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Reproductive Isolation in Treefrogs Distributed in Japan, Korea, Europe and America

By

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ABSTRACT

Hybridization experiments were performed among the Hiroshima, Tsushima and Korea populations of *Hyla arborea japonica, Hyla a. arborea* from Luxembourg and *Hyla regilla* from California. In reciprocal crosses between the Hiroshima and Tsushima, between the Hiroshima and Korea and between the Tsushima and Korea populations, more than 88.5% of the eggs cleaved normally and 32.5~85.9% became normally metamorphosed frogs. The hybrids were lower in rate of metamorphosis than the controls, except those between a female of the Korea population and a male of the Hiroshima population. In the crosses between females of these three populations and a male *H. a. arborea,* 87.0~98.0% of the eggs cleaved normally, and 20.0~47.8% became normally metamorphosed frogs. In the crosses between females of the three populations and a male *H. regilla,* all the eggs died before the gastrula stage, although 24.2~57.6% of the eggs cleaved normally.

Of juvenile and mature hybrids among the Hiroshima, Tsushima and Korea populations and between females of these populations and a male *H. a. arborea,* 39.6~60.3% were males. The males of reciprocal hybrids between the Hiroshima and Tsushima populations are somewhat inferior to those of the control Hiroshima and Tsushima populations in reproductive capacity. The female hybrids between a female of the Hiroshima population and a male of the Tsushima population are remarkably inferior, while those of the reciprocal combination are somewhat inferior to the controls in this respect. The males of reciprocal hybrids between the Hiroshima and Korea populations are somewhat inferior to the controls in reproductive capacity. The male and female hybrids between a female of the Korea population and a male of the Tsushima population are almost similar to the control Tsushima population in this respect. Thus, it has been found that the Hiroshima and Tsushima populations at least are not similar to each other in reproductive physiology. In fact, the frogs of the Tsushima population are larger in body length and egg size, and deep green in contrast to the Hiroshima population.

The mature male and female hybrids between females of the three populations of *H. a. japonica* and a male *H. a. arborea* are completely sterile. This seems to evidently show that *H. a. japonica* is a valid species.

INTRODUCTION

Japanese treefrog was first named *Hyla arborea* var. *japonica* by GUENTHER (1858) and STEJNEGER (1907) changed the position as a variety into *Hyla arborea japonica*

GUENTHER. Although the Japanese treefrog closely resembles European treefrogs, it is an interesting problem to confirm the existence of any reproductively isolating mechanisms between them. DAITO (1968) made crosses in our laboratory between two female *H. a. japonica* from Hiroshima and a male *H. a. sarda* from Corsica Island. The result of crosses showed that the male and female hybrids are probably quite sterile. Thus, it was evident that the two subspecies are genetically isolated from each other in spite of their similarity in morphological characters.

As there are six subspecies including the standard *H. a. arborea* in Europe from Portugal to Caucasus, the present authors made a plan to cross *H. a. japonica* with *H. a. arborea* from the middle Europe and at the same time to cross the Hiroshima, Tsushima and Korea populations of *H. a. japonica* with each other and *H. a. arborea.* They also developed a plan to cross the Japanese treefrogs with *H. regilla* distributed along the Pacific coast of California. A preliminary note has been reported in 1972 by KAWAMURA, NISHIOKA and UEDA.

MATERIALS AND METHODS

Hybridization experiments were performed in the breeding seasons of 1971 and 1972, by making use of the following treefrogs: two females and one male of *Hyla arborea arborea* (*arb* L) collected from Luxembourg and shipped by Mr. R. THORN in October, 1970, five females and one male of *Hyla arborea japonica* (*jap* T) collected from Tsushima, Japan in November, 1970, two males of *Hyla regilla* (*reg* A) collected from California and shipped by Dr. L. E. MAXSON in April, 1971, two females and two males of *Hyla arborea japonica* (*jap* K) collected from Seoul, Korea in May, 1971, and five females and one male of *Hyla arborea japonica* (*jap* H) collected from the suburbs of Hiroshima in June, 1970.

Ovulation was induced by injecting the pituitaries of *Rana nigromaculata.* Insemination was made with sperm suspension in tap water. Tadpoles were fed on boiled spinach in a concrete water tank. Metamorphosed frogs were fed on houseflies. The gonads of immature and mature frogs were preserved in NAVASHIN's fluid, embedded in paraffin, cut into sections and stained with HEIDENHAIN's iron-hematoxylin.

OBSERVATION

I. Characteristics of treefrogs used in crossings

The Hiroshima population of *Hyla arborea japonica* is yellowish green, the Tsushima population is deep green, the Korea population is somewhat dirty green and *Hyla a. arborea* is deep green. The Tsushima population is fairly larger in body size and lay larger eggs than the other populations which are nearly similar to each other in these respects.

1. Female

When five females of the Hiroshima population, being 31.5~38.0 mm in body length, were injected with pituitaries, ovulation occurred normally in three of them. The eggs were 440~818 in number, and 1.1~1.2 mm in diameter. The jelly envelopes were 1.9~2.5 mm in diameter one hour after insemination. When five females of the Tsushima population, being 40.0~48.0 mm in body length, were injected with pituitaries, ovulation occurred normally in two of them. The eggs of the two females were 1.8 mm and 1.9 mm in diameter, and 409 and 898 in number. The jelly envelopes were 2.1 mm in diameter one hour after insemination. When two females of the Korea population, being 34.0 mm and 37.0 mm in body length, were injected with pituitaries, ovulation occurred in the smaller female. The eggs were 1.0 mm in diameter and 430 in number. The jelly envelopes were 1.4 mm in diameter one hour after insemination. When two females of *H. a. arborea,* being 36.0 mm and 37.5 mm in body length, were injected with pituitaries, ovulation occurred normally in the two females. The eggs were 1.0 mm in diameter and 738 and 953 in number. The jelly envelopes were 1.5~1.6 mm in diameter one hour after insemination.

2. Male

The testes of a male *H. a. japonica* from Hiroshima, being 37.5 mm in body length, were yellowish white and 4.2 mm×2.0 mm and 4.0 mm×2.0 mm in size. The testes of a male *H. a. japonica* from Tsushima, being 43.0 mm in body length, were yellowish white and 6.5 mm×2.0 mm and 6.5 mm×2.5 mm in size. The testes of a male *H. a. japonica* from Korea, being 36.0 mm in body length, were yellowish white and 6.0 mm×2.0 mm and 6.0 mm×2.5 mm in size. The testes of a male *H. a. arborea* from Luxembourg, being 36.0 mm in body length, were yellowish white and 3.5 mm×2.0 mm and 3.5 mm×2.0 mm in size. The testes of a male *H. regilla* from California, being 37.5 mm in body length, were black and 4.0 mm×3.0 mm and 4.0 mm×3.0 mm in size.

II. Developmental capacity of hybrids

1. Inter- and intraspecific hybrids between females of the Hiroshima population of *Hyla arborea japonica* and males of four kinds of *Hyla*

a. Control series, *jap* H♀, Nos. 1~3×*jap* H♂, No. 1

In 1971, the control matings were made between three females (Nos. 1~3) and one male (No. 1) of the Hiroshima population of *Hyla a. japonica.* As shown in Table 1, 302 (95.0%) of the 318 eggs cleaved normally. Ten eggs died of abnormalities by the blastula stage and 33 eggs were incomplete in invagination at the gastrula stage. At the hatching stage, four eggs died of ill-development. Eventually, 255 eggs (80.2%) became normally hatched tadpoles. After 50 tadpoles died of edema or ill-development, the remaining 205 (64.5%) normally completed metamorphosis. This number corresponded to 67.9% of the normally

TABLE 1

Results of matings between female *Hyla arborea japonica* from Hiroshima and males of five populations of *Hyla arborea japonica, H. a. arborea* and *H. regilla*

Parents		No. of eggs	No. of normal cleavages	No. of gastrulae		No. of hatched tadpoles		No. of metamorphosed frogs
Female	Male			Normal	Ab.	Normal	Ab.	
jap H, Nos. 1~3	*jap* H, No. 1	318	302 (95.0%)	260 (81.8%)	33 (10.4%)	255 (80.2%)	4 (1.3%)	205 (64.5%)
	jap T, No. 1	400	354 (88.5%)	266 (66.5%)	88 (22.0%)	222 (55.5%)	30 (7.5%)	130 (32.5%)
	jap K, No. 1	306	273 (89.2%)	240 (78.4%)	32 (10.5%)	214 (69.9%)	22 (7.2%)	160 (52.3%)
	arb L, No. 1	439	382 (87.0%)	322 (73.3%)	60 (13.7%)	291 (66.3%)	14 (3.2%)	210 (47.8%)
	reg A, No. 1	327	79 (24.2%)	0	56 (17.1%)	0	0	0

cleaved eggs (Table 1).

b. Experimental series

1) *jap* H♀, Nos. 1~3×*jap* T♂, No. 1

In 1971, crossings were made between the above three females (Nos. 1~3) of the Hiroshima population and a male (No. 1) of the Tsushima population. Of the 400 eggs, 354 (88.5%) cleaved normally. Of the latter, 88, 14 and 30 died of incomplete invagination at the gastrula stage, edema or some other abnormalities at the tail-bud stage and edema or ill-development at the hatching stage, respectively. Eventually, 222 eggs (55.5%) became normally hatched tadpoles. After 92 tadpoles died of edema or ill-development without taking food, the remaining 130 (32.5%) normally completed metamorphosis. This number corresponded to 36.7% of the normally cleaved eggs (Table 1).

2) *jap* H♀, Nos. 1~3×*jap* K♂, No. 1

In 1971, crossings were made between the above three females (Nos. 1~3) of the Hiroshima population and a male (No. 1) of the Korea population. Of the 306 eggs, 273 (89.2%) cleaved normally. Of the normally cleaved eggs, one, 32, four and 22 died of edema, ill-development or some other abnormalities at the blastula, gastrula, tail-bud and hatching stages, respectively. Eventually, 214 eggs (69.9%) hatched normally. While 207 tadpoles began to eat, 47 of them died of edema, ill-development or some other abnormalities and the other 160 (52.3%) normally completed metamorphosis. This number corresponded to 58.6% of the normally cleaved eggs (Table 1).

3) *jap* H♀, Nos. 1~3×*arb* L♂, No. 1

In 1971, crossings were made between the above three females (Nos. 1~3) of the Hiroshima population and a male (No. 1) of *H. a. arborea*. Of the 439 eggs, 382

(87.0%) cleaved normally. Of these eggs, 60, 17 and 14 died of edema, ill-development or some other abnormalities at the gastrula, tail-bud and hatching stages, respectively, while 291 eggs (66.3%) became normally hatched tadpoles. However, 37 of them died of edema or ill-development without taking food and 44 others gradually died of edema, ill-development or some other abnormalities. Eventually, 210 (47.8%) tadpoles normally completed metamorphosis. This number corresponded to 55.0% of the normally cleaved eggs (Table 1).

4) *jap* H♀, Nos. 1~3×*reg* A♂, No. 1

In 1971, crossings were made between the above three females (Nos. 1~3) of the Hiroshima population and a male (No. 1) of *H. regilla*. Of the 327 eggs, 79 (24.2%) cleaved normally. Of the normally cleaved eggs, 23 became abnormal and died as partial blastulae, while the other 56 became normal blastulae. However, 13 of them died as exogastrulae, and the remaining 43 stopped their development at the late blastula stage and died (Table 1).

2. Inter- and intraspecific hybrids between females of the Tsushima population of *Hyla arborea japonica* and males of four kinds of *Hyla*

a. Control series, *jap* T♀, Nos. 1 and 2×*jap* T♂, No. 1

In 1971, the control matings were made between two females (Nos. 1 and 2) and one male (No. 1) of the Tsushima population. Of the 230 eggs, 229 (99.6%) cleaved normally. After five, six and seven eggs died of incomplete invagination at the gastrula stage, edema at the tail-bud stage and edema or ill-development at the hatching stage, respectively, 211 eggs (91.7%) hatched normally. Thereafter, 40 tadpoles died of ill-development by metamorphosis and 171 (74.3%) completed metamorphosis. This number corresponded to 74.7% of the normally cleaved eggs (Table 2).

TABLE 2

Results of matings between female *Hyla arborea japonica* from Tsushima and males of five populations of *Hyla arborea japonica*, *H. a. arborea* and *H. regilla*

Parents		No. of eggs	No. of normal cleavages	No. of gastrulae		No. of hatched tadpoles		No. of metamorphosed frogs
Female	Male			Normal	Ab.	Normal	Ab.	
jap T, Nos. 1, 2	*jap* T, No. 1	230	229 (99.6%)	224 (97.4%)	5 (2.2%)	211 (91.7%)	7 (3.0%)	171 (74.3%)
	jap H, No. 1	274	270 (98.5%)	263 (96.0%)	7 (2.6%)	258 (94.2%)	5 (1.8%)	140 (51.1%)
	jap K, No. 1	293	287 (98.0%)	266 (90.8%)	21 (7.2%)	249 (85.0%)	14 (4.8%)	106 (36.2%)
	arb L, No. 1	305	299 (98.0%)	206 (67.5%)	93 (30.5%)	66 (21.6%)	120 (39.3%)	61 (20.0%)
	reg A, No. 1	205	118 (57.6%)	0	118 (57.6%)	0	0	0

b. Experimental series

1) *jap* T♀, Nos. 1 and 2×*jap* H♂, No. 1

In 1971, crossings were made between the above two females (Nos. 1 and 2) of the Tsushima population and a male (No. 1) of the Hiroshima population. Of the 274 eggs, 270 (98.5%) cleaved normally. While seven and five of the normally cleaved eggs died of incomplete invagination at the gastrula stage and edema at the hatching stage, respectively, 258 (94.2%) became normally hatched tadpoles. However, 42 tadpoles died of edema without taking food, and 76 others thereafter died of edema, ill-development or some other abnormalities by the completion of metamorphosis. Eventually, 140 (51.1%) tadpoles attained the completion of metamorphosis. This number corresponded to 51.9% of the normally cleaved eggs (Table 2).

2) *jap* T♀, Nos. 1 and 2×*jap* K♂, No. 1

In 1971, crossings were made between the above two females (Nos. 1 and 2) of the Tsushima population and a male (No. 1) of the Korea population. Of the 293 eggs, 287 (98.0%) cleaved normally. Twenty-one, three and 14 of the normally cleaved eggs died of incomplete invagination at the gastrula stage, edema at the tail-bud stage and ill-development or edema at the hatching stage, respectively, and 249 (85.0%) became normally hatched tadpoles. Thereafter, 51 tadpoles died of edema or ill-development, and 198 tadpoles began to eat normally. However, 92 tadpoles died of edema or some other abnormalities, and eventually, 106 (36.2%) tadpoles attained completion of metamorphosis. This number corresponded to 36.9% of the normally cleaved eggs (Table 2).

3) *jap* T♀, Nos. 1 and 2×*arb* L♂, No. 1

In 1971, crossings were made between the above two females (Nos. 1 and 2) of the Tsushima population and a male (No. 1) of *H. a. arborea*. While 299 (98.0%) of 305 eggs cleaved normally, 93 died of incomplete invagination at the gastrula stage, 20 died of edema of the haploid type or some other abnormalities at the tail-bud stage, 40 died of edema of the haploid type and 80 died of the other type of abnormalities or ill-development, respectively, at the hatching stage. Eventually, 66 (21.6%) became normally hatched tadpoles. After five tadpoles died of edema or some other abnormalities, 61 (20.0%) completed metamorphosis. This number corresponded to 20.4% of the normally cleaved eggs (Table 2).

