Studies on the Ichinoseki Population of *Rana japonica*

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(With 19 Text-figures and 4 Plates)

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INTRODUCTION

Rana japonica Günther is widely distributed in Japan excluding Hokkaido, Aomori Prefecture and the northern part of Iwate Prefecture. Although there are some individual differences in color and pattern of this species, no local variations have been reported hitherto in external characters.

Recently, the present author (1979) reported that the Ichinoseki population distributed in the northern extremity of the range of Rana japonica remarkably differed from the Hiroshima population in the results of crossing experiments with Rana tsushimensis. This suggests that differentiation has occurred in some degree between the Hiroshima and

Ichinoseki populations of *Rana japonica*.

In order to examine the existence of reproductive isolating mechanisms between the Hiroshima and Ichinoseki populations, the present author carried out crossing experiments between them as well as compared these two populations morphologically, biochemically and karyologically. Preliminary reports of this research were made previously (1979, 1980).

MATERIALS AND METHODS

Rana japonica Günther were collected in the suburbs of Hiroshima City, Hiroshima Prefecture and Ichinoseki City, Iwate Prefecture. The Hiroshima specimens were caught late in October, 1978, and kept in an outdoor container during hibernation. The Ichinoseki specimens were caught early in August, 1978, and reared in the laboratory until the following year. Crossing experiments were performed in Feburary and March, 1979 and 1980. Ovulation was induced by implantation of *Rana catesbeiana* pituitaries. Reciprocal crosses were made by the routine method of artificial fertilization. A part of the fertilized eggs, about 20 eggs in each series, were kept at 18°C until the completion of metamorphosis for comparison with those of the other series in developmental velocity. In this paper, the embryonic stages follow those of Tahara's table (1959), while the tadpole stages follow those of Taylor and Kollros's table (1946). Tadpoles fed on boiled spinach, while metamorphosed frogs fed on crikets. Histological observation of gonads was made after fixed in Navashin's fluid, sectioned at 10 or 12μ and stained with Heidenhain's iron hematoxylin. Electrophoretic analyses were performed for ten enzymes and two blood proteins. They were carried out by starch gel electrophoresis, basically in accordance with Brewer's methods (1970). The two populations of *Rana japonica* were compared with each other in sixteen loci of these proteins.

Chromosomes were observed in the tail-tips of tadpoles by Makino and Nishimura's squash method (1952), as well as in bone marrow cells by Omura's vapor fixation method (1967). The karyotype of each population was examined by analyzing 54 metaphase spreads obtained from bone marrow cells. These spreads were also utilized to calculate the relative chromosome length and the centromere position of each chromosome. The karyotypes of two populations were compared with each other by the method of Hubbs and Hubbs (1953).

OBSERVATION

I. Developmental capacity

1. Hybrids between Hiroshima females and Ichinoseki males

(i) On 5 Feburary 1979, a field-caught female of the

Hiroshima population(H♀1) was mated with a field-caught male of the Hiroshima population(H♂1) and a field-caught male of the Ichinoseki population(I♂1) (Table 1, Fig.1-a,b). Of 120 eggs in the control series, 111(92.5%) cleaved normally, 102 (85.0%) hatched normally and 96(80.0%) metamorphosed normally, while of 157 eggs in the experimental series, 147(93.6%) cleaved normally, 144(91.7%) hatched normally and 138(87.9%) metamorphosed normally.

(ii) On 10 March 1980, six field-caught females of the Hiroshima population(H♀2~7) were mated with six field-caught males of the Hiroshima population(H♂2~7) and six one-year-old males of the Ichinoseki population(I♂2~7) (Table 1, Fig.1-a,b). In six control series, the proportions of normal cleavages were comparatively high, being 81.8~97.2%, 89.2% on the average. While only a small number of normally cleaved eggs died of various abnormalities, 77.3 ~ 96.5%, 87.2% on the average, of the respective total number of eggs, hatched normally. During the tadpole stage, only a few individuals died; 74.2 ~ 93.6%, 83.9% on the average, of the respective total number of eggs, metamorphosed normally. In six experimental series, 78.7 ~ 98.5%, 90.7% on the average, of the respective total number of eggs cleaved normally. While a few normally cleaved eggs died of various abnormalities, 77.0 ~ 96.3%, 88.3% on the average, hatched normally. A few tadpoles died of under-development or edema, and eventually 72.7 ~ 91.2%, 81.6% on the average, metamorphosed normally. No significant differences were found between the control and experimental series in mortality at each developmental stage and in morphological defects.

2. Hybrids between Ichinoseki females and Hiroshima males

(i) On 5 Feburary 1979, a field-caught female of the Ichinoseki population(I♀1) was mated with field-caught males of both populations stated above(H♂1 and I♂1) (Table 1, Fig. 1-c,d). Of 165 eggs in the control series, 147(89.1%) cleaved normally. After normally cleaved eggs died of various kinds of abnormalities at various developmental stages from gastrula to hatching, 96(58.2%) hatched normally. During the tadpole stage, some individuals died of edema or underdevelopment, and eventually 88(53.3%) completed metamorphosis. In the experimental series, 211(86.5%) of 244 eggs cleaved normally. The normally cleaved eggs were nearly the same as the controls in developmental capacity, that is, 148(60.7%) hatched normally and 127(52.0%) completed metamorphosis. Morphological defects found in many embryos or tadpoles were similar to those of the controls.

(ii) On 10 March 1980, three one-year-old females of the Ichinoseki population(I♀2∼4) were mated with the same males as those stated above(H♂2∼4 and I♂2∼4) (Table 1, Fig.1-c,d). In three control series, 74.5 ∼ 83.5%, 79.1% on the average, of the respective total number of eggs cleaved normally, and afterwards many of the normally cleaved eggs died of various abnormalities at various embryonic stages from gastrula to hatching. After 55.5~63.0%, 59.4% on the average, hatched normally, some tadpoles also died of underdevelopment or edema, and eventually 40.1~44.9%, 41.6% on the average, completed metamorphosis. In three experimental series, 76.3~86.1%, 81.5% on the average, of the respective total number of eggs cleaved normally. After many of normally cleaved eggs died of similar abnormalities as those of the controls at various embryonic stages, 54.9~56.5%, 55.5% on the average, hatched normally. During the tadpole stage, some individuals died of underdevelopment; 42.2~44.3%, 43.3% on the average, completed metamorphosis.

II. Developmental velocity

The Hiroshima and Ichinoseki populations were compared with each other in developmental velocity at 18°C (Tables 2 and 3). There was a slight difference in this respect between the two populations during the early embryonic stage. Three hours after insemination, the Hiroshima eggs began to cleave (stage 3E), while the Ichinoseki eggs had almost completed the first cleavage and were at the 2-cell stage (stage 3). Five hours after insemination, the Hiroshima embryos started the third cleavage (stage 5E), while the Ichinoseki embryos had completed the third cleavage and were at the 8-cell stage(stage 5). Eight hours after insemination, the Ichinoseki embryos were at the morula stage(stage 7b), while the Hiroshima embryos were not yet at the same stage (stage 7aL). Twenty two hours after insemination, invagination of the blastopore was slightly observed in both populations (stage 10a). Thirty hours after insemination, the yolk plug was formed in the Hiroshima embryos(stage 12E), while there was still a horseshoe-shaped blastopore in the Ichinoseki embryos(stage 11). Sixty nine hours after insemination, the Hiroshima embryos were at the tail-bud stage(stage 17), while the Ichinoseki embryos were at the early tail-bud stage (stage 17E). Thereafter the two populations proceeded almost identically in development until TK stage III. Eight days after insemination, the external gills degenerated almost completely(stage 25), and 10 days later they attained TK stage III. After this stage a difference in developmental velocity appeared again between the tadpoles of the two populations. The Hiroshima and Ichinoseki tadpoles required 76.2 days and 89.3 days on the average, respectively, to attain TK stage XX, that is, fore-limb protrusion.

No observations were made on developmental velocity at 18°C in reciprocal hybrids between the two populations. Thus, only the ages of the reciprocal hybrids were observed at the time of attaining TK stage XX at room temperature. At room temperature, 87 Hiroshima eggs required 78～87 days, 81.1 days on the average, to attain TK stage XX, while 85 Ichinoseki eggs required 85～94 days, 87.5 days on the average, to attain the same stage. On the other hand, 138 hybrids between Hiroshima females and Ichinoseki males and 127 reciprocal ones required 87～97 days, 89.3 days on the average, and 86～100 days, 89.4 days on the average, to attain TK stage XX, respectively.

III. Morphology

A. Measurements

The Hiroshima population, the Ichinoseki population and reciprocal hybrids between them were mutually compared by measuring several body sites at Shumway stage 25, TK stage XX, 3 months after metamorphosis and the age of one year (Tables 4～7).

(a) Embryos at stage 25

Measurements were made on ten embryos from each series (Table 4). The Hiroshima embryos were larger than the Ichinoseki embryos in total length, body length and tail length. Reciprocal hybrids were smaller than or almost equal to the embryos of the parental population which were smaller in every respect.

(b) Tadpoles at stage XX

Ten tadpoles of each series were measured (Table 5).

The Ichinoseki tadpoles were somewhat larger than the Hiroshima tadpoles in total length, body length, head width and tail height. Hybrids between a female of the Hiroshima population and a male of the Ichinoseki population were somewhat smaller than or almost similar to the Hiroshima tadpoles in each of these respects. The reciprocal hybrids were similar to the Hiroshima tadpoles in total length and body length, while they were similar to the Ichinoseki tadpoles in head width and tail height.

(c) Froglets three months after metamorphosis

Measurements were made on 20 froglets of each series (Table 6). It was found that the Hiroshima froglets were comparatively larger than the Ichinoseki froglets in hind leg length and tibia length, while they were somewhat smaller than the latter in body length. The ratio of hind leg length to body length in the Ichinoseki population was considerably smaller than that in the Hiroshima population. Furthermore, the head length was nearly similar to the head width in the Ichinoseki population, while the head length was slightly larger than the head width in the Hiroshima population. Therefore, the head of the Hiroshima froglets appeared to be more slender than that of the Ichinoseki froglets. The hybrids between a female of the Hiroshima population and a male of the Ichinoseki population were nearly the same as the Hiroshima froglets in body length, head length and head width, and similar to the Ichinoseki froglets in hind leg length. The reciprocal hybrids were slightly smaller than the Hiroshima froglets in body length, while they were nearly the same as the above hybrids in size of each body site.

(d) One-year-old frogs

Measurements were made on 10 male frogs of each series at the age of one year (Table 7). The Hiroshima frogs were smaller than the Ichinoseki frogs in body length and size of the metatarsal tubercle. The ratio of hind leg length to body length in the Ichinoseki frogs was somewhat smaller than that in the Hiroshima frogs. Reciprocal hybrids were similar to each other and to the Hiroshima frogs in various respects, although they were somewhat smaller in snout length and larger in size of the metatarsal tubercle. They were more similar to the Hiroshima frogs than the Ichinoseki frogs in the ratio of hind leg length to body length.

(e) Field-caught frogs

Measurements were made on the four field-caught frogs which were used in the mating experiments performed in 1979 (Table 8). The Hiroshima and Ichinoseki frogs evidently differed from each other in two respects, ratio of hind leg length to body length and size of the metatarsal tubercle. The hind legs of the Hiroshima frogs were larger than those of the Ichinoseki frogs, while the metatarsal tubercle of the Hiroshima frogs was smaller than that of the Ichinoseki frogs. When measurements were made on six females and three males of the Ichinoseki population and ten females and five males of the Hiroshima population, similar findings were obtained. The Hiroshima frogs were 53.06±0.80mm in body length, 2.44±0.12mm in size of the metatarsal tubercle and 1.80±0.02 in ratio of hind leg length to body length. On the other hand, the Ichinoseki frogs were 55.43±0.41mm in body length, 3.23±0.02mm in size of the metatarsal tubercle and 1.62±0.03 in ratio of hind leg length to body length.

B. External characters

(a) Dental formulae of tadpoles

According to the dental formulae of Rana japonica tadpoles described by Kawamura (1943, 1950), the upper and lower jaw formulae were 1:2+2 and 2:1+1, respectively, although an extra rudimentary upper or lower row was found in some tadpoles. As shown in Table 9, the dental formulae of the Hiroshima tadpoles examined by the present author were also 1:2+2 or 1:3+3 in the upper jaw and 2:1+1 or 3:1+1 in the lower jaw. On the other hand, the dental formulae of the Ichinoseki tadpoles were always 1:2+2 in the upper jaw and 1:2+2 or 2:1+1 in the lower jaw in most tadpoles. In other words, the Ichinoseki tadpoles somewhat differed from the Hiroshima tadpoles in the dental formula of the lower jaw. In contrast to the Hiroshima tadpoles, some of the Ichinoseki tadpoles had a dental formula of the lower jaw, where the second low was interrupted in the middle. The dental formulae of reciprocal hybrids were variable, as shown in Table 9. The dental formula of the lower jaw was mostly 2:1+1 and 3:1+1, although there were some tadpoles whose dental formula of the lower jaw was 1:2+2 or 2:2+2.

(b) Adult frogs (Plate I)

Hiroshima frogs resembled Ichinoseki frogs so closely in appearance that they could not be definitely distinguished from the latter. However, some vague differences could be recognized in several external characters between the two kinds of frogs. The external characters were mainly observed on 3 males and 6 females from Ichinoseki and 5 males and 10 females from Hiroshima. All these frogs were those which had been collected in the field and used for crossing experiments in 1979.

(i) Dorsal surface

Although the dorsal surfaces changed in color according to physiological conditions, they were generally light brown in the Hiroshima frogs, while they were dark brown in the Ichinoseki frogs. Besides a V-shaped spot found in the center of the back, there were no or grey vague spots on the back of the Hiroshima frogs, while there were no or small black spots, fused irregular black spots and grey obscure spots in the Ichinoseki frogs. While the body surfaces of the Hiroshima frogs seemed to be comparatively smooth and firm, those of the Ichinoseki frogs seemed to be coarse and loose. The snout was somewhat slender and pointed in the Hiroshima frogs, while it was thick and not so pointed in the Ichinoseki frogs. In both populations, the dorso-lateral ridges were pale yellowish-brown with parallel lines running nearly straight from the posterior ends of the upper eyelids to the level of the hind legs.

(ii) Ventral surface

The ventral surfaces of the Hiroshima and Ichinoseki frogs were white, pale yellow or rarely orange, and there were no remarkable differences in ground coloration between the two populations. However, innumerable minute grey spots were sometimes distributed all over the ventral surfaces in the Ichinoseki population, while such spots were scarcely found in the Hiroshima population.

IV. Biochemical characters

In order to compare the two populations biochemically, ten enzymes, lactate dehydrogenase(LDH), malate dehydrogenase (MDH), α-glycerophosphate dehydrogenase(α-GDH), isocitrate dehydrogenase(IDH), aspartate aminotransferase(AAT), phosphoglucomutase(PGM), glucose-phosphate isomerase(GPI), superoxide dismutase(SOD), creatine kinase(CK) and esterase (Est) and two blood proteins, serum albumin(Ab), hemoglobin (Hb) were analyzed by starch gel electrophoresis, using

15 frogs of the Hiroshima population and 9 frogs of the Ichinoseki population which were collected in the field. The electrophoretic patterns of these proteins are shown in Figs.2 and 3. The comparison of the two populations in 16 loci which control these proteins is shown in Table 10. In twelve loci controlling LDH-A,B, MDH-A, IDH-A,B, AAT-A, PGM, GPI, SOD, CK, Est-1 and Hb among the 16 loci analyzed, no differences were observed between the two populations; only a single allele common to both populations was expressed and no variant was found in each locus except for GPI locus (Figs. 2 and 3). In three other loci controlling MDH-B, α-GDH and AAT-B, the two populations slightly differed. Although they had a single common major allele, a variant specific to the Hiroshima population was found in each locus with a low frequency. In the remaining one locus of serum albumin, a distinct difference was found between the two populations. The Hiroshima frogs had two albumin alleles, a and b, while the Ichinoseki frogs had two alleles, b and c. The major allele was b in the Hiroshima population, while it was c in the Ichinoseki population.

V. Karyological characters

Chromosomes were examined in metaphase plates of bone marrow cells of six-month-old frogs in the experimental and control series performed in 1979. A total of 284 metaphase plates from 10 frogs of two series in the Hiroshima population, and a total of 197 metaphase plates from 17 frogs of 3 series in the Ichinoseki population were observed (Table 11).

1. Karyotype of the Hiroshima population

The chromosomes of the Hiroshima population are 26 in diploid number. Its karyotype is shown in Fig. 4, where the 13 pairs of chromosomes are arranged in order of length. The relative length and centromere position of each chromosome are presented in Tables 12 and 13, respectively. The chromosomes were divided into two groups according to their size. Group 1 consisted of five large chromosomes, Nos. 1 to 5, while group 2 consisted of eight small chromosomes Nos. 6 to 13. On the other hand, the 13 pairs of chromosomes were divided into three types, median(m), submedian(sm) and subterminal(st), according to the numerical values of their centromere position, 50.0～37.5, 37.5～25.0 and 25.0～12.5, respectively. The largest chromosome (No.1) of group 1 was of m type, while chromosome No. 2 was slightly smaller than No. 1 and was of sm type near m type. Chromosomes Nos. 3 and 4 were similar to each other in relative length, but they differed from each other in the position of centromere; No. 3 was of sm type, while No. 4 was of m type. Chromosome No. 5 was the smallest of group 1 and of m type. Among the chromosomes of group 2, No. 6 was the largest and followed by No. 7, while these two were of m type. Chromosomes

Nos. 8 and 9 were similar to each other in relative length but they were distinguishable in shape. While chromosome No. 8 was of sm type, No. 9 was of st type. Chromosomes Nos. 10 and 11 resembled each other in relative length, but No. 10 was of sm type and peculiar in having a distinct secondary constriction in the long arm. Chromosome No. 11 was of m type. Chromosome No. 12 was slightly smaller than No. 11 and of sm type. Chromosome No. 13 was the smallest and of sm type.

2. Karyotype of the Ichinoseki population

The chromosomes of the Ichinoseki population were very similar to those of the Hiroshima population; they were 26 in diploid number and divided into two groups according to their size. The karyotype of this population is shown in Fig. 5 and the relative length and centromere position of each chromosome are presented in Tables 12 and 13. Chromosome No. 1 was the largest among the five chromosomes of group 1 and of m type. Chromosome No. 2 was slightly smaller than No. 1 and of m type near sm type. Although chromosome No. 4 was slightly larger than chromosome No. 3 in relative length, they were arranged as those of the Hiroshima population on the basis of centromere position. Chromosome No. 5 was the smallest in group 1 and of m type. Chromosome No. 6 was the largest among the eight chromosomes of group 2, and No. 7 was slightly smaller than No. 6; both chromosomes were of m type. Chromosomes Nos. 8～11 were very similar to each other in relative length. As Nos. 8 and 9 were of sm type, they were often indistinguishable from each other. Chromosome No. 10 was of sm type and had a secondary constriction in the long arm. Accordingly, it could be easily distinguished from No. 10 which was of m type. Chromosome No. 12 was smaller than No. 11 and of sm type. Chromosome No. 13 was the smallest and of sm type.

3. Comparison of the karyotypes of the two populations

Slight differences in relative length and centromere position of chromosomes were found between the karyotypes of the two populations, as shown in Figs. 6～8. In relative length, there were statistically significant differences in 7 chromosomes, Nos. 1, 3, 7, 10, 11, 12 and 13 (Fig. 6). While there were no statistically significant differences in centromere positions of the five large chromosomes, Nos. 1～5, the two populations were significantly different from each other in chromosomes Nos. 6 and 9 (Fig. 7). While chromosome No. 9 was of st type in the Hiroshima population, it was of sm type in the Ichinoseki population. The numerical values of the centromere positions in the Hiroshima and Ichinoseki populations were 20.65±0.30 and 29.79±0.28, respectively. Although chromosome No. 6 was of m type in the two populations, the numerical values of the centromeres were also 39.70±0.29 and 46.75±0.27, respectively. In the other six small chromosomes, Nos. 7, 8, 10, 11, 12 and 13, there were either no or slightly significant differences between the two populations (Fig. 7).

4. Karyotypes of reciprocal hybrids

Chromosomes of reciprocal hybrids were observed in 63 metaphase plates obtained from 10 individuals, as shown in Table 10. The karyotypes of these hybrids are shown in Figs. 9 and 10. The two homologous chromosomes of each of two pairs, Nos. 6 and 9, were usually found to be distinctly different in centromere position.

