Descendants of Reciprocal Hybrids between Two Japanese Pond-frog Species, *Rana nigromaculata* and *Rana brevipoda*

By

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(With 14 Figures)

INTRODUCTION

Japanese pond- or water-frogs presented a taxonomic confusion like the *Rana esculenta* group in Europe, though of lesser complexity.   They were first given the name *Rana esculenta* by Schlegel in 1838.   While the present name *Rana nigromaculata* was given by Hallowell in 1860, they had been called by such names as *Rana esculenta* var. *japonica*, *Rana esculenta nigromaculata* or *Rana esculenta* var. *chinensis* until Stejneger adopted *Rana nigromaculata* in his book "Herpetology of Japan and Adjacent Territory" (1907).   Although the name *Tomopterna porosa* was given to pond frogs collected from the Kanto district by Cope (1868) and this name was later corrected to *Rana porosa* by Boulenger (1882), Stejneger (1907) used this as a synonym of *Rana nigromaculata*.   Terentjev (1927) and Okada (1931) placed all the Japanese pond frogs under *Rana nigromaculata nigromaculata* as the result of the description given to a new subspecies distributed in China or Korea.

Incidentally, Ito (1941) recognized the existence of two types of pond frogs in the Nagoya district and described the smaller, short-legged one as *Rana nigromaculata brevipoda* in a local journal of biology.   Moriya (1951, 1954, 1960a, 1960b) divided Japanese pond frogs into five races, that is, *nigromaculata* common, Niigata intermediate, Tokyo intermediate, Nagoya *brevipoda* and Okayama *brevipoda* race on the basis of morphological and distributional differences.   According to Moriya (1951, 1960b), the Okayama *brevipoda* race is nearly completely isolated from the sympatric *nigromaculata* common race by male hybrid sterility in addition to seasonal, ecological and sexual isolations.   While there is no hybrid sterility between the Okayama *brevipoda* race and the allopatric Nagoya *brevipoda* or two intermediate races, male hybrids between the Okayama *brevipoda* race and the two intermediate races are somewhat abnormal in spermatogenesis.   Subsequently, Moriya and the present authors have found that the Niigata race is a mixture of the Tokyo intermediate race, natural hybrids between the Tokyo intermediate race and the *nigromaculata* common race, and their descendants.   As a result,

KAWAMURA (1962) proposed a classification of the Japanese pond frogs into the following three taxa: *Rana nigromaculata* HALLOWELL, *Rana brevipoda* ITO and *Rana brevipoda porosa* (COPE). If the International Rules of Zoological Nomenclature is observed, the latter two should be *Rana porosa brevipoda* and *Rana porosa porosa*. However, this classification does not seem to be practical, because natural hybrids and their descendants are inevitably included in *Rana porosa porosa*. KAWAMURA, then, ventured to give the name *Rana brevipoda brevipoda* to the well-defined population and the name *Rana bervipoda porosa* to the complicated intermediate one from a practical standpoint.

The relationship between the two Japanese pond-frog species is very similar to that between *Rana ridibunda* and *Rana lessonae* in Europe. As in the European species, the two Japanese species are incompletely isolated by hybrid sterility. According to BERGER (1964, 1966, 1967, 1968, 1970, 1977,), *Rana esculenta* is not a real species, but is a hybrid formed either by crossing between *Rana lessonae* and *Rana ridibunda* or by backcrossing of *Rana esculenta* with *Rana lessonae*. This interpretation has been supported by many investigaters (GÜNTHER, 1968, 1973, 1974; TUNNER, 1970, 1972, 1973, 1974, TUNNER and DOBROWSKY, 1976; BLANKENHORN, HEUSSER and VOGEL, 1971; ENGELMANN, 1972, 1973; HALFMANN and MÜLLER, 1972; BLANKENHORN, 1973, 1977; HEMMER, 1973; HOTZ, 1974; UZZEL and BERGER, 1975; UZZEL, BERGER and GÜNTHER, 1975; EBENDAL, 1976, 1977, 1978; VOGEL and CHEN, 1976, 1977; VOGEL, 1977). The reproductive capacity of *Rana esculenta* does not seem to change with repeated backcrosses. In Japan, natural hybrids between *Rana nigromaculata* and *Rana brevipoda porosa* are also frequently found in two local areas which are peculiar in climate (KAWAMURA and NISHIOKA, 1977). It is evident that numerous offspring are produced from repeated backcrosses with parental species in these areas, although it is not easy to distinguish them from the latter by external characters.

