

Interspecific Hybrids between *Bombina orientalis* (BOULENGER) and *B. variegata* (L.)

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(With 4 Plates)

INTRODUCTION

The genus *Bombina* has only five species. While two species, *B. bombina* and *B. variegata*, are distributed in Europe, the other three, *B. orientalis*, *B. maxima* and *B. microdeladigitata*, are distributed in Asia (NIKOL'SKII, 1918; LIU, 1950; SHANNON, 1956). MICHALOWSKI (1966) and MICHALOWSKI and MADEJ (1969) produced interspecific hybrids of the two European species to clarify their relationship. RAEHMEL (1974) reported that interspecific hybrids were obtained between a female *B. orientalis* and a male *B. variegata* in an aquaterrarium.

In order to examine the existence of reproductive isolating mechanisms between European and Korean *Bombina* species, the author carried out hybridization experiments in 1969 and 1970 by making use of *B. variegata* and *B. orientalis*. A preliminary report of this research was made previously (1970).

MATERIALS AND METHODS

Bombina orientalis (BOULENGER) were collected from the vicinity of Seoul in 1963. Three males and two females were caught by Professor G. Goo and were soon brought to this laboratory. The two females and two pairs of their progeny were utilized as materials in the present study. Two pairs of *Bombina variegata* (L.) collected from West Germany were sent to this laboratory in 1969 by Mr. R. THORN, Luxemburg.

On July 4, 1969, two pairs of *Bombina orientalis* and a female and two male *Bombina variegata* were injected with suspension of pituitaries of *Rana nigromaculata* at the rate of one pituitary per head. Each of the females was coupled at once with a male of the other species in a vessel which contained water at a depth of 5~6 cm. This vessel was kept dark and at a temperature of about 25°C. However, no amplexus occurred, although the male was actively giving a mating call and trying to clasp the female for about six hours after the treatment. If a male and a female of the same species are placed in such a condition after pituitary injection, they are always induced to amplexus and would lay fertilized eggs (KAWAMURA, NISHIOKA and UEDA, 1972). About twelve hours after pituitary

injection, eggs were taken out of the cloaca by pressing the abdomen of each female, since she began to spawn without amplexus. These eggs were inseminated with sperm of the other species as well as of the same species.

On July 24, 1970, reciprocal hybrids between *Bombina orientalis* and *B. variegata* were again produced by artificial insemination. In this experiment, a male and a female of each species were used. The ovulation of each female was accelerated by injection of frog pituitary suspension. In order to compare the reciprocal hybrids with the controls in developmental velocity, twenty eggs in each of the experimental and the control series were raised at 25°C from 20 minutes after the insemination to the latest tadpole stage in which the fore-legs began to protrude.

Tadpoles were fed on boiled soft vegetables like Japanese spinach or chard. Metamorphosed toads were mainly reared with worms of Tubificidae, such as *Limnodrilus* or *Tubifex*. Gonads were fixed in NAVASHIN's fluid. Sections were cut at 12 μ and stained with HEIDENHAIN's iron hematoxylin.

OBSERVATION

I. Viability

1. Hybrids of *Bombina orientalis* ♀ × *B. variegata* ♂

In 1969, 296 of 426 eggs obtained from two female *orientalis* were inseminated with sperm of a male *variegata*, while the other 130 were inseminated with sperm of a male *orientalis* (Table 1). As a result, 239 (80.7%) and 119 (91.5%) eggs cleaved normally in the experimental and the control series, respectively. Besides them, 38 (12.8%) eggs in the experimental series cleaved abnormally, while only 4 (3%) in the control series did so. In the experimental series, 213 (72.0%) and 203 (68.6%) eggs became normal gastrulae and tail-bud embryos, respectively, and 198 (66.9%) hatched normally. The other embryos died of edema, microcephaly or some other abnormalities. In the control series, 107 (82.3%),

TABLE 1
Results of crosses between

Year	Parents		Diameter of eggs (mm)	No. of eggs	No. of Normal (%)
	Female	Male			
1969	<i>orient. I</i>	<i>orient. I</i>	2.5	77	74 (96.1)
		<i>varieg. I</i>		196	169 (86.2)
1969	<i>orient. II</i>	<i>orient. I</i>	2.5	53	45 (84.9)
		<i>varieg. I</i>		100	70 (70.0)
1970	<i>orient. III</i>	<i>orient. II</i>	2.5	15	13 (86.7)
		<i>varieg. II</i>		66	58 (87.9)
1969	<i>varieg. I</i>	<i>varieg. I</i>	2.0	135	128 (94.8)
		<i>orient. I</i>		87	85 (97.7)
1970	<i>varieg. II</i>	<i>varieg. II</i>	2.0	62	57 (91.9)
		<i>orient. II</i>		57	50 (87.7)

101 (77.7%) and 98 (75.4%) eggs became normal gastrulae, tail-bud embryos and hatched tadpoles, respectively. During the tadpole stage, the hybrids did not differ from the controls in viability. In the experimental series, 175 (59.1%) tadpoles metamorphosed normally, while 87 (66.9%) did so in the control series.