VI. Sex and gonads

A. Sex ratios

The gonads of reciprocal hybrids and their controls were observed in juvenile frogs within one month after metamorphosis and six-month-old frogs with thumb pads. The sex ratios of these frogs are presented in Table 14. While the sex of control Rana japonica was almost differentiated one month after metamorphosis, there were comparatively numerous hermaphrodites in the hybrids at this stage. Thus, the sex of these juvenile frogs was divided into the following five categories on the basis of the inner structure of gonads, according to Kawamura and Nishioka (1972).

(1) Normal female (♀). The gonads are ovaries filled with growing auxocytes.

(2) Hermaphrodite type 1 (⚥$_1$), at the beginning of sex-reversal. Multiplication of rete cells is found in the medullary parts of the gonads. In the cortical parts, there are aboundant oogonia and young oocytes.

(3) Hermaphrodite type 2 (⚥$_2$), at the middle stage of sex-reversal. Owing to distinct multiplication of rete cells, the gonads are testicular in structure in the inner part at least. However, somewhat wide areas of ovarian structure mostly remain in the outer part.

(4) Hermaphrodite type 3 (⚥$_3$), at the last stage of sex-reversal. The gonads are testes as a whole. Nearly all the gonia are surrounded with rete cells and there are no ovarian cavities, although there are small groups of oocytes.

(5) Normal male (♂). The gonads are typical testes. However, some males of this category have a few testis-ova or oocytes in their testes.

(a) Juvenile frogs dead or killed within one month after metamorphosis

In the controls, there were 252 females, 1 hermaphrodite of type 3 and 242 males among 495 juveniles obtained from 7 matings in the Hiroshima population, while there were 57 females, 1 hermaphrodite of type 2 and 50 males among 108 juveniles obtained from 4 matings in the Ichinoseki population. On the other hand, in the hybrids between Hiroshima females and Ichinoseki males, there were 90 females, 31 hermaphrodites and 495 males among 616 juveniles obtained from 7 matings. Of these hermaphrodites, 12 were of type 1, 15 of type 2 and 4 of type 3. In the reciprocal hybrids, there were 13 females, 1 hermaphrodite of type 2 and 112 males among 126 juveniles obtained from 4 matings. While the sex ratio of the control frogs was almost 1:1, the overwhelming majority were males in reciprocal hybrids between the two populations and there were comparatively many hermaphrodites in the hybrids between Hiroshima females and Ichinoseki males.

(b) Six-month-old frogs

Of the 306 control frogs obtained from 5 matings in the Hiroshima population, 171 were females and 135 were males, while of the 136 control frogs obtained from four matings in the Ichinoseki population, 61 were females and 75 were males. On the other hand, of the 269 hybrids obtained from five matings between Hiroshima females and Ichinoseki males, only 25 were females and 244 were males, while of the 157 hybrids obtained from four matings of the reciprocal combination, only 18 were females and 139 were males.

When the hermaphrodites were counted as males, 47.2% and 51.6% of the control frogs were males in the Hiroshima and Ichinoseki populations, respectively, while 87.0% and 89.0% of the hybrids were males in the crosses between Hiroshima females and Ichinoseki males and the reciprocal ones, respectively.

B. Structure of gonads

(a) Juvenile frogs three months after metamorphosis

(i) Males

The size, shape, color and inner structure of testes were observed in four male frogs belonging to each of four kinds, reciprocal hybrids and Hiroshima and Ichinoseki controls at three months after metamorphosis. The testes of reciprocal hybrids were almost same in external aspects as those of the controls at this stage. They were oval in shape, whitish, greyish or blackish in color and 1.4~3.2mm in length and 0.7~2.3mm in width (PlateII, Table 15). The inner structure of the gonads of these four kinds of juvenile frogs was as follows.

Hiroshima controls. In three of the four males, the seminiferous tubules contained first and second spermatocytes, spermatids and bundles of spermatozoa. Along the inner walls, there were primary and secondary spermatogonia. In the other male, the seminiferous tubules were filled with spermatogonia; spermatocytes and spermatids were scarce.

Ichinoseki controls. In all the four males, the seminiferous tubules contained primary and secondary spermatogonia, first and second spermatocytes, spermatids and bundles of spermatozoa, as observed in the three males of the Hiroshima population.

Hybrids, Hiroshima♀ x Ichinoseki♂. The seminiferous tubules in the four male hybrids were somewhat similar in differentiation to those in the control frogs. There were primary and secondary spermatogonia along the inner walls of the seminiferous tubules. There were also comparatively many first spermatocytes at the metaphase of the first reduction division. In contrast with the control males, normal germ cells such as second spermatocytes, spermatids and spermatozoa were scarce, while a few large abnormal spermatozoa were found in some tubules together with some degenerating germ cells and pycnotic nuclei.

Hybrids, Ichinoseki♀ x Hiroshima♂. The testes of the four male hybrids were different from one another in differentiation of seminiferous tubules. In one male, nearly all the seminiferous tubules were filled with first and second spermatogonia except that there were a few spermatocytes at the prophase of the first reduction division. In another male, there were a considerably large number of spermatocytes at the prophase of the first reduction division, besides abundant first and second spermatogonia. In still another male, there were some first spermatocytes at the metaphase in many seminiferous tubules. In some seminiferous tubules, there were a few large abnormal spermatozoa and pycnotic nuclei. In the remaining male hybrid, there were many first spermatocytes at the prophase and metaphase and also a small number of second spermatocytes. In some seminiferous tubules, there were a small number of large abnormal spermatozoa and pycnotic nuclei.

(ii) Females

The ovaries of juvenile frogs were observed in four females belonging to each of four kinds, reciprocal hybrids and Hiroshima and Ichinoseki controls 3 months after metamorphosis. As shown in Table 16, the size of ovaries was relative to the body length and there seemed to be no significant difference in size of ovaries between the hybrids and the controls. Auxocytes could be observed by the naked eye on the surfaces of these ovaries.

(b) Mature frogs

(i) Males

In the breeding season of 1980, one-year-old males of reciprocal hybrids and the controls attained sexual maturity. After the testes of these males were measured, the left testes were fixed and used for histological observation, while the right testes were used for examining reproductive ability. The testes were classified into the following five types on the basis of abnormality in inner structure according to Kawamura and Nishioka (1972). The size and type of the testes of each male are shown in Table 17.

Type 1. The testis is quite normal in inner strucure. Seminiferous tubules are filled with close bundles of normal spermatozoa. A small number of pycnotic nuclei are found

(Plate III-13).

Type 2. Spermatozoa are far fewer than those of type 1. Each bundle of spermatozoa is small and coarse. Among the bundles, there are considerably numerous abnormal spermatozoa and pycnotic nuclei (Plate III-15, 16).

Type 3. Seminiferous tubules are mostly filled with abnormal spermatozoa and pycnotic nuclei. Besides, there are a few small bundles of normal spermatozoa here and there (Plate IV-17, 18).

Type 4. Seminiferous tubules are filled with abnormal spermatozoa and pycnotic nuclei. However, there are a few normal spermatozoa distributed sparsely (Plate IV-19, 20).

Type 5. No normal spermatozoa are found in seminiferous tubules, which are filled with numerous abnormal spermatozoa and pycnotic nuclei (Plate III-14).

Hiroshima controls. Six males were 43.2～45.8mm, 44.0 mm on the average, in body length. Their testes were 3.7～5.7mm, 4.4mm on the average, in length and 2.8～4.2mm, 3.5mm on the average, in width. All of them were of type 1 in inner structure; seminiferous tubules were filled with close bundles of normal spermatozoa.

Ichinoseki controls. Six males were 46.2～51.2mm, 48.3 mm on the average, in body length. Their testes were almost same in size as those of the Hiroshima population. They were 3.2～6.6mm, 4.6mm on the average, in length and 2.7～4.4

mm, 3.6mm on the average, in width. All of them were of type 1 in inner structure. However, there were comparatively numerous pycnotic nuclei in seminiferous tubules of some males.

Hybrids, Hiroshima♀ x Ichinoseki♂. Twelve males were 43.2～49.7mm, 46.4mm on the average, in body length. Their testes were 3.2～5.6mm, 4.3mm on the average, in length and 2.2～3.8mm, 3.0mm on the average, in width. Their testes were considerably abnormal in inner structure; seminiferous tubules were filled with remarkably numerous pycnotic nuclei. Of the twelve males, two were of type 3, nine of type 4 and the remainder of type 5 in inner structure of the testis.

Hybrids, Ichinoseki♀ x Hiroshima♂. Twelve males were 40.3～46.8mm, 43.8mm on the average, in body length. Their testes were almost same in size as those of the reciprocal hybrids, that is, 3.2～5.7mm, 4.4mm on the average, in length and 2.2～5.0mm, 3.0mm on the average, in width. They were not so abnormal in inner structure as those of the reciprocal hybrids. Of the twelve males, seven were of type 2, three of type 3 and the remaining two of type 4 in inner structure of the testis.

(ii) Females

By pituitary injection, all mature females of reciprocal hybrids and the controls could spawn. The number and size of eggs are shown in Table 18.

Hiroshima controls. Three females were 41.8～53.4mm, 46.8mm on the average, in body length. After pituitary injection, all these three females discharged 659～971 eggs, 758.7 eggs on the average, which were 1.73±0.01～1.80±0.01mm in diameter.

Ichinoseki controls. Two females were 50.7mm and 53.2mm, 52.0mm on the average, in body length. These two females discharged 971 and 1063 eggs, 1017 eggs on the average, which were 1.70±0.02, 1.85±0.02mm in diameter, respectively.

Hybrids, Hiroshima♀ x Ichinoseki♂. Four females were 42.2～50.5mm, 47.4mm on the average, in body length. After pituitary injection, all of them discharged 600～968 eggs, 744 eggs on the average, which were 1.67±0.01～1.93±0.02mm in diameter.

Hybrids, Ichinoseki♀ x Hiroshima♂. Six females were 40.0～52.3mm, 46.7mm on the average, in body length. After pituitary injection, all of them discharged 609～1202 eggs, 848.5 eggs on the average, which were 1.78±0.02～1.88±0.02mm in diameter.

VII. Reproductive capacity

1. Male hybrids and the controls

Males of reciprocal hybrids and the controls obtained in 1979 (Table 17) were backcrossed with field-caught females of the Hiroshima population (Table 19) in the breeding season of 1980. The results are presented in Table 19 and shown in Fig. 11. It was evident that male hybrids were almost sterile or remarkably inferior to the controls in reproductive capacity.

(i) Controls

By the artificial insemination method, six males of the Hiroshima population and six males of the Ichinoseki population which were produced from control matings in 1979 were backcrossed with six females of the Hiroshima population collected in the field.

In six matings (Nos. 1～6) using the six control males of the Hiroshima population, 83.3～95.6%, 87.6% on the average, of the respective total number of eggs cleaved normally (Table 20, Fig. 11-a). Most of the normally cleaved eggs developed normally, and 81.0～93.3%, 86.5% on the average, hatched normally and became normally feeding tadpoles. Thereafter, all the tadpoles from two matings (Nos. 2 and 3) were preserved. In the other matings, 80.5～88.9%, 84.9% on the average, metamorphosed normally.

In six matings (Nos. 1～6), using six males of the Ichinoseki population, 81.6～95.7%, 89.4% on the average, of the respective total number of eggs cleaved normally (Table 20, Fig. 11-b). While only a few of the normally cleaved eggs died of various abnormalities, 76.3～93.6%, 86.6% on the average, hatched normally and 76.3～91.5%, 85.4%

on the average, became normally feeding tadpoles. Thereafter, all the tadpoles from two matings (Nos. 2 and 3) were preserved. In the other matings, 81.5~89.4%, 85.3% on the average, metamorphosed normally.

(ii) Reciprocal hybrids

Twelve mature males of reciprocal hybrids produced from crossing experiments in 1979 were backcrossed with twelve females of the Hiroshima population collected in the field.

Hybrids, Hiroshima♀No.1 x Ichinoseki♂No.1. In four (Nos. 1, 3, 10 and 11) of twelve matings (Nos. 1~12) using twelve male hybrids, no eggs cleaved normally nor abnormally. In the other four matings, Nos. 2, 4, 5 and 8, one(0.2%), four(1.1%), one(0.3%) and eight(4.3%) eggs cleaved abnormally, respectively, while there were no eggs which cleaved normally. In the remaining four matings, Nos. 6, 7, 9 and 12, only two (0.8%), two(0.8%), one(0.9%) and one(0.8%) egg cleaved normally, respectively. All of these normally cleaved eggs hatched normally and then metamorphosed normally (Table 20, Fig. 11-c).

Hybrids, Ichinoseki♀No.1 x Hiroshima♂No.1. In only one (No. 4) of twelve matings (Nos. 1~12) using twelve male hybrids, no eggs cleaved normally, while only three(1.1%) eggs cleaved abnormally. In the other eleven matings, 1.0~66.2%, 23.9% on the average, of the respective total number of eggs cleaved normally. Almost all of the normally cleaved eggs developed normally and became normally feeding tadpoles. While the tadpoles from matings Nos. 1, 2, 8 and 9 were preserved, those from the other seven matings were continuously reared. In these matings, 1.9∼58.4%, 22.4% on the average, metamorphosed normally (Table 20, Fig. 11-d).

2. Female hybrids and the controls

Females of reciprocal hybrids and the controls obtained in 1979 (Table 18) were mated with males of the Hiroshima population collected in the field and the Ichinoseki population produced in the laboratory (Table 21) by artificial insemination in the breeding season of 1980. The results are presented in Table 21 and shown in Fig. 12. It was evident that the female hybrids were essentially fertile, though some females were slightly inferior to the controls in reproductive capacity.

(i) Controls

Eggs of three females of the Hiroshima population which were produced from control matings in 1979 were inseminated with sperm of three males of the Hiroshima or Ichinoseki population. In three matings (Nos. 1∼3) with the Hiroshima males, 82.4∼91.9%, 86.1% on the average, cleaved normally (Table 22, Fig. 12-a). While some of the normally cleaved eggs became abnormal at various embryonic stages, 57.1∼74.3%, 66.0% on the average, became normally feeding tadpoles. After some tadpoles died of underdevelopment, 54.6～69.3%, 62.6% on the average, metamorphosed normally. In three matings (Nos. 1～3) with the Ichinoseki males, 78.9～88.2%, 84.8% on the average, cleaved normally. While some of the normally cleaved eggs died of various abnormalities at the embryonic stage, 51.2～70.7%, 61.4% on the average, grew into normally feeding tadpoles. After some tadpoles died of underdevelopment or edema, 48.8～66.4%, 56.6% on the average, metamorphosed normally (Table 22, Fig. 12-a).

On the other hand, two females of the Ichinoseki population which were produced from control matings in 1979 were mated with two males of the Hiroshima or Ichinoseki population. In two matings (Nos. 1 and 2) with the Hiroshima males, 64.5% and 75.6% of the respective total number of eggs cleaved normally. While many of the normally cleaved eggs became abnormal at the embryonic stage, 38.3% and 45.6% became normally feeding tadpoles and eventually 33.4% and 40.1% metamorphosed normally (Table 22, Fig. 12-b). In two matings (Nos. 1 and 2) with the Ichinoseki males, 68.9% and 82.6% cleaved normally. While some of the normally cleaved eggs died of various abnormalities at the embryonic stage, 44.8% and 44.9% became normally feeding tadpoles. After some tadpoles died of underdevelopment, 34.9% and 43.3% metamorphosed normally (Table 22, Fig. 12-b).

(ii) Reciprocal hybrids

Hybrids, Hiroshima♀No.1 × Ichinoseki♂No.1. Eggs of four female hybrids produced from crossing experiments in 1979 were inseminated with sperm of four males of the Hiroshima or Ichinoseki population. In one (No. 2) of four matings (Nos. 1~4) backcrossed with the Hiroshima males, 10.1% of the total eggs cleaved normally, while 79.9~89.2%, 84.4% on the average, in the other three matings did so (Table 22, Fig. 12-c). While a small number of normally cleaved eggs died of various abnormalities at the embryonic stage, 5.5% in mating No. 2, and 59.4~68.8%, 63.6% on the average, in matings Nos. 1, 3 and 4 grew into normally feeding tadpoles. Thereafter, all the tadpoles from mating No. 2 were preserved. While some tadpoles died of underdevelopment in the other matings, 41.1~62.0%, 52.3% on the average, metamorphosed normally.

In one (No. 2) of four matings (Nos. 1~4) with Ichinoseki males, 18.9% of the total number of eggs cleaved normally, while 83.4~91.0%, 86.4% on the average, did so in the other three matings (Table 22, Fig. 12-c). After some of the normally cleaved eggs died of various abnormalities at the embryonic stage, 10.5% in mating No. 2 and 57.6~73.1%, 65.2% on the average, in the other three matings became normally feeding tadpoles. All the tadpoles of mating No. 2 were preserved. In the other three matings, 53.0~64.4%, 59.3% on the average, completed metamorphosis.

Hybrids, Ichinoseki♀No.1 x Hiroshima♂No.1. Six female hybrids produced from crossing experiments in 1979

(Table 18) were mated with six males of the Hiroshima or Ichinoseki population. In six matings (Nos. 1～6) with six Hiroshima males, 74.3～86.9%, 82.4% on the average, cleaved normally (Table 22, Fig. 12-d). While many of the normally cleaved eggs died of various abnormalities at the embryonic stage, 16.9～51.7%, 33.1% on the average, grew into normally feeding tadpoles. A part of tadpoles in matings Nos. 2, 4 and 5 was used to examine the chromosomes in the tail-tips after colchichine treatment. In the other matings, some tadpoles died of underdevelopment and 21.4～38.1%, 32.3% on the average, completed metamorphosis. In six matings (Nos. 1～6) with five males of the Ichinoseki population, 52.8～89.0%, 78.3% on the average, cleaved normally (Table 22, Fig. 12-d). While many of the normally cleaved eggs died of various abnormalities at the embryonic stage, 14.3～53.0%, 33.7% on the average, became normally feeding tadpoles. A part of tadpoles of matings Nos. 2, 4 and 5 were used to examine the chromosomes in the tail-tips after colchichine treatment. In the other three matings (Nos. 1, 3 and 6), some of the tadpoles died of underdevelopment or edema, while 39.8～44.1%, 42.6% on the average, metamorphosed normally.

VIII. Chromosome aberrations in backcrosses of reciprocal hybrids

Chromosomes were observed by the squash method in the tail-tips of normally shaped tadpoles, 20~60 days old, which had been produced from backcrossing of reciprocal hybrids. The results are presented in Tables 23 and 24 and shown in Figs. 13~19.

1. Backcrosses of male hybrids and the controls

(i) Controls

Seventy tadpoles produced from four matings using four control males of the Hiroshima population and 65 tadpoles from four matings using four control males of the Ichinoseki population were examined. All of them were normal diploids (Table 23).

(ii) Backcrosses

Six tadpoles produced from four matings using four male hybrids, Hiroshima♀ x Ichinoseki♂, were all normal diploids. Of 252 tadpoles produced from nine matings using nine male hybrids, Ichinoseki♀ x Hiroshima♂, 251 were diploids, while the remaining one was a triploid (Table 23).

2. Backcrosses of female hybrids and the controls

(i) Controls

Of 179 tadpoles produced from three matings using three control females of the Hiroshima population, 175 were diploids and four were triploids. All 153 tadpoles produced from two matings using two control females of the Ichinoseki population were diploids (Table 24).

(ii) Backcrosses

In three matings (Nos. 1, 3 and 4) using three female hybrids, Hiroshima♀ x Ichinoseki♂, 177 of 180 tadpoles were diploids and the other three were triploids (Table 23). All the tadpoles produced from the other mating (No. 2) were preserved without observing the chromosomes.

In three matings (Nos. 1, 3 and 6) using three female reciprocal hybrids, Ichinoseki♀ x Hiroshima♂, 179 of 180 tadpoles were diploids and the remaining one was a triploid. In the other three matings (Nos. 2, 4 and 5), the chromosomes of some tadpoles were observed by the squash method after colchichine pretreatment. It was found that 41 tadpoles produced from two matings (Nos. 2 and 5) were all normal diploids, while in the other mating (No. 4), 26 of 50 tadpoles were normal diploids and the other 24 were all hyperdiploids. Of the latter, 12 were trisomics, ten were tetrasomics and the remaining two were pentasomics (Table 24). The karyotypes of each of these hyperdiploids were analyzed on 2～9 metaphase plates. Some trisomic tadpoles contained chromosome No. 5 (Fig. 13) or chromosome No. 7 in addition (Fig. 14). In tetrasomic tadpoles, the additional two chromosomes were Nos. 1 and 12 (Fig. 15), Nos. 6 and 8 (Fig. 16) or Nos. 7 and 10 (Fig. 17). The two pentasomic tadpoles contained three chromosomes Nos. 6, 11 and 12 (Fig. 18) or Nos. 6, 8 and 9 in addition (Fig. 18).