Thus, the present authors considered it interesting to examine the change of reproductive capacity in the descendants of reciprocal hybrids between the two Japanese pond-frog species in order to compare these hybrids with *Rana esculenta*.

MATERIALS AND METHODS

While there is *Rana nigromaculata nigromaculata* HALLOWELL alone in the pond frogs distributed in the Hiroshima district, this species is sympatric with *Rana brevipoda brevipoda* ITO in the Okayama district which is about 100 km away from Hiroshima. *Rana nigromaculata nigromaculata* used for crossing experiments were collected from the suburbs of Hiroshima, and *Rana brevipoda brevipoda* were obtained from the vicinity of Okayama. Ovulation was accelerated by injection of frog pituitary suspension. Crossing was made by the routine method of artificial fertilization. Embryos, tadpoles and frogs were reared at the room temperature. While tadpoles fed on boiled spinach or chard, frogs were mainly given domestic flies and bagworms. Karyotyping was made by the squash method in the tail tips clipped from tadpoles after the tadpoles had been kept in 0.005% colchicine

solution for about 18 hours.

RESULTS

Reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda* were produced in 1962. In each experimental series, eggs of a female were inseminated by sperm of a male. In each kind of backcrossing, three females and two or three males were removed from each of two experimental series where more than 90% of the respective total number of eggs became normal, metamorphosed frogs, and backcrossed in 1964 with male or female *Rana nigromaculata* or *Rana brevipoda* collected from the field. Backcrossing was repeated three times in 1966, 1968 and 1971. When the hybrids between female *Rana nigromaculata* and male *Rana brevipoda* are repeatedly backcrossed by *Rana nigromaculata,* the nuclei of the hybrids must gradually be restored to *Rana nigromaculata* nuclei in the cytoplasm of this species (Restoration line). When they were repeatedly backcrossed by *Rana brevipoda,* the nuclei of the hybrids must gradually be substituted with *Rana brevipoda* nuclei (Substitution line). A similar phenomenon occurs in the hybrids between female *Rana brevipoda* and male *Rana nigromaculata* when they are repeatedly backcrossed with *Rana brevipoda* or *Rana nigromaculata.* The results of repeated backcrosses in the two Japanese pond-frog species are diagrammatically shown in Figs. 1, 2, 3 and 4. Each rectangle in these figures represents the number of eggs utilized in each mating as 100%. The actual number of eggs was about $250 \sim 940$, 443.8 on the average. In each rectangle, the number of un-fertilized or uncleaved eggs, abnormal embryos, abnormal tadpoles and normal, metamorphosed frogs is shown in percentage. In some rectangles, the number of abnormal, metamorphosed frogs is also shown in percentage, as peculiar frogs with ill-developed forelegs appeared from the matings.

