, in body length, were injected with frog pituitaries to accelerate ovulation in the breeding season of *japonicus*. The results indicated that ovulation occurred in seven of them (Table 17). However, the eggs obtained from each of these female hybrids usually were remarkably fewer than those laid by each of the control female *japonicus* and *bufo*. While two hybrids (Nos. 3 and 7) laid 1467 and 1402 eggs, the other five did only 8～956 eggs. Moreover, another female (No. 4) laid a mixture of large and normal-sized eggs. Of 511 eggs, 62 were large, being 2.43±0.02 mm and 449 were of normal size, being 2.00±0.07 mm in diameter. In the remaining six females, the eggs laid by each female were of nearly the same size. Those of female No. 3 which laid the largest number (1467) of eggs were 1.88±0.01 mm in diameter. The gelatinous strings including eggs laid by this female were about 6.5 mm in thickness when they were measured about 5 hours after immersed in water. The gelatinous sheath surrounding eggs was composed of a single homogeneous layer like that of *japonicus*, although it was intermediate between those of the two parental subspecies in solidity. The animal half of each egg was dark brown, although the color was slightly lighter than that of *japonicus* eggs. The gelatinous strings including eggs laid by female No. 4 were about 8.5 mm in thickness. The gelatinous sheath surrounding the eggs was composed of a single homogeneous layer and was intermediate in solidity between those of the two subspecies like that of female No. 3. The eggs were also the same as those of female No. 3 in color.

Two four-year-old female hybrids (Nos. 8 and 9), 90.0 mm and 73.5 mm, average of 81.8 mm, in body length, laid 1843 and 1004 eggs after pituitary injection (Table 17). The gelatinous strings including these eggs of the two females were 13.0 mm and 11.5 mm in thickness when they were measured about 5 hours after immersed in water. The eggs laid by each female were sorted into two kinds by size, normal and large. The large eggs were always far fewer than the normal-sized ones.

iii) Hybrids, *Bufo bufo bufo*♀ No. 1 × *Bufo bufo japonicus*♂ No. 3

Five one-year-old female hybrids, 80.0～99.0 mm, average of 92.6 mm, in body length, had ovaries which were 11.5 mm × 6.0 mm～13.0 mm × 5.5 mm in size. These values showed that their ovaries were remarkably smaller than those of the reciprocal hybrids and the controls (Fig. 6d). When the ovaries of the one-year-old female hybrids were microscopically examined, it was found that they were more abnormal in inner structure than those of the reciprocal hybrids. There were only a few growing auxocytes which were about 220 μ in diameter. Some of these oocytes were degenerating. Each ovary mostly contained oogonia, young oocytes and the remnants of follicles whose auxocytes had degenerated (Plate IX, 61).

Five two-year-old female hybrids, 83.5～115.5 mm, average of 107.5 mm, in body length were injected with frog pituitaries. It was found that only one of them laid 737 eggs; no ovulation occurred in the other four female (Table 17).

In contrast with two-year-old female hybrids, six four-year-old females (Nos. 2～7), 89.0～103.0 mm, average of 96.3 mm, in body length, all laid 2259～3867 eggs (Table 17). The gelatinous strings were 7.5～11.5 mm in thickness when they were measured about 5 hours after immersed in water. A small number of eggs laid by each female were remarkably large in size, being 2.57～2.84 mm in mean diameter, while the others were of normal size, being 2.11～2.42 mm.

2. Hybrids produced in 1977 between Japanese and European toads

a. Male

Some of the male hybrids produced from crosses between a female *Bufo bufo japonicus* and two male *Bufo bufo* from France and Greece revealed secondary sexual characters at the age of one year. The testes and BIDDER's organs of these males were observed.

i) Hybrids, *Bufo bufo japonicus*♀ No. 3 × *Bufo bufo* from France♂ No. 1

Five one-year-old male hybrids which revealed secondary sexual characters were 76.0～86.0 mm, average of 80.3 mm, in body length. Their testes were 5.5～10.5 mm in length and 2.5～4.5 mm in width (Table 18, Fig. 7a). When these testes were compared with those of four control males produced from the same mother, *Bufo bufo japonicus*♀ No. 3, by mating with a male *Bufo bufo japonicus* No. 4 in 1977 (Table 20), it was found that they were, roughly speaking, one half of the latter in length, although the one-year-old male *japonicus* were somewhat larger in body length. They were nearly the same in relative size as those of one-year-old male hybrids produced from reciprocal crosses between *japonicus* and *bufo* from Portugal in 1976 (Table 16). BIDDER's organs of the five hybrids between the female *japonicus* and the male *bufo* from France were nearly normal in size, except that the right organ was remarkably small in each of three hybrids Nos. 1, 2 and 3 (Table 18).

The testes of the five male hybrids were abnormal in inner structure. No normal spermatozoa were produced in their testes. In four males (Nos. 1, 2, 4

TABLE 18

Testes and BIDDER's organs of mature male hybrids between *Bufo bufo japonicus* and *Bufo bufo* from France or Greece and between *Bufo bufo* from Portugal and France
All the toads were produced in 1977

Parents		Individual no.	Age	Body length	Size of testes		Size of BIDDER's organs	
Female	Male		year(s)	mm	Left mm	Right mm	Left mm	Right mm
jap. W, No. 3	*bufo* F. W, No. 1	1	1	86.0	5.5×2.5	6.5×3.5	8.5×2.5	2.5×2.0
		2	1	82.0	8.5×4.0	8.5×4.0	12.0×3.0	1.5×2.5
		3	1	80.0	8.0×3.5	10.5×4.5	4.0×1.5	1.5×1.5
		4	1	77.5	6.5×2.5	5.5×2.5	6.5×2.5	8.0×2.5
		5	1	76.0	9.0×4.0	7.5×4.5	8.0×3.0	8.0×2.5
jap. W, No. 3	*bufo* G. W, No. 1	1	1	80.5	4.0×2.5	5.0×4.5	5.0×2.0	7.5×3.0
		2	1	83.5	5.5×3.5	6.5×3.5	6.0×2.5	8.5×2.5
		3	1	82.0	2.0×2.0	5.0×4.5	5.5×3.5	4.5×3.0
		4	1	79.0	6.0×3.5	4.5×3.0	7.5×2.0	8.5×3.5
		5	1	74.0	3.5×2.0	Very small	11.0×6.0	11.5×6.5
bufo P. W, No. 2	*bufo* F. W, No. 1	1	3	78.0	7.0×7.0		5.0×3.5	

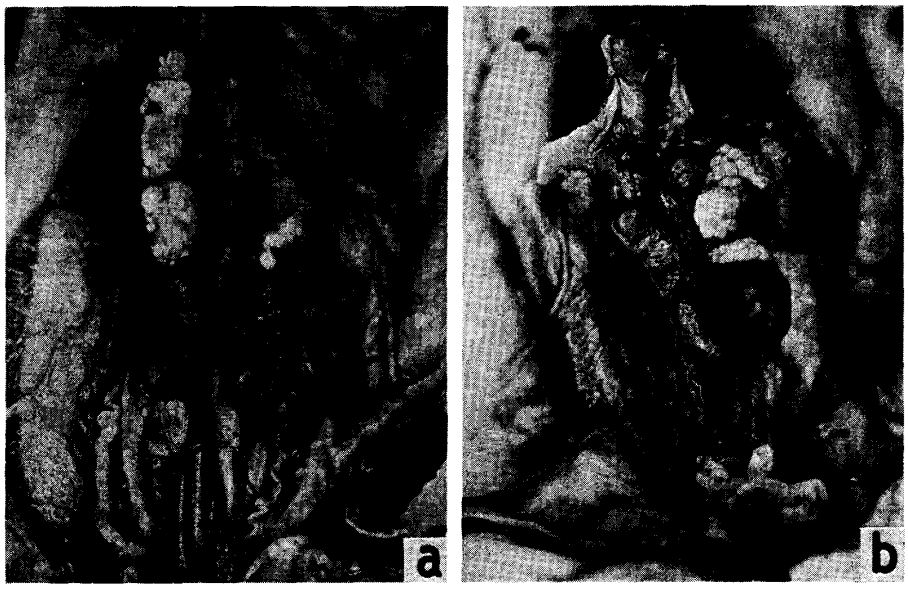

Fig. 7. Testes of one-year-old male hybrids between a female *Bufo bufo japonicus* and a male *Bufo bufo* from France or Greece. ×2.0

a. Hybrid No. 2, *japonicus* ♀ No. 3 × *bufo* from France ♂ No. 1
b. Hybrid No. 2, *japonicus* ♀ No. 3 × *bufo* from Greece ♂ No. 1

and 5), the cross sections of seminiferous tubules were distinctly small in diameter and the reduction divisions of germ cells hardly advanced beyond the first metaphase. Many first spermatocytes at this stage fused with one another to form a few large masses and degenerated sooner or later (Plate X, 63). The seminiferous tubules mostly contained spermatogonia along the walls. In male hybrid No. 3, the cross sections of seminiferous tubules were larger in diameter and the reduction divisions seemed mostly to become abnormal at the first

anaphase. There were a few abnormal spermatozoa and many pycnotic nuclei in the seminiferous tubules. Along the walls of the latter there were many spermatogonia (Plate X, 64).

BIDDER's organs of the hybrids somewhat differed from the control *japonicus* in inner structure (Plate XX, 103). Enlarged oocytes like those found in the control female toads were very few, while there were usually many small oocytes. There was a great variety in size among these oocytes. BIDDER's organ on one side had no enlarged oocytes. The animal halves of a few enlarged oocytes were somewhat dark, owing to deposition of melanin.

ii) Hybrids, *Bufo bufo japonicus*♀ No. 3 × *Bufo bufo* from Greece♂ No. 1

Five one-year-old male hybrids with clear, secondary sexual characters were 74.0～83.5 mm, average of 79.8 mm, in body length and had testes which were 2.0～6.5 mm in length and 2.0～4.5 mm in width (Fig. 7b). These testes were generally smaller than those of the male hybrids between *japonicus* and *bufo* from Portugal or France. Especially, the left testis of male hybrid No. 3 was only 2.0 mm long and 2.0 mm wide. The right testis of male hybrid No. 5 was also extremely small. The testes of the five male hybrids were very similar in inner structure to those of the male hybrids produced from the same mother by mating with a male *bufo* from France. No normal spermatozoa were found in these testes. In male hybrid No. 1, the first spermatocytes degenerated at the metaphase. Some of them formed a few large masses by fusing with one another before degeneration, as found in four male hybrids derived from male *bufo* from France No. 1. The seminiferous tubules of three other males (Nos. 2～4) contained some abnormal spermatozoa and pycnotic nuclei besides spermatogonia and first spermatocytes (Plate X, 66). In these testes, second spermatocytes were very scarce. The remaining male hybrid (No. 5) had no germ cells in seminiferous tubules. The latter were slender and empty, while wide spaces between them were filled with compact stromatic tissues (Plate X, 65).

BIDDER's organs of the five male hybrids were 4.5 mm × 3.0 mm～11.5 mm × 6.5 mm in size. There were no male hybrids which had remarkably small BIDDER's organs like those found in some male hybrids between the same mother and a male *bufo* from France and between a female *bufo* from Portugal and a male *japonicus*. BIDDER's organs of male hybrids Nos. 1 and 4 were nearly the same as those of the control *japonicus* (Table 20) in inner structure. They were almost filled with enlarged oocytes which were nearly of the same size (Plate XX, 104). Male hybrid No. 2 had no enlarged oocytes in BIDDER's organ, while male hybrid No. 3 had a small number of enlarged oocytes which varied in size. BIDDER's organs of male hybrid No. 5 contained a few oocytes which were extremely large.

b. Female

A one-year-old female hybrid between female *Bufo bufo japonicus* No. 3 and male *Bufo bufo* from France No. 1 was 76.0 mm in body length. The left and right ovaries of this female were 15.0 mm × 5.0 mm and 17.5 mm × 4.0 mm in

size, respectively. Four one-year-old female hybrids between the same mother and male *bufo* from Greece No. 1 were 80.0~89.5 mm, average of 86.4 mm, in body length. Their ovaries were 15.0 mm × 4.0 mm ~ 17.5 mm × 7.0 mm in size. The ovaries of these two kinds of female hybrids were filled with growing auxocytes, although they were slightly inferior in differentiation to the control female *japonicus* produced from the same mother.

Five three-year-old female hybrids (Nos. 1~5) between female *Bufo japonicus* Nos. 3~5 and male *Bufo bufo* from France No. 1 were 99.0~123.0 mm in body length (Table 19). Of these female hybrids, three (Nos. 1~3) laid 4228~6163 eggs after pituitary injection. The gelatinous strings including these eggs were 13.5~15.0 mm in thickness about five hours after immersed in water. Most of the eggs were 2.19~2.27 mm in mean diameter, and besides them there were always a small number of larger eggs, 2.68~2.86 mm in mean diameter. Five three-year-old female hybrids (Nos. 1~5) between female *Bufo bufo japonicus* No. 3 and male *Bufo bufo* from Greece No. 1 were 95.0~118.5 mm in body length (Table 19). After pituitary injection three (Nos. 1~3) of them laid 476~3137 eggs, most of which were 2.14~2.18 mm in mean diameter. In addition to these normal-sized eggs, there were always a small number of large eggs that were 2.58~2.65 mm in mean diameter. The gelatinous strings including the eggs were 13.5~15.0 mm in thickness about five hours after immersed in water.

TABLE 19

Eggs of mature female hybrids between *Bufo bufo japonicus* and *Bufo bufo* from France or Greece and between *Bufo bufo* from Portugal and France
All the toads were produced in 1977

Parents		Individual no.	Age	Body length	No. of eggs	Mean diameter of eggs	
						Normal (50 eggs)	Large (10~30 eggs)
Female	Male		year(s)	mm		mm	mm
jap. W, No. 3	*bufo* F. W, No. 1	1	3	104.5	4228	2.19±0.01	2.72±0.02
jap. W, No. 5	*bufo* F. W, No. 1	2	3	120.0	6163	2.27±0.01	2.86±0.02
jap. W, No. 5	*bufo* F. W, No. 1	3	3	123.0	5255	2.19±0.01	2.68±0.02
jap. W, No. 3	*bufo* F. W, No. 1	4	3	116.0	0		
jap. W, No. 4	*bufo* F. W, No. 1	5	3	99.0	0		
bufo P. W, No. 2	*bufo* F. W, No. 1	1	3	90.0	5332	2.20±0.00	
		2	3	90.5	Many	2.20±0.00	
		3	3	91.0	5015	2.18±0.01	
		4	3	88.0	Many	2.19±0.01	
		5	3	91.0	Many	2.13±0.01	
		6	3	91.5	5368		
		7	3	90.5	4713		
		8	3	95.5	5893		
jap. W, No. 3	*bufo* G. W, No. 1	1	3	98.5	3006	2.14±0.01	2.62±0.01
		2	3	95.0	476	2.18±0.01	2.65±0.01
		3	3	118.5	3137	2.14±0.01	2.58±0.03
		4	3	105.0	0		
		5	3	100.0	0		

3. Hybrids produced in 1977 between different populations of European *Bufo bufo*

a. Male

Of the male hybrids produced from the cross between a female *Bufo bufo bufo* from Portugal (No. 2) and a male *Bufo bufo* from France (No. 1), only two attained the age of three years. The left testis of one of them, 78 mm in body length, was removed from the body cavity and measured. It was spherical, 7 mm × 7 mm in size (Table 18). BIDDER's organ on the left side was 5.0 mm × 3.5 mm.

b. Female

Eight female hybrids (Nos. 1～8), 88.0～95.5 mm, average of 91.0 mm, in body length, produced from the same cross as the above were injected with frog pituitaries (Table 19). The results showed that all of them laid numerous eggs which were considered to be completely normal in number and size. Although the number of eggs laid by three females (Nos. 2, 4 and 5) was not counted, the egg size was measured. The other five females laid 4713～5893 eggs, average of 5264.2 eggs. The eggs of five female hybrids (Nos. 1～5) were 2.13～2.20 mm in mean diameter of 50 eggs; there were no large eggs like those that were laid by female intersubspecific hybrids together with normal-sized eggs. The gelatinous strings including the eggs of these five females were 9.0～10.0 mm in thickness when they were measured about 5 hours after immersed in water.

4. Hybrids produced in 1977 from two Japanese toad species

Male and female hybrids between a female *Bufo bufo japonicus* and a male *Bufo torrenticola* and the controls obtained from the same mother by mating with a male *Bufo bufo japonicus* matured sexually at the age of one year. In contrast, female hybrids produced from the reciprocal cross and the control females obtained from the same female by mating with a male *torrenticola* could sexually mature at the age of two years, while males matured at the age of one year.

a. Male

i) Controls, *Bufo bufo japonicus* and *Bufo torrenticola*

The testes of mature toads obtained from two matings, *Bufo bufo japonicus*♀ No. 3 × *Bufo bufo japonicus*♂ No. 4, and *Bufo torrenticola*♀ No. 1 × *Bufo torrenticola*♂ No. 2, were measured at the age of one, two or three years (Table 20). BIDDER's organs of some of these toads were also observed. Four one-year-old male *japonicus* (Nos. 1～4), 92.5～98.0 mm, average of 95.5 mm, in body length, had testes which were 14.5～20.5 mm in length and 4.0～6.0 mm in width. BIDDER's organs of two (Nos. 3 and 4) of these males were 3.0 mm × 2.0 mm～6.5 mm × 3.5 mm in size. Two two-year-old male *japonicus* (Nos. 5 and 6) were 118.0 mm and 119.0 mm in body length. The left testes of these males were 21.0 mm × 6.0 mm and 22.5 mm × 5.0 mm in size. Their left BIDDER's organs

TABLE 20

Testes and BIDDER's organs of mature male hybrids between *Bufo bufo japonicus* and *Bufo torrenticola* and the controls. All the toads were produced in 1977

Parents		Individual no.	Age	Body length	Size of testes		Size of BIDDER's organs	
Female	Male		year(s)	mm	Left mm	Right mm	Left mm	Right mm
jap. W, No. 3	*jap.* W, No. 4	1	1	98.0	15.5×4.5	15.0×4.5		
		2	1	92.5	14.5×4.0	15.0×4.0		
		3	1	96.0	20.5×6.0	17.0×5.0	5.0×3.0	3.0×2.0
		4	1	95.5	19.5×6.0	19.0×5.5	6.5×3.5	3.0×3.0
		5	2	118.0	21.0×6.0		6.0×4.0	
		6	2	119.0	22.5×5.0		7.5×5.0	
		7	3	125.0	30.5×5.5	29.0×5.5	7.5×8.0	8.5×7.0
		8	3	119.0	29.5×6.5	29.0×6.5		
tor. W, No. 1	*tor.* W, No. 2	1	1	81.5	12.0×4.5	12.0×3.5	4.5×2.0	6.0×2.5
		2	1	82.0	12.5×4.0	14.5×4.0	4.5×4.0	6.5×4.0
		3	1	80.5	12.5×4.5	11.5×4.0	6.5×4.5	8.5×4.5
		4	1	79.5	12.5×4.0	9.0×4.0	7.0×4.5	7.0×4 5
		5	1	75.0	9.0×4.5	7.0×4.5	6.0×6.0	7.5×6.5
		6	2	97.5	11.5×4.5		8.0×3.5	
		7	2	94.5	13.0×4.0		5.5×5.0	
		8	3	99.0	16.5×4.5	16.0×4.5	7.5×4.0	8.5×6.0
jap. W, No. 3	*tor.* W, No. 1	1	1	93.0	15.0×5.5	15.0×6.0		
		2	1	86.5	10.5×5.0	10.0×5.5		
		3	1	88.0	8.5×5.0	7.5×5.0		
		4	1	82.5	12.5×6.0	3.5×2.5		
		5	1	73.5	7.5×5.0	7.0×4.5		
		6	1	93.5	16.5×6.0	14.5×6.0	6.0×5.5	6.5×5.0
		7	2	114.5	15.0×5.0		6.0×4.5	
		8	3	109.0	15.0×5.5	16.0×5.5	6.0×7.0	5.0×4.0
tor. W, No. 1	*jap.* W, No. 5	1	1	84.0	13.5×4.5	13.5×4.5	6.5×3.0	6.5×3.0
		2	1	85.5	16.5×3.5	8.5×4.0	6.5×4.5	9.0×4.0
		3	1	82.5	10.5×3.0	12.5×3.5	10.0×4.5	10.0×4.0
		4	1	80.0	13.0×3.5	13.0×3.5	6.5×3.0	2.5×3.0
		5	1	84.5	14.5×3.5	14.5×3.0	6.5×3.0	4.0×3.0
		6	2	104.5	21.0×4.0		5.0×4.0	
		7	2	93.0	18.5×4.0		6.0×3.5	
		8	2	100.5	19.0×4.0		6.0×3.5	
		9	2	96.5	16.5×3.5		4.5×3.0	
		10	2	102.0	18.0×4.0		4.0×3.5	
		11	2	94.0	14.0×3.0		6.0×3.0	
		12	3	101.5	21.0×6.0	21.5×6.5		

were 6.0 mm×5.0 mm and 7.5 mm×5.0 mm. The right testes and BIDDER's organs were not measured, as they were removed one year ago for the purpose of artificial insemination.

Two three-year-old male *japonicus* were 125.0 mm and 119.0 mm in body length. Their testes were 29.0~30.5 mm in length and 5.5~6.5 mm in width. The right and left BIDDER's organs of one of these males were 8.5 mm×7.0 mm and 7.5 mm×8.0 mm in size, respectively.

Five one-year-old male *torrenticola* (Nos. 1~5) were 75.0~82.0 mm, average

of 79.7 mm, in body length. Their testes were 7.0~14.5 mm in length and 3.5~4.5 mm in width (Table 20). BIDDER's organs of these five males were 4.5 mm×2.0 mm~7.5 mm×6.5 mm in size. Two two-year-old male *torrenticola* (Nos. 6 and 7) were 97.5 mm and 94.5 mm in body length. Their left testes were 11.5 mm×4.5 mm and 13.0 mm×4.0 mm in size. The left BIDDER's organs of these two males were 8.0 mm×3.5 mm and 5.5 mm×5.0 mm in size.

One three-year-old male *torrenticola*, 99.0 mm in body length, had testes, of which the right one was 16.0 mm×4.5 mm and the left one 16.0 mm×4.5 mm in size. The right and left BIDDER's organs were 8.5 mm×6.0 mm and 7.5 mm ×4.0 mm in size, respectively (Fig. 8).

When the testes of the male *torrenticola* were compared with those of the male *japonicus* for the above measurements, it was found that there was a slight difference in the following respects. The testes of *torrenticola* were generally smaller than those of *japonicus* in the ratio of length to width as well as in the ratio of testis size to body length. BIDDER's organs of the male *torrenticola* did not remarkably differ in size from those of the male *japonicus*.

The testes of the male *torrenticola* were the same as those of the male *japonicus* in inner structure. The seminiferous tubules were filled with compact bundles of normal spermatozoa. Along the walls there were many germ cells at various stages. Abnormal spermatozoa and pycnotic nuclei were very scarce (Plate XI, 67 and 68).

ii) Reciprocal hybrids between *Bufo bufo japonicus* and *Bufo torrenticola*

The testes of mature male hybrids produced from reciprocal crosses between *Bufo bufo japonicus* and *Bufo torrenticola* were measured at the age of one, two or three years (Table 20). Six hybrids (Nos. 1~6) produced from a cross, *Bufo bufo japonicus*♀ No. 3×*Bufo torrenticola*♂ No. 1, were 73.5~93.5 mm, average of 86.2 mm, in body length and had testes which were 3.5~16.5 mm in length and 2.5~6.0 mm in width. Male hybrid No. 4 had an exceptionally small right testis which was 3.5 mm×2.5 mm in size, while the left one was 12.5 mm×6.0 mm. The testes of the six hybrids were generally smaller than those of the two parental species in the ratio of length to width, while they were slightly larger or nearly the same as those of the control *torrenticola* in the ratio of testis size to body length. BIDDER's organs were measured in one (No. 6) of the six hybrids. They were 6.0 mm×5.5 mm and 6.5 mm×5.0 mm in size.

One two-year-old male hybrid (No. 7) produced from a mating, *Bufo bufo japonicus*♀ No. 3×*Bufo torrenticola*♂ No. 1, was 114.5 mm in body length. The left testis of this male was 15.0 mm in length and 5.0 mm in width, that is, nearly the same in shape as those of the control *torrenticola*. BIDDER's organ on the left side was 6.0 mm×4.5 mm in size.

One three-year-old male hybrid produced from the same parents as the above was 109.0 mm in body length and had testes that were similar in size and shape to those of some of the above stated one- and two-year-old hybrids (Fig. 8c). BIDDER's organs were normal in development.

Five one-year-old male hybrids (Nos. 1～5) produced from a cross, *Bufo torrenticola*♀ No. 1×*Bufo bufo japonicus*♂ No. 5, were 80.0～85.5 mm, average of 83.3 mm, in body length. Their testes were 8.5～16.5 mm in length and 3.0～4.5 mm in width. These were more slender than those of the reciprocal hybrids at the same age. They were nearly the same as those of the control *japonicus* as a whole in the ratio of length to width, while they were generally similar to those of the control *torrenticola* in the ratio of testis size to body length. BIDDER's organs of these hybrids were 2.5 mm×3.0 mm～10.0 mm×4.5 mm in size.

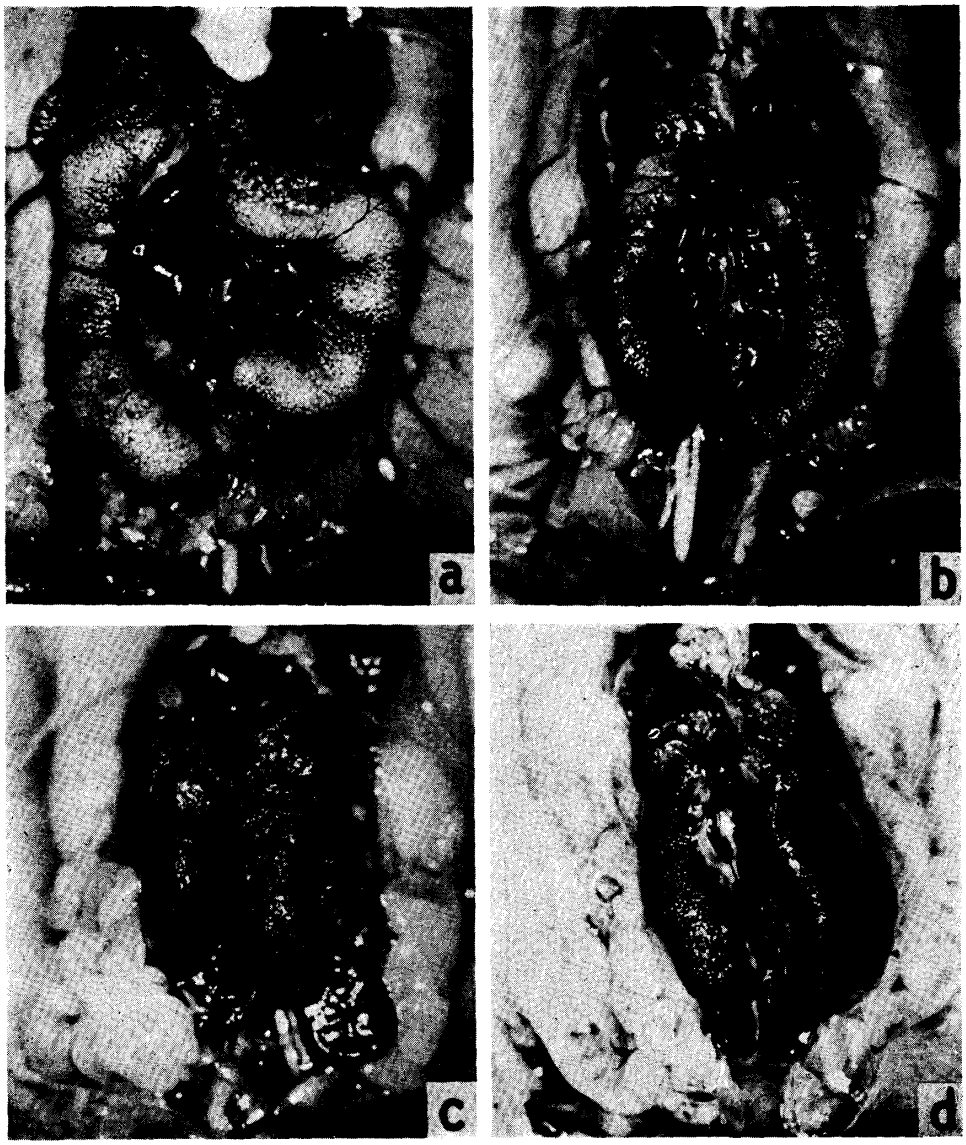

Fig. 8. Testes of three-year-old male hybrids between *Bufo japonicus* and *Bufo torrenticola* and the controls. ×2.0

a. Control No. 7, *japonicus*♀ No. 3×*japonicus*♂ No. 4
b. Control No. 8, *torrenticola*♀ No. 1×*torrenticola*♂ No. 2
c. Hybrid No. 8, *japonicus*♀ No. 3×*torrenticola*♂ No. 1
d. Hybrid No. 12, *torrenticola*♀ No. 1×*japonicus*♂ No. 5

They did not remarkably differ in shape and size from those of the control *japonicus* as well as *torrenticola*.

Six two-year-old male hybrids produced from the same cross, *Bufo torrenticola*♀ No. 1 × *Bufo bufo japonicus*♂ No. 5, were 93.0～104.5 mm, average of 98.4 mm, in body length (Table 20). Their left testes were 14.0～21.0 mm in length and 3.0～4.0 mm in width. These testes were somewhat larger as a whole in the ratio of length to width than those of the control *japonicus*, *torrenticola* and the reciprocal hybrid at the age of two years. The left BIDDER's organs were 4.5 mm × 3.0 mm ～6.0 mm × 3.5 mm in size. They scarcely differed in size from those of these three kinds of toads.

One three-year-old male hybrid produced from the same parents as the above was 101.5 mm in body length and had testes that were similar in size and shape to those of some of the one- and two-year-old control *japonicus*.

The testes of male reciprocal hybrids between *japonicus* and *torrenticola* at the age of one or two years were completely the same as those of the control *japonicus* and *torrenticola* in inner structure. The seminiferous tubules were filled with compact bundles of normal spermatozoa. Along the walls of the seminiferous tubules, there were many first and second spermatocytes at various stages and some spermatogonia (Plate XI, 69 and 70). Abnormal spermatozoa and pycnotic nucle were very scarce. BIDDER's organs of male reciprocal hybrids at the age of one or two years were also the same as those of the control *japonicus* and *torrenticola* in inner structure. They were filled for the most part with enlarged oocytes which were nearly of the same size (Plate XX, 105 and 106).

b. Female

i) Controls, *Bufo bufo japonicus* and *Bufo torrenticola*

Five one-year-old females produced from a mating, *Bufo bufo japonicus*♀ No. 3 × *Bufo bufo japonicus*♂ No. 4, were 99.5～100.5 mm, average of 100.1 mm, in body length. Their ovaries were large and contained numerous full-grown ova. In contrast, five one-year-old females produced from a mating, *Bufo torrenticola*♀ No. 1 × *Bufo torrenticola*♂ No. 2, were 81.0～90.5 mm, average of 86.6 mm, in body length and sexually immature. Their ovaries were 16.0 mm × 7.0 mm～17.5 mm × 8.0 mm in size and filled with growing auxocytes.

The body length and the number of eggs of two-year-old females produced from the above two matings were as follows (Table 21). Three female *japonicus* (Nos. 1～3) were 120.5～125.0 mm, average of 122.5 mm, in body length and laid 6012～6535 eggs after pituitary injection. The eggs of female No. 1 were 2.41±0.01 mm in diameter; the animal half of each egg was dark brown. The gelatinous strings including eggs were about 9.5 mm in thickness 5 hours after immersed in water; the outer gelatinous sheath consisted of a single homogeneous layer. Two female *torrenticola* (Nos. 1 and 2) were 97.0 mm and 97.5 mm in body length and laid 2467 and 2595 eggs. These eggs were very similar to those of the *japonicus* in size and color. The eggs of female *torrenticola* No. 1 were 2.40±0.01 mm in diameter; the animal half was dark brown. The gelationus

TABLE 21
Eggs of mature female hybrids between *Bufo bufo japonicus* and *Bufo torrenticola* and the controls. All the toads were produced in 1977

Parents		Individual no.	Age year(s)	Body length mm	No. of eggs	Mean diameter of eggs mm
Female	Male					
jap. W, No. 3	*jap.* W, No. 4	1	2	125.0	6012	2.41±0.01
		2	2	122.0	6535	
		3	2	120.5	6106	
tor. W, No. 1	*tor.* W, No. 2	1	2	97.5	2595	2.40±0.01
		2	2	97.0	2467	
jap. W, No. 3	*tor.* W, No. 1	1	2	110.0	5190	2.27±0.01
		2	2	115.5	5241	
		3	2	112.0	3808	
		4	2	113.0	4018	
		5	2	108.5	4810	
tor. W, No. 1	*jap.* W, No. 5	1	2	98.5	3686	2.29±0.01
		2	2	96.0	3772	2.28±0.01
		3	2	97.0	2013	
		4	2	96.0	3133	

strings including eggs were about 6.0 mm in thickness 5 hours after immersed in water. The outer gelatinous sheath consisted of a single homogeneous layer which was somewhat softer than that of *japonicus*.

ii) Reciprocal hybrids between *Bufo bufo japonicus* and *Bufo torrenticola*

Five one-year-old female hybrids produced from a cross, *Bufo bufo japonicus*♀ No. 3×*Bufo torrenticola*♂ No. 1, were 90.0~94.5 mm, average of 92.9 mm, in body length. Their ovaries were large and contained many full-grown ova. In contrast, five one-year-old female hybrids produced from a cross, *Bufo torrenticola* ♀ No. 1×*Bufo bufo japonicus*♂ No. 5, were 77.5~87.5 mm, average of 82.4 mm, in body length and immature. Their ovaries were 14.0 mm×9.5 mm~15.5 mm ×10.0 mm in size and filled with normal growing auxocytes.

Five two-year-old female hybrids produced from the cross, *Bufo bufo japonicus*♀ No. 3×*Bufo torrenticola*♂ No. 1, were 108.5~115.5 mm, average of 111.8 mm, in body length and laid 3808~5241 eggs after pituitary injection (Table 21). The eggs laid by each female were almost of an equal size and somewhat smaller than those of the control *japonicus* and *torrenticola*. The eggs of female hybrid No. 1 were 2.27±0.01 mm in diameter. The gelatinous strings including eggs were about 9.0 mm in thickness 5 hours after immersed in water. Four two-year-old female hybrids produced from the reciprocal cross, *Bufo torrenticola*♀ No. 1×*Bufo bufo japonicus*♂ No. 5, were 96.0~98.5 mm, average of 96.9 mm, in body length and laid 2013~3772 eggs after pituitary injection. These eggs were nearly the same in size as those of the reciprocal hybrids. The eggs of female hybrid No. 1 were 2.29±0.01 mm in diameter, while those of female hybrid No. 2 were 2.28 ±0.01 mm. The animal half of each egg was dark brown. The gelatinous strings were about 8.0 mm in thickness 5 hours after immersed in water.

It was noteworthy that the eggs laid by the two kinds of hybrids were intermediate in number between those laid by the two parental species. It was also interesting that the female hybrids produced from the cross, *Bufo bufo japonicus*♀ × *Bufo torrenticola*♂, were larger than those produced from the reciprocal cross in body length as well as in number of eggs.

5. Hybrids produced in 1977 from Japanese and American toads

a. Controls

Males and females produced from a mating, *Bufo americanus*♀ No. 1 × *Bufo americanus*♂ No. 1, matured sexually at the age of one year, as those of *Bufo bufo japonicus* produced as controls in 1977. Male *americanus* revealed secondary sexual characters. The body length and the size of testes were measured at the age of one or two years. They are presented in Table 22, while those of the control *japonicus* are shown in Table 20. As compared with the testes of the latter, those of the *americanus* were remarkably small in length and width. The testes of two one-year-old males, 69.0 mm and 70.0 mm in body length, were 7.5～9.5 mm in length and 2.5～3.5 mm in width, while those of two two-year-old males, 79.5 mm and 80.0 mm in body length, were 7.5～12.0 mm in length and 3.0～3.5 mm in width (Fig. 9a).

TABLE 22

Gonads and BIDDER's organs of mature hybrids between a female *Bufo americanus* and a male *Bufo bufo japonicus* and the controls. All the toads were produced in 1977

Parents		Sex	Individual no.	Age	Body length	Size of gonads		Size of BIDDER's organs	
Female	Male			year(s)	mm	Left mm	Right mm	Left mm	Right mm
ame. W, No. 1	*ame.* W, No. 1	♂	1	1	69.0	8.0×3.5	7.5×3.5	4.5×3.0	4.0×3.0
		♂	2	1	70.0	9.5×3.0	8.5×2.5	3.5×2.0	3.0×2.0
		♂	3	2	80.0	8.0×3.5	12.0×3.5		
		♂	4	2	79.5	8.0×3.5	7.5×3.0		
		♀	1	1	75.5	41.5×26.5	46.5×25.5		
		♀	2	1	75.0	43.0×25.5	46.5×25.5		
ame. W, No. 1	*jap.* W, No. 4	♂	1	1	72.5	6.0×1.0	6.5×1.0	0	0
		♂	2	1	71.0	7.0×2.0	7.5×4.0	0	0
		♂	3	1	73.0	6.5×1.0	6.0×1.0	0	0
		♀	1	1	82.5	Very small	Very small	0	0
		♀	2	1	69.0	Very small	Very small	0	0
		♀	3	1	72.0	7.5×2.0	4.5×3.0	0	0

The testes of the one- or two-year-old male *americanus* were normal in inner structure. The seminiferous tubules were filled with compact bundles of normal spermatozoa. There were many first and second spermatocytes at various stages and a small number of spermatogonia (Plate XII, 71 and 72).

The ovaries of one- or two-year-old female *americanus* were filled with full-grown ova. Two one-year-old females, 75 mm and 75.5 mm in body length, had ovaries which were 41.5 mm × 26.5 mm～46.5 mm × 25.5 mm in size. The eggs were

small; those of a two-year-old female were 1.41 ± 0.01 mm in diameter, while they were extremely large in number. The animal half of each egg was purple black. The gelatinous string including eggs was about 5.0 mm in thickness when they were measured about 5 hours after immersed in water. The gelatinous sheath of the string consisted of a single homogeneous layer as that of *japonicus*, although it was far softer than the latter. In contrast with the strings of many other *Bufo*, those of *americanus* had a tendency to spiral.

b. Hybrids

Of one-year-old hybrids produced from a cross, *Bufo americanus* ♀ No. 1 × *Bufo bufo japonicus* ♂ No. 4, three male (Nos. 1~3) and three females (Nos. 1~3) were examined. The three males were 71.0~73.0 mm, average 72.2 mm, in body length and revealed secondary sexual characters. Their testes were generally smaller and slender than those of the control males; they were 6.0~7.0 mm in length and 1.0~2.0 mm in width, except for the right testis of male hybrid No. 2, which was 7.5 mm × 4.0 mm. BIDDER's organs were scarcely formed in these male hybrids.

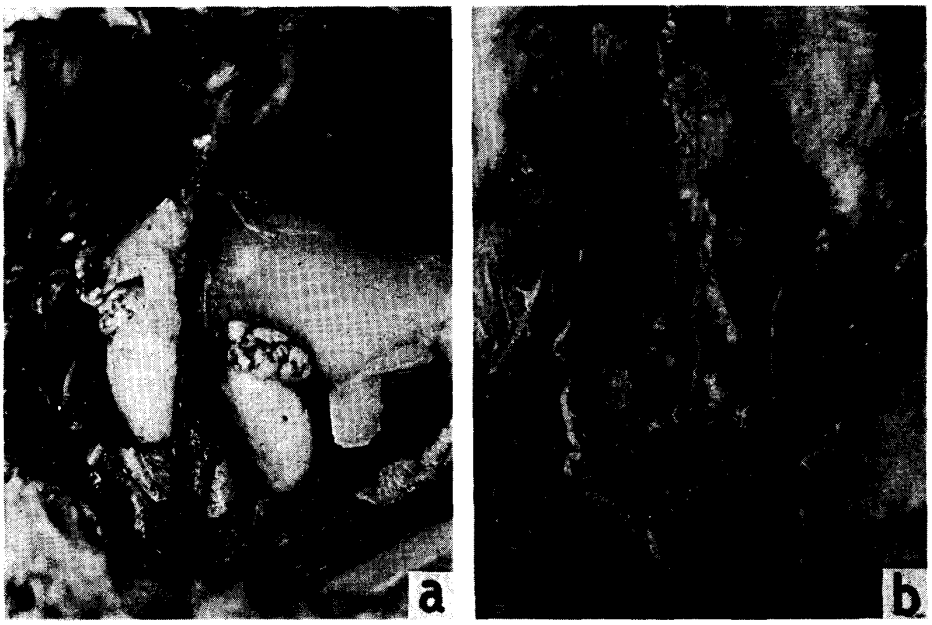

Fig. 9. Gonads of a one-year-old hybrid between a female *Bufo americanus* and a male *Bufo bufo japonicus* and the control. ×2.0

a. Male control No. 1, *americanus* ♀ No. 1 × *americanus* ♂ No. 1

b. Female hybrid No. 1, *americanus* ♀ No. 1 × *japonicus* ♂ No 4

The testes of the three male hybrids were very abnormal in inner structure. They were constructed of slender seminiferous tubules and abundant stromatic tissue. No germ cells were contained in the seminiferous tubules, while a few remnants of degenerated germ cells were found here and there (Plate XII, 73).

Three one-year-old female hybrids were 69.0~82.5 mm, average of 74.5 mm, in body length. Two of them had very small ovaries which were extremely

degenerative (Fig. 9b). The ovaries of the remaining female (No. 3) were nearly equal to the testes of the one-year-old males in length and width, although they were distinctly flat. The ovaries of all the three females were very abnormal in inner structure; they contained no germ cells. However, ovarian cavities were observable (Plate XII, 74).

6. Hybrids produced in 1978 among Japanese and European toads

a. Controls

i) *Bufo bufo japonicus*

Of one-year-old males produced from a mating, *Bufo bufo japonicus*♀ No. 6 ×*Bufo bufo japonicus*♂ No. 6, two (Nos. 1 and 2) were observed. These two

TABLE 23

Testes and BIDDER's organs of mature male hybrids between *Bufo bufo miyakonis* and *Bufo bufo japonicus* or *Bufo torrenticola*, and mature male *Bufo bufo japonicus*, *Bufo bufo miyakonis*, *Bufo viridis* and *Bufo torrenticola*. All the toads except four were produced in 1978

Parents		Individual no.	Age	Body length	Size of testes		Size of BIDDER's organs	
Female	Male		year(s)	mm	Left mm	Right mm	Left mm	Right mm
jap. W, No. 6	*jap.* W, No. 6	1	1	90.5	14.0×4.0		6.0×4.0	
		2	1	92.0	16.5×4.5	15.5×4.9		
miy. W, No. 2	*miy.* W, No. 1	1*	1	82.0	11.5×2.5	12.0×2.5		
vir. W, No. 1	*vir.* W, No. 1	1	1	86.5	9.5×5.5	10.5×6.5	9.5×6.0	9.5×5.0
		2	1	90.0	12.0×4.5	10.0×4.5		
miyakonis	*miyakonis*	1	Wild	97.0	28.5×4.5	25.0×4.0		
		2	Wild	96.5	16.5×3.0		3.5×4.0	
torrenticola	*torrenticola*	4	Wild	97.0	18.0×4.0	18.0×4.0		
miy. W, No. 1	*jap.* W, No. 6	1	1	85.0	11.5×4.5	14.0×5.0	5.0×4.0	5.5×4.0
		2	1	83.0	17.0×5.5	17.5×4.5	4.5×4.0	4.5×4.0
		3	1	89.0	11.5×4.0	12.0×2.5	4.5×4.0	5.0×4.0
		4	1	89.5	16.5×4.0	16.0×4.0	5.5×4.5	5.5×4.0
		5	1	90.0	17.0×4.0	17.0×4.0	6.5×4.0	6.0×3.5
		6	1	74.5	13.5×4.5	14.0×4.0	5.5×4.0	5.0×4.0
		7	2	83.5	20.5×3.5			
		8	2	92.0	16.0×3.5			
		9	2	78.0	17.0×3.0			
		10	2	81.5	11.5×3.5			
		11	2	79.0	15.0×3.5			
miy. W, No. 1	*tor.* W, No. 3	1	1	75.0	10.0×4.5	11.5×4.0	3.5×3.0	4.5×3.0
		2	1	74.0	15.5×5.0	14.0×4.5	5.0×3.0	5.5×4.0
		3	1	80.5	18.0×5.0	12.0×5.0	8.5×4.0	4.0×4.0
		4	1	82.5	12.5×5.0	11.5×3.5	6.5×4.0	6.0×5.0
		5	1	79.5	9.0×4.0	12.0×4.0	3.5×4.0	6.5×3.0
		6	1	68.0	12.5×3.0	12.5×3.0	2.0×1.5	6.0×3.0
		7	1	72.5	13.5×4.5	13.0×4.0	6.0×4.5	4.5×4.0
		8	2	87.0	11.5×3.0			
		9	2	79.0	12.0×3.5			
		10	2	78.5	11.0×2.5			
		11	2	76.0	12.0×3.5			
		12	2	74.5	13.5×3.5			

* This mating was made in 1979.

males were 90.5 mm and 92.0 mm in body length and revealed their secondary sexual characters. Their testes were large and cylindrical (Table 23).