IX. Sex ratio in backcrosses of reciprocal hybrids

The sex of the offspring of male and female hybrids and the controls was examined in juveniles within one month after metamorphosis and in 6-month-old frogs. The results are presented in Tables 25 and 26.

1. Backcrosses of male hybrids and the controls

The sex ratio in the backcrosses produced from matings between field-caught females of the Hiroshima population and males of reciprocal hybrids and in the controls is presented in Table 25.

(a) Juveniles within one month after metamorphosis

(i) Controls

Of 160 juveniles produced from four matings between the field-caught females and control males of the Hiroshima population, 84 were females and 76 were males, while there were 27 females, 3 hermaphrodites and 160 males among 190 juveniles produced from four matings between the same female parents and control males of the Ichinoseki population (Table 25). Of these three hermaphrodites, one was of type 1 and the other two were of type 2 in inner structure of the gonads.

(ii) Backcrosses

Of four juveniles produced from two male hybrids, Hiroshima♀ x Ichinoseki♂, two were females and two were males (Table 25). Of 206 juveniles produced from seven male hybrids, Ichinoseki♀ x Hiroshima♂, 84 were females, one was a hermaphrodite of type 3 and 121 were males.

(b) Six-month-old frogs

Two frogs produced from two matings (Nos. 9 and 12) using male hybrids, Hiroshima♀x Ichinoseki♂ were males. Of 87 frogs produced from four matings (Nos. 3, 6, 7 and 12) using male hybrids, Ichinoseki♀x Hiroshima♂, 33 were females and 54 were males (Table 25).

When the hermaphrodites were counted as males, 47.5% and 85.8% of the respective total number of frogs were males in the offspring produced from field-caught females of the Hiroshima population by mating with Hiroshima and Ichinoseki males, respectively, while 66.7% and 60.1% were males in the backcrosses of male hybrids, Hiroshima♀x Ichinoseki♂, and those of the reciprocal crosses, mated with Hiroshima females, respectively. In the backcrosses, the males seemed to be higher in percentage than those of controls but not so large in percentage as those of reciprocal hybrids (Tables 14 and 25).

2. Backcrosses of female hybrids and the controls

The sex ratio in the backcrosses produced from females of reciprocal hybrids and males of the Hiroshima and Ichinoseki populations is presented in Table 26.

(a) Juveniles within one month after metamorphosis

(i) Controls

Of 536 juveniles produced from three matings between control females and field-caught males of the Hiroshima population, 273 were females and 263 were males, while there were 60 females, 14 hermaphrodites and 340 males among 414 juveniles produced from three matings between the same female parents of the Hiroshima population and males of the Ichinoseki population (Table 26). Of the 14 hermaphrodites, seven were of type 1 and the other seven were of type 2 in inner structure of the gonads. Of 172 juveniles produced from two matings between females and males of the Ichinoseki population, 67 were normal females, one was a female with underdeveloped ovaries, three were hermaphrodites and 101 were males. Of the three hermaphrodites, one was of type 1 and the other two were of type 2. Among 89 juveniles produced from two matings between the same females of the Ichinoseki population and field-caught males of the Hiroshima population, there were 8 females, 5 hermaphrodites and 76 males. Of the five hermaphrodites, one was of type 1, three were of type 2 and the remaining one was of type 3.

(ii) Backcrosses

Among 279 juveniles produced from three matings between female hybrids, Hiroshima♀x Ichinoseki♂, and field-caught males of the Hiroshima population, there were 133 females, 4 hermaphrodites of type 2 and 142 males, while there were 61 females, 19 hermaphrodites and 278 males among 358 juveniles produced from three matings between the same female hybrids and Ichinoseki males (Table 26). Of the 19 hermaphrodites, eight were of type 1, five of type 2 and the remaining six of type 3.

Of 195 juveniles produced from three matings between female hybrids, Ichinoseki♀ x Hiroshima♂, and field-caught males of Hiroshima population, 45 were females, one was a hermaphrodite of type 1 and 149 were males, while there were 104 normal females, one was a female with underdeveloped ovaries, three were hermaphrodites and 170 were males among 278 juveniles produced from three matings using the same female hybrids, Ichinoseki♀ x Hiroshima♂ and Ichinoseki males (Table 26). Of the three hermaphrodites, one was of type 1 and the other two were of type 2.

(b) Six-month-old frogs

(i) Controls

Among 96 frogs produced from two matings between females and males of the Ichinoseki population, there were 49 females and 47 males, while there were 13 females and 105 males among 118 frogs produced from two matings between the same females of the Ichinoseki population and field-caught males of the Hiroshima population (Table 26).

(ii) Backcrosses

Of 160 frogs produced from three matings between female hybrids, Hiroshima♀ x Ichinoseki♂, and field-caught males of the Hiroshima population, 65 were females and 95 were males, while there were 38 females and 122 males among

160 frogs produced from three matings between the same female hybrids and males of the Ichinoseki population (Table 26). Among 111 frogs produced from three matings between female hybrids, Ichinoseki♀ x Hiroshima♂, and field-caught males of the Hiroshima population, there were 24 females and 87 males, while there were 69 females and 83 males among 152 frogs produced from three matings between the same female hybrids and males of the Ichinoseki population.

When the hermaphrodites were counted as males, 48.7% and 85.8% of the respective total number of frogs were males in the offspring produced from Hiroshima females by mating with Hiroshima and Ichinoseki males, respectively, while 89.9% and 56.3% were males in the offspring produced from Ichinoseki females and the same males of both populations, respectively. On the other hand, 54.9% and 80.9% were males in the offspring produced from female hybrids, Hiroshima♀ x Ichinoseki♂, by mating with field-caught males of the Hiroshima and the Ichinoseki population, respectively, while 77.5% and 59.5% were males in the offspring produced from female hybrids, Ichinoseki♀ x Hiroshima♂, by mating with the same males of the Hiroshima and the Ichinoseki population, respectively.

In the backcrosses of female hybrids, there were remarkable differences in percentage of males between those backcrossed with males of the maternal population and those backcrossed with males of the paternal population. While the percentages of males were 54.9% or 59.5% in the backcrosses of female reciprocal hybrids mated with males of the maternal population, those were 80.9% or 77.5% in the backcrosses of female reciprocal hybrids mated with males of the paternal population, being, considerably higher than those in the former backcrosses.

DISCUSSION

Hybridization experiments in brown frogs were performed by Pflüger (1882), Pflüger and Smith (1883) and Born (1883, 1886) for the first time. They obtained viable hybrids from crosses between female Rana arvaris and male Rana fusca (= temporaria). This kind of hybrids was thoroughly studied by Dürken (1935, 1938), who reported that they were all males and completely sterile. In Japanese brown frogs, Kawamura (1942) also reported that mature hybrids between female Rana japonica from Hiroshima and male Rana temporaria (=chensinensis) from Hokkaido were all sterile males. The same findings have been described in hybrids between female Rana japonica and male Rana ornativentris by Kawamura (1950), in reciprocal hybrids between Rana ornativentris and Rana temporaria (= chensinensis) from Hokkaido by Kawamura and Kobayashi (1959) and in hybrids between Japanese brown frog species, Rana japonica and Rana ornativentris, and European brown frog species, Rana arvalis and Rana temporaria (Kawamura and Kobayashi, 1960). Thereafter, it was confirmed that all the hybrids between female Rana dybowskii and male Rana japonica, ornativentris and chensinensis also became sterile males (Kawamura and Nishioka, 1977). All the interspecific hybrids stated above were those produced from two species which were either not isolated or incompletely isolated from each other by gametic isolation or hybrid inviability. Besides, there were many combinations of brown frog species which were completely isolated by hybrid inviability (Kawamura and Nishioka, 1977).

In the present study, it was found that the intraspecific hybrids between the Hiroshima population and the Ichinoseki population of Rana japonica were not the same in sex ratio as the controls, while there was neither gametic isolation nor hybrid inviability between these two populations. An overwhelming majority of reciprocal hybrids were males, whereas there was nearly an equal number of males and females in the control frogs belonging to each of the two populations. However, these intraspecific hybrids evidently differed from the interspecific hybrids stated above in that females usually appeared in a small percentage, although 54 hybrids were all males in one of eleven reciprocal crosses. In four other crosses, more than 91% of hybrids were males. All the males of reciprocal hybrids examined were abnormal in a greater or lesser degree in inner structure of testes, while the females were normal in size and number of eggs laid after pituitary injection. The male intraspecific hybrids also differed from the interspecific hybrids in that they were not always completely sterile. In this respect, the two kinds of reciprocal hybrids differed from each other. While all the 12 Hiroshima♀ x Ichinoseki♂ hybrids were either completely or nearly completely sterile, only half of the reciprocal hybrids was completely so and the other half was fertile in some degree, although there were no hybrids which were normal in fertility. In contrast with the males of intraspecific hybrids, there were no completely sterile females. While one of four Hiroshima♀ x Ichinoseki♂ females was extremely low in fertility, the other three were nearly the same as the control Hiroshima females. While three of six female Ichinoseki♀ x Hiroshima♂ hybrids were distinctively low in fertility, the other three were similar to the control Ichinoseki females.

The backcrosses of male or female intraspecific hybrids were not normal in sex ratios. Of the backcrosses of seven male Ichinoseki♀ x Hiroshima♂ hybrids mated with Hiroshima females, 60.1% were males, while 47.5% of the Hiroshima controls were males. Although the sex of the backcrosses of male Hiroshima♀ x Ichinoseki♂ hybrids mated with Hiroshima females was examined only in six individuals, four of them were males. Of the backcrosses of female reciprocal hybrids mated with males of the maternal population, 54.9% or 59.5% were males, but 80.9% or 77.5% of the backcrosses of female reciprocal hybrids mated with males of the paternal population were males. It was found that males were remarkably more numerous in the latter backcrosses than those in the former.

Adult Rana japonica collected from Ichinoseki resembled those from Hiroshima so closely in appearance that the two populations could not be definitely distinguished from each other, although slight differences were usually found in color and pattern and shape of the snout. The snout of the Hiroshima frogs appeared to be more slender and more pointed than that of the Ichinoseki frogs. When the individuals of the two populations were reared under the same condition, the Hiroshima tadpoles attained completion of metamorphosis more quickly than the Ichinoseki tadpoles did. When they were compared three months after metamorphosis and at the age of one year, the Ichinoseki frogs were larger in body length than the Hiroshima frogs. The Ichinoseki population was not the same as the Hiroshima population in the dental formula of tadpoles. While Hiroshima tadpoles were mostly 2:1+1 or 3:1+1 in the lower jaw, Ichinoseki tadpoles were mostly 2:1+1 or 1:2+2. While the formula of 1:2+2 was not found in the Hiroshima tadpoles, that of 3:1+1 was exceptional in the Ichinoseki tadpoles.

Ichinoseki frogs were compared with Hiroshima frogs in electrophoretic patterns of twelve proteins including ten enzymes (LDH, MDH, α-GDH, IDH, AAT, PGM, GPI, SOD, CK and Est) and two blood proteins (serum albumin and hemoglobin). It was found that the two populations were nearly the same in the loci of these proteins except for only one locus of serum albumin. The Hiroshima population had alleles a and b, and the Ichinoseki population had alleles b and c; the two populations had allele b in common.

Kawamura (1939) has reported that the chromosomes of Rana japonica are 26 in spermatogonia of normal males. This number of chromosomes was also counted by him (1940) in oogonia of diploid parthenogenetic females. Thereafter, Kawamura (1943) described that the metaphase plates of spermatogonia contained five pairs of large chromosomes and eight pairs of small chromosomes. After a long time, the karyotype of Rana japonica was observed by Seto (1965), Nishioka et al. (1972) and Kuramoto et al. (1973). These authors clarified that chromosome No. 10 had a conspicuous secondary constriction. The present author compared the karyotypes of the two Rana japonica populations with each other and found that they differed slightly in centromere position of chromosome Nos. 6 and 9. A similar difference in centromere position of chromosome No. 7 was reported by Nishioka (1972) between two Japanese pond frog species, Rana nigromaculata and Rana brevipoda.

As stated above, there is no doubt that the Ichinoseki population slightly differs morphologically, developmentally, biochemically and karyologically from the Hiroshima population. Above all, it is evident that the two populations are isolated from each other by hybrid sterility, although this is not complete. Thus, it seems reasonable to give a position as a sibling species of Rana japonica to the Ichinoseki population. However, there is a problem to be solved between the Ichinoseki population and Rana temporaria martensi Boulenger. The latter was first described by Boulenger (1886) as Rana martensi, then placed as Rana temporaria var. martensi by Okada and Kawano (1923) and lastly changed into Rana temporaria martensi by Okada (1931). According to Okada (1931, 1966), this subspecies closely resembles Rana japonica, except that the snout is not so pointed and that the dorso-lateral glandular folds somewhat flare out to the upper margin of the tympanum. Okada (1966) has described that this frog inhabits the plains near mountains together with Rana japonica in Honshu, Shikoku and Kyushu, that is, all over Japan except Hokkaido. Kawamura (1962) and Nakamura and Ueno (1963) placed Rana temporaria martensi as a synonym of Rana japonica in accordance with Stejneger (1907) who identified Rana martensi with Rana japonica. While the Ichinoseki population of Rana japonica somewhat resembles Rana temporaria martensi described by Okada (1966) in shape of the snout and some other characters, it differs distinctively from the latter in the shape of dorso-lateral glandular folds. The question whether the Ichinoseki population is a part of the brown frog species called *Rana martensi* will be answered hereafter, together with the problem on the spread of this population.

SUMMARY

1. The existence of a speciation from *Rana japonica* was examined between two populations, Ichinoseki and Hiroshima, by hybridization experiments together with morphological and karyological observations and electrophoretic analysis.

2. Ichinoseki frogs resembled Hiroshima frogs in appearance so closely that the two kinds of frogs could not be definitely distinguished from each other.

3. Hiroshima tadpoles attained completion of metamorphosis more rapidly than Ichinoseki tadpoles did. Ichinoseki frogs were larger in body length than Hiroshima frogs three months after metamorphosis and at the age of one year.

4. A part of Hiroshima tadpoles had the same dental formula as that found in a part of Ichinoseki tadpoles. The remaining Hiroshima tadpoles mostly differed from the remaining Ichinoseki tadpoles.

5. Hiroshima frogs were nearly the same as Ichinoseki frogs in the electrophoretic patterns of eleven of twelve proteins examined. In the remaining protein, serum albumin, there was a difference between the two populations. While the Hiroshima frogs had two alleles, a and b, the Ichinoseki frogs had b and c. While the major allele was b in the Hiroshima population, it was c in the Ichinoseki population.

6. The karyotypes of the two populations were very similar to each other. However, they differed slightly in centromere position of chromosomes Nos. 6 and 9.

7. Although there was no hybrid inviability between the two populations, there was remarkable preponderance of males in number in reciprocal hybrids. Male reciprocal hybrids were completely or incompletely sterile. Female reciprocal hybrids were almost completely fertile.

8. One of six female hybrids, Ichinoseki♀ x Hiroshima♂, produced many hyperdiploids in company with normal diploids by backcrossing with a Hiroshima or Ichinoseki male.

9. In the backcrosses of male reciprocal hybrids mated with Hiroshima females, males were slightly more numerous than females. In the backcrosses of female reciprocal hybrids mated with males belonging to the paternal population, males were remarkably more numerous than females.

10. It seems reasonable to give a position as a sibling species of *Rana japonica* to the Ichinoseki population.

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TABLE 1

Results of crosses between the Hiroshima (H) and Ichinoseki (I) populations of *Rana japonica*

Year	Parents		No. of eggs	No. of normally cleaved eggs	No. of normal tail-bud embryos	No. of normally hatched tadpoles	No. of normally feeding tadpoles	No. of meta-morphosed frogs
	Female	Male						
1979	H 1	H 1	120	111 (92.5)	103 (85.8)	102 (85.0)	101 (84.2)	96 (80.0)
1980	H 2	H 2	208	177 (85.1)	170 (81.7)	170 (81.7)	170 (81.7)	164 (78.8)
	H 3	H 3	132	108 (81.8)	102 (77.3)	102 (77.3)	102 (77.3)	98 (74.2)
	H 4	H 4	155	142 (91.6)	142 (91.6)	140 (90.3)	139 (89.7)	137 (88.4)
	H 5	H 5	155	136 (87.7)	135 (87.1)	135 (87.1)	132 (85.2)	128 (82.6)
	H 6	H 6	104	98 (94.2)	98 (94.2)	97 (93.3)	97 (93.3)	92 (88.5)
	H 7	H 7	141	137 (97.2)	136 (96.5)	136 (96.5)	136 (96.5)	132 (93.6)
1979	H 1	I 1	157	147 (93.6)	145 (92.4)	144 (91.7)	144 (91.7)	138 (87.9)
1980	H 2	I 2	96	77 (80.2)	77(80.2)	75 (78.1)	75 (78.1)	71 (74.0)
	H 3	I 3	183	144 (78.7)	142 (77.6)	141 (77.0)	140 (76.5)	133 (72.7)
	H 4	I 4	310	293 (94.5)	284 (91.6)	283 (91.3)	275 (88.7)	252 (81.3)
	H 5	I 5	184	178 (96.7)	172 (93.5)	170 (92.4)	164 (89.1)	157 (85.3)
	H 6	I 6	136	134 (98.5)	132 (97.1)	131 (96.3)	130 (95.6)	124 (91.2)
	H 7	I 7	157	141 (89.8)	141 (89.8)	141 (89.8)	140 (89.2)	133 (84.7)
1979	I 1	H 1	244	211 (86.5)	172 (70.5)	148 (60.7)	134 (54.9)	127 (52.0)
1980	I 2	H 2	131	100 (76.3)	80 (61.1)	72 (55.0)	62 (47.3)	57 (43.5)
	I 3	H 3	122	105 (86.1)	86 (70.5)	67 (54.9)	61 (50.0)	54 (44.3)
	I 4	H 4	147	121 (82.3)	95 (64.6)	83 (56.5)	66 (44.9)	62 (42.2)
1979	I 1	I 1	165	147 (89.1)	121 (73.3)	96 (58.2)	95 (57.6)	88 (53.3)
1980	I 2	I 2	137	102 (74.5)	84 (61.3)	76 (55.5)	68 (49.6)	55 (40.2)
	I 3	I 3	127	106 (83.5)	86 (67.7)	80 (63.0)	73 (57.5)	57 (44.9)
	I 4	I 4	157	125 (79.6)	101 (64.3)	94 (59.9)	79 (50.3)	63 (40.1)

TABLE 2

Stages of embryos at definite ages in the two *Rana japonica* populations at 18 °C (1979)

Kind \ Age(hrs)	3	5	8	22	30	45	54	69	78	117	144.5	199
Hiroshima population	3E	5E	7aL	10a	12E	13bL	15	17	19E	21	23	25
Ichinoseki population	3	5	7b	10a	11	13b	15E	17E	19E	21	23	25

TABLE 3

Stages of larvae at definite ages in the two *Rana japonica* populations at 18 ℃ (1979)

Kind \ Age(days)	18	38	49	67	76	83	89
Hiroshima population	III	V ~ VIII	X ~ XII	XVII ~ XVIII	XX(76.2)		
Ichinoseki population	III	IV ~ V	VII ~ IX	XIII ~ XV	XV ~ XVIII	XVIII ~ XX	XX(89.3)

TABLE 4

Measurements of reciprocal hybrids between the Hiroshima and Ichinoseki populations of *Rana japonica* and the controls at Shumway stage 25

Parents		Tadpoles immediately before feeding (Shumway stage 25)				
Female	Male	Total length (mm)	Body length (mm)	Head width (mm)	Tail length (mm)	Tail height (mm)
H 1	H 1	13.6 ± 0.10	5.6 ± 0.02	2.9 ± 0.01	8.0 ± 0.07	2.8 ± 0.03
H 1	I 1	12.7 ± 0.08	5.2 ± 0.04	2.6 ± 0.03	7.5 ± 0.06	2.7 ± 0.03
I 1	H 1	12.3 ± 0.26	5.2 ± 0.08	2.9 ± 0.08	7.0 ± 0.19	2.9 ± 0.06
I 1	I 1	12.8 ± 0.16	5.2 ± 0.06	3.0 ± 0.03	7.6 ± 0.13	2.9 ± 0.04

TABLE 5

Measurements of reciprocal hybrids between the Hiroshima and Ichinoseki populations of *Rana japonica* and the controls at TK stage XX