In 1970, 66 of 81 eggs taken out of a female *orientalis* were inseminated with sperm of a male *variegata*, while the other 15 eggs were done with sperm of a male *orientalis*. In the experimental series, 58 (87.9%) eggs cleaved normally, while 13 (86.7%) did so in the control series. Most of the normally cleaved eggs in the experimental series developed normally; 44 (66.7%) hatched normally and 42 (63.6%) became normally metamorphosed toads. In the control series, 12 (80.0%) eggs hatched normally and 11 (73.3%) became normal toads.

2. Hybrids of *Bombina variegata* ♀ × *B. orientalis* ♂

In 1969 and 1970, 341 eggs obtained from two female *variegata* were inseminated with sperm of the same males which were used for insemination of the eggs of the three female *orientalis* stated above. In the two experimental series, 135 (93.8%) of 144 eggs cleaved normally by insemination with sperm of the two male *orientalis*, while 185 (93.9%) of 197 eggs did so by insemination with sperm of the two male *variegata* stated above. While 167 (84.8%), 163 (82.7%) and 146 (74.1%) eggs became normal gastrulae, normally hatched tadpoles and normally metamorphosed toads, in the control series, respectively, 127 (88.2%), 126 (87.5%) and 117 (81.3%) of eggs became normal gastrulae, normally hatched tadpoles and normally metamorphosed toads in the experimental series, respectively.

II. Developmental velocity

Reciprocal hybrids and the controls were compared with one another in the developmental velocity at the temperature of 25°C (Tables 2 and 3). Two hours after insemination all the eggs in the experimental and the control series completed their first cleavage. The subsequent cleavage of the eggs proceeded almost simultaneously until the 32-cell stage, five hours after insemination. Since

Bombina orientalis and *B. variegata*

cleaved eggs Abnormal (%)	No. of normal gastrulae (%)	No. of normal tail-bud embryos (%)	No. of normal hatched larvae (%)	No. of metamor- phosed toads (%)
2 (2.6)	65 (84.4)	60 (77.9)	58 (75.3)	52 (67.5)
23 (11.7)	145 (74.0)	143 (73.0)	143 (73.0)	123 (62.8)
2 (3.8)	42 (79.2)	41 (77.4)	40 (75.5)	35 (66.0)
15 (15.0)	68 (68.0)	60 (60.0)	55 (55.0)	52 (52.0)
0 (0)	12 (80.0)	12 (80.0)	12 (80.0)	11 (73.3)
6 (9.1)	50 (75.8)	50 (75.8)	44 (66.7)	42 (63.6)
2 (1.5)	112 (83.0)	108 (80.0)	108 (80.0)	99 (73.3)
0 (0)	80 (92.0)	80 (92.0)	80 (92.0)	75 (86.2)
1 (1.6)	55 (88.7)	55 (88.7)	55 (88.7)	47 (75.8)
1 (1.8)	47 (82.5)	46 (80.7)	46 (80.7)	42 (73.7)

TABLE 2
Embryonic stages of reciprocal hybrids between *Bombina orientalis* and
B. variegata and the controls at definite ages at 25°C (1970)

Kind	Age (hours)									
	2	5	10	24	29	50	79	96	120	144
<i>orient.</i> × <i>orient.</i>	3L	7	9	14E	15	19	21L	24	25	
<i>orient.</i> × <i>varieg.</i>	3	7	9E	13L	15	19	21	23L	25	
<i>varieg.</i> × <i>orient.</i>	3	7	8L	13	14L	19E	21	23E	25E	25
<i>varieg.</i> × <i>varieg.</i>	3	7	8	12L	14	19E	21E	22L	24E	25

E, Early. L, Late. Each stage corresponds to that of SHUMWAY's table.

TABLE 3
Larval stages of reciprocal hybrids between *Bombina orientalis* and *B. variegata*
and the controls at definite ages at 25°C (1970)

Kind	Age (days)							
	10	12	21	25	28	31	43	
<i>orient.</i> × <i>orient.</i>	IV	VI	XIV	XX				
<i>orient.</i> × <i>varieg.</i>	III	V	XII	XIV	XVII	XX		
<i>varieg.</i> × <i>orient.</i>	II	V	XII	XV	XX			
<i>varieg.</i> × <i>varieg.</i>	II	IV	IX	XII	XV	XVII	XX	

Each stage corresponds to that of TAYLOR and KOLLROS' table.

that time, slight differences began to appear in developmental velocity among both kinds of hybrids and the controls. Twenty-four hours after insemination, the *orientalis* embryos revealed distinct neural folds, while the *variegata* embryos were at the late gastrula stage. Although both kinds of hybrids were intermediate between the two species in developmental stage, the *orientalis* ♀ × *variegata* ♂ hybrids were somewhat more advanced than the reciprocal hybrids.