The testes of the two males contained many compact bundles of normal spermatozoa in the seminiferous tubules. They were the same in inner structure as those of the control male *japonicus* produced in 1976. BIDDER's organs were also similar in size and inner structure to those of the latter.

ii) *Bufo bufo miyakonis*

A male *Bufo bufo miyakonis* produced in 1979 from a mating, *Bufo bufo miyakonis* ♀ No. 2 × *Bufo bufo miyakonis* ♂ No. 1, is placed here in order to compare with hybrids derived from a female *miyakonis*, as the control *miyakonis* could not be produced in 1978 owing to absence of mature males. This male was one year old and 82.0 mm in body length. The testes were 11.5 mm or 12.0 mm in length and 2.5 mm in width.

Two field-caught males (Nos. 1 and 2) were also observed in order to compare with male hybrids. Their real age was unknown, although they were assumed to be two years old. They were 97.0 mm and 96.5 mm in body length and had cylindrical testes. The testes of male No. 1 were 28.5 mm × 4.5 mm and 25.0 mm × 4.0 mm in size. The left testis of male No. 2 was 16.5 mm × 3.0 mm, while the right one had been removed before the measurement. The left BIDDER's organ was 3.5 mm × 4.0 mm in size.

The testes of these males contained many compact bundles of normal spermatozoa in the seminiferous tubules. Along the walls of the latter there were numerous first and second spermatocytes at various stages and a few spermatogonia (Plate XV, 83). BIDDER's organs were filled with enlarged oocytes.

iii) *Bufo viridis*

Males of the control *Bufo viridis* produced from a mating, *Bufo viridis* ♀ No. 1 × *Bufo viridis* ♂ No. 1, matured at the age of one year; they revealed secondary sexual characters. Two (Nos. 1 and 2) of them were 86.5 mm and 90.0 mm in body length and had testes which were 9.5 ~ 12.0 mm in length and 4.5 ~ 6.5 mm in width. These testes were shorter and thicker than those of the control male *japonicus*. BIDDER's organs of male hybrid No. 1 were 9.5 mm × 6.0 mm and 9.5 mm × 5.0 mm in size (Fig. 12a).

The testes of the two males had many compact bundles of normal spermatozoa in the seminiferous tubules. Along the walls of the latter there were many first and second spermatocytes at various stages and some spermatogonia (Plate XV, 84). BIDDER's organs were filled with numerous enlarged oocytes which varied in size (Plate XXII, 112).

iv) *Bufo torrenticola*

A field-caught male (No. 4) that was assumed to be two years old was observed in order to compare with male hybrids. This male was 97.0 mm in body length and had cylindrical testes which were similar to those of the above male *japonicus*

and *miyakonis*. The testes were 18.0 mm in length and 4.0 mm in width and contained many compact bundles of normal spermatozoa in addition to numerous first and second spermatocytes at various stages and a few spermatogonia.

b. Hybrids between a female *Bufo bufo miyakonis* and male Japanese toads

One-year-old hybrids produced from a cross between a female *Bufo bufo miyakonis* and a male *Bufo bufo japonicus* or *Bufo torrenticola* were examined. Male hybrids all matured sexually and revealed secondary sexual characters, while female ones were immature.

i) Hybrids, *Bufo bufo miyakonis* ♀ No. 1 × *Bufo bufo japonicus* ♂ No. 6

Six one-year-old male hybrids (Nos. 1～6) were 74.5～90.0 mm, average of 85.2 mm, in body length and had testes which were 11.5～17.5 mm in length and 2.5～5.5 mm in width (Table 23). Their testes were cylindrical and similar to those of the control *japonicus* in shape as well as in the relative size to body length (Fig. 10a). BIDDER's organs were also normal in size, being 4.5 mm × 4.0 mm～6.5 mm × 4.0 mm.

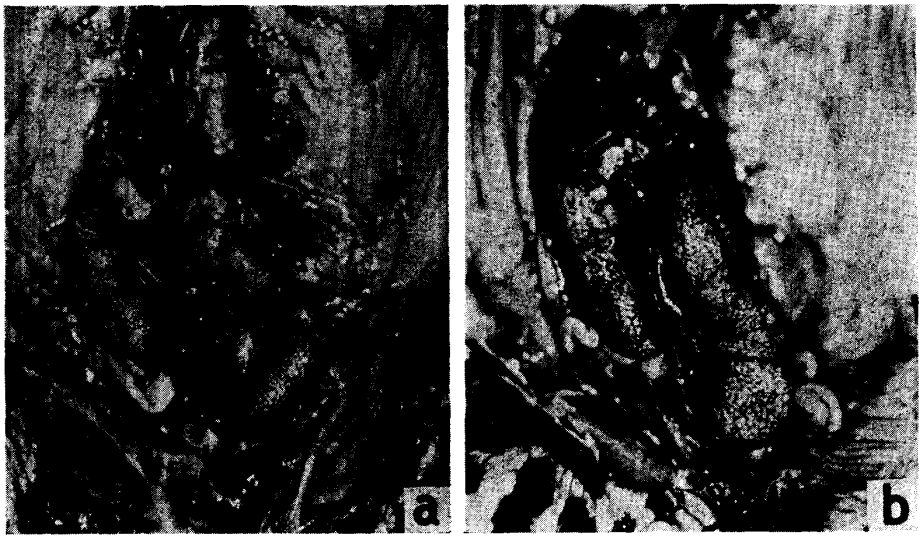

Fig 10. Testes of one-year-old male hybrids between a female *Bufo bufo miyakonis* and a male *Bufo bufo japonicus* or *Bufo torrenticola*. ×2.0

a. Hybrid No. 2, *miyakonis* ♀ No. 1 × *japonicus* ♂ No. 6

b. Hybrid No. 3, *miyakonis* ♀ No. 1 × *torrenticola* ♂ No. 3

The testes of these male hybrids were not always normal in inner structure. Three males (Nos. 1, 4 and 6) had many large and small pycnotic nuclei in addition to several loose bundles of normal spermatozoa in the cross-sections of seminiferous tubules (Plate XIII, 75). Along the walls of seminiferous tubules, there were many first and second spermatocytes and a few spermatogonia. While some germ cells underwent normal spermatogenesis, the others seemed mostly to become abnormal and degenerate at the first metaphase. In contrast to these three males, the other three (Nos. 2, 3 and 5) were more abnormal in

inner structure of the testes (Plate XIII, 76). The seminiferous tubules scarcely contained normally shaped spermatozoa; they were filled with numerous large and small pycnotic nuclei and a few abnormal spermatozoa. Along the walls of seminiferous tubules, there were many first and second spermatocytes and a few spermatogonia. BIDDER's organs were filled with enlarged oocytes (Plate XXI, 107).

Five two-year-old male hybrids (Nos. 7~11) were 78.0~92.0 mm in body length. Their testes were 11.5~20.5 mm in length and 3.0~3.5 mm in width (Table 23).

Female hybrids produced from the cross, *Bufo bufo miyakonis*♀ No. 1 × *Bufo bufo japonicus*♂ No. 6, were immature at the age of one year, as the control *japonicus* were. Their ovaries were normal in inner structure as well as in appearance; they were filled with growing auxocytes.

TABLE 24

Eggs of mature female hybrids between a female *Bufo bufo miyakonis* and a male *Bufo bufo japonicus* or *Bufo torrenticola*. All the toads were produced in 1978

Parents		Individual no.	Age year(s)	Body length mm	No. of eggs	Mean diameter of eggs mm
Female	Male					
miy. W, No. 1	*jap.* W, No. 6	1	2	99.5	5919	2.17±0.01
		2	2	98.5	7287	2.01±0.01
		3	2	104.5	7042	2.06±0.01
		4	2	104.0	4234	2.01±0.01
		5	2	105.5	8833	1.91±0.01
miy. W, No. 1	*tor.* W, No. 3	1	2	93.5	4132	2.00±0.00
		2	2	90.5	3004	1.99±0.01
		3	2	89.5	3077	1.98±0.01
		4	2	91.0	2116	1.97±0.01
		5	2	84.0	0	

Five two-year-old female hybrids (Nos. 1~5) were 98.5~105.5 mm in body length. They laid 4234~8833 eggs after pituitary injection. The eggs were 1.91~2.17 mm in mean diameter; those of each female were almost uniform in size. The gelatinous strings including eggs were 6.5~8.0 mm in thickness when they were measured about 5 hours after immersed in water (Table 24).

ii) Hybrids, *Bufo bufo miyakonis*♀ No. 1 × *Bufo torrenticola*♂ No. 3

Seven one-year-old male hybrids (Nos. 1~7) were 68.0~82.5 mm, average of 76.0 mm, in body length and revealed secondary sexual characters. Their testes were 9.0~18.0 mm in length and 3.0~5.0 mm in width (Table 23). They were cylindrical and nearly the same as those of the control *japonicus* in shape and size (Fig. 10b). BIDDER's organs were normal, that is, 3.5 mm × 3.0 mm~8.5 mm × 4.0 mm in size, except for the left one of male hybrid No. 6 which was 2.0 mm × 1.5 mm.

The testes of five (Nos. 1~5) of the seven male hybrids were sectioned to examine their inner structure. It was found that those of four males (Nos. 1~4)

had several loose bundles of normal spermatozoa and many large and small pycnotic nuclei in the seminiferous tubules (Plate XIII, 77). Besides, there were many first and second spermatocytes and spermatogonia along the walls of the seminiferous tubules. In the testes of the remaining male hybrid (No. 5), there were a few large, normally shaped spermatozoa and some large or small pycnotic nuclei in addition to many first and second spermatocytes and spermatogonia (Plate XIII, 78). BIDDER's organs were filled with enlarged oocytes (Plate XXI, 108).

Five two-year-old male hybrids (Nos. 8～12) were 74.5～87.0 mm in body length. Their left testes were 11.0～13.5 mm in length and 2.5～3.5 mm in width (Table 23).

Female hybrids produced from the same cross, *miyakonis*♀ No. 1 × *torrenticola*♂ No. 3, were immature at the age of one year. However, the ovaries were normal in inner structure and filled with growing auxocytes.

Five two-year-old female hybrids (Nos. 1～5) were 84.0～93.5 mm in body length. After pituitary injection, four of them laid 2116～4132 eggs that were 1.97～2.00 mm in mean diameter. The eggs of each female were almost uniform in size. The gelatinous strings including these eggs were 5.5 mm or 6.0 mm in thickness when measured about 5 hours after immersed in water (Table 24).

c. Hybrids between a female *Bufo bufo miyakonis* and male European toads

One-year-old hybrids produced from a cross between the same female *Bufo bufo miyakonis* (No. 1) as that used in the above crossing experiments and a male *Bufo bufo bufo* from Portugal♂ No. 5 or *Bufo viridis*♂ No. 1 were examined. While all the male hybrids derived from the male *bufo* matured sexually, some male hybrids drived from the male *viridis* could mature at the age of one year. The female hybrids produced from the cross with the male *bufo* had ovaries with some full-grown ova. No females were obtained from the cross with the male *viridis*.

i) Hybrids, *Bufo bufo miyakonis*♀ No. 1 × *Bufo bufo bufo*♂ No. 5

All one-year-old male hybrids matured and revealed secondary sexual characters. Three (Nos. 1～3) of them, 59.0～68.0 mm, average of 62.3 mm, in body length, had testes (Fig. 11a) which were 3.0～7.5 mm in length and 2.0～5.0 mm in width (Table 25). These testes seemed to be similar to those of one-year-old male *bufo* from Portugal in shape and the relative size to body length, although the right testes of male hybrid No. 1 was exceptionally small, that is, 3.0 mm × 2.0 mm in size.

The testes of the three male hybrids were abnormal in inner structure. The left testis of male hybrid No. 1 contained a small number of normally and abnormally shaped spermatozoa and pycnotic nuclei in the seminiferous tubules, which were almost filled with first spermatocytes at various stages and spermatogonia (Plate XIV, 80). In the seminiferous tubules of the left testis of male hybrid No. 2, there were a few abnormal spermatozoa and abundant pycnotic

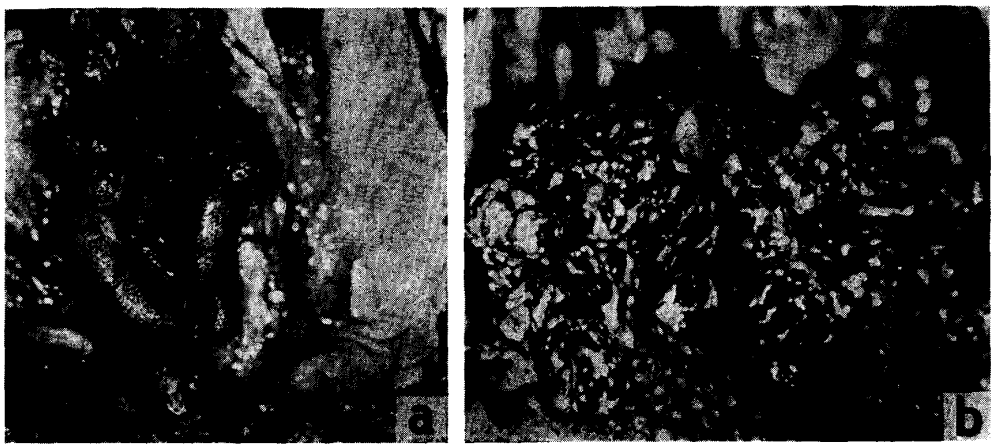

Fig. 11. Gonads of one-year-old hybrids between a female *Bufo bufo miyakonis* and a male *Bufo bufo bufo* from Portugal. ×2.0

a. Male hybrid No. 1, *miyakonis* ♀ No. 1 × *bufo* from Portugal ♂ No. 5

b. Female hybrid No. 1, *miyakonis* ♀ No. 1 × *bufo* from Portugal ♂ No. 5

TABLE 25

Testes and BIDDER's organs of mature male hybrids among *Bufo bufo miyakonis*, *Bufo bufo japonicus*, *Bufo torrenticola*, *Bufo bufo bufo* from Portugal and *Bufo viridis*

All the toads were produced in 1978

Parents		Individual no.	Age	Body length	Size of testes		Size of BIDDER's organs	
Female	Male		year(s)	mm	Left mm	Right mm	Left mm	Right mm
miy. W, No. 1	*bufo* P. W, No. 5	1	1	59.0	6.0×4.0	3.0×2.0	3.5×3.0	9.5×3.0
		2	1	68.0	7.5×4.0	7.0×5.0	5.5×3.5	6.0×4.0
		3	1	60.0	5.5×4.5	7.0×2.5	5.5×4.0	4.5×4.0
		4	2	63.0	4.5×3.0		11.5×6.5	
		5	2	63.0	6.0×3.0		6.5×3.5	
		6	2	63.5	5.5×3.0		7.0×4.0	
miy. W, No. 1	*vir.* W, No. 1	1	1	74.0	7.5×4.0		5.0×7.0	
		2	1	75.0	5.0×3.5		6.0×5.0	
		3	1	75.5	4.0×2.0		7.5×5.0	
		4	1	76.5	7.0×4.5		6.5×4.0	
		5	1	72.5	10.0×5.5		5.0×4.5	
		6	1	66.5	7.5×5.0	6.0×4.0	0	8.0×7.0
		7	1	65.0	7.0×4.0	7.0×4.0	5.0×7.0	7.0×4.0
		8	1	57.0	4.5×1.0	7.0×2.0	5.5×4.5	4.0×2.5
		9	1	74.0	8.0×2.0	7.0×2.5	11.5×9.5	4.0×4.0
		10	1	75.5	4.5×3.5	5.5×3.0	10.0×7.5	5.0×3.0
jap. W, No. 6	*vir.* W, No. 1	1	1	89.0	9.0×4.0	7.0×4.0	5.0×5.0	10.5×7.0
		2	1	85.5	9.0×4.5	6.5×4.0	0	0
		3	1	83.5	7.0×4.0	12.0×2.5	10.5×5.0	7.5×3.0
		4	1	77.0	Very small	Very small	6.5×3.0	4.0×2.5
		5	1	85.5	Very small	Very small	0	0
		6	1	72.0	Very small	Very small	0	0
bufo P. W, No. 3	*tor.* W, No. 3	1	1	81.5	3.5×4.0	4.5×3.5	4.5×8.5	0
		2	1	76.0	5.0×3.0	Very small	4.0×1.5	0
		3	2	78.5	6.5×4.0		1.0×0.5	
		4	2	86.5	5.5×3.5		4.5×3.0	
		5	2	85.5	5.0×3.5		7.0×5.0	
		6	2	81.0	6.0×3.5		8.0×7.0	
		7	2	75.0	5.0×3.5		6.0×5.5	

nuclei. Along the walls of seminiferous tubules there were some first spermatocytes and spermatogonia (Plate XIV, 81). The left testis of male hybrid No. 3 had many abnormally large spermatozoa which seemed almost normal in shape. In addition to these spermatozoa, there were some first spermatocytes and spermatogonia (Plate XIV, 82). BIDDER's organs were nearly the same in inner structure as those of the male hybrids produced in 1976 from a female *bufo* and a male *japonicus* (Plate XXI, 109).

Three two-year-old male hybrids (Nos. 4~6) were 63.0 mm or 63.5 mm in body length. Their testes were small; the left ones were 4.5~6.0 mm in length and 3.0 mm in width. BIDDER's organs on the left side were 6.5 mm×3.5 mm ~11.5 mm×6.5 mm in size (Table 25).

Two one-year-old female hybrids, 74.0 mm and 72.0 mm in body length, matured sexually. Their ovaries were almost filled with full-grown ova which varied in size. Besides, there were many growing auxocytes (Fig. 11b, Plate XIV, 79).

ii) Hybrids, *Bufo bufo miyakonis*♀ No. 1×*Bufo viridis*♂ No. 1

Ten one-year-old mature male hybrids (Nos. 1~10) were 57.0~76.5 mm, average of 71.2 mm, in body length (Table 25). Their testes and BIDDER's organs were measured, although the right testes and BIDDER's organs of five (Nos. 1~5) of these males were not measured, as they had been removed in order to utilize for fertilization test. The results showed that the testes were 4.0~10.0 mm in length and 1.0~5.5 mm in width, and that BIDDER's organs were 4.0 mm×2.5 mm~11.5 mm×9.5 mm in size. Male hybrid No. 6 had no BIDDER's organ on the left side. While five (Nos. 1 and 4~7) of the ten male

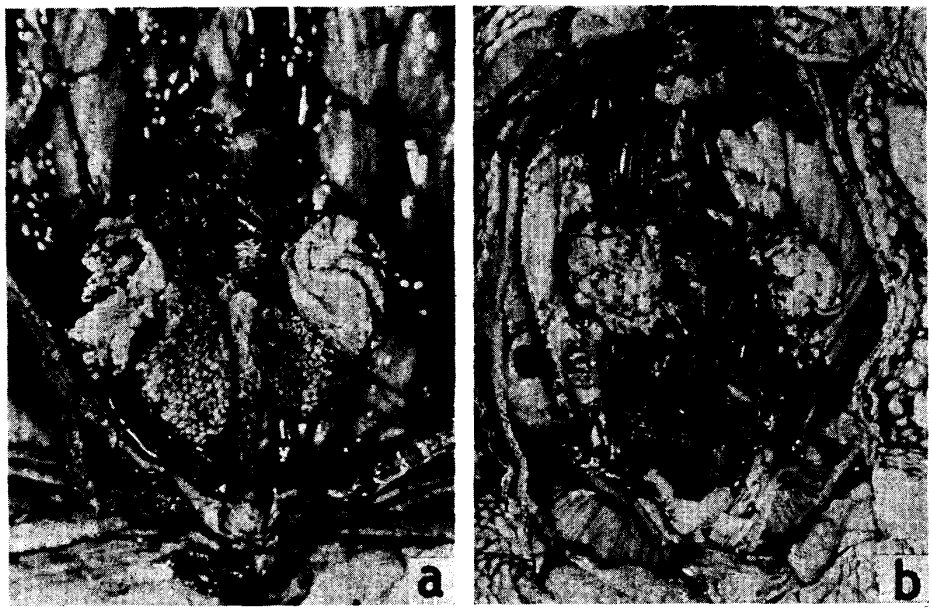

Fig. 12. Testes of a one-year-old male hybrid between a female *Bufo bufo miyakonis* and a male *Bufo viridis* and the control. ×2.0

a. Control No. 1, *viridis*♀ No. 1×*viridis*♂ No. 1

b. Hybrid No. 9, *miyakonis*♀ No. 1×*viridis*♂ No. 1

hybrids had testes that were nearly the same as or slightly smaller than those of the control male *viridis* (Table 23), the testes of the other five male hybrids (Nos. 2, 3 and 8～10) were remarkably smaller or more slender than the latter (Fig. 12b). BIDDER's organs of the ten male hybrids were nearly normal in size.

The inner structure of testes was examined in five (Nos. 1～5) of the ten male hybrids. The results indicated that these testes were all abnormal with no normal spermatozoa. Although there were numerous first spermatocytes at various stages, they hardly proceeded beyond the metaphase. Many pycnotic nuclei were found in the seminiferous tubules (Plate XV, 85 and 86). Along the walls of the latter, there were some spermatogonia. BIDDER's organs contained a considerable number of growing auxocytes, some of which were degenerating (Plate XXII, 113).

d. Hybrids between a female *Bufo bufo japonicus* and a male *Bufo viridis*

A total of six one-year-old male hybrids produced from a mating, *Bufo bufo japonicus*♀ No. 6×*Bufo viridis*♂ No. 1, were observed. Of these males, secondary sexual characters were evident in three (Nos. 1～3), while the other three (Nos. 4～6) looked as if they were females owing to the absence of such characters. The former three male hybrids were 83.5～89.0 mm, average of 86.0 mm, in body length and had testes which were 6.5～12.0 mm in length and 2.5～4.5 mm in width (Table 25). These testes were somewhat smaller as a whole than those of the control *viridis*, although they were almost similar to the latter in shape. The right testis of one (No. 3) of these male hybrids was exceptionally cylindrical like the testes of the control *japonicus*. The remaining three male hybrids (Nos. 4～6) were 72.0～85.5 mm, average of 78.2 mm, in body length. The testes of these males were so small that they could be hardly measured. While three of the six male hybrids had no BIDDER's organs, the other three had those which were 4.0 mm×2.5 mm～10.5 mm×7.0 mm in size.

The testes of the male hybrids were very abnormal in inner structure; there were neither normal nor abnormal spermatozoa in the seminiferous tubules. In the three male hybrids with testes of some size, spermatogenesis became abnormal at the first meiotic division and hardly proceeded further. Along the walls of seminiferous tubules, there were a considerable number of spermatogonia (Plate XVI, 87 and 88). The vestigial testes of the other three male hybrids had no germ cells (Plate XVI, 89 and 90). BIDDER's organs of three male hybrids (Nos. 1, 3 and 4) were degenerative in inner structure, although there were still many enlarged oocytes (Plate XXII, 114).

e. Hybrids between a female *Bufo bufo bufo* and a male *Bufo torrenticola*

Of one-year-old hybrids produced from a cross, *Bufo bufo bufo* from Portugal♀ No. 3×*Bufo torrenticola*♂ No. 3, two largest males and two largest females were observed. The male hybrids (Nos. 1 and 2) distinctly revealed secondary sexual characters. They were 81.5 mm and 76.0 mm in body length; their testes were remarkably smaller than those of the parental species at the same age (Table 25).

In one (No. 2) of the male hybrids, the right testis was extremely small. Both male hybrids had no BIDDER's organ on the right side, while the left BIDDER's organs were 4.5 mm × 8.5 mm and 4.0 mm × 1.5 mm in size.

The testes of these males were very abnormal in inner structure and similar in this respect to those of male hybrids produced in 1976 from a female *bufo* and a male *japonicus*. There were no normal spermatozoa in the seminiferous tubules. The latter contained a few abnormal spermatozoa and some pycnotic nuclei (Plate XVII, 93 and 94). Along the walls of seminiferous tubules there were many spermatogonia. BIDDER's organs were nearly the same in inner structure as those of the male hybrids produced in 1976 from a female *bufo* and a male *japonicus* (Plate XXI, 110).

Five two-year-old male hybrids (Nos. 3～7) were 75.0～86.5 mm in body length and had small testes. Their left testes were 5.0～6.5 mm in length and 3.5 mm or 4.0 mm in width. BIDDER's organs on the left side were 1.0 mm × 0.5 mm～8.0 mm × 7.0 mm in size (Table 25).

The two one-year-old females were 86.5 mm and 91.0 mm in body length. Their ovaries were very similar in size and inner structure to those of one-year-old female hybrids produced in 1976 from a cross of a female *bufo* and a male *japonicus*. Only a few growing auxocytes were found in these ovaries (Plate XVII, 91 and 92).

7. Hybrids produced in 1979 from European and American toads

The gonads of hybrids produced in 1979 from crosses among *Bufo bufo bufo* from Portugal, *Bufo viridis* and *Bufo americanus* were observed at the age of one year.

a. Hybrids, *Bufo bufo bufo*♀ Nos. 4 and 5 × *Bufo viridis*♂ No. 1

Five one-year-old male hybrids (Nos. 1～5) were 73.5～81.0 mm, average of 76.0 mm, in body length. Their testes were small and extremely slender, being 2.0～5.5 mm in length and 1.0～2.0 mm in width (Table 26). They were very abnormal in inner structure; the seminiferous tubules contained no germ cells, although a few remnants of degenerated germ cells were occasionally found. These findings were very similar to those in the testes of the male hybrids obtained from the cross, *americanus*♀ × *japonicus*♂ (Plate XVIII, 95). All the male hybrids had no BIDDER's organs.

b. Hybrids, *Bufo viridis*♀ No. 1 × *Bufo bufo bufo*♂ No. 5

Two one-year-old female hybrids (Nos. 1 and 2) were 43.0 mm and 40.5 mm in body length. Their ovaries degenerated almost completely and had no germ cells (Plate XVIII, 97 and 98). There were no BIDDER's organs in these hybrids.

c. Hybrids, *Bufo americanus*♀ Nos. 2 and 3 × *Bufo viridis*♂ No. 1

Five one-year-old male hybrids (Nos. 1～5) were 67.0～72.0 mm in body length. Their testes were 5.0～6.5 mm in length and 1.0～1.5 mm in width.

TABLE 26

Gonads of mature hybrids between *Bufo bufo bufo* from Portugal and *Bufo viridis* and between female *Bufo americanus* and a male *Bufo viridis*, and a mature male *Bufo bufo miyakonis*. All the toads were produced in 1979

Parents		Sex	Individual no.	Age	Body length	Size of gonads		Size of BIDDER's organs	
Female	Male			year(s)	mm	Left mm	Right mm	Left mm	Right mm
miy. W, No. 2	*miy.* W, No. 1	♂	1	1	82.0	11.5×2.5	12.0×2.5		
bufo P. W, Nos. 4, 5	*vir.* W, No. 1	♂	1	1	81.0	5.5×2.0	4.0×1.5	0	0
		♂	2	1	73.5	3.0×1.5	4.0×1.5	0	0
		♂	3	1	74.0	2.0×1.0	2.0×1.0	0	0
		♂	4	1	73.5	2.0×1.0	2.5×1.0	0	0
		♂	5	1	78.0	4.0×2.0	4.0×2.0	0	0
vir. W, No. 1	*bufo* P. W, No. 5	♀	1	1	43.0	Very small	Very small	0	0
		♀	2	1	40.5	Very small	Very small	0	0
ame. W, Nos. 2, 3	*vir.* W, No. 1	♂	1	1	72.0	7.0×1.5	6.0×1.5	0	0
		♂	2	1	70.0	6.0×1.0	6.0×1.0	0	0
		♂	3	1	70.0	6.5×1.5	6.5×1.0	0	0
		♂	4	1	68.5	6.0×1.5	5.5×1.5	0	0
		♂	5	1	67.0	5.5×1.0	5.0×1.0	0	0

They were very slender as those of the male hybrids produced from the same male by crossing with a female *bufo* from Portugal, although the former were somewhat longer than the latter. They were very abnormal in inner structure; there were no germ cells in the testes of four of the five male hybrids (Plate XVIII, 96), as found in those of the hybrids, *americanus*♀ ×*japonicus*♂ and *bufo*♀ ×*viridis*♂. In the testes of the remaining male, the seminiferous tubules contained a few spermatogonia and remnants of degenerated germ cells. All these male hybrids had no BIDDER's organs.

V. Reproductive capacity

1. Hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo*

a. Backcrosses of male hybrids with female *Bufo bufo japonicus*

i) Controls

Four one-year-old males (Nos. 1~4) produced in 1976 from a mating, *Bufo bufo japonicus*♀ No. 1 ×*Bufo bufo japonicus*♂ No. 1, and three one-year-old males (Nos. 1~3) produced in 1976 from a mating, *Bufo bufo bufo*♀ No. 1 ×*Bufo bufo bufo*♂ No. 3 (Table 16), were mated with three female *Bufo bufo japonicus* (Nos. 9~11) collected from the field. The results indicated that 92.5% of 864 eggs in total cleaved normally, 78.6% hatched normally, 75.9% became feeding tadpoles and eventually 69.7% became normally metamorphosed toads by inseminating with *japonicus* sperm, while 85.5%, 76.7%, 57.4% and 53.2% of 941 eggs in total normally cleaved, hatched, began to eat and metamorphosed, respectively, by inseminating with *bufo* sperm (Table 27).

TABLE 27

Developmental capacity of the backcrosses of male hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo* from Portugal and the controls. All the backcrosses were produced in 1977 and 1979

Parents		No. of eggs	No. of cleavages		No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally metamorphosed toads	PMC
Female	Male		Normal	Abnormal				
jap. W, Nos. 9～11	76(*jap.* ♀1×*jap.* ♂1) Nos. 1～4	864	799 (92.5%)	0	679 (78.6%)	656 (75.9%)	602 (69.7%)	75.4
	76(*jap.* ♀1×*bufo* P. ♂1) Nos. 1～5	3061	0	29 (0.9%)	0	0	0	
	76(*bufo* P. ♀1×*jap.* ♂3) Nos. 1～5	4550	0	35 (0.8%)	0	0	0	
	76(*bufo* P. ♀1×*bufo* P. ♂3) Nos. 1～3	941	805 (85.5%)	0	722 (76.7%)	540 (57.4%)	501 (53.2%)	62.2
76(*jap.* ♀1×*jap.* ♂1) Nos. 3～5	76(*jap.* ♀1×*jap.* ♂1) Nos. 5～8	818	794 (97.1%)	0	789 (96.5%)	693 (84.7%)	662 (80.9%)	83.4
	76(*jap.* ♀1×*bufo* P. ♂3) Nos. 6～10	5868	5 (0.09%)	31 (0.5%)	2 (0.03%)	0	0	
	76(*bufo* P. ♀1×*jap.* ♂3) Nos. 6～10	5997	12 (0.2%)	42 (0.7%)	10 (0.17%)	4 (0.07%)	1 (0.02%)	8.3
	76(*bufo* P. ♀1×*bufo* P. ♂3) Nos. 4, 5	764	627 (82.1%)	0	549 (71.9%)	501 (65.6%)	453 (59.3%)	72.2

PMC, Percentage of metamorphosed toads to normally cleaved eggs

Four two-year-old males (Nos. 5～8) produced in 1976 from *japonicus*♀ No. 1 ×*japonicus*♂ No. 1 and two two-year-old males (Nos. 4 and 5) produced in 1976 from *bufo*♀ No. 1×*bufo*♂ No. 3 (Table 16), were mated with three two-year-old females (Nos. 3～5) produced in 1976 from *japonicus*♀ No. 1×*japonicus* ♂No. 1. It was found that 97.1% of 818 eggs in total cleaved normally, 96.5% hatched normally, 84.7% became feeding tadpoles and 80.9% became normally metamorphosed toads by inseminating with *japonicus* sperm, while 82.1%, 71.9%, 65.6% and 59.3% of 764 eggs in total normally cleaved, hatched, began to eat and metamorphosed, respectively, by inseminating with *bufo* sperm (Table 27).

ii) Reciprocal hybrids

Five one-year-old male hybrids (Nos. 1～5) produced in 1976 from a cross, *Bufo bufo japonicus*♀ No. 1×*Bufo bufo bufo*♂ No. 1, and five one-year-old male hybrids (Nos. 1～5) produced in 1976 from a cross, *Bufo bufo bufo*♀ No. 1×*Bufo bufo japonicus*♂ No. 3 (Table 16), were mated with three field-caught female *Bufo bufo japonicus* (Nos. 9～11). No normally cleaved eggs were obtained by these crosses. Only 29 (0.9%) of 3061 eggs in total cleaved abnormally by inseminating with sperm of male hybrids between the female Japanese and the male European toad, while 35 (0.8%) of 4550 eggs in total cleaved abnormally by inseminating with sperm of the reciprocal hybrids. All these abnormally cleaved eggs died without attaining the blastula stage (Table 27).

Five two-year-old male hybrids (Nos. 6～10) produced in 1976 from the cross, *japonicus*♀ No. 1×*bufo*♂ No. 1 and five two-year-old male hybrids produced in 1976 from *bufo*♀ No. 1×*japonicus*♂ No. 3 (Table 16) were mated with three two-year-old females (Nos. 3～5) obtained from *japonicus*♀ No. 1×*japonicus*♂

No. 1. The results showed that only five (0.09%) of 5868 eggs in total cleaved normally and 31 others (0.5%) did abnormally by inseminating with sperm of the hybrids between the female Japanese and the male European toad. While the abnormally cleaved eggs died without attaining the blastula stage and three of the normally cleaved ones died at the gastrula stage, the remaining two developed normally during the embryonic stage and hatched normally. However, they died without taking food. Of 5997 eggs in total, 12 (0.2%) and 42 (0.7%) cleaved normally and abnormally, respectively, by inseminating with sperm of male hybrids between the female European and the male Japanese toad. The abnormally cleaved eggs all died before the blastula stage. While two of the normally cleaved eggs died at the gastrula stage, the other ten developed normally during the embryonic stage and hatched. However, six tadpoles died without taking food. Three of the other four died of ill-development before metamorphosis. The remaining one tadpole barely completed metamorphosis. This backcross toad was small and thin, and died shortly after the completion of metamorphosis without taking food (Table 27).

b. Backcrosses of female hybrids with males of the parental species

i) Controls

Two two-year-old females (Nos. 1 and 2) obtained in 1976 from a mating, *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo japonicus*♂ No. 1 (Table 17), were mated with a two-year-old male (No. 5) obtained in 1976 from *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo japonicus*♂ No. 1 and a two-year-old male (No. 4) obtained in 1976 from *Bufo bufo bufo*♀ No. 1 × *Bufo bufo bufo*♂ No. 3 (Table 16). It was found that 99.2%, 95.1% and 80.9% of 528 eggs in total normally cleaved, hatched and metamorphosed, respectively, by inseminating with sperm of the male *japonicus*, while 96.7%, 69.0%, 67.3% and 67.0% of 581 eggs in total normally cleaved, hatched, began to eat and metamorphosed, respectively, by inseminating with sperm of the male *bufo* (Table 28).

Three two-year-old female (Nos. 1~3) obtained in 1976 from *bufo*♀ No. 1 × *bufo*♂ No. 3 (Table 17) were mated with the same two males as in the above control matings. By inseminating with sperm of male *bufo* No. 4, 70.4%, 61.3%, 57.7% and 51.1% of 442 eggs in total normally cleaved, hatched, began to eat and metamorphosed, respectively, while 94.3%, 80.3%, 78.3% and 73.2% of 314 eggs in total normally cleaved, hatched, began to eat and metamorphosed, respectively, by inseminating with sperm of male *japonicus* No. 5 (Table 28).

ii) Hybrids, *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo bufo*♂ No. 1

Twenty-five two-year-old female hybrids produced in 1976 from a cross, *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo bufo*♂ No. 1, were injected with frog pituitary suspension. Ovulation occurred in seven (Nos. 1~7) of them (Table 17). These females were mated with male No. 5 obtained in 1976 from *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo japonicus*♂ No. 1 and male No. 4 obtained in 1976

TABLE 28

Developmental capacity of the backcrosses of female hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo* from Portugal and the controls

All the backcrosses were produced in 1978

Parents		No. of eggs	No. of cleavages		No. of normal neurulae	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally metamorphosed toads	PMC
Female	Male		Normal	Abnormal					
76(*jap.* ♀ 1 × *jap.* ♂ 1) Nos. 1, 2	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 5	528	524 (99.2%)	0	509 (96.4%)	502 (95.1%)	495 (93.8%)	427 (80.9%)	81.5
	76(*bufo* P. ♀ 1 × *bufo* P. ♂ 3) No. 4	581	562 (96.7%)	6 (1.0%)	413 (71.1%)	401 (69.0%)	391 (67.3%)	389 (67.0%)	69.2
76(*bufo* P. ♀ 1 × *bufo* P. ♂ 3) Nos. 1～3	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 5	314	296 (94.3%)	17 (5.4%)	277 (88.2%)	252 (80.3%)	246 (78.3%)	230 (73.2%)	77.7
	76(*bufo* P. ♀ 1 × *bufo* P. ♂ 3) No. 4	442	311 (70.4%)	19 (4.3%)	298 (67.4%)	271 (61.3%)	255 (57.7%)	226 (51.1%)	72.7
76(*jap.* ♀ 1 × *bufo* P. ♂ 1) No. 1	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 5	125	72 (57.6%)	8 (6.4%)	30 (24.0%)	8 (6.4%)	1 (0.8%)	1 (0.8%)	1.4
76(*jap.* ♀ 1 × *bufo* P. ♂ 1) No. 2	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 5	640	462 (72.2%)	118 (18.4%)	242 (37.8%)	42 (6.6%)	8 (1.3%)	8 (1.3%)	1.7
	76(*bufo* P. ♀ 1 × *bufo* P. ♂ 3) No. 4	316	185 (58.5%)	56 (17.7%)	74 (23.4%)	26 (8.2%)	1 (0.3%)	0	
76(*jap.* ♀ 1 × *bufo* P. ♂ 1) No. 3	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 5	709	575 (81.1%)	77 (10.9%)	327 (46.1%)	104 (14.7%)	15 (2.1%)	5 (0.7%)	0.9
	76(*bufo* P. ♀ 1 × *bufo* P. ♂ 3) No. 4	696	556 (79.9%)	37 (5.3%)	201 (28.9%)	53 (7.6%)	11 (1.6%)	4 (0.6%)	0.7
76(*jap.* ♀ 1 × *bufo* P. ♂ 1) No. 4	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 5	326	210 (64.4%)	58 (17.8%)	17 (5.2%)	10 (3.1%)	2 (0.6%)	1 (0.3%)	0.5
	76(*bufo* P. ♀ 1 × *bufo* P. ♂ 3) No. 4	185	131 (70.8%)	21 (11.4%)	11 (5.9%)	2 (1.1%)	0	0	
76(*jap.* ♀ 1 × *bufo* P. ♂ 1) No. 5	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 5	8	4 (50.0%)	0	2 (25.0%)	2 (25.0%)	0	0	
76(*jap.* ♀ 1 × *bufo* P. ♂ 1) No. 6	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 5	91	56 (61.5%)	19 (20.9%)	22 (24.2%)	6 (6.6%)	1 (1.1%)	1 (1.1%)	1.8
	76(*bufo* P. ♀ 1 × *bufo* P. ♂ 3) No. 4	124	53 (42.7%)	26 (21.0%)	13 (10.5%)	2 (1.6%)	1 (0.8%)	0	
76(*jap.* ♀ 1 × *bufo* P. ♂ 1) No. 7	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 5	790	421 (53.3%)	97 (12.3%)	164 (20.8%)	84 (10.6%)	6 (0.8%)	1 (0.1%)	0.2
	76(*bufo* P. ♀ 1 × *bufo* P. ♂ 3) No. 4	612	164 (26.8%)	62 (10.1%)	56 (9.2%)	13 (2.1%)	2 (0.3%)	2 (0.3%)	1.2

PMC, Percentage of metamorphosed toads to normally cleaved eggs

from *Bufo bufo bufo*♀ No. 1 × *Bufo bufo bufo*♂ No. 3 (Table 28).