Parents		Tadpoles immediately after protrusion of fore-legs (TK stage XX)				
Female	Male	Total length (mm)	Body length (mm)	Head width (mm)	Tail length (mm)	Tail height (mm)
H 1	H 1	48.2 ± 0.45	16.8 ± 0.33	8.3 ± 0.16	31.4 ± 0.34	5.9 ± 0.17
H 1	I 1	47.6 ± 0.54	16.6 ± 0.19	8.1 ± 0.13	31.0 ± 0.43	6.1 ± 0.09
I 1	H 1	48.5 ± 0.52	16.9 ± 0.13	9.3 ± 0.15	31.5 ± 0.45	7.0 ± 0.09
I 1	I 1	49.5 ± 0.41	18.0 ± 0.16	9.4 ± 0.12	31.5 ± 0.44	7.3 ± 0.14

TABLE 6

Measurements of reciprocal hybrids between the Hiroshima and Ichinoseki populations of *Rana japonica* and the controls 3 months after metamorphosis

Parents		Froglets 3 months after metamorphosis							
Female	Male	Body length (a) (mm)	Head length (mm)	Head width (mm)	Snout length (mm)	Fore-leg length (mm)	Hind leg length (b) (mm)	Tibia length (mm)	b/a
H 1	H 1	37.2 ± 0.43	13.6 ± 0.25	12.7 ± 0.24	5.8 ± 0.13	21.9 ± 0.52	60.3 ± 0.83	18.6 ± 0.45	1.60 ± 0.01
H 1	I 1	37.5 ± 0.46	13.4 ± 0.29	12.6 ± 0.25	5.9 ± 0.12	22.2 ± 0.46	58.6 ± 0.85	17.7 ± 0.39	1.56 ± 0.01
I 1	H 1	35.4 ± 0.45	13.2 ± 0.21	12.3 ± 0.22	5.8 ± 0.08	21.6 ± 0.51	57.7 ± 0.92	17.6 ± 0.40	1.63 ± 0.01
I 1	I 1	39.0 ± 0.46	13.8 ± 0.16	13.5 ± 0.15	6.0 ± 0.08	22.6 ± 0.29	58.6 ± 0.68	17.7 ± 0.23	1.51 ± 0.02

TABLE 7

Measurements of reciprocal hybrids between the Hiroshima and Ichinoseki populations of *Rana japonica* and the controls at the age of one year

Parents		One-year-old males							
Female	Male	Body length (a) (mm)	Head length (mm)	Head width (mm)	Snout length (mm)	Hind leg length (b) (mm)	Diameter of tympanic membrane (mm)	Metatarsal tubercle (mm)	b/a
H 1	H 1	44.5 ± 0.49	15.9 ± 0.39	15.6 ± 0.25	7.3 ± 0.05	67.3 ± 0.67	3.8 ± 0.10	2.14 ± 0.03	1.53 ± 0.01
H 1	I 1	45.0 ± 0.58	15.3 ± 0.29	15.9 ± 0.27	6.8 ± 0.13	69.7 ± 1.33	3.8 ± 0.09	2.85 ± 0.09	1.50 ± 0.01
I 1	H 1	43.8 ± 0.57	15.2 ± 0.15	15.5 ± 0.18	7.0 ± 0.12	68.7 ± 1.45	3.6 ± 0.07	2.80 ± 0.04	1.55 ± 0.02
I 1	I 1	47.4 ± 0.77	16.3 ± 0.58	16.9 ± 0.29	7.3 ± 0.10	67.8 ± 0.93	3.8 ± 0.08	2.95 ± 0.06	1.43 ± 0.01

TABLE 8

Measurements of field-caught frogs of the Hiroshima and Ichinoseki populations of *Rana japonica*

Kind	Hiroshima population		Ichinoseki population	
Individual no.	978 (H 1)	974 (H 1)	982 (I 1)	977 (I 1)
Sex	♀	♂	♀	♂
Body length (a) (mm)	51.7	44.7	70.3	51.7
Head length (mm)	17.0	14.7	21.0	15.8
Head width (mm)	14.7	13.9	20.2	15.9
Snouth length (c) (mm)	6.8	6.0	8.6	7.1
Head width at anterior edge of upper eyelid (d) (mm)	9.8	9.3	14.5	11.1
Fore-leg length (mm)	29.0	24.6	37.4	28.6
Hind leg length (b)(mm)	88.2	76.5	109.2	79.2
Diameter of tympanic membrane (mm)	3.4	3.0	4.2	3.6
Metatarsal tubercle (mm)	2.3	2.1	3.5	3.0
b/a	1.71	1.71	1.55	1.53
c/d	0.69	0.65	0.59	0.64

TABLE 9

Dental formulae of reciprocal hybrids and the controls at TK stage X (1979)

Parents		No. of tadpoles	Kind of dental formula							
Female	Male		1:2+2 / 2:1+1	1:2+2 / 3:1+1	1:2+2 / 2:2+2	1:2+2 / 1:2+2	1:3+3 / 3:1+1	1:2+2 / 3:0+0	1:2+2 / 4:0+0	1:2+2 / 0:3+3
H 1	H 1	50	12	37			1			
H 2	H 2	50	9	37			3		1	
H 1	I 1	50	8	26	6	1	8		1	
I 1	H 1	50	28	19	2	1				
I 1	I 1	50	38			4		7		1
I 2	I 2	50	14	1	3	29				3
I 3	I 3	37	7			24				6

TABLE 10

Comparison in 16 loci of 12 proteins between the Hiroshima and Ichinoseki populations of *Rana japonica*

Kind \ Locus	LDH		MDH		α-GDH	IDH		AAT		PGM	GPI	SOD	CK	Est-1	Hb	Ab
	A	B	A	B		A	B	A	B							
Hiroshima population	a	b	a	b, <u>c</u>	a, <u>b</u>	a	a	a	<u>b</u>, d	b	a, <u>b</u>	b	a	b	a	a, <u>b</u>
Ichinoseki population	a	b	a	c	b	a	a	a	b	b	a, <u>b</u>	b	a	b	a	b, <u>c</u>

Underline shows the major allele.

TABLE 11

Number of frogs and mitotic figures examined for chromosome analysis in 1979

Parents		No. of analyzed frogs	No. of analyzed mitoses	No. of measured mitoses
Female	Male			
H 1	H 1	7	239	27
H 2	H 2	3	45	27
I 1	I 1	7	93	9
I 2	I 1	8	99	45
I 3	I 2	2	5	0
H 1	I 1	10	63	–
I 1	H 1	10	63	–

TABLE 12

Relative lengths of metaphase chromosomes in the Hiroshima and Ichinoseki populations

Hiroshima population				Ichinoseki population			
Chromosome no.	Minimum	Maximum	Mean	Chromosome no.	Minimum	Maximum	Mean
1	13.76	16.76	14.90 ± 0.11	1	12.87	16.10	14.09 ± 0.09
2	11.91	14.53	12.78 ± 0.09	2	11.26	13.95	12.56 ± 0.08
3	10.46	12.58	11.54 ± 0.07	3	9.93	11.85	10.92 ± 0.06
4	10.04	12.51	11.35 ± 0.07	4	10.10	12.29	11.15 ± 0.07
5	9.23	10.77	9.89 ± 0.05	5	8.83	11.22	9.81 ± 0.07
6	5.59	7.10	6.29 ± 0.04	6	5.75	6.94	6.36 ± 0.03
7	4.82	6.18	5.60 ± 0.04	7	5.01	6.65	5.83 ± 0.04
8	4.73	5.88	5.21 ± 0.04	8	4.86	5.84	5.22 ± 0.03
9	4.63	5.71	5.07 ± 0.04	9	4.45	5.58	5.13 ± 0.03
10	3.82	5.41	4.79 ± 0.05	10	4.49	5.73	5.14 ± 0.04
11	3.89	5.01	4.60 ± 0.04	11	4.42	5.77	5.09 ± 0.05
12	3.58	4.89	4.29 ± 0.04	12	3.89	5.31	4.65 ± 0.04
13	3.15	4.25	3.70 ± 0.03	13	3.33	4.81	4.14 ± 0.04

Relative chromosome length: $\frac{\text{Each chromosome length}}{\text{Genome length}} \times 100$

± Standard error of the mean

TABLE 13

Centromere positions represented by numerical values and types of metaphase chromosomes in the Hiroshima and Ichinoseki populations

Hiroshima population					Ichinoseki population				
Chromosome no.	Minimum	Maximum	Mean	Type	Chromosome no.	Minimum	Maximum	Mean	Type
1	43.29	49.65	46.69 ± 0.19	m	1	41.74	48.47	46.01 ± 0.17	m
2	33.08	41.52	37.43 ± 0.20	sm	2	32.03	41.84	38.05 ± 0.27	m
3	30.92	37.09	34.35 ± 0.20	sm	3	29.75	39.95	35.29 ± 0.26	sm
4	37.52	46.04	40.30 ± 0.20	m	4	37.00	45.69	42.01 ± 0.23	m
5	42.16	48.69	45.16 ± 0.21	m	5	42.18	48.12	45.53 ± 0.16	m
6	35.26	44.17	39.70 ± 0.29	m	6	40.59	49.60	46.75 ± 0.27	m
7	36.88	44.86	41.15 ± 0.25	m	7	38.00	49.43	43.20 ± 0.34	m
8	24.38	32.98	27.92 ± 0.33	sm	8	21.72	31.58	27.13 ± 0.29	sm
9	15.45	26.18	20.65 ± 0.30	st	9	25.41	35.38	29.79 ± 0.28	sm
10	33.16	41.98	37.16 ± 0.22	sm	10	32.23	41.05	36.71 ± 0.28	sm
11	37.28	46.97	43.00 ± 0.29	m	11	38.57	45.13	41.96 ± 0.24	m
12	30.73	42.31	35.88 ± 0.32	sm	12	32.49	42.58	37.47 ± 0.27	sm
13	29.52	40.00	35.65 ± 0.30	sm	13	30.66	38.79	35.28 ± 0.25	sm

Numerical value of the centromere position: $\frac{\text{Short-arm length}}{\text{Chromosome length}} \times 100$

± Standard error of the mean

	NVC	Type
Chromosome type:	50.0 ~ 37.5	m
	37.5 ~ 25.0	sm
	25.0 ~ 12.5	st
	12.5 ~ 0.0	t

TABLE 14

Sex of reciprocal hybrids between the Hiroshima and Ichinoseki populations of *Rana japonica*

Parents		No. of metamor-phosed frogs	Sex of frogs dead or killed within 1 month after metamorphosis						Sex of 6-month-old frogs			Sex of all frogs examined		
Female	Male		No. of frogs	♀	⚥ 1	⚥ 2	⚥ 3	♂(%)	No. of frogs	♀	♂	Total	♀	♂(%)
H 1	H 1	96	41	21				20 (48.8)	53	35	18	94	56	38 (40.4)
H 2	H 2	164	106	59				47 (44.3)	49	26	23	155	85	70 (45.2)
H 3	H 3	98	38	16				22 (57.9)	51	27	24	89	43	46 (51.7)
H 4	H 4	137	19	9			1	9 (47.4)	115	60	55	134	69	65 (48.5)
H 5	H 5	128	114	66				48 (42.1)	–	–	–	114	66	48 (42.1)
H 6	H 6	92	88	43				45 (51.1)	–	–	–	88	43	45 (51.1)
H 7	H 7	132	89	38				51 (57.3)	38	23	15	127	61	66 (52.0)
Total		847	495	252			1	242 (48.9)	306	171	135	801	423	378 (47.2)
H 1	I 1	138	27	4		1		22 (81.5)	93	4	89	120	8	112 (93.3)
H 2	I 2	71	36	9		1		26 (72.2)	30	5	25	66	14	52 (78.8)
H 3	I 3	133	75	4	3	1		67 (89.3)	37	1	36	112	5	107 (95.5)
H 4	I 4	252	141	30	4	6	3	98 (69.5)	73	13	60	214	43	171 (79.9)
H 5	I 5	157	147	13	4	1		129 (87.8)	–	–	–	147	13	134 (91.2)
H 6	I 6	124	115	27	1	4	1	82 (71.3)	–	–	–	115	27	88 (76.5)
H 7	I 7	133	75	3		1		71 (94.7)	36	2	34	111	5	106 (95.5)
Total		1008	616	90	12	15	4	495 (80.4)	269	25	244	885	115	770 (87.0)
I 1	H 1	127	28	3				25 (89.3)	89	13	76	117	16	101 (86.3)
I 2	H 2	57	38	3		1		34 (89.5)	19	3	16	57	6	51 (89.5)
I 3	H 3	54	21	0				21 (100)	33	0	33	54	0	54 (100)
I 4	H 4	62	39	7				32 (82.1)	16	2	14	55	9	46 (83.6)
Total		300	126	13		1		112 (88.9)	157	18	139	283	31	252 (89.0)
I 1	I 1	88	34	21				13 (38.2)	50	22	28	84	43	41 (48.8)
I 2	I 2	55	36	17		1		18 (50.0)	18	7	11	54	24	30 (55.6)
I 3	I 3	57	21	12				9 (42.9)	30	12	18	51	24	27 (52.9)
I 4	I 4	63	17	7				10 (58.8)	38	20	18	55	27	28 (50.9)
Total		263	108	57		1		50 (46.3)	136	61	75	244	118	126 (51.6)

⚥, Hermaphrodites

TABLE 15

Testes of male reciprocal hybrids and the controls 3 months after metamorphosis

Kind	Individual no.	Body length (mm)	Size of testes	
			Left (mm)	Right (mm)
H1 x H1	1	37.7	2.6 x 1.8	3.1 x 1.8
	2	34.0	2.9 x 1.4	2.8 x 1.8
	3	33.3	1.9 x 1.4	2.1 x 1.2
	4	32.9	1.6 x 0.8	1.4 x 0.7
H1 x I1	1	38.5	3.2 x 2.3	3.2 x 2.1
	2	36.7	2.9 x 2.1	2.9 x 2.3
	3	34.0	2.8 x 1.8	2.8 x 1.9
	4	33.4	2.2 x 1.6	2.0 x 1.8
I1 x H1	1	34.8	2.6 x 1.6	3.1 x 1.6
	2	34.7	2.2 x 1.1	2.4 x 1.0
	3	32.4	1.9 x 1.4	2.0 x 1.3
	4	30.2	1.6 x 1.4	1.7 x 1.3
I1 x I1	1	38.4	3.1 x 2.3	3.1 x 2.1
	2	36.0	2.6 x 1.9	2.5 x 1.9
	3	35.8	2.5 x 1.8	2.6 x 1.7
	4	35.1	2.3 x 1.8	2.6 x 1.8

TABLE 16

Ovaries of female reciprocal hybrids and the contrals dead or killed within 3 months after metamorphosis

Kind	Individual no.	Body length (mm)	Size of ovaries	
			Left (mm)	Right (mm)
H1 x H1	1	33.7	8.8 x 4.1	10.2 x 4.3
	2	33.1	7.6 x 4.2	7.5 x 4.3
	3	32.2	6.6 x 4.3	6.8 x 4.0
	4	29.3	6.6 x 3.0	6.7 x 3.6
H1 x I1	1	33.1	9.2 x 4.8	9.8 x 5.1
	2	32.7	7.3 x 3.6	8.2 x 3.2
	3	30.5	6.8 x 4.1	7.0 x 3.9
	4	25.8	5.7 x 3.8	5.8 x 3.7
I1 x H1	1	32.0	6.3 x 4.1	6.8 x 3.3
	2	24.0	3.7 x 2.4	3.6 x 1.7
	3	23.8	3.2 x 2.2	3.8 x 2.2
I1 x I1	1	36.7	10.1 x 7.1	11.6 x 6.0
	2	33.6	9.4 x 6.3	9.8 x 6.4
	3	30.1	7.2 x 4.0	8.4 x 3.6
	4	28.9	6.8 x 5.2	7.7 x 4.3

TABLE 17

Testes of male reciprocal hybrids and the controls at the age of one year

Kind	Individual no.	Body length (mm)	Size of testes		Type
			Left (mm)	Right (mm)	
H1 x H1	1	43.2	4.7 x 4.2	5.7 x 4.2	1
	2	44.8	4.7 x 4.2	5.0 x 4.2	1
	3	43.2	3.7 x 3.1	3.8 x 3.1	1
	4	45.8	3.9 x 3.2	4.6 x 3.2	1
	5	43.0	3.8 x 2.8	4.3 x 3.1	1
	6	43.7	4.0 x 3.2	4.2 x 3.3	1
H1 x I1	1	49.2	4.5 x 3.5	4.3 x 3.3	4
	2	46.9	4.1 x 3.2	4.1 x 3.1	4
	3	45.7	3.6 x 3.0	4.7 x 3.0	4
	4	46.2	4.8 x 2.7	5.0 x 2.8	5
	5	49.7	4.0 x 3.0	4.8 x 2.9	4
	6	46.8	5.2 x 3.3	5.6 x 3.8	3
	7	45.1	4.8 x 2.7	5.2 x 2.7	4
	8	43.2	4.0 x 2.2	3.8 x 2.4	4
	9	47.3	3.2 x 2.3	3.4 x 2.8	4
	10	48.1	3.2 x 2.7	3.8 x 3.3	4
	11	44.0	4.6 x 2.8	4.8 x 3.0	4
	12	44.5	3.9 x 3.0	4.0 x 3.0	3
I1 x H1	1	44.3	4.1 x 3.0	4.0 x 3.0	2
	2	46.8	4.7 x 3.2	4.8 x 3.2	2
	3	46.2	4.6 x 2.5	4.6 x 2.9	2
	4	41.6	5.2 x 3.7	5.4 x 3.9	4
	5	43.9	4.7 x 3.5	4.2 x 3.2	2
	6	43.7	5.1 x 4.7	5.7 x 5.0	3
	7	44.8	3.2 x 2.6	3.7 x 2.7	2
	8	44.1	3.7 x 2.4	3.3 x 2.3	2
	9	46.0	4.7 x 2.8	4.6 x 3.0	3
	10	42.1	4.2 x 2.6	4.9 x 3.0	4
	11	42.2	3.7 x 2.3	3.7 x 2.3	2
	12	40.3	4.0 x 2.2	4.3 x 2.7	3
I1 x I1	1	51.2	6.6 x 4.4	6.4 x 4.4	1
	2	48.0	4.8 x 4.1	4.7 x 3.8	1
	3	46.2	4.9 x 3.9	4.8 x 3.9	1
	4	48.6	4.3 x 3.2	3.8 x 3.2	1
	5	47.0	4.0 x 3.1	3.5 x 3.0	1
	6	48.8	3.2 x 2.7	4.2 x 3.0	1

TABLE 18

Eggs of female reciprocal hybrids and the controls
at the age of one year

Kind	Individual no.	Body length (mm)	No. of eggs	Mean diameter of 20 eggs (mm)
H1 x H1	1	53.4	971	1.73 ± 0.01
	2	45.2	646	1.80 ± 0.01
	3	41.8	659	1.75 ± 0.01
H1 x I1	1	48.5	968	1.77 ± 0.01
	2	50.5	804	1.93 ± 0.02
	3	48.2	600	1.79 ± 0.02
	4	42.2	604	1.67 ± 0.01
I1 x H1	1	45.8	869	1.85 ± 0.01
	2	43.4	742	1.88 ± 0.02
	3	49.0	1202	1.85 ± 0.01
	4	40.0	609	1.78 ± 0.02
	5	49.4	827	1.83 ± 0.01
	6	52.3	842	1.88 ± 0.02
I1 x I1	1	53.2	971	1.85 ± 0.02
	2	50.7	1063	1.70 ± 0.02

TABLE 19

Eggs of twelve field-caught females of the Hiroshima population used for backcross experiments in 1980

Kind	Individual no.	Body length (mm)	No. of eggs	Mean diameter of 20 eggs (mm)
Hiroshima population	1	60.6	1309	2.07 ± 0.02
	2	55.7	1471	1.78 ± 0.02
	3	51.4	748	2.09 ± 0.02
	4	49.5	973	1.67 ± 0.01
	5	52.0	1013	1.77 ± 0.01
	6	48.8	838	1.72 ± 0.02
	7	54.8	1213	1.66 ± 0.01
	8	50.6	869	1.64 ± 0.01
	9	47.7	626	1.74 ± 0.02
	10	46.0	867	1.63 ± 0.01
	11	45.8	593	1.80 ± 0.01
	12	46.8	759	1.64 ± 0.01

TABLE 20

Developmental capacity of the backcrosses of male reciprocal hybrids between the Hiroshima and Ichinoseki populations