The *orientalis* and the *orientalis* ♀ × *variegata* ♂ hybrids began to take food five days after insemination, while the *variegata* and the *variegata* ♀ × *orientalis* ♂ hybrids did so one day later. To attain the stage in which the length of the limb bud is twice its diameter, the *orientalis*, the *variegata* and both kinds of hybrid tadpoles required 11, 13 and 12 days after insemination, respectively. Fore-limbs protruded in more than half the tadpoles of *orientalis*, *variegata* ♀ × *orientalis* ♂, *orientalis* ♀ × *variegata* ♂ and *variegata* at the ages of 25, 28, 31 and 43 days, re-

TABLE 4
Measurements of reciprocal hybrids between

Parents		Tadpoles immediately before feeding (Stage 25)				Tadpoles with (Stage)	
Female	Male	Total length (mm)	Body length (mm)	Head width (mm)	Tail height (mm)	Total length (mm)	Body length (mm)
<i>orient.</i>	<i>orient.</i>	14.9 ± 0.05	7.0 ± 0.01	4.0 ± 0.03	4.0 ± 0.03	25.0 ± 0.1	12.2 ± 0.1
<i>orient.</i>	<i>varieg.</i>	15.0 ± 0.02	7.0 ± 0.02	4.0 ± 0.03	4.0 ± 0.03	24.5 ± 0.3	11.4 ± 0.3
<i>varieg.</i>	<i>orient.</i>	13.5 ± 0.05	6.2 ± 0.05	3.5 ± 0.03	3.8 ± 0.06	22.6 ± 0.1	11.1 ± 0.1
<i>varieg.</i>	<i>varieg.</i>	12.9 ± 0.04	6.1 ± 0.05	3.5 ± 0.03	3.8 ± 0.05	21.5 ± 0.4	10.3 ± 0.2

spectively.

In the experiments carried out under room temperature, *orientalis* ♀ × *variegata* ♂ hybrids required 38.2 days in 1969 and 31.8 days in 1970 on the average, while *variegata* ♀ × *orientalis* ♂ required 38.8 days in 1969 and 32.3 days in 1970 to attain the stage of fore-limb protrusion from the insemination. On the other hand, *orientalis* required 35.3 days in 1969 and 26.7 days in 1970 on the average, while *variegata* required 45.3 days in 1969 and 34.2 days in 1970 to attain the same stage.

III. Morphology

1. Measurements

The eggs of *orientalis* are somewhat larger than those of *variegata*. During the embryonic stages, reciprocal hybrids were almost equal to the embryos of their maternal species in size; the size of embryos corresponded with that of the eggs. However, at the stage immediately before feeding, the *variegata* ♀ × *orientalis* ♂ hybrids were a little larger than the *variegata* tadpoles in total length (Table 4). When measurements were made on 20 tadpoles of each series at the limb-bud stage, the *orientalis* were the largest and the *variegata* were the smallest in total length among the four kinds of tadpoles. The *orientalis* ♀ × *variegata* ♂ hybrids were almost similar to the *orientalis* in total length, while the *variegata* ♀ × *orientalis* ♂ hybrids were a little larger than the *variegata* and a little smaller than the reciprocal hybrids. While the *variegata* were slightly larger than the *orientalis* in head width, both kinds of hybrids were nearly equal to or somewhat larger than the *variegata* in this respect. In tail height, the *variegata* were the largest, both kinds of hybrids were the next and the *orientalis* were the smallest.

At the stage of fore-limb extrusion, the *orientalis* were the largest, the *orientalis* ♀ × *variegata* ♂ hybrids the second, the *variegata* ♀ × *orientalis* ♂ hybrids the third and the *variegata* the smallest in body length as well as in total length. At the age of nine or 12 months, the four kinds of toads were arranged in the same order as the above in body length (Table 4).

2. External feature

One-year-old toads in each of the four series were examined in terms of ex-

Bombina orientalis and *B. variegata* and the controls

limb-buds V)		Tadpoles immediately after protrusion of fore-limbs (Stage XX)		9-month- old toads	1-year- old toads
Head width (mm)	Tail height (mm)	Total length (mm)	Body length (mm)	Body length (mm)	Body length (mm)
5.7 ± 0.06	5.3 ± 0.07	38.7 ± 0.4	14.5 ± 0.1	34.1 ± 0.6	38.5 ± 0.23
6.2 ± 0.1	5.7 ± 0.1	36.6 ± 0.2	14.0 ± 0.07	32.9 ± 0.6	36.7 ± 0.83
6.1 ± 0.03	5.8 ± 0.1	35.8 ± 0.3	13.9 ± 0.09	27.6 ± 0.5	35.7 ± 1.23
6.0 ± 0.1	6.0 ± 0.1	34.5 ± 0.3	13.4 ± 0.09	29.5 ± 0.4	33.5 ± 0.39

ternal features. The *orientalis* in the control series were dull yellow-green in ground color of the dorsal surface; the *variegata* were grayish brown. Most of the *orientalis* ♀ × *variegata* ♂ hybrids were almost uniformly pale yellow-brown, while the others were a mosaic of pale yellow-brown and dull yellow-green parts. The *variegata* ♀ × *orientalis* ♂ hybrids were all yellowish brown.