The results indicated that 53.3～81.1%, 65.0% on the average, of 91～790 eggs obtained from six of the seven females cleaved normally by inseminating with sperm of male *japonicus* No. 5, while 26.8～79.9%, 55.7% on the average, of 124～696 eggs obtained from five of the seven females, did so by inseminating with sperm of male *bufo* No. 4. However, the normally cleaved eggs mostly died of various abnormalities like edema, blisters and ill-development during the embryonic stage (Fig. 13c, d). A total of 256 (9.5%) and a total of 96 (5.0%) embryos hatched normally in the series derived from male *japonicus* No. 5 and *bufo* No. 4, respectively. Most of the tadpoles died of abnormalities in the gills or some other organs. Only 33 (1.2%) and 15 (0.8%) hybrids began to eat and 17 (0.6%) and 6 (0.3%) metamorphosed normally in the series of male *japonicus* No. 5 and male *bufo* No. 4, respectively, although they were very small and weak as compared with the controls. Eventually, a single toad derived from

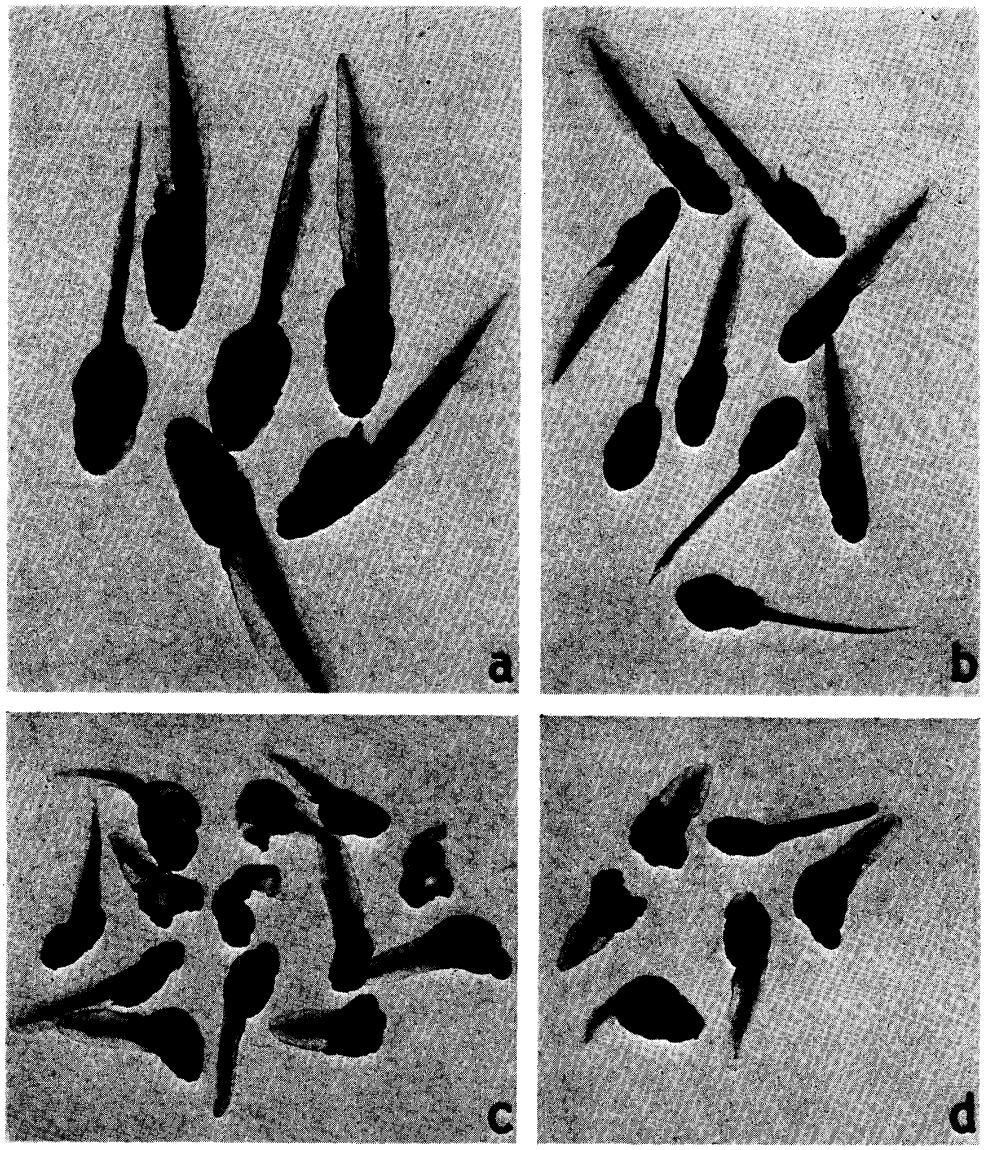

Fig. 13. Abnormal tadpoles in the backcrosses of a female hybrid between a female *Bufo bufo japonicus* and a male *Bufo bufo bufo* from Portugal. ×2.5

a. Controls, (*japonicus* ♀ No. 1 × *japonicus* ♂ No. 1) ♀ No. 1 × (*japonicus* ♀ No. 1 × *japonicus* ♂ No. 1) ♂ No. 5
b. Controls, (*bufo* from Portugal ♀ No. 1 × *bufo* from Portugal ♂ No. 3) ♀ No. 1 × (*bufo* from Portugal ♀ No. 1 × *bufo* from Portugal ♂ No. 3) ♂ No. 4
c. Hybrids, (*japonicus* ♀ No. 1 × *bufo* from Portugal ♂ No. 1) ♀ No. 2 × (*japonicus* ♀ No. 1 × *japonicus* ♂ No. 1) ♂ No. 5
d. Hybrids, (*japonicus* ♀ No. 1 × *bufo* from Portugal ♂ No. 1) ♀ No. 2 × (*bufo* from Portugal ♀ No. 1 × *bufo* from Portugal ♂ No. 3) ♂ No. 4

an egg of female hybrid No. 2 inseminated with a spermatozoon of male *japonicus* No. 5 and a single toad from an egg of female hybrid No. 3 inseminated with a spermatozoon of male *bufo* No. 4 grew normally and were 116.0 mm and 98.5 mm in body length, respectively, at the age of two years. These two toads were a female and a male. All the other toads died of ill-development, as they

were mostly unable to take food, owing to abnormality of the mouth.

In 1980, two four-year-old females (Nos. 8 and 9) produced in 1976 from *japonicus*♀ No. 1 × *bufo*♂ No. 1 were backcrossed to males of the parental species. These males were *Bufo bufo japonicus*♂ No. 9 obtained in 1976 from *japonicus*♀ No. 1 × *japonicus*♂ No. 1 and *Bufo bufo bufo*♂ No. 6 obtained in 1976 from *bufo*♀ No. 1 × *bufo*♂ No. 3. Female hybrids Nos. 8 and 9 were 90.0 mm and 73.5 mm in body length and laid 1843 and 1004 eggs, respectively, after pituitary injection (Table 17). The eggs of female hybrid No. 8 were divided into two groups. One group was inseminated with sperm of male *japonicus* No. 9, while the other was inseminated with sperm of male *bufo* No. 6. The eggs of female hybrid No. 9 were inseminated with sperm of male *japonicus* No. 9. The results indicated that 74.8% of 1033 eggs of female hybrid No. 8 and 80.8% of 1004 eggs of female hybrid No. 9 cleaved normally after inseminating with sperm of the male *japonicus*. In contrast, only 25.2% of 810 eggs of female hybrid No. 8 cleaved normally after inseminating with sperm of male *bufo* No. 6.

Most of the normally cleaved eggs obtained from the two female hybrids died of abnormality during the early embryonic stage, 138 (13.4%) and 137 (13.6%) of

TABLE 29

Developmental capacity of the backcrosses of female hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo* from Portugal. All the backcrosses were produced in 1980

Parents		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally metamorphosed toads	PMC
Female	Male						
76(*jap.* ♀1 × *bufo* P. ♂1) No. 8	76(*jap.* ♀1 × *jap.* ♂1) No. 9	1033	773 (74.8%)	138 (13.4%)	25 (2.4%)	16 (1.5%)	2.1
	76(*bufo* P. ♀1 × *bufo* P. ♂3) No. 6	810	204 (25.2%)	27 (3.3%)	3 (0.4%)	3 (0.4%)	1.5
76(*jap.* ♀1 × *bufo* P. ♂1) No. 9	76(*jap.* ♀1 × *jap.* ♂1) No. 9	1004	811 (80.8%)	137 (13.6%)	12 (1.2%)	4 (0.4%)	0.5
76(*bufo* P. ♀1 × *jap.* ♂3) No. 2	76(*bufo* P. ♀1 × *bufo* P. ♂3) No. 6	1787	1206 (67.5%)	22 (1.2%)	1 (0.1%)	1 (0.1%)	0.1
	76(*jap.* ♀1 × *jap.* ♂1) No. 9	2080	1811 (87.1%)	139 (6.7%)	5 (0.2%)	5 (0.2%)	0.3
76(*bufo* P. ♀1 × *jap.* ♂3) No. 3	76(*bufo* P. ♀1 × *bufo* P. ♂3) No. 6	1238	807 (65.2%)	39 (3.2%)	18 (1.5%)	14 (1.1%)	1.7
	76(*jap.* ♀1 × *jap.* ♂1) No. 9	1036	813 (78.5%)	93 (9.0%)	27 (2.6%)	21 (2.0%)	2.6
76(*bufo* P. ♀1 × *jap.* ♂3) No. 4	76(*bufo* P. ♀1 × *bufo* P. ♂3) No. 6	1166	678 (58.1%)	73 (6.3%)	7 (0.6%)	4 (0.3%)	0.6
	76(*jap.* ♀1 × *jap.* ♂1) No. 9	1093	794 (72.6%)	164 (15.0%)	19 (1.7%)	14 (1.3%)	1.8
76(*bufo* P. ♀1 × *jap.* ♂3) No. 5	76(*bufo* P. ♀1 × *bufo* P. ♂3) No. 6	1362	724 (53.2%)	83 (6.1%)	5 (0.4%)	4 (0.3%)	0.6
	76(*jap.* ♀1 × *jap.* ♂1) No. 9	1543	1261 (81.7%)	266 (17.2%)	6 (0.4%)	2 (0.1%)	0.2
76(*bufo* P. ♀1 × *jap.* ♂3) No. 6	76(*bufo* P. ♀1 × *bufo* P. ♂3) No. 6	1985	1770 (89.2%)	135 (6.8%)	26 (1.3%)	25 (1.3%)	1.4
	76(*jap.* ♀1 × *jap.* ♂1) No. 9	1341	863 (64.4%)	217 (16.2%)	43 (3.2%)	32 (2.4%)	3.7
76(*bufo* P. ♀1 × *jap.* ♂3) No. 7	76(*bufo* P. ♀1 × *bufo* P. ♂3) No. 6	1067	340 (31.9%)	10 (0.9%)	1 (0.1%)	1 (0.1%)	0.3
	76(*jap.* ♀1 × *jap.* ♂1) No. 9	1596	1009 (63.2%)	148 (9.3%)	13 (0.8%)	11 (0.7%)	1.1

PMC, Percentage of metamorphosed toads to normally cleaved eggs

the eggs inseminated with sperm of the male *japonicus* and 27 (3.3%) of those inseminated with sperm of the male *bufo* hatched normally. Of these 302 hatched embryos in total, 91 were remarkably larger than the others. They were all raised from large eggs which had been laid together with normal-sized eggs by the two female hybrids. Eventually, only 40 embryos raised from large eggs became feeding tadpoles and 23 of the latter became normally metamorphosed toads, while all the others died of various abnormalities (Table 29).

iii) Hybrids, *Bufo bufo bufo*♀ No. 1 × *Bufo bufo japonicus*♂ No. 3

In 1978, a single two-year-old female hybrid produced in 1976 from a cross, *Bufo bufo bufo*♀ No. 1 × *Bufo bufo japonicus*♂ No. 3, was not mated with a male *bufo* nor *japonicus*, although ovulation occurred by pituitary injection. The eggs were too precociously laid to be utilized in artificial insemination (Table 17).

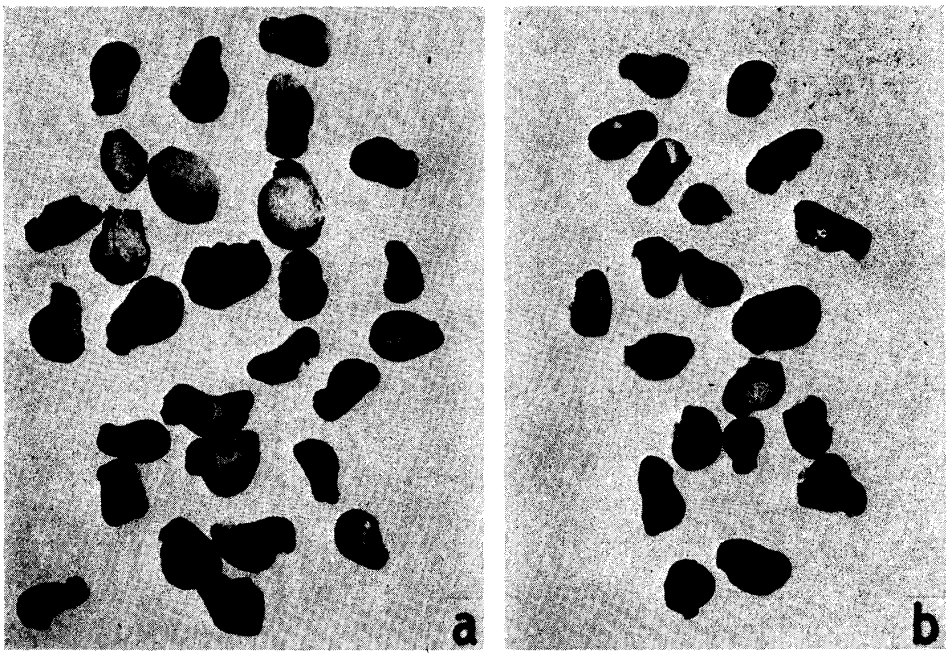

Fig. 14. Abnormal embryos in the backcrosses of a female hybrid between a female *Bufo bufo bufo* from Portugal and a male *Bufo bufo japonicus*. ×2.5

a. Backcrosses, (*bufo* from Portugal♀ No. 1 × *japonicus*♂ No. 3)♀ No. 2 × (*japonicus*♀ No. 1 × *japonicus*♂ No. 1)♂ No. 9

b. Backcrosses, (*bufo* from Portugal♀ No. 1 × *japonicus*♂ No. 3)♀ No. 2 × (*bufo* from Portugal♀ No. 1 × *bufo* from Portugal♂ No. 3)♂ No. 6

In 1980, six four-year-old female hybrids (Nos. 2～7) produced in 1976 from the same cross, *bufo*♀ No. 1 × *japonicus*♂ No. 3, were mated with a male *bufo* and a male *japonicus*. The female hybrids were 89.0～103.0 mm, average of 96.3 mm, in body length and laid 2259～3867 eggs, average of 2882.3 eggs, after pituitary injection (Table 17). The male *bufo* (No. 6) was obtained in 1976 from *bufo*♀ No. 1 × *bufo*♂ No. 3, while the male *japonicus* (No. 9) was obtained in 1976 from *japonicus*♀ No. 1 × *japonicus*♂ No. 1.

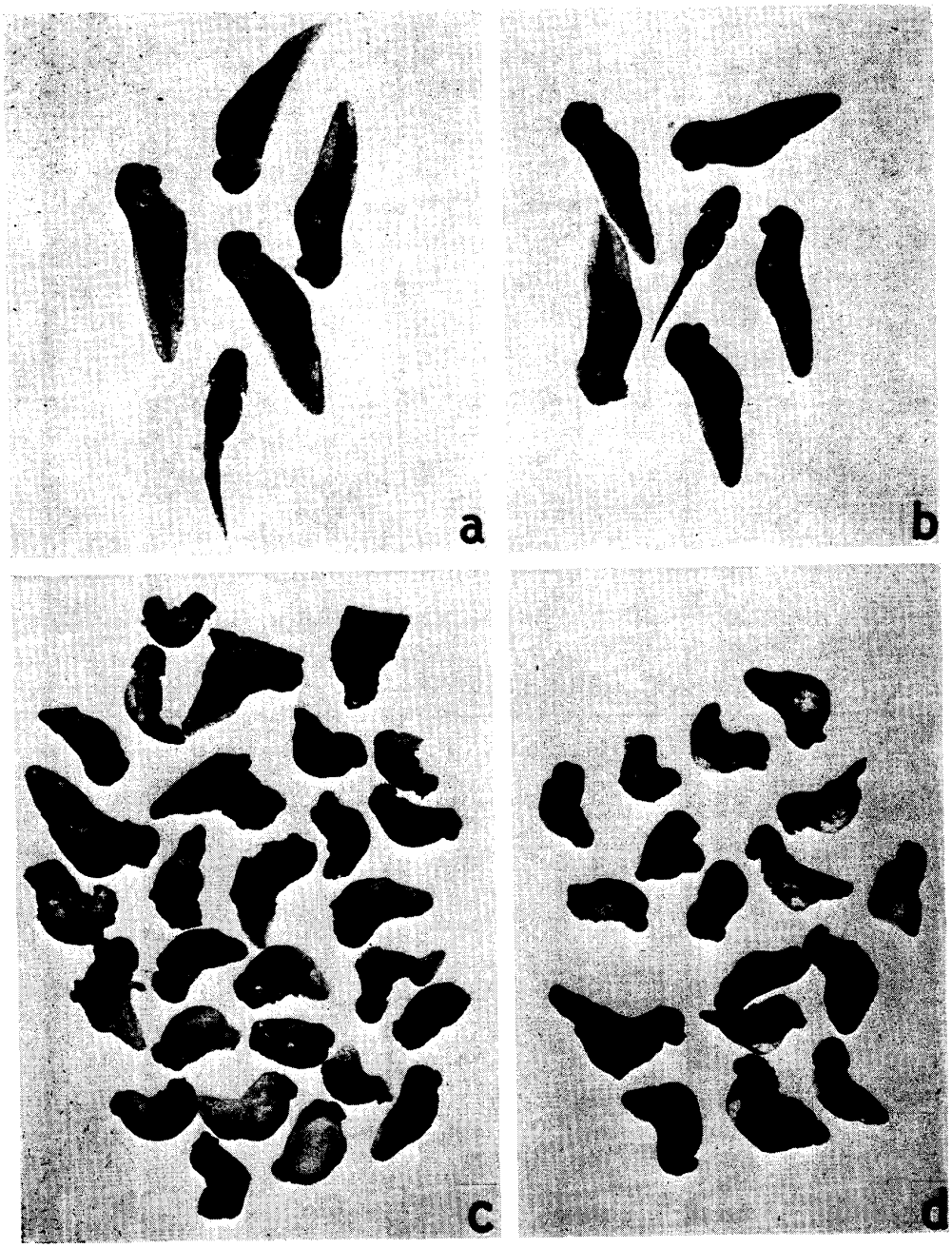

Fig. 15. Abnormal individuals at the post-hatching stage in the backcrosses of a female hybrid between a female *Bufo bufo bufo* from Portugal and a male *Bufo bufo japonicus*. ×2.5

a, c. Nearly normal and abnormal backcrosses, (*bufo* from Portugal ♀ No. 1 × *japonicus* ♂ No. 3) ♀ No. 6 × (*japonicus* ♀ No. 1 × *japonicus* ♂ No. 1) ♂ No. 9

b, d. Nearly normal and abnormal backcrosses, (*bufo* from Portugal ♀ No. 1 × *japonicus* ♂ No. 3) ♀ No. 6 × (*bufo* from Portugal ♀ No. 1 × *bufo* from Portugal ♂ No. 3) ♂ No. 6

The eggs of each female hybrid were divided into two groups. One group was inseminated with sperm of the male *bufo*, while the other group was with sperm of the male *japonicus*. It was found that 31.9～89.2%, average of 60.9%, of 1067～1985 eggs cleaved normally by inseminating with sperm of the male *bufo*, while 63.2～87.1%, average of 74.6%, of 1036～2080 eggs did so by inseminating

with sperm of the male *japonicus* (Table 29). More than three-fourths of the normally cleaved eggs in each series died of abnormality during the early embryonic stage (Figs. 14a, b; 15c, d); 0.9~6.8% of the eggs inseminated with sperm of the male *bufo* and 6.7~17.2% of the eggs inseminated with sperm of the male *japonicus* hatched normally (Fig. 15a, b). Of 362 hatched embryos in total produced from matings of the six female hybrids with the male *bufo*, 120 were raised from large eggs, while 287 of 1027 hatched embryos in total produced from matings of the female hybrids with the male *japonicus* were also raised from large eggs. Eventually, 1 (0.1%)~26 (1.3%) eggs, 58 in total, became feeding tadpoles in each of the series derived from the male *bufo*, while 5 (0.2%)~43 (3.2%) eggs, 113 in total, did so in each of the series derived from the male *japonicus*. It was remarkable that all these feeding tadpoles were raised from large eggs. Of the tadpoles in the series of the male *bufo*, 49 completed metamorphosis normally, while 85 of the tadpoles in the series of the male *japonicus* did so (Table 29).

TABLE 30

Chromosomes of backcrosses of female hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo* from Portugal. All the backcrosses were produced in 1980

Parents		Number of tadpoles								
			Number of chromosomes							
Female	Male	Analyzed	22 (2n)	23	31	32	33 (3n)	36	55 (5n)	60	Unknown
76(*jap.* ♀ 1 × *bufo* P. ♂ 1) No. 8	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 9	18	2				15	1			
	76(*bufo* P. ♀ 1 × *bufo* P. ♂ 3) No. 6	3					3				
76(*jap.* ♀ 1 × *bufo* P. ♂ 1) No. 9	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 9	10	1	3			6				
76(*bufo* P. ♀ 1 × *jap.* ♂ 3) No. 2	76(*bufo* P. ♀ 1 × *bufo* P. ♂ 3) No. 6	1					1				
	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 9	5	2				3				
76(*bufo* P. ♀ 1 × *jap.* ♂ 3) No. 3	76(*bufo* P. ♀ 1 × *bufo* P. ♂ 3) No. 6	16					16				
	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 9	26					25				1
76(*bufo* P. ♀ 1 × *jap.* ♂ 3) No. 4	76(*bufo* P. ♀ 1 × *bufo* P. ♂ 3) No. 6	7					5				2
	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 9	16					16				
76(*bufo* P. ♀ 1 × *jap.* ♂ 3) No. 5	76(*bufo* P. ♀ 1 × *bufo* P. ♂ 3) No. 6	5			1		4				
	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 9	4		2			2				
76(*bufo* P. ♀ 1 × *jap.* ♂ 3) No. 6	76(*bufo* P. ♀ 1 × *bufo* P. ♂ 3) No. 6	26				1	21	2	2		
	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 9	43		1			41			1	
76(*bufo* P. ♀ 1 × *jap.* ♂ 3) No. 7	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 9	13		1	1	4	6		1		

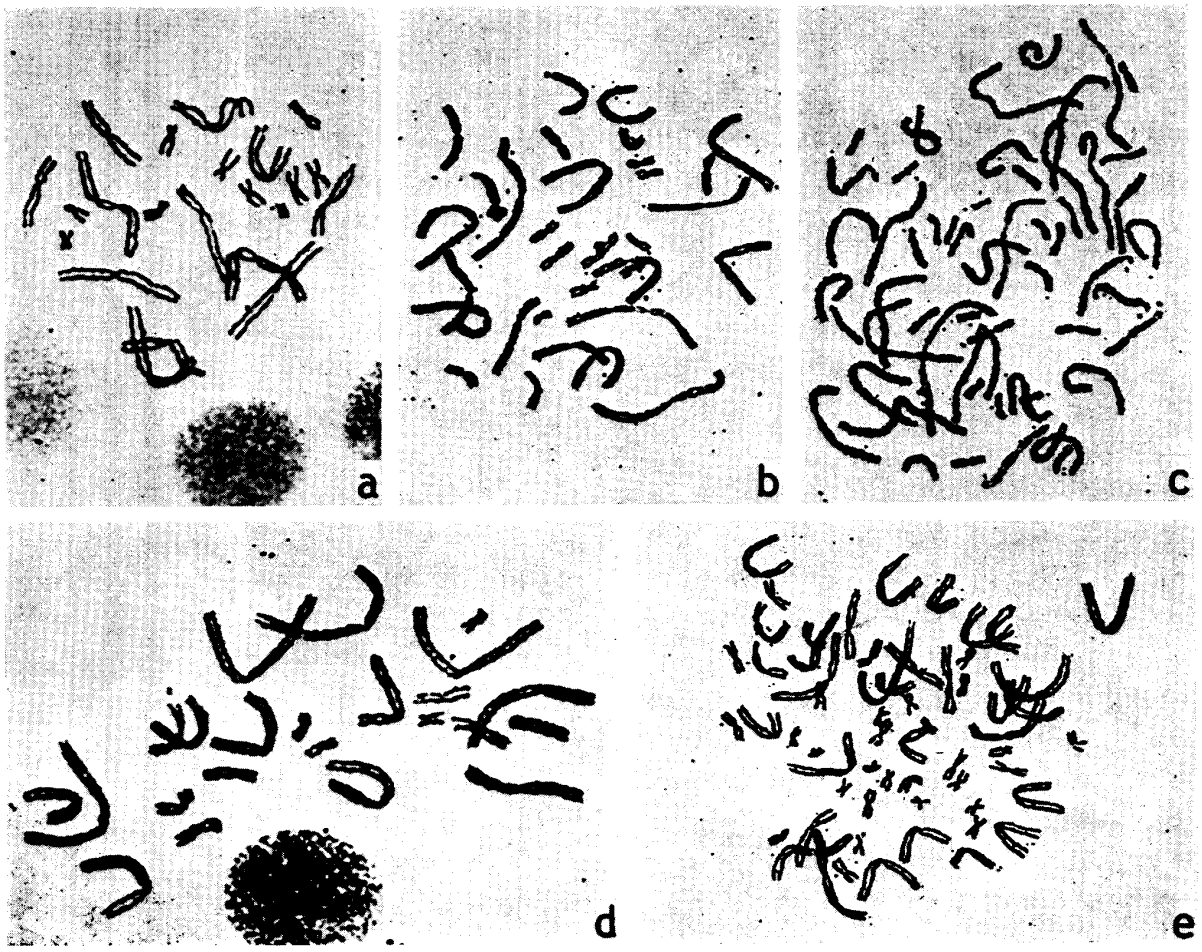

Fig. 16. Chromosomes of normally feeding tadpoles in the backcrosses of female hybrids between female *Bufo bufo bufo* from Portugal No. 1 and male *Bufo bufo japonicus* No. 3 with male *Bufo bufo japonicus* No. 9 or *Bufo bufo bufo* from Portugal No. 6.

a. Diploid metaphase spread of a backcross raised from a large egg of female hybrid No. 2 by mating with male *Bufo bufo japonicus* No. 9 ×1000
b. Triploid metaphase spread of a backcross raised from a large egg of female hybrid No. 6 by mating with male *Bufo bufo bufo* from Portugal No. 6 ×1000
c. Pentaploid metaphase spread of another backcross raised from a large egg in the same way as (b) ×800
d. Hyperdiploid (2n+1) metaphase spread of a backcross raised from a large egg of female hybrid No. 6 by mating with male *Bufo bufo japonicus* No. 9 ×1000
e. Hyperpentaploid (5n+5) metaphase spread of another backcross raised from a large egg in the same way as (d) ×800

c. Chromosomes of the backcrosses produced from female hybrids, *Bufo bufo japonicus* and *Bufo bufo bufo* from Portugal

Chromosomes were examined in 31 tadpoles produced from two females (Nos. 8 and 9) of the hybrids, *Bufo bufo japonicus*♀ × *Bufo bufo bufo* from Portugal♂, and 162 tadpoles produced from six females (Nos. 2~7) of the reciprocal hybrids by backcrossing with a male of each parental species (Table 30). All the tadpoles were those that had been raised from larger eggs. Of the 31 tadpoles produced from the two female hybrids, *japonicus*♀ × *bufo*♂, 24 were triploids, while three

were diploids, three others were hyperdiploids (2n+1=23) and the remaining one was hypertriploid (3n+3). Of the 162 tadpoles produced from the six female hybrids, *bufo*♀×*japonicus*♂, 140 were triploids, while two were diploids, four hyperdiploids (2n+1), seven hypotriploids (3n−2 or 3n−1), two hypertriploids (3n+3), three pentaploids and one hyperpentaploid (5n+5). The ploidy of the remaining three could not be determined owing to paucity of analyzable mitoses (Fig. 16).

2. Hybrids between female *Bufo bufo japonicus* and a male *Bufo bufo* from France or Greece

a. Backcrosses and some other crosses of female hybrids derived from a male *Bufo bufo* from France

A total of five female hybrids produced in 1977 from crosses, *Bufo bufo japonicus* ♀ Nos. 3 and 5×*Bufo bufo* from France♂ No. 1, matured sexually in the breeding season of 1980 (Table 19). They were 99.0~123.0 mm, average of 112.5 mm, in body length. Three of them laid 4228~6163 eggs, average of 5215 eggs, after pituitary injection, while the other two laid no eggs. Some of these eggs were divided into two groups; one group was inseminated with sperm of a male *japonicus* (No. 8) produced in 1977 from *japonicus*♀ No. 3×*japonicus*♂ No. 4 and the other was with sperm of a male *bufo* (No. 7) produced in the same year from *bufo* from Portugal♀ No. 1×*bufo* from Portugal♂ No. 3. A small number of the eggs laid by each female were distinctly larger than the others.

It was found that 92.5% of 1073 eggs of female hybrid No. 1 derived from female *japonicus* No. 3, 94.6% of 1288 eggs of female hybrid No. 2 derived from female *japonicus* No. 5 and 89.8% of 1270 eggs of female hybrid No. 3 derived from the same female (No. 5) cleaved normally after inseminating with sperm of male *japonicus* (No. 8), while 81.2% of 1217 eggs of female hybrid No. 1 derived from female *japonicus* No. 3, 93.3% of 1205 eggs of female hybrid No. 2 derived from female *japonicus* No. 5 and 93.1% of 1222 eggs of female hybrid No. 3 derived from the same female (No. 5) did so after inseminating with sperm of male *bufo* No. 7 (Table 31). Most of these normally cleaved eggs died of abnormality during the embryonic stage (Fig. 17); only 1.3~10.6% hatched normally and 0~0.8% became feeding tadpoles.

As observed in the matings of female hybrids produced from crosses between a female *japonicus* and a male *bufo* from France, large eggs were remarkably superior to the others in developmental capacity. In the backcross series of female hybrid No. 1 derived from female *japonicus* No. 3, all, three, two and two of six large eggs cleaved normally, hatched normally, began to eat and became normally metamorphosed toads, respectively, after inseminating with sperm of the male *japonicus* (No. 8), while four of eight large eggs inseminated with sperm of the male *bufo* No. 7 cleaved normally and died during the early embryonic stage. In the backcross series of female hybrid No. 2 derived from female *japonicus* No. 5, twenty, ten, two and two of 26 large eggs cleaved normally, hatched normally, began to eat and became normally metamorphosed toads,

TABLE 31

Developmental capacity of backcrosses and some other offspring of female hybrids between female *Bufo bufo japonicus* and male *Bufo bufo* from France or Greece
All the backcrosses were produced in 1980

Parents		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally metamorphosed toads	PMC
Female	Male						
77(*jap.* ♀3×*bufo* F. ♂1) No. 1	77(*jap.* ♀3×*jap.* ♂4) No. 8	1073	993 (92.5%)	90 (8.4%)	2 (0.2%)	2 (0.2%)	0.2
	76(*bufo* P. ♀1×*bufo* P. ♂3) No. 7	1217	988 (81.2%)	34 (2.8%)	0	0	
77(*jap.* ♀5×*bufo* F. ♂1) No. 2	77(*jap.* ♀3×*jap.* ♂4) No. 8	1288	1218 (94.6%)	108 (8.4%)	3 (0.2%)	2 (0.2%)	0.2
	76(*bufo* P. ♀1×*bufo* P. ♂3) No. 7	1205	1124 (93.3%)	16 (1.3%)	0	0	
77(*jap.* ♀5×*bufo* F. ♂1) No. 3	77(*jap.* ♀3×*jap.* ♂4) No. 8	1270	1140 (89.8%)	134 (10.6%)	10 (0.8%)	8 (0.6%)	0.7
	76(*bufo* P. ♀1×*bufo* P. ♂3) No. 7	1222	1138 (93.1%)	48 (3.9%)	6 (0.5%)	5 (0.4%)	0.4
77(*jap.* ♀3×*bufo* G. ♂1) No. 1	77(*jap.* ♀3×*jap.* ♂4) No. 8	1037	1003 (96.7%)	158 (15.2%)	9 (0.9%)	8 (0.8%)	0.8
	76(*bufo* P. ♀1×*bufo* P. ♂3) No. 7	1007	845 (83.9%)	51 (5.1%)	4 (0.4%)	1 (0.1%)	0.1
77(*jap.* ♀3×*bufo* G. ♂1) No. 2	77(*jap.* ♀3×*jap.* ♂4) No. 8	246	186 (75.6%)	16 (6.5%)	1 (0.4%)	1 (0.4%)	0.5
	76(*bufo* P. ♀1×*bufo* P. ♂3) No. 7	230	89 (38.7%)	0	0	0	
77(*jap.* ♀3×*bufo* G. ♂1) No. 3	77(*jap.* ♀3×*jap.* ♂4) No. 8	1589	1390 (87.5%)	154 (9.7%)	44 (2.8%)	15 (0.9%)	1.1
	76(*bufo* P. ♀1×*bufo* P. ♂3) No. 7	1548	1206 (77.9%)	76 (4.9%)	27 (1.7%)	26 (1.7%)	2.2

PMC, Percentage of metamorphosed toads to normally cleaved eggs

respectively, after inseminating with sperm of male *japonicus* (No. 8), while eight and three of 19 large eggs cleaved normally and hatched normally, respectively, after inseminating with sperm of male *bufo* No. 7. No feeding tadpoles were produced in the latter series. In the backcross series of female hybrid No. 3 derived from the same female (No. 5), 57 (86.4%), 33 (50.0%), nine (13.6%) and eight (12.1%) of 66 large eggs cleaved normally, hatched normally, began to eat and became normally metamorphosed toads, respectively, after inseminating with sperm of male *japonicus* (No. 8), while 38 (71.7%), 20 (37.7%), six (11.3%) and five (9.4%) of 53 large eggs did so, respectively, after inseminating with sperm of male *bufo* (No. 7). Of 21 feeding tadpoles in total produced from all the matings of the three female hybrids, 19 were obtained from large eggs; 17 of the latter became normally metamorphosed toads.

b. Backcrosses and some other crosses of female hybrids derived from a male *Bufo bufo* from Greece

Five female hybrids (Nos. 1～5) produced in 1977 from a cross, *Bufo bufo japonicus*♀ No. 3×*Bufo bufo* from Greece♂ No. 1, matured in the season of 1980. They were 95.0～118.5 mm, average of 103.4 mm, in body length. Three of them laid eggs after pituitary injection, while the other two did not. Female hybrids Nos. 1 and 3 laid 3006 and 3137 eggs, respectively, and No. 2 laid only

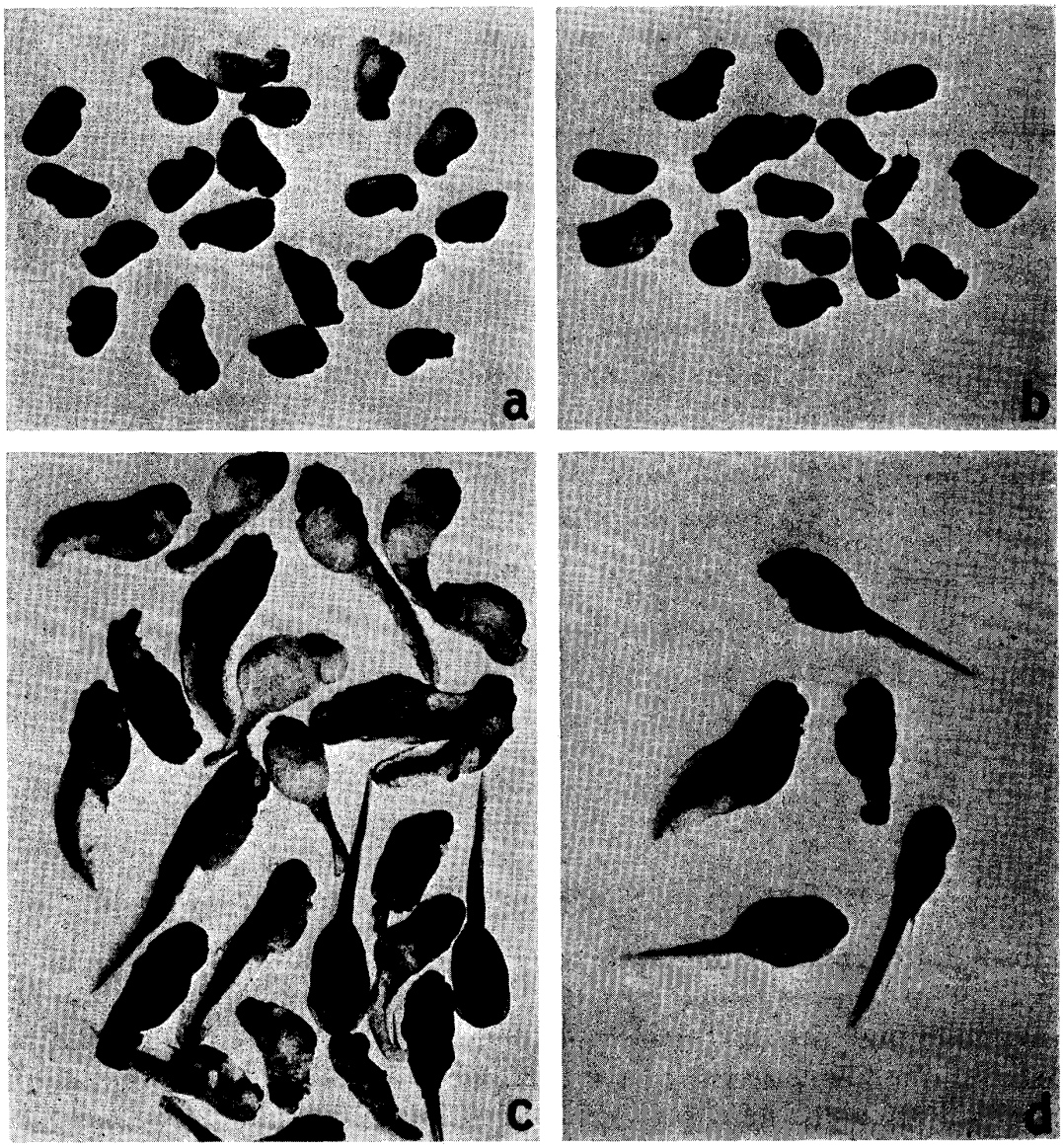

Fig. 17. Abnormal embryos and tadpoles in the offspring of a female hybrid between a female *Bufo bufo japonicus* and a male *Bufo bufo* from France. ×2.5

a, c. Backcrosses, (*japonicus* ♀ No. 5 × *bufo* from France ♂ No. 1) ♀ No. 3 × (*japonicus* ♀ No. 3 × *japonicus* ♂ No. 4) ♂ No. 8

b, d. Second-generation offspring, (*japonicus* ♀ No. 5 × *bufo* from France ♂ No. 1) ♀ No. 3 × (*bufo* from Portugal ♀ No. 1 × *bufo* from Portugal ♂ No. 3) ♂ No. 7

476 eggs. Among the eggs laid by each female there were always a small number of distinctly larger eggs (Table 19). Most of the eggs obtained from female hybrid No. 1 as well as all the eggs of female hybrids Nos. 2 and 3 were divided into two groups. One group was inseminated with sperm of male *japonicus* No. 8, while the other group was with sperm of male *bufo* No. 7 (Table 31).

The results indicated that 96.7% of 1037 eggs from female hybrid No. 1, 75.6% of 246 eggs from female hybrid No. 2 and 87.5% of 1589 eggs from female hybrid No. 3 cleaved normally after inseminating with sperm of male *japonicus* (No. 8), while 83.9% of 1007 eggs from female hybrid No. 1, 38.7% of 230 eggs

from female hybrid No. 2 and 77.9% of 1548 eggs from female hybrid No. 3 did so after inseminating with sperm of male *bufo* No. 7 (Table 31). However, most of the normally cleaved eggs died of abnormality during the embryonic stage (Fig. 18); 0~15.2% hatched normally and 0~2.8% became feeding tadpoles. The eggs inseminated with sperm of the male *bufo* were somewhat inferior to those backcrossed with sperm of the male *japonicus* in developmental capacity. It was noteworthy that almost all of the feeding tadpoles were produced from large eggs. In the series of female hybrid No. 1 backcrossed with the male *japonicus*, 40 (58.0%), 32 (46.4%), nine (13.0%) and eight (11.6%) of 69 large eggs cleaved normally, hatched normally, began to eat and became normally metamorphosed toads, respectively, while in the series of the same female mated with the male *bufo*, 33 (46.5%), 16 (22.5%), four (5.6%) and one (1.4%) of 71 large eggs did so, respectively. In the series of female hybrid No. 2 backcrossed with the male *japonicus*, all, two, one and one of three large eggs cleaved normally, hatched normally, began to eat and became normally metamorphosed toad, respectively, while in the series of the same female mated with the male *bufo*, two large eggs could not cleave normally. In the series of female hybrid No. 3 backcrossed with the male *japonicus*, 203 (73.6%), 86 (31.2%), 27 (9.8%) and 15 (5.4%) of 276 large eggs cleaved normally, hatched normally, began to eat and became normally metamorphosed toads, respectively, while in the series of the same female mated with the male *bufo*, 206 (80.2%), 57 (22.2%), 27 (10.5%) and 26 (10.1%) of 257 large eggs did so, respectively. Of 85 feeding tadpoles in total produced from all the backcrossings of the three female hybrids (Nos. 1~3), 82 were obtained from the large eggs.

c. Chromosome number of backcrosses

The chromosomes of feeding tadpoles raised from the eggs of female hybrids between *Bufo bufo japonicus*♀ Nos. 3~5 and *Bufo bufo* from France♂ No. 1 or Greece♂ No. 1 by inseminating with sperm of a male *japonicus* or *bufo* were examined in the tail tips by the squash method. In addition to these tadpoles, abnormal ones which could not eat at all were examined by the same method.

i) Feeding tadpoles

Eight of the ten tadpoles raised from the eggs of female hybrid No. 3, *japonicus*♀ No. 5 × *bufo* from France♂ No. 1, by inseminating with sperm of the male *japonicus* and all the six tadpoles raised from the eggs of the same female by inseminating with sperm of the male *bufo* from France were completely triploids, that is, 33 in chromosome number. The chromosome number of the other two was unknown (Table 32).