Parents: Female	Parents: Male	No. of eggs	No. of normally cleaved eggs	No. of normal tail-bud embryos	No.of normally hatched tadpoles	No. of normally feeding tadpoles	No. of metamorphosed frogs
H 1	HI 1	380	0 (0)	0	0	0	0
H 2	HI 2	498	0 (0)	0	0	0	0
H 3	HI 3	213	0 (0)	0	0	0	0
H 4	HI 4	380	0 (0)	0	0	0	0
H 5	HI 5	328	0 (0)	0	0	0	0
H 6	HI 6	253	2 (0.8)	2 (0.8)	2 (0.8)	2 (0.8)	2 (0.8)
H 7	HI 7	243	2 (0.8)	2 (0.8)	2 (0.8)	2 (0.8)	2 (0.8)
H 8	HI 8	185	0 (0)	0	0	0	0
H 9	HI 9	115	1 (0.9)	1 (0.9)	1 (0.9)	1 (0.9)	1 (0.9)
H 10	HI 10	174	0 (0)	0	0	0	0
H 11	HI 11	110	0 (0)	0	0	0	0
H 12	HI 12	131	1 (0.8)	1 (0.8)	1 (0.8)	1 (0.8)	1 (0.8)
H 1	IH 1	524	230 (43.9)	218 (41.6)	214 (40.8)	214 (40.8)	–
H 2	IH 2	520	182 (35.0)	172 (33.1)	170 (32.7)	165 (31.7)	–
H 3	IH 3	269	178 (66.2)	173 (64.3)	170 (63.2)	170 (63.2)	157 (58.4)
H 4	IH 4	264	0 (0)	0	0	0	0
H 5	IH 5	240	60 (25.0)	60 (25.0)	60 (25.0)	60 (25.0)	56 (23.3)
H 6	IH 6	220	21 (9.5)	19 (8.6)	18 (8.2)	18 (8.2)	16 (7.3)
H 7	IH 7	198	53 (26.8)	53 (26.8)	53 (26.8)	52 (26.3)	46 (23.2)
H 8	IH 8	191	2 (1.0)	2 (1.0)	2 (1.0)	2 (1.0)	–
H 9	IH 9	136	7 (5.1)	7 (5.1)	7 (5.1)	7 (5.1)	–
H 10	IH 10	161	3 (1.9)	3 (1.9)	3 (1.9)	3 (1.9)	3 (1.9)
H 11	IH 11	112	45 (40.2)	44 (39.3)	44 (39.3)	44 (39.3)	40 (35.7)
H 12	IH 12	152	12 (7.9)	12 (7.9)	12 (7.9)	12 (7.9)	11 (7.2)
H1~6	H*	207	190 (91.8)	190 (91.8)	190 (91.8)	189 (91.3)	–
H 7	H 1	57	50 (87.7)	50 (87.7)	50 (87.7)	50 (87.7)	48 (84.2)
H 8	H 2	42	35 (83.3)	35 (83.3)	34 (81.0)	34 (81.0)	–
H 9	H 3	54	45 (83.3)	45 (83.3)	45 (83.3)	45 (83.3)	–
H 10	H 4	41	35 (85.4)	35 (85.4)	35 (85.4)	35 (85.4)	33 (80.5)
H 11	H 5	50	45 (90.0)	44 (88.0)	44 (88.0)	44 (88.0)	43 (86.0)
H 12	H 6	45	43 (95.6)	43 (95.6)	42 (93.3)	42 (93.3)	40 (88.9)
H 7	I 1	54	48 (88.9)	47 (87.0)	47 (87.0)	46 (85.2)	44 (81.5)
H 8	I 2	79	65 (82.3)	64 (81.0)	63 (79.7)	63 (79.7)	–
H 9	I 3	76	62 (81.6)	60 (78.9)	58 (76.3)	58 (76.3)	–
H 10	I 4	62	59 (95.2)	57 (91.9)	57 (91.9)	55 (88.7)	52 (83.9)
H 11	I 5	47	45 (95.7)	44 (93.6)	44 (93.6)	43 (91.5)	42 (89.4)
H 12	I 6	66	61 (92.4)	60 (90.9)	60 (90.9)	60 (90.9)	57 (86.4)

H*- Field-caught male of Hiroshima population

TABLE 21

Testes of six field-caught males of the Hiroshima population and five one-year-old males of the Ichinoseki population

Kind	Individual no.	Body length (mm)	Size of testes	
			Left (mm)	Right (mm)
Hiroshima population	1	50.7	4.1 x 2.9	4.6 x 2.8
	2	49.2	3.7 x 2.4	3.4 x 2.5
	3	51.3	4.0 x 2.4	4.3 x 2.5
	4	48.7	3.3 x 2.5	3.2 x 2.2
	5	44.0	3.4 x 2.2	3.2 x 2.0
	6	43.3	3.7 x 2.3	4.2 x 2.3
Ichinoseki population	1	53.0	3.8 x 3.2	3.8 x 3.2
	2	45.0	3.7 x 2.1	3.8 x 2.2
	3	46.7	3.9 x 3.1	4.3 x 3.2
	4	48.2	4.5 x 3.2	3.7 x 3.0
	5	51.5	4.2 x 3.4	4.2 x 3.2

TABLE 22

Developmental capacity of the backcrosses of female reciprocal hybrids between the Hiroshima and Ichinoseki populations

Parents		No. of eggs	No. of normally cleaved eggs	No. of normal tail-bud embryos	No. of normally hatched tadpoles	No. of normally feeding tadpoles	No. of meta-morphosed frogs
Female	Male						
H 1	H 1	459	422 (91.9)	370 (80.6)	359 (78.2)	341 (74.3)	318 (69.3)
H 2	H 2	320	269 (84.1)	226 (70.6)	218 (68.1)	213 (66.6)	204 (63.8)
H 3	H 3	324	267 (82.4)	198 (61.1)	193 (59.6)	185 (57.1)	177 (54.6)
H 1	I 1	482	425 (88.2)	364 (75.5)	356 (73.9)	341 (70.7)	320 (66.4)
H 2	I 2	242	191 (78.9)	157 (64.9)	153 (63.2)	151 (62.4)	132 (54.5)
H 3	I 3	283	247 (87.3)	165 (58.3)	151 (53.4)	145 (51.2)	138 (48.8)
I 1	H 1	311	235 (75.6)	176 (56.6)	141 (45.3)	119 (38.3)	104 (33.4)
I 2	H 2	287	185 (64.5)	152 (53.0)	134 (46.7)	131 (45.6)	115 (40.1)
I 1	I 1	459	379 (82.6)	264 (57.5)	222 (48.4)	206 (44.9)	160 (34.9)
I 2	I 2	344	237 (68.9)	193 (56.1)	172 (50.0)	154 (44.8)	149 (43.3)
HI 1	H 1	387	323 (83.5)	258 (66.7)	248 (64.1)	230 (59.4)	159 (41.1)
HI 2	H 2	347	35 (10.1)	22 (6.3)	20 (5.8)	19 (5.5)	–
HI 3	H 3	239	191 (79.9)	173 (72.4)	160 (66.9)	153 (64.0)	140 (58.6)
HI 4	H 4	295	263 (89.2)	211 (71.5)	208 (70.5)	203 (68.8)	183 (62.0)
HI 1	I 1	481	401 (83.4)	307 (63.8)	297 (61.7)	277 (57.6)	255 (53.0)
HI 2	I 2	392	74 (18.9)	46 (11.7)	45 (11.5)	41 (10.5)	–
HI 3	I 3	312	284 (91.0)	249 (79.8)	240 (76.9)	228 (73.1)	201 (64.4)
HI 4	I 4	309	267 (86.4)	228 (73.8)	222 (71.5)	214 (69.3)	198 (64.1)
IH 1	H 1	375	326 (86.9)	231 (61.6)	200 (53.3)	165 (44.0)	143 (38.1)
IH 2	H 2	312	249 (79.8)	160 (51.3)	111 (35.6)	86 (27.6)	–
IH 3	H 3	477	406 (85.1)	324 (67.9)	261 (54.7)	176 (36.9)	102 (21.4)
IH 4	H 4	320	264 (82.6)	183 (57.2)	82 (25.6)	54 (16.9)	(18)
IH 5	H 5	343	255 (74.3)	103 (30.0)	83 (24.2)	73 (21.3)	–
IH 6	H 6	480	410 (85.4)	334 (69.6)	287 (59.8)	248 (51.7)	180 (37.5)
IH 1	I 1	354	294 (83.1)	190 (53.7)	169 (47.7)	153 (43.2)	141 (39.8)
IH 2	I 2	350	289 (82.6)	182 (52.0)	131 (37.4)	100 (28.6)	–
IH 3	I 3	500	445 (89.0)	372 (74.4)	324 (64.8)	265 (53.0)	220 (44.0)
IH 4	I 4	329	282 (85.7)	201 (61.1)	87 (26.4)	47 (14.3)	(16)
IH 5	I 5	284	150 (52.8)	65 (22.9)	55 (19.4)	48 (16.9)	–
IH 6	I 5	222	170 (76.6)	141 (63.5)	130 (58.6)	103 (46.4)	98 (44.1)

TABLE 23

Chromosome analysis of backcrosses of male reciprocal hybrids between the Hiroshima and Ichinoseki populations and the controls

Parents		No. of analyzed tadpoles	No. of tadpoles	
Female	Male		Diploid (2n=26)	Triploid (3n=39)
H 7	H 1	20	20	—
H 10	H 4	15	15	—
H 11	H 5	20	20	—
H 12	H 6	15	15	—
H 7	I 1	23	23	—
H 10	I 4	12	12	—
H 11	I 5	20	20	—
H 12	I 6	10	10	—
H 6	HI 6	2	2	—
H 7	HI 7	2	2	—
H 9	HI 9	1	1	—
H 12	HI 12	1	1	—
H 1	IH 1	70	69	1
H 2	IH 2	30	30	—
H 3	IH 3	30	30	—
H 5	IH 5	30	30	—
H 6	IH 6	18	18	—
H 7	IH 7	30	30	—
H 10	IH 10	3	3	—
H 11	IH 11	30	30	—
H 12	IH 12	12	12	—

TABLE 24

Chromosome analysis of backcrosses of female reciprocal hybrids between the Hiroshima and Ichinoseki populations and the controls

Parents		No. of analyzed tadpoles	No. of tadpoles				
			Normal diploid (2n=26)	Hyperdiploid			Normal triploid (3n=39)
Female	Male			2n=27	2n=28	2n=29	
H 1	H 1	29	26	–	–	–	3
H 2	H 2	30	29	–	–	–	1
H 3	H 3	30	30	–	–	–	–
H 1	I 1	30	30	–	–	–	–
H 2	I 2	30	30	–	–	–	–
H 3	I 3	30	30	–	–	–	–
I 1	H 1	41	41	–	–	–	–
I 2	H 2	26	26	–	–	–	–
I 1	I 1	57	57	–	–	–	–
I 2	I 2	29	29	–	–	–	–
HI 1	H 1	30	28	–	–	–	2
HI 2	H 2	–					
HI 3	H 3	30	30	–	–	–	–
HI 4	H 4	30	29	–	–	–	1
HI 1	I 1	30	30	–	–	–	–
HI 2	I 2	–					
HI 3	I 3	30	30	–	–	–	–
HI 4	I 4	30	30	–	–	–	–
IH 1	H 1	30	30	–	–	–	–
IH 2	H 2	12	12	–	–	–	–
IH 3	H 3	30	30	–	–	–	–
IH 4	H 4	21	9	3	7	2	–
IH 5	H 5	9	9	–	–	–	–
IH 6	H 6	30	30	–	–	–	–
IH 1	I 1	30	29	–	–	–	1
IH 2	I 2	11	11	–	–	–	–
IH 3	I 3	30	30	–	–	–	–
IH 4	I 4	29	17	9	3	–	–
IH 5	I 5	9	9	–	–	–	–
IH 6	I 5	30	30	–	–	–	–

TABLE 25

Sex of the backcrosses of male reciprocal hybrids between the Hiroshima and Ichinoseki populations

Parents		No. of metamorphosed frogs	Sex of frogs dead or killed within one month after metamorphosis						Sex of 6-month-old frogs			Sex of all frogs examined		
Female	Male		No. of frogs	♀	⚥ 1	⚥ 2	⚥ 3	♂ (%)	No. of frogs	♀	♂	Total	♀	♂ (%)
H 7	H 1	48	48	26				22 (45.8)	–	–	–	48	26	22 (45.8)
H 10	H 4	33	31	17				14 (45.2)	–	–	–	31	17	14 (45.2)
H 11	H 5	43	42	22				20 (47.6)	–	–	–	42	22	20 (47.6)
H 12	H 6	40	39	19				20 (51.3)	–	–	–	39	19	20 (51.3)
Total		164	160	84				76 (47.5)	–	–	–	160	84	76 (47.5)
H 7	I 1	44	44	9				35 (79.5)	–	–	–	44	9	35 (79.5)
H 10	I 4	52	52	5	1			46 (88.5)	–	–	–	52	5	47 (90.4)
H 11	I 5	42	42	10		1		31 (73.8)	–	–	–	42	10	32 (76.2)
H 12	I 6	57	52	3		1		48 (92.3)	–	–	–	52	3	49 (94.2)
Total		195	190	27	1	2		160 (84.2)	–	–	–	190	27	163 (85.8)
H 6	HI 6	2	2	1				1 (50.0)	–	–	–	2	1	1 (50.0)
H 7	HI 7	2	2	1				1 (50.0)	–	–	–	2	1	1 (50.0)
H 9	HI 9	1	–	–				–	1	0	1	1	0	1 (100)
H 12	HI 12	1	–	–				–	1	0	1	1	0	1 (100)
Total		6	4	2				2 (50.0)	2	0	2	6	2	4 (66.7)
H 3	IH 3	157	88	38				50 (56.8)	49	22	27	137	60	77 (56.2)
H 5	IH 5	56	56	14				42 (75.0)	–	–	–	56	14	42 (75.0)
H 6	IH 6	16	5	3				2 (40.0)	11	1	10	16	4	12 (75.0)
H 7	IH 7	46	14	7			1	6 (42.9)	23	9	14	37	16	21 (56.8)
H 10	IH 10	3	3	2				1 (33.3)	–	–	–	3	2	1 (33.3)
H 11	IH 11	40	34	18				16 (47.1)	–	–	–	34	18	16 (47.1)
H 12	IH 12	11	6	2				4 (66.6)	4	1	3	10	3	7 (70.0)
Total		329	206	84			1	121 (58.7)	87	33	54	293	117	176 (60.1)

⚥, Hermaphrodites

TABLE 26

Sex of the backcrosses of female reciprocal hybrids between the Hiroshima and Ichinoseki populations

Parents		No. of metamorphosed frogs	Sex of frogs dead or killed within one month after metamorphosis							Sex of 6-month-old frogs			Sex of all frogs examined		
Female	Male		No. of frogs	♀		⚥			♂ (%)	No. of frogs	♀	♂	Total	♀	♂ (%)
				N	U	1	2	3							
H 1	H 1	318	176	93					83 (47.2)	–	–	–	176	93	83 (47.2)
H 2	H 2	204	198	99					99 (50.0)	–	–	–	198	99	99 (55.0)
H 3	H 3	177	162	81					81 (50.0)	–	–	–	162	83	79 (48.8)
Total		699	536	273					263 (49.1)	–	–	–	536	275	261 (48.7)
H 1	I 1	320	164	35		6	2		121 (73.8)	–	–	–	164	35	129 (78.7)
H 2	I 2	132	131	9		1	4		117 (89.3)	–	–	–	131	9	122 (93.1)
H 3	I 3	138	119	16			1		102 (85.7)	–	–	–	119	16	103 (86.6)
Total		590	414	60		7	7		340 (82.1)	–	–	–	414	60	354 (85.5)
I 1	H 1	104	36	0					36 (100)	56	1	55	92	1	91 (98.9)
I 2	H 2	115	53	8		1	3	1	40 (75.5)	62	12	50	115	20	95 (82.6)
Total		219	89	8		1	3	1	76 (85.4)	118	13	105	207	21	186 (89.9)
I 1	I 1	160	105	40	1		1		63 (60.0)	40	16	24	145	57	88 (60.7)
I 2	I 2	149	67	27		1	1		38 (56.7)	56	33	23	123	60	63 (51.2)
Total		309	172	67	1	1	2		101 (58.7)	96	49	47	268	117	151 (56.3)
HI 1	H 1	159	100	43			3		54 (54.0)	36	11	25	136	54	82 (60.3)
HI 3	H 3	140	69	33			1		35 (50.7)	66	26	40	135	59	76 (56.3)
HI 4	H 4	183	110	57					53 (48.2)	58	28	30	168	85	83 (49.4)
Total		482	279	133			4		142 (50.9)	160	65	95	439	198	241 (54.9)
HI 1	I 1	255	186	42		6	4	5	129 (69.4)	35	9	26	221	51	170 (76.9)
HI 3	I 3	201	63	17		2			44 (69.8)	60	28	32	123	45	78 (63.4)
HI 4	I 4	198	109	2			1	1	105 (96.3)	65	1	64	174	3	171 (98.3)
Total		654	358	61		8	5	6	278 (77.7)	160	38	122	518	99	419 (80.9)
IH 1	H 1	143	47	16		1			30 (63.8)	60	17	43	107	33	74 (69.2)
IH 3	H 3	102	59	15					44 (74.6)	35	4	31	94	19	75 (79.8)
IH 6	H 6	180	89	14					75 (84.3)	16	3	13	105	17	88 (83.8)
Total		425	195	45		1			149 (76.4)	111	24	87	306	69	237 (77.5)
IH 1	I 1	141	70	28			1		41 (58.6)	65	22	43	135	50	85 (63.0)
IH 3	I 3	220	151	60	1	1	1		88 (58.3)	55	35	20	206	96	110 (53.4)
IH 6	I 6	98	57	16					41 (71.9)	32	12	20	89	28	61 (68.5)
Total		459	278	104	1	1	2		170 (61.2)	152	69	83	430	174	256 (59.5)

♀N, Females with normal ovaries ♀U, Females with underdeveloped ovaries ⚥, Hermaphrodites

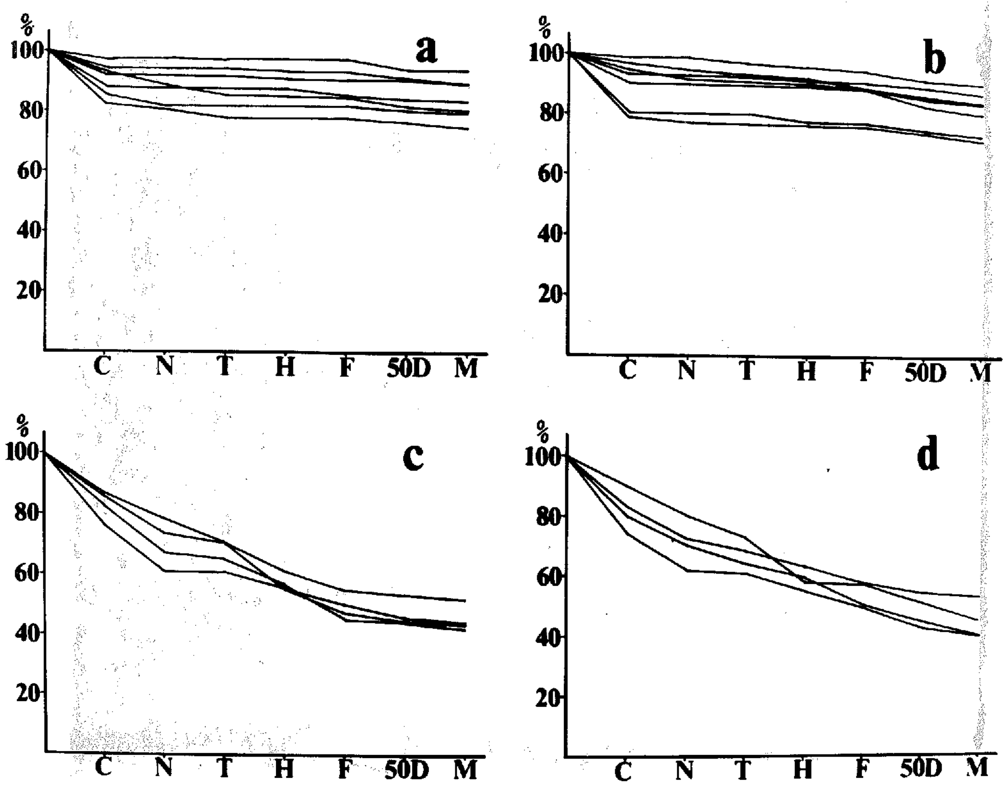


Fig. 1. Survival curves of reciprocal hybrids between the Hiroshima and Ichinoseki populations of <u>Rana japonica</u>.