1. Descendants of hybrids between female *Rana nigromaculata* and male *Rana brevipoda*

a) Backcrossing with the mother species, *Rana nigromaculata*

While five male hybrids had almost no fertilizing sperm, six female hybrids were fertile to some extent (Fig. 1). In the six B_1 series derived from these females, $75 \sim 97\%$, 84.5% on the average, of the respective total number of eggs cleaved normally, and later $25 \sim 77\%$, 47.5% on the average, became normal, metamorphosed frogs. Of mature frogs produced by B_1, six females and six males were removed for obtaining the B_2 generation from two series which had produced the most numerous frogs. While only two of the six females were slightly superior to their mothers in the production of metamorphosed frogs, the others were more or less inferior to their mothers. In the six B_2 series, $72 \sim 97\%$, 86.1% on the average, of the respective total number of eggs were normally fertilized, and later $33 \sim 75\%$, 58.6% on the average, became normal, metamorphosed frogs. On the other hand, two of the six males had almost no fertilizing sperm, while the other four produced some progeny. In the four B_2 series derived from the males, $10 \sim$
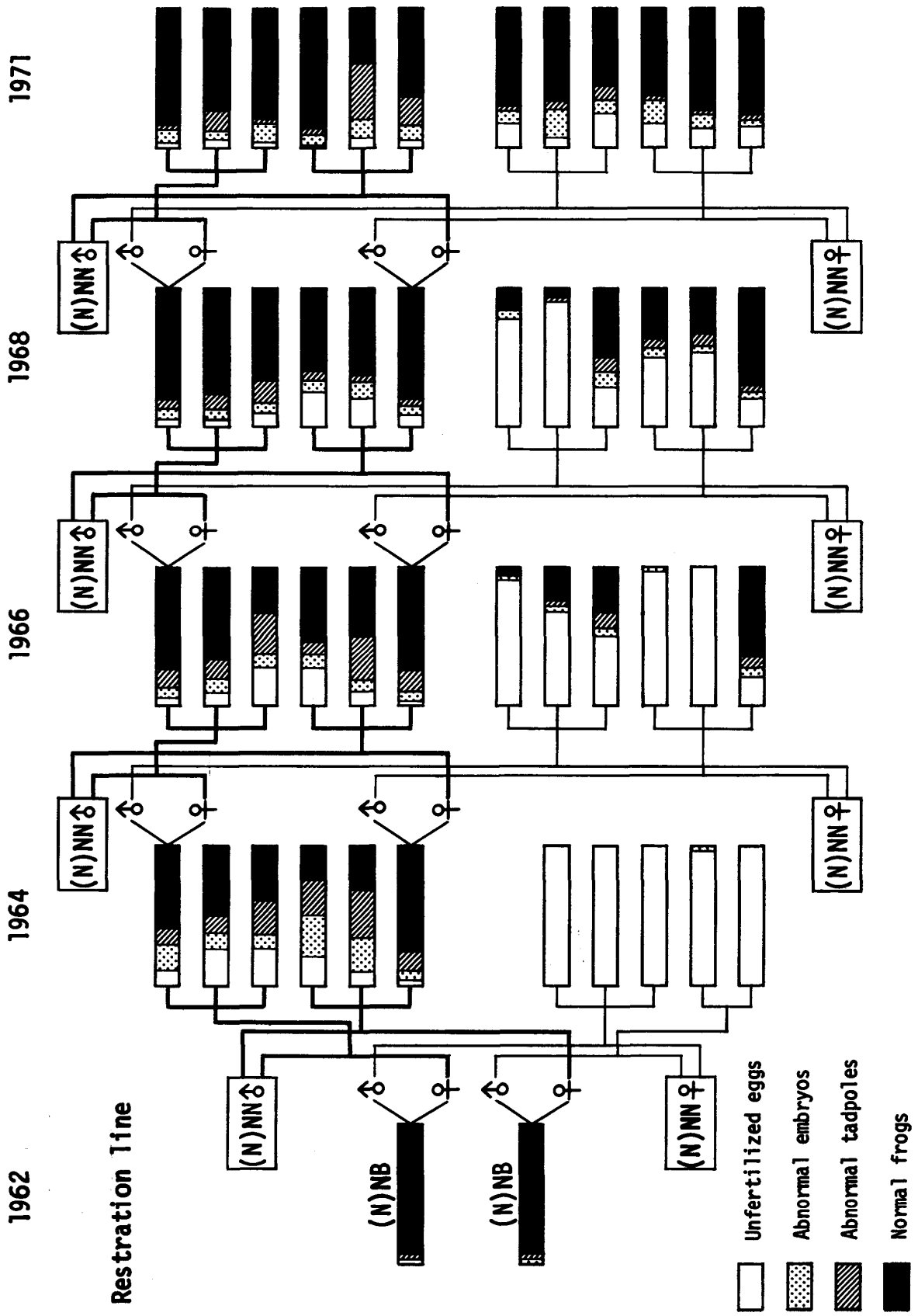
Fig. 1.   Reproductive capacity of hybrids, *Rana nigromaculata* ♀ × *Rana brevipoda* ♂, and their backcrosses. I
(N)NN— *Rana nigromaculata*   (N)NB— Hybrid

80% of the respective total number of eggs cleaved normally and later 7~65% became normal, metamorphosed frogs.

Six females and six males were removed from among mature frogs belonging to two series which were the best in the percentage of normal, metamorphosed frogs among the six B_2 series and used for obtaining the B_3 generation. Of these females, three were slightly superior to their mothers in reproductive capacity, while the other three were somewhat inferior to the latter. In the six B_3 series, the normal, metamorphosed frogs did not increase as a whole, as compared with the foregoing two series, that is, 75~95%, 88.5% on the average, of the respective total number of eggs cleaved normally and later 60~80%, 71.2% on the average, became normal, metamorphosed frogs. The six males were distinctly inferior to the females in reproductive capacity, although all of them were more or less fertile; 10~80%, 47.0% on the average, cleaved normally and 7~70%, 34.5% on the average, became normal, metamorphosed frogs. In order to produce the B_4 generation, six males and six females were removed from two series which were the best in the percentage of normal, metamorphosed frogs and mated with *Rana nigromaculata*. Five of these six females were nearly the same as their mothers in reproductive capacity, while the remainder was remarkably inferior to her mother. In the six series, 94~98%, 96.0% on the average, of the respective total number of eggs cleaved normally and 39~86%, 75.6% on the average, became normal, metamorphosed frogs. It was noteworthy that the six males were fertile to a large extent, that is, 76~92%, 84.3% on the average, cleaved normally in contrast with the males used for producing the B_1, B_2 or B_3 generation. The normal, metamorphosed frogs corresponded to 56~76%, 67.3% on the average, of the respective total number of eggs in the six series.