Eight of nine tadpoles raised from the eggs of female hybrid No. 1, *japonicus*♀ No. 3 × *bufo* from Greece♂ No. 1, by inseminating with sperm of the male *japonicus* and three of the four tadpoles raised from the eggs of the same female hybrid by inseminating with sperm of the male *bufo* were completely triploids, that is, 33 in chromosome number. The chromosome number of the remaining two tadpoles

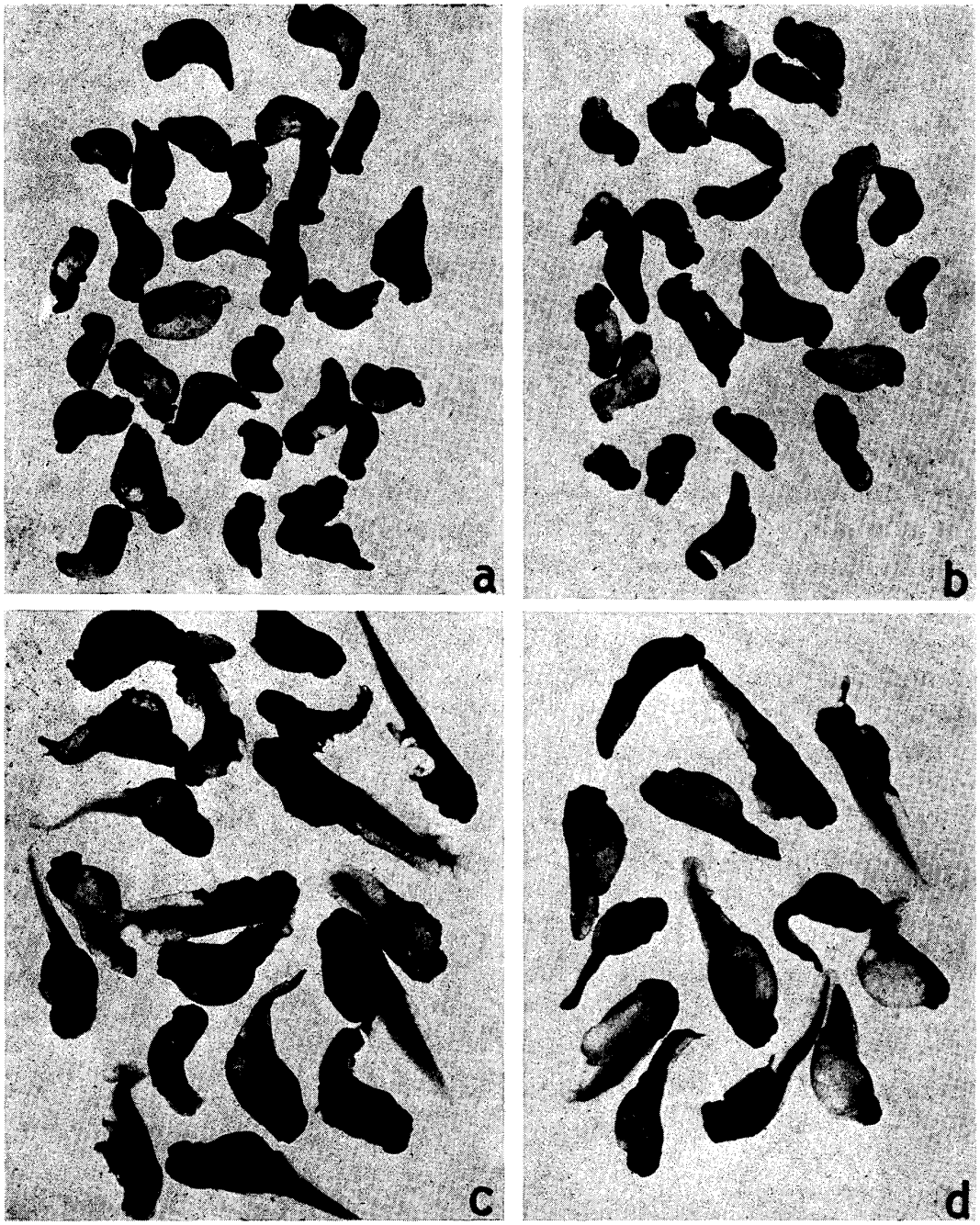

Fig. 18. Abnormal embryos and tadpoles in the offspring of a female hybrid between a female *Bufo bufo japonicus* and a male *Bufo bufo* from Greece. ×2.5

a, c. Backcrosses, (*japonicus* ♀ No. 3 × *bufo* from Greece ♂ No. 1) ♀ No. 1 × (*japonicus* ♀ No. 3 × *japonicus* ♂ No. 4) ♂ No. 8

b, d. Second-generation offspring, (*japonicus* ♀ No. 3 × *bufo* from Greece ♂ No. 1) ♀ No. 1 × (*bufo* from Portugal ♀ No. 1 × *bufo* from Portugal ♂ No. 3) ♂ No. 7

was unknown. The chromosome number of the feeding tadpoles raised from the eggs of female hybrid No. 3, *japonicus* ♀ No. 3 × *bufo* from Greece ♂ No. 1, by inseminating with sperm of the male *japonicus* or *bufo* was examined in 37 of the 44 and 26 of the 27 individuals, respectively. It was found that thirty, one and

TABLE 32

Chromosomes of backcrosses and some other offspring of female hybrids between female *Bufo bufo japonicus* and male *Bufo bufo* from France or Greece
All the backcrosses were produced in 1980

Parents		Kinds	Number of tadpoles									
			Ana-lyzed	Number of chromosomes								
Female	Male			22 (2n)	23～27 (2n+)	28～32 (3n−)	33 (3n)	34～38 (3n+)	39～43 (4n−)	44 (4n)	M	Un-known
77(*jap.* ♀5 ×*bufo* F. ♂1) No. 3	77(*jap.* ♀3 ×*jap.* ♂4) No. 8	LF	10				8					2
	76(*bufo* P. ♀1 ×*bufo* P. ♂3) No. 7	LF	6				6					
77(*jap.* ♀3 ×*bufo* G. ♂1) No. 1	77(*jap.* ♀3 ×*jap.* ♂4) No. 8	LF	9				8					1
	77(*bufo* P. ♀1 ×*bufo* P. ♂3) No. 7	LF	4				3					1
77(*jap.* ♀3 ×*bufo* G. ♂1) No. 3	77(*jap.* ♀3 ×*jap.* ♂4) No. 8	LF	37			1	30	1				5
		LL	20			10	6	2	1	1		
		LN	26	6	15	3	1				1	
	77(*bufo* P. ♀1 ×*bufo* P. ♂3) No. 7	LF	26				25					1

M, Mosaics LF, Normal feeding tadpoles produced from large eggs
LL, Lethal tadpoles produced from large eggs
LN, Lethal tadpoles produced from normal-sized eggs

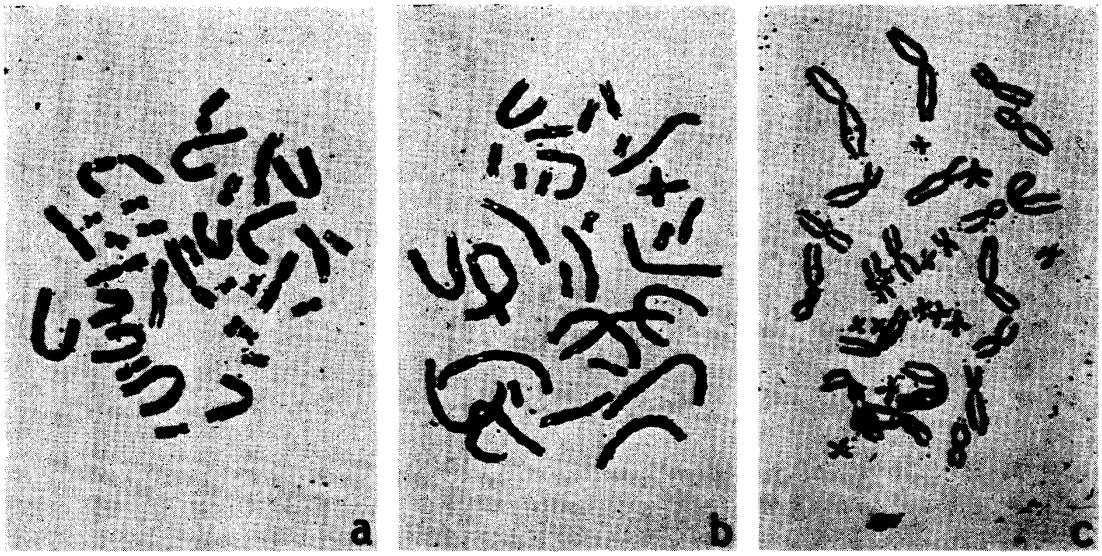

Fig. 19. Chromosomes of normally feeding tadpoles in the backcrosses of female hybrid No. 3 between female *Bufo bufo japonicus* No. 3 and male *Bufo bufo* from Greece No. 1 mated with male No. 8 between female *Bufo bufo japonicus* No. 3 and male *Bufo bufo japonicus* No. 4. ×1000

a. Triploid metaphase spread of a backcross raised from a large egg
b. Hypotriploid (3n−1) metaphase spread of another backcross raised from a large egg
c. Hypertriploid (3n+1) metaphase spread of still another backcross raised from a large egg

one of the former were triploids, a hypertriploid (3n+1) and a hypotriploid (3n−1), respectively (Fig. 19), while 25 of the latter were triploids. The chromosome number of the remaining five and one tadpoles, respectively, was unknown (Table 32).

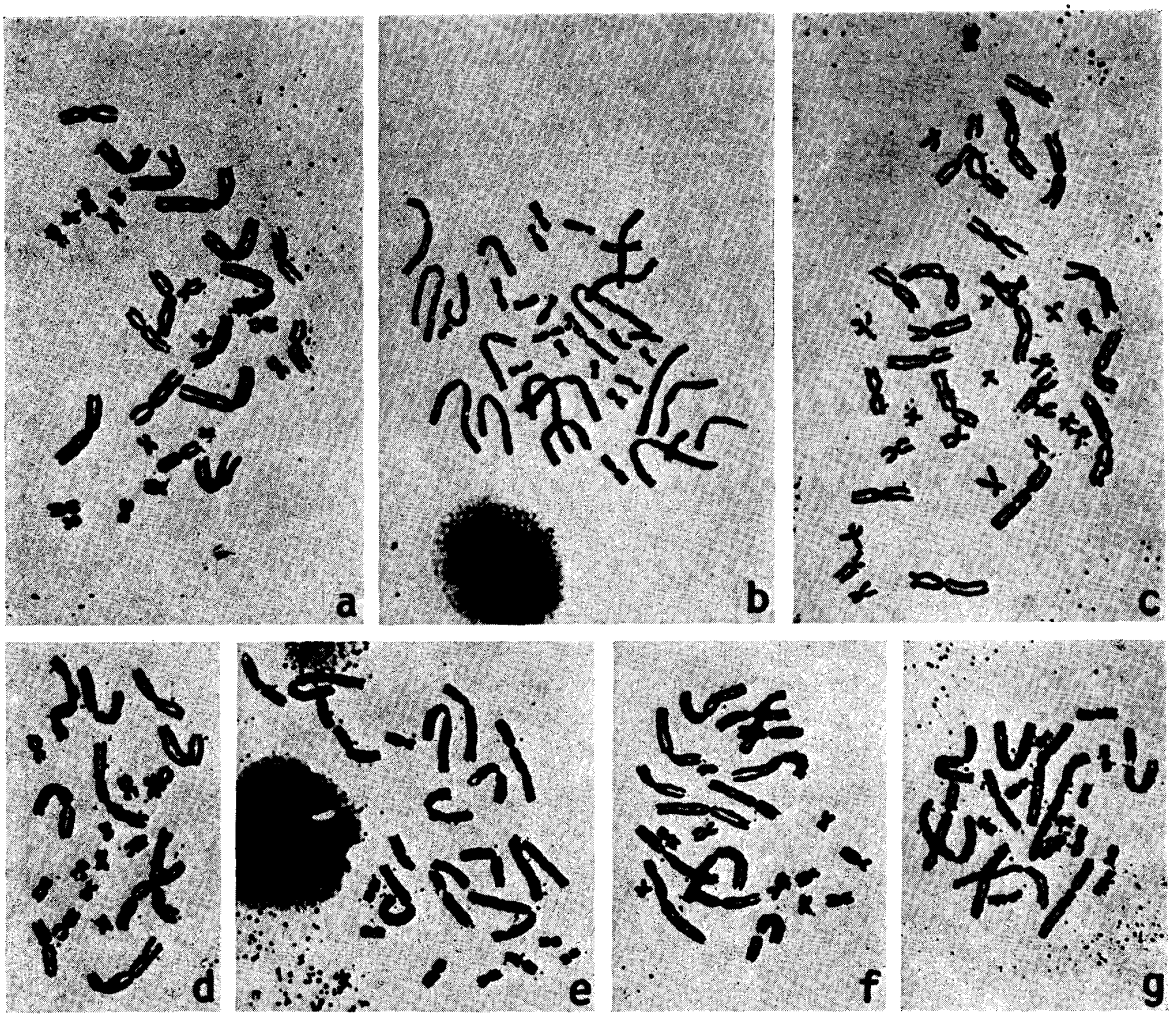

Fig. 20. Chromosomes of lethal tadpoles in the backcrosses of female hybrid No. 3 between female *Bufo bufo japonicus* No. 3 and male *Bufo bufo* from Greece No. 1 mated with male No. 8 between female *Bufo bufo japonicus* No. 3 and male *Bufo bufo japonicus* No. 4.

a. Hypotriploid (3n−2) metaphase spread of a backcross raised from a large egg ×1000
b. Hypertriploid (3n+4) metaphase spread of another backcross raised from a large egg ×800
c. Hypotetraploid (4n−3) metaphase spread of still another backcross raised from a large egg ×1000
d, e. Abnormally diploid metaphase spreads of two backcrosses raised from normal-sized eggs ×1000
f. Hyperdiploid (2n+2) metaphase spread of another backcross raised from a normal-sized egg ×1000
g. Hyperdiploid (2n+1) metaphase spread of still another backcross raised from a normal-sized egg ×1000

ii) Lethal tadpoles

As described earlier, some of the normally hatched embryos produced by backcrossing were raised from larger eggs, while the others were from normal-sized eggs. Almost all the embryos raised from normal-sized eggs as well as most of the embryos raised from large eggs were inviable; they could not develop into feeding tadpoles.

At the stage when viable tadpoles began to eat, there were 65 and 45 lethal tadpoles raised from normal-sized eggs and large ones, respectively, in the backcross series of female hybrid No. 3, *japonicus*♀ No. 3 × *bufo* from Greece♂ No. 1, and the male *japonicus*. Chromosome number was determined in 26 and 20 lethal tadpoles raised from normal-sized eggs and large ones, respectively. Of the former tadpoles, eight had a thin body and ill-developed external gills, 13 were microcephalic and edematous, and the remaining five were almost normal in appearance but did not take food. The chromosome number of these lethal tadpoles raised from normal-sized eggs showed a wide range of aberrations from diploid to triploid (Table 32). Only six tadpoles were abnormal diploids (Fig. 20d, e), another was a mosaic consisting of a mixture of trisomic and monosomic cells, and still another was a triploid. The remaining 18 tadpoles were intermediate between diploid and triploid (Fig. 20f, g), although most of them were 23, 24, 25 and 27 in chromosome number. In contrast, the lethal tadpoles raised from large eggs had more numerous chromosomes than those raised from normal-sized eggs (Table 32). One tadpole had 28 chromosomes, while another was a tetraploid, having 44 chromosomes. The other 18 tadpoles were intermediate in chromosome number between these two (Fig. 20a~c). Most of them were approximately triploids; they had 31, 32, or 33 chromosomes.

3. Hybrids between a female *Bufo bufo bufo* and a male *Bufo bufo* from France

a. Backcrosses of female hybrids with a male *Bufo bufo bufo*

Five female hybrids (Nos. 1~5) produced in 1977 from a cross, *Bufo bufo bufo*♀ No. 2 × *Bufo bufo* from France♂ No. 1, matured sexually in the season of 1980. They were 88.0~91.0 mm, average of 90.1 mm, in body length. Of these female hybrids, No. 1 and No. 3 laid 5332 and 5015 eggs, respectively, after pituitary injection (Table 19). The other three females (Nos. 2, 4 and 5) also laid numerous eggs which were similar in number to those of the above two, although the exact number of their eggs was not counted. The male *Bufo bufo bufo* used in the backcrossing was produced in 1976 from a mating, *Bufo bufo bufo*♀ No. 1 × *Bufo bufo bufo*♂ No. 3 (Table 1).

It was found that 62.2~96.7%, average of 79.2%, of 407~442 eggs cleaved normally. Of these normally cleaved eggs, those of female hybrids Nos. 2 and 5 mostly developed normally during the embryonic stage; 59.4% and 67.1% hatched normally, 50.7% and 46.9% began to eat, and 37.5% and 33.9% became normally metamorphosed toads. Many of the normally cleaved eggs of female hybrids Nos. 1, 3 and 4 died of oxygen shortage attributable to want of care. In these three backcross series, 27.8~46.0% hatched normally, 11.8~40.5% began to eat, and 2.9~26.7% became normally metamorphosed toads (Table 33).

b. Crosses of female hybrids with various kinds of male hybrids

Three other female hybrids (Nos. 6~8) produced in 1977 from the same cross

TABLE 33

Developmental capacity of backcrosses of female hybrids and F_2 between a female a *Bufo bufo bufo* from Portugal and a male *Bufo bufo* from France, and sterility of male hybrids between a female *Bufo bufo bufo* from Portugal and a male *Bufo torrenticola* and between a female *Bufo bufo miyakonis* and a male *Bufo bufo bufo* from Portugal. All the offspring of the hybrids were produced in 1980

Parents		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally metamorphosed toads	PMC
Female	Male						
77(*bufo* P. ♀2 ×*bufo* F. ♂1) No. 1	76(*bufo* P. ♀1 ×*bufo* P. ♂3) No. 6	420	406 (96.7%)	193 (46.0%)	170 (40.5%)	112 (26.7%)	27.6
77(*bufo* P. ♀2 ×*bufo* F. ♂1) No. 2	76(*bufo* P. ♀1 ×*bufo* P. ♂3) No. 6	416	314 (75.5%)	247 (59.4%)	211 (50.7%)	156 (37.5%)	49.7
77(*bufo* P. ♀2 ×*bufo* F. ♂1) No. 3	76(*bufo* P. ♀1 ×*bufo* P. ♂3) No. 6	442	275 (62.2%)	123 (27.8%)	52 (11.8%)	13 (2.9%)	4.7
77(*bufo* P. ♀2 ×*bufo* F. ♂1) No. 4	76(*bufo* P. ♀1 ×*bufo* P. ♂3) No. 6	411	318 (77.4%)	157 (38.2%)	122 (29.7%)	76 (18.5%)	23.9
77(*bufo* P. ♀2 ×*bufo* F. ♂1) No. 5	76(*bufo* P. ♀1 ×*bufo* P. ♂3) No. 6	407	342 (84.0%)	273 (67.1%)	191 (46.9%)	138 (33.9%)	40.4
77(*bufo* P. ♀2 ×*bufo* F. ♂1) No. 6	77(*bufo* P. ♀2 ×*bufo* F. ♂1) No. 1	174	170 (97.7%)	133 (76.4%)	116 (66.7%)	97 (55.7%)	57.1
77(*bufo* P. ♀2 ×*bufo* F. ♂1) No. 7	77(*bufo* P. ♀2 ×*bufo* F. ♂1) No. 1	281	268 (95.4%)	251 (89.3%)	204 (72.6%)	192 (68.3%)	71.6
77(*bufo* P. ♀2 ×*bufo* F. ♂1) No. 8	77(*bufo* P. ♀2 ×*bufo* F. ♂1) No. 1	250	247 (98.8%)	215 (86.0%)	200 (80.0%)	132 (52.8%)	53.4
77(*bufo* P. ♀2 ×*bufo* F. ♂1) Nos. 6~8	78(*bufo* P. ♀3 ×*tor.* ♂3) No. 3	1322	0	0	0	0	
	78(*bufo* P. ♀3 ×*tor.* ♂3) No. 4	1192	0	0	0	0	
	78(*bufo* P. ♀3 ×*tor.* ♂3) No. 5	1327	0	0	0	0	
	78(*bufo* P. ♀3 ×*tor.* ♂3) No. 6	1212	0	0	0	0	
	78(*bufo* P. ♀3 ×*tor.* ♂3) No. 7	1196	0	0	0	0	
77(*bufo* P. ♀2 ×*bufo* F. ♂1) Nos. 6~8	78(*miy.* ♀1 ×*bufo* P. ♂5) No. 4	1259	0	0	0	0	
	78(*miy.* ♀1 ×*bufo* P. ♂5) No. 5	1429	0	0	0	0	
	78(*miy.* ♀1 ×*bufo* P. ♂5) No. 6	1232	0	0	0	0	

PMC, Percentage of metamorphosed toads to normally cleaved eggs

as the above matured sexually in the season of 1980. These toads were 90.5~95.5 mm, average of 92.5 mm, in body length and laid 4713~5893 eggs, average of 5324.7 eggs, after pituitary injection. They were mated with three kinds of male hybrids by the routine method of artificial insemination.

One of two mature male hybrids obtained in 1977 from a cross, *Bufo bufo bufo*♀ No. 2 × *Bufo bufo* from France ♂ No. 1, was used to produce F_2 offspring. This male toad was 78.0 mm in body length (Table 18). The right testis was 7.0 mm in both length and width; the right BIDDER's organ was 5.0 mm × 3.5 mm in size. By inseminating with sperm of this male hybrid, 95.4~98.8%, average of 97.3%, of 174~281 eggs obtained from female hybrids Nos. 6~8 cleaved normally. A small part of the normally cleaved eggs was removed in each series, while the other part was continuously reared. It was found that 76.4%, 89.3% and 86.0%, average of 83.9%, of the respective number of eggs hatched

normally, 66.7%, 72.6% and 80.0%, average of 73.1%, began to eat and 55.7%, 68.3% and 52.8%, average of 58.9%, became normally metamorphosed toads, in the series of female hybrids Nos. 6, 7 and 8, respectively.

Five male hybrids (Nos. 3~7) produced in 1978 from a cross, *Bufo bufo bufo*♀ No. 3 × *Bufo torrenticola*♂ No. 3 and three male hybrids (Nos. 4~6) produced in 1978 from a cross, *Bufo bufo miyakonis*♀ No. 1 × *Bufo bufo bufo*♂ No. 5, were 75.0~86.5 mm, average of 81.3 mm, and 63.0~63.5 mm, average of 63.2 mm, in body length, respectively. The left testes of the male hybrids, *bufo*♀ × *torrenticola*♂, were 5.0~6.5 mm, average of 5.6 mm, in length and 3.5~4.0 mm, average of 3.6 mm, in width. Their left BIDDER's organs were 1.0 mm × 0.5 mm~8.0 mm × 7.0 mm in size. The left testes of the male hybrids, *miyakonis*♀ × *bufo*♂, were 4.5~6.0 mm, average of 5.3 mm, in length and 3.0 mm in width. The right BIDDER's organs were 6.5 mm × 3.5 mm~11.5 mm × 6.5 mm in size. These measurements indicated that the testes of the male hybrids between Japanese and European toads were remarkably smaller than that of the above male hybrid between the two European populations of *Bufo bufo* (Table 25).

The results of matings between the three female hybrids, *bufo bufo*♀ × *bufo* from France♂, and the five male hybrids, *bufo*♀ × *torrenticola*♂, or the three male hybrids, *miyakonis*♀ × *bufo*♂, indicated that all these male hybrids were completely sterile. No normally cleaved eggs were obtained in each matings (Table 33).

4. Hybrids between *Bufo bufo japonicus* and *Bufo torrenticola*

a. Backcrosses of male hybrids with female *Bufo bufo japonicus* in 1978

i) Controls

Two one-year-old males (Nos. 1 and 2) obtained in 1977 from a mating, *Bufo bufo japonicus*♀ No. 3 × *Bufo bufo japonicus*♂ No. 4 (Table 20), and two one-year-old males (Nos. 1 and 2) obtained in 1977 from *Bufo torrenticola*♀ No. 1 × *Bufo torrenticola*♂ No. 2 (Table 20) were mated with two two-year-old females (Nos. 6 and 7) obtained in 1976 from a mating, *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo japonicus*♂ No. 1. It was found that by inseminating with sperm of the male *japonicus* 99.6% of 798 eggs in total cleaved normally, while by inseminating with sperm of *torrenticola* 94.4% of 574 eggs did so. In the former case, 93.2% hatched normally, 74.9% began to eat and 65.5% became normally metamorphosed toads, while in the latter case 78.6% hatched normally, 64.8% began to eat and 43.9% became normally metamorphosed toads (Table 34).

ii) Hybrids, *Bufo bufo japonicus*♀ No. 3 × *Bufo torrenticola*♂ No. 1

Five one-year-old male hybrids (Nos. 1~5) produced in 1977 from a cross, *Bufo bufo japonicus*♀ No. 3 × *Bufo torrenticola*♂ No. 1 (Table 20), were mated with the same female *japonicus* (Nos. 6 and 7) as those used in the control matings. The results indicated that 95.7~98.5%, average of 97.5%, of 554~800 eggs, cleaved normally by inseminating with sperm of the male hybrids (Table 34). While a considerable number of normally cleaved eggs died of various abnormalities, 78.5~90.5%, average of 83.2%, hatched normally. After some in-

TABLE 34

Developmental capacity of backcrosses of male hybrids between *Bufo bufo japonicus* and *Bufo torrenticola* and the controls. All the backcrosses were produced in 1978 and 1979

Parents		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally metamorphosed toads	PMC
Female	Male						
76(*jap.* ♀1×*jap.* ♂1) Nos. 6, 7	77(*jap.* ♀3×*jap.* ♂4) Nos. 1, 2	798	795 (99.6%)	744 (93.2%)	598 (74.9%)	523 (65.5%)	65.8
	77(*tor.* ♀1×*tor.* ♂2) Nos. 1, 2	574	542 (94.4%)	451 (78.6%)	372 (64.8%)	252 (43.9%)	46.5
	77(*jap.* ♀3×*tor.* ♂1) No. 1	769	757 (98.4%)	696 (90.5%)	568 (73.9%)	519 (67.5%)	68.6
	77(*jap.* ♀3×*tor.* ♂1) No. 2	800	788 (98.5%)	628 (78.5%)	483 (60.4%)	443 (55.4%)	56.2
	77(*jap.* ♀3×*tor.* ♂1) No. 3	554	530 (95.7%)	467 (84.3%)	392 (70.8%)	349 (63.0%)	65.8
	77(*jap.* ♀3×*tor.* ♂1) No. 4	800	775 (96.9%)	661 (82.6%)	519 (64.9%)	481 (60.1%)	62.1
	77(*jap.* ♀3×*tor.* ♂1) No. 5	800	784 (98.0%)	642 (80.3%)	465 (58.1%)	439 (54.9%)	56.0
77(*jap.* ♀3×*jap.* ♂4) Nos. 1, 2	77(*jap.* ♀3×*jap.* ♂4) No. 5	791	726 (91.8%)	566 (71.6%)	495 (62.6%)	456 (57.6%)	62.8
	77(*tor.* ♀1×*tor.* ♂2) No. 6	782	719 (91.9%)	577 (73.8%)	524 (67.0%)	473 (60.5%)	65.8
	77(*tor.* ♀1×*jap.* ♂5) No. 6	788	703 (89.2%)	549 (69.7%)	447 (56.7%)	409 (51.9%)	58.2
	77(*tor.* ♀1×*jap.* ♂5) No. 7	786	738 (93.9%)	562 (71.5%)	489 (62.2%)	354 (45.0%)	48.0
	77(*tor.* ♀1×*jap.* ♂5) No. 8	798	754 (94.5%)	594 (74.4%)	524 (65.7%)		
	77(*tor.* ♀1×*jap.* ♂5) No. 9	796	730 (91.7%)	565 (71.0%)	508 (63.8%)		
	77(*tor.* ♀1×*jap.* ♂5) No. 10	800	713 (89.1%)	618 (77.3%)	583 (72.9%)		
	77(*tor.* ♀1×*jap.* ♂5) No. 11	800	765 (95.6%)	652 (81.5%)	597 (74.6%)	578 (72.3%)	75.6
77(*tor.* ♀1×*tor.* ♂2) Nos. 1, 2	77(*jap.* ♀3×*jap.* ♂4) No. 5	398	388 (97.5%)	314 (78.9%)	228 (57.3%)	187 (47.0%)	48.2
	77(*tor.* ♀1×*tor.* ♂2) No. 6	383	383 (100 %)	339 (88.5%)	273 (71.3%)	209 (54.6%)	54.6
	77(*tor.* ♀1×*jap.* ♂5) No. 6	398	372 (93.5%)	302 (75.9%)	256 (64.3%)	235 (59.0%)	63.2
	77(*tor.* ♀1×*jap.* ♂5) No. 7	392	388 (99.0%)	334 (85.2%)	262 (66.8%)	199 (50.8%)	51.3
	77(*tor.* ♀1×*jap.* ♂5) No. 8	295	291 (98.6%)	238 (80.7%)	197 (66.8%)		
	77(*tor.* ♀1×*jap.* ♂5) No. 9	383	380 (99.2%)	302 (78.9%)	258 (67.4%)		
	77(*tor.* ♀1×*jap.* ♂5) No. 10	291	291 (100 %)	273 (93.8%)	239 (82.1%)		
	77(*tor.* ♀1×*jap.* ♂5) No. 11	373	373 (100 %)	340 (91.2%)	309 (82.8%)	278 (74.5%)	74.5

PMC, Percentage of metamorphosed toads to normally cleaved eggs

dividuals died of ill-development, 58.1~73.9%, average of 65.6%, began to eat and 54.9~67.5%, average of 60.2%, became normally metamorphosed toads (Table 34).

Accordingly, it was found that the male hybrids did not remarkably differ from the control male *japonicus* in reproductive capacity.

b. Backcrosses of male hybrids with female *Bufo bufo japonicus* in 1979

i) Controls

A two-year-old male (No. 5) obtained in 1977 from a mating, *Bufo bufo japonicus*♀ No. 3×*Bufo bufo japonicus*♂ No. 4 and a two-year-old male (No. 6) obtained in 1977 from a mating, *Bufo torrenticola*♀ No. 1×*Bufo torrenticola*♂ No. 2 (Table 20), were mated with two two-year-old females (Nos. 1 and 2) obtained in 1977 from a mating, *Bufo bufo japonicus*♀ No. 3×*Bufo bufo japonicus*♂ No. 4 (Table 21). Of 791 eggs in total, 91.8% cleaved normally by inseminating with sperm of the male *japonicus*, 71.6% hatched normally, 62.6% began to eat and 57.6% metamorphosed normally. On the other hand, 91.9% of 782 eggs cleaved normally by inseminating with sperm of the male *torrenticola*. Afterwards, 73.8% hatched normally, 67.0% began to eat and 60.5% became normally metamorphosed toads (Table 34).

ii) Hybrids, *Bufo torrenticola*♀ No. 1×*Bufo bufo japonicus*♂ No. 5

Six two-year-old male hybrids (Nos. 6～11) produced in 1977 from a cross, *Bufo torrenticola*♀ No. 1×*Bufo bufo japonicus*♂ No. 5 (Table 20), were mated with the same two two-year-old female *japonicus* (Nos. 1 and 2) as those used in the control matings. The results showed that 89.1～95.6%, average of 92.3%, of 786～800 eggs in total cleaved normally by inseminating with sperm of the male hybrids (Table 34). After a considerable number of normally cleaved eggs died of various abnormalities, 69.7～81.5%, average of 74.2%, of the respective number of eggs hatched normally and 56.7～74.6%, average of 66.0%, began to eat. While the tadpoles produced by inseminating with sperm of three male hybrids (Nos. 8, 9 and 10) were preserved before metamorphosis, those produced by the other three male hybrids (Nos. 6, 7 and 11) were continuously reared. It was found that 45.0～72.3%, average of 56.4%, of the respective number of eggs became normally metamorphosed toads (Table 34).

Consequently, it was found that the male hybrids were nearly the same as the control male *japonicus* in reproductive capacity.

c. Backcrosses of male hybrids with female *Bufo torrenticola* in 1979

i) Control males

The same two-year-old male *Bufo bufo japonicus* (No. 5) and *Bufo torrenticola* (No. 6) as those used in the control matings of the above experimental series (b) were mated with two two-year-old females (Nos. 1 and 2) obtained in 1977 from a cross, *Bufo torrenticola*♀ No. 1×*Bufo torrenticola*♂ No. 2 (Table 21). The results showed that 97.5% of 398 eggs in total cleaved normally by inseminating with sperm of male *japonicus* No. 5, while 100% of 383 eggs in total did so by inseminating with sperm of male *torrenticola* No. 6. In the former series, 78.9% hatched normally, 57.3% began to eat and 47.0% became normally metamorphosed toads, while in the latter series 88.5% hatched normally, 71.3% began to eat and 54.6% completed normal metamorphosis (Table 34).

ii) Hybrids, *Bufo torrenticola*♀ No. 1 × *Bufo bufo japonicus*♂ No. 5

The same six two-year-old male hybrids (Nos. 6~11) as those used in the above experimental series (b) were mated with the same two female *torrenticola* as those used in the control matings. The results indicated that 93.5~100%, average of 98.4%, of 291~398 eggs in total cleaved normally by inseminating with sperm of the male hybrids. After a small number of normally cleaved eggs died of various abnormalities, 75.9~93.8%, average of 84.3%, hatched normally and 64.3~82.8%, average of 71.7%, began to eat. While the tadpoles produced by three male hybrids (Nos. 8~10) were preserved before metamorphosis, those produced by the other three male hybrids (Nos. 6, 7 and 11) were continuously reared. Eventually, 50.8~74.5%, average of 61.4%, of eggs in total became normally metamorphosed toads (Table 34).

Accordingly it was evident that the male hybrids were scarcely inferior to the control male *Bufo torrenticola* in reproductive capacity.

d. Matings of female hybrids with male *Bufo bufo japonicus*, *Bufo torrenticola* and hybrids in 1979

i) Controls

A two-year-old female (No. 3) obtained in 1977 from a mating, *Bufo bufo japonicus*♀ No. 3 × *Bufo bufo japonicus*♂ No. 4 (Table 21), was mated with a two-year-old male (No. 6) obtained in 1977 from a mating, *Bufo bufo japonicus*♀ No. 3 × *Bufo bufo japonicus*♂ No. 4, a two-year-old male (No. 7) obtained in 1977 from *Bufo torrenticola*♀ No. 1 × *Bufo torrenticola*♂ No. 2, a two-year-old male hybrid (No. 7) produced in 1977 from a cross, *Bufo bufo japonicus*♀ No. 3 × *Bufo torrenticola*♂ No. 1 and a two-year-old male (No. 11) produced in 1977 from a cross, *Bufo torrenticola*♀ No. 1 × *Bufo bufo japonicus*♂ No. 5 (Table 20). It was found that 98.1~99.7%, average of 99.0%, of 377~400 eggs cleaved normally by inseminating with sperm of these four kinds of males (Table 35). While many of the normally cleaved eggs died of various abnormalities, 51.2~84.0%, average of 64.4%, hatched normally. After a small number of individuals died of ill-development or some other abnormality during the tadpole stage, 47.8~74.5%, average of 58.9%, began to eat and eventually 43.5~68.5%, average of 53.1%, became normally metamorphosed toads (Table 35).

ii) Hybrids, *Bufo bufo japonicus*♀ No. 3 × *Bufo torrenticola*♂ No. 1

Five two-year-old female hybrids (Nos. 1~5) produced in 1977 from a cross, *Bufo bufo japonicus*♀ No. 3 × *Bufo torrenticola*♂ No. 1 (Table 21), were mated with the same four kinds of males as those used in the control matings (Table 35). The results indicated that 80.0~99.7%, average of 91.3%, of 145~400 eggs cleaved normally by inseminating with sperm of male *japonicus* No. 6. Of these eggs, 48.8~97.8%, average of 72.2%, hatched normally, 38.6~86.2%, average of 59.4%, began to eat and eventually 35.0~75.1%, average of 53.0%, became normally metamorphosed toads. On the other hand, 82.8~100%, average of 93.1%, cleaved normally by inseminating with sperm of male *torrenticola* No. 7.

TABLE 35

Developmental capacity of backcrosses of male and female hybrids and F_2 between *Bufo bufo japonicus* and *Bufo torrenticola* and the controls. All the backcrosses and F_2 were produced in 1979

Parents		No. of eggs	No. of normal cleavages	No. of normal hatched embryos	No. of normal feeding tadpoles	No. of normal metamorphosed toads	PMC
Female	Male						
77(*jap.* ♀3×*jap.* ♂4) No. 3	77(*jap.* ♀3×*jap.* ♂4) No. 6	400	396 (99.0%)	336 (84.0%)	298 (74.5%)	274 (68.5%)	69.2
	77(*tor.* ♀1×*tor.* ♂2) No. 7	377	370 (98.1%)	214 (56.8%)	202 (53.6%)	179 (47.5%)	48.4
	77(*jap.* ♀3×*tor.* ♂1) No. 7	391	390 (99.7%)	200 (51.2%)	187 (47.8%)	170 (43.5%)	43.6
	77(*tor.* ♀1×*jap.* ♂5) No. 11	400	397 (99.3%)	262 (65.5%)	238 (59.5%)	211 (52.8%)	53.1
77(*jap.* ♀3×*tor.* ♂1) No. 1	77(*jap.* ♀3×*jap.* ♂4) No. 6	400	386 (96.5%)	304 (76.0%)	267 (66.8%)	249 (62.3%)	64.5
	77(*tor.* ♀1×*tor.* ♂2) No. 7	386	386 (100 %)	337 (87.3%)	310 (80.3%)	252 (65.3%)	65.3
	77(*jap.* ♀3×*tor.* ♂1) No. 7	397	395 (99.5%)	353 (88.9%)	284 (71.5%)	215 (54.2%)	54.4
	77(*tor.* ♀1×*jap.* ♂5) No. 11	400	398 (99.5%)	337 (84.3%)	273 (68.3%)	230 (57.5%)	57.8
77(*jap.* ♀3×*tor.* ♂1) No. 2	77(*jap.* ♀3×*jap.* ♂4) No. 6	383	342 (89.3%)	187 (48.8%)	148 (38.6%)	134 (35.0%)	39.2
	77(*tor.* ♀1×*tor.* ♂2) No. 7	314	306 (97.5%)	174 (55.4%)	149 (47.5%)	126 (40.1%)	41.2
	77(*jap.* ♀3×*tor.* ♂1) No. 7	398	349 (87.7%)	202 (50.8%)	163 (41.0%)		
	77(*tor.* ♀1×*jap.* ♂5) No. 11	391	333 (85.2%)	154 (39.4%)	146 (37.3%)		
77(*jap.* ♀3×*tor.* ♂1) No. 3	77(*jap.* ♀3×*jap.* ♂4) No. 6	370	369 (99.7%)	362 (97.8%)	319 (86.2%)	278 (75.1%)	75.3
	77(*tor.* ♀1×*tor.* ♂2) No. 7	392	391 (99.7%)	372 (94.9%)	315 (80.4%)	241 (61.5%)	61.6
	77(*jap.* ♀3×*tor.* ♂1) No. 7	300	294 (98.0%)	287 (95.7%)	232 (77.3%)	164 (54.7%)	55.8
	77(*tor.* ♀1×*jap.* ♂5) No. 11	384	377 (98.2%)	358 (93.2%)	296 (77.1%)	224 (58.3%)	59.4
77(*jap.* ♀3×*tor.* ♂1) No. 4	77(*jap.* ♀3×*jap.* ♂4) No. 6	145	116 (80.0%)	105 (72.4%)	75 (51.7%)	74 (51.0%)	63.8
	77(*tor.* ♀1×*tor.* ♂2) No. 7	220	188 (85.5%)	168 (76.4%)	126 (57.3%)	103 (46.8%)	54.8
	77(*jap.* ♀3×*tor.* ♂1) No. 7	205	148 (72.2%)	139 (67.8%)	102 (49.8%)		
	77(*tor.* ♀1×*jap.* ♂5) No. 11	381	349 (91.6%)	290 (76.1%)	213 (55.9%)		
77(*jap.* ♀3×*tor.* ♂1) No. 5	77(*jap.* ♀3×*jap.* ♂4) No. 6	390	354 (90.8%)	257 (65.9%)	209 (53.6%)	162 (41.5%)	45.8
	77(*tor.* ♀1×*tor.* ♂2) No. 7	378	313 (82.8%)	305 (80.7%)	129 (34.1%)	117 (31.0%)	37.4
	77(*jap.* ♀3×*tor.* ♂1) No. 7	396	390 (98.5%)	270 (68.2%)	191 (48.2%)		
	77(*tor.* ♀1×*jap.* ♂5) No. 11	342	318 (93.0%)	204 (59.6%)	159 (46.5%)		

PMC, Percentage of metamorphosed toads to normally cleaved eggs

Of these eggs, 55.4%~94.9%, average of 79.0%, hatched normally, 34.1~80.4%, average of 59.9%, began to eat and 31.0~65.3%, average of 48.9%, became normally metamorphosed toads.

The results of matings between the five female hybrids (Nos. 1~5) and the two male hybrids obtained by reciprocal crosses of *japonicus* and *torrenticola* were

as follows (Table 35). While 72.2~99.5%, average of 91.2%, of 205~398 eggs cleaved normally, 50.8~95.7%, average of 74.3%, hatched normally, and 41.0~77.3%, average of 57.6%, began to eat by inseminating with sperm of male hybrid No. 7 produced by *japonicus*♀ × *torrenticola*♂, 85.2~99.5%, average of 93.5%, of 342~400 eggs cleaved normally, 39.4~93.2%, average of 70.5%, hatched normally and 37.3~77.1%, average of 57.0%, began to eat by inseminating with sperm of male hybrid No. 11 produced by *torrenticola*♀ × *japonicus*♂. The tadpoles raised from eggs of two females Nos. 1 and 3 were continuously reared, while the other were preserved before metamorphosis. In these two series, 54.2% and 54.7% of the respective number of eggs inseminated with sperm of male hybrid No. 7 became normally metamorphosed toads, while 57.5% and 58.3% of the respective number of eggs inseminated with sperm of male hybrid No. 11 did so.

From these results, it was evident that the female hybrids produced from a cross between a female *japonicus* and a male *torrenticola* scarcely differed from the control female *japonicus* in reproductive capacity, and also that the male hybrids produced from reciprocal crosses between *japonicus* and *torrenticola* did not distinctly differ from the control male *japonicus* or *torrenticola* in this respect.

iii) Hybrids, *Bufo torrenticola*♀ No. 1 × *Bufo bufo japonicus*♂ No. 5

Four two-year-old female hybrids (Nos. 1~4) produced in 1977 from a cross *torrenticola*♀ × *japonicus*♂, were mated with the same four kinds of males as those used in the control matings (Table 36). It was found that 83.6~98.3%, average of 92.0%, of 300~400 eggs cleaved normally by inseminating with sperm of male *japonicus* No. 6, while 87.3~95.5%, average of 91.9%, of 300~400 eggs did so by inseminating with sperm of male *torrenticola* No. 7. In the former series, 52.5~94.0%, average of 78.8%, hatched normally and 39.0~81.8%, average of 62.8%, began to eat, while 56.4~84.5%, average of 71.8%, hatched normally and 43.1~67.7%, average of 54.4%, began to eat in the latter series. By inseminating with sperm of male hybrid No. 7 produced from a cross, *japonicus*♀ × *torrenticola*♂, 89.0~93.1%, average of 91.7%, of 272~400 eggs cleaved normally, 68.0~86.3%, average of 76.3%, hatched normally, and 44.7~67.4%, average of 56.4%, began to eat. On the other hand, 80.5~99.3%, average of 91.7%, of 300~400 eggs, cleaved normally, 59.8~95.0%, average of 82.2%, hatched normally, and 43.2~75.0%, average of 65.1%, began to eat by inseminating with sperm of male hybrid No. 11 produced from a cross, *torrenticola*♀ × *japonicus*♂.

The tadpoles raised from the eggs of females Nos. 1 and 4 were continuously reared, while those raised from the eggs of females Nos. 2 and 3 were all preserved before metamorphosis. Of the respective number of eggs of female No. 1 inseminated with sperm of male *japonicus* and *torrenticola*, 49.8% and 46.0% became normally metamorphosed toads. On the other hand, 55.5% and 45.5% did so by inseminating with sperm of male hybrid No. 7 obtained from *japonicus*♀ × *torrenticola*♂ and of male hybrid No. 11 from the reciprocal cross, respectively.

TABLE 36

Developmental capacity of backcrosses of female hybrids and F_2 between *Bufo bufo japonicus* and *Bufo torrenticola*

All the backcrosses and F_2 were produced in 1979

Parents		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally metamorphosed toads	PMC
Female	Male						
77(*tor.* ♀ 1 × *jap.* ♂ 5) No. 1	77(*jap.* ♀ 3 × *jap.* ♂ 4) No. 6	400	356 (89.0%)	326 (81.5%)	243 (60.8%)	199 (49.8%)	55.9
	77(*tor.* ♀ 1 × *tor.* ♂ 2) No. 7	400	382 (95.5%)	338 (84.5%)	221 (55.3%)	184 (46.0%)	48.2
	77(*jap.* ♀ 3 × *tor.* ♂ 1) No. 7	400	371 (92.8%)	345 (86.3%)	259 (64.8%)	222 (55.5%)	59.8
	77(*tor.* ♀ 1 × *jap.* ♂ 5) No. 11	400	358 (89.5%)	332 (83.0%)	293 (73.3%)	182 (45.5%)	50.8
77(*tor.* ♀ 1 × *jap.* ♂ 5) No. 2	77(*jap.* ♀ 3 × *jap.* ♂ 4) No. 6	341	285 (83.6%)	179 (52.5%)	133 (39.0%)		
	77(*tor.* ♀ 1 × *tor.* ♂ 2) No. 7	385	336 (87.3%)	217 (56.4%)	166 (43.1%)		
	77(*jap.* ♀ 3 × *tor.* ♂ 1) No. 7	272	242 (89.0%)	185 (68.0%)	132 (48.5%)		
	77(*tor.* ♀ 1 × *jap.* ♂ 5) No. 11	338	272 (80.5%)	202 (59.8%)	146 (43.2%)		
77(*tor.* ♀ 1 × *jap.* ♂ 5) No. 3	77(*jap.* ♀ 3 × *jap.* ♂ 4) No. 6	300	295 (98.3%)	282 (94.0%)	209 (69.7%)		
	77(*tor.* ♀ 1 × *tor.* ♂ 2) No. 7	300	271 (90.3%)	207 (69.0%)	154 (51.3%)		
	77(*jap.* ♀ 3 × *tor.* ♂ 1) No. 7	300	276 (92.0%)	220 (73.3%)	134 (44.7%)		
	77(*tor.* ♀ 1 × *jap.* ♂ 5) No. 11	300	298 (99.3%)	285 (95.0%)	225 (75.0%)		
77(*tor.* ♀ 1 × *jap.* ♂ 5) No. 4	77(*jap.* ♀ 3 × *jap.* ♂ 4) No. 6	400	388 (97.0%)	348 (87.0%)	327 (81.8%)	298 (74.5%)	74.5
	77(*tor.* ♀ 1 × *tor.* ♂ 2) No. 7	350	330 (94.3%)	271 (77.4%)	237 (67.7%)	211 (60.3%)	63.9
	77(*jap.* ♀ 3 × *tor.* ♂ 1) No. 7	350	326 (93.1%)	272 (77.7%)	236 (67.4%)	205 (58.6%)	62.9
	77(*tor.* ♀ 1 × *jap.* ♂ 5) No. 11	350	341 (97.4%)	318 (90.9%)	241 (68.9%)	190 (54.3%)	55.7

PMC, Percentage of metamorphosed toads to normally cleaved eggs

Of the respective number of eggs of female No. 4 inseminated with sperm of male *japonicus* No. 6 and male *torrenticola* No. 7, 74.5% and 60.3% became normally metamorphosed toads, respectively. From the eggs of the same female which were inseminated with sperm of male hybrid No. 7 produced from *japonicus*♀ × *torrenticola*♂ and of male hybrid No. 11 from the reciprocal cross, 58.6% and 54.3% became normally metamorphosed toads, respectively.