4) *jap* T♀, Nos. 1 and 2×*reg* A♂, No. 1

In 1971, crossings were made between the above two females (Nos. 1 and 2) of the Tsushima population and a male (No. 1) of *H. regilla*. Of the 205 eggs, 118 (57.6%) cleaved normally and developed until the late blastula stage. However, all of them stopped their development and died without attaining the gastrula stage (Table 2).

TABLE 3
Results of matings between a female *Hyla arborea japonica* from Korea and males of five populations of *Hyla arborea japonica*, *H. a. arborea* and *H. regilla*

Parents		No. of eggs	No. of normal cleavages	No. of gastrulae		No. of hatched tadpoles		No. of metamorphosed frogs
Female	Male			Normal	Ab.	Normal	Ab.	
jap K, No. 1	*jap* K, No. 1	132	129 (97.7%)	123 (93.2%)	6 (4.5%)	121 (91.7%)	2 (1.5%)	105 (79.5%)
	jap H, No. 1	85	83 (97.6%)	81 (95.3%)	0	81 (95.3%)	0	73 (85.9%)
	jap T, No. 1	68	67 (98.5%)	66 (97.1%)	1 (1.5%)	65 (95.6%)	1 (1.5%)	47 (69.1%)
	arb L, No. 1	96	93 (96.9%)	84 (87.5%)	9 (9.4%)	81 (84.4%)	3 (3.1%)	39 (40.6%)
	reg A, No. 1	49	21 (42.9%)	0	21 (42.9%)	0	0	0

3. Inter- and intraspecific hybrids between a female of the Korea population of *Hyla arborea japonica* and males of four kinds of *Hyla*

a. Control series, *jap* K♀, No. 1×*jap* K♂, No. 1

In 1971, a mating was made between a female (No. 1) and a male (No. 1) of the Korea population and 129 (97.7%) of the 132 eggs cleaved normally. Six and two of the normally cleaved eggs died of incomplete invagination at the gastrula stage and ill-development at the hatching stage, respectively, and 121 eggs (91.7%) became normally hatched tadpoles. While 16 tadpoles died of edema or ill-development by the metamorphosis, 105 (79.5%) attained completion of metamorphosis. This number corresponded to 81.4% of the normally cleaved eggs (Table 3).

b. Experimental series

1) *jap* K♀, No. 1×*jap* H♂, No. 1

In 1971, a crossing was made between the above female (No. 1) of the Korea population and a male (No. 1) of the Hiroshima population, and 83 (97.6%) of the 85 eggs cleaved normally. After two of the normally cleaved eggs died of partial blastula, 81 eggs (95.3%) became normally hatched tadpoles. Three tadpoles died without taking food, and five others died immediately before metamorphosis. Eventually, 73 (85.9%) normally completed metamorphosis. This number corresponded to 88.0% of the normally cleaved eggs (Table 3).

2) *jap* K♀, No. 1×*jap* T♂, No. 1

In 1971, a crossing was made between the above female (No. 1) of the Korea population and a male (No. 1) of the Tsushima population, and 67 (98.5%) of the 68 eggs cleaved normally. After one and one eggs died of abnormalities at the gastrula and hatching stages, respectively, 65 (95.6%) became normally hatched

tadpoles. While seven tadpoles died of edema or ill-development without taking food, the other 58 began to eat. However, after 11 tadpoles died of ill-development, edema or some other abnormalities, the other 47 (69.1%) normally completed metamorphosis. This number corresponded to 70.1% of the normally cleaved eggs (Table 3).

3) *jap* K♀, No. 1×*arb* L♂, No. 1

In 1971, a crossing was made between the above female (No. 1) of the Korea population and a male (No. 1) of *H. a. arborea,* and 93 (96.9%) of the 96 eggs cleaved normally. After nine of the normally cleaved eggs died of incomplete invagination at the gastrula stage, and three others died of abnormalities at the hatching stage, 81 (84.4%) became normally hatched tadpoles. Of these tadpoles, 17 died of edema or ill-development without taking food, while the other 64 began to eat. However, after 25 of them died of edema or ill-development, 39 (40.6%) normally completed metamorphosis. This number corresponded to 41.9% of the normally cleaved eggs (Table 3).

4) *jap* K♀, No. 1×*reg* A♂, No. 1

In 1971, a crossing was made between the above female (No. 1) of the Korea population and a male (No. 1) of *H. regilla,* and 21 (42.9%) of the 49 eggs cleaved normally. While 21 of them developed normally until the blastula stage, all of them stopped their development at the late blastula stage and died without attaining the gastrula stage (Table 3).

4. Inter- and intraspecific hybrids between female *Hyla a. arborea* and males of four kinds of *Hyla*

a. Control series, *arb* L♀, Nos. 1 and 2×*arb* L♂, No. 1

In 1971, matings were made between two females (Nos. 1 and 2) and a male (No. 1) of *H. a. arborea.* As the jelly envelopes of eggs became opaque by infection, they were sterilized with H_2O_2 and diluted alcohol. Of the 507 eggs, 325 (64.1%) cleaved normally. While 285 eggs became normal gastrulae, the other 40 died of incomplete invagination. Thereafter, 52 and 27 eggs died of abnormalities at the tail-bud and hatching stages, respectively. Although 206 (40.6%) became normally hatched tadpoles, 67 of them died of edema or ill-development by completion of metamorphosis and eventually 139 (27.4%) normally completed metamorphosis. This number corresponded to 42.8% of the normally cleaved eggs.

b. Experimental series

When the above two females (Nos. 1 and 2) of *H. a. arborea* were crossed with a male (No. 1) of each of the Hiroshima, Tsushima and Korea populations, 241 (49.8%) of the 484 eggs in total, 147 (51.8%) of the 284 eggs in total and 111 (49.8%) of the 223 eggs in total cleaved normally and became normal gastrulae, respectively. However, as the cloudiness of the jelly envelopes progressed, the

embryos suffered from oxygen starvation and all of them died at the tail-bud stage.

When the above two females (Nos. 1 and 2) of *H. a. arborea* were crossed with a male (No. 1) of *H. regilla*, 113 (51.4%) of the 220 eggs in total became normal blastulae. However, only two of them began gastrulation, while the other 111 did not develop beyond the blastula stage.

III. Body length of hybrids

1. Controls

Among the Hiroshima, Tsushima and Korea populations of *H. a. japonica*, the Tsushima was the largest in body length. At one year of age, the females were 36.0~47.0 mm, 42.4 mm on the average, while the males were 34.0~41.5 mm, 37.4 mm on the average. The other two populations were nearly equal to each other.

TABLE 4

Body lengths of the hybrids among three populations of *Hyla arborea japonica* and a population of *H. a. arborea*

Parents		Female		Male	
Female	Male	No. of frogs	Body length (mm)	No. of frogs	Body length (mm)
jap H	*jap* H	20	30.0~36.0 (32.4)	20	27.0~34.5 (31.4)
	jap T	15	34.0~45.0 (37.6)	13	30.5~38.0 (35.0)
	jap K	15	30.0~40.0 (35.3)	12	29.0~35.0 (32.6)
	arb L	29	26.0~36.0 (30.2)	9	26.5~34.5 (32.3)
jap T	*jap* T	15	36.0~47.0 (42.4)	10	34.0~41.5 (37.4)
	jap H	12	36.5~49.0 (40.5)	8	33.0~49.5 (37.8)
	jap K	15	30.0~39.5 (33.2)	10	27.5~37.5 (30.6)
	arb L	13	26.0~34.5 (31.4)	16	31.0~37.5 (34.6)
jap K	*jap* K	10	32.5~37.5 (34.4)	7	29.5~39.5 (33.0)
	jap H	5	32.0~34.5 (33.0)	8	31.0~37.0 (33.3)
	jap T	7	32.5~40.5 (37.8)	8	33.5~36.5 (35.2)
	arb L	5	27.5~36.5 (31.0)	6	29.0~39.0 (32.0)

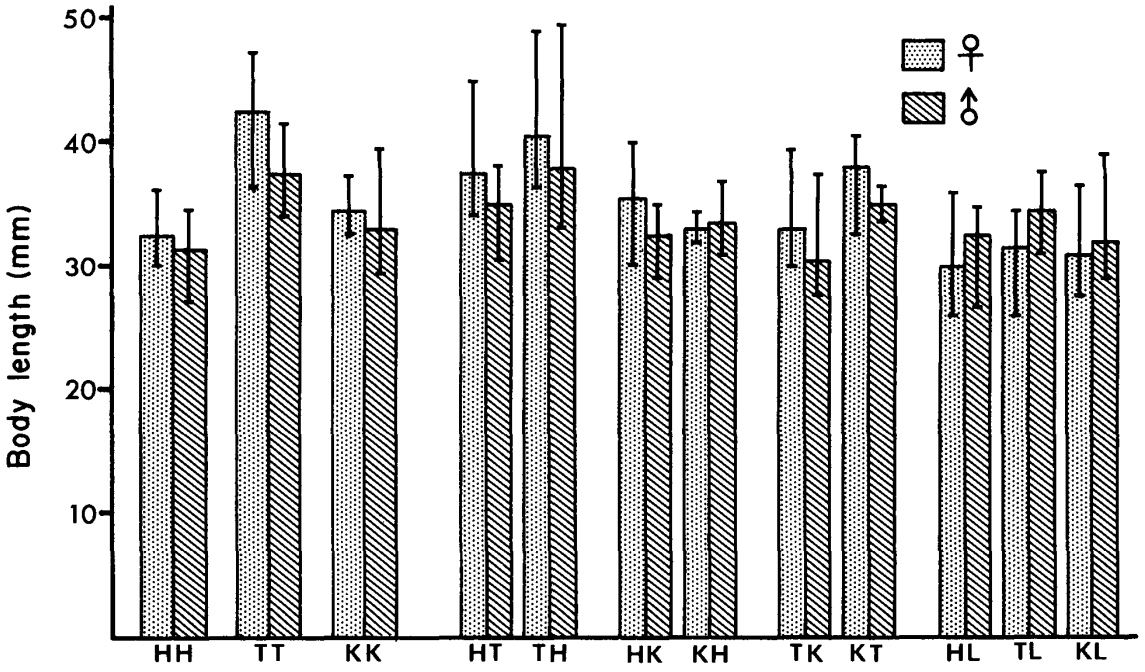

Fig. 1. Bar-graphs showing the body lengths of one-year-old hybrids among four populations of *Hyla arborea japonica* and *H. a. arborea* and the controls. Abbreviation shows each population or each kind of hybrids.

HH. *Hyla a. japonica* from Hiroshima (*jap* H)
TT. *Hyla a. japonica* from Tsushima (*jap* T)
KK. *Hyla a. japonica* from Korea (*jap* K)
HT. Hybrids, *jap* H♀ × *jap* T♂
TH. Hybrids, *jap* T♀ × *jap* H♂
HK. Hybrids, *jap* H♀ × *jap* K♂
KH. Hybrids, *jap* K♀ × *jap* H♂
TK. Hybrids, *jap* T♀ × *jap* K♂
KT. Hybrids, *jap* K♀ × *jap* T♂
HL. Hybrids, *jap* H♀ × *arb* L♂
TL. Hybrids, *jap* T♀ × *arb* L♂
KL. Hybrids, *jap* K♀ × *arb* L♂

In the Hiroshima population, the females were 30.0~36.0 mm, 32.4 mm on the average, while the males were 27.0~34.5 mm, 31.4 mm on the average. In the Korea population, the females were 32.5~37.5 mm, 34.4 mm on the average, while the males were 29.5~39.5 mm, 33.0 mm on the average (Table 4; Fig. 1).

2. Hybrids between the Hiroshima and Tsushima populations of *H. a. japonica*

At one year of age, the hybrids were intermediate in body length between the two populations. Of the hybrids between three females of the Hiroshima population and a male of the Tsushima population, the females were 34.0~45.0 mm, 37.6 mm on the average, while the males were 30.5~38.0 mm, 35.0 mm on the average. The females of the reciprocal hybrids were 36.5~49.0 mm, 40.5 mm on the average, while the males were 33.0~49.5 mm, 37.8 mm on the average (Table 4; Fig. 1).

3. Hybrids between the Hiroshima and Korea populations of *H. a. japonica*

At one year of age, the female hybrids between three females of the Hiroshima

population and a male of the Korea population were 30.0~40.0 mm, 35.3 mm on the average, while the male hybrids were 29.0~35.0 mm, 32.6 mm on the average. The females of the reciprocal hybrids were 32.0~34.5 mm, 33.0 mm on the average, while the males were 31.0~37.0 mm, 33.3 mm on the average. Reciprocal hybrids between the two populations were almost similar in body length to each other and to the parental populations (Table 4; Fig. 1).

4. Hybrids between the Tsushima and Korea populations of *H. a. japonica*

At one year of age, the female hybrids between two females of the Tsushima population and a male of the Korea population were 30.0~39.5 mm, 33.2 mm on the average, while the male hybrids were 27.5~37.5 mm, 30.6 mm on the average. The females of the reciprocal hybrids were 32.5~40.5 mm, 37.8 mm on the average, while the males were 33.5~36.5 mm, 35.2 mm on the average. It was found that the hybrids between a female of the Korea population and a male of the Tsushima population were larger than the reciprocal hybrids (Table 4; Fig. 1).

5. Hybrids between females of the Hiroshima, Tsushima and Korea populations of *H. a. japonica* and a male of *H. a. arborea*

At one year of age, the female hybrids between three females of the Hiroshima population and a male of *H. a. arborea* were 26.0~36.0 mm, 30.2 mm on the average, while the male hybrids were 26.5~34.5 mm, 32.3 mm on the average. The female hybrids between two females of the Tsushima population and a male *H. a. arborea* were 26.0~34.5 mm, 31.4 mm on the average, while the male hybrids were 31.0~37.5 mm, 34.6 mm on the average. The female hybrids between a female of the Korea population and a male *H. a. arborea* were 27.5~36.5 mm, 31.0 mm on the average, while the male hybrids were 29.0~39.0 mm, 32.0 mm on the average. It was found that the male hybrids were always larger than the female hybrids in contrast to the hybrids of the other combinations (Table 4; Fig. 1).

IV. Sex and inner structures of gonads

1. Hybrids between females of the Hiroshima population of *Hyla arborea japonica* and males of three kinds of *Hyla*

a. Control series, *jap* H♀ × *jap* H♂

Of the 205 normally metamorphosed frogs produced from the control matings of the Hiroshima population, 177 juvenile and mature ones were examined for sex. Of the 117 juvenile frogs, 56 had normal ovaries (Fig. 2a), 60 had normal testes (Fig. 2d) and the remainder was a hermaphrodite. Of 60 mature frogs, 27 were normal females and 33 were normal males. When the only hermaphrodite was counted as a male, the 177 frogs in total consisted of 83 females and 94 (53.1%) males (Table 5).