Both *orientalis* and *variegata* have numerous round tubercles on the entire dorsal surfaces. In the control *orientalis*, these tubercles were mostly arranged in longitudinal rows. Especially in six places situated in the two rows along the median line, two to several tubercles were connected with one another to form an elongate wart which was colored jet-black. The other tubercles had usually a small black spot (Plate I, 1). In the control *variegata*, the tubercles on the back were usually isolated from one another; elongate warts were very few. The tubercles of this species were generally a little larger than those of the *orientalis* and not colored black, although they were somewhat dark (Plate I, 7).

The two kinds of hybrids in the experimental series were intermediate between the two species in appearance. Although all the hybrids had some elongate warts constructed from more than two tubercles, the connection between the tubercles was not so close as in the *orientalis*. Moreover, the elongate warts were not colored black (Plate I, 3, 5).

The ventral surfaces of *orientalis* collected from the field are reddish orange with clear-cut black patches. Differing from these specimens, the toads raised in this laboratory were dull yellow-orange in ground color at the age of one year. Although this color gradually increased in tinge as they became older, it was bright orange even at the age of six years. The ventral surfaces of *variegata* collected from the field are vivid yellow with clear-cut gray patches and minute dark dots. The toads of this species raised in this laboratory were yellowish white in ground color of the ventral surfaces at the age of one year, and became light yellow at the age of six years (Plate I, 2, 8).

The two kinds of hybrids in the experimental series were intermediate between the two species of the control series in ground color of the ventral surfaces; they were pale yellow at the age of one year. However, the *orientalis* ♀ × *variegata* ♂ hybrids somewhat differed from the reciprocal hybrids in color at the age of six years. The former were bright yellowish-orange, while the latter were light yellow. The clear-cut gray patches on the ventral surfaces of reciprocal hybrids were similar in color to those of *variegata*, when observed at the age of one year. However, they became black and similar to those of *orientalis* at the age of six years. In contrast with these patches, minute dark spots distributed on the ventral surfaces of the *variegata* were always found in the two kinds of hybrids.

All the male hybrids which attained sexual maturity revealed secondary sexual characters in the breeding season. They had a pad-like swelling on the inner side of the first finger. Their dorsal surfaces were rougher than those of the female hybrids; their dermal tubercles were somewhat pointed.

IV. Sex

1. Gonads of juvenile toads

The testes of male hybrids killed shortly after metamorphosis did not distinctly differ from those of males in the control series in size, shape and inner structure. The ovaries of female hybrids at the same stage were nearly normal in inner structure, although they contained no growing oocytes, differing from those of the control females.

The sex ratio of juvenile hybrids was almost 1:1. Of 60 hybrids between three female *orientalis* and two male *variegata*, 30 were females and 30 were males. Of 48 hybrids between two female *variegata* and two male *orientalis*, 21 were females and 27 were males. In the control series, 14 of 25 *orientalis* were females and 11 were males, while 22 of 51 *variegata* were females and 29 were males (Table 5).

TABLE 5
Sex of reciprocal hybrids between *Bombina orientalis* and *B. variegata* and the controls

Parents		Toads shortly after metamorphosis		Mature toads				Total	
Female	Male	Female	Male	Female		Male		Female	Male (%)
				With normal ovaries	With abnormal ovaries	With normal testes	With abnormal testes		
<i>orient. I</i>	<i>orient. I</i>	7	6	13	0	16	0	20	22 (52.4)
<i>orient. I</i>	<i>varieg. I</i>	11	9	0	42	0	55	53	64 (54.7)
<i>orient. II</i>	<i>orient. I</i>	6	5	8	0	10	0	14	15 (51.8)
<i>orient. II</i>	<i>varieg. I</i>	12	16	0	10	0	11	22	27 (55.1)
<i>orient. III</i>	<i>orient. II</i>	1	0	4	0	3	0	5	3 (37.5)
<i>orient. III</i>	<i>varieg. II</i>	7	5	0	14	0	13	21	18 (46.2)
<i>varieg. I</i>	<i>varieg. I</i>	2	5	39	0	38	0	41	43 (51.2)
<i>varieg. I</i>	<i>orient. I</i>	3	6	0	38	0	25	41	31 (43.1)
<i>varieg. II</i>	<i>varieg. II</i>	20	24	0	0	0	0	20	24 (54.5)
<i>varieg. II</i>	<i>orient. II</i>	18	21	0	0	0	0	18	21 (53.8)

2. Gonads of mature toads

Male and female *orientalis* or *variegata* in the control series matured at the age of one year. The ovaries of the females had abundant mature eggs (Plate II, 13, 16). In the testes of the males, the seminal tubules were filled with bundles of spermatozoa (Plate IV, 21, 22). The gonads of reciprocal hybrids at the age of one year were very abnormal.

a. *Bombina orientalis* ♀ × *B. variegata* ♂

The ovaries of female hybrids were similar to those of 3-month-old females of the control series in size and shape, although the female hybrids had well-developed oviducts (Plate II, 14). The ovaries were very abnormal in inner structure; they contained numerous cysts of germ cells in place of auxocytes (Plate II,

17, 18). These cysts were roundish or polygonal, being about $50\sim 70\ \mu$ in largest diameter. Each of them was surrounded with a cyst wall. The germ cells in each cyst were mostly primary or secondary oögonia. Young oocytes were found in some cysts, together with actively dividing germ cells. Degenerating germ cells were usually abundant in each cyst. In the marginal portion of the ovary, numerous primary oögonia were arranged along the theca externa. Two of 15 females whose ovaries were sectioned had a few growing auxocytes. The latter were all degenerating or showed signs of degeneration. After degeneration of auxocytes, their follicle cells became like undifferentiated cells having spherical nuclei without any increase in number.