b) Backcrossing with the father species, *Rana brevipoda*

Male hybrids had almost no fertilizing sperm (Fig. 2). On the other hand, in the six B_1 series derived from six female hybrids, 50~90%, 67.7% on the average, of the respective total number of eggs cleaved normally, and later 17~77%, 40.7% on the average, became normal, metamorphosed frogs. Six females and six males were removed from two B_1 series which were the best in the production of metamorphosed frogs, and were utilized for producing the B_2 generation. While one of these females was the same as her mother in reproductive capacity, the others were more or less inferior to their mothers. In the six B_2 series, 67~90%, 75.1% on the average, cleaved normally, and 33~67%, 47.5% on the average, became normal, metamorphosed frogs. Unlike the females, the males were very low in reproductive capacity. While one of them had no fertilizing sperm, 27.8% of the total number of eggs cleaved normally and 17.5% became normal, metamorphosed frogs by the other five males.

In order to produce the B_3 generation, six females and six males were removed from two series which were the best in the production of normal, metamorphosed frogs among the six B_2 series derived from two females, and were mated with *Rana brevipoda*. While only one of the six females was remarkably superior
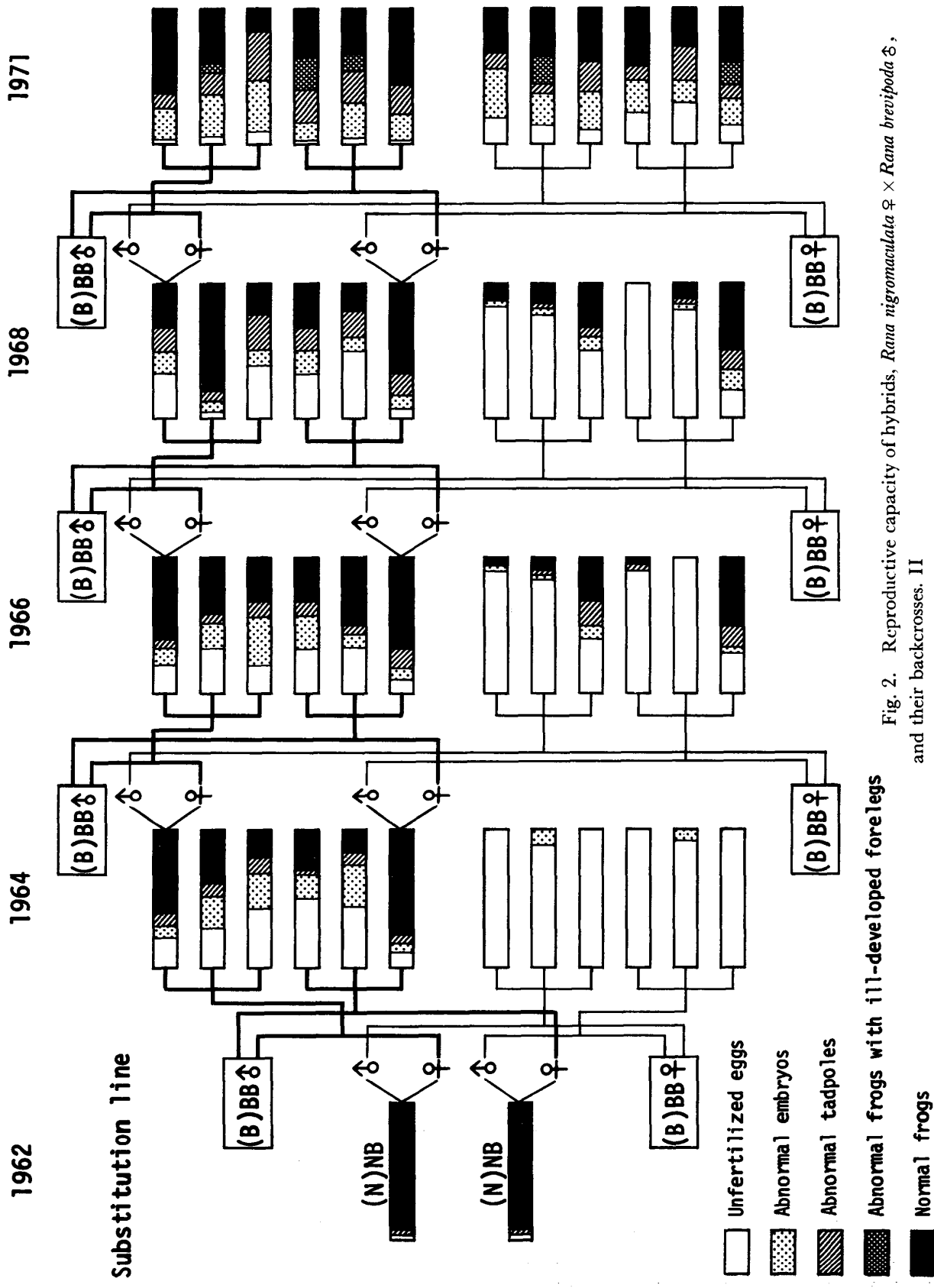
Fig. 2.   Reproductive capacity of hybrids, *Rana nigromaculata* ♀ × *Rana brevipoda* ♂, and their backcrosses. II