The ovaries of all the 27 mature females were filled with normal ova. Four of these females were used in mating experiments (Table 6). When the testes of five of the 33 mature males were cut into sections to examine the inner structures, the

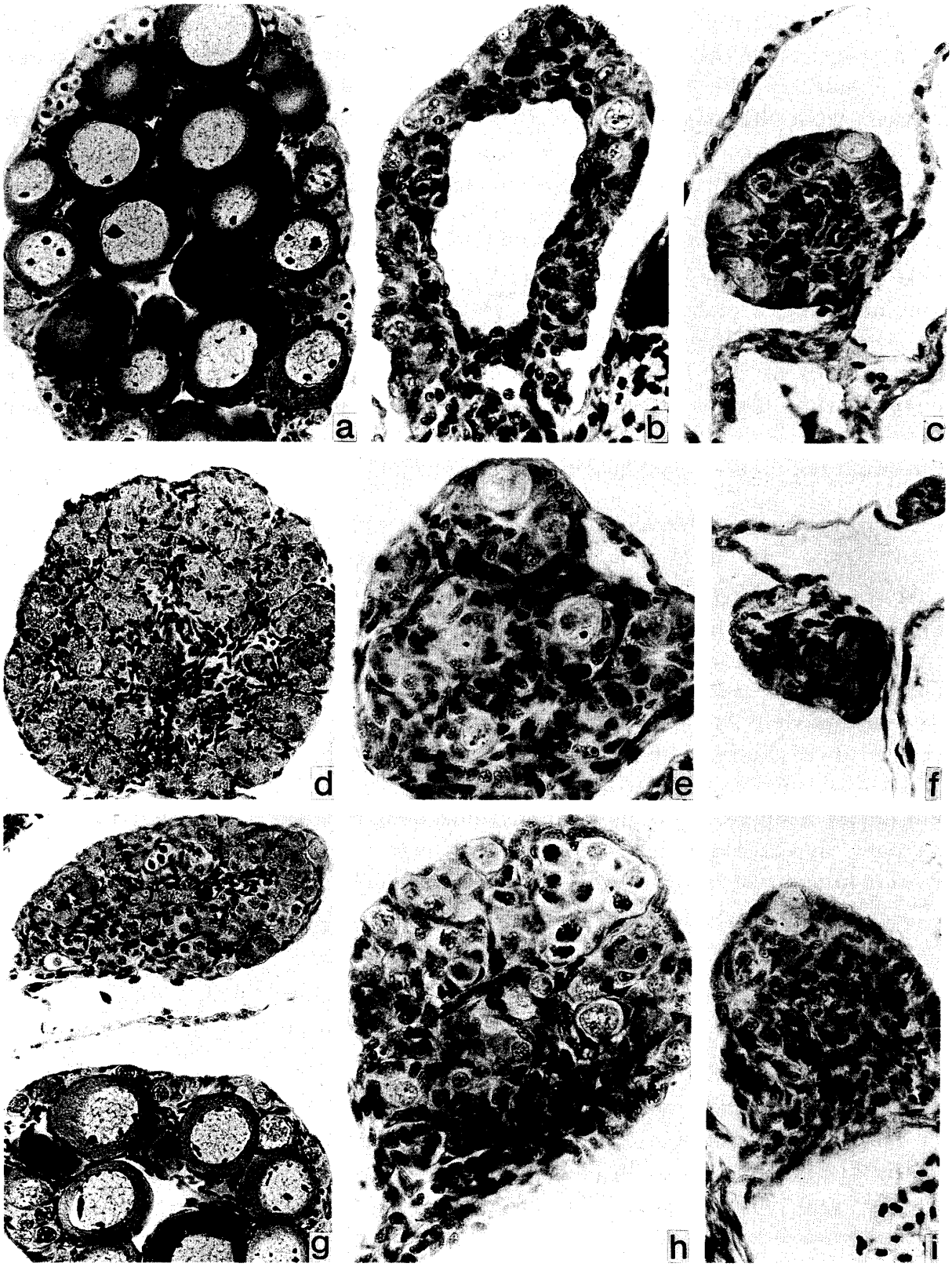

Fig. 2. Cross-sections of six types of gonads of juvenile frogs in *Hyla arborea japonica* and its hybrids.

a. Normal ovary (♀N), (*jap* H♀×*jap* H♂)♀. ×156
b. Underdeveloped ovary (♀U), (*jap* H♀×*arb* L♂)♀. ×315
c. Degenerated ovary (♀D), (*jap* H♀×*arb* L♂)♀. ×315
d. Normal testis (♂N), (*jap* H♀×*jap* H♂)♂. ×156
e. Rudimentary testis (♂R), (*jap* H♀×*arb* L♂)♂. ×315
f. Rudimentary testis (♂R), (*jap* H♀×*arb* L♂)♂. ×315
g. Hermaphrodite (bilateral) (⚥), (*jap* H♀×*jap* T♂)⚥. ×156
h. Hermaphrodite (⚥), (*jap* H♀×*arb* L♂)⚥. ×315
i. Hermaphrodite (⚥), (*jap* H♀×*arb* L♂)⚥. ×315

TABLE 5

Numbers and sexes of juvenile and mature offspring produced from matings among four populations of *Hyla arborea japonica* and *H. a. arborea*

Parents		No. of frogs	Juvenile frogs							Mature frogs								All frogs examined		
											Females			Males						
Female	Male		No.	♀N	♀U	♀D	☤	♂R	♂N	Total	No.	♀N	♀D	No.	♂N	♂1	♂2	No.	♀	♂(%)
jap H	*jap* H	205	117	56	0	0	1	0	60	60	27	27	0	33	9	0	0	177	83	94(53.1)
	jap T	130	82	43	2	0	5	0	32	37	22	22	0	15	1	7	0	119	67	52(43.7)
	jap K	160	98	49	7	0	6	0	36	38	23	23	0	15	0	6	0	136	79	57(41.9)
	arb L	210	131	0	46	27	17	25	16	38	29	0	29	9	0	0	9	169	102	67(39.6)
jap T	*jap T*	171	75	37	0	0	2	0	36	72	36	36	0	36	8	0	0	147	73	74(50.3)
	jap H	140	63	29	4	0	3	4	23	53	13	13	0	40	0	8	0	116	46	70(60.3)
	jap K	106	27	9	2	0	3	5	8	45	24	24	0	21	4	4	0	72	35	37(51.4)
	arb L	61	29	0	10	5	6	5	3	29	13	0	13	16	0	0	16	58	28	30(51.7)
jap K	*jap K*	105	30	14	0	0	1	0	15	47	28	28	0	19	8	0	0	77	42	35(45.5)
	jap H	73	29	13	0	0	1	1	14	35	16	15	1	19	0	8	0	64	29	35(54.7)
	jap T	47	17	8	0	0	2	1	6	18	7	7	0	11	1	7	0	35	15	20(57.1)
	arb L	39	14	0	5	2	2	2	3	20	9	0	9	11	0	0	11	34	16	18(52.9)

♀N, Normal ovary. ♀U, Underdeveloped ovary. ♀D, Degenerated ovary. ☤, Hermaphrodite.
♂R, Rudimentary testis. ♂N, Normal testis. ♂1, Abnormal testis of type 1. ♂2, Abnormal testis of type 2.

TABLE 6

Body lengths and egg diameters of female hybrids among three populations of *Hyla arborea japonica*, used for backcrosses

Individual no.		Body length (mm)	No. of eggs	Egg diameter (mm)
(*jap* H×*jap* H)♀,	No. 1	31.5	361	1.2
	No. 2	33.0	366	1.2
	No. 3	33.5	472	1.2
	No. 4	34.5	629	1.4
(*jap* T×*jap* T)♀,	No. 1	44.5	429	1.9
	No. 2	46.0	421	1.9
	No. 3	48.0	444	1.8
(*jap* H×*jap* T)♀,	No. 1	42.0	71	1.6
	No. 2	41.0	315	1.6
(*jap* T×*jap* H)♀,	No. 1	44.5	69	1.7
	No. 2	42.5	117	1.7
(*jap* K×*jap* T)♀,	No. 1	40.5	232	1.7
	No. 2	36.0	214	1.7

seminiferous tubules were filled with bundles of normal spermatozoa. When sperm suspension was made from the testes of each of four other mature males (Table 7), normal spermatozoa were abundantly observed. These testes were called normal testes (♂N) (Fig. 3a, b).

TABLE 7

Boby lengths and testicular sizes of male hybrids among four populations of *Hyla arborea japonica* and *H. a. arborea* and the controls

Individual no.		Body length (mm)	Size of testes	
			Right (mm)	Left (mm)
(*jap* H×*jap* H) ♂,	No. 1	34.5	5.0×2.5	5.0×2.5
	No. 2	31.0	4.5×2.0	4.5×3.0
	No. 3	31.5	4.0×2.5	4.0×2.5
	No. 4	30.0	4.0×2.5	4.0×2.0
(*jap* T×*jap* T) ♂,	No. 1	38.0	7.0×2.0	6.0×2.5
	No. 2	37.5	6.0×2.5	5.5×2.5
	No. 3	40.0	6.5×2.5	6.0×2.5
(*jap* K×*jap* K) ♂,	No. 1	39.5	6.0×2.0	6.0×2.0
	No. 2	34.5	5.5×2.0	5.5×2.0
(*jap* H×*jap* T) ♂,	No. 1	38.0	5.0×2.5	3.5×1.5
	No. 2	37.5	5.5×3.0	4.0×3.0
	No. 3	36.0	5.0×2.0	4.5×2.5
(*jap* T×*jap* H) ♂,	No. 1	49.5	5.5×3.5	4.5×2.5
	No. 2	35.0	6.0×2.0	4.5×2.5
	No. 3	44.0	5.0×2.5	5.0×2.5
(*jap* H×*jap* K) ♂,	No. 1	35.0	3.5×1.5	4.0×2.0
(*jap* K×*jap* H) ♂,	No. 1	33.0	5.0×2.5	4.5×2.0
	No. 2	37.0	5.5×3.0	4.5×2.5
	No. 3	35.5	6.5×2.5	4.0×2.5
(*jap* K×*jap* T) ♂,	No. 1	36.5	5.5×2.0	4.0×2.5
	No. 2	33.5	4.0×3.0	4.0×3.0
	No. 3	34.0	6.0×2.0	4.5×2.0
(*jap* H×*arb* L) ♂,	No. 1	37.0	2.0×2.0	2.0×2.0
	No. 2	33.5	2.0×2.0	2.0×2.0
	No. 3	34.5	3.0×2.0	2.5×2.0
	No. 4	33.0	2.0×1.0	2.0×1.0
(*jap* T×*arb* L) ♂,	No. 1	37.5	2.0×2.0	3.0×2.0
	No. 2	31.0	2.0×2.0	2.0×2.0
	No. 3	35.0	0	5.0×1.5
	No. 4	35.0	4.0×2.0	3.0×2.0
(*jap* K×*arb* L) ♂,	No. 1	39.0	1.0×0.5	2.0×0.5
	No. 2	33.5	2.0×1.0	2.0×1.0
	No. 3	30.5	1.5×0.5	1.0×0.5
	No. 4	30.0	1.5×1.0	1.0×0.5

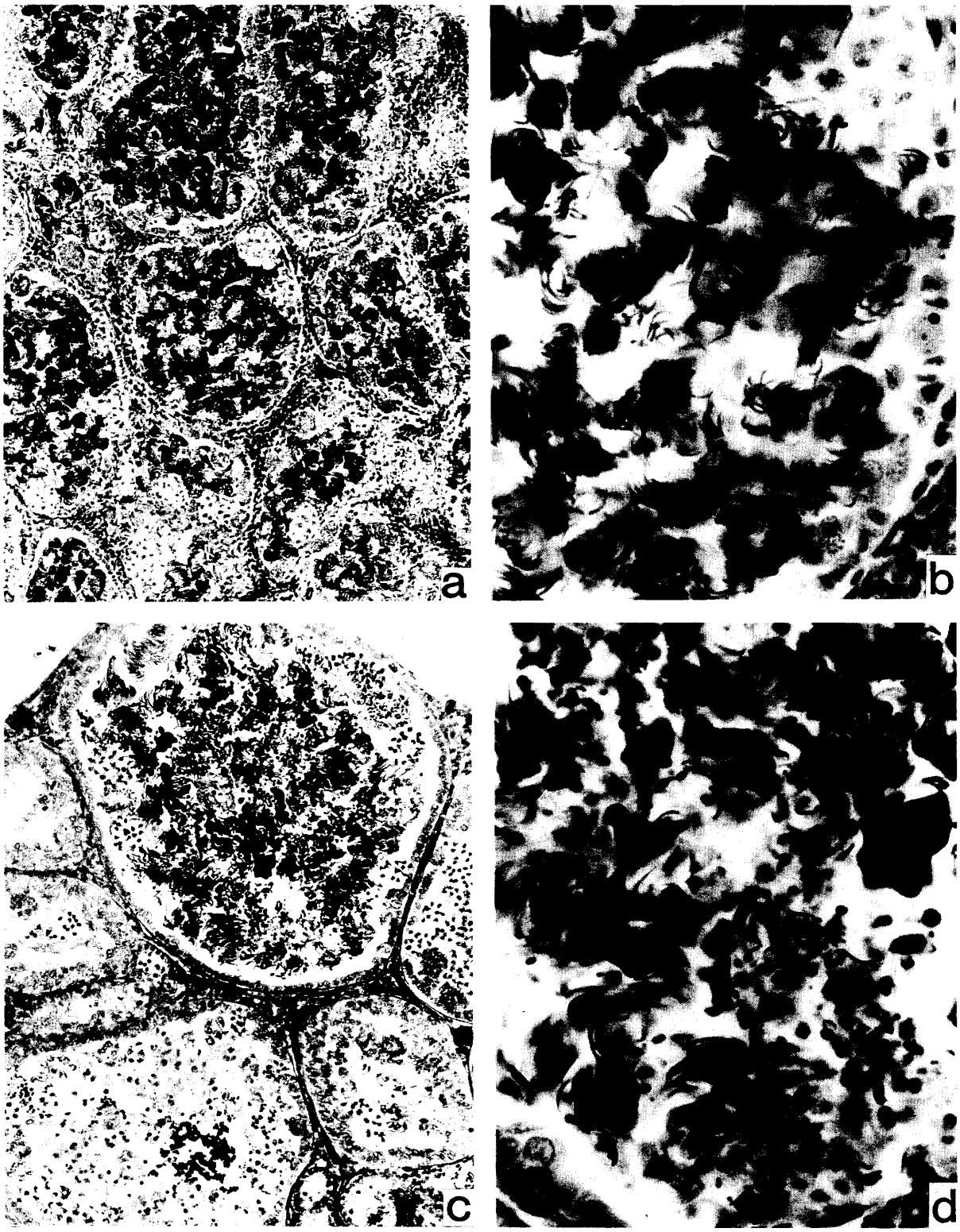

Fig. 3. Cross-sections of the testes of a one-year-old male hybrid between a female *Hyla arborea japonica* from Hiroshima and a male *H. a. japonica* from Korea and the control.

a. Normal testis (♂N) of a control male, (*jap* H♀ ×*jap* H♂)♂, No. 3. ×110
b. ditto ×450
c. Abnormal testis of type 1 (♂1) of a male hybrid, (*jap* H♀ ×*jap* K♂)♂, No. 5. ×110
d. ditto ×450