These results indicated that the female hybrids between a female *japonicus* and a male *torrenticola* scarcely differed in reproductive capacity from the control female *japonicus* as well as the female hybrids produced from the reciprocal cross (Tables 35 and 36). It was also evident that the four kinds of males, *japonicus*, *torrenticola* and reciprocal hybrids between these two species, did not remarkably differ from one another in reproductive capacity.

TABLE 37

Developmental capacity of backcrosses and some other offspring of male hybrids between a female *Bufo bufo miyakonis* and a male *Bufo bufo japonicus* and the controls. All the second-generation offspring were produced in 1979 and 1980

Parents		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally metamorphosed toads	PMC
Female	Male						
miy. W, Nos. 4, 5	*miy.* W, No. 2	693	676 (97.5%)	625 (90.2%)	566 (81.7%)	514 (74.2%)	76.0
	78(*miy.* ♀1 × *jap.* ♂6) No. 1	506	478 (94.5%)	369 (72.9%)	281 (55.5%)	253 (50.0%)	52.9
	78(*miy.* ♀1 × *jap.* ♂6) No. 2	526	16 (3.0%)	4 (0.8%)	0	0	
	78(*miy.* ♀1 × *jap.* ♂6) No. 3	420	18 (4.3%)	14 (3.3%)	5 (1.2%)	0	
	78(*miy.* ♀1 × *jap.* ♂6) No. 4	534	526 (98.5%)	482 (90.3%)	461 (86.3%)	384 (71.9%)	73.0
	78(*miy.* ♀1 × *jap.* ♂6) No. 5	534	28 (5.2%)	17 (3.2%)	13 (2.4%)	0	
77(*jap.* ♀3 × *jap.* ♂4) Nos. 4, 5	77(*jap.* ♀3 × *jap.* ♂4) No. 6	430	368 (85.6%)	263 (61.2%)	237 (55.1%)	213 (49.5%)	57.9
	78(*miy.* ♀1 × *jap.* ♂6) No. 1	413	304 (73.6%)	252 (61.0%)	226 (54.7%)	201 (48.7%)	66.1
	78(*miy.* ♀1 × *jap.* ♂6) No. 2	438	3 (0.7%)	0	0	0	
	78(*miy.* ♀1 × *jap.* ♂6) No. 3	459	19 (4.1%)	10 (2.2%)	3 (0.7%)	0	
	78(*miy.* ♀1 × *jap.* ♂6) No. 4	463	385 (83.2%)	330 (71.3%)	305 (65.9%)	268 (57.9%)	69.6
	78(*miy.* ♀1 × *jap.* ♂6) No. 5	438	17 (3.9%)	5 (1.1%)	2 (0.5%)	0	
76(*jap.* ♀1 × *jap.* ♂1) No. 8	76(*jap.* ♀1 × *jap.* ♂1) No. 10	177	162 (91.5%)	145 (81.9%)	134 (75.7%)	113 (63.8%)	69.8
	78(*miy.* ♀1 × *jap.* ♂6) No. 7	152	151 (99.3%)	139 (91.4%)	136 (89.5%)	125 (82.3%)	82.8
	78(*miy.* ♀1 × *jap.* ♂6) No. 8	163	10 (6.1%)	8 (4.9%)	4 (2.5%)	3 (1.8%)	30.0
	78(*miy.* ♀1 × *jap.* ♂6) No. 9	106	99 (93.4%)	86 (81.1%)	83 (78.3%)		
	78(*miy.* ♀1 × *jap.* ♂6) No. 10	118	42 (35.6%)	37 (31.4%)	15 (12.7%)	6 (5.1%)	14.3
	78(*miy.* ♀1 × *jap.* ♂6) No. 11	116	100 (86.2%)	93 (80.2%)	90 (77.6%)		
gar. W, No. 1	*gar.* W, No. 1	349	343 (98.3%)	235 (67.3%)	162 (46.4%)		
	78(*miy.* ♀1 × *jap.* ♂6) No. 7	327	326 (99.7%)	260 (79.5%)	202 (61.8%)		
	78(*miy.* ♀1 × *jap.* ♂6) No. 8	392	7 (1.8%)	2 (0.5%)	0		
	78(*miy.* ♀1 × *jap.* ♂6) No. 9	335	258 (77.0%)	216 (64.5%)	170 (50.7%)		
	78(*miy.* ♀1 × *jap.* ♂6) No. 10	230	147 (63.9%)	98 (42.6%)	15 (6.5%)		
	78(*miy.* ♀1 × *jap.* ♂6) No. 11	242	242 (100 %)	199 (82.2%)	156 (64.5%)		
gar. W, No. 2	*gar.* W, No. 2	424	417 (98.3%)	378 (89.2%)	371 (87.5%)		
	78(*miy.* ♀1 × *jap.* ♂6) No. 7	427	426 (99.8%)	412 (96.5%)	394 (92.3%)		
	78(*miy.* ♀1 × *jap.* ♂6) No. 8	463	41 (8.9%)	27 (5.8%)	14 (3.0%)	7 (1.5%)	17.1
	78(*miy.* ♀1 × *jap.* ♂6) No. 9	531	498 (93.8%)	473 (89.1%)	310 (58.4%)		
	78(*miy.* ♀1 × *jap.* ♂6) No. 10	411	264 (64.2%)	122 (29.7%)	13 (3.2%)	4 (1.0%)	1.5
	78(*miy.* ♀1 × *jap.* ♂6) No. 11	523	507 (96.9%)	477 (91.2%)	419 (80.1%)		

PMC, Percentage of metamorphosed toads to normally cleaved eggs

5. Hybrids between a female *Bufo bufo miyakonis* and male *Bufo bufo japonicus*

Male hybrids produced in 1978 from a cross between female *Bufo bufo miyakonis* No. 1 and male *Bufo bufo japonicus* No. 6 matured sexually in the breeding season of 1979 (Table 23). Five of them (Nos. 1~5) were mated with two field-caught female *Bufo bufo miyakonis* (Nos. 4 and 5) and two female *Bufo bufo japonicus* (Nos. 4 and 5) obtained in 1977 from a mating between female *Bufo bufo japonicus* No. 3 and male *Bufo bufo japonicus* No. 4.

a. Backcrosses of male hybrids with field-caught female *Bufo bufo miyakonis*

i) Control

As a control male *Bufo bufo miyakonis*, a field-caught toad (No. 2) was used (Table 23). This male was mated with the two field-caught female *miyakonis* (Table 37). It was found that 97.5%, 90.2% and 81.7% of 693 eggs cleaved normally, hatched normally, and began to eat, respectively. Eventually, 74.2% became normally metamorphosed toads.

ii) Hybrids, *Bufo bufo miyakonis*♀ No. 1 × *Bufo bufo japonicus*♂ No. 6

The five male hybrids (Nos. 1~5) were sorted into two groups in reproductive capacity. One group consisting of males Nos. 2, 3 and 5 was extremely inferior in this respect, while the other group consisting of males Nos. 1 and 4 scarcely differed from the control (Table 37). Of 420~534 eggs of female *miyakonis* Nos. 4 and 5 only 3.0~5.2%, average of 4.2%, cleaved normally, 0.8~3.3%, average of 2.4%, hatched normally by inseminating with sperm of males Nos. 2, 3 and 5. Although there were 35 individuals at this stage, only 18 of them began to eat, while the others died of ill-development without taking food. All the tadpoles which were taking food also died of ill-development sooner or later. No tadpoles could attain the metamorphosing stage.

In contrast to these three mating series, 94.5% of 506 eggs and 98.5% of 534 eggs of the female *miyakonis* cleaved normally by inseminating with sperm of male hybrids Nos. 1 and 4, respectively. In the mating series of male No. 1, 72.9% hatched normally, 55.5% began to eat, and 50.0% became normally metamorphosed toads, while 90.3% hatched normally, 86.3% began to eat, and 71.9% became normally metamorphosed toads in the mating series of male No. 4.

b. Backcrosses of male hybrids with female *Bufo bufo japonicus*

i) Controls

A male (No. 6) obtained in 1977 from a mating between female *Bufo bufo japonicus* No. 3 and male *Bufo bufo japonicus* No. 4 (Table 20) was mated with the two female *japonicus* (Table 37). The results showed that 85.6% of 430 eggs cleaved normally by inseminating with sperm of the male. Afterwards, 61.2% hatched normally, 55.1% began to eat, and 49.5% became normally metamorphosed toads.

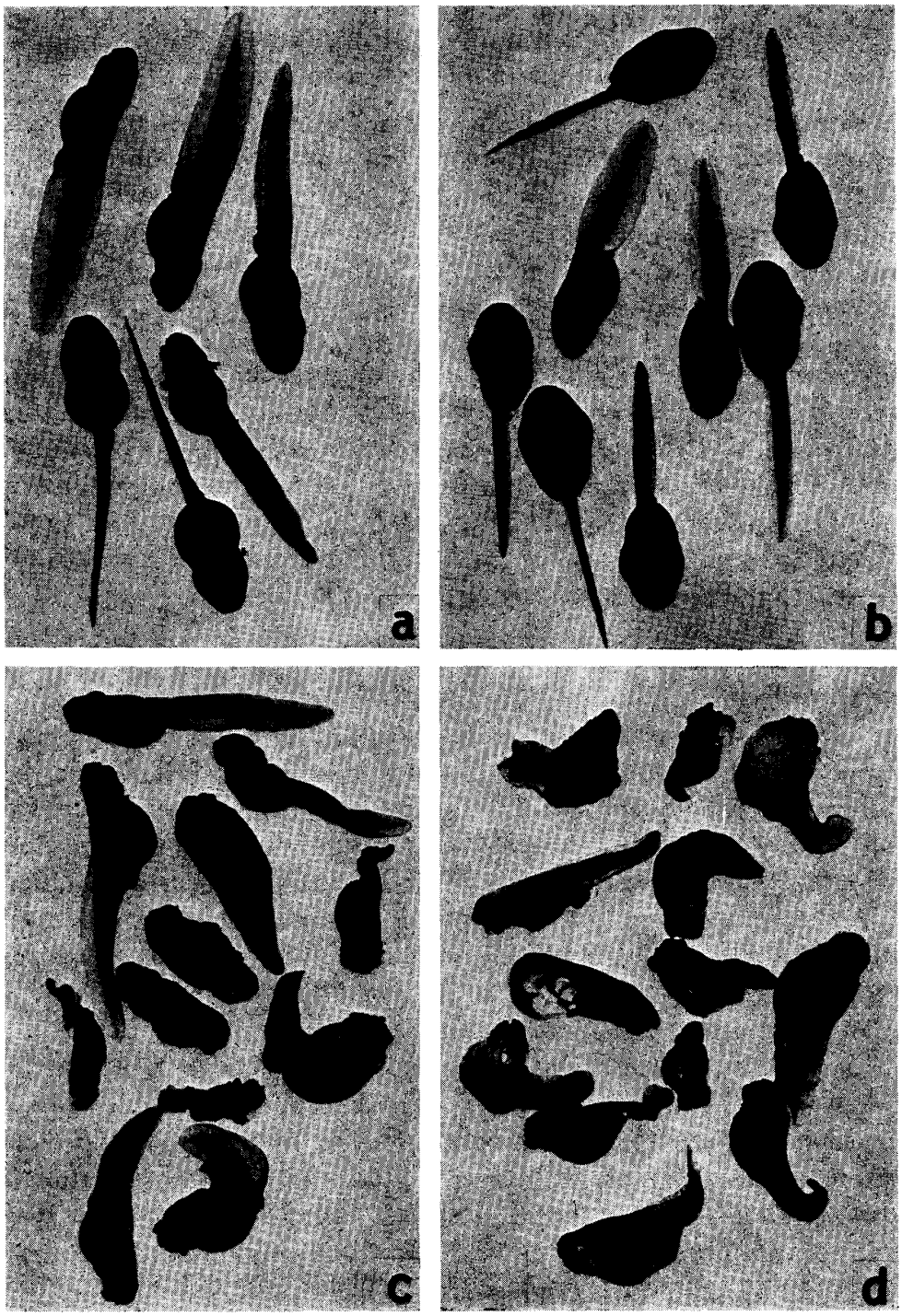

Fig. 21. Abnormal tadpoles in the backcrosses of male hybrids between a female *Bufo bufo miyakonis* and a male *Bufo bufo japonicus* mated with a female *Bufo bufo japonicus* and the controls.

a. Controls, (*japonicus* ♀ No. 3 × *japonicus* ♂ No. 4) ♀ No. 4 × (*japonicus* ♀ No. 3 × *japonicus* ♂ No. 4) ♂ No. 6

b. Controls, *miyakonis* ♀ No. 4 × *miyakonis* ♂ No. 2

c. Backcrosses, (*japonicus* ♀ No. 3 × *japonicus* ♂ No. 4) ♀ No. 4 × (*miyakonis* ♀ No. 1 × *japonicus* ♂ No. 6) ♂ No. 1

d. Backcrosses, (*japonicus* ♀ No. 1 × *japonicus* ♂ No. 1) ♀ No. 8 × (*miyakonis* ♀ No. 1 × *japonicus* ♂ No. 6) ♂ No. 10

ii) Hybrids, *Bufo bufo miyakonis*♀ No. 1 × *Bufo bufo japonicus*♂ No. 6

Nearly the same results as those in the matings of the male hybrids with female *miyakonis* were obtained in the mating with female *japonicus* (Table 37). Of 438~459 eggs of female *japonicus* Nos. 4 and 5, only 0.7~4.1% cleaved normally by inseminating with sperm of males Nos. 2, 3 and 5. After the normally cleaved eggs mostly died of various abnormalities, 0~2.2% hatched normally. Although five tadpoles in total began to eat after hatching, all of them died of ill-development without attaining the metamorphosing stage.

In contrast to these three male hybrids, the other two (Nos. 1 and 4) were nearly the same as the control male (No. 6) in reproductive capacity. By inseminating with sperm of male hybrid No. 1, 73.6%, 61.0%, 54.7% and 48.7% of 413 eggs cleaved normally, hatched normally, began to eat and became normally metamorphosed toads, respectively. On the other hand, 83.2%, 71.3%, 65.9% and 57.9% of 463 eggs cleaved normally, hatched normally, began to eat and became normally metamorphosed toads, respectively, by inseminating with sperm of male hybrid No. 4.

In 1980, five two-year-old male hybrids (Nos. 7~11) produced in 1978 from a cross, *Bufo bufo miyakonis*♀ No. 1 × *Bufo bufo japonicus*♂ No. 6, were backcrossed with a female *Bufo bufo japonicus* (No. 8) which had been produced in 1976 from a mating, *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo japonicus*♂ No. 1. The male hybrids were 78.0~92.0 mm, average of 82.8 mm, in size. Their right testes were 11.5~20.5 mm, average of 16.0 mm, in length and 3.0~3.5 mm, average of 3.4 mm, in width. The results of backcrossing showed that 86.2~99.3% of

TABLE 38

Developmental capacity of backcrosses of female hybrids between a female *Bufo bufo miyakonis* and a male *Bufo bufo japonicus*. All the backcrosses were produced in 1980

Parents		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally metamorphosed toads	PMC
Female	Male						
78(*miy.* ♀ 1 × *jap.* ♂ 6) No. 1	79(*miy.* ♀ 2 × *miy.* ♂ 1) No. 1	414	348 (84.1%)	222 (53.6%)	174 (42.0%)	146 (35.3%)	42.0
	77(*jap.* ♀ 3 × *jap.* ♂ 4) No. 7	403	332 (82.4%)	160 (39.7%)	133 (33.0%)	118 (29.3%)	35.5
78(*miy.* ♀ 1 × *jap.* ♂ 6) No. 2	79(*miy.* ♀ 2 × *miy.* ♂ 1) No. 1	446	433 (97.1%)	359 (80.5%)	344 (77.1%)	292 (65.5%)	67.4
	77(*jap.* ♀ 3 × *jap.* ♂ 4) No. 7	406	397 (97.8%)	306 (75.4%)	252 (62.1%)	237 (58.4%)	59.7
78(*miy.* ♀ 1 × *jap.* ♂ 6) No. 3	79(*miy.* ♀ 2 × *miy.* ♂ 1) No. 1	432	423 (97.9%)	342 (79.2%)	306 (70.8%)	268 (62.0%)	63.4
	77(*jap.* ♀ 3 × *jap.* ♂ 4) No. 7	417	410 (98.3%)	337 (80.8%)	279 (66.9%)	237 (56.8%)	57.8
78(*miy.* ♀ 1 × *jap.* ♂ 6) No. 4	79(*miy.* ♀ 2 × *miy.* ♂ 1) No. 1	420	378 (90.0%)	296 (70.5%)	246 (58.6%)	229 (54.5%)	60.6
	77(*jap.* ♀ 3 × *jap.* ♂ 4) No. 7	421	373 (88.6%)	284 (67.5%)	246 (58.4%)	214 (50.8%)	57.4
78(*miy.* ♀ 1 × *jap.* ♂ 6) No. 5	79(*miy.* ♀ 2 × *miy.* ♂ 1) No. 1	414	410 (99.0%)	397 (95.9%)	321 (77.5%)	283 (68.4%)	69.0
	77(*jap.* ♀ 3 × *jap.* ♂ 4) No. 7	412	406 (98.5%)	353 (85.7%)	275 (66.7%)	235 (57.0%)	57.9

PMC, Percentage of metamorphosed toads to normally cleaved eggs

106～152 eggs cleaved normally by inseminating with sperm of male hybrids Nos. 7, 9 and 11, while 6.1% of 163 eggs and 35.6% of 118 eggs did so by inseminating with sperm of male hybrids Nos. 8 and 10, respectively (Table 37). In the former group of backcross series, 80.2～91.4% hatched normally and 77.6～89.5% began to eat. Of the tadpoles in the series of male hybrid No. 7, 82.3% completed metamorphosis normally, while the tadpoles in the series of male hybrids Nos. 9 and 11 were preserved before metamorphosis. In the two backcross series of the latter group, 4.9% and 31.4% hatched normally, 2.5% and 12.7% began to eat, and 1.8% and 5.1% became normally metamorphosed toads. About half of the normally hatched embryos became abnormal tadpoles without taking food and shortly died (Fig. 21d).

When 177 control eggs of the female *japonicus* (No. 8) were inseminated with sperm of a male *japonicus* (No. 10) obtained in 1976 from a mating, *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo japonicus*♂ No. 1, 91.5%, 81.9%, 75.7% and 63.8% cleaved normally, hatched normally, began to eat and became normally metamorphosed toads, respectively.

c. Mating of male hybrids with field-caught female *Bufo bufo gargarizans*

In 1980, the five male hybrids (Nos. 7～11) used in backcrossing with a female *japonicus* were mated with two field-caught female *gargarizans* (Nos. 1 and 2) in order to compare the latter subspecies with *japonicus* and *miyakonis*.

It was found that male hybrids Nos. 8 and 10 were remarkably inferior in reproductive capacity to the other three (Nos. 7, 9 and 11), as found in back-

Bufo bufo japonicus♂ No. 4, while the other group was with sperm of a male *Bufo bufo miyakonis* obtained in 1979 from a mating, *Bufo bufo miyakonis*♀ No. 2 × *Bufo bufo miyakonis*♂ No. 1 (Table 23). It was found that 84.1～99.0%, average of 93.6%, of 414～446 eggs and 82.4～98.5%, average of 93.1%, of 403～421 eggs cleaved normally by inseminating with sperm of the male *miyakonis* and *japonicus*, respectively (Table 38). While one (No. 1) of the five female hybrids was somewhat inferior in reproductive capacity, the other were nearly normal in this respect. Of the eggs of female hybrid No. 1, 53.6% and 39.7% hatched normally, 42.0% and 33.0% began to eat and 35.3% and 29.3% became normally metamorphosed toads by inseminating with sperm of the male *miyakonis* and *japonicus*, respectively. There were many tadpoles which became abnormal without taking food after they hatched normally (Fig. 22a, b). In contrast, 70.5～95.9%, average of 81.5%, of the respective number of eggs of the other four female hybrids hatched normally, 58.6～77.5%, average of 71.0%, began to eat and 54.5～68.4%, average of 62.6%, became normally metamorphosed toads by inseminating with sperm of the male *miyakonis*, while 67.5～85.7%, average of 77.4%, hatched normally, 58.4～66.9%, avergae of 63.5%, began to eat and 50.8～58.4%, avergae of 55.8%, became normally metamorphosed toads by inseminating with sperm of the male *japonicus*.

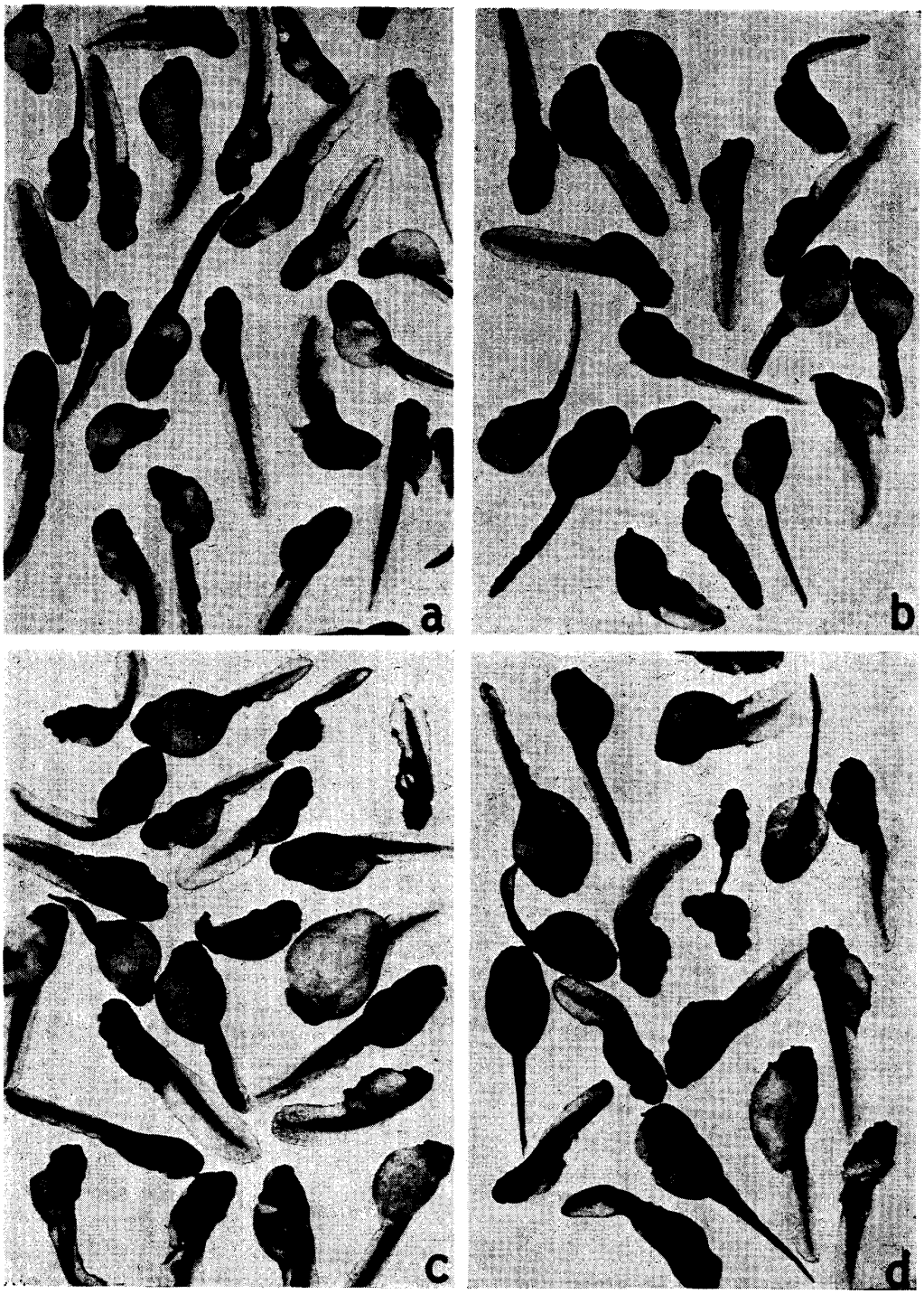

Fig. 22. Abnormal tadpoles in the backcrosses of female hybrids between a female *Bufo bufo miyakonis* and a male *Bufo bufo japonicus* or *Bufo torrenticola*. ×2.5

a. Backcrosses, (*miyakonis* ♀ No. 1 × *japonicus* ♂ No. 6) ♀ No. 1 × (*japonicus* ♀ No. 3 × *japonicus* ♂ No. 4) ♂ No. 7

b. Backcrosses, (*miyakonis* ♀ No. 1 × *japonicus* ♂ No. 6) ♀ No. 1 × (*miyakonis* ♀ No. 2 × *miyakonis* ♂ No. 1) ♂ No. 1

c. Backcrosses, (*miyakonis* ♀ No. 1 × *torrenticola* ♂ No. 3) ♀ No. 4 × *torrenticola* W, ♂ No. 5

d. Backcrosses, (*miyakonis* ♀ No. 1 × *torrenticola* ♂ No. 3) ♀ No. 4 × (*miyakonis* ♀ No. 2 × *miyakonis* ♂ No. 1) ♂ No. 1

Bufo bufo japonicus♂ No. 4, while the other group was with sperm of a male *Bufo bufo miyakonis* obtained in 1979 from a mating, *Bufo bufo miyakonis*♀ No. 2 × *Bufo bufo miyakonis*♂ No. 1 (Table 23). It was found that 84.1~99.0%, average of 93.6%, of 414~446 eggs and 82.4~98.5%, average of 93.1%, of 403~421 eggs cleaved normally by inseminating with sperm of the male *miyakonis* and *japonicus*, respectively (Table 38). While one (No. 1) of the five female hybrids was somewhat inferior in reproductive capacity, the other were nearly normal in this respect. Of the eggs of female hybrid No. 1, 53.6% and 39.7% hatched normally, 42.0% and 33.0% began to eat and 35.3% and 29.3% became normally metamorphosed toads by inseminating with sperm of the male *miyakonis* and *japonicus*, respectively. There were many tadpoles which became abnormal without taking food after they hatched normally (Fig. 22a, b). In contrast, 70.5~95.9%, average of 81.5%, of the respective number of eggs of the other four female hybrids hatched normally, 58.6~77.5%, average of 71.0%, began to eat and 54.5~68.4%, average of 62.6%, became normally metamorphosed toads by inseminating with sperm of the male *miyakonis*, while 67.5~85.7%, average of 77.4%, hatched normally, 58.4~66.9%, avergae of 63.5%, began to eat and 50.8~58.4%, avergae of 55.8%, became normally metamorphosed toads by inseminating with sperm of the male *japonicus*.

e. Chromosomes of the offspring of male hybrids, *Bufo bufo miyakonis*♀ No. 1 × *Bufo bufo japonicus*♂ No. 6

TABLE 39

Chromosomes of backcrosses and some other offspring of male hybrids between a female *Bufo bufo miyakonis* and a male *Bufo bufo japonicus* or *Bufo torrenticola*

All the second-generation offspring were produced in 1980

Parents		Number of tadpoles											
			Number of chromosomes										
Female	Male	Analyzed	22 (2n)	23~27 (2n+)	28~32 (3n−)	33 (3n)	34~38 (3n+)	39~43 (4n−)	44 (4n)	45~49 (4n+)	50~54 (5n−)	56~60 (5n+)	66 (6n)
76(*jap.*♀1 × *jap.*♂1) No. 8	76(*jap.*♀1 × *jap.*♂1) No. 10	60				60							
76(*jap.*♀1 × *jap.*♂1) No. 8	78(*miy.*♀1 × *jap.*♂6) No. 7	20				20							
	78(*miy.*♀1 × *jap.*♂6) No. 8	4				3	1						
	78(*miy.*♀1 × *jap.*♂6) No. 10	15					8	2		2	1	1	1
gar. W, Nos. 1 and 2	*gar.* W, Nos. 1 and 2	40	39			1							
gar. W, Nos. 1 and 2	78(*miy.*♀1 × *jap.*♂6) No. 7	20	20										
	78(*miy.*♀1 × *jap.*♂6) No. 8	12	6	2	4								
	78(*miy.*♀1 × *jap.*♂6) No. 10	25		3	5	2	9	6					
76(*jap.*♀1 × *jap.*♂1) No. 8	78(*miy.*♀1 × *tor.*♂3) No. 8	20				20							
	78(*miy.*♀1 × *tor.*♂3) No. 11	8			1	4	2		1				
gar. W, Nos. 1 and 2	78(*miy.*♀1 × *tor.*♂3) No. 11	17	9	2	2	1	3						

As there were two kinds of male hybrids which extremely differed from each other in reproductive capacity, the chromosomes of three male hybrids (Nos. 7, 8 and 10) and the control male *japonicus* (No. 10) as well as those of the offspring at the feeding tadpole stage produced from these males by mating with a female *japonicus* (No. 8) and two female *gargarizans* (Nos. 1 and 2) were observed in order to elucidate the cause of such a difference (Table 39).

Of the feeding tadpoles produced from the control mating between female *japonicus* No. 8 and male *japonicus* No. 10, 60 were examined for chromosome number. It was found that all these tadpoles were triploids. As the male *japonicus* was confirmed to be a diploid by examining the chromosomes of bone marrow cells, female *japonicus* No. 8 was assumed to be a tetraploid which had been produced in the laboratory. In contrast with this female, two females (Nos. 1 and 2) and two males (Nos. 1 and 2) of *gargarizans* used in producing

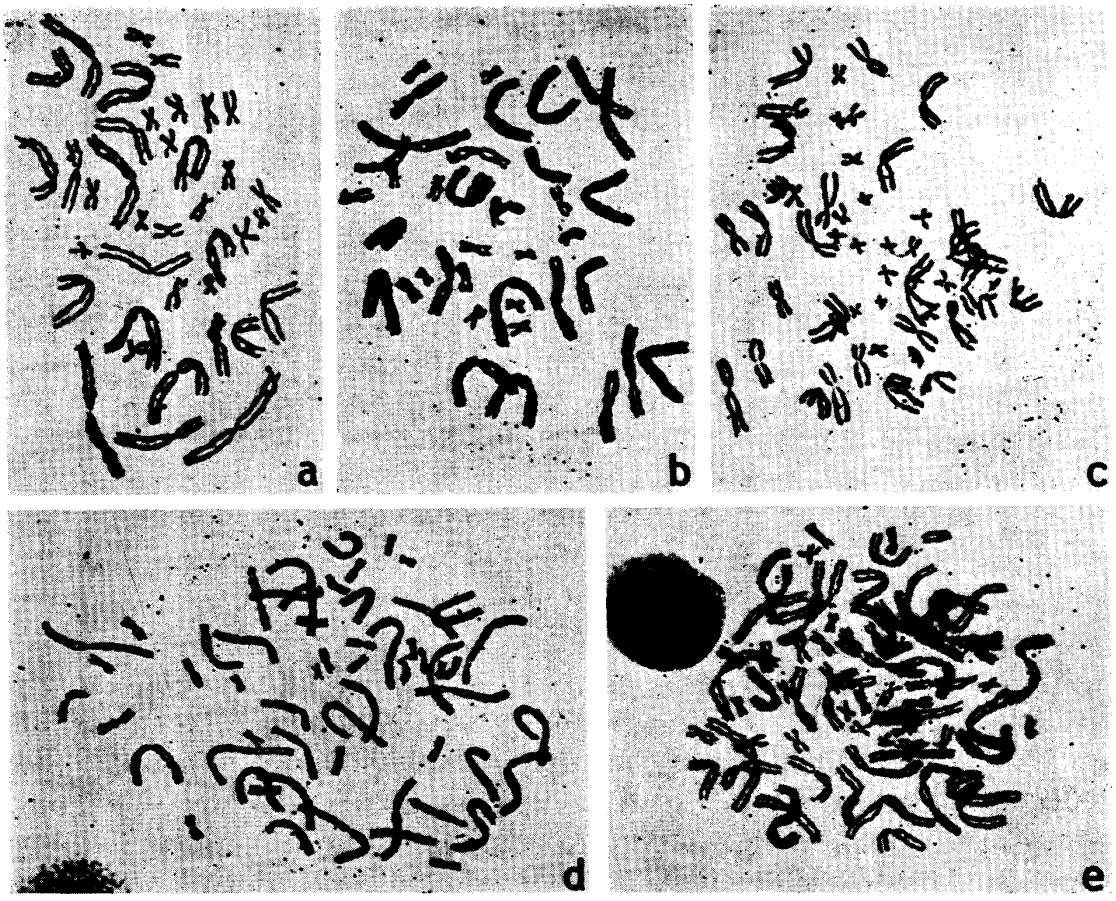

Fig. 23. Chromosomes of feeding tadpoles in the backcrosses of male hybrid No. 10 between female *Bufo bufo miyakonis* No. 1 and male *Bufo bufo japonicus* No. 6 mated with female No. 8 between female *Bufo bufo japonicus* No. 1 and male *Bufo bufo japonicus* No. 1.

a. Hypertriploid (3n+5) metaphase spread ×1000
b. Hypotetraploid (4n−4)metaphase spread ×1000
c. Hypopentaploid (5n−5) metaphase spread ×800
d. Hyperpentaploid (5n+2) metaphase spread ×800
e. Hexaploid metaphase spread ×800

the controls were diploids, since 39 of 40 controls were diploids and the remaining one was a triploid.

The chromosomes of the three male hybrids (Nos. 7, 8 and 10) used in producing backcrosses were observed in bone-marrow cells. It was found that male hybrid No. 7 was a diploid, while the other two (Nos. 8 and 10) were triploids. When male hybrid No. 7 was backcrossed with female *japonicus* No. 8, 20 tadpoles examined were all triploids, while 20 tadpoles produced from the same male by mating with female *gargarizans* No. 1 or 2 were all diploids. When male hybrids Nos. 8 and 10 were backcrossed with female *japonicus* No. 8, 19 offspring were triploids, hypertriploids, hyper- or hypoteraploids, hyper- or hypopentaploids, or hexaploid (Fig. 23). When these two male hybrids were mated with female *gargarizans* Nos. 1 and 2, 37 offspring were diploids, hyperdiploids, triploids, hyper- or hypotriploids, or hypotetraploids.

6. Hybrids between a female *Bufo bufo miyakonis* and a male *Bufo torrenticola*

a. Backcrosses of male hybrids with female *Bufo bufo miyakonis*

Male hybrids produced in 1978 from a cross between female *Bufo bufo miyakonis* No. 1 and male *Bufo torrenticola* No. 3 matured sexually in the breeding season of 1979 (Table 23). Five of them (Nos. 1～5) were mated with field-caught female *Bufo bufo miyakonis* Nos. 4 and 5 (Table 40). It was found that three male hybrids Nos. 1～3 scarcely differed in reproductive capacity from field-caught male *miyakonis* No. 2 (Table 37). Of 476～658 eggs, 96.2～99.8%, average of 98.1%, cleaved normally, 90.0～93.0%, average of 91.3%, hatched normally, and 83.6～89.5%, average of 87.5%, became feeding tadpoles. There were a small number of embryos which became abnormal tadpoles without taking food after hatching. While tadpoles raised from the eggs inseminated with sperm of male hybrid No. 3 were preserved before metamorphosis, those derived from sperm of male hybrids Nos. 1 and 2 were continuously reared. In these two series, 71.6% and 66.1% of the respective number of eggs became normally metamorphosed toads.

In the backcross series of male hybrid No. 4, 67.5% of 529 eggs cleaved normally. This percentage was comparatively low as compared with that in the control. However, the normally cleaved eggs did not distinctly differ in developmental ability from those of the above three backcross series; 59.5% hatched normally and 57.8% became feeding tadpoles. All the tadpoles were preserved at this stage. In contrast to male hybrids Nos. 1～4, male hybrid No. 5 was nearly sterile; only nine (1.3%) of 716 eggs cleaved normally by inseminating with sperm of this male. All these normally cleaved eggs died of edema, ill-development or some other abnormalities during the embryonic and tadpole stages.

b. Mating of male hybrids with a female *Bufo bufo japonicus*

In 1980, five male hybrids (Nos. 8～12) produced in 1978 from the same

TABLE 40

Developmental capacity of backcrosses and some other offspring of male hybrids between a female *Bufo bufo miyakonis* and a male *Bufo torrenticola* and the controls, and sterility of male hybrids between the female *Bufo bufo miyakonis* and a male *Bufo viridis* or *Bufo bufo bufo* from Portugal. All the second-generation offspring were produced in 1979 and 1980

Parents		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally meta-morphosed toads	PMC
Female	Male						
miy. W, Nos. 4, 5	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 1	476	458 (96.2%)	433 (91.0%)	425 (89.3%)	341 (71.6%)	74.5
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 2	658	646 (98.2%)	592 (90.0%)	550 (83.6%)	435 (66.1%)	67.3
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 3	560	559 (99.8%)	521 (93.0%)	501 (89.5%)		
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 4	529	357 (67.5%)	315 (59.5%)	306 (57.8%)		
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 5	716	9 (1.3%)	5 (0.7%)	3 (0.4%)	0	
76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 8	76(*jap.* ♀ 1 × *jap.* ♂ 1) No. 10	177	162 (91.5%)	145 (81.9%)	134 (75.7%)	113 (63.8%)	69.8
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 8	152	148 (97.4%)	69 (45.4%)	61 (40.1%)	52 (34.2%)	35.1
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 9	163	160 (98.2%)	80 (49.1%)	79 (48.5%)		
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 10	41	40 (97.6%)	40 (97.6%)	33 (80.5%)		
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 11	105	11 (10.5%)	11 (10.5%)	9 (8.6%)	4 (3.8%)	36.4
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 12	82	73 (89.0%)	68 (82.9%)	68 (82.9%)		
gar. W, No. 1	*gar.* W, No. 1	349	343 (98.3%)	235 (67.3%)	162 (46.4%)		
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 8	320	311 (97.2%)	250 (78.1%)	168 (52.5%)		
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 9	495	484 (97.8%)	373 (75.4%)	267 (53.9%)		
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 10	383	362 (94.5%)	274 (71.5%)	221 (57.7%)		
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 11	318	59 (18.6%)	12 (3.8%)	7 (2.2%)	1 (0.3%)	1.7
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 12	299	288 (96.3%)	186 (62.2%)	126 (42.1%)		
gar. W, No. 2	*gar.* W, No. 2	424	417 (98.3%)	378 (89.2%)	371 (87.5%)		
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 8	375	372 (99.2%)	332 (88.5%)	312 (83.2%)		
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 9	386	383 (99.2%)	381 (98.7%)	352 (91.2%)		
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 10	378	369 (97.6%)	339 (89.7%)	321 (84.9%)		
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 11	442	52 (11.8%)	36 (8.1%)	12 (2.7%)		
	78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 12	520	520 (100 %)	504 (96.9%)	445 (85.6%)		
miy. W, Nos. 4, 5	78(*miy.* ♀ 1 × *vir.* ♂ 1) Nos. 1～5	3719	0	0	0		
	78(*miy.* ♀ 1 × *bufo* P. ♂ 5) Nos. 1～3	1830	0	0	0		

PMC, Percentage of metamorphosed toads to normally cleaved eggs

cross as the above were mated with a female *Bufo bufo japonicus* (No. 8) obtained in 1976 from a mating between *Bufo bufo japonicus*♀ No. 1 × *Bufo bufo japonicus*♂ No. 1 in order to compare this subspecies with *Bufo bufo miyakonis* and *Bufo tor-*

renticola and to test the reproductive capacity of these male hybrids.

The results showed that there were two kinds of male hybrids in reproductive capacity, as found in the above backcrosses. Of 105 eggs of the female *japonicus* (No. 8), 10.5% cleaved and hatched normally, 8.6% began to eat and 3.8% became normally metamorphosed toads by inseminating with sperm of male hybrid No. 11, while of 41~163 eggs, 89.0~98.2%, average of 95.6%, cleaved normally, 45.4~97.6%, average of 68.8%, hatched normally and 40.1~82.9%, average of 63.0%, became feeding tadpoles by inseminating with sperm of male hybrids Nos. 8, 9, 10 and 12. While tadpoles raised from eggs inseminated with sperm of male hybrids Nos. 9, 10 and 12 were preserved before metamorphosis, those derived from sperm of male hybrid No. 8 were continuously reared. In this series, 34.2% became normally metamorphosed toads. Of 177 control eggs inseminated with sperm of a male *Bufo bufo japonicus* obtained from a mating, *Bufo bufo japonicus*♀ No. 1×*Bufo bufo japonicus*♂ No. 1, 91.5%, 81.9%, 75.7% and 63.8% cleaved normally, hatched normally, began to eat and became normally metamorphosed toads, respectively.

c. Matings of male hybrids with field-caught female *Bufo bufo gargarizans*

In 1980, the same five male hybrids (Nos. 8~12) as used in the above matings were mated with two field-caught female *gargarizans* (Nos. 1 and 2) in order to compare the latter subspecies with *miyakonis* and *torrenticola* and to test the reproductive capacity of these male hybrids (Table 40).

The results were very similar to those of the matings between the male hybrids and the female *japonicus*. Of 318 eggs of *gargarizans* No. 1, only 18.6%, 3.8% and 2.2% cleaved normally, hatched normally and became feeding tadpoles, respectively, by inseminating with sperm of male hybrid No. 11 (Table 40). In contrast, 94.5~97.8%, average of 96.5%, 62.2~78.1%, average of 71.8%, and 42.1~57.7%, average of 51.6%, of 299~495 eggs of *gargarizans* No. 1 cleaved normally, hatched normally and became feeding tadpoles, respectively, by inseminating with sperm of male hybrids Nos. 8, 9, 10 and 12. Similar results were obtained from matings between the male hybrids and female *gargarizans* No. 2. Of 442 eggs of this female, 11.8%, 8.1% and 2.7% cleaved normally, hatched normally and became feeding tadpoles, respectively, by inseminating with sperm of male hybrid No. 11, while 97.6~100.0%, average of 99.0%, 88.5~98.7%, average of 93.5%, and 83.2~91.2%, average of 86.2%, of 375~520 eggs did so, respectively, by inseminating with sperm of the four male hybrids.

Of 773 control eggs inseminated with sperm of field-caught male *Bufo bufo gargarizans* Nos. 1 and 2, 98.3% cleaved normally, 79.3% hatched normally and 69.0% became feeding tadpoles.

d. Backcrosses of female hybrids with males of the parental species

Five female hybrids (Nos. 1~5) produced in 1978 from a mating between *Bufo bufo miyakonis*♀ No. 1 and *Bufo torrenticola*♂ No. 3 matured in the season of 1980. They were 84.0~93.5 mm, average of 89.8 mm, in body length (Table

24). Four of them laid 2116~4132 eggs, average of 3082 eggs, after pituitary injection, while the remaining one (No. 5) which was the smallest in body length laid no eggs. The eggs laid by each of four females, about 360~420 in number, were divided into two groups; one group was inseminated with sperm of a male *Bufo torrenticola* collected from the field, and the other group was with sperm of a male *Bufo bufo miyakonis* produced in 1979 from a mating, *Bufo bufo miyakonis*♀ No. 2 × *Bufo bufo miyakonis*♂ No. 1 (Table 41). The results showed that 97.8~99.2%, average of 98.8%, of 360~421 eggs cleaved normally by inseminating with sperm of the male *torrenticola*, while 88.9~94.9%, average of 93.1%, did so by inseminating with sperm of the male *miyakonis*. Although 88.5~98.6%, average of 93.5%, and 84.3~90.0%, average of 86.6%, hatched normally by inseminating with sperm of the male *torrenticola* and *miyakonis*, respectively, most of them died of edema or ill-development shortly after hatching. (Fig. 22c, d) Eventually, only 7.8~15.6%, average of 12.6%, and 10.4~17.9%, average of 14.4%, of the respective number of eggs became feeding tadpoles, and 1.9~3.8%, average of 2.7%, and 1.6~4.7%, average of 3.1%, attained the completion of metamorphosis, respectively.