The testes of male *orientalis* ♀ × *variegata* ♂ hybrids were similar to those of the control *variegata* in shape, while they were more slender than those of the control *orientalis*. The male *orientalis* in the control series had small vestiges of Müllerian ducts at the anterior ends of the kidneys, while the male *variegata* had slender Müllerian ducts which attained the level of anterior one-third to two-thirds of the kidney. The Müllerian ducts of the male *orientalis* ♀ × *variegata* ♂ were rather well-developed, although they were similar to those of the male *variegata* in length (Plate II, 9, 10, 12). In the testes of the male hybrids, the seminal tubules were mostly filled with secondary spermatogonia and first spermatocytes. A small number of primary spermatogonia with polymorphic nuclei were arranged along the walls of the seminal tubules. Some spermatogonia repeated mitoses without making cytoplasmic division and became huge cells having a large nucleus. These cells degenerated sooner or later. Some other secondary spermatogonia degenerated at their latest stage. Although first spermatocytes were numerous in most seminal tubules, they did not make normal reduction division. Most of them degenerated before the completion of the first reduction division, while a few became large abnormal spermatozoa after abortive first reduction division (Plate IV, 23).

It was quite clear that the male and female *orientalis* ♀ × *variegata* ♂ hybrids were completely sterile, as their gonads were extremely abnormal in inner structure. The sex ratio of the one-year-old hybrids produced from the crosses between three female *orientalis* and two male *variegata* was nearly the same as that of the one-year-old controls obtained by mating the same three females with two male *orientalis*. Of 145 hybrids, 66 were females and 79 (54.5%) were males, while 25 were females and 29 (53.7%) were males among 54 control toads.

b. *Bombina variegata* ♀ × *B. orientalis* ♂

The ovaries of female hybrids at the age of one year were similar in appearance to those of the control females at one month after metamorphosis. However, the female hybrids had well-developed oviducts (Plate II, 15). The ovaries were simple in inner structure; they consisted of cortical portions surrounding ovarian cavities. The cortical portions and the walls of the ovarian cavities were somewhat hypertrophied with growth of connective tissues. A small number of oögonia were found in the cortical portions (Plate III, 19, 20).

The testes of male hybrids were similar to those of male *variegata* of the control series in size and shape. The Müllerian ducts of the male hybrids were rather well-developed, although they were similar to those of the control male *variegata* in length (Plate II, 11). All the ten males whose testes were sectioned had only a few spermatogonia in abnormal seminal tubules that were slender and solid. These seminal tubules were supported with abundant stroma cells besides rete apparatus (Plate IV, 24).

From the abnormal structures of gonads stated above, it was quite clear that the male and female *variegata* ♀ × *orientalis* ♂ hybrids were completely sterile, as were the reciprocal hybrids. The sex ratio of the one-year-old hybrids produced from the cross between a female *variegata* and a male *orientalis* was somewhat different from that of the one-year-old controls obtained by mating the same female with a male *variegata*. While 39 of 77 control *variegata* were females and 38 (49.4%) were males, 38 of 63 hybrid toads were females and 25 (39.7%) were males (Table 5).

DISCUSSION

Interspecific hybrids of the two European *Bombina* species were already reported by HERON-ROYER as early as 1891. When a couple of a female *Bombina bombina* and a male *Bombina variegata* as well as a couple of the reciprocal combination was kept in an aquarium, amplexus occurred and the female spawned. Ten *bombina* ♀ × *variegata* ♂ and 18 *variegata* ♀ × *bombina* ♂ hybrids metamorphosed normally. While almost all of them perished in a storm, three of the former and one of the latter remained alive and grew normally. HERON-ROYER's experiment was repeated by MICHALOWSKI (1966) and MICHALOWSKI and MADEJ (1969) to clarify the relationship of *Bombina bombina* and *Bombina variegata*. They obtained 40 metamorphosed hybrids, of which 22 were produced from a cross between a female *variegata* and a male *bombina*, and 18 were from a cross of the reciprocal combination. Of the former hybrids, 12 were females and 10 were males, while 5 of the latter were females and 13 were males. According to MICHALOWSKI and MADEJ, the interspecific hybrids of the two *Bombina* species were fertile, although the details of backcross experiments carried out by MICHALOWSKI were not published.

Regarding the interspecific hybrids between Asian and European *Bombina* species, RAEHMEL (1974) published a short report for the first. A female *Bombina orientalis* kept in couple with a male *Bombina variegata* in an aquaterrarium laid eggs after amplexus. Although a few eggs were not fertilized, the other developed normally, and eventually 40 normal tadpoles and some young toads were produced. From the results obtained by the author in the present study, it seems quite clear that there is no remarkable gametic isolation between *Bombina orientalis* and *Bombina variegata*, as the percentages of normally cleaved eggs in reciprocal crosses performed by artificial insemination were nearly the same as those of the control series. However, the existence of sexual isolation is not

always denied. While amplexus occurred between a female *orientalis* and a male *variegata* in RAEHMEL's experiment, this did not occur in two couples of the same combination in the present study, in spite of the injection of frog pituitaries.