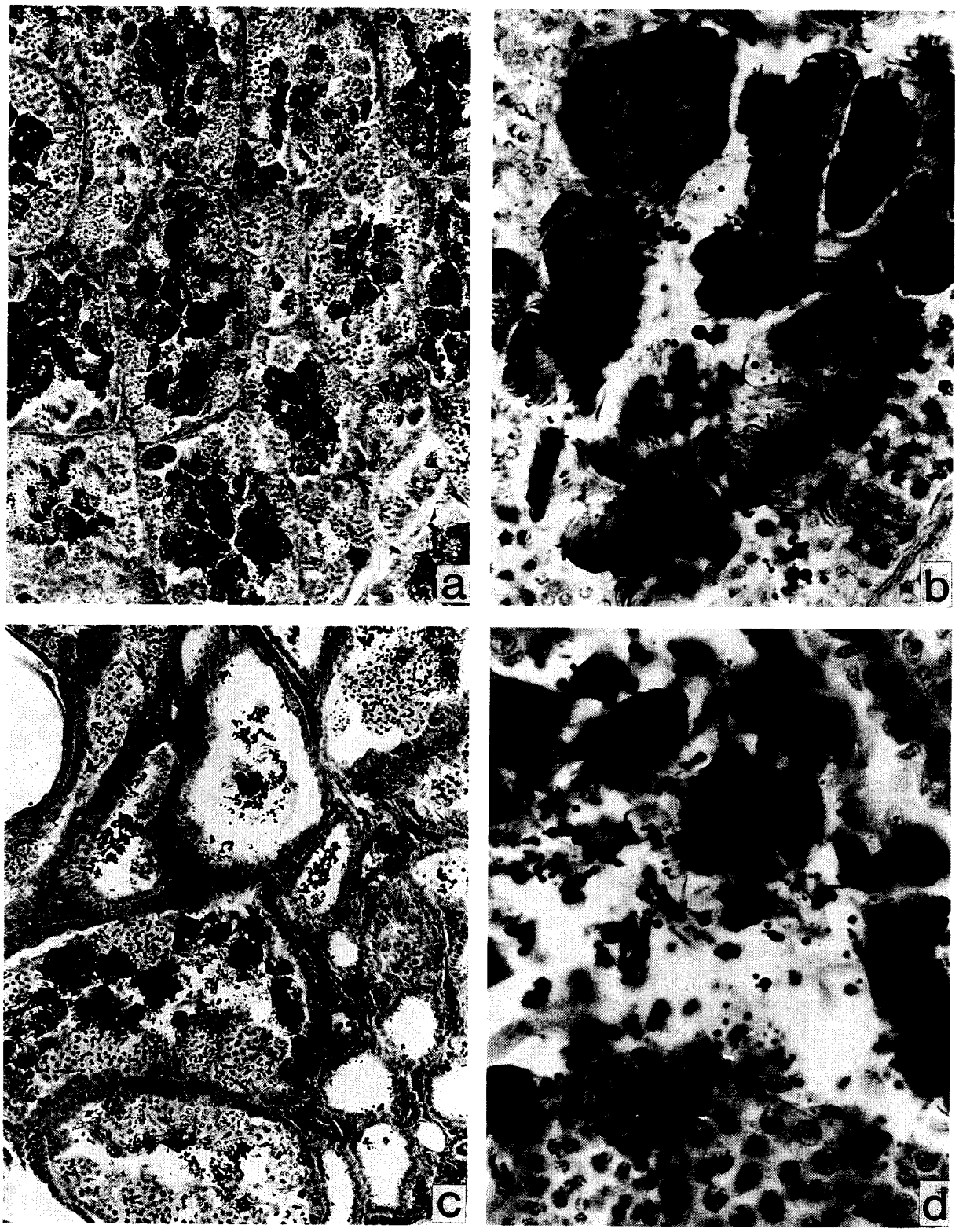

Fig. 4. Cross-sections of the testes of one-year-old male hybrids between a female *Hyla arborea japonica* from Hiroshima and a male *H. a. japonica* from Tsushima.

a. Normal testis (♂N) of a male hybrid, (*jap* H♀ × *jap* T♂)♂, No. 1. ×110
b. ditto ×450
c. Abnormal testis of type 1 (♂1) of a male hybrid, (*jap* H♀ × *jap* T♂)♂, No. 5. ×110
d. ditto ×450

b. Experimental series

1) *jap* H♀ × *jap* T♂

Sex was examined in 119 juvenile and mature frogs of the 130 normally metamorphosed ones obtained from crossings between females of the Hiroshima population and a male of the Tsushima population. The results showed that 43 of the 82 juvenile frogs had normal ovaries, 32 had normal testes, two had underdeveloped ovaries and the other five were hermaphrodites (Fig. 2g). Of 37 mature frogs, 22 were females of normal appearance, and 15 were males. When the hermaphrodites were counted as males, there were 67 females and 52 (43.7%) males in total (Table 5).

The 22 mature females all contained abundant normal eggs. Two of these females (Table 6) were used in backcrosses. When the testes of five of the 15 mature males were examined by making sections, one male had normal testes like those of the control males. The seminiferous tubules were filled with bundles of normal spermatozoa (♂N), while abnormal spermatozoa and pycnotic nuclei were very scarce (Fig. 4a, b). In the testes of the other four males, there were some parts of seminiferous tubules which contained abundant abnormal spermatozoa and pycnotic nuclei but no normal spermatozoa (Fig. 4c, d). Normal spermatozoa contained in these testes were less than one half in amount as compared with those in the control frogs. Such testes were called abnormal testes of type 1 (♂1). Three other males (Table 7) were used to test the reproductive capacity. In contrast to the control males, the testes of these males contained fairly abundant abnormal spermatozoa and pycnotic nuclei in addition to normal spermatozoa (♂1).

2) *jap* H♀ × *jap* K♂

Sex was examined in 136 juvenile and mature frogs of the 160 normally metamorphosed ones obtained from crossings between females of the Hiroshima population and a male of the Korea population. The results showed that 49 of the 98 juvenile frogs had normal ovaries, 36 had normal testes, seven had underdeveloped ovaries and the remaining six were hermaphrodites. Of the 38 mature frogs, 23 were females and 15 were males. These females and males were apparently normal. When the hermaphrodites were counted as males, there were 79 females and 57 (41.9%) males in total (Table 5).

All the 23 mature females contained normal eggs abundantly (Table 5). When the testes of five of the 15 mature males were examined, there were abundant abnormal spermatozoa and pycnotic nuclei which were less than one half of the normal spermatozoa in amount. However, the testes of these five males had some seminiferous tubules which contained abundant abnormal spermatozoa and pycnotic nuclei but no normal spermatozoa. They corresponded to abnormal testes of type 1 (♂1) (Fig. 3c, d). The left testis of another male (Table 7) was used to test the reproductive capacity. When the inner structures of the right testis were examined by sections, they were the same as those of testes of the

foregoing five males. This testis was of type 1 (♂1).

3) *jap* H♀×*arb* L♂

Sex was examined in 169 juvenile and mature frogs of the 210 normally metamorphosed ones produced from crossings between females of the Hiroshima population and a male of *H. a. arborea*. The results showed that 46 of the 131 juvenile frogs had underdeveloped ovaries (Fig. 2b), 27 had degenerated ovaries (Fig. 2c), 17 were transforming from females to males (Fig. 2h, i), 25 had rudimentary testes (Fig. 2e, f) and the remaining 16 had nearly normal testes. While 29 of the 38 mature frogs were females, nine were males. When the hermaphrodites were counted as males, there were 102 females and 67 (39.6%) males in total (Table 5).

The ovaries of the 29 mature females were very small and degenerated. They were semitransparent and wavy in shape. The ovaries of two females were 5.0~5.5 mm in length and 2.0~2.5 mm in width. Each ovary contained several auxocytes and fairly numerous oocytes and oogonia. There was a well-developed ovarian cavity (Fig. 5a). The ovaries of 10 other females had small ovaries which were 3.0~4.0 mm in length and 0.5~1.5 mm in width and contained no auxocytes. However, there were numerous oocytes and a distinct ovarian cavity in each cross section (Fig. 5b). In the other 17 females, the inner structures of the ovaries were very abnormal and had no auxocytes, although there were some females whose ovaries contained a few oogonia in the cortical parts (Table 5; Fig. 5c).

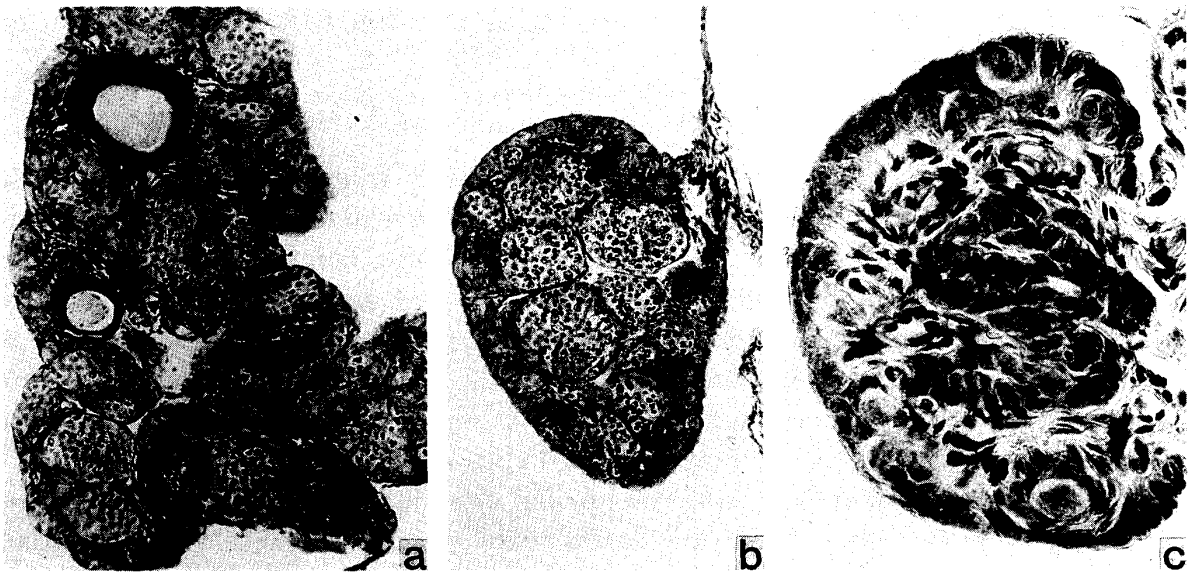

Fig. 5. Cross-sections of the ovaries of one-year-old female hybrids between female *Hyla arborea japonica* from Hiroshima and a male *H. a. arborea* from Luxembourg.

a. Degenerated ovary (♀D) of fairly large size in a female hybrid, (*jap* H♀×*arb* L♂)♀, No. 1, having a few auxocytes. ×110

b. Degenerated ovary (♀D) of medium size in a female hybrid, (*jap* H♀×*arb* L♂)♀, No. 12, having no auxocytes. ×110

c. Degenerated ovary (♀D) of very small size in a female hybrid, (*jap* H♀×*arb* L♂)♀, No. 24, having a few oogonia. ×450

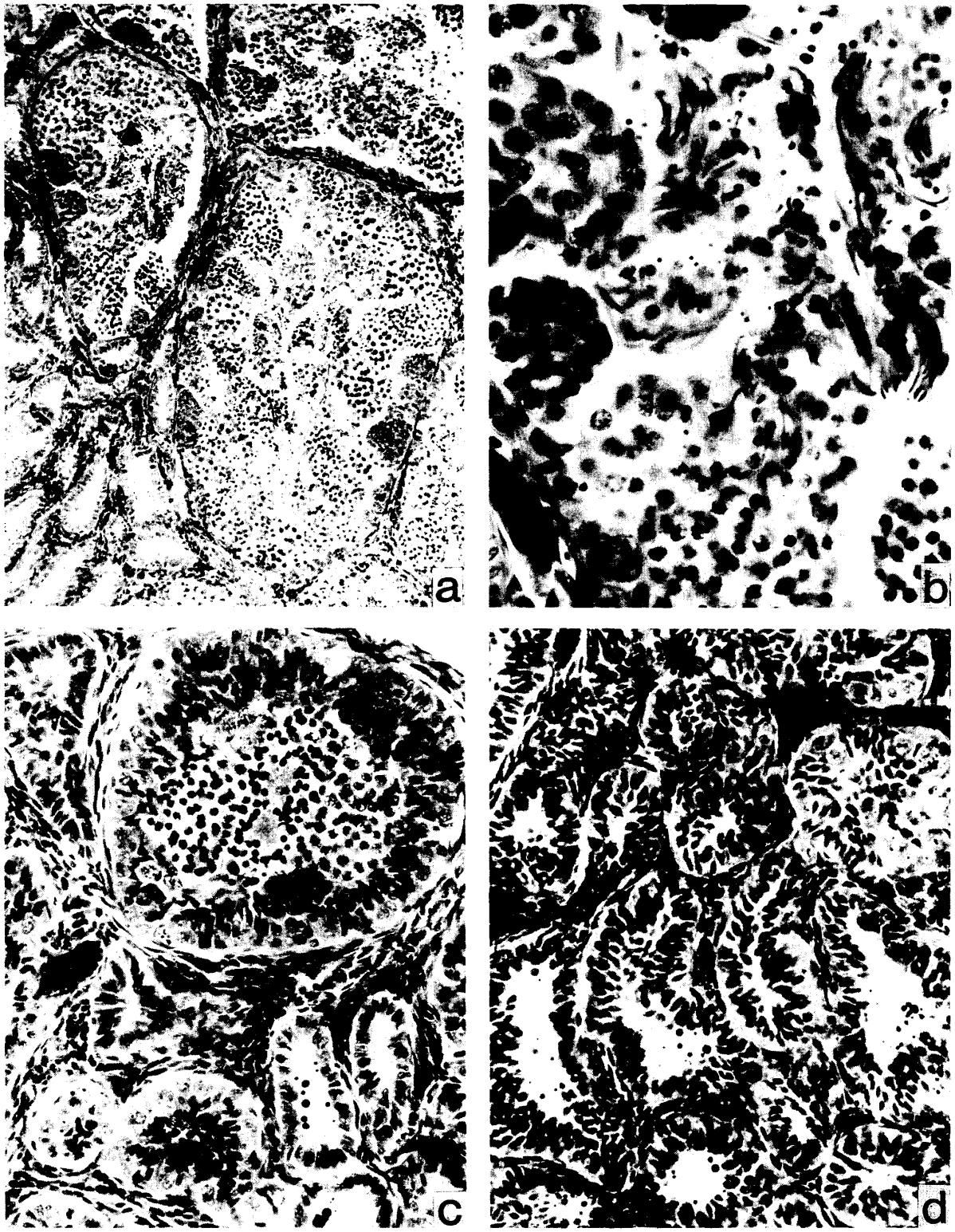

Fig. 6. Cross-sections of the testes of one-year-old male hybrids between female *Hyla arborea japonica* from Hiroshima and a male *H. a. arborea* from Luxembourg.

a. Abnormal testis of type 2 (♂2) of a male hybrid, (*jap* H♀×*arb* L♂)♂, No. 2. ×110
b. ditto ×450
c. Abnormal testis of type 2 (♂2) of a male hybrid, (*jap* H♀×*arb* L♂)♂, No. 4. ×225
d. Abnormal testis of type 2 (♂2) of a male hybrid, (*jap* H♀×*arb* L♂)♂, No. 5. ×225

Nine mature frogs were males. Four of them (Table 7) had testes which were 2.0×1.0 mm~3.0×2.0 mm in size. The left testes of these males were used in backcrosses to test their reproductive capacity. Their right testes were sectioned to examine their inner structures. The results showed that the seminiferous tubules in the testes of three males contained a few normal and abnormal spermatozoa together with many abnormal spermatocytes and pycnotic nuclei. Small bundles of spermatozoa were also rarely found (Fig. 6a, b). The testis of the other male contained no normal spermatozoa, while there were pycnotic nuclei distributed abundantly or sparsely in the seminiferous tubules (Fig. 6c). Such testes were called abnormal testes of type 2 (♂2). The other five males had rudimentary testes which were very small and abnormal in inner structure. Seminiferous tubules contained no normal spermatozoa, but pycnotic nuclei were scarcely found (♂2) (Table 5; Fig. 6d).

2. Hybrids between females of the Tsushima population of *Hyla arborea japonica* and males of three kinds of *Hyla*

a. Control series, *jap* T♀×*jap* T♂

Sex was examined in 147 juvenile and mature frogs of the 171 normally metamorphosed ones produced from matings between females and a male of the Tsushima population. The results showed that 37 of the 75 juvenile frogs had normal ovaries, 36 had normal testes and the remaining two were hermaphrodites. Of the 72 mature frogs, 36 were normal females and 36 were normal males. When the hermaphrodites were counted as males, 73 were females and 74 (50.3%) were males in total (Table 5).