a. H♀ x H♂
b. H♀ x I♂
c. I♀ x H♂
d. I♀ x I♂

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

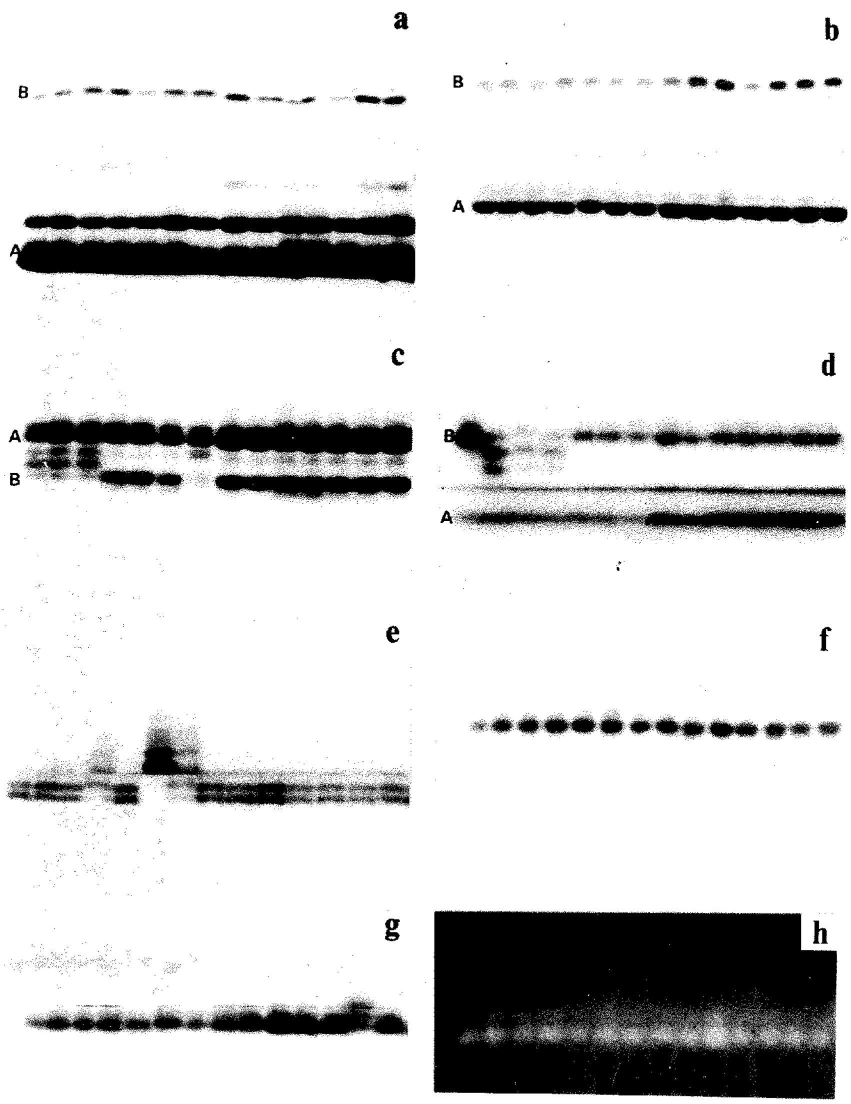


Fig. 2. Electrophoretic patterns of eight enzymes in the Hiroshima and Ichinoseki populations of <u>Rana japonica</u>. Left seven samples are from the Hiroshima population. Right seven samples are from the Ichinoseki population.

a. Lactate dehydrogenase (LDH)
b. Isocitrate dehydrogenase (IDH)
c. Malate dehydrogenase (MDH)
d. Aspartate aminotransferase (AAT)
e. α-Glycerophosphate dehydrogenase (α-GDH)
f. Creatine kinase (CK)
g. Glucose-phosphate isomerase (GPI)
h. Superoxide dismutase (SOD)

Fig. 3. Electrophoretic patterns of two enzymes and two blood proteins in the Hiroshima and Ichinoseki populations of Rana japonica. Left seven (a) or five (b~d) samples are from the Hiroshima population. Right seven (a) or six (b~d) samples are from the Ichinoseki population.

a. Phosphoglucomutase (PGM)
b. Esterase (Est)
c. Hemoglobin (Hb)
d. Serum albumin (Ab)

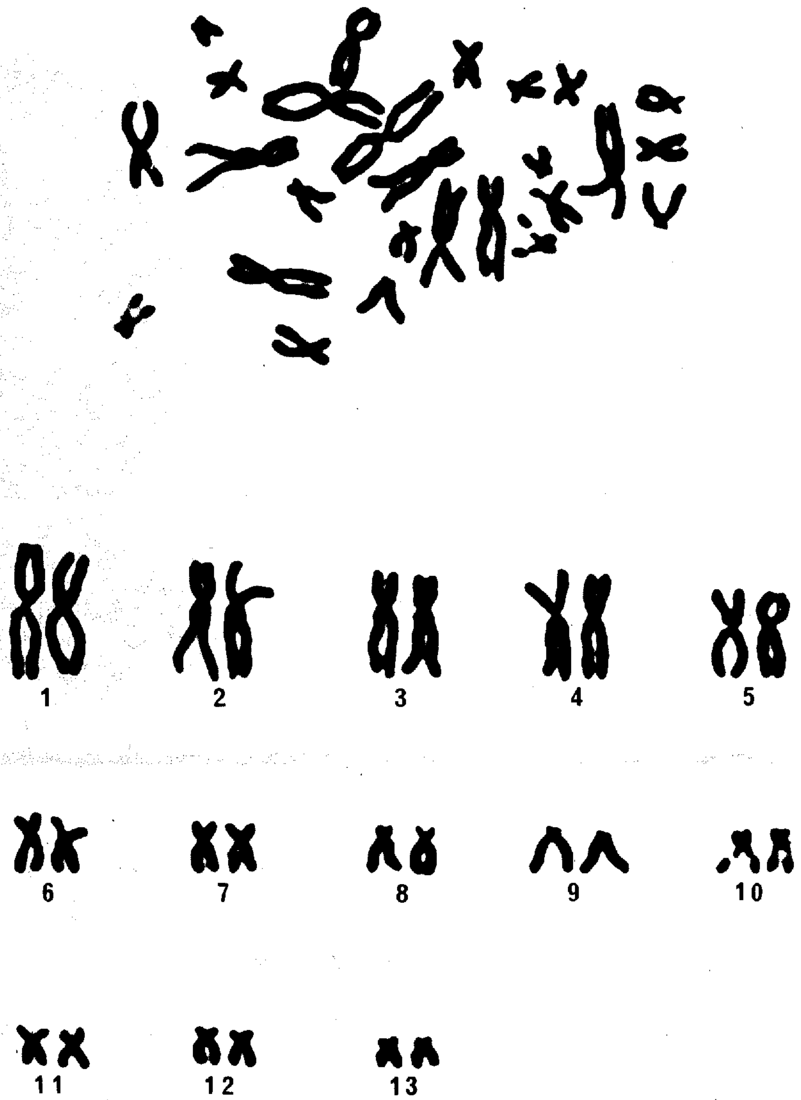


Fig. 4. Metaphase spread and the karyotype of an epidermal cell from a Hiroshima tadpole.

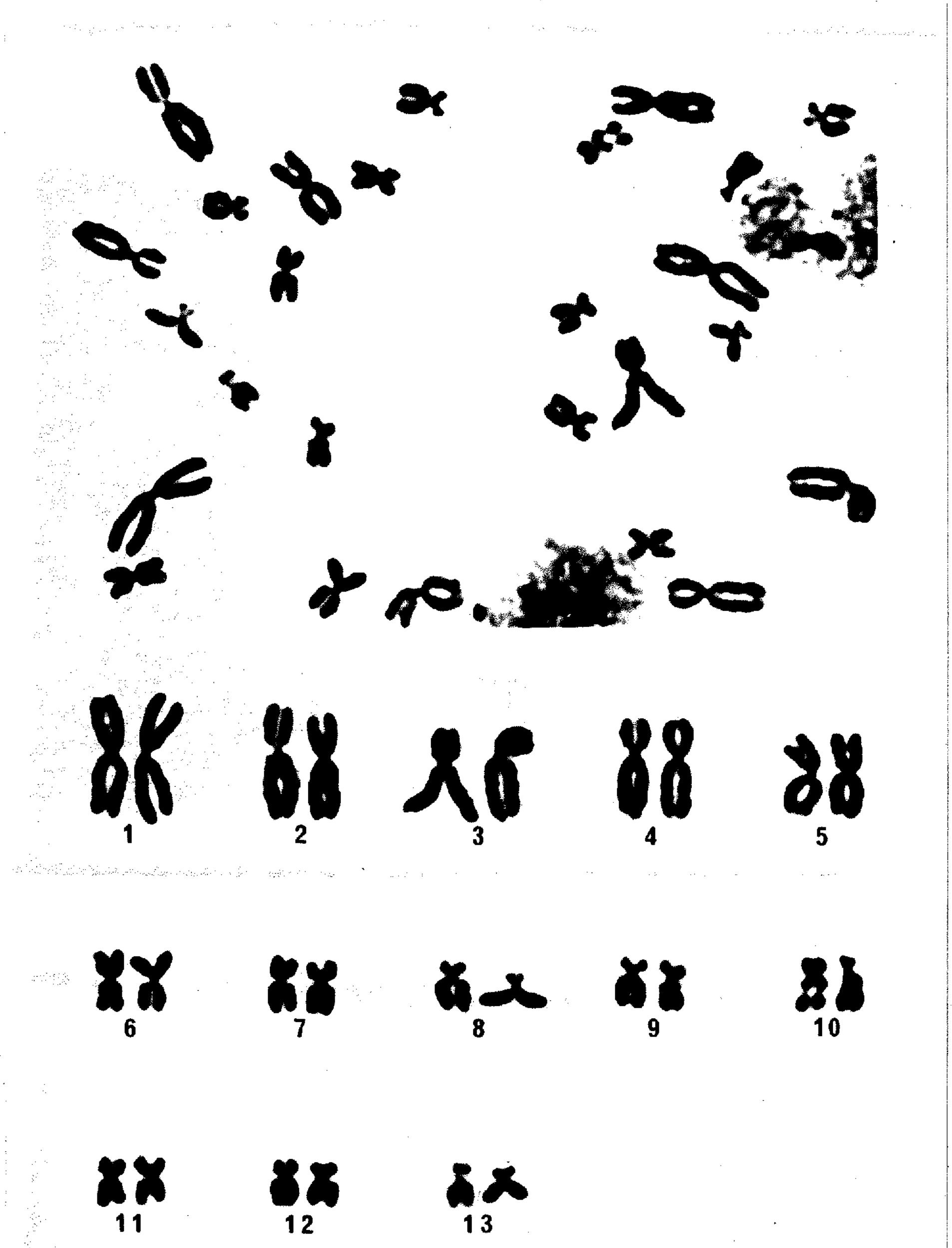


Fig. 5. Metaphase spread and the karyotype of an epidermal cell from an Ichinoseki tadpole.

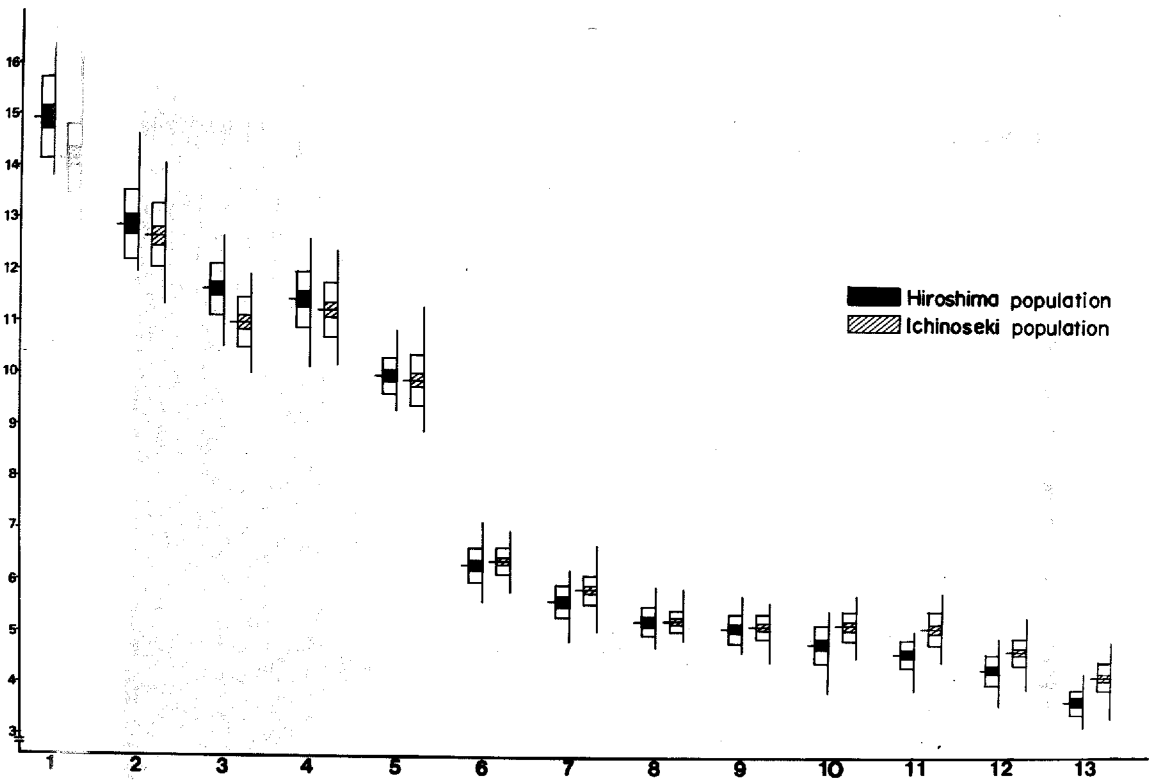


Fig. 6. A graph showing differences in relative chromosome length between the two populations of *Rana japonica*.

The left and right of each pair in the graph represent a Hiroshima and an Ichinoseki chromosome, respectively. A vertical line shows the range of relative chromosome lengths, a short horizontal line, the mean of the latter; an open rectangle on each side of the horizontal line, the standard deviation of the mean; a black or an oblique rectangle on each side of the horizontal line, two times the standard error of the mean. In general, if a black rectangle does not overlap an oblique rectangle, the difference between the two chromosomes is statistically significant.

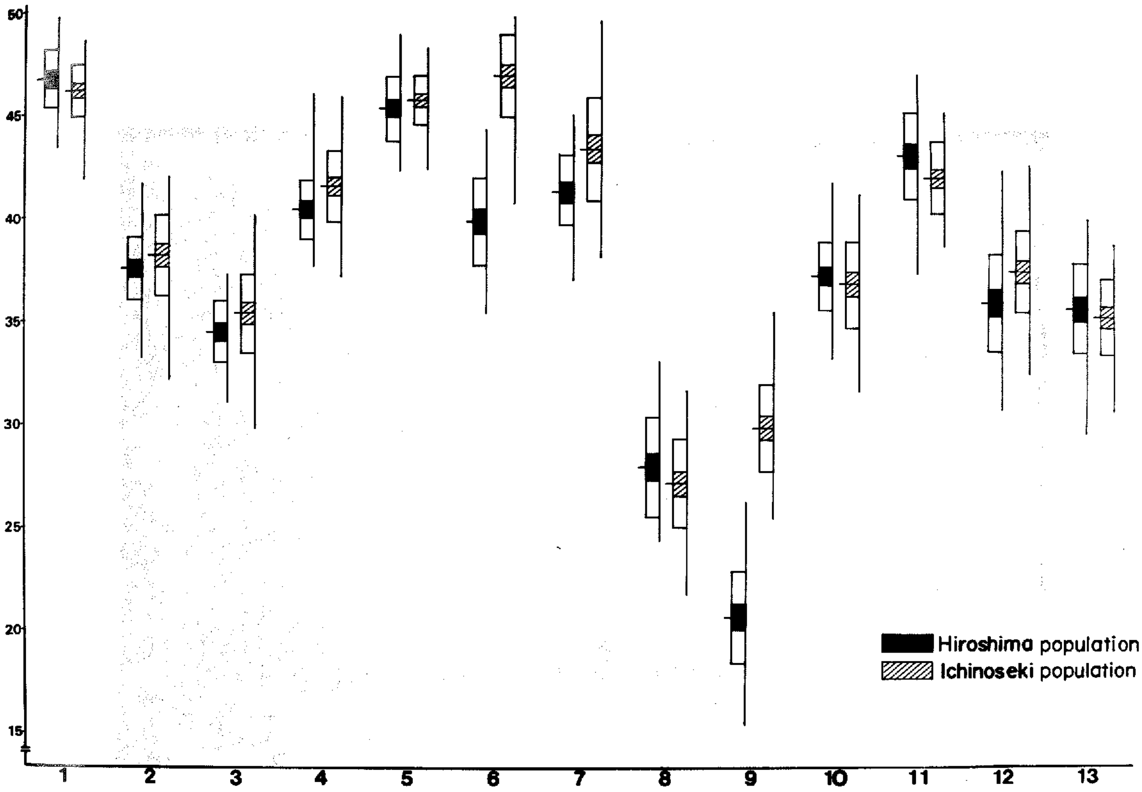


Fig. 7. A graph showing differences in centromere position between the two populations of Rana japonica.

The left and right of each pair in the graph represent a Hiroshima and an Ichinoseki chromosome, respectively. A vertical line shows the range of numerical values of the centromere position; a short horizontal line, the mean of the numerical values; an open rectangle on each side of the horizontal line, the standard deviation of the mean; a black or an oblique rectangle on each side of the horizontal line, two times the standard error of the mean.

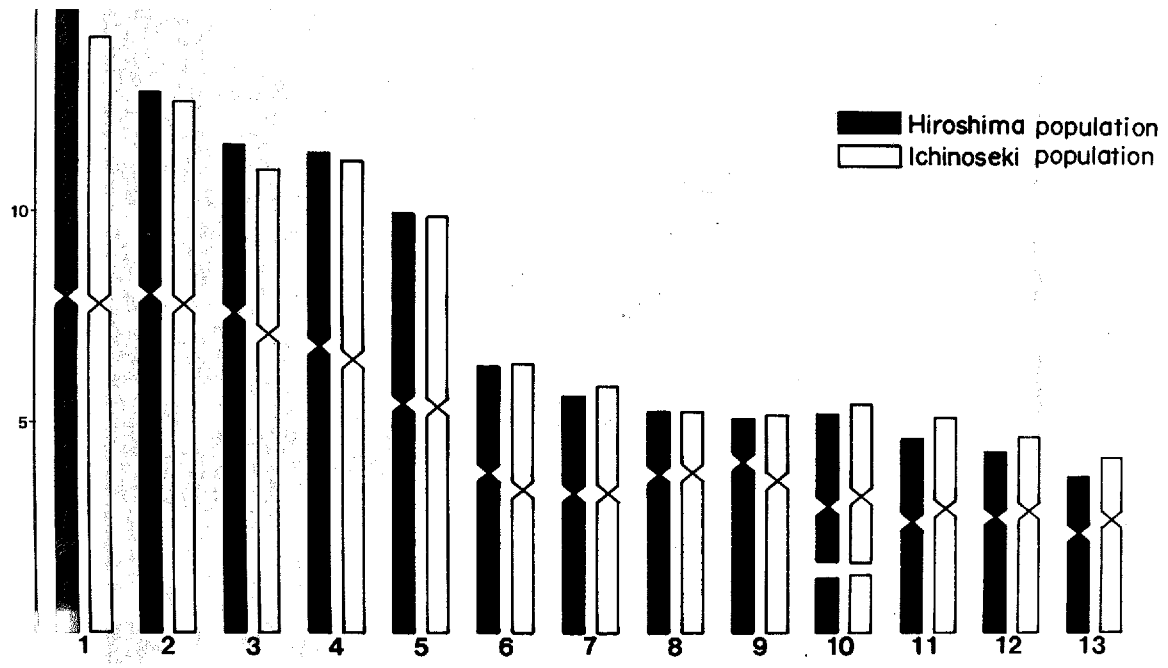


Fig. 8. Composite ideogram showing differences in relative chromosome length and centromere position between the two populations of <u>Rana japonica</u>. Constrictions indicate the centromeres. A gap in chromosome No. 10 indicates the secondary constriction.