The Asian and European *Bombina* species are not isolated from each other by hybrid inviability. Reciprocal hybrids between the two species develop normally during embryonic and tadpole stages nearly as the controls do. They metamorphose normally and grow into mature toads. However, these two species seem to be completely isolated from each other by hybrid sterility, differing from the relationship between the two European species. Both males and females of reciprocal hybrids have extremely abortive gonads.

The existence of isolating mechanisms between East Asian and European species, subspecies or populations in some other anurans has been reported by KAWAMURA and KOBAYASHI (1960), KAWAMURA and NISHIOKA (1962, '73, '75), KAWAMURA, NISHIOKA and KURAMOTO (1972), NISHIOKA (1972) and DAITO (1968). In pond frogs, Japanese *Rana nigromaculata* and *R. brevipoda* and Korean *Rana nigromaculata* and *R. plancyi chosonica* are completely isolated from European *Rana esculenta* (= *lessonae*) and *R. ridibunda ferezi* by hybrid inviability or hybrid sterility. In brown frogs, Japanese *Rana japonica*, *R. ornativentris* and *R. chensinensis* are completely isolated from European *Rana arvalis* and *R. temporaria* by gametic isolation, hybrid inviability or hybrid sterility. *Rana chensinensis* distributed in Hokkaido, Japan is so similar to European *Rana temporaria temporaria* in morphological characters as having been identified with the latter subspecies for many years. This name was changed into *Rana chensinensis* by KAWAMURA (1962), mainly on the basis of isolating mechanisms. DAITO (1968) reported that the male and female hybrids between female Japanese tree-frogs, *Hyla arborea japonica* and a male Corsican *Hyla arborea sarda* were sterile. It is very probable that all the East Asian anurans are completely isolated from allied European anurans by physiological isolating mechanisms, even if they are similar to the latter in appearance, although nothing has yet been known about the *Bufo* group.

SUMMARY

1. Crosses were made between *Bombina orientalis* from Korea and *Bombina variegata* from West Germany. Reciprocal hybrids were produced by the method of artificial fertilization.

2. Although the two *Bombina* species are not isolated from each other by gametic isolation nor by hybrid inviability, they are completely isolated by hybrid sterility.

3. Interspecific hybrids are intermediate between the two parental species in the developmental velocity at embryonic and tadpole stages at 25°C, as well as in the morphological characters of tadpoles and toads. However, slight differences are found between the two kinds of hybrids in these two respect.

4. Nearly equal numbers of male and female hybrids are produced. Both

males and females have extremely abortive gonads.

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EXPLANATION OF PLATES

PLATE I

Bombina orientalis, *B. variegata* and their reciprocal hybrids at the age of one year. × 1.2

1, 2. *Bombina orientalis* (♀).

3, 4. Hybrid (♀) between a female *Bombina orientalis* and a male *B. variegata*.

5, 6. Hybrid (♀) between a female *Bombina variegata* and a male *B. orientalis*.

7, 8. *Bombina variegata* (♀).