The ovaries of all the 36 mature females were filled with normal ova. Three of these females were used in mating experiments (Table 6). The testes of five of the 36 males were cut into sections to observe the inner structures. Three others (Table 7) were used for crossing experiments by making sperm suspensions from their testes. The results showed that the testes of the eight males abundantly contained normal spermatozoa. The inner structures of the sectioned testes were completely normal, and the seminiferous tubules were filled with bundles of normal spermatozoa (♂N) (Fig. 7a, b).

b. Experimental series

1) *jap* T♀×*jap* H♂

Sex was examined in 116 juvenile and mature frogs of the 140 normally metamorphosed ones produced from crossings between females of the Tsushima population and a male of the Hiroshima population. The results showed that 29 of the 63 juvenile frogs had normal ovaries, four had underdeveloped ovaries, 23 had normal testes, four had rudimentary testes and three were hermaphrodites. Of the 53 mature frogs, 13 were normal females and 40 were males with testes of normal appearance. When the hermaphrodites were counted as males, the 116 frogs in total consisted of 46 females and 70 (60.3%) males (Table 5).

The 13 mature females all contained abundant normal ova. Two (Table 6) of

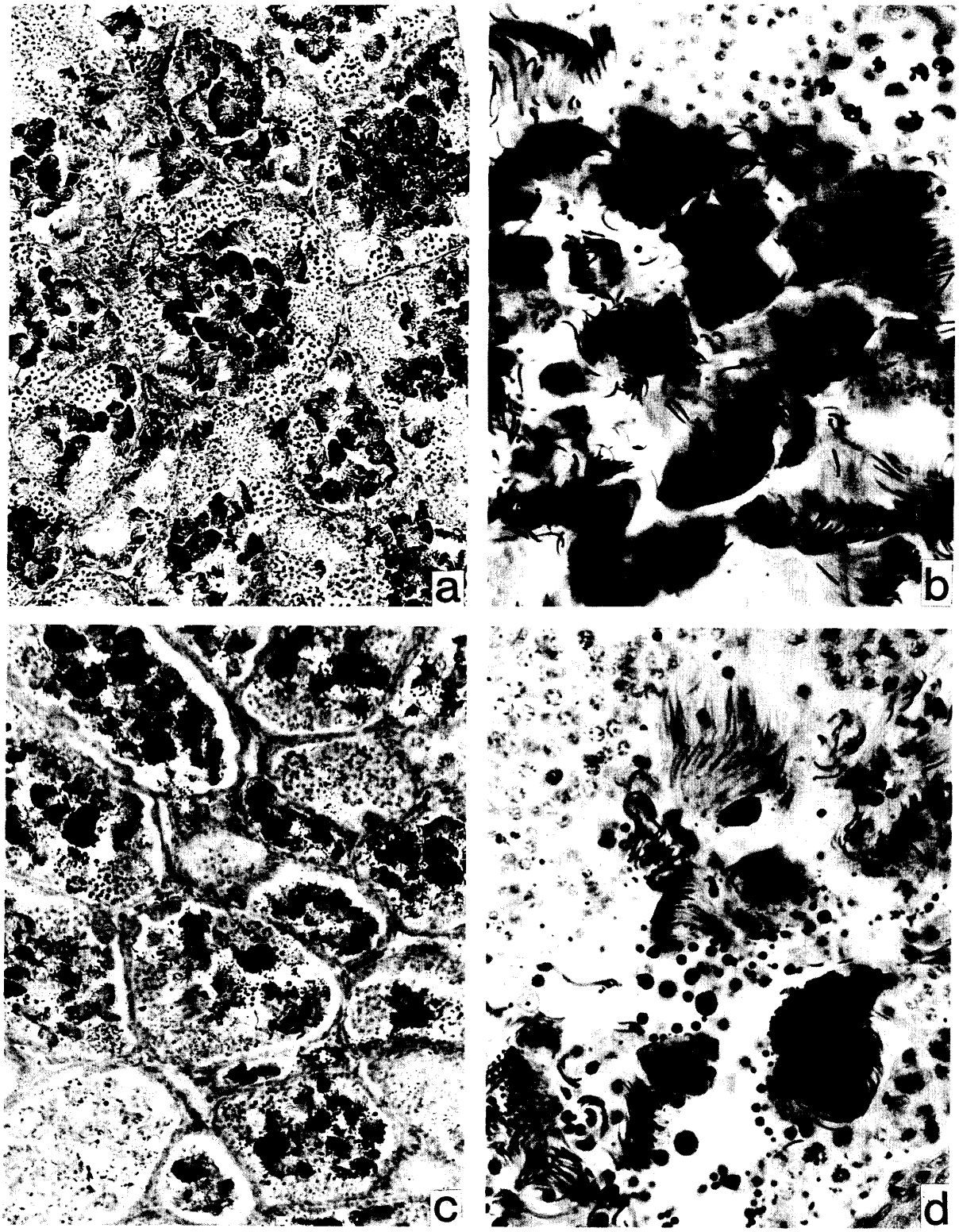

Fig. 7. Cross-sections of the testes of a one-year-old male hybrid between a female *Hyla arborea japonica* from Tsushima and a male *H. a. japonica* from Hiroshima and the control.

a. Normal testis (♂N) of a control male, (*jap* T♀ × *jap* T♂) ♂, No. 3. ×110
b. ditto ×450
c. Abnormal testis of type 1 (♂1) of a male hybrid, (*jap* T♀ × *jap* H♂) ♂, No. 5. ×110
d. ditto ×450

these females were used for crossing experiments. Forty others were males with testes of normal appearance. The inner structures of testes were examined in five of these males by sectioning their testes. While bundles of normal spermatozoa were found in all the seminiferous tubules of the five males, they were nearly half in amount as compared with those in the testes of the control frogs (Fig. 7c, d). Moreover, there were numerous abnormal spermatozoa and pycnotic nuclei. In some tubules, degenerating spermatocytes were found everywhere (♂1) (type 1). Sperm suspensions were made from the testes of three other mature males (Table 7) for crossing experiments. There were many abnormal spermatozoa and pycnotic nuclei in addition to normal spermatozoa in the suspensions (♂1).

2) *jap* T♀ × *jap* K♂

Sex was examined in 72 juvenile and mature frogs of the 106 produced from crossings between females of the Tsushima population and a male of the Korea population. The results showed that nine of the 27 juvenile frogs were females whose ovaries contained numerous normal auxocytes, two were females with underdeveloped ovaries, eight were males with normal testes, five were males with rudimentary testes and the remaining three were hermaphrodites. Of the 45 mature frogs, 24 were females and 21 were males having testes of normal appearance. When the hermaphrodites were counted as males, the 72 frogs in total consisted of 35 females and 37 (51.4%) males (Table 5).

All the 45 mature frogs were ill-developed and died before backcrosses. However, the ovaries of the 24 mature females were filled with many normal ova. In eight of the 21 mature males, the inner structures of the testes were observed. It was found that those of four males were almost normal (♂N) and similar to those of the control males, although they contained a few abnormal spermatozoa and pycnotic nuclei (Table 5; Fig. 8a, b). The testes of the other four males contained fairly numerous abnormal spermatozoa and pycnotic nuclei. In the cross sections of these testes, some seminiferous tubules had no spermatozoa, while the others contained bundles of normal spermatozoa here and there, although the spermatozoa were very few, being less than half of those of the control males in amount. Then, these four males were named abnormal type 1 (♂1) (Table 5; Fig. 8c, d).

3) *jap* T♀ × *arb* L♂

Sex was examined in 58 juvenile and mature frogs of the 61 produced from crossings between females of the Tsushima population and a male of *H. a. arborea*. The results showed that ten of the 29 juvenile frogs were females whose ovaries were underdeveloped and contained no auxocytes, five were females whose ovaries were degenerated and contained no germ cells, three were males whose testes were almost normal in inner structure, five were males whose testes were rudimentary and contained no germ cells, and the remaining six were hermaphrodites. Of the 29 mature frogs, 13 were females and 16 were males. When the hermaphrodites were counted as males, the 58 frogs in total consisted of 28 females and 30 (51.7%)

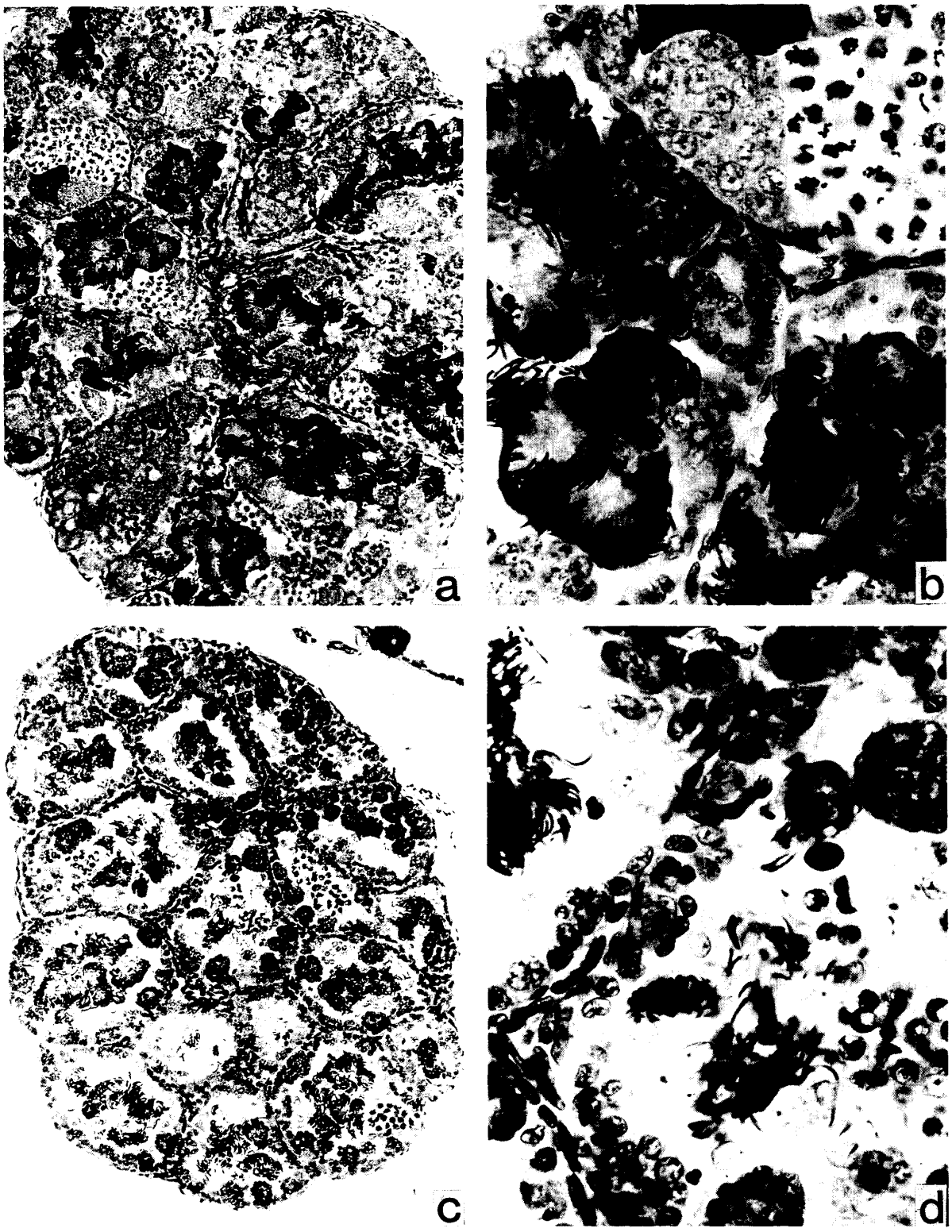

Fig. 8. Cross-sections of the testes of one-year-old male hybrids between female *Hyla arborea japonica* from Tsushima and a male *H. a. japonica* from Korea.

a. Normal testis (♂N) of a male hybrid, (*jap* T♀ ×*jap* K♂)♂, No. 3. ×110
b. ditto ×450
c. Abnormal testis of type 1 (♂1) of a male hybrid, (*jap* T♀ ×*jap* K♂)♂, No. 5. ×110
d. ditto ×450

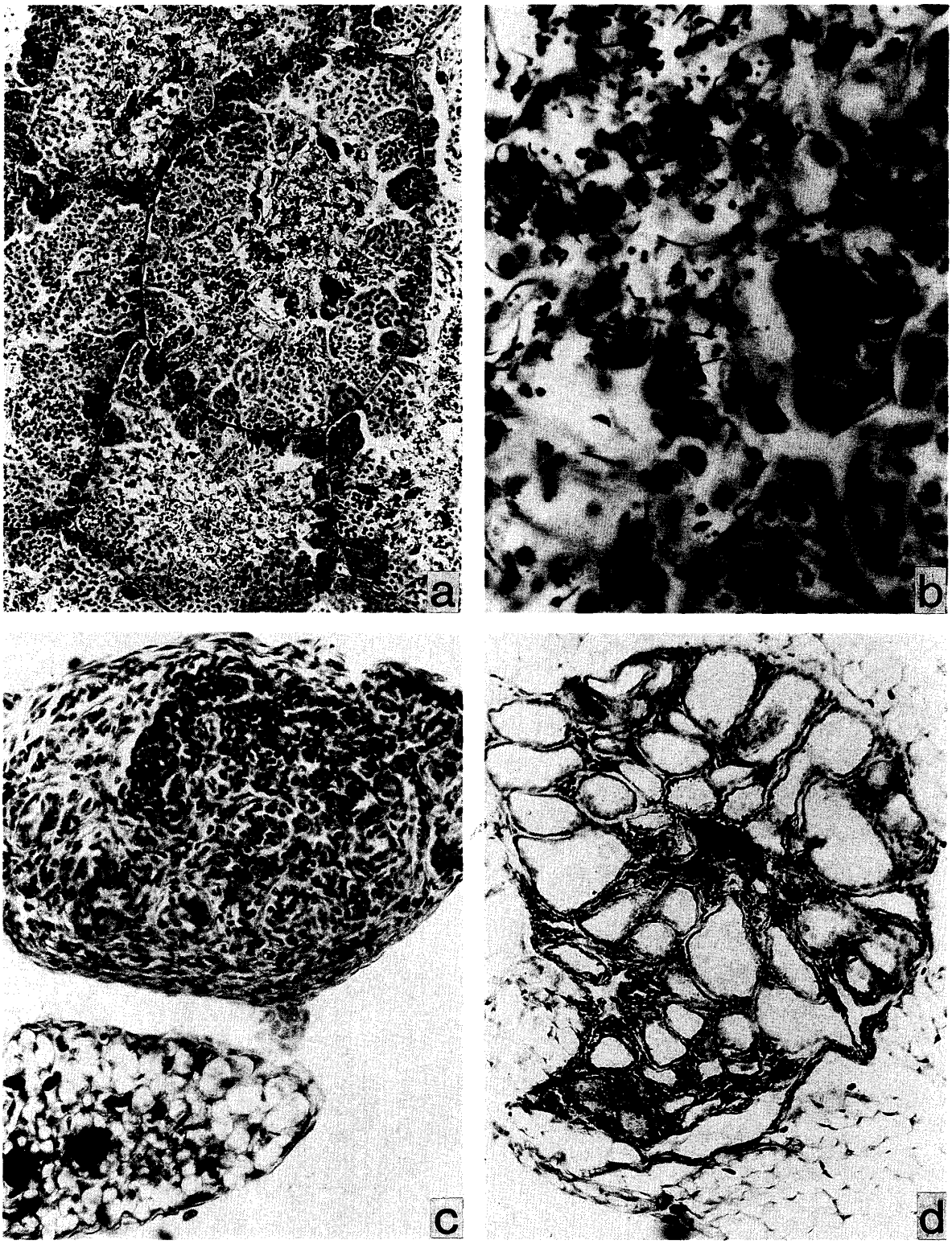

Fig. 9. Cross-sections of the gonads of one-year-old hybrids between female *Hyla arborea japonica* from Tsushima and a male *H. a. arborea* from Luxembourg.

a. Abnormal testis of type 2 (♂2) of a male hybrid, (*jap* T♀×*arb* L♂)♂, No. 3. ×110
b. ditto ×450
c. Abnormal testis of type 2 (♂2) of a male hybrid, (*jap* T♀×*arb* L♂)♂, No. 6. ×225
d. Degenerated ovary (♀D) of a female hybrid, (*jap* T×*arb* L♂)♀, No. 9. ×110

males (Table 5).