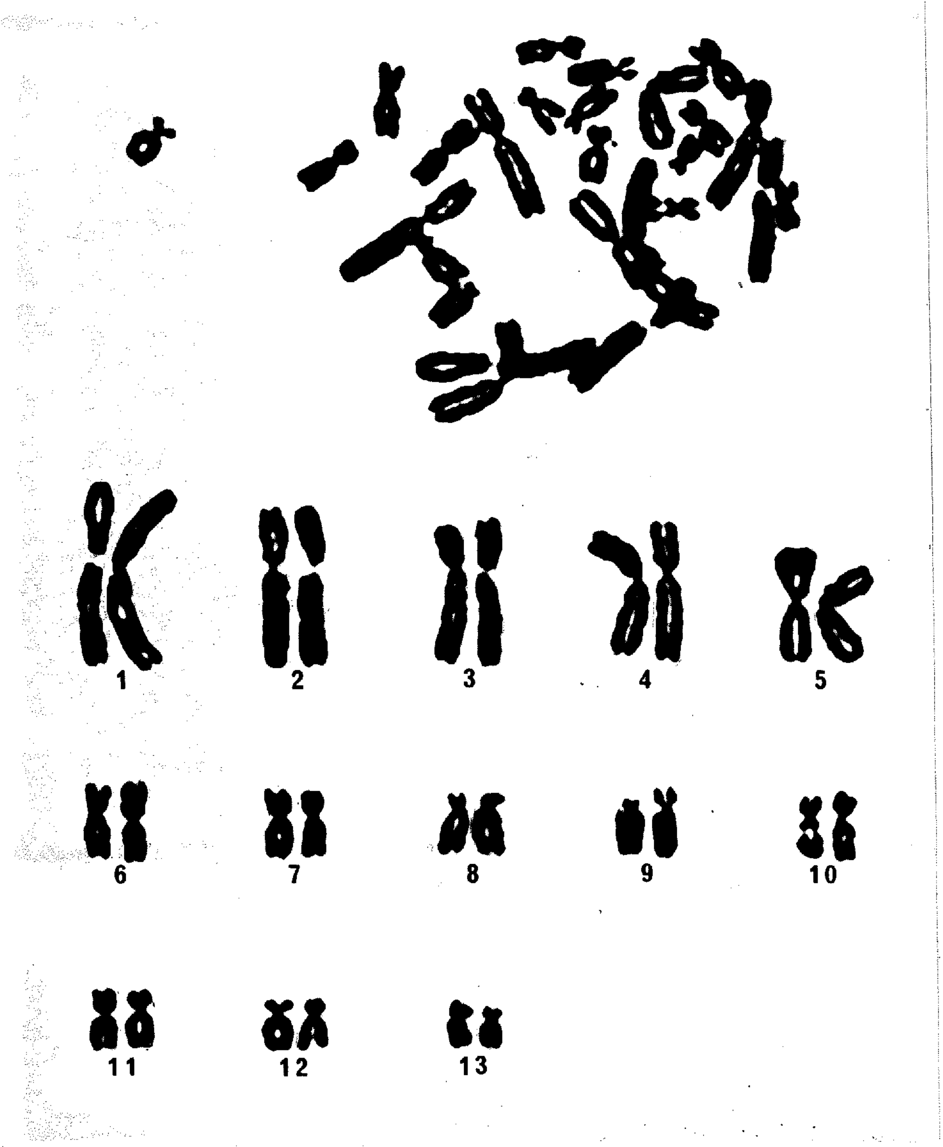


Fig. 9. Metaphase spread and the karyotype of an epidermal cell from a hybrid tadpole between a Hiroshima female and an Ichinoseki male.

Fig. 10. Metaphase spread and the karyotype of an epidermal cell from a hybrid tadpole between an Ichinoseki female and a Hiroshima male.

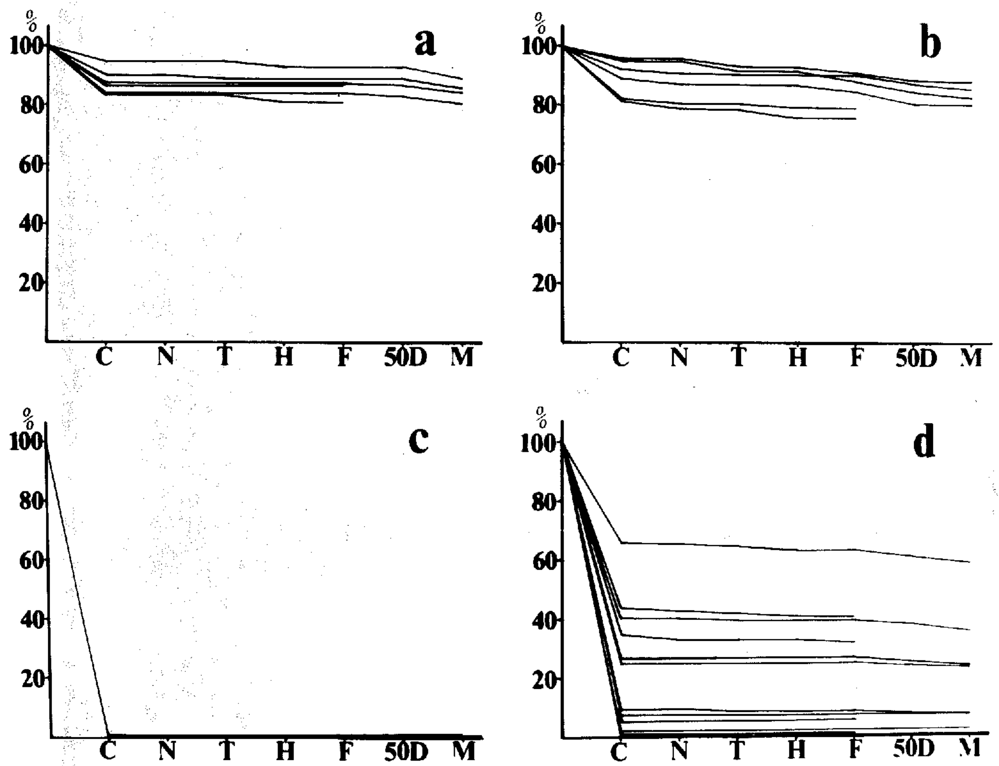


Fig. 11. Survival curves of the backcrosses produced from male reciprocal hybrids between the Hiroshima and Ichinoseki populations and the controls.

a. H♀ x (H♀ x H♂)♂, Nos.1～6

b. H♀ x (I♀ x I♂)♂, Nos.1～6

c. H♀ x (H♀ x I♂)♂, Nos.1～6

d. H♀ x (I♀ x H♂)♂, Nos.1～6

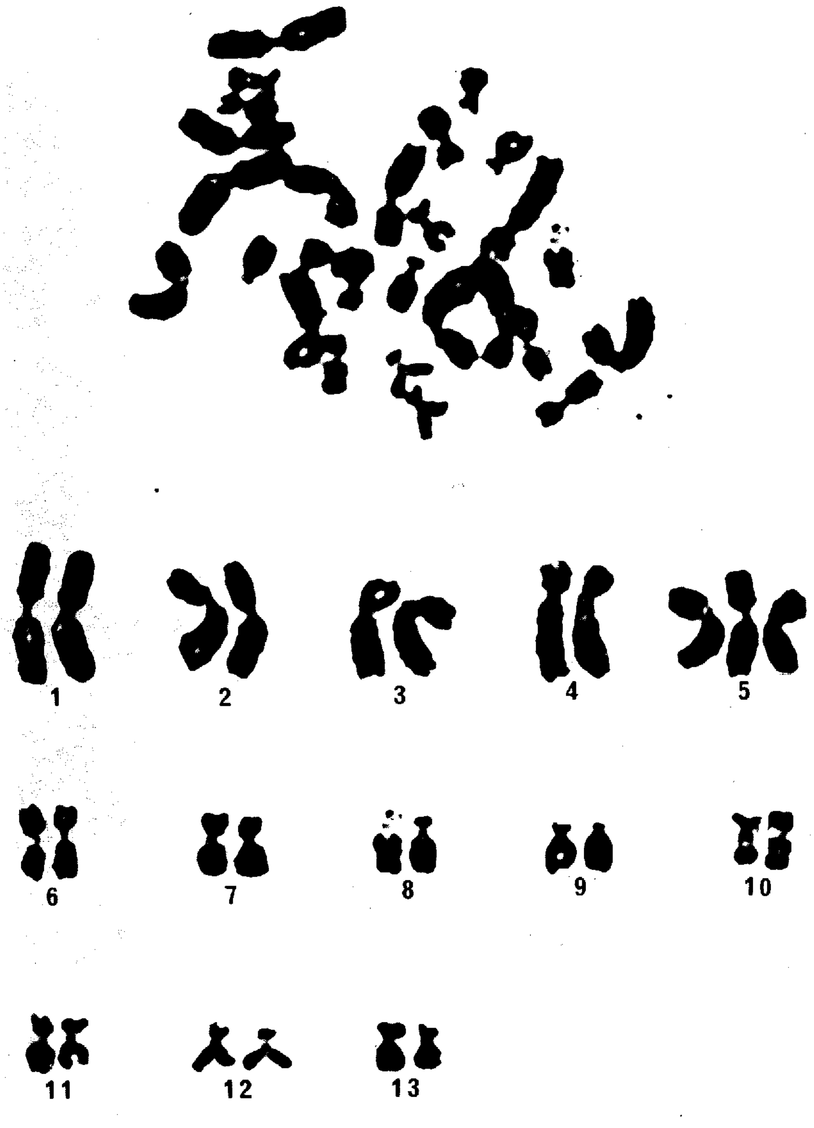


Fig. 13. Chromosome aberration (2n=27) in a normally shaped tadpole produced from a mating, (I♀ x H♂)♀4 x I♂4.

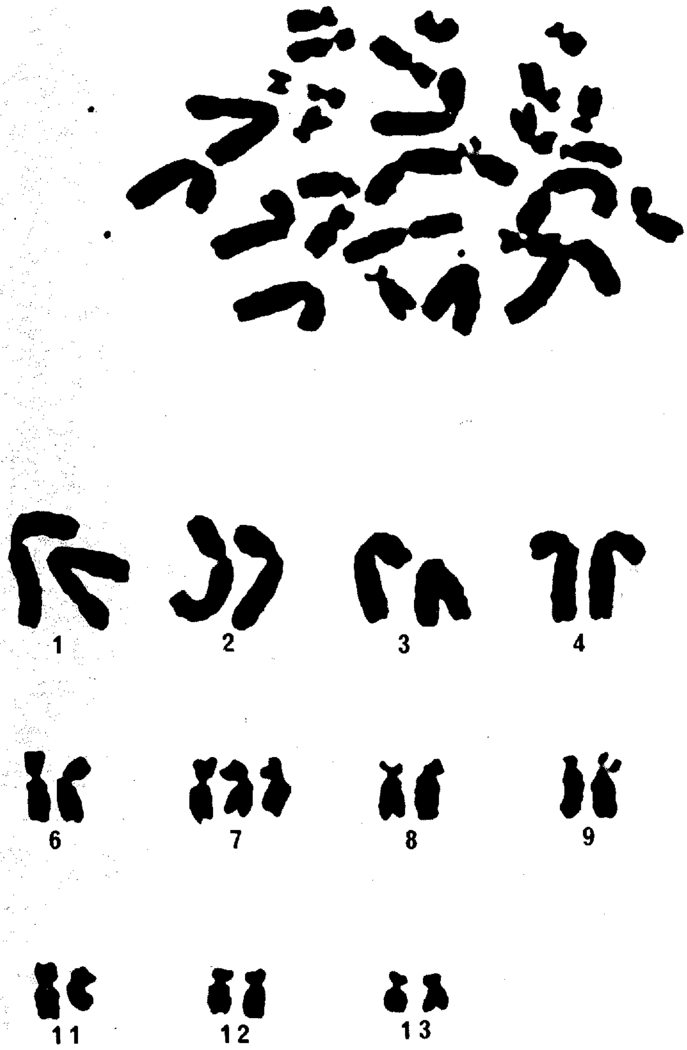


Fig. 14. Chromosome aberration (2n=27) in a normally shaped tadpole produced from a mating, (I♀ x H♂)♀4 x H♂4.

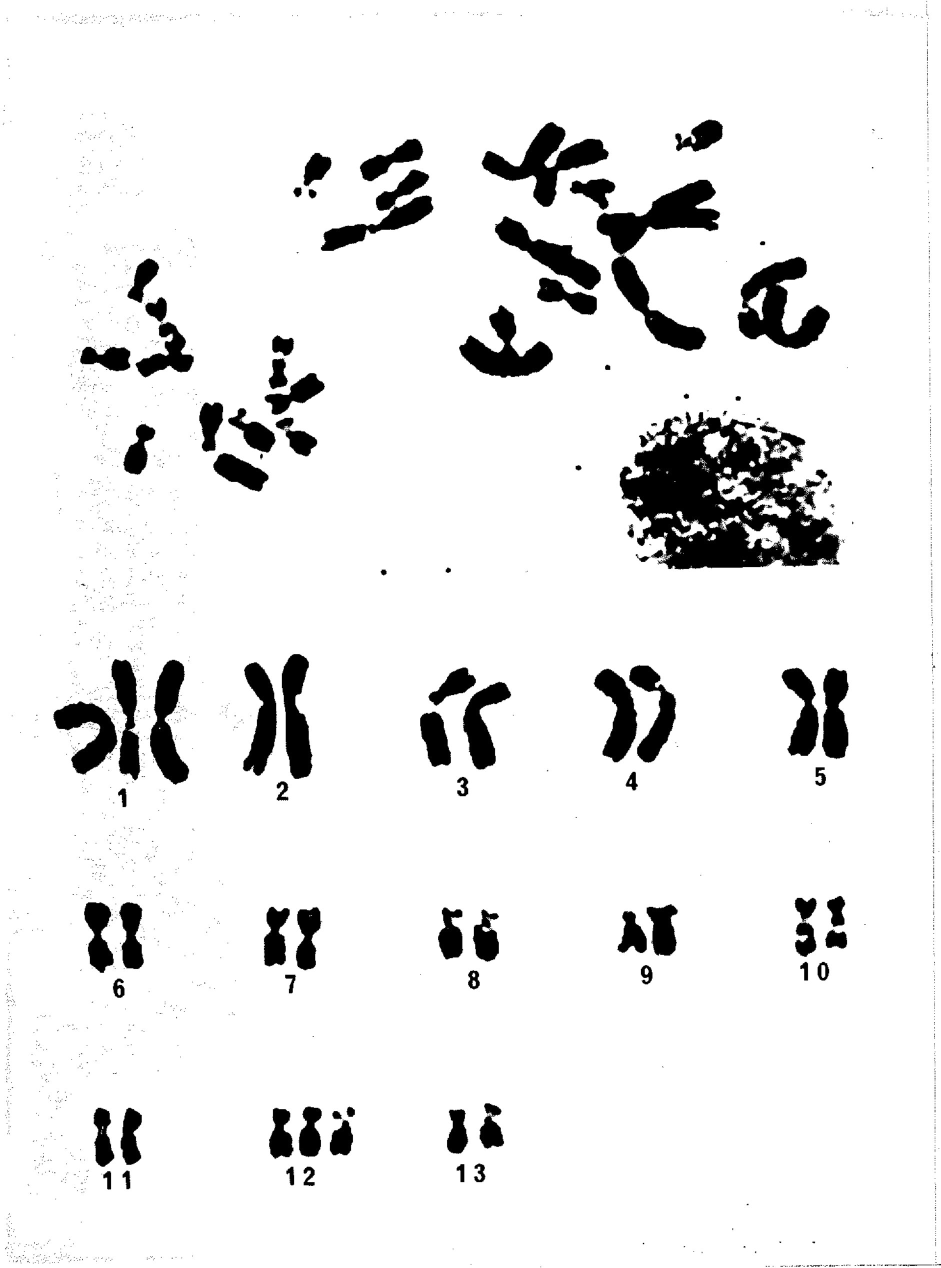


Fig. 15. Chromosome aberration (2n=28) in a normally shaped tadpole produced from a mating, (I♀ x H♂) ♀4 x I♂4.

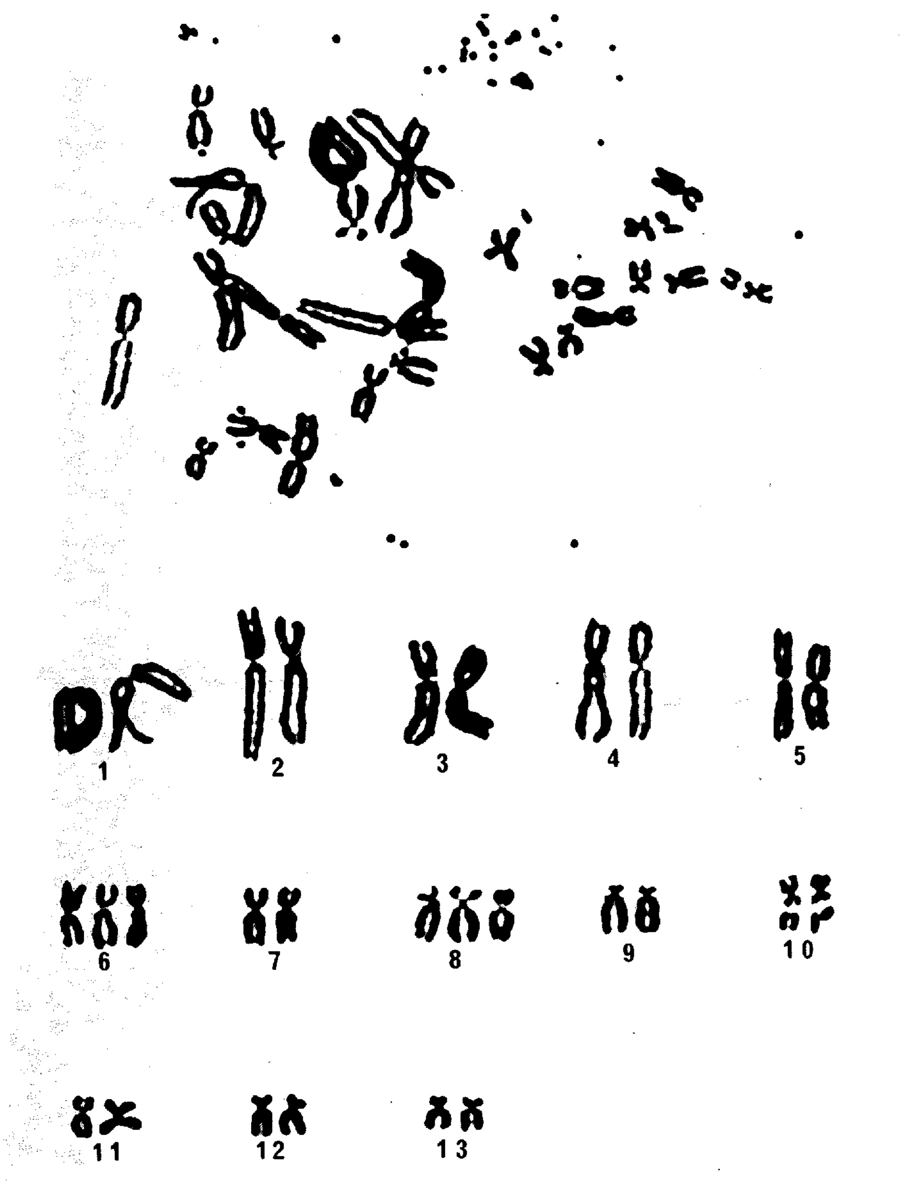


Fig. 16. Chromosome aberration (2n=28) in a normally shaped tadpole produced from a mating, (I♀ x H♂) ♀4 x H♂4.

Fig. 17. Chromosome aberration (2n=28) in a normally shaped tadpole produced from a mating, (I♀ x H♂)♀4 x H♂4.