H. UEDA

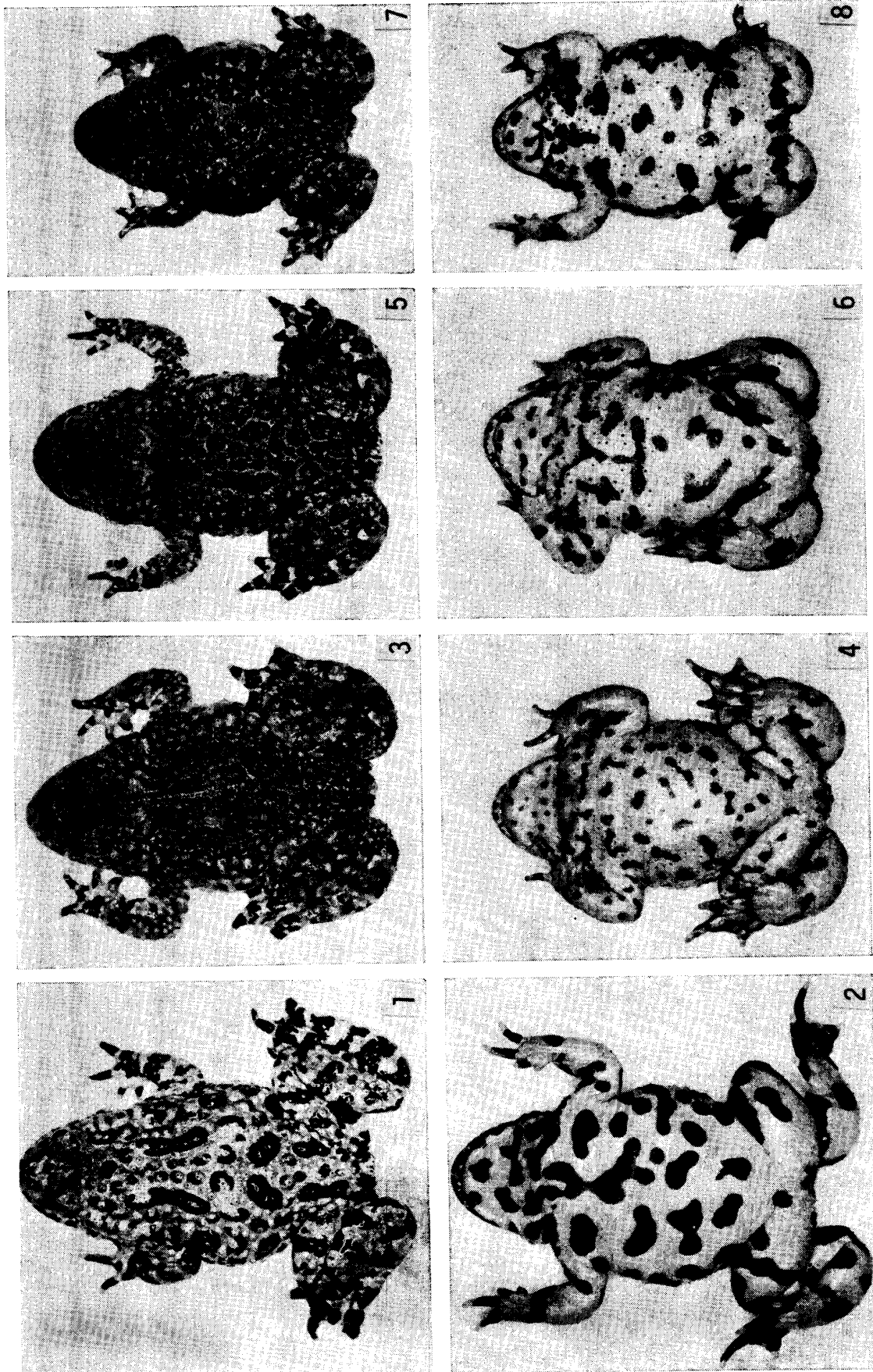


PLATE II

Gonads of *Bombina orientalis*, *B. variegata* and their reciprocal hybrids at the age of one year.

9. Testes of a male *Bombina orientalis*. × 3.8
10. Testes of a male hybrid between a female *Bombina orientalis* and a male *B. variegata*.
× 3.8
11. Testes of a male hybrid between a female *Bombina variegata* and a male *B. orientalis*.
× 3.8
12. Testes of a male *Bombina variegata*. × 3.8
13. Ovaries of a female *Bombina orientalis*. × 2.0
14. Ovaries of a female hybrid between a female *Bombina orientalis* and a male *B. variegata*.
× 3.8
15. Ovaries of a female hybrid between a female *Bombina variegata* and a male *B. orientalis*.
× 3.8
16. Ovaries of a female *Bombina variegata*. × 2.0

H. UEDA

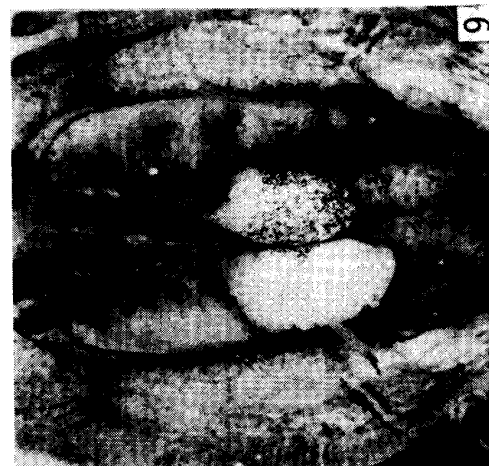
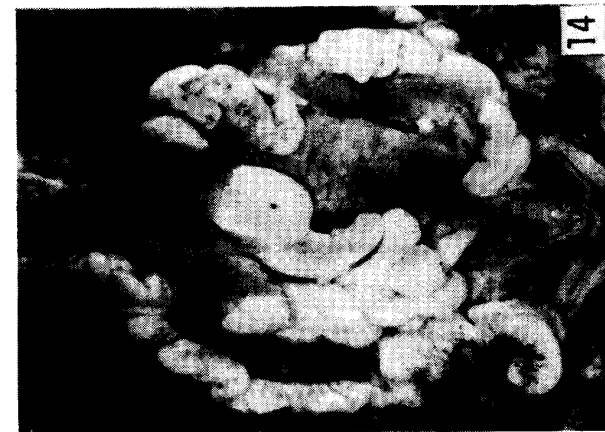
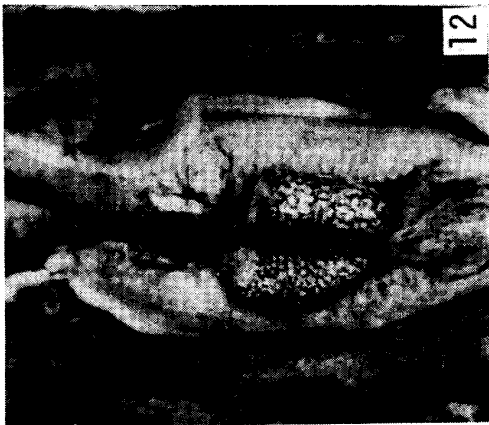


PLATE III

Cross-sections of the ovaries of female hybrids between *Bombina orientalis* and *B. variegata* at the age of one year.

17. A female hybrid between a female *Bombina orientalis* and a male *B. variegata*. × 60.
18. Ditto. × 270.
19. A female hybrid between a female *Bombina variegata* and a male *B. orientalis*. × 110.
20. Ditto. × 270.

H. UEDA

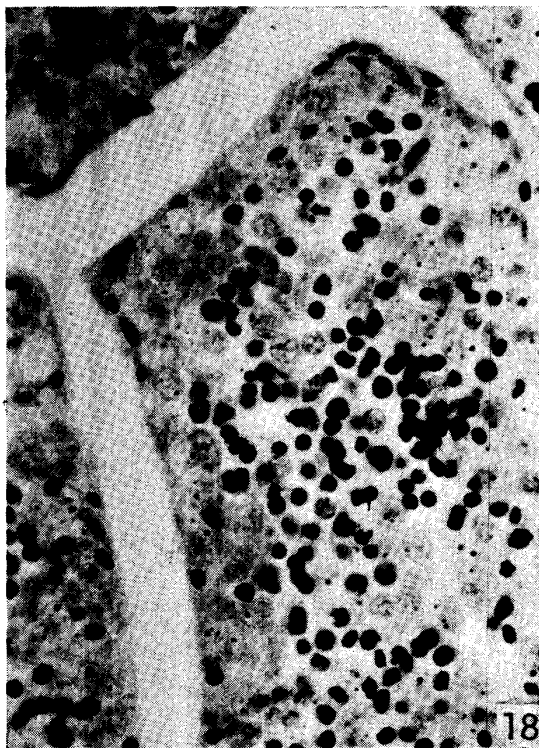
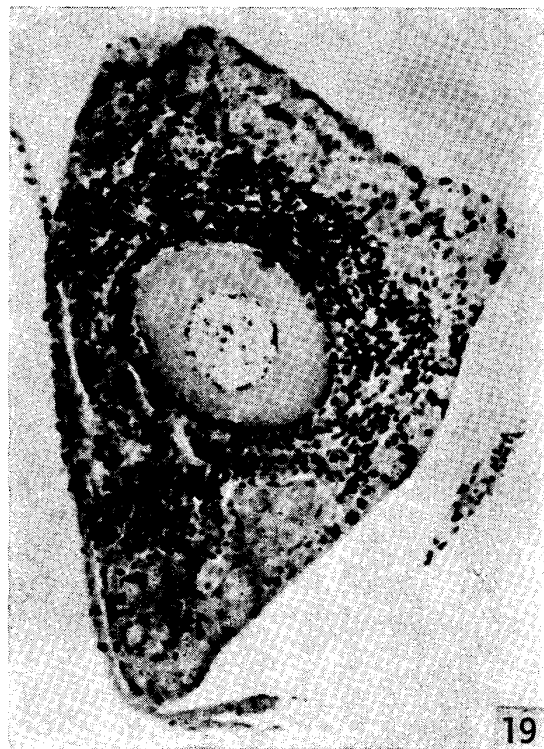
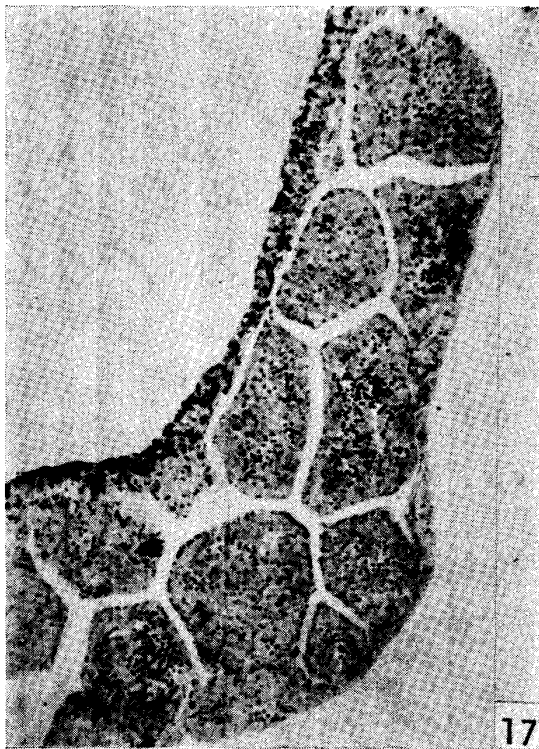


PLATE IV

Cross-sections of the testes of *Bombina orientalis*, *B. variegata* and their reciprocal hybrids at the age of one year. $\times 270$.

21. A male *Bombina orientalis*.
22. A male *Bombina variegata*.
23. A male hybrid between a female *Bombina orientalis* and a male *B. variegata*.
24. A male hybrid between a female *Bombina variegata* and a male *B. orientalis*.

H. UEDA