The ovaries of 13 mature females were extremely degenerated and recognized as ovaries by existence of ovarian cavities (Fig. 9d). Twelve were males whose testes were very small and extremely degenerated (♂2) (Fig. 9c), and the remaining four were males whose testes were almost normal in appearance, being 2.0~5.0 mm in length and 1.5~2.0 mm in width (Table 7). The left testes of these four males were crushed to make sperm suspensions for crossings. There were abnormal spermatozoa and pycnotic nuclei in addition to a few normal spermatozoa in the suspensions. The right testes were examined by making sections. Two of these four right testes were rudimentary and contained no germ cells, and the remaining two contained abnormal spermatozoa and fairly numerous pycnotic nuclei (♂2) (Table 5; Fig. 9a, b).

3. Hybrids between a female of the Korea population of *Hyla arborea japonica* and males of three kinds of *Hyla*

a. Control series, *jap* K♀ × *jap* K♂

Sex was examined in 77 juvenile and mature frogs of the 105 produced from a mating between a female and a male of the Korea population. The results showed that 14 of the 30 juveniles were females with normal auxocytes, 15 others were males with normal testes and the remainder was a hermaphrodite. Of the 47 mature frogs, 28 were normal females and 19 were males having testes of normal appearance. When the only hermaphrodite was counted as a male, the frogs in total consisted of 42 females and 35 (45.5%) males (Table 5).

The 28 mature females all contained abundant normal eggs. The testes of six of these 19 mature males were cut into sections, while those of two other males were made into sperm suspensions for crossing experiments (Table 7). The testes of these eight males were all filled with normal spermatozoa (♂N) (Table 5; Fig. 10a, b).

b. Experimental series

1) *jap* K♀ × *jap* H♂

Sex was examined in 64 juvenile and mature frogs of the 73 produced from a crossing between a female of the Korea population and a male of the Hiroshima population. The results showed that 13 of the 29 juvenile frogs had normal ovaries filled with normal auxocytes, 14 had normal testes, one had rudimentary testes and the remainder was a hermaphrodite. Of the 35 mature frogs, 16 were females and 19 were males. When the only hermaphrodite was counted as a male, the 64 frogs in total consisted of 29 females and 35 (54.7%) males (Table 5).

The ovaries of 15 mature females were filled with normal ova and one was a female with degenerated ovaries (Table 5). The testes of five of the 19 mature males were cut into sections to observe their inner structures. Abnormal spermatozoa and pycnotic nuclei were fairly abundant in addition to bundles of normal spermatozoa in seminiferous tubules (♂1) (Fig. 10c, d). When the testes of three other males (Table 7) whose testes were the largest were crushed to make sperm

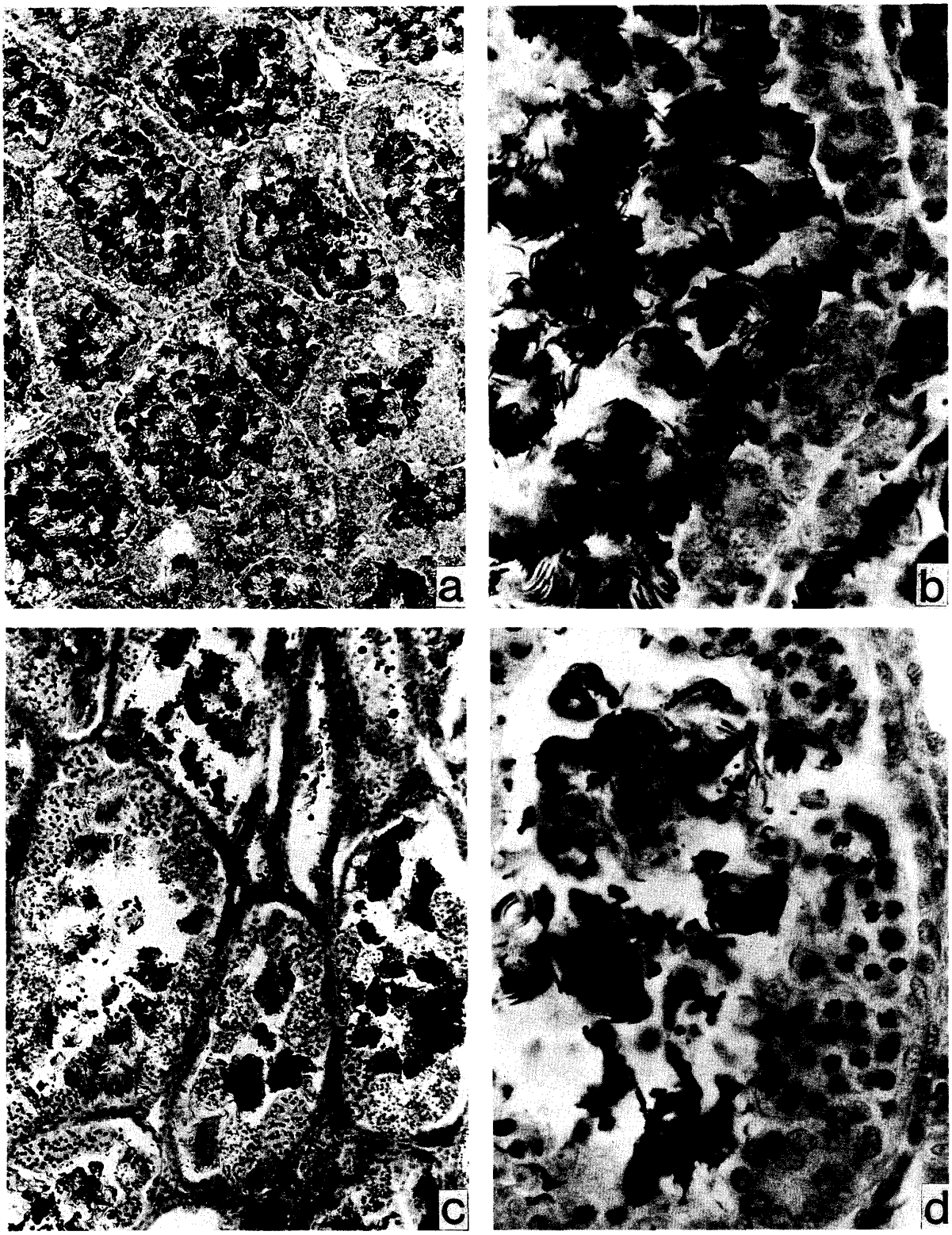

Fig. 10. Cross-sections of the testes of a one-year-old male hybrid between a female *Hyla arborea japonica* from Korea and a male *H. a. japonica* from Hiroshima and the control.

a. Normal testis (♂N) of a control male, (*jap* K♀ × *jap* K♂)♂, No. 5. ×110
b. ditto ×450
c. Abnormal testis of type 1 (♂1) of a male hybrid, (*jap* K♀ × *jap* H♂)♂, No. 3. ×110
d. ditto ×450

suspensions, there were fairly numerous abnormal spermatozoa and pycnotic nuclei in addition to many normal spermatozoa (♂ 1).

2) *jap* K♀ × *jap* T♂

Sex was examined in 35 juvenile and mature frogs of the 47 produced from a crossing between a female of the Korea population and a male of the Tsushima population. The results showed that eight of the 17 juvenile frogs were normal females whose ovaries contained normal auxocytes, six were males with normal testes, one was a male with rudimentary testes and the remaining two were hermaphrodites. Seven of the 18 mature frogs were normal females and the other 11 were males whose testes were of normal appearance. When the two hermaphrodites were counted as males, the 35 frogs in total consisted of 15 females and 20 (57.1%) males (Table 5).

The ovaries of the seven mature females all contained abundant normal eggs. Two of these females (Table 6) were used in backcrosses. When the testes of five of the 11 mature males were cut into sections to observe their inner structures, it was found that the testes of one male were normal and did not differ from those of the control males (Table 5). The seminiferous tubules were filled with bundles of normal spermatozoa and scarcely contained abnormal spermatozoa and pycnotic nuclei (♂ N) (Fig. 11a, b). In contrast to the testes of this male, those of the other four males contained fairly abundant abnormal spermatozoa and pycnotic nuclei in addition to normal spermatozoa (♂ 1) (Fig. 11c, d). The testes of three other males (Table 7) were crushed into pieces to make sperm suspensions. The latter contained fairly numerous abnormal spermatozoa and pycnotic nuclei together with numerous normal spermatozoa (♂ 1).

3) *jap* K♀ × *arb* L♂

Sex was examined in 34 juvenile and mature frogs of the 39 hybrids produced from a crossing between a female of the Korea population and a male *H. a. arborea*. The results showed that five of the 14 juvenile frogs were females whose ovaries were underdeveloped and had no auxocytes, two were females whose ovaries were completely degenerated, two were hermaphrodites, two had rudimentary testes and the remaining three were males whose testes were of nearly normal appearance. Of the 20 mature frogs, nine were females and 11 were males. When the hermaphrodites were counted as males, the 34 frogs in total consisted of 16 females and 18 (52.9%) males (Table 5).

The ovaries of nine mature females were degenerated at various degrees. The ovaries of some females were leaflike or filiform and contained no auxocytes (Fig. 12d). They were filled with oogonia and first oocytes, or had no germ cells. Eleven frogs were males with extremely small testes being 0.2~2.0 mm in length and 0.1~1.0 mm in width. The left testes of four mature males which had comparatively well-developed testes, being 1.0~2.0 mm in length and 0.5~1.0 mm in width, were cut into sections to observe the inner structures (Table 7). It was found that each of the four males had not both normal and abnormal spermatozoa.

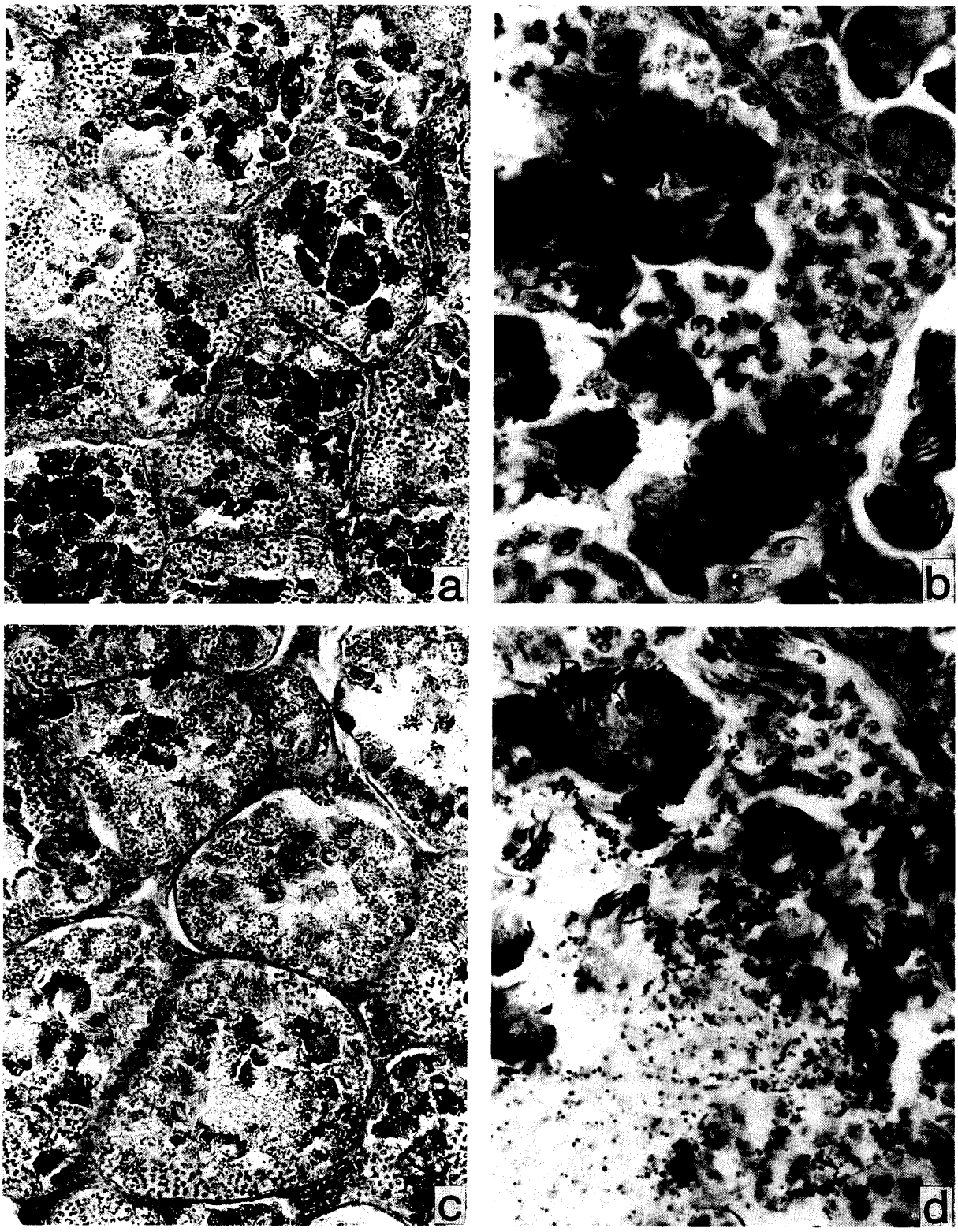

Fig. 11. Cross-sections of the testes of one-year-old male hybrids between a female *Hyla arborea japonica* from Korea and a male *H. a. japonica* from Tsushima.

a. Normal testis (♂N) of a male hybrid, (*jap* K♀ × *jap* T♂)♂, No. 4. ×110
b. ditto ×450
c. Abnormal testis of type 1 (♂1) of a male hybrid, (*jap* K♀ × *jap* T♂)♂, No. 7. ×110
d. ditto ×450