TABLE 41

Developmental capacity of backcrosses of female hybrids between a female *Bufo bufo miyakonis* and a male *Bufo torrenticola*. All the backcrosses were produced in 1980

Parents		No. of eggs	No. of normal cleavages	No. of normally hatched embryos	No. of normally feeding tadpoles	No. of normally metamorphosed toads	PMC
Female	Male						
78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 1	*tor.* W, No. 5	412	408 (99.0%)	376 (91.3%)	32 (7.8%)	8 (1.9%)	2.0
	79(*miy.* ♀ 2 × *miy.* ♂ 1) No. 1	385	361 (93.8%)	332 (86.2%)	62 (16.1%)	12 (3.1%)	3.3
78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 2	*tor.* W, No. 5	392	389 (99.2%)	347 (88.5%)	61 (15.6%)	15 (3.8%)	3.9
	79(*miy.* ♀ 2 × *miy.* ♂ 1) No. 1	422	400 (94.8%)	380 (90.0%)	44 (10.4%)	20 (4.7%)	5.0
78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 3	*tor.* W, No. 5	421	417 (99.0%)	415 (98.6%)	53 (12.6%)	8 (1.9%)	1.9
	79(*miy.* ♀ 2 × *miy.* ♂ 1) No. 1	369	350 (94.9%)	311 (84.3%)	66 (17.9%)	6 (1.6%)	1.7
78(*miy.* ♀ 1 × *tor.* ♂ 3) No. 4	*tor.* W, No. 5	360	352 (97.8%)	344 (95.6%)	51 (14.2%)	12 (3.3%)	3.4
	79(*miy.* ♀ 2 × *miy.* ♂ 1) No. 1	377	335 (88.9%)	323 (85.7%)	50 (13.3%)	11 (2.9%)	3.3

PMC, Percentage of metamorphosed toads to normally cleaved eggs

e. Chromosomes of the offspring of male hybrids, *Bufo bufo miyakonis*♀ No. 1 × *Bufo torrenticola*♂ No. 3

Of five male hybrids (Nos. 8~12) obtained from a cross between a female *Bufo bufo miyakonis* (No. 1) and a male *Bufo torrenticola* (No. 3), one (No. 11) was extremely inferior to the others in reproductive capacity. In order to elucidate the cause of this inferiority, the chromosomes of these male hybrids and the offspring produced from two (Nos. 8 and 11) of them by mating with female *Bufo bufo japonicus* No. 8 and *Bufo bufo gargarizans* Nos. 1 and 2 were observed.

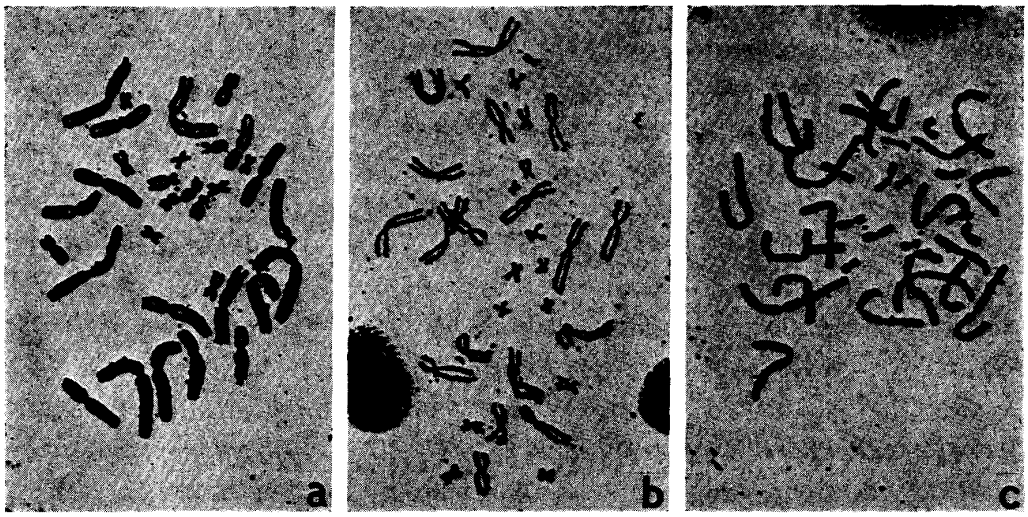

Fig. 24. Chromosomes of feeding tadpoles in the backcrosses of male hybrid No. 11 between female *Bufo bufo miyakonis* No. 1 and male *Bufo torrenticola* No. 3 mated with female No. 8 between *Bufo bufo japonicus* No. 1 and male *Bufo bufo japonicus* No. 1.

a. Hypotriploid (3n−1) metaphase spread ×1000
b. Triploid metaphase spread ×800
c. Hypertriploid (3n+2) metaphase spread ×1000

It was already stated that the female *japonicus* was probably a tetraploid and the two *gargarizans* were diploids (cf. pp. 104~106).

Male hybrids Nos. 8, 9, 10 and 12 were diploids, and 20 tadpoles produced from male hybrid No. 8 by mating with female *japonicus* No. 8 were all triploids. In contrast, male hybrid No. 11 was a triploid. Of eight offspring at the feeding tadpole stage obtained from this male by mating with female *japonicus* No. 8, four were triploids, two others hypertriploids, still another a hypotriploid and the remaining one a tetraploid (Fig. 24). Of 17 tadpoles produced from the same male by mating with the two female *gargarizans*, nine were diploids, two hyperdiploids, one a triploid, two hypotriploids and three hypertriploids (Table 39).

7. Hybrids between a female *Bufo bufo miyakonis* and a male *Bufo viridis*

As male hybrids produced in 1978 from a cross, *Bufo bufo miyakonis*♀ No. 1 ×*Bufo viridis*♂ No. 1, matured sexually in the breeding season of 1979 (Table 25), five of them (Nos. 1~5) were mated with two field-caught female *miyakonis* Nos. 4 and 5. However, none of 3719 eggs in total cleaved normally or even abnormally by the routine method of artificial insemination (Table 40).

8. Hybrids between a female *Bufo bufo miyakonis* and a male *Bufo bufo bufo*

Three male hybrids produced in 1978 from a cross, *Bufo bufo miyakonis*♀ No. 1 ×*Bufo bufo bufo*♂ No. 5, matured sexually in the breeding season of 1979 (Table 25). These male hybrids were mated with the same two female *miyakonis* as

those used in the above experiments. It was found that none of 1830 eggs in total cleaved normally or abnormally by artificial insemination (Table 40).

DISCUSSION

1. *Bufo bufo japonicus*

STEJNEGER (1907) described three species, *Bufo bufo japonicus, Bufo formosus* and *Bufo smithi,* in the toads distributed in the main island of Japan and OKADA (1966) recognized two plain-living subspecies, *Bufo bufo japonicus* and *Bufo bufo formosus,* and one mountainous subspecies, *Bufo bufo montanus.* NAKAMURA and UENO (1963) united these three subspecies into *Bufo bufo japonicus,* as they are continuous in morphological characteristics and, moreover, their distribution range overlaps with one another in many districts. MATSUI (1976 a) added a new mountainous species, *Bufo torrenticola,* to the three subspecies which had been classified by OKADA. In any case, it has been generally accepted by Japanese taxonomists that all the Japanese toads excepting *Bufo torrenticola* belong to the same species as the European *Bufo bufo.*

The present authors had concurred with NAKAMURA and UENO in uniting the two subspecies, *Bufo bufo japonicus* and *Bufo bufo formosus,* into one taxon and tentatively giving them the subspecific name *Bufo bufo japonicus.* But, the real taxonomic position of *Bufo bufo japonicus* was reexamined on the basis of the results of hybridization experiments.

CEI (1975) has confirmed by his serological tests that European specimens of *Bufo bufo* lie at a stronger serological distance from *Bufo bufo japonicus,* stressing a true interspecific relationship. MATSUI (1976 b) crossed a female *Bufo bufo japonicus* with a male *Bufo bufo spinosus* from southern France by natural hybridization. Juvenile hybrid toads shortly after metamorphosis were released in an institute garden and recaptured two to four months later. A male had unripe testes containing a few abnormal spermatogonia, while the ovaries of a female hybrid seemed to be degenerative. He has stated that *Bufo bufo japonicus* may be separated as a full species from *Bufo bufo spinosus.* A preliminary report has been made by NISHIOKA and UEDA (1978) on the reproductive isolating mechanisms as well as the biochemical differences between Japanese, European and American toads.

In the present study, the authors clarified that the Japanese toads were barely or considerably isolated from European toads by hybrid inviability, while they were almost completely isolated from the latter by hybrid sterility. Reciprocal hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo* were not inferior to the controls in viability. While there was nearly an equal number of males and females in the hybrids, *Bufo bufo japonicus* ♀ × *Bufo bufo bufo* ♂, about two-thirds of the reciprocal hybrids were males. Male hybrids produced from reciprocal crosses were almost completely sterile, while female hybrids were barely fertile and produced only a few mature triploid offspring raised from larger eggs. W. F.

BLAIR (1972) has listed numerous kinds of interspecific hybrids, in which the females produced triploid offspring alone. On the basis of hybrid sterility, *Bufo bufo japonicus* should be given a position of species, as CEI suggested from his serological tests. As the name *Bufo bufo japonicus* was changed from *Bufo vulgaris japonicus* SCHLEGEL 1838 by STEJNEGER (1907) and this is the oldest name given to a subspecies of *Bufo bufo* distributed in the East, it seems proper to change the name *Bufo bufo japonicus* SCHLEGEL into *Bufo japonicus japonicus* SCHLEGEL.

According to MERTENS and WERMUTH (1960), there are four subspecies of *Bufo bufo, B. b. bufo, B. b. gredosicola, B. b. spinosus* and *B. b. verrucosissimus*, in Europe. Although it is undetermined whether or not the male French and Greek toads used in the present study belong to *Bufo bufo bufo*, they behaved themselves fairly like *Bufo bufo bufo* from Portugal in postmating isolating mechanisms against female *Bufo bufo japonicus*. The hybrids, *Bufo bufo japonicus*♀ × *Bufo bufo* from France or Greece♂, were somewhat or slightly inferior to the controls in viability. While there was nearly an equal number of males and females in the hybrids produced from a Greek male, the hybrids produced from a French male gave a ratio of one female to nine males. These two kinds of hybrids were very similar to the hybrids produced from the male *Bufo bufo bufo* from Portugal in reproductive capacity. They were almost completely sterile except that female hybrids produced a few triploid backcrosses which metamorphosed normally and attained sexual maturity.

The hybrids between female *Bufo bufo japonicus* and a male *Bufo viridis* were slightly inferior to the controls in viability, while the reciprocal hybrids were remarkably inferior in this respect. The hybrids, *Bufo bufo japonicus*♀ × *Bufo viridis*♂, were all males which were completely sterile. This strange shift of sex may be explained by the assumption that the male was YY in sex-chromosome constitution, since all the offspring of this male mated with a female *Bufo bufo miyakonis* or *Bufo viridis* were also males. As no female hybrids were obtained from the cross, *Bufo bufo japonicus*♀ × *Bufo viridis*♂, it is unknown if they are the same as the hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo* in the degree of sterility. However, it is evident that *Bufo bufo japonicus* is isolated from *Bufo viridis* by hybrid sterility as it is from *Bufo bufo bufo*.

While the hybrids, *Bufo bufo japonicus*♀ × *Bufo americanus*♂, were completely inviable, the reciprocal hybrids were viable, although the latter were inferior to the controls in viability. The males and females of these hybrids were all completely sterile. Thus, *Bufo bufo japonicus* seems to be more remotely related to *Bufo americanus* than to *Bufo bufo bufo*.

2. *Bufo torrenticola*

While MATSUI (1976a) gave a species position to this mountainous toad on the basis of some morphological and ecological characteristics, he (1980) had recognized that this species is closely related to *Bufo bufo japonicus* in karyotype. In the present study, it has been found that *Bufo bufo japonicus* and *Bufo torrenticola* are isolated neither by hybrid inviability nor by hybrid sterility. There was

nearly an equal number of males and females in reciprocal hybrids between these two species. Thus, it seems reasonable that *Bufo torrenticola* is recognized as a subspecies of *Bufo japonicus* and named *Bufo japonicus torrenticola.*

As reviewed earlier, the findings that natural hybridization usually occurs between allied species or subspecies of *Bufo* distributed in the same region have been reported by many American workers. Although natural hybridization in Japanese toads has not yet been reported, the status that the three subspecies, *B. b. japonicus, B. b. formosus* and *B. b. montanus,* can not be clearly distinguished from one another owing to the existence of intermediary individuals seems to indicate the frequent occurrence of natural hybridization among these subspecies at the contact zones of their distribution areas. When *Bufo torrenticola* distributed in the central part of Japan come to contact with mountain dwellers of *Bufo bufo japonicus* such as *Bufo bufo montanus* distributed in the northern part of Japan, it is believed that natural hybridization will inevitably occur between these two species and produce fertile hybrids which are intermediate in morphological and ecological characters.

Bufo torrenticola is very similar to *Bufo bufo japonicus* in postmating isolation against European toads, *Bufo bufo bufo* and *Bufo viridis.* While the hybrids between female *Bufo bufo bufo* and male *Bufo torrenticola* were not inferior to the controls in viability, the reciprocal hybrids were inviable to some extent. The males of the former hybrids were completely sterile. Reciprocal hybrids between *Bufo torrenticola* and *Bufo viridis* were inviable to a large extent. *Bufo torrenticola* is also very similar to *Bufo bufo japonicus* in postmating isolation against *Bufo americanus.* Reciprocal hybrids between *Bufo torrenticola* and *Bufo americanus* were completely or almost completely inviable.

3. *Bufo bufo miyakonis* and *Bufo bufo yakushimensis*

a. *Bufo bufo miyakonis*

The distribution of *Bufo bufo miyakonis* is confined to Miyakojima, a small island situated near the southwestern end of the Ryukyu Islands. This subspecies described by OKADA (1931, 1966) was considered to be identical with *Bufo bufo gargarizans* by NAKAMURA and UENO (1963). It is evident from the present study that *Bufo bufo miyakonis* and *Bufo bufo japonicus* belong to the same species, as they are not isolated from each other by hybrid inviability nor by hybrid sterility. However, it was noteworthy that about half of the male hybrids produced from a female *Bufo bufo miyakonis* and a male *Bufo bufo japonicus* were triploids and nearly sterile, and the other half were diploids whose testes were somewhat abnormal in spermatogenesis in spite of their almost normal fertility. BOGART (1972) has reported that polyploids occurred in many cross combinations of *Bufo* species, although they occurred even in control crosses. According to him, triploids were most common, but some pentaploids were also discovered among the polyploids. As he has already stated, the triploidy of the male hybrids, *Bufo bufo miyakonis*♀ × *Bufo bufo japonicus*♂, seems to be attributable to fertilization of a diploid egg with a haploid spermatozoon. It is the present authors' belief

that such a diploid egg may be produced by the fault in releasing the second polar body nucleus as a result of feeble surface reaction of the egg caused by a foreign spermatozoon.

Bufo bufo miyakonis is nearly the same as *Bufo bufo japonicus* in postmating isolation against European and American toads. Reciprocal hybrids between *Bufo bufo miyakonis* and *Bufo bufo bufo* were not always inferior to the controls in viability. In the hybrids between a female *Bufo bufo miyakonis* and a male *Bufo bufo bufo*, there was nearly an equal number of males and females. While male hybrids were completely sterile, the female hybrids laid a small number of large eggs in addition to many normal-sized ones. Reciprocal hybrids between *Bufo bufo miyakonis* and *Bufo viridis* were barely or somewhat inferior to the controls in viability. The hybrids, *Bufo bufo miyakonis*♀ × *Bufo viridis*♂, were all sterile males, like those, *Bufo bufo japonicus*♀ × *Bufo viridis*♂. As stated above, this extreme shift of sex ratio seemed to be attributable to the fact that the male *Bufo viridis* (No. 1) was accidentally YY in sex-chromosome constitution.

Reciprocal hybrids between *Bufo bufo miyakonis* and *Bufo torrenticola* were completely viable as the controls were. However, two of ten male hybrids produced from a female *Bufo bufo miyakonis* and a male *Bufo torrenticola* were triploids and almost sterile. The other male hybrids were diploids and almost normal in reproductive capacity, although their testes were not completely normal in inner structure. Five female hybrids produced from the same cross were all triploids. One of them laid no eggs. The others were almost sterile, although they laid fairly numerous eggs.

From the above-stated results of crossing experiments, it is evident that *Bufo bufo miyakonis* belongs to the same species as that of *Bufo bufo japonicus*, although it can not be determined at present whether or not *Bufo bufo miyakonis* is the same subspecies as *Bufo bufo gargarizans*. While one of the three female *Bufo bufo miyakonis* used in the present hybridization experiments produced many triploid hybrids together with diploid ones, it has not yet been examined if the other two females produced triploid hybrids. The problem whether or not female *Bufo bufo miyakonis* are especially apt to produce triploids by hybridization will be solved some time later.

b. *Bufo bufo yakushimensis*

Bufo bufo yakushimensis described by OKADA (1927, 1931, 1966) is found on two small islands, Yakushima and Tanegashima, situated in the south of Kagoshima Prefecture. This subspecies is distinguished from *Bufo bufo japonicus* by several morphological characteristics. In the present study, a male *Bufo bufo yakushimensis* was crossed with a female *Bufo bufo japonicus*, *Bufo bufo miyakonis*, *Bufo torrenticola*, or *Bufo viridis*. It was found that all these kinds of hybrids were slightly inferior to the controls in viability. While the hybrids produced from a female *Bufo bufo japonicus* metamorphosed nearly at the same time as the control *Bufo bufo japonicus* did and were almost equal to the latter in body length immediately after metamorphosis, the other three kinds of hybrids were all delayed in metamorphosis as

compared with the controls and were comparatively small in body length in spite of the long tadpole stage.

These findings seem to show that *Bufo bufo yakushimensis* somewhat differ reproductively from *Bufo bufo japonicus*. This assumption will be confirmed by crossing male and female *Bufo bufo yakushimensis* with the other subspecies and allied species, and by examining the viability and reproductive capacity of the hybrids obtained. As *Bufo bufo yakushimensis* resembles closely *Bufo bufo japonicus* in appearance, it seems proper for the present that the subspecific name is changed to *Bufo japonicus yakushimensis* OKADA.

4. European *Bufo bufo* and *Bufo viridis*

The findings that reciprocal hybrids between European *Bufo bufo* and *Bufo viridis* differ from each other in viability have been reported by several authors, as already reviewed in the introduction. According to W. F. BLAIR (1972), only 6.1% of fertilized eggs metamorphosed in one of three *Bufo viridis*♀ × *Bufo bufo*♂ crossings, while all the fertilized eggs stopped as larvae in the other two. In the present study, the hybrids, *Bufo bufo bufo*♀ × *Bufo viridis*♂, were superior to the reciprocal hybrids in viability in one of two crossing experiments and produced some mature toads which were all sterile males. As stated above, the production of males alone seems to be attributable to the fact that the single male *Bufo viridis* (No. 1) was YY in sex-chromosome constitution. Although nine metamorphosed toads were produced from a cross, *Bufo viridis*♀ × *Bufo bufo bufo*♂, they were feeble and only two of them could live for one year.

The intraspecific hybrids between a female *Bufo bufo bufo* and a male *Bufo bufo* from France or Greece were nearly the same as the control *Bufo bufo bufo* in viability. The males and females of the hybrids, *Bufo bufo bufo*♀ × *Bufo bufo* from France♂, were almost normal in reproductive capacity. However, these hybrids were unusual in sex ratio. They gave a ratio of seven females to one male. This unbalance in sex ratio seemed to be attributable to a nature peculiar to this French male (No. 1), as the hybrids produced from the same male by mating with three female *Bufo bufo japonicus* gave a ratio of one female to nine males, that is, an unbalanced sex ratio in the opposite direction. This strange nature of the male *Bufo bufo* from France will be analyzed in detail in the near future.

W. F. BLAIR (1972) has made some hybridization experiments between four species of the *Bufo americanus* group and two European species, *Bufo bufo* and *Bufo viridis*. The hybrids, *Bufo americanus*♀ × *Bufo bufo*♂, were barely viable; only 1.1% of fertilized eggs metamorphosed in two of four crossing experiments. While the hybrids, *Bufo viridis*♀ × *Bufo americanus*♂, stopped as gastrula or neurula, a few of the reciprocal hybrids could metamorphose. Similar results were obtained by the present authors. In three crosses, *Bufo americanus*♀ × *Bufo bufo bufo*♂, 0~0.1% of normally cleaved eggs became metamorphosed toads, while none could reach the hatching stage in three of the reciprocal crosses. While the hybrids, *Bufo viridis*♀ × *Bufo americanus*♂, could not develop beyond

the hatching stage, the reciprocal hybrids were not distinctly inferior to the controls in viability. Many individuals grew normally passing over the metamorphosing stage. All of them were sterile males owing probably to the fact that the male parent *Bufo viridis* was YY in sex-chromosome constitution, as stated above repeatedly.

SUMMARY

1. Hybridization experiments were made on Japanese, European and American toads by the artificial fertilization method in order to ascertain the existence of postmating, isolating mechanisms among them. In each of the combinations between different species or subspecies except a female *Bufo viridis,* a very or fairly high percentage of eggs cleaved normally.

2. Female *Bufo bufo japonicus* were crossed with a male or males of *Bufo bufo yakushimensis, Bufo bufo miyakonis, Bufo torrenticola, Bufo bufo* from Portugal, France or Greece, *Bufo viridis* or *Bufo americanus.* While the *Bufo bufo japonicus* eggs fertilized with sperm of *Bufo bufo miyakonis* or *Bufo torrenticola* were superior to the controls in production of feeding tadpoles and metamorphosed toads, the eggs of the same females fertilized with sperm of male *bufo* from Europe or *viridis* were nearly equal or somewhat inferior to the controls in this respect. The fertilized eggs between a female *japonicus* and a male *yakushimensis* were nearly equal to the controls in production of metamorphosed toads. No eggs of *japonicus* fertilized with sperm of a male *americanus* attained the hatching stage, although normal cleavages occurred in about half the number of eggs.

3. Female *Bufo bufo miyakonis* were crossed with one or more males of *Bufo bufo japonicus, Bufo bufo yakushimensis, Bufo torrenticola, Bufo bufo bufo* from Portugal, *Bufo viridis* or *Bufo americanus.* While the *miyakonis* eggs fertilized with sperm of *japonicus* or *torrenticola* were nearly equal to the controls in production of metamorphosed toads, those fertilized with sperm of *bufo* from Portugal or *viridis* were nearly the same or somewhat inferior to the controls in this respect. The fertilized eggs between female *Bufo bufo miyakonis* and a male *Bufo bufo yakushimensis* were somewhat inferior to the controls in production of metamorphosed toads, although a very high percentage of them became feeding tadpoles.

4. Female *Bufo torrenticola* were crossed with a male *Bufo bufo japonicus, Bufo bufo yakushimensis, Bufo bufo miyakonis, Bufo bufo bufo* from Portugal, *Bufo viridis* or *Bufo americanus.* While the *torrenticola* eggs fertilized with sperm of male *japonicus* or *miyakonis* were nearly equal to the controls in production of feeding tadpoles and metamorphosed toads, those fertilized with sperm of a male *bufo* from Portugal or *viridis* were remarkably inferior to the controls in this respect. The fertilized eggs between a female *torrenticola* and a male *yakushimensis* were slightly inferior to the controls in production of metamorphosed toads, although they were nearly the same as the controls in becoming feeding tadpoles. The *torrenticola* eggs fertilized with *americanus* sperm could not attain the hatching stage.

5. Female *Bufo bufo bufo* from Portugal were crossed with one or more males

of *Bufo bufo japonicus, Bufo bufo miyakonis, Bufo torrenticola, Bufo bufo* from France or Greece, *Bufo viridis* or *Bufo americanus*. The eggs of female *bufo* from Portugal fertilized with sperm of one or two male *japonicus, miyakonis* or *torrenticola* were not inferior to the controls in production of metamorphosed toads, while those fertilized with sperm of a male *bufo* from France or Greece were nearly the same as the controls in this respect. The Portuguese *bufo* eggs fertilized with sperm of male *viridis* were remarkably inferior to the controls in production of metamorphosed toads, while no Portuguese *bufo* eggs fertilized with sperm of male *americanus* could hatch normally.

6. A female *Bufo viridis* was crossed with one or more male *Bufo bufo japonicus, Bufo bufo yakushimensis, Bufo bufo miyakonis, Bufo torrenticola, Bufo bufo bufo* from Portugal or *Bufo americanus*. Only 12～43% of the respective number of eggs cleaved normally by insemination with sperm of each kind of the males, although the normal cleavages of the control eggs were also low, that is, they occurred in 32% of them. While about one-third of the normally cleaved control eggs became normally metamorphosed toads, about one-tenth to one-fourth of normally cleaved eggs did so in the series derived from the male *japonicus, yakushimensis, miyakonis* or *torrenticola*. Only a few metamorphosed toads were obtained from the cross, *viridis*♀ × *bufo* from Portugal♂. No feeding tadpoles were produced from the cross, *viridis*♀ × *americanus*♂.

7. Female *Bufo americanus* were crossed with one to three males of *Bufo bufo japonicus, Bufo bufo miyakonis, Bufo torrenticola, Bufo bufo* from Portugal, France or Greece, or *Bufo viridis*. The *americanus* eggs fertilized with sperm of *japonicus, miyakonis* or *viridis* were remarkably inferior to the controls in production of metamorphosed toads. From the crosses with the male *torrenticola* or *bufo* from Portugal, France or Greece, metamorphosed toads were scarcely produced.

8. Reciprocal hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo* from Portugal were as follows after metamorphosis.

a. The control *japonicus* metamorphosed earlier and were smaller in body length immediately after metamorphosis than the control *bufo*. However, the former were larger than the latter when measured at the age of about one year. Reciprocal hybrids between these two subspecies were intermediate in these respects, although they were more similar to the maternal subspecies than to the paternal. While there was nearly an equal number of males and females in three kinds of mature toads, *japonicus, bufo* and hybrids produced from the female *japonicus,* males were more numerous than females in the reciprocal hybrids.

b. The two subspecies distinctly differed from each other in many external characters. Reciprocal hybrids were intermediate between the two subspecies in appearance as a whole, although each hybrid resembled closely the paternal or maternal subspecies or was intermediate between the two subspecies in each of the external characters. However, reciprocal hybrids were usually distinguishable from each other, as they resembled their maternal subspecies more closely in some characters, such as size of tympanum, shape and location of parotoid glands, and color and pattern of parotoid glands and flanks.

c. The testes of *japonicus* differed remarkably from those of *bufo* in size and shape; the former were larger and longer than the latter. Although the testes of reciprocal hybrids generally appeared to resemble those of the control *bufo* in shape, some of them were remarkably smaller than the latter. All the testes of reciprocal hybrids were distinctly abnormal in inner structure; they contained no normal spermatozoa.

The ovaries of one-year-old female hybrids produced from reciprocal crosses were nearly equal to or somewhat smaller than those of the control females, and were underdeveloped or abnormal in inner structure. Some auxocytes were degenerating. A small number of the two-year-old female hybrids produced from reciprocal crosses laid eggs after pituitary injection, while all the control females did so. These hybrids usually laid fewer eggs than the controls did. One of the female hybrids laid two kinds of eggs in size. All four-year-old females of reciprocal hybrids injected with pituitaries laid two kinds of eggs in size. The number of eggs laid by each female was nearly equal to or somewhat smaller than that of eggs laid by the control two-year-old females.

d. Male hybrids produced from reciprocal crosses were nearly completely sterile. Although a few *japonicus* eggs cleaved normally by inseminating with sperm of a few male hybrids produced from reciprocal crosses, almost all of them died before the completion of metamorphosis. Female hybrids produced from reciprocal crosses were barely fertile; only a few eggs cleaved normally by inseminating with sperm of males of the two parental subspecies. While an overwhelming majority of the fertilized eggs died before the hatching stage, a few of them became feeding tadpoles. Some of the latter metamorphosed normally and attained sexual maturity. Almost all the feeding tadpoles developed from larger eggs were triploids.

9. Reciprocal hybrids between *Bufo bufo japonicus* and *Bufo torrenticola* were as follows after metamorphosis.

a. The control *torrenticola* metamorphosed earlier and were larger in body length immediately after metamorphosis than the control *japonicus*. Reciprocal hybrids were similar to the maternal species in the age of metamorphosis and intermediate between the two species in body length. At the age of about one year, *torrenticola* were smaller in body length than *japonicus*. While the hybrids derived from female *japonicus* were intermediate between the two species, those derived from female *torrenticola* were somewhat smaller than the control *torrenticola*. In one-year-old hybrids produced from reciprocal crosses, there was nearly an equal number of males and females.

b. The control *torrenticola* differed from the control *japonicus* in several external characters. Reciprocal hybrids were intermediate between the two species in appearance as a whole. They were intermediate in diameter of tympanum, one of the most characteristic features. They resembled more closely *torrenticola* in relative arm length, while they were intermediate between the two species in the relative length of parotoid glands. They resembled more closely the maternal species in the dorsal skin of the body and its main tubercles.

c. The testes of male *japonicus* differed slightly from those of male *torrenticola* in shape and size; the former were generally larger than the latter in the ratio of length to width as well as in the ratio of their size to body length. The testes of reciprocal hybrids closely resembled those of the control male *torrenticola* in size, while they did more closely those of the paternal species in shape. All of them were the same as those of the control *japonicus* and *torrenticola* in inner structure.

d. The ovaries of reciprocal hybrids were completely normal. While the eggs laid by females of reciprocal hybrids were intermediate in number between those laid by the two parental species, the female hybrids produced from the cross, *japonicus*♀ × *torrenticola*♂, were larger than those produced from the reciprocal cross in number of eggs, as they were in body length.

e. Male and female hybrids produced from reciprocal crosses scarcely differed from the control males and females of the two parental species in reproductive capacity.

10. Reciprocal hybrids between *Bufo bufo japonicus* and *Bufo bufo miyakonis* were as follows after metamorphosis.

a. The control *japonicus* metamorphosed earlier and were smaller in body length immediately after metamorphosis than the control *miyakonis*. While the hybrids between a female *japonicus* and a male *miyakonis* metamorphosed earlier than the control *japonicus*, they were larger in body length than the latter. The hybrids between female *miyakonis* and a male *japonicus* were intermediate between the two subspecies in these two respects. In the hybrids between a female *miyakonis* and a male *japonicus*, there was nearly an equal number of males and females.

b. The hybrids between a female *miyakonis* and a male *japonicus* were intermediate in appearance as a whole. The male hybrids had testes that were similar to those of the control *japonicus* in size and shape. However, their testes were not always normal in inner structure; spermatogenesis was abnormal to a greater or lesser extent. Five of eleven male hybrids were probably triploids. The ovaries of one-year-old female hybrids were normal in inner structure, although they were immature. Two-year-old female hybrids laid as many eggs as female *japonicus*, although their eggs appeared to be somewhat smaller than those of the latter.

c. Of ten male hybrids between a female *miyakonis* and a male *japonicus*, five were nearly sterile and produced no or only a few metamorphosed toads by mating with female *miyakonis*, *japonicus* or *gargarizans*, while the other five were nearly the same as the control males of the three subspecies in reproductive capacity. It was confirmed that the former five male hybrids were triploids, while the latter five were diploids. Moreover, it was confirmed that the female *japonicus* (No. 8) used in the backcrossings in 1980 was a tetraploid, as all the offspring produced from this female by mating with the male diploid hybrids or *japonicus* were triploids as far as they were karyologically examined.

Female hybrids between a female *miyakonis* and a male *japonicus* were almost normal in reproductive capacity.

11. Reciprocal hybrids between *Bufo torrenticola* and *Bufo bufo miyakonis* were as follows after metamorphosis.

a. While the hybrids between a female *miyakonis* and a male *torrenticola* were intermediate between the parental species in the age of metamorphosis, the reciprocal hybrids metamorphosed earlier than the two kinds of controls. The four kinds of toads, *miyakonis, torrenticola* and reciprocal hybrids, were similar with one another in body length immediately after metamorphosis.

b. The hybrids between a female *miyakonis* and a male *torrenticola* had tympanums which were larger in diameter than those of *torrenticola* and field-caught *miyakonis*. The testes of twelve male hybrids were similar to those of male *torrenticola* in size and shape. The testes of four of the five male hybrids examined were normal in inner structure. Those of the remaining male hybrid were abnormal and had no normal spermatozoa.

Of five female hybrids injected with pituitaries, four laid eggs that were almost normal in number and size. The remaining female hybrid laid no eggs.

c. Ten male hybrids between a female *miyakonis* and a male *torrenticola* were mated with two female *miyakonis,* a female *japonicus* and two female *gargarizans*. While eight of the male hybrids were almost normal in reproductive capacity, the other two were nearly sterile. It was confirmed that the former eight were diploids and the latter two were triploids.

Four females of the hybrids were mated with a male *torrenticola* and a male *miyakonis*. Although normal cleavages always occurred in very high percentages of eggs, only a few percentage attained the completion of metamorphosis in each backcrossing.

12. Reciprocal hybrids between *Bufo bufo japonicus* and *Bufo viridis* were as follows after metamorphosis.

a. The control *viridis* metamorphosed definitely later and were larger in body length immediately after metamorphosis than the control *japonicus*. Reciprocal hybrids were similar to the maternal species in the age of metamorphosis and intermediate between the control *japonicus* and *viridis* in body length. The hybrids between a female *japonicus* and a male *viridis* were definitely smaller than the control *japonicus* at the age of about one year. All these hybrids were males in contrast to the control *japonicus*.

b. The hybrids between a female *japonicus* and a male *viridis* were intermediate between the two species in appearance as a whole. Three of six hybrids revealed secondary sexual characters and had testes that were somewhat small and very abnormal in inner structure. The other three revealed no secondary sexual characters and their testes were so small that they could hardly be measured. All the male hybrids were completely sterile, as they had neither normal nor abnormal spermatozoa.

13. Hybrid between female *Bufo americanus* and male *Bufo bufo japonicus* were as follows after metamorphosis.

a. The control *americanus* metamorphosed remarkably later and were distinctly larger in body length immediately after metamorphosis than the control

japonicus. The hybrids were intermediate between the two species in the age of metamorphosis and nearly similar to the control *japonicus* in body length. At the age of about one year, the hybrids were intermediate in body length between the two kinds of controls. There was nearly an equal number of males and females in the hybrids as well as in the two kinds of controls.

b. The hybrids were intermediate between the two species in appearance as a whole. The testes of three male hybrids were generally smaller and slender than those of the control *americanus*. They were very abnormal in inner structure. The ovaries of three female hybrids were very small and degenerative. Both male and female hybrids contained no germ cells and were completely sterile.

14. Hybrids between female *Bufo bufo japonicus* and a male *Bufo bufo* from France or Greece were as follows after metamorphosis.

a. They metamorphosed nearly at the same time as the control *japonicus* did and were distinctly larger in body length immediately after metamorphosis. However, they were somewhat smaller than the controls at the age of about one year. Of the hybrids derived from a French male, about 90% were males, while there was nearly an equal number of males and females in the hybrids derived from a Greek male.

b. The hybrids were intermediate between their parents in appearance as a whole. The testes of male hybrids were remarkably smaller than those of the control *japonicus* and very abnormal in inner structure. No normal spermatozoa were produced.

The ovaries of one-year-old female hybrids were filled with growing auxocytes, although they were slightly inferior in differentiation to the control *japonicus*. Of five three-year-old female hybrids between a female *japonicus* and a male *bufo* from Greece, three laid fairly many or a few eggs after pituitary injection, while the other two laid no eggs. Of five three-year-old female hybrids between three female *japonicus* and a male *bufo* from France, three laid numerous eggs which were almost normal in number after pituitary injection, while the other two laid no eggs. Some of the eggs laid by each of the above six female hybrids were remarkably larger than the other normal-sized ones.

c. Three female hybrids between a female *japonicus* and a male *bufo* from Greece and three female hybrids between two female *japonicus* and a male *bufo* from France were mated with a male *japonicus* and a male *bufo* from Portugal. While a great majority of eggs usually cleaved normally in each mating, no or only a few eggs became feeding tadpoles. Almost all of the latter had been raised from larger eggs and were generally triploids. Most of them metamorphosed normally.

15. Hybrids between female *Bufo bufo bufo* from Portugal and a male *Bufo bufo* from France or Greece were as follows after metamorphosis.

a. Both kinds of hybrids metamorphosed somewhat earlier than the control *bufo* from Portugal, although the hybrids derived from the French male metamorphosed a little earlier than those derived from the Greek male. The two kinds of hybrids were nearly the same as the control *bufo* from Portugal in body

length immediately after metamorphosis. The hybrids derived from the French male were also similar to the control *bufo* from Portugal in body length at the age of about one year. However, 42 of 48 hybrids were females, while there were six females and four males in the control *bufo* from Portugal.

b. The hybrids derived from the French male were very similar in appearance to the control *bufo* from Portugal. The gonads of male and female hybrids were normal. Each of eight females laid abundant eggs that were normal in number and size.

c. Five of the eight female hybrids were backcrossed with a male *bufo* from Portugal and the other three were mated with one of their brothers. In the brother and sister matings, the development of eggs were always normal and most eggs became normally metamorphosed toads. It was evident that there was no postmating isolation between *bufo* from Portugal and *bufo* from France. Although the development of eggs was not so good in the backcrossings, their inferiority in developmental capacity was probably attributable to overripeness of the eggs.

16. Reciprocal hybrids between *Bufo bufo miyakonis* and *Bufo bufo bufo* from Portugal were as follows after metamorphosis.

a. Reciprocal hybrids between the two subspecies metamorphosed earlier than the control *bufo* from Portugal and nearly at the same time as or somewhat earlier than the control *miyakonis.* They were somewhat smaller than the control *bufo* from Portugal and nearly the same as or a little smaller than the control *miyakonis* in body length immediately after metamorphosis. The hybrids between a female *miyakonis* and a male *bufo* from Portugal were remarkably smaller than the control *bufo* from Portugal in body length at the age of about one year. In these hybrids there was nearly an equal number of males and females, as in the controls.

b. The hybrids between a female *miyakonis* and a male *bufo* from Portugal were intermediate between the two parental subspecies in appearance as a whole. The testes of one- and two-year-old male hybrids were similar in shape to those of one-year-old male *bufo* from Portugal and nearly the same as or somewhat smaller than the latter in relative size to body length. The testes of the male hybrids were abnormal in inner structure; no or only a few normally shaped spermatozoa were contained in the seminiferous tubules. The ovaries of two one-year-old female hybrids were filled with full-grown ova that varied in size.

c. The male hybrids were completely sterile when mated with female *miyakonis* or female hybrids between a female *bufo* from Portugal and a male *bufo* from France.

17. Reciprocal hybrids between *Bufo bufo miyakonis* and *Bufo viridis* were as follows after metamorphosis.

a. While the control *miyakonis* metamorphosed normally at the same time as the control *viridis,* the hybrids between two female *miyakonis* and a male *viridis* did earlier and those between a female *viridis* and a male *miyakonis* did remarkably later than the two controls. Reciprocal hybrids were intermediate between the

two controls in body length immediately after metamorphosis, while the control *viridis* were distinctly larger than the control *miyakonis*. It was strange that 128 one-year-old hybrids between a female *miyakonis* and a male *viridis* as well as six control *viridis* were all males. This was considered to be attributable to the fact that the male *viridis* was accidentally YY in sex-chromosome constitution.

b. The hybrids between a female *miyakonis* and a male *viridis* were intermediate between the two species in appearance as a whole. While the testes of five of ten male hybrids were nearly the same as or slightly smaller than those of the control *viridis*, those of the other were remarkably smaller or slender. The testes of all the male hybrids were abnormal in inner structure and contained no normal spermatozoa.

c. Five male hybrids between a female *miyakonis* and a male *viridis* were mated with two female *miyakonis*. It was confirmed that the male hybrids were completely sterile.

18. Reciprocal hybrids between *Bufo bufo bufo* from Portugal and *Bufo torrenticola* were as follows after metamorphosis.

a. While the control *torrenticola* metamorphosed remarkably earlier than the control *bufo* from Portugal, reciprocal hybrids were somewhat similar to the maternal species in the age of metamorphosis, although they were intermediate in this respect between the two species. While the control *bufo* from Portugal were somewhat larger than the control *torrenticola* in body length immediately after metamorphosis, reciprocal hybrids were nearly the same as the paternal species. Of the 36 hybrids produced from the cross between a female *bufo* from Portugal and a male *torrenticola*, 15 were females and 21 were males, while there was nearly an equal number of males and females in the control *bufo* from Portugal.

b. The hybrids between a female *bufo* from Portugal and a male *torrenticola* were intermediate between the two species in appearance as a whole. The testes of male hybrids were remarkably smaller than those of the parental species and very abnormal in inner structure. The ovaries of female hybrids were similar in size and inner structure to those of hybrids produced from a female *bufo* from Portugal and a male *japonicus*.

c. Five male hybrids between a female *bufo* from Portugal and a male *torrenticola* were mated with three female hybrids between a female *bufo* from Portugal and a male *bufo* from France. It was found that the male hybrids were completely sterile.

19. On the basis of postmating isolating mechanisms, *Bufo bufo japonicus* SCHLEGEL and *Bufo torrenticola* M. MATSUI should be changed in nomenclature into *Bufo japonicus japonicus* SCHLEGEL and *Bufo japonicus torrenticola* M. MATSUI, respectively. *Bufo bufo miyakonis* OKADA and *Bufo bufo yakushimensis* OKADA should be also changed into *Bufo japonicus miyakonis* OKADA and *Bufo japonicus yakushimensis* OKADA, respectively, until their systematic position are confirmed by further hybridization experiments.