(B)BB—— *Rana brevipoda*   (N)NB—— Hybrid

to her mother in reproductive capacity, another was similar and the remainders were distinctly inferior to their mothers. In the six B_3 series, $50 \sim 95\%$, 72.5% on the average, of the respective total number of eggs cleaved normally, and $20 \sim 80\%$, 42.7% on the average, became normal, metamorphosed frogs. The males were very similar to those in the foregoing generation in reproductive capacity. While one of them had no fertilizing sperm, the others were slightly fertile. In these five B_3 series, only 30.7% of the total number of eggs cleaved normally, and 18.2% became normal, metamorphosed frogs. The B_4 generation was produced from six females and six males removed from two series which were the second and the third in abundance of normal, metamorphosed frogs among the six B_3 series derived from females of two B_2 series. Of the six females, only two were superior to their mother in reproductive capacity, while the others were distinctly inferior. In the six B_4 series, $91 \sim 98\%$, 95.8% on the average, of the respective total number of eggs cleaved normally, and $17 \sim 63\%$, 41.3% on the average, became normal, metamorphosed frogs. Thus, there was no improvement as a whole in the reproductive capacity of females. On the other hand, the males somewhat improved in reproductive capacity, as compared with those of the foregoing generation. None of the six males was sterile. In the six B_4 series, $69 \sim 90\%$, 81.2% on the average, cleaved normally, and $28 \sim 43\%$, 36.8% on the average, became normal, metamorphosed frogs. It was curious to observe that some abnormal frogs having ill-developed forelegs were produced in five series of the B_4 generation.

2. Descendants of hybrids between female *Rana brevipoda* and male *Rana nigromaculata*

a) Backcrossing with the mother species, *Rana brevipoda*

Like the reciprocal hybrids, male hybrids had almost no fertilizing sperm, while females were fertile to some extent (Fig. 3). In the six B_1 series derived from six female hybrids, $73 \sim 90\%$, 82.8% on the average, of the respective total number of eggs cleaved normally, and $20 \sim 60\%$, 42.3% on the average, became normal, metamorphosed frogs. Six females and six males were removed from two series which were the best and the third in the reproduction of normal, metamorphosed frogs among the six B_1 series derived from female hybrids mated with *Rana brevipoda*. Of these females, four were somewhat improved in reproductive capacity as compared with their mothers, while the others were somewhat inferior to the latter. In the six B_2 series, $63 \sim 95\%$, 81.7% on the average, of the respective total number of eggs cleaved normally, and $37 \sim 80\%$, 61.7% on the average, became normal, metamorphosed frogs. The males were more or less fertile in contrast with the male hybrids, although they were distinctly lower than the females in reproductive capacity; only $10 \sim 80\%$, 39.8% on the average, cleaved normally, and $6 \sim 63\%$, 26.3% on the average, became normal, metamorphosed frogs in the six B_2 series.

Six females and six males were removed from two B_2 series which were the best in the production of normal, metamorphosed frogs, in order to produce the