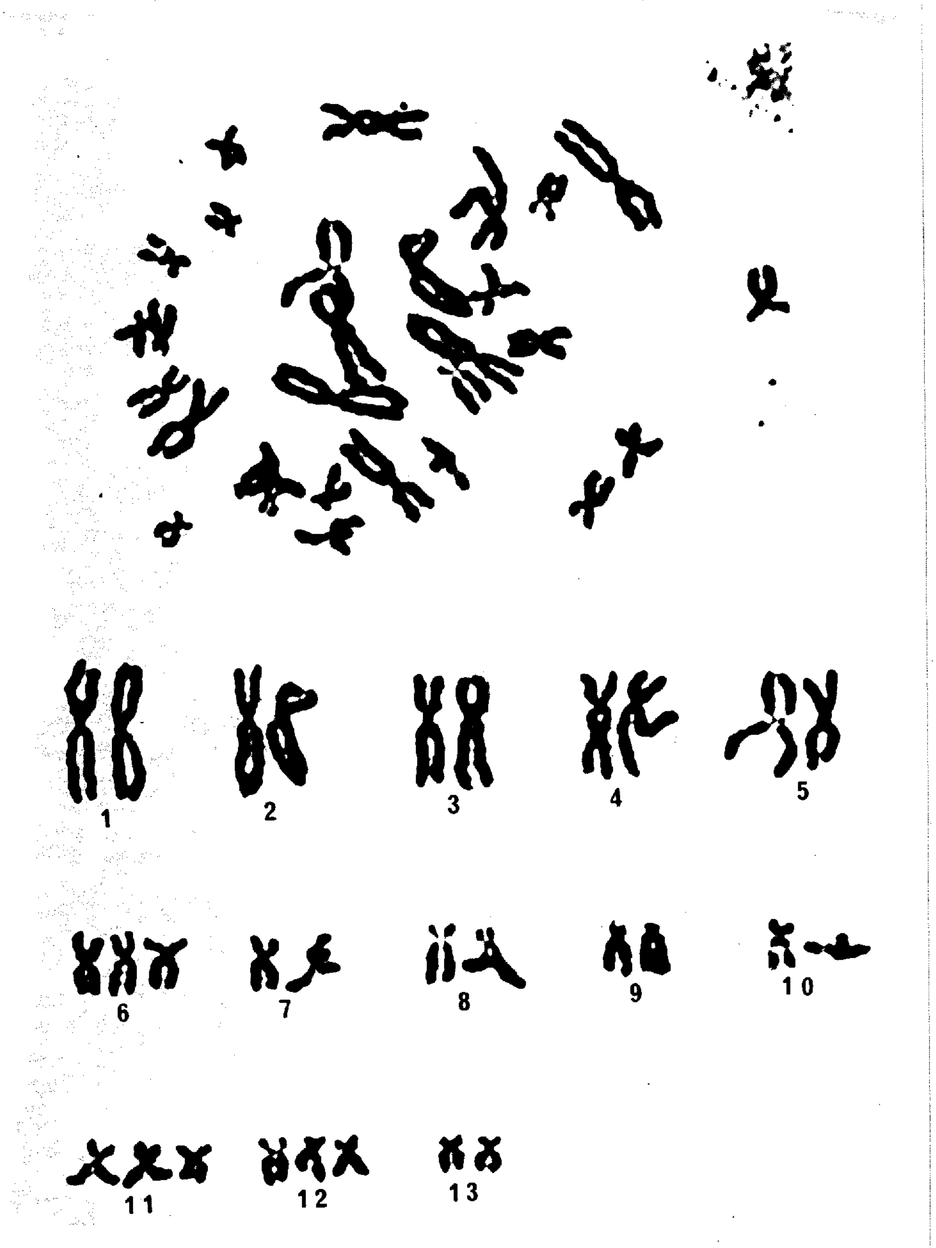


Fig. 18. Chromosome aberration (2n=29) in a normally shaped tadpole produced from a mating, (I♀ x H♂)♀4 x H♂4.

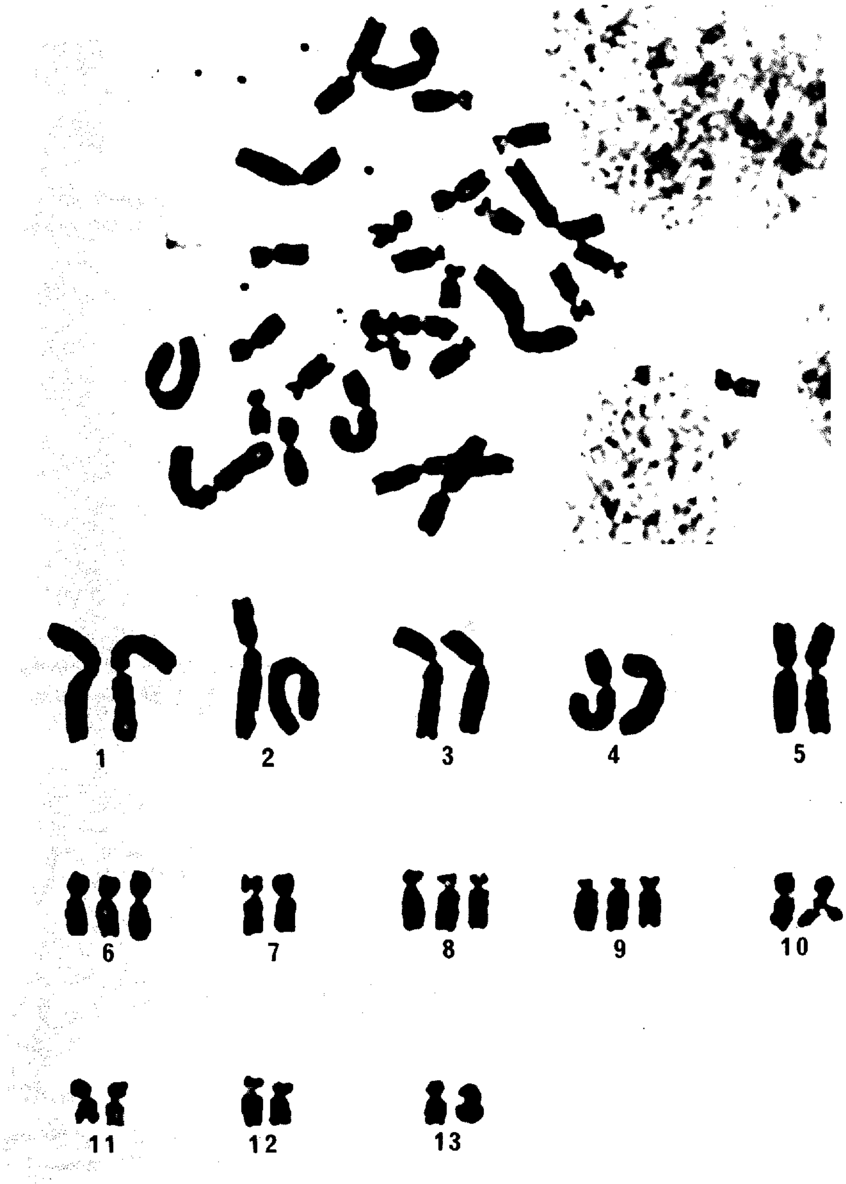


Fig. 19. Chromosome aberration (2n=29) in a normally shaped tadpole produced from a mating, (I♀ x H♂)♀4 x H♂4.

EXPLANATION OF PLATES

PLATE I

Adult frogs of the two populations of *Rana japonica* at the age of one year x 1.0

1, 2. A female of the Ichinoseki population

3, 4. A male of the Ichinoseki population

5, 6. A female of the Hiroshima population

7, 8. A male of the Hiroshima population

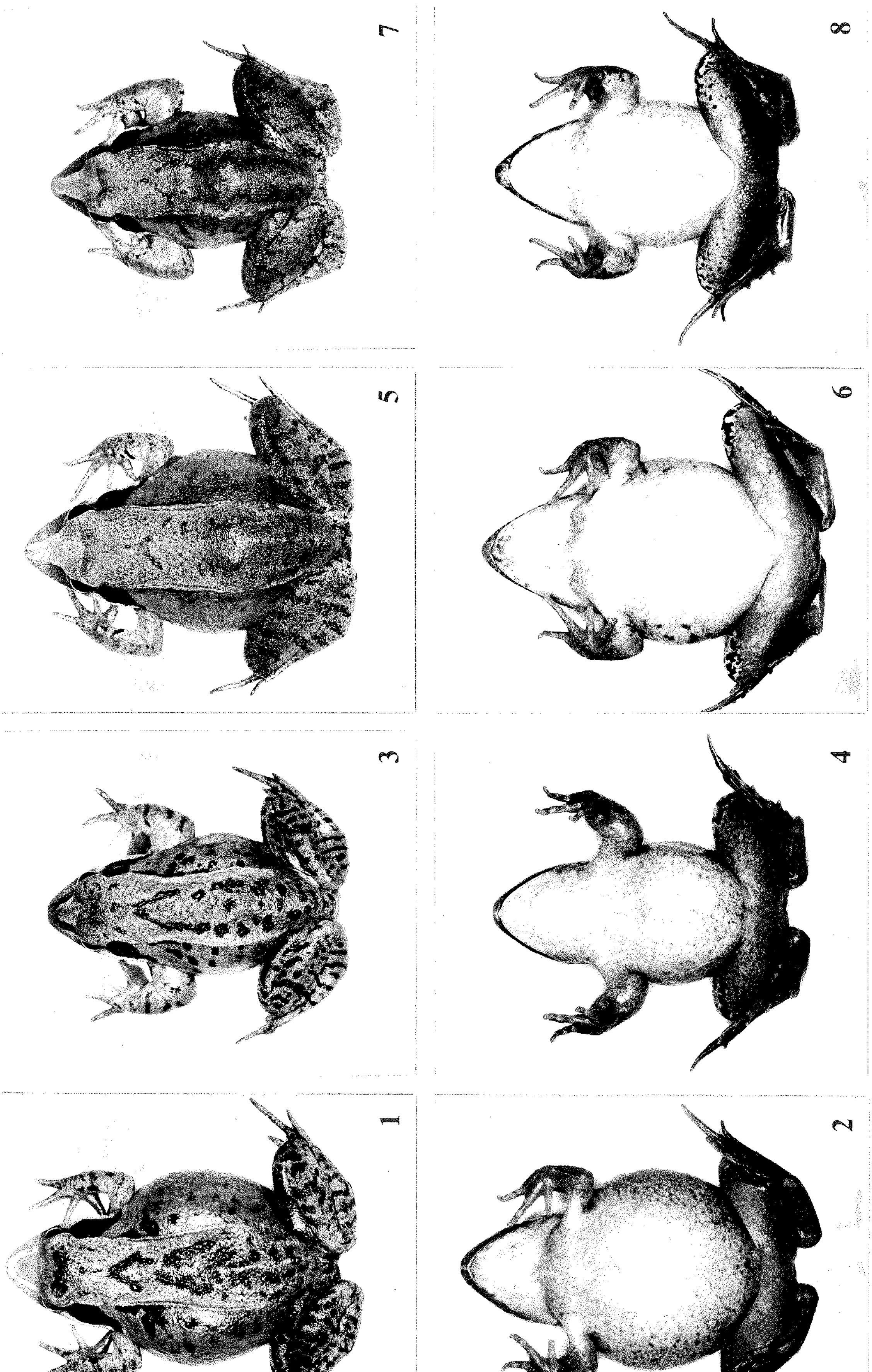
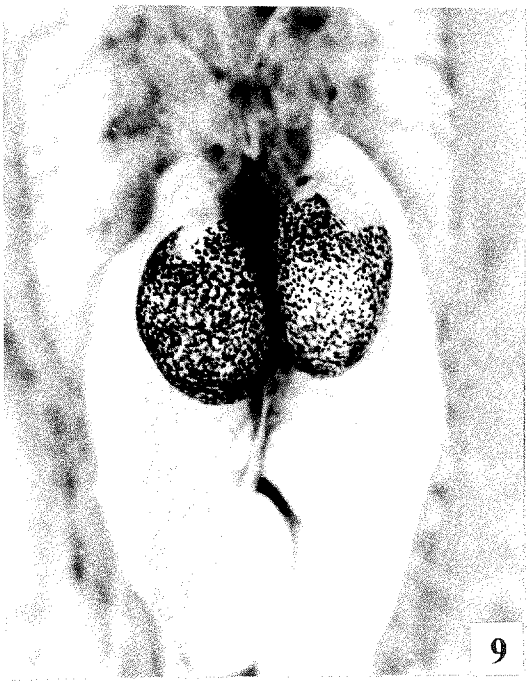


PLATE II

Testes of males of the two *Rana japonica* populations and their reciprocal hybrids at the age of three months x 6.0

9. A male of the Hiroshima population
10. A male hybrid between a Hiroshima female and a Ichinoseki male
11. A male hybrid between a Ichinoseki female and a Hiroshima male
12. A male of the Ichinoseki population

PLATE II



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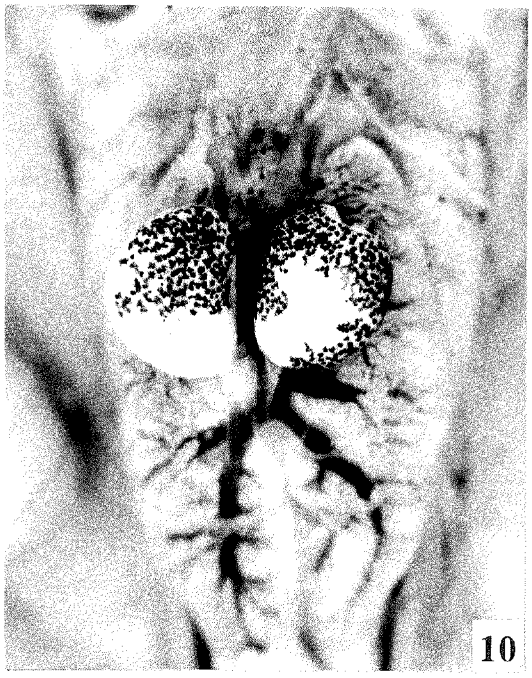

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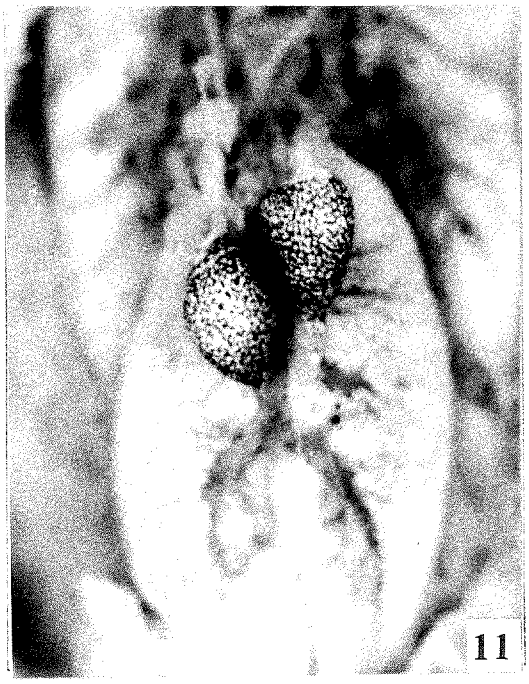

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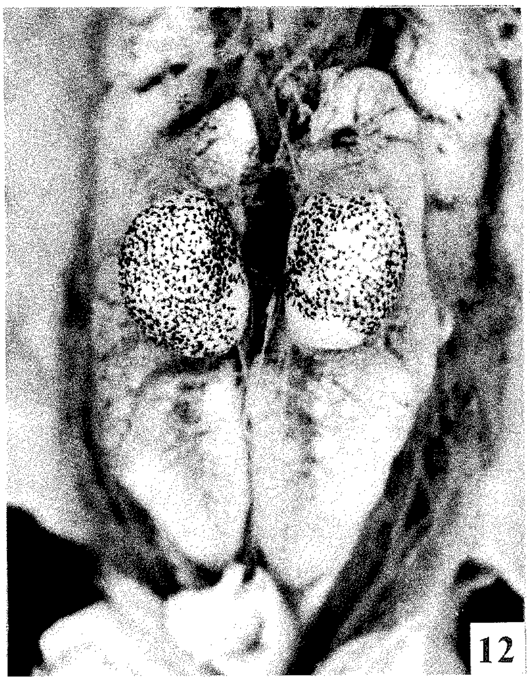

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PLATE III

Cross-sections of seminal tubules of the testes of mature hybrids at the age of one year

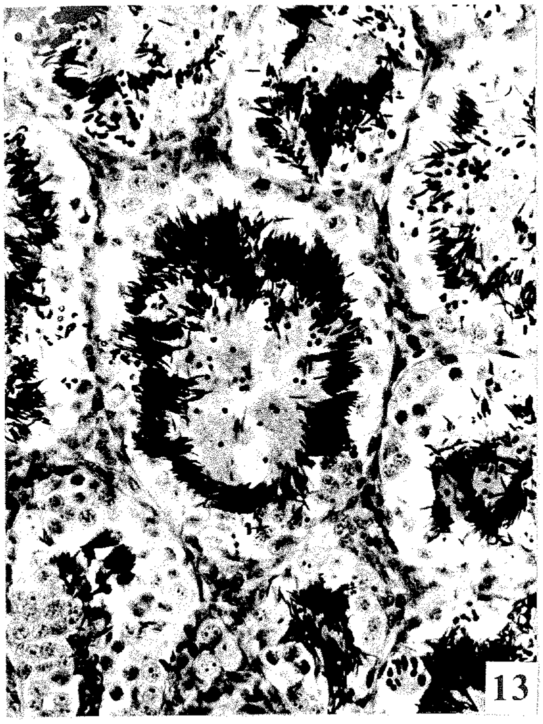
13. Type 1 Testis of control male No.5 produced from a mating, Ichinoseki population♀ x Ichinoseki population♂ x 280

14. Type 5 Testis of male hybrid No.4 produced from a mating, Hiroshima population♀ x Ichinoseki population♂ x 280

15. Type 2 Testis of male hybrid No.7 produced from a mating, Ichinoseki population♀ x Hiroshima population♂ x 280

16. Ditto x 140

PLATE III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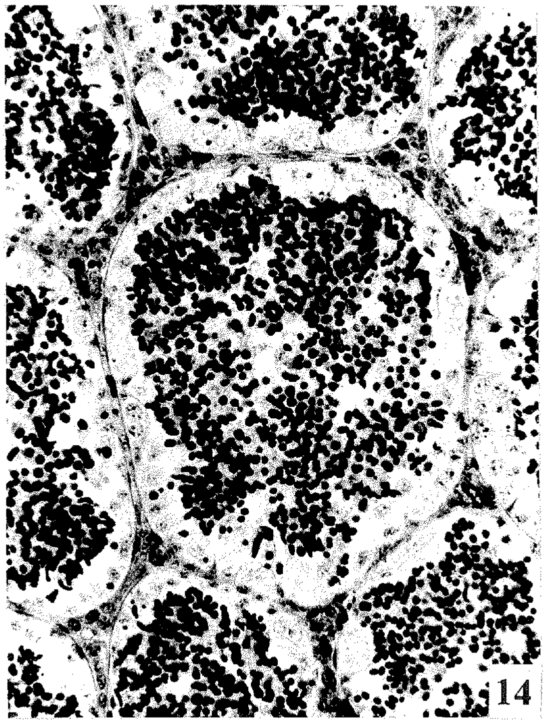
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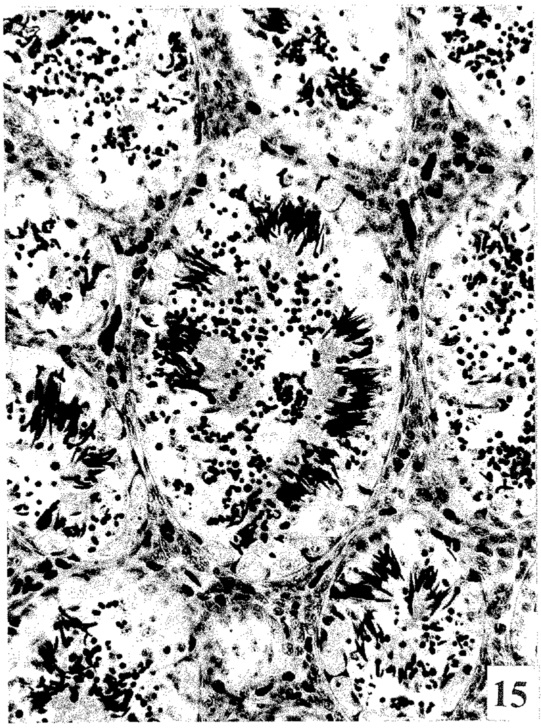
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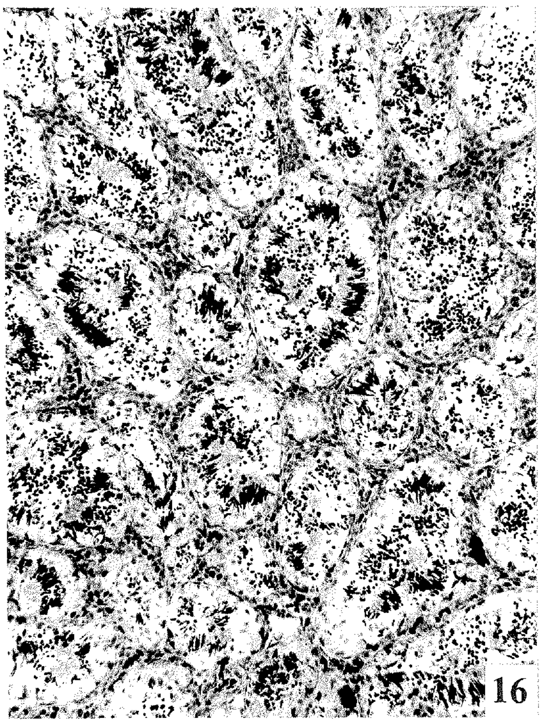
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PLATE IV

Cross-sections of seminal tubules of the testes of mature hybrids at the age of one year

17. Type 3 Testis of male hybrid No.9 produced from a mating, Ichinoseki population♀ x Hiroshima population♂ x 280
18. Ditto x 140
19. Type 4 Testis of male hybrid No.9 produced from a mating, Hiroshima population♀ x Ichinoseki population♂ x 280
20. Ditto x 140

PLATE IV