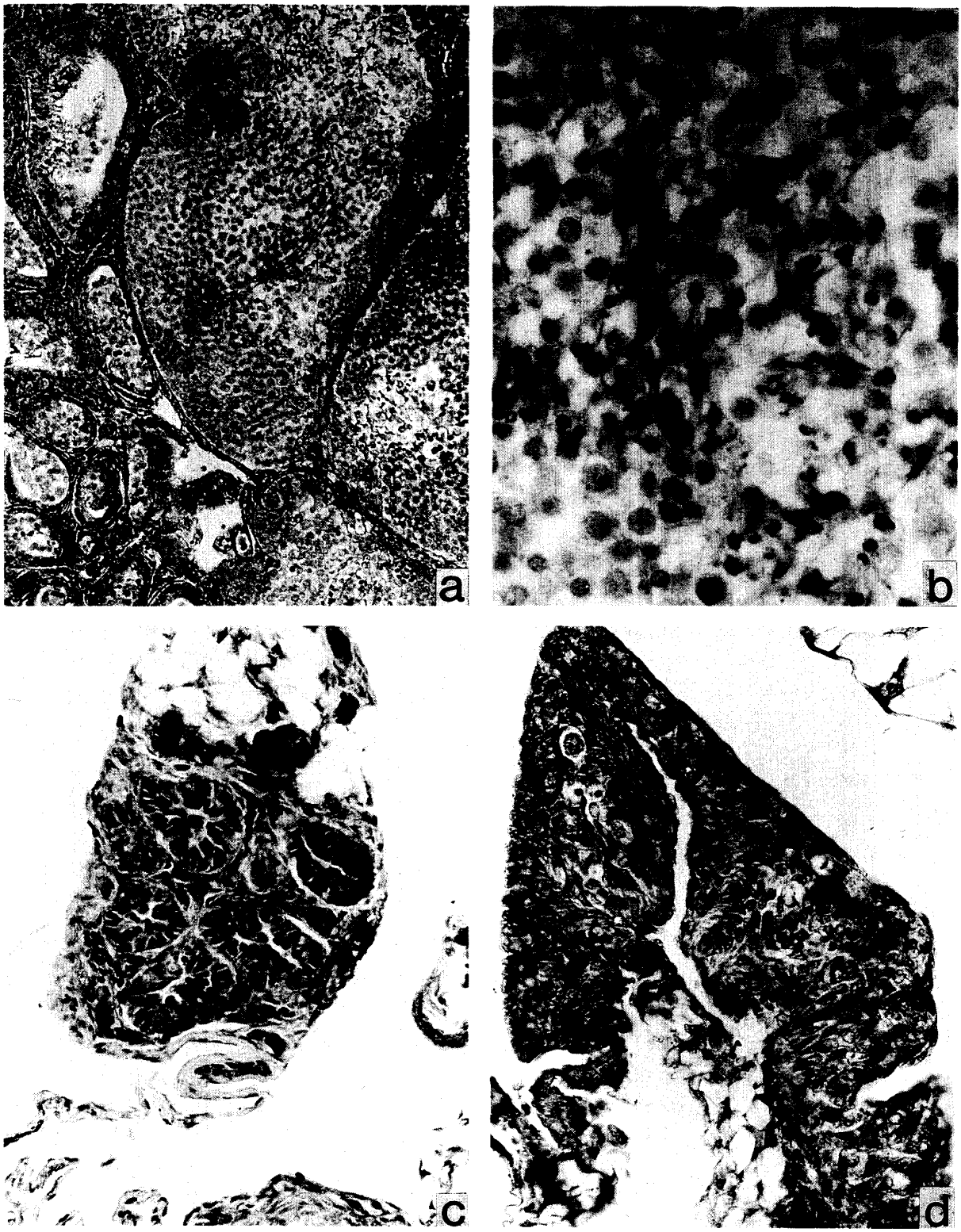

Fig. 12. Cross-sections of the gonads of one-year-old hybrids between a female *Hyla arborea japonica* from Korea and a male *H. a. arborea* from Luxembourg.

a. Abnormal testis of type 2 (♂2) of a male hybrid, (*jap* K♀ × *arb* L♂)♂, No. 5. ×110
b. ditto ×450
c. Abnormal testis of type 2 (♂2) of a male hybrid, (*jap* K♀ × *arb* L♂)♂, No. 8. ×225
d. Degenerated ovary (♀D) of a female hybrid, (*jap* K♀ × *arb* L♂)♀, No. 7. ×225

Thus, these males were not used for backcrosses and the testes of the remaining seven males were also cut into sections to examine the inner structures. While the testes of one male contained a few spermatozoa (Fig. 12a, b), those of the other six males had no germ cells (Fig. 12c). These males were named Type 2 (♂2).

V. Reproductive capacity of the hybrids between the Hiroshima and Tsushima populations of Hyla arborea japonica

1. Male hybrids between a female of the Hiroshima population and a male of the Tsushima population

a. Control series, (*jap* H♀ × *jap* H♂)♀, Nos. 1~4 × (*jap* H♀ × *jap* H♂)♂, Nos. 1~4

As female and male offspring obtained in 1971 from matings between three females and a male of the Hiroshima population attained sexual maturity in 1972, four control matings were made between four females (Nos. 1~4) and four males (Nos. 1~4) of the Hiroshima population. The results showed that 354 (84.7%) of the 418 eggs in total cleaved normally and 41 eggs cleaved abnormally. Of the normally cleaved eggs, 52, five and 23 became abnormal and died at the gastrula, tail-bud and hatching stages, respectively, while 274 (65.6%) hatched normally. Of the normally hatched tadpoles, 42 died without taking food, and 45 died by completion of metamorphosis. Eventually, 187 (44.7%) of the total eggs completed metamorphosis. This number corresponded to 52.8% of the normally cleaved eggs (Table 8).

b. Experimental series, (*jap* H♀ × *jap* H♂)♀, Nos. 1~3 × (*jap* H♀ × *jap* T♂)♂, Nos. 1~3

In 1972, backcrosses were made between three control females (Nos. 1~3) of the Hiroshima population and three one-year-old male hybrids, (*jap* H♀ × *jap* T♂)♂, Nos. 1~3. The results showed that 174(52.6%) of the 331 eggs in total cleaved normally, while 29 eggs cleaved abnormally. Of the normally cleaved eggs, 15, four and four became abnormal and died at the gastrula, tail-bud and hatching stages, respectively, while 151 (45.6%) hatched normally. Of these tadpoles, 21 died without taking food, and 63 became abnormal and died by completion of metamorphosis. Eventually, 67 (20.2%) tadpoles completed metamorphosis. This number corresponded to 38.5% of the normally cleaved eggs (Table 8).

2. Male hybrids between a female of the Tsushima population and a male of the Hiroshima population

a. Control series, (*jap* T♀ × *jap* T♂)♀, Nos. 1~3 × (*jap* T♀ × *jap* T♂)♂, Nos. 1~3

As female and male offspring obtained in 1971 from matings of two females and a male of the Tsushima population attained sexual maturity in 1972, three control matings were made between three females (Nos. 1~3) and three males (Nos. 1~3).

TABLE 8
Reproductive capacity of male hybrids between the Hiroshima and Tsushima populations of *Hyla arborea japonica* and their controls

Parents		No. of eggs	No. of cleavages		No. of tail-bud embryos		No. of hatched tadpoles		No. of feeding tadpoles		No. of metamorphosed frogs
Female	Male		Normal	Ab	Normal	Ab	Normal	Ab	Normal	Ab	
jap H♀ ×*jap* H♂, No. 1	*jap* H♀ ×*jap* H♂, No. 1	54	49 (90.7%)	2	47 (87.0%)	2	47 (87.0%)	0	43 (79.6%)	4	40 (74.1%)
jap H♀ ×*jap* H♂, No. 2	*jap* H♀ ×*jap* H♂, No. 2	48	40 (83.3%)	3	35 (72.9%)	2	33 (68.8%)	2	29 (60.4%)	4	25 (52.1%)
jap H♀ ×*jap* H♂, No. 3	*jap* H♀ ×*jap* H♂, No. 3	53	39 (73.6%)	4	35 (66.0%)	0	33 (62.3%)	2	23 (43.4%)	10	20 (37.7%)
jap H♀ ×*jap* H♂, No. 4	*jap* H♀ ×*jap* H♂, No. 4	263	226 (85.9%)	32	180 (68.4%)	1	161 (61.2%)	19	137 (52.1%)	24	102 (38.8%)
jap T♀ ×*jap* T♂, No. 1	*jap* T♀ ×*jap* T♂, No. 1	82	78 (95.1%)	1	72 (87.8%)	4	68 (82.9%)	4	51 (62.2%)	17	44 (53.7%)
jap T♀ ×*jap* T♂, No. 2	*jap* T♀ ×*jap* T♂, No. 2	12	9 (75.0%)	1	8 (66.7%)	0	8 (66.7%)	0	6 (50.0%)	2	5 (41.7%)
jap T♀ ×*jap* T♂, No. 3	*jap* T♀ ×*jap* T♂, No. 3	26	23 (88.5%)	2	21 (80.8%)	1	18 (69.2%)	3	15 (57.7%)	3	14 (53.8%)
jap H♀ ×*jap* H♂, No. 1	*jap* H♀ ×*jap* T♂, No. 1	100	90 (90.0%)	3	82 (82.0%)	1	82 (82.0%)	0	78 (78.0%)	4	42 (42.0%)
jap H♀ ×*jap* H♂, No. 2	*jap* H♀ ×*jap* T♂, No. 2	120	22 (18.3%)	3	19 (15.8%)	1	17 (14.2%)	2	14 (11.7%)	3	5 (4.2%)
jap H♀ ×*jap* H♂, No. 3	*jap* H♀ ×*jap* T♂, No. 3	111	62 (55.9%)	23	54 (48.6%)	2	52 (46.8%)	2	38 (34.2%)	14	20 (18.0%)
jap T♀ ×*jap* T♂, No. 1	*jap* T♀ ×*jap* H♂, No. 1	147	138 (93.9%)	3	120 (81.6%)	8	112 (76.2%)	8	89 (60.5%)	23	46 (31.3%)
jap T♀ ×*jap* T♂, No. 2	*jap* T♀ ×*jap* H♂, No. 2	125	120 (96.0%)	3	115 (92.0%)	2	95 (76.0%)	20	87 (69.6%)	8	41 (32.8%)
jap T♀ ×*jap* T♂, No. 3	*jap* T♀ ×*jap* H♂, No. 3	124	104 (83.9%)	12	60 (48.4%)	12	53 (42.7%)	7	31 (25.0%)	22	26 (21.0%)

The results showed that 110 (91.7%) of the 120 eggs in total cleaved normally, while four eggs cleaved abnormally. Of the normally cleaved eggs, four, five and seven became abnormal and died at the gastrula, tail-bud and hatching stages, respectively, while 94 (78.3%) hatched normally. Thereafter, 22 of them died without taking food and nine became abnormal and died by completion of metamorphosis. Eventually, 63 (52.5%) of the total eggs completed metamorphosis. This number corresponded to 57.3% of the normally cleaved eggs (Table 8).

b. Experimental series, (*jap* T♀ ×*jap* T♂)♀, Nos. 1~3×(*jap* T♀ ×*jap* H♂)♂, Nos. 1~3

In 1972, backcrosses were made between three control females (Nos. 1~3) of the Tsushima population and three one-year-old male hybrids, (*jap* T♀ ×*jap* H♂)♂, Nos. 1~3. The results showed that 362 (91.4%) of the 396 eggs in total cleaved normally, and 18 eggs cleaved abnormally. While 45, 22 and 35 of the normally cleaved eggs became abnormal and died at the gastrula, tail-bud and hatching

stages, respectively, 260 (65.7%) hatched normally. Thereafter, 53 tadpoles died without taking food and 94 others died by the completion of metamorphosis. Eventually, 113 (28.5%) completed metamorphosis. This number corresponded to 31.2% of the normally cleaved eggs (Table 8).

3. Female hybrids between a female of the Hiroshima population and a male of the Tsushima population

When ovulation was stimulated by injection with pituitaries of *Rana nigromaculata* in 1972, two (Nos. 1 and 2) of the five one-year-old female hybrids (*jap* H♀ × *jap* T♂)♀ laid 71 and 315 eggs, respectively. These eggs were 1.6 mm in diameter, being almost intermediate between those of the parental populations (Table 6).

a. (*jap* H♀ × *jap* T♂)♀, Nos. 1 and 2 × (*jap* H♀ × *jap* H♂)♂, No. 1

Two backcrosses were made between two female hybrids (*jap* H♀ × *jap* T♂)♀, Nos. 1 and 2 and a male of the Hiroshima population (*jap* H♀ × *jap* H♂)♂, No. 1. The results showed that 114 (51.1%) of the 223 eggs in total cleaved normally, and 103 cleaved abnormally. Of the normally cleaved eggs, 18, 43 and 15 became abnormal and died at the gastrula, tail-bud and hatching stages, respectively, while 38 (17.0%) hatched normally. Thereafter, 13 tadpoles died without taking food, and 11 became abnormal and died by the completion of metamorphosis. Eventually, 14 (6.3%) tadpoles completed metamorphosis. This number corresponded to 12.3% of the normally cleaved eggs (Table 9).

b. (*jap* H♀ × *jap* T♂)♀, Nos. 1 and 2 × (*jap* T♀ × *jap* T♂)♂, No. 1

The above two female hybrids, (*jap* H♀ × *jap* T♂)♀, Nos. 1 and 2, were

TABLE 9

Reproductive capacity of female hybrids between the Hiroshima and Tsushima populations of *Hyla arborea japonica*

Parents		No. of eggs	No. of cleavages		No. of tail-bud embryos		No. of hatched tadpoles		No. of feeding tadpoles		No. of metamorphosed frogs
Female	Male		Normal	Ab	Normal	Ab	Normal	Ab	Normal	Ab	
jap H♀ × *jap* T♂, No. 1	*jap* H♀ × *jap* H♂, No. 1	43	12 (27.9%)	31	8 (18.6%)	2	8 (18.6%)	0	5 (11.6%)	3	2 (4.7%)
	jap T♀ × *jap* T♂, No. 1	28	3 (10.7%)	23	3 (10.7%)	0	3 (10.7%)	0	2 (7.1%)	1	0
jap H♀ × *jap* T♂, No. 2	*jap* H♀ × *jap* H♂, No. 1	180	102 (56.7%)	72	45 (25.0%)	41	30 (16.7%)	15	20 (11.1%)	10	12 (6.7%)
	jap T♀ × *jap* T♂, No. 1	135	72 (53.3%)	56	18 (13.3%)	38	17 (12.6%)	1	8 (5.9%)	9	5 (3.7%)
jap T♀ × *jap* H♂, No. 1	*jap* T♀ × *jap* T♂, No. 1	69	25 (36.2%)	47	25 (36.2%)	0	24 (34.8%)	1	22 (31.9%)	2	17 (24.6%)
jap T♀ × *jap* H♂, No. 2	*jap* T♀ × *jap* T♂, No. 1	117	62 (53.0%)	40	60 (51.3%)	2	56 (47.9%)	4	52 (44.4%)	4	36 (30.8%)

backcrossed with a male of the Tsushima population, (*jap* T ♀ ×*jap* T ♂) ♂, No. 1. The results showed that 75 (46.0%) of the 163 eggs in total cleaved normally, and 79 eggs cleaved abnormally. Of the normally cleaved eggs, 16, 38 and one became abnormal and died at the gastrula, tail-bud and hatching stages, respectively, while 20 (12.3%) hatched normally. Thereafter, 10 tadpoles died without taking food and five others became abnormal and died by the completion of metamorphosis. Eventually, only five (3.1%) completed metamorphosis. This number corresponded to 6.7% of the normally cleaved eggs (Table 9).

When the two female hybrids (Nos. 1 and 2) were compared with each other in reproductive capacity, there were no remarkable difference between them. Female No. 1 laid 71 eggs, of which 15 (21.1%) cleaved normally and two (2.8%) became normally metamorphosed frogs, while female No. 2 laid 315 eggs, of which 174 (55.2%) cleaved normally and 17 (5.4%) became normally metamorphosed frogs.

4. Female hybrids between a female of the Tsushima population and a male of the Hiroshima population

When ovulation was stimulated by injection of pituitaries of *Rana nigromaculata* in 1972, two (Nos. 1 and 2) of the five female hybrids (*jap* T♀ ×*jap* H♂) ♀ laid 69 and 117 eggs, respectively. These eggs were 1.7 mm in diameter, being almost intermediate between those of the two parental populations. Two backcrosses were made between two female hybrids and a male of the Tsushima population (*jap* T♀ ×*jap* T♂) ♂, No. 1. The results showed that 87 (46.8%) of the 186 eggs in total cleaved normally, while 87 eggs cleaved abnormally. Of the normally cleaved eggs, two and five became abnormal and died at the tail-bud and hatching stages, respectively, and 80 (43.0%) hatched normally. Six tadpoles died without taking food and 21 became abnormal and died by the completion of metamorphosis. Eventually, 53 (28.5%) tadpoles normally completed metamorphosis. This number corresponded to 60.9% of the normally cleaved eggs (Table 9).