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EXPLANATION OF PLATES

PLATE I

Bufo bufo japonicus, *Bufo bufo bufo* from Portugal and reciprocal hybrids between these two subspecies at the age of two years. ×0.4

1, 2. *Bufo bufo japonicus* ♀ No. 1, *japonicus* ♀ No. 1 × *japonicus* ♂ No. 1
3, 4. Hybrid ♀ No. 3, *japonicus* ♀ No. 1 × *bufo* from Portugal ♂ No. 1
5, 6. Hybrid ♀ No. 1, *bufo* from Portugal ♀ No. 1 × *japonicus* ♂ No. 3
7, 8. *Bufo bufo bufo* from Portugal ♀ No. 2, *bufo* from Portugal ♀ No. 1 × *bufo* from Portugal ♂ No. 3

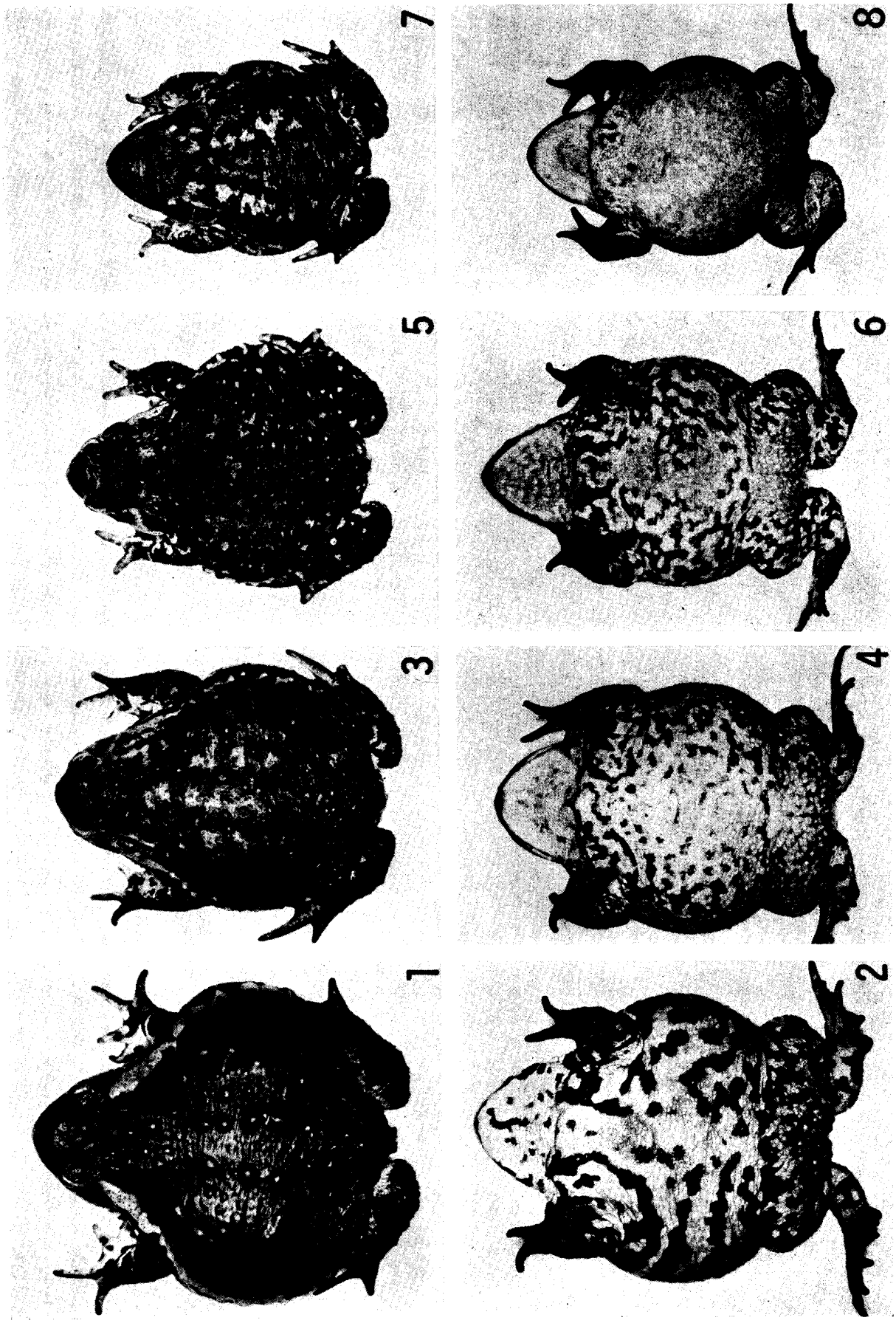
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PLATE II

Bufo bufo japonicus, *Bufo torrenticola*, and reciprocal hybrids between these two species at the age of two years. ×0.4

9, 10. *Bufo bufo japonicus* ♀ No. 1, *japonicus* ♀ No. 3 × *japonicus* ♂ No. 4
11, 12. Hybrid ♀ No. 2, *japonicus* ♀ No. 3 × *torrenticola* ♂ No. 1
13, 14. Hybrid ♀ No. 4, *torrenticola* ♀ No. 1 × *japonicus* ♂ No. 5
15, 16. *Bufo torrenticola* ♀ No. 1, *torrenticola* ♀ No. 3 × *torrenticola* ♂ No. 2

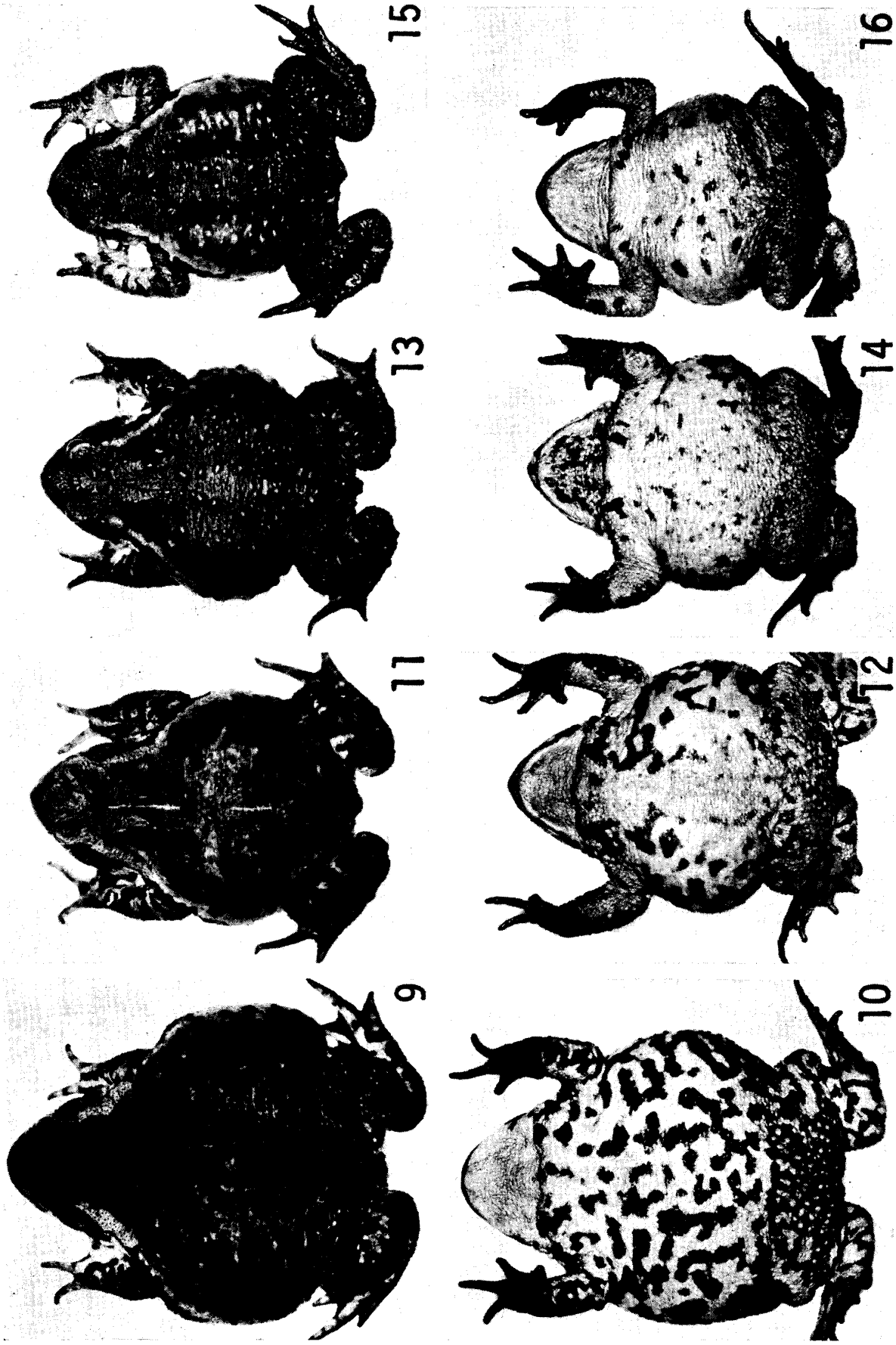
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PLATE III

Bufo bufo japonicus, Bufo americanus and a hybrid between these two species at the age of two years. ×0.4

17, 18. *Bufo bufo japonicus* ♀ No. 2, *japonicus* ♀ No. 3 × *japonicus* ♂ No. 4

19, 20. Hybrid ♀ No. 4, *americanus* ♀ No. 1 × *japonicus* ♂ No. 4

21, 22. *Bufo americanus* ♀ No. 3, *americanus* ♀ No. 1 × *americanus* ♂ No. 1

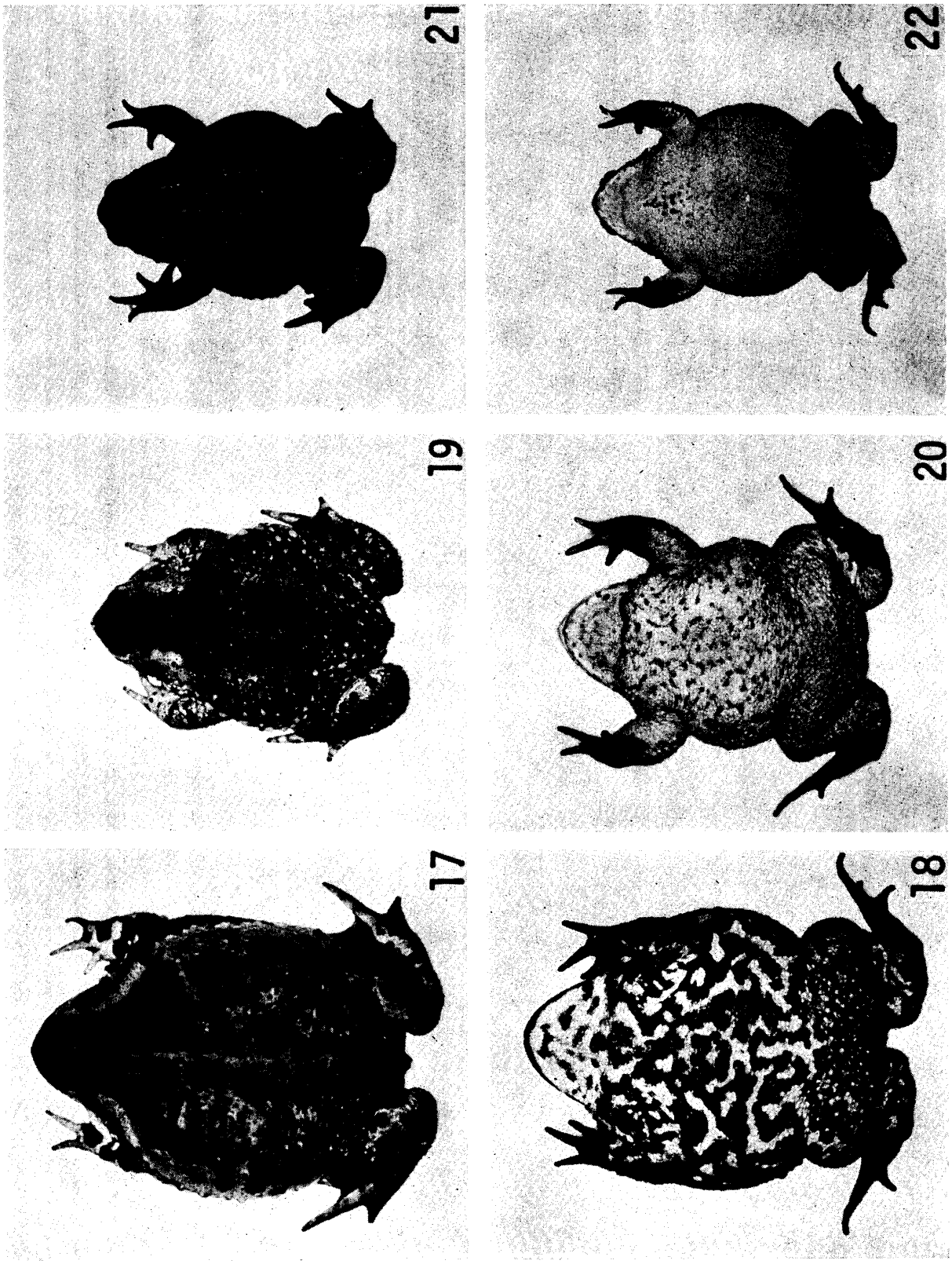
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PLATE IV

Bufo bufo from Greece and France and hybrids between these toads and a *Bufo bufo japonicus*. The hybrids were two years old. ×0.4

23, 24. *Bufo bufo* from Greece ♂ No. 1, field-caught
25, 26. Hybrid ♀ No. 3, *japonicus* ♀ No. 3 × *bufo* from Greece ♂ No. 1
27, 28. *Bufo bufo* from France ♂ No. 1, field-caught
29, 30. Hybrid ♀ No. 2, *japonicus* ♀ No. 3 × *bufo* from France ♂ No. 1

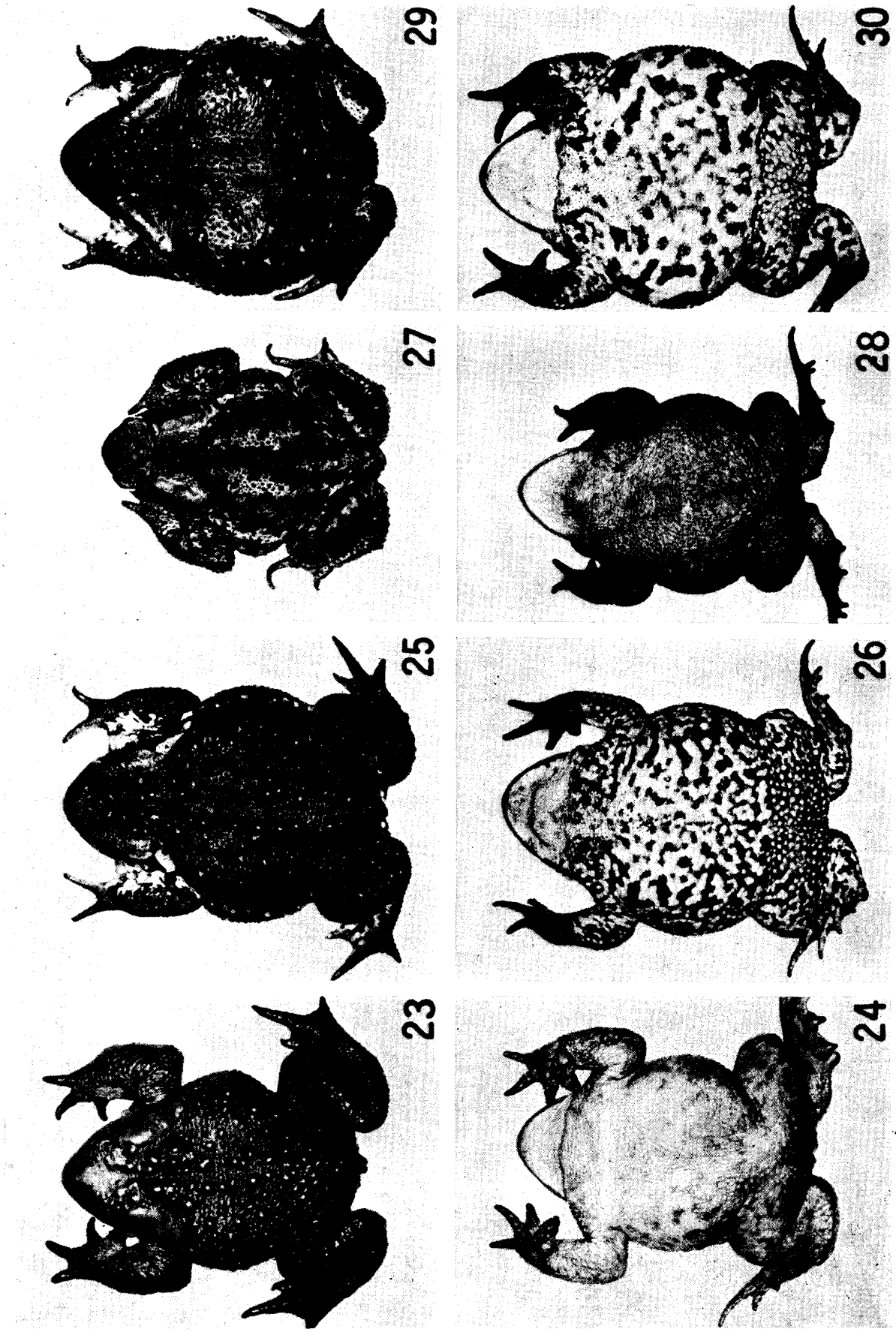
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PLATE V

Bufo bufo bufo from Portugal, *Bufo bufo miyakonis,* and hybrids between a female or male *Bufo bufo bufo* from Portugal and a male *Bufo bufo* from France or a female *Bufo bufo miyakonis*. ×0.4

31, 32. *Bufo bufo bufo* from Portugal ♀ No. 2, *bufo* from Portugal ♀ No. 2 × *bufo* from Portugal ♂ No. 4, at the age of two years

33, 34. Hybrid ♀ No. 3, *bufo* from Portugal ♀ No. 2 × *bufo* from France ♂ No. 1, at the age of two years

35, 36. Hybrid ♀ No. 1, *miyakonis* ♀ No. 1 × *bufo* from Portugal ♂ No. 5, at the age of two years

37, 38. *Bufo bufo miyakonis* ♀ No. 4, field-caught

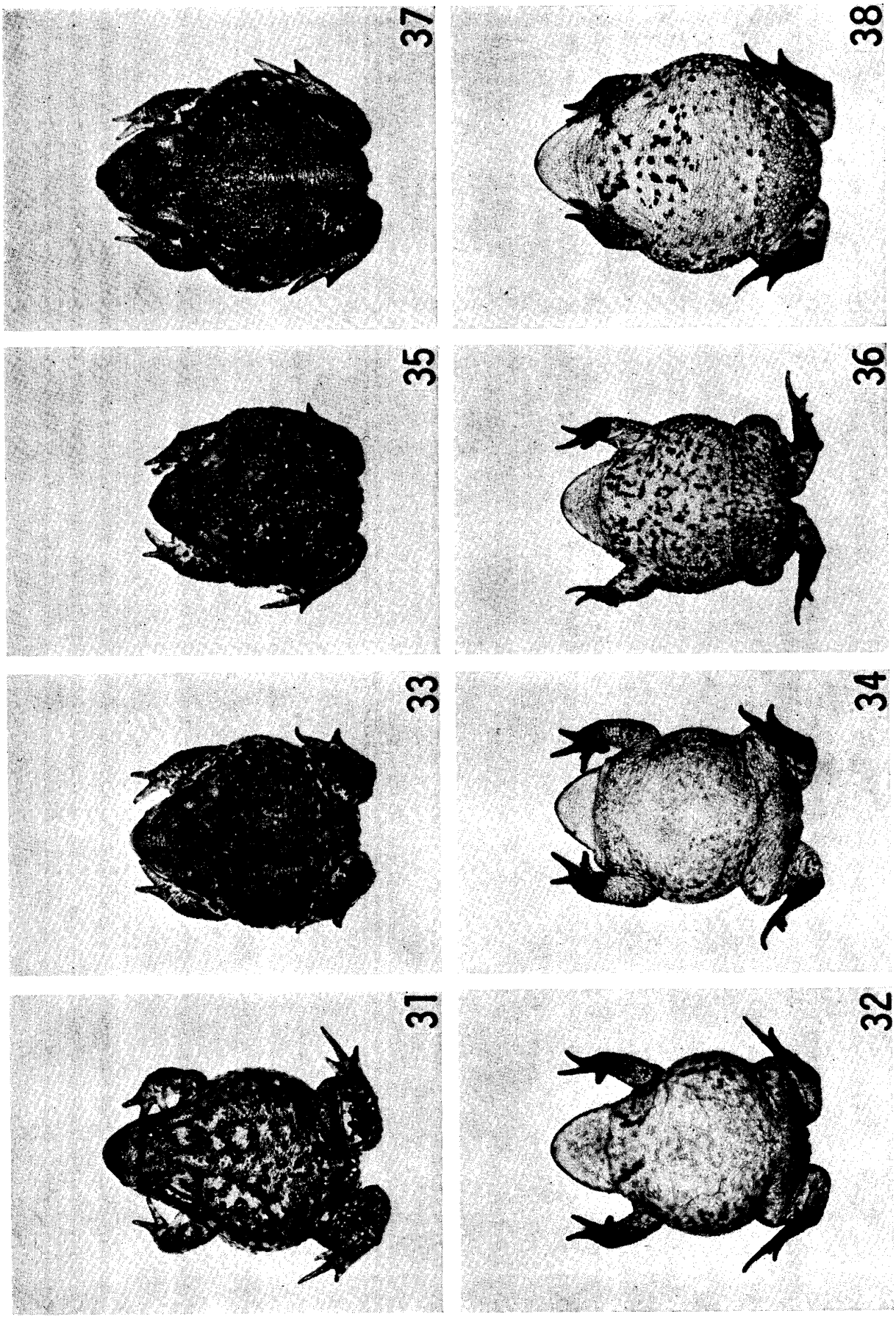
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PLATE VI

Bufo bufo japonicus, *Bufo torrenticola*, and hybrids between a female *Bufo bufo miyakonis* and a male *Bufo bufo japonicus* or *Bufo torrenticola*. All the toads were two years old, excepting *Bufo torrenticola*. ×0.4

39, 40. *Bufo bufo japonicus* ♀ No. 1, *japonicus* ♀ No. 6 × *japonicus* ♂ No. 6
41, 42. Hybrid ♀ No. 4, *miyakonis* ♀ No. 1 × *japonicus* ♂ No. 6
43, 44. Hybrid ♀ No. 2, *miyakonis* ♀ No. 1 × *torrenticola* ♂ No. 3
45, 46. *Bufo torrenticola* ♀ No. 4, field-caught

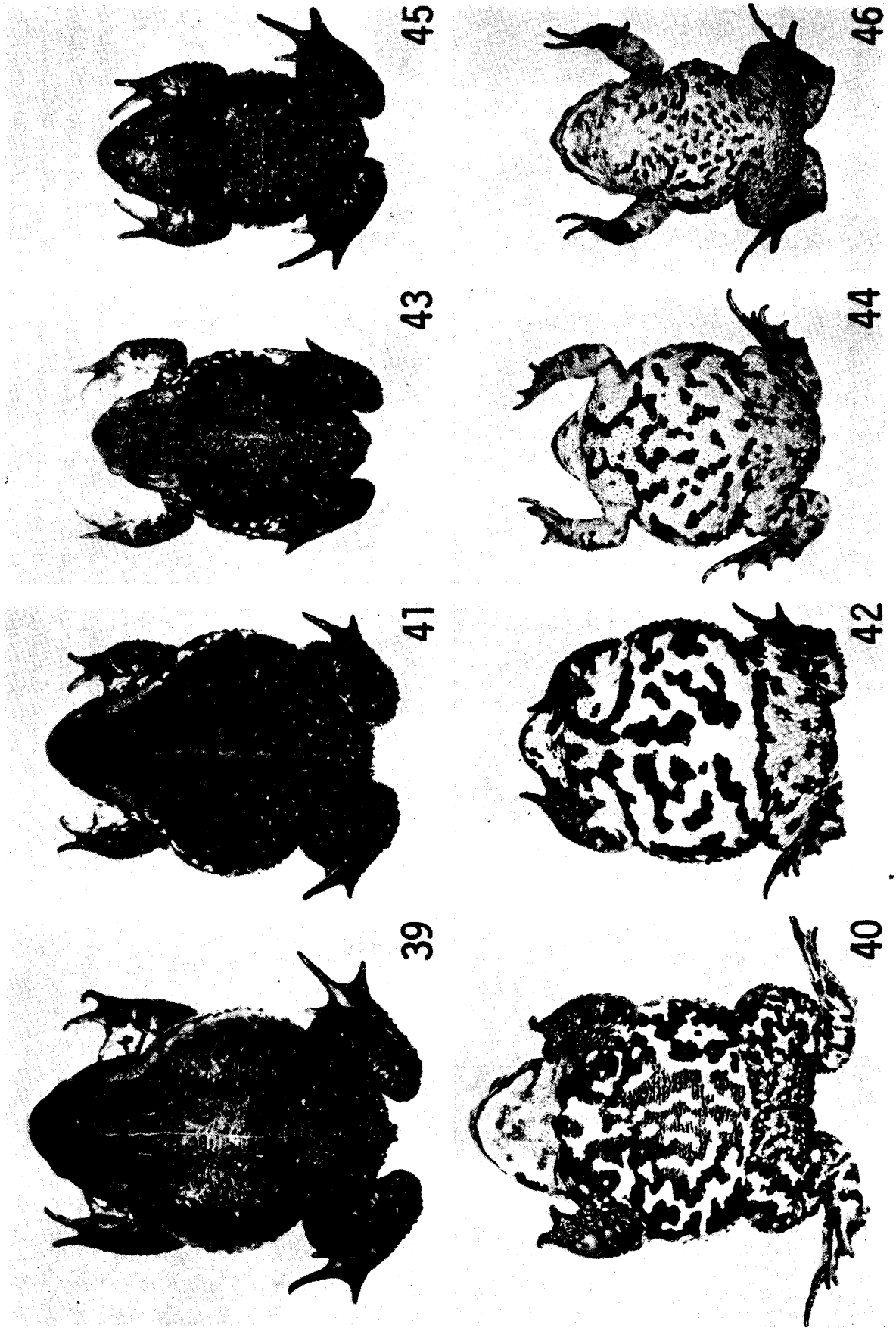
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PLATE VII

Bufo viridis, hybrids between a female *Bufo bufo miyakonis* or *Bufo bufo japonicus* and a male *Bufo viridis* and a hybrid between a female *Bufo bufo bufo* from Portugal and a male *Bufo torrenticola*. All the toads were two years old. ×0.4

47, 48. *Bufo viridis* ♂ No. 7, *viridis* ♀ No. 1 × *viridis* ♂ No. 1
49, 50. Hybrid ♂ No. 12, *miyakonis* ♀ No. 1 × *viridis* ♂ No. 1
51, 52. Hybrid ♂ No. 10, *japonicus* ♀ No. 7 × *viridis* ♂ No. 1
53, 54. Hybrid ♀ No. 8, *bufo* from Portugal ♀ No. 3 × *torrenticola* ♂ No. 3

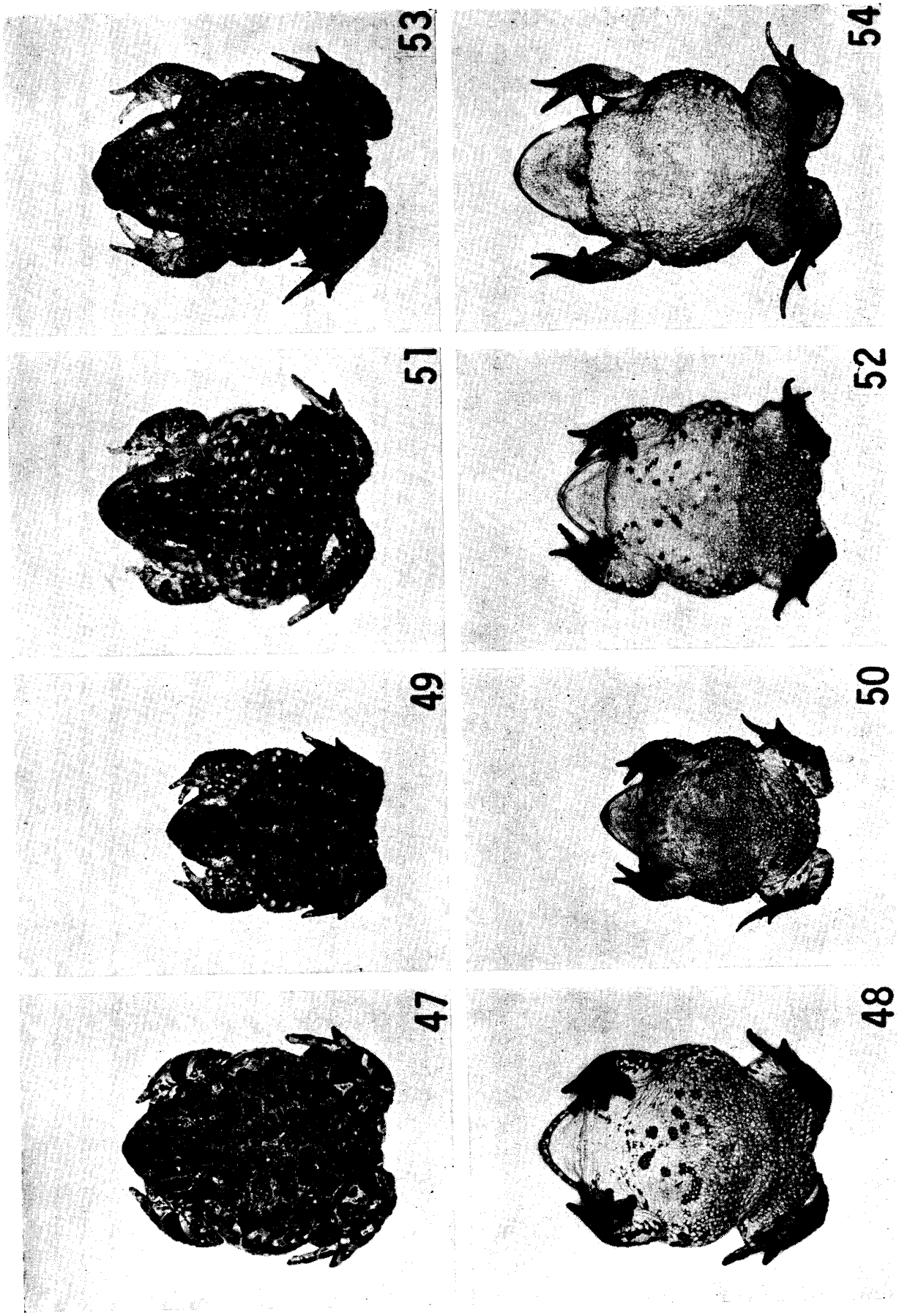
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PLATE VIII

Cross-sections of the testes of *Bufo bufo japonicus*, *Bufo bufo bufo* from Portugal and reciprocal hybrids between the two subspecies at the age of one year. ×225

55. *Bufo bufo japonicus* ♂ No. 1, *japonicus* ♀ No. 1 × *japonicus* ♂ No. 1
56. *Bufo bufo bufo* ♂ No. 1, *bufo* from Portugal ♀ No. 1 × *bufo* from Portugal ♂ No. 3
57. Hybrid ♂ No. 1, *japonicus* ♀ No. 1 × *bufo* from Portugal ♂ No. 1
58. Hybrid ♂ No. 2, *bufo* from Portugal ♀ No. 1 × *japonicus* ♂ No. 3

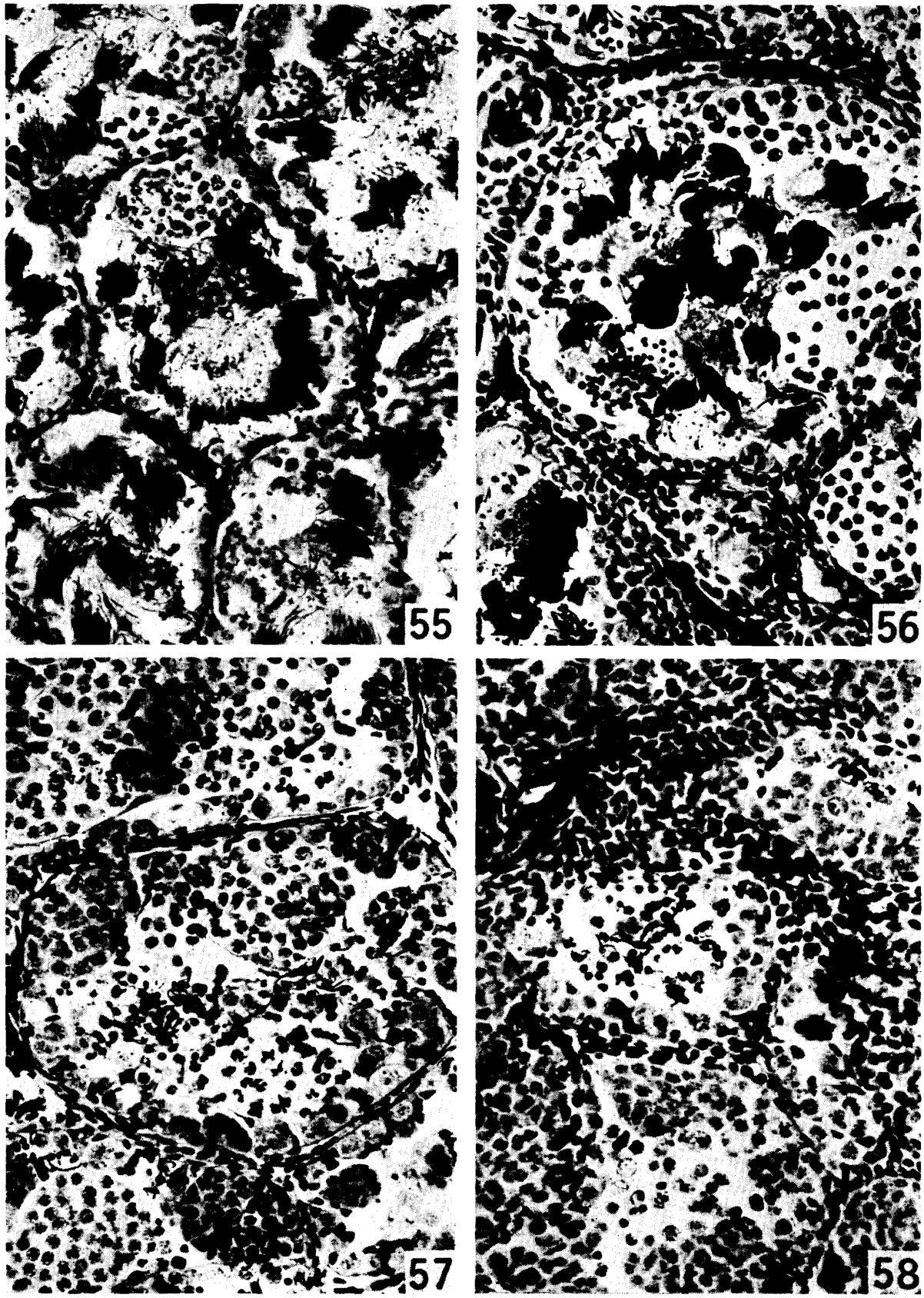
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PLATE IX

Cross-sections of the ovaries of female *Bufo bufo bufo* from Portugal, reciprocal hybrids between *Bufo bufo japonicus* and *Bufo bufo bufo* from Portugal and a female hybrid between a female *Bufo bufo japonicus* and a male *Bufo bufo* from France. All the toads were one year old. ×45

59. *Bufo bufo bufo* ♀, *bufo* from Portugal ♀ No. 1 × *bufo* from Portugal ♂ No. 3
60. Hybrid ♀, *japonicus* ♀ No. 1 × *bufo* from Portugal ♂ No. 1
61. Hybrid ♀, *bufo* from Portugal ♀ No. 1 × *japonicus* ♂ No. 3
62. Hybrid ♀, *japonicus* ♀ No. 3 × *bufo* from France ♂ No. 1

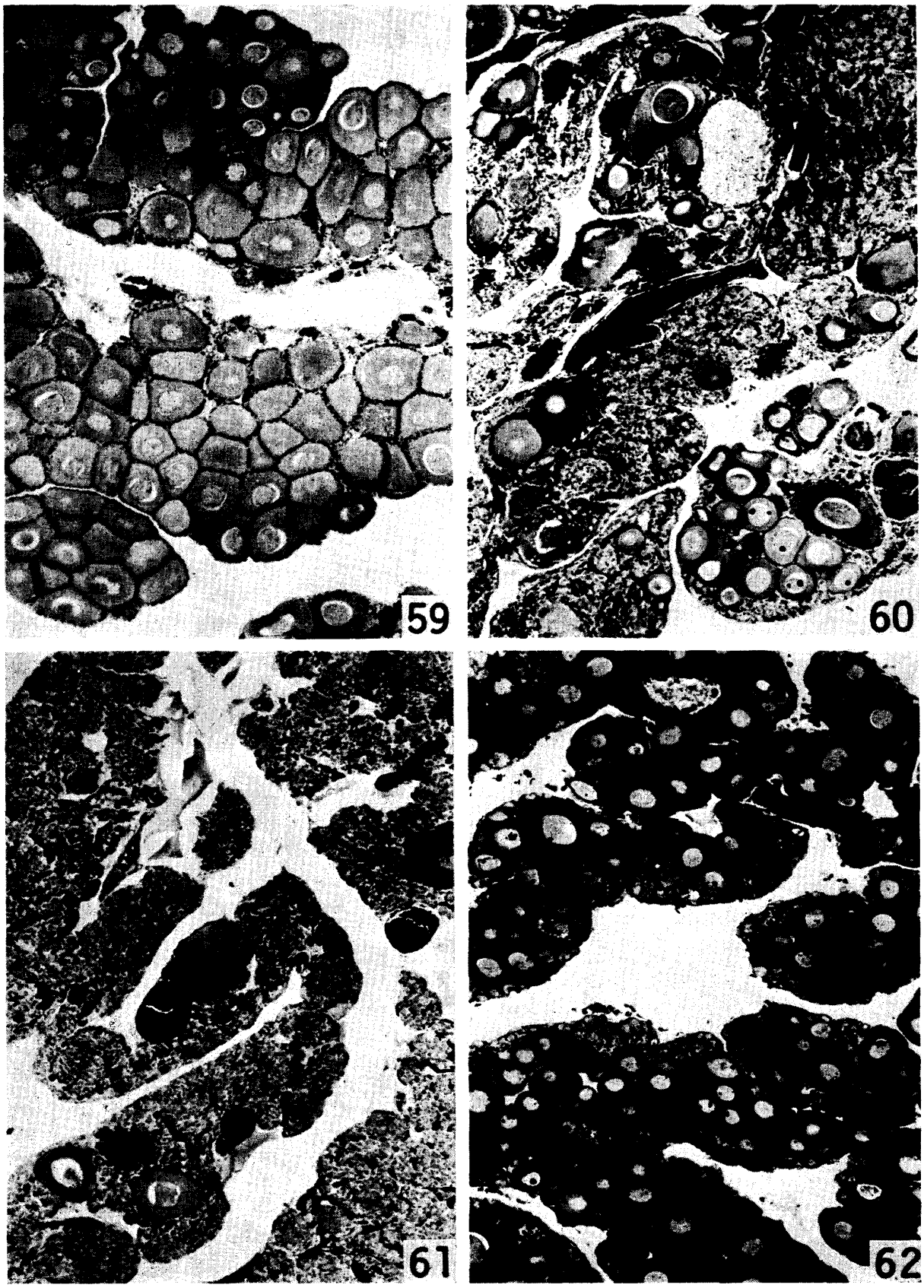
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PLATE X

Cross-sections of the testes of male hybrids between a female *Bufo bufo japonicus* and a male *Bufo bufo* from France or Greece. All the hybrids were one year old. ×225

63. Hybrid ♂ No. 1, *japonicus* ♀ No. 3 × *bufo* from France ♂ No. 1
64. Hybrid ♂ No. 3, *japonicus* ♀ No. 3 × *bufo* from France ♂ No. 1
65. Hybrid ♂ No. 5, *japonicus* ♀ No. 3 × *bufo* from Greece ♂ No. 1
66. Hybrid ♂ No. 4, *japonicus* ♀ No. 3 × *bufo* from Greece ♂ No. 1

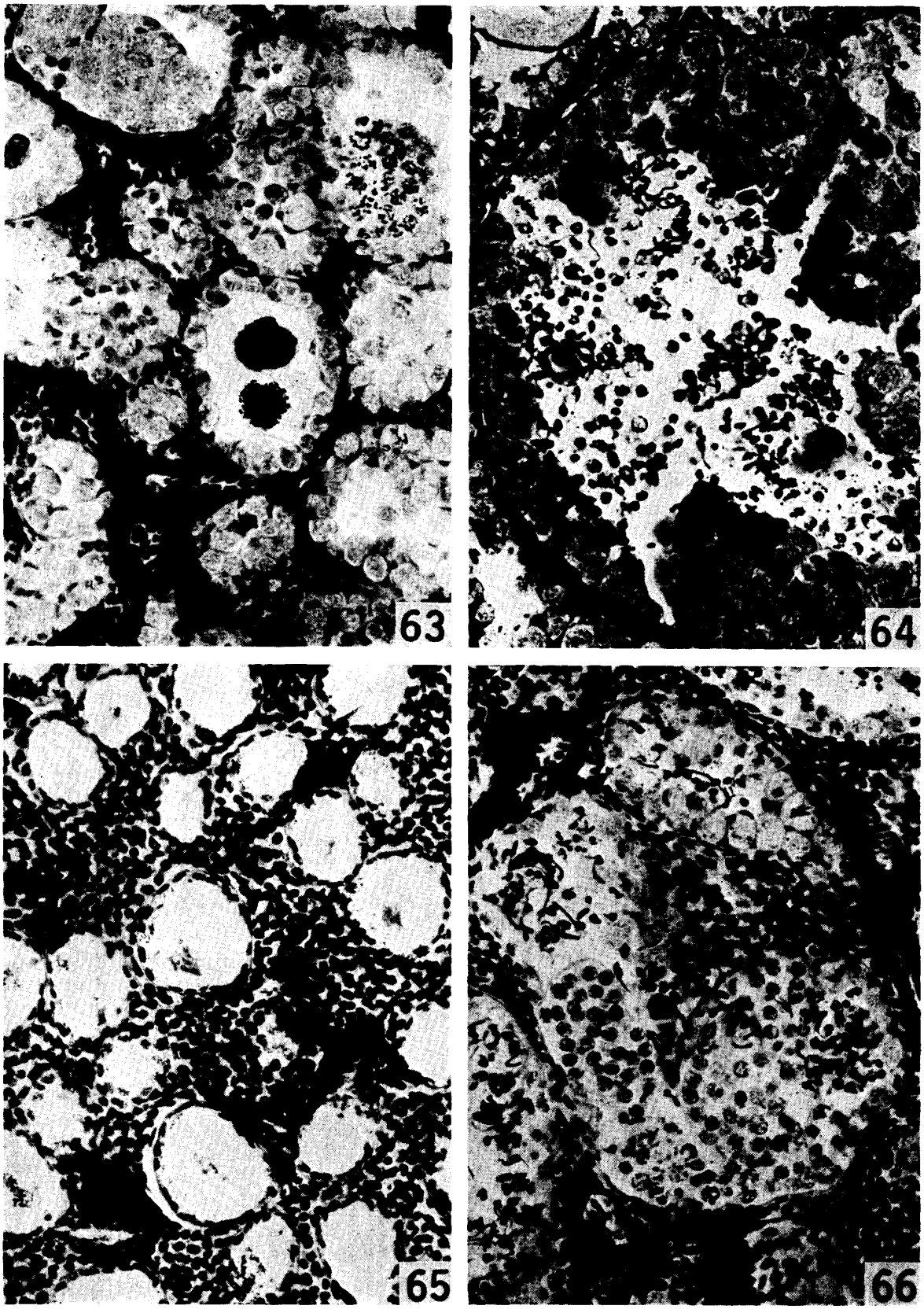
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PLATE XI

Cross-sections of the testes of male *Bufo bufo japonicus, Bufo torrenticola* and reciprocal hybrids between the two species at the age of one year. ×225

67. *Bufo bufo japonicus* ♂ No. 3, *japonicus* ♀ No. 3 × *japonicus* ♂ No. 4
68. *Bufo torrenticola* ♂ No. 2, *torrenticola* ♀ No. 1 × *torrenticola* ♂ No. 2
69. Hybrid ♂ No. 1, *japonicus* ♀ No. 3 × *torrenticola* ♂ No. 1
70. Hybrid ♂ No. 4, *torrenticola* ♀ No. 1 × *japonicus* ♂ No. 5