VI. Reproductive capacity of the hybrids between the Hiroshima and Korea populations of Hyla arborea japonica

1. Male hybrids between a female of the Hiroshima population and a male of the Korea population

In 1972, a backcross was made between a control female (*jap* H♀ ×*jap* H♂) ♀, No. 4 and a one-year-old male hybrid, (*jap* H♀ ×*jap* K♂) ♂, No. 1. The result showed that 185 (67.5%) of the 274 eggs cleaved normally and 70 cleaved abnormally. Of the normally cleaved eggs, 15, six and 38 became abnormal and died at the gastrula, tail-bud and hatching stages, respectively, while 126 (46.0%) hatched normally. Of these tadpoles, eight died without taking food and 76 died by the completion of metamorphosis. Eventually, 42 (15.3%) tadpoles completed metamorphosis. This number corresponded to 22.7% of the normally cleaved eggs (Table 10).

TABLE 10
Reproductive capacity of male hybrids between the Hiroshima and Korea populations of *Hyla arborea japonica*

Parents		No. of eggs	No. of cleavages		No. of tail-bud embryos		No. of hatched tadpoles		No. of feeding tadpoles		No. of metamorphosed frogs
Female	Male		Normal	Ab	Normal	Ab	Normal	Ab	Normal	Ab	
jap H♀×*jap* H♂, No. 4	*jap* H♀×*jap* K♂, No. 1	274	185 (67.5%)	70	164 (59.9%)	6	126 (46.0%)	38	118 (43.1%)	8	42 (15.3%)
jap H♀×*jap* H♂, No. 1	*jap* K♀×*jap* H♂, No. 1	149	74 (49.7%)	42	61 (40.9%)	6	61 (40.9%)	0	42 (28.2%)	19	21 (14.1%)
jap H♀×*jap* H♂, No. 2	*jap* K♀×*jap* H♂, No. 2	114	60 (52.6%)	31	51 (44.7%)	5	47 (41.2%)	4	40 (35.1%)	7	25 (21.9%)
jap H♀×*jap* H♂, No. 3	*jap* K♀×*jap* H♂, No. 3	187	150 (80.2%)	28	107 (57.2%)	8	80 (42.8%)	27	72 (38.5%)	8	37 (19.8%)

2. Male hybrids between a female of the Korea population and a male of the Hiroshima population

In 1972, three backcrosses were made between three control females, (*jap* H♀×*jap* H♂)♀, Nos. 1~3, and three one-year-old male hybrids, (*jap* K♀×*jap* H♂)♂, Nos. 1~3. The results showed that 284 (63.1%) of the 450 eggs in total cleaved normally, and 101 cleaved abnormally. Of the normally cleaved eggs, 46, 19 and 31 became abnormal and died at the gastrula, tail-bud and hatching stages, respectively, while 188 (41.8%) hatched normally. Thereafter, 34 tadpoles died without taking food, and 71 died by completion of metamorphosis. Eventually, 83 (18.4%) completed metamorphosis. This number corresponded to 29.2% of the normally cleaved eggs (Table 10).

VII. Reproductive capacity of the hybrids between the Korea and Tsushima populations of Hyla arborea japonica

1. Male hybrids between a female of the Korea population and a male of the Tsushima population

In 1972, three backcrosses were made between three control females, (*jap* T♀×*jap* T♂)♀, Nos. 1~3, and three one-year-old male hybrids, (*jap* K♀×*jap* T♂)♂, Nos. 1~3. The results showed that 230 (90.9%) of the 253 eggs in total cleaved normally, and 16 cleaved abnormally. Of the normally cleaved eggs, 12, five and 10 became abnormal and died at the gastrula, tail-bud and hatching stages, respectively, while 203 (80.2%) hatched normally. Thereafter, 43 tadpoles died without taking food, and 55 died by the completion of metamorphosis. Eventually, 105 (41.5%) completed metamorphosis. This number corresponded to 45.7% of the normally cleaved eggs (Table 11).

TABLE 11

Reproductive capacity of male and female hybrids between the Tsushima and Korea populations of *Hyla arborea japonica*

Parents		No. of eggs	No. of cleavages		No. of tail-bud embryos		No. of hatched tadpoles		No. of feeding tadpoles		No. of metamorphosed frogs
Female*	Male		Normal	Ab	Normal	Ab	Normal	Ab	Normal	Ab	
jap T♀ ×*jap* T♂, No. 1	*jap* K♀ ×*jap* T♂, No. 1	64	61 (95.3%)	3	57 (89.1%)	0	55 (85.9%)	2	45 (70.3%)	10	34 (53.1%)
jap T♀ ×*jap* T♂, No. 2	*jap* K♀ ×*jap* T♂, No. 2	89	75 (84.3%)	10	67 (75.3%)	3	61 (68.5%)	6	47 (52.8%)	14	40 (44.9%)
jap T♀ ×*jap* T♂, No. 3	*jap* K♀ ×*jap* T♂, No. 3	100	94 (94.0%)	3	89 (89.0%)	2	87 (87.0%)	2	68 (68.0%)	19	31 (31.0%)
jap K♀ ×*jap* T♂, No. 1	*jap* T♀ ×*jap* T♂, No.1	94	85 (90.4%)	7	84 (89.4%)	1	82 (87.2%)	2	49 (52.1%)	33	32 (34.0%)
	jap K♀ ×*jap* K♂, No. 1	138	129 (93.5%)	7	113 (81.9%)	7	112 (81.2%)	1	109 (79.0%)	3	65 (47.1%)
jap K♀ ×*jap* T♂, No. 2	*jap* T♀ ×*jap* T♂, No. 1	93	90 (96.8%)	1	89 (95.7%)	1	82 (88.2%)	7	70 (75.3%)	12	43 (46.2%)
	jap K♀ ×*jap* K♂, No. 1	121	107 (88.4%)	3	102 (84.3%)	5	99 (81.8%)	3	92 (76.0%)	7	62 (51.2%)

2. Female hybrids between a female of the Korea population and a male of the Tsushima population

In 1972, when ovulation was stimulated in five one-year-old hybrid females by injection of pituitaries of *Rana nigromaculata,* two of them, (*jap* K♀ ×*jap* T♂)♀, Nos. 1 and 2, laid 232 and 214 eggs, respectively. These eggs were 1.7 mm in diameter, being intermediate between those of the two parental populations (Table 6).

a. (*jap* K♀ ×*jap* T♂)♀, Nos. 1 and 2×(*jap* T♀ ×*jap* T♂)♂, No. 1

Two backcrosses were made between two female hybrids, (*jap* K♀ ×*jap* T♂)♀, Nos. 1 and 2, and a control male of the Tsushima population, (*jap* T♀ ×*jap* T♂)♂, No. 1. The results showed that 175 (93.6%) of the 187 eggs in total cleaved normally, and eight cleaved abnormally. Of the normally cleaved eggs, two and nine became abnormal and died at the tail-bud and hatching stages, respectively, while 164 (87.7%) hatched normally. Thereafter, 45 tadpoles died without taking food, and 44 became abnormal and died by the completion of metamorphosis. Eventually, 75 (40.1%) completed metamorphosis. This number corresponded to 42.9% of the normally cleaved eggs (Table 11).

b. (*jap* K♀ ×*jap* T♂)♀, Nos. 1 and 2×(*jap* K♀ ×*jap* K♂)♂, No. 1

Two backcrosses were made between two female hybrids, (*jap* K♀ ×*jap* T♂)♀, Nos. 1 and 2, and a control male of the Korea population, (*jap* K♀ ×*jap* K♂)♂, No. 1. The results showed that 236 (91.1%) of the 259 eggs in total cleaved normally and 10 eggs cleaved abnormally. Of the normally cleaved eggs,

nine, 12 and four became abnormal and died at the gastrula, tail-bud and hatching stages, respectively, while 211 (81.5%) hatched normally. Thereafter, 10 tadpoles died without taking food, and 74 became abnormal and died by completion of metamorphosis. Eventually, 127 (49.0%) tadpoles completed metamorphosis. This number corresponded to 53.8% of the normally cleaved eggs (Table 11).

VIII. Reproductive capacity of the hybrids between Hyla arborea japonica and H. a. arborea

1. Female hybrids

Females of the hybrids between female *H. a. japonica* from the Hiroshima, Tsushima and Korea populations and a male *H. a. arborea* were completely sterile, as the ovaries of mature female hybrids were extremely ill-developed and contained no ova. However, only two female hybrids between a female *H. a. japonica* from Hiroshima and a male *H. a. arborea* contained a few auxocytes in their ovaries.

2. Male hybrids

a. (*jap* H♀×*jap* H♂)♀, Nos. 1~4×(*jap* H♀×*arb* L♂)♂, Nos. 1~4

Four backcrosses were made between four control females of the Hiroshima population, (*jap* H♀×*jap* H♂)♀, Nos. 1~4, and four male hybrids, (*jap* H♀×*arb* L♂)♂, Nos. 1~4 (Table 7). These males were those which had the largest testes among the nine mature male hybrids between a female of the Hiroshima population of *H. a. japonica* and a male *H. a. arborea*. The results showed that no normally cleaved eggs were obtained from a total of 355 eggs of the four females. In three of these four female hybrids, 37 of the 58 eggs, 53 of the 84 eggs and 95 of the 121 eggs cleaved abnormally. However, all abnormally cleaved eggs died without attaining the blastula stage.

b. (*jap* T♀×*jap* T♂)♀, Nos. 1~3×(*jap* T♀×*arb* L♂)♂, Nos. 1~4

Four backcrosses were made between three control females of the Tsushima population of *H. a. japonica*, (*jap* T♀×*jap* T♂)♀, Nos. 1~3, and four male hybrids, (*jap* T♀×*arb* L♂)♂, Nos. 1~4 (Table 7). These four males were those which had the largest testes among the 16 mature male hybrids between a female of the Tsushima population of *H. a. japonica* and a male *H. a. arborea*. The results showed that no normally cleaved eggs were obtained from a total of 525 eggs of the three females.

c. (*jap* K♀×*arb* L♂)

The testes of the four male hybrids which had the largest testes among the 11 mature male hybrids between a female of the Korea population of *H. a. japonica* and a male *H. a. arborea* were very ill-developed, being 1.0~2.0 mm in length and 0.5~1.0 mm in width (Table 7). Thus, no attempt was made to backcross all

these male hybrids with the control females.

DISCUSSION

Numerous reports have been published on artificial interspecific crosses in treefrogs (BLAIR, 1958; MECHAM, 1960a, 1965; PYBURN, 1960; PYBURN and KENNEDY, 1960, 1961; MOORE, 1961; LITTLEJOHN, 1961; PAILLETTE, 1967; RALIN, 1976, 1977; DAITO, 1968; KURAMOTO, 1984, *etc.*). Natural interspecific hybrids have been also reported by several authors, such as MECHAM(1960a, 1960b), GERHARDT, GUTTMAN and KARLIN (1980) and ANDERSON and MOLER (1986).

In 1954, Moore has reported on the results of intraspecific crosses between the eastern and western populations of *Hyla aurea* as well as *Crinia signifera* in Australia. The hybrids between a female *H. aurea aurea* from New South Wales (N.S.W.) and male *H. aurea raniformis* from west Australia all died at the late embryonic stage. According to MOORE, this result indicates that the N.S.W. individuals belong to a different genetic species from those in west Australia. Then, the latter should be named *H. raniformis*. Hybridization between the east Australia population and the southwest Australia population of *Crinia signifera* also results in considerable inviability of the progeny. As the two populations are considered to be genetically different species, MOORE gave a new name, *Crinia insignifera,* to the south-west Australia population of *Crinia signifera.*

DAITO (1968) in our laboratory made intersubspecific crosses between female Japanese treefrogs, *H. arborea japonica* GUENTHER, and a male *H. arborea sarda* DE BETTA from Corsica Island. The results showed that the males and females of the almost mature hybrids were probably sterile. Accordingly, he assumed that the two subspecies are genetically isolated from each other, in spite of their similarity in morphological characters, and suggested that *H. arborea japonica* is a valid species with some reservations. KAWAMURA, NISHIOKA and UEDA made crossing experiments between female *H. arborea japonica* from Hiroshima, Tsushima and Korea and a male *H. a. arborea* from Luxembourg and confirmed the results obtained by DAITO. While all the hybrids between the female *H. arborea japonica* and the male *H. a. arborea* attained sexual maturity, the gonads of both male and female hybrids were degenerated and infertile. It was evident that *H. arborea japonica* was completely isolated from *H. a. arborea* by hybrid sterility. KURAMOTO (1984) made also crossing experiments between female *H.* (*arborea*) *japonica* from Fukuoka, Japan, and male *H. arborea arborea* from Austria and Italy and *H. arborea sarda* from Corsica Island. He stated that the presumed low hybrid fertility of the hybrids supports the specific status of *H.* (*arborea*) *japonica,* as all the female hybrids obtained by him had abnormal ovaries at metamorphosis. Hereafter, the present authors will use the specific name, *Hyla japonica* GUENTHER in place of *Hyla arborea japonica* GUENTHER, too.

It was especially interesting to us to clarify the genetic difference among different populations of *H. japonica.* We made reciprocal hybridization experiments among the Hiroshima, Tsushima and Korea populations. It was found

that the inter-populational hybrids were somewhat lower in rate of metamorphosis than the controls, except the hybrids between females of the Korea population and males of the Hiroshima population. The six kinds of hybrids among the three populations did not remarkably differ from the controls in sex ratio, as the males in juvenile and mature hybrids were 41.9~60.3%, while the controls were 45.5 ~53.1%. However, it was evident that males and females of each kind of hybrids were remarkably inferior than the controls in reproductive capacity. In backcrosses of female hybrids between females of the Hiroshima population and males of the Tsushima population with males of the Hiroshima or Tsushima population, less than 6.7% of the eggs attained the completion of metamorphosis. In two backcrosses of female hybrids of the reciprocal combination with males of the Tsushima population, 24.6% and 30.8% of the eggs completed metamorphosis. In backcrosses of males of reciprocal hybrids between the Hiroshima and Tsushima populations with females of the Hiroshima or Tsushima population, 4.2 ~42.0% of the eggs attained completion of metamorphosis. Backcrosses of males of reciprocal hybrids between the Hiroshima and Korea populations with females of the Hiroshima population were also inferior in production of metamorphosed frogs. In these backcrosses, 14.1~21.9% of the eggs became metamorphosed frogs. In contrast to these male and female hybrids, 37.7~74.1% of the eggs became metamorphosed frogs in the control Hiroshima and Tsushima populations. In backcrosses of the hybrids between the Korea and Tsushima populations with the Tsushima population, 31.0~53.1% of the eggs attained completion of metamorphosis. This suggests that the genetic relationship between the Hiroshima population and Tsushima or Korea population is fairly remote and that the Tsushima and Korea populations are comparatively close to each other.

NISHIOKA, SUMIDA and BORKIN (1990) have reported on the results of electrophoretic examinations of enzymes and blood proteins extracted from frogs of 13 populations of *Hyla* in the Far East. Of the genetic distances estimated among these populations, those between the Hiroshima and Tsushima populations, between the Hiroshima and the Korea populations and between the Tsushima and the Korea populations were 0.050, 0.137 and 0.059, respectively. These values of genetic distances seem to show that the three populations of *H. japonica* are within the rank of subspecies, although the Tsushima and Korea populations are somewhat isolated from the Hiroshima population by incomplete hybrid sterility.

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