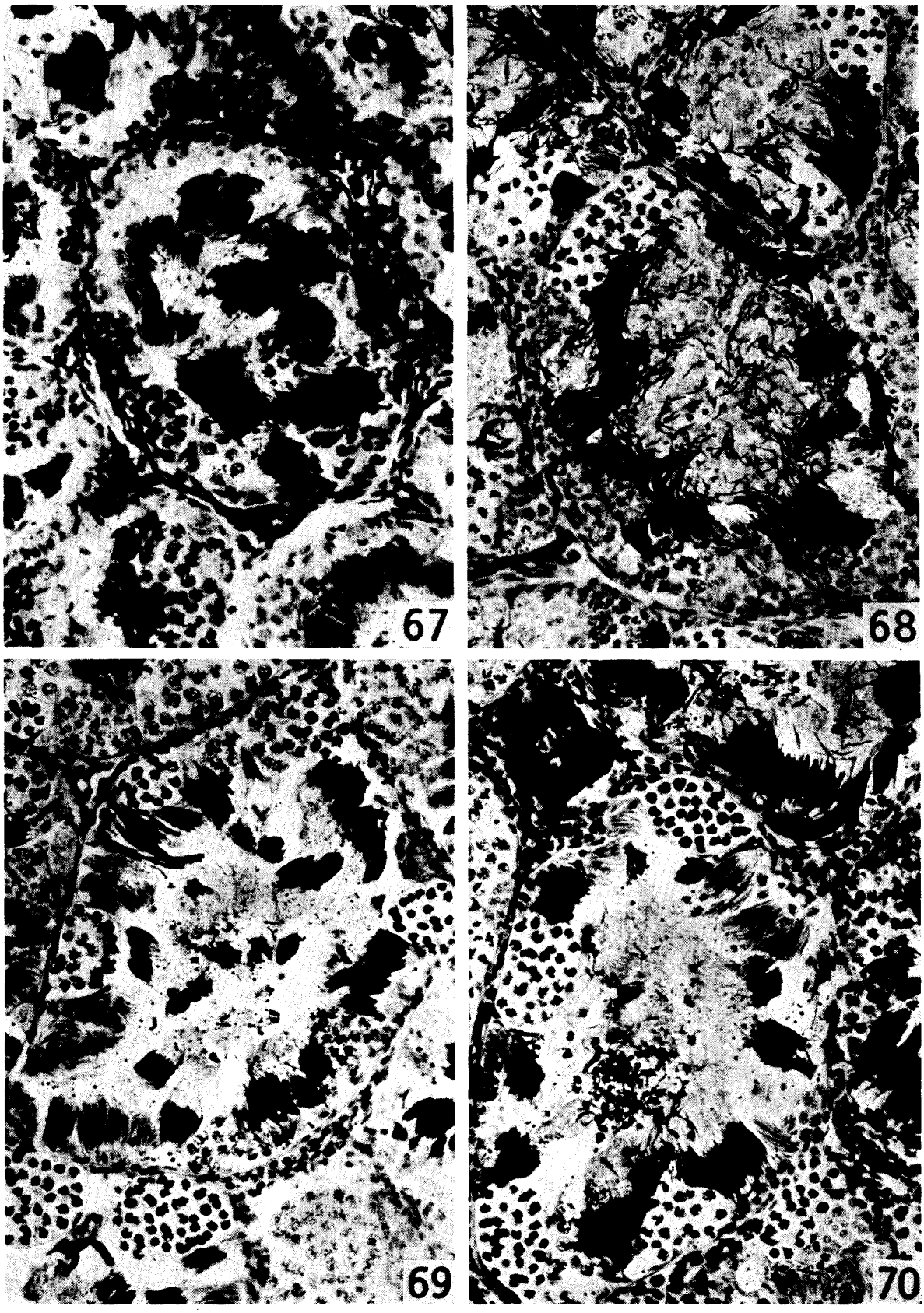
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PLATE XII

Cross-sections of the gonads of *Bufo bufo japonicus*, *Bufo americanus* and hybrids between a female *Bufo americanus* and a male *Bufo bufo japonicus*. All the toads were one year old. ×225

71. Testis of *Bufo bufo japonicus* ♂ No. 2, *japonicus* ♀ No. 3 × *japonicus* ♂ No. 4
72. Testis of *Bufo americanus* ♂ No. 1, *americanus* ♀ No. 1 × *americanus* ♂ No. 1
73. Testis of hybrid ♂ No. 1, *americanus* ♀ No. 1 × *japonicus* ♂ No. 4
74. Ovary of hybrid ♀ No. 3, *americanus* ♀ No. 1 × *japonicus* ♂ No. 4

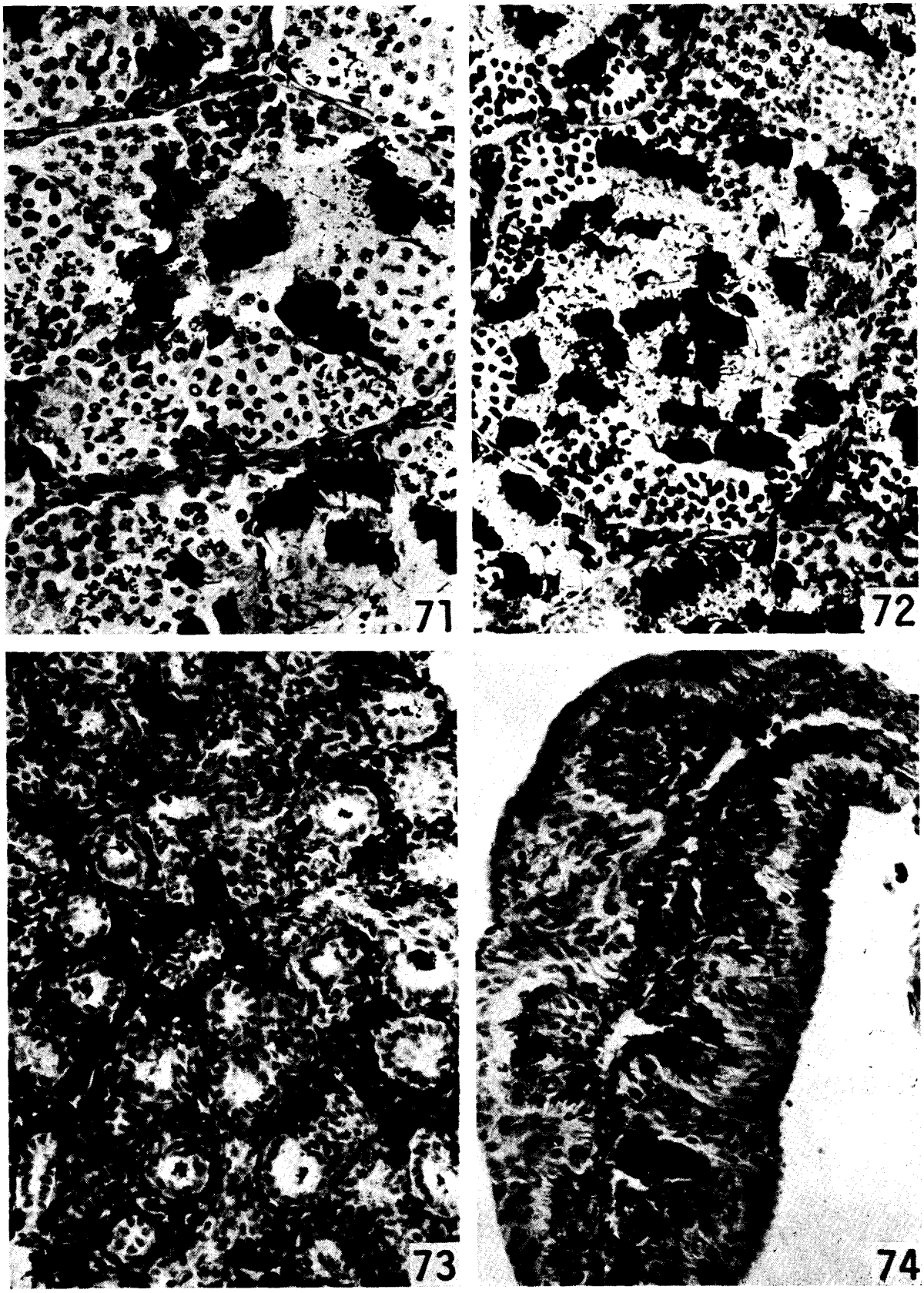
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PLATE XIII

Cross-sections of the testes of male hybrids between a female *Bufo bufo miyakonis* and a male *Bufo bufo japonicus* or *Bufo torrenticola*. All the hybrids were one year old. ×225

75. Hybrid ♂ No. 1, *miyakonis* ♀ No. 1 × *japonicus* ♂ No. 6
76. Hybrid ♂ No. 2, *miyakonis* ♀ No. 1 × *japonicus* ♂ No. 6
77. Hybrid ♂ No. 1, *miyakonis* ♀ No. 1 × *torrenticola* ♂ No. 3
78. Hybrid ♂ No. 5, *miyakonis* ♀ No. 1 × *torrenticola* ♂ No. 3

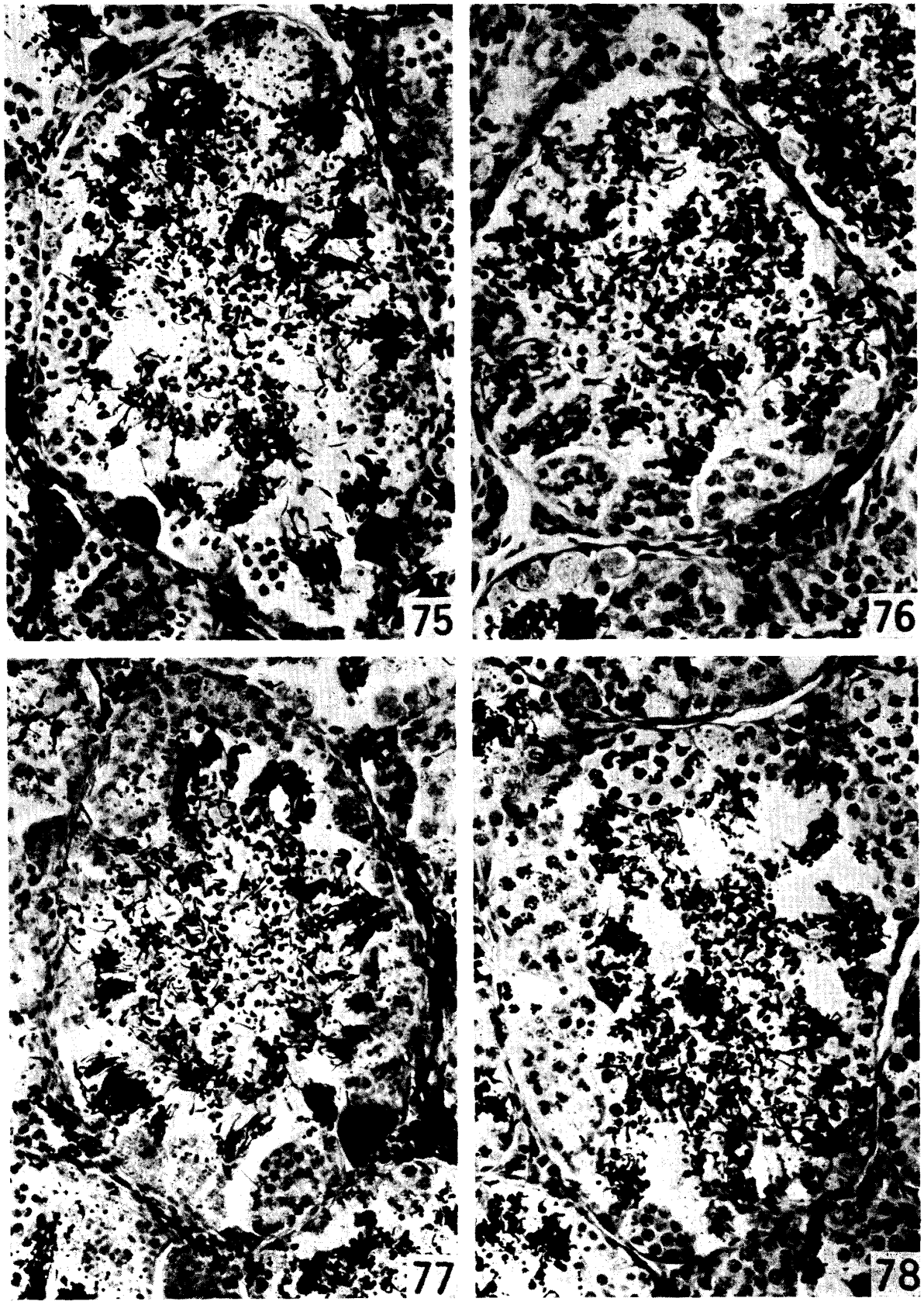
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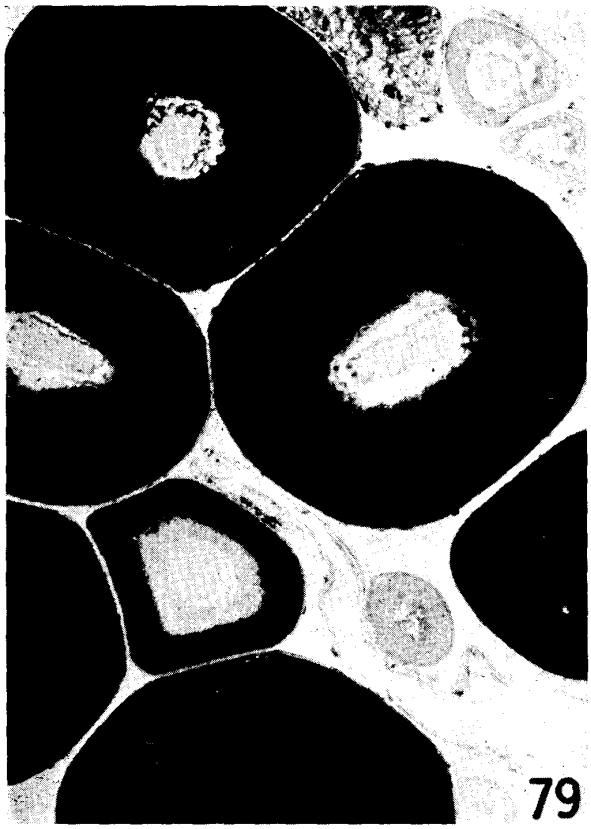
PLATE XIV

Cross-sections of the gonads of hybrids between female *Bufo bufo miyakonis* No. 1 and male *Bufo bufo bufo* from Portugal No. 5. All the toads were one year old.

79. Ovary of hybrid ♀ No. 1 × 45
80. Testis of hybrid ♂ No. 1 ×225
81. Testis of hybrid ♂ No. 2 ×225
82. Testis of hybrid ♂ No. 3 ×225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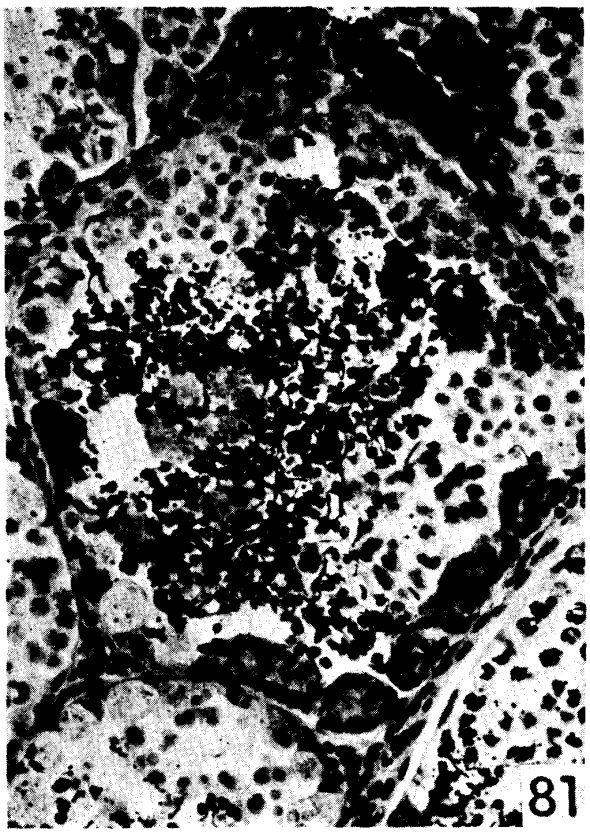
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PLATE XV

Cross-sections of the testes of male *Bufo bufo miyakonis, Bufo viridis* and hybrids between these two species at the age of one year. ×225

83. *Bufo bufo miyakonis* ♂ No. 1, *miyakonis* ♀ No. 2 × *miyakonis* ♂ No. 1
84. *Bufo viridis* ♂ No. 1, *viridis* ♀ No. 1 × *viridis* ♂ No. 1
85. Hybrid ♂ No. 2, *miyakonis* ♀ No. 1 × *viridis* ♂ No. 1
86. Hybrid ♂ No. 4, *miyakonis* ♀ No. 1 × *viridis* ♂ No. 1

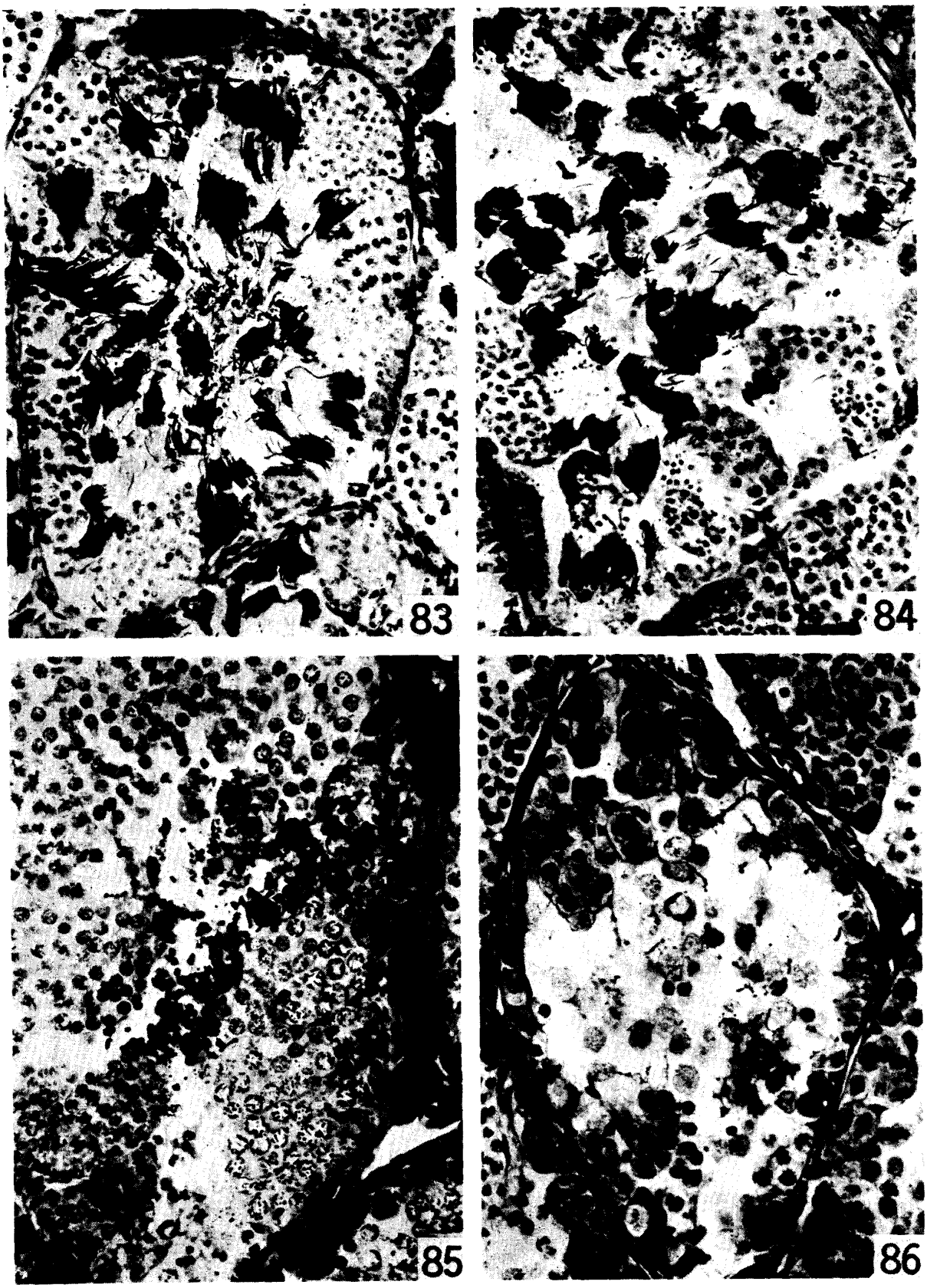
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PLATE XVI

Cross-sections of the testes of male hybrids between female *Bufo bufo japonicus* No. 6 and male *Bufo viridis* No. 1. All the hybrids were one year old.

87. Hybrid ♂ No. 1 ×225
88. The same as (87) ×450
89. Hybrid ♂ No. 4 ×110
90. The same as (89) ×225


87


88


89

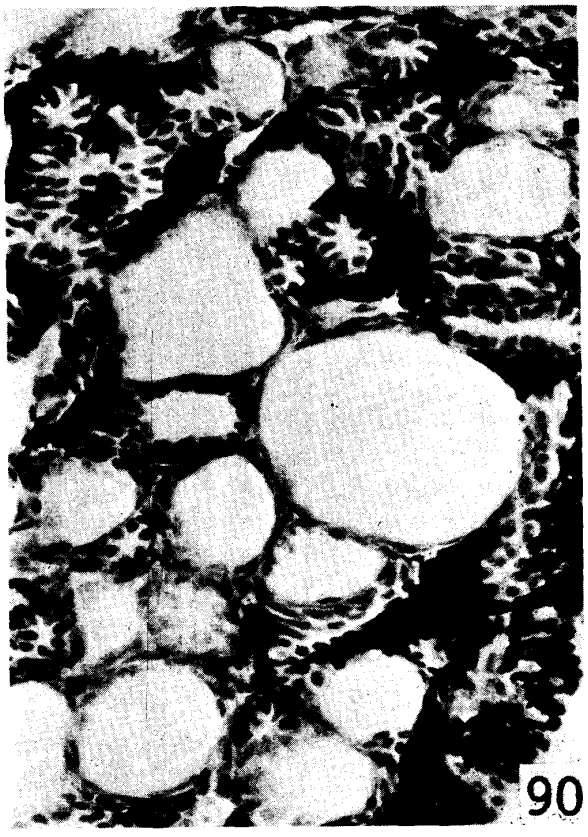

90

PLATE XVII

Cross-sections of the gonads of hybrids between female *Bufo bufo bufo* from Portugal No 3 and male *Bufo torrenticola* No. 3. All the hybrids were one year old.

91. Ovary of hybrid ♀ No. 1 × 45
92. The same as (91) ×225
93. Testis of hybrid ♂ No. 1 ×225
94. The same as (93) ×450

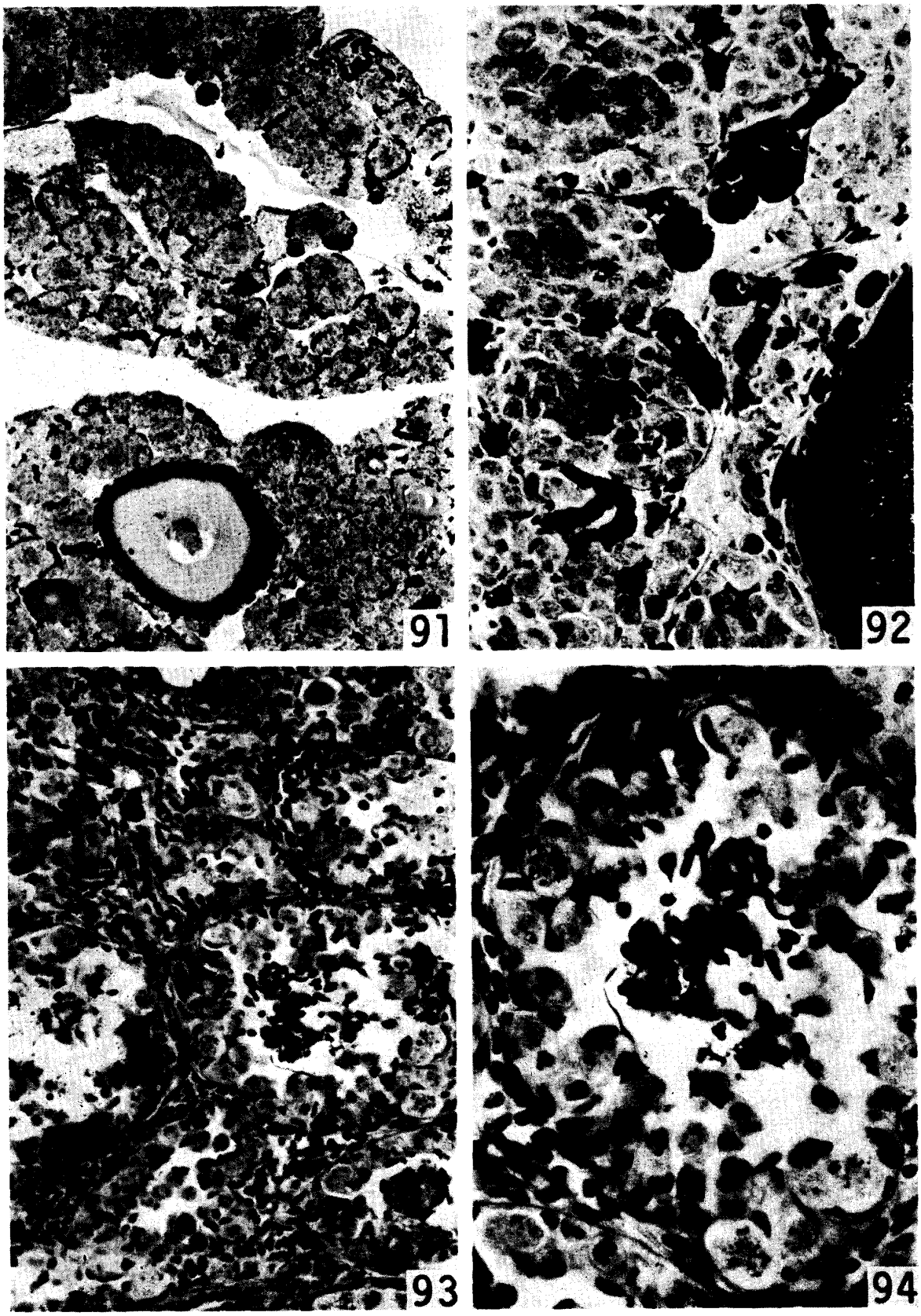

91
92
93
94

PLATE XVIII

Cross-sections of the gonads of hybrids between a female *Bufo bufo bufo* from Portugal or *Bufo americanus* and a male *Bufo viridis*, and between a female *Bufo viridis* and a male *Bufo bufo bufo* from Portugal. All the hybrids were one year old.

95. Testis of male hybrid ♂ No. 3, *bufo* from Portugal ♀ No. 4 × *viridis* ♂ No. 1 × 225
96. Testis of male hybrid ♂ No. 4, *americanus* ♀ No. 3 × *viridis* ♂ No. 1 × 225
97. Rudimentary ovary of female hybrid ♀ No. 1, *viridis* ♀ No. 1 × *bufo* from Portugal ♂ No. 5 × 225
98. The same as (97) × 450


95


96


97


98

PLATE XIX

Cross-sections of BIDDER's organs of male *Bufo bufo japonicus*, *Bufo bufo bufo* from Portugal and reciprocal hybrids between the two subspecies. All the toads were one year old. ×45

99. *Bufo bufo japonicus* ♂ No. 1, *japonicus* ♀ No. 1 × *japonicus* ♂ No. 1
100. *Bufo bufo bufo* ♂ No. 1, *bufo* from Portugal ♀ No. 1 × *bufo* from Portugal ♂ No. 3
101. Hybrid ♂ No. 1, *japonicus* ♀ No. 1 × *bufo* from Portugal ♂ No. 1
102. Hybrid ♂ No. 2, *bufo* from Portugal ♀ No. 1 × *japonicus* ♂ No. 3

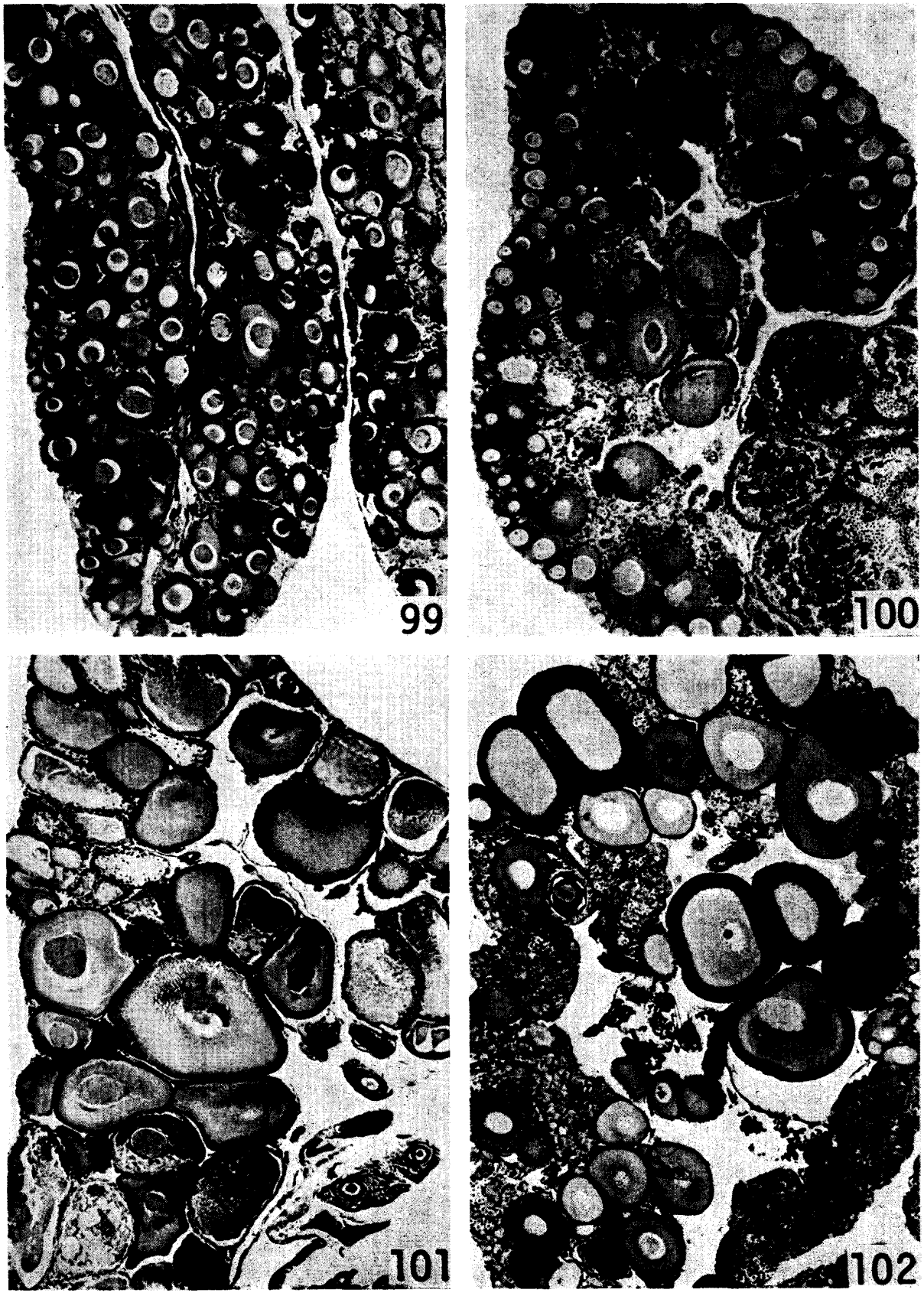
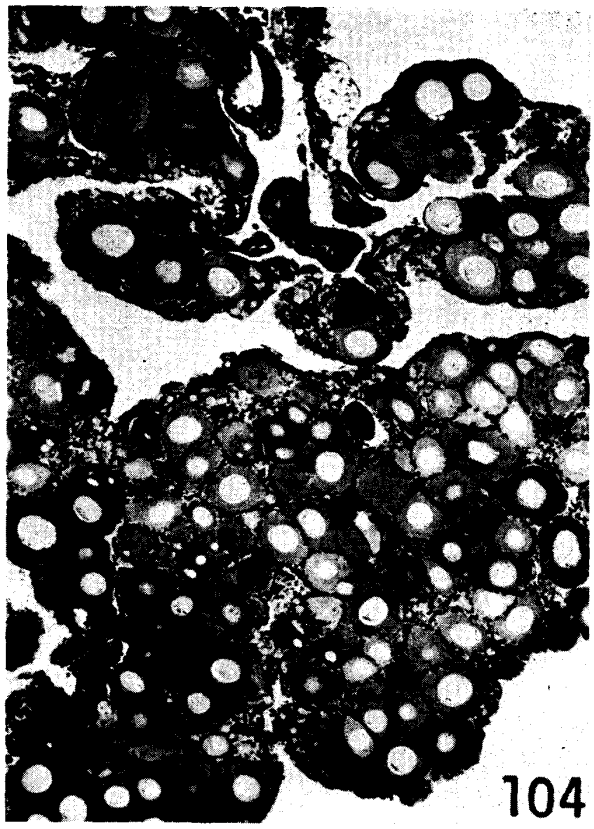

99
100
101
102

PLATE XX

Cross-sections of BIDDER's organs of male hybrids between a female *Bufo bufo japonicus* and a male *Bufo bufo* from France or Greece, and reciprocal hybrids between *Bufo bufo japonicus* and *Bufo torrenticola*. All the hybrids were one year old. ×45

103. Hybrid ♂ No. 1, *japonicus* ♀ No. 3 × *bufo* from France ♂ No. 1
104. Hybrid ♂ No. 4, *japonicus* ♀ No. 3 × *bufo* from Greece ♂ No. 1
105. Hybrid ♂ No 1, *japonicus* ♀ No 3 × *torrenticola* ♂ No. 1
106. Hybrid ♂ No. 3, *torrenticola* ♀ No. 1 × *japonicus* ♂ No. 5


103


104


105

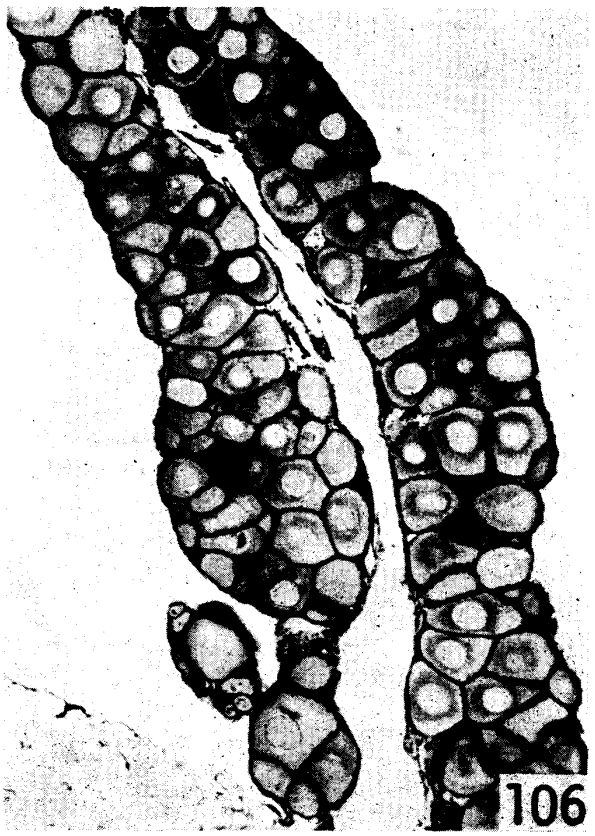

106

PLATE XXI

Cross-sections of BIDDER's organs of male hybrids between a female *Bufo bufo miyakonis* and a male *Bufo bufo japonicus, Bufo torrenticola* or *Bufo bufo bufo* from Portugal, and between a female *Bufo bufo bufo* from Portugal and a male *Bufo torrenticola*. All the hybrids were one year old. ×45

107. Hybrid ♂ No. 4, *miyakonis* ♀ No. 1 × *japonicus* ♂ No. 6
108. Hybrid ♂ No. 1, *miyakonis* ♀ No. 1 × *torrenticola* ♂ No. 3
109. Hybrid ♂ No. 2, *miyakonis* ♀ No. 1 × *bufo* from Portugal ♂ No. 5
110. Hybrid ♂ No. 1, *bufo* from Portugal ♀ No. 3 × *torrenticola* ♂ No. 3

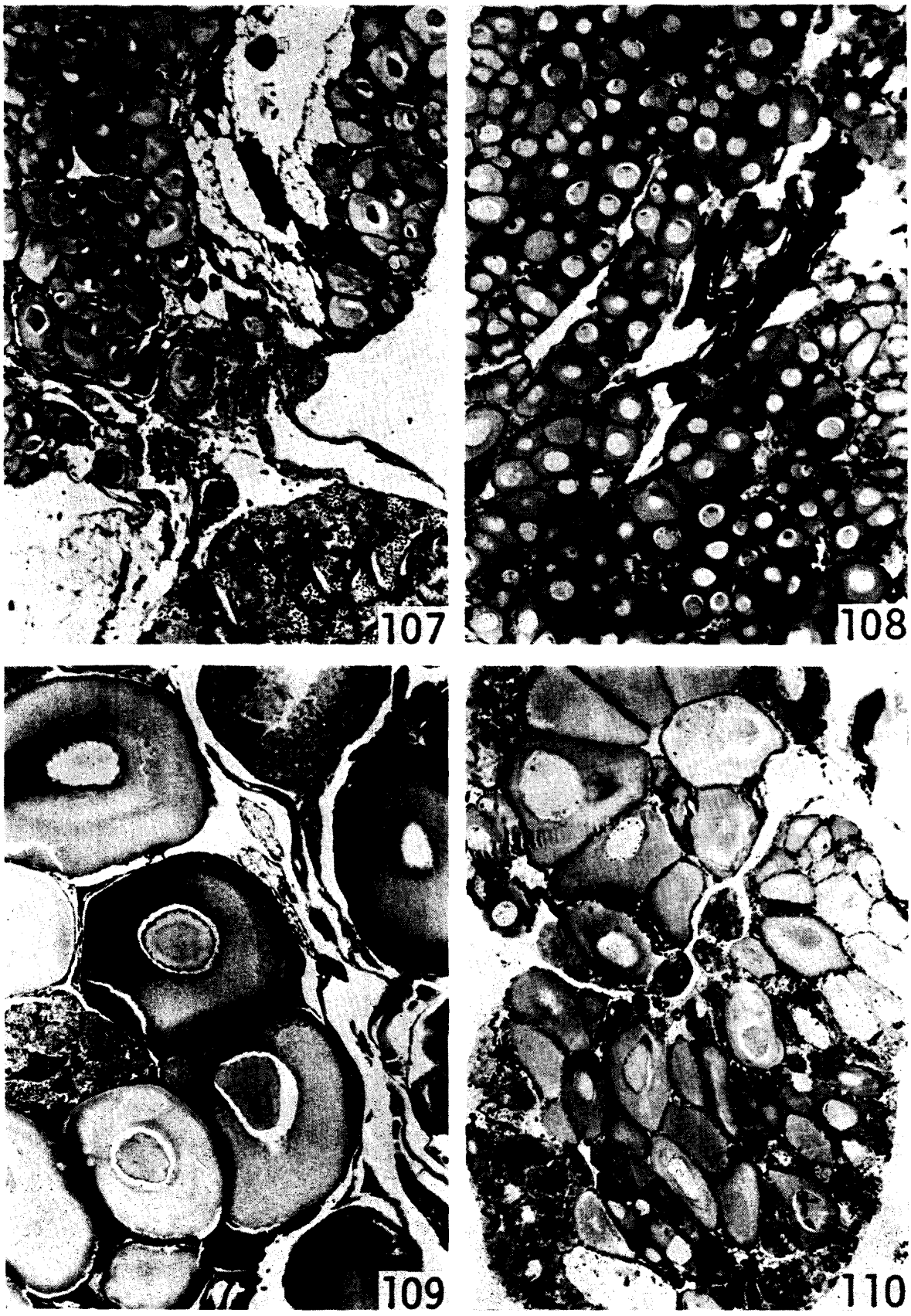
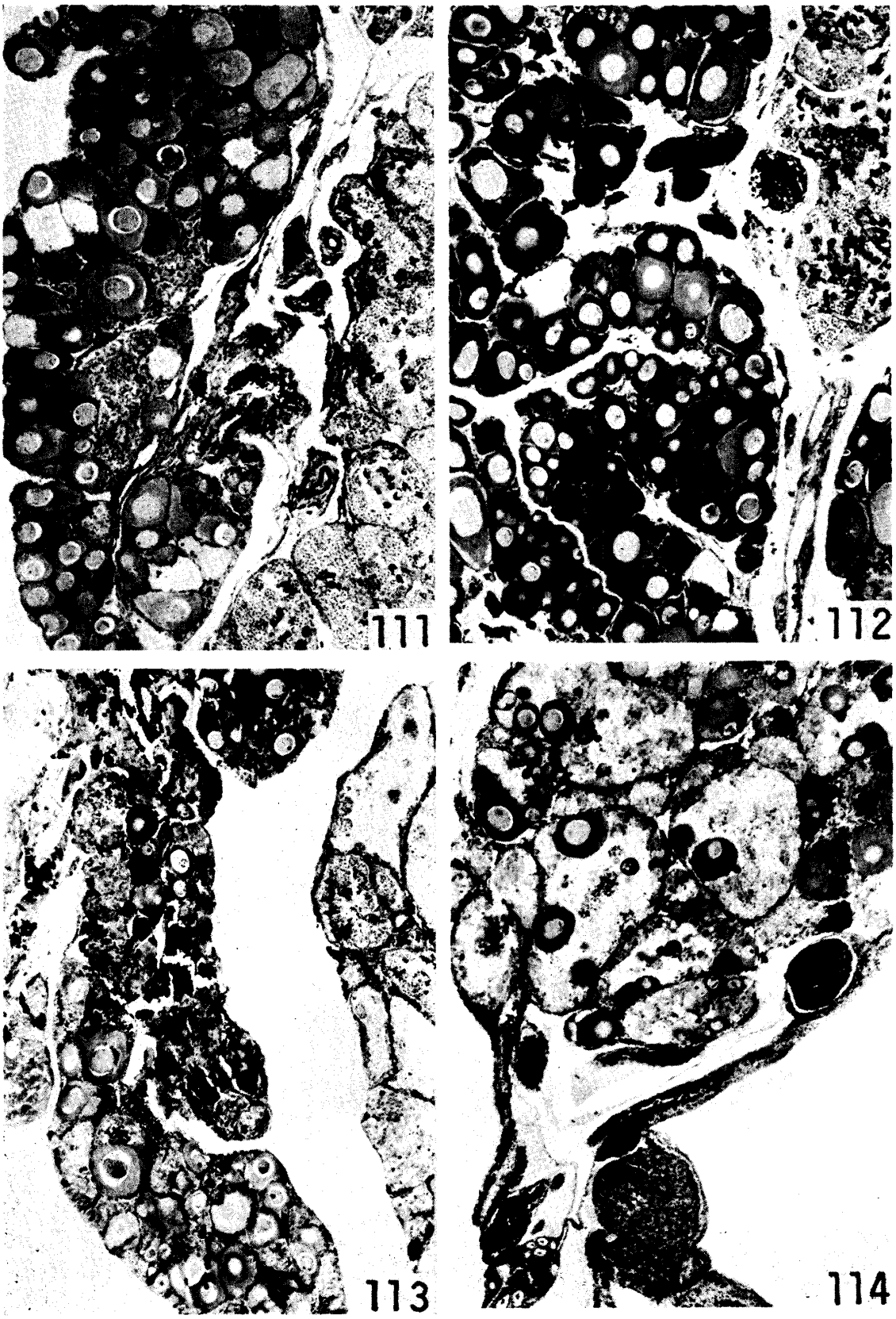

107
108
109
110

PLATE XXII

Cross-sections of BIDDER's organs of male *Bufo bufo japonicus*, *Bufo viridis* and hybrids between a female *Bufo bufo miyakonis* or *Bufo bufo japonicus* and a male *Bufo viridis*. All the toads were one year old. ×45

111. *Bufo bufo japonicus* ♂ No. 2, *japonicus* ♀ No. 6 × *japonicus* ♂ No. 6
112. *Bufo viridis* ♂ No. 1, *viridis* ♀ No. 1 × *viridis* ♂ No. 1
113. Hybrid ♂ No. 4, *miyakonis* ♀ No. 1 × *viridis* ♂ No. 1
114. Hybrid ♂ No. 4, *japonicus* ♀ No. 6 × *viridis* ♂ No. 1


111
112
113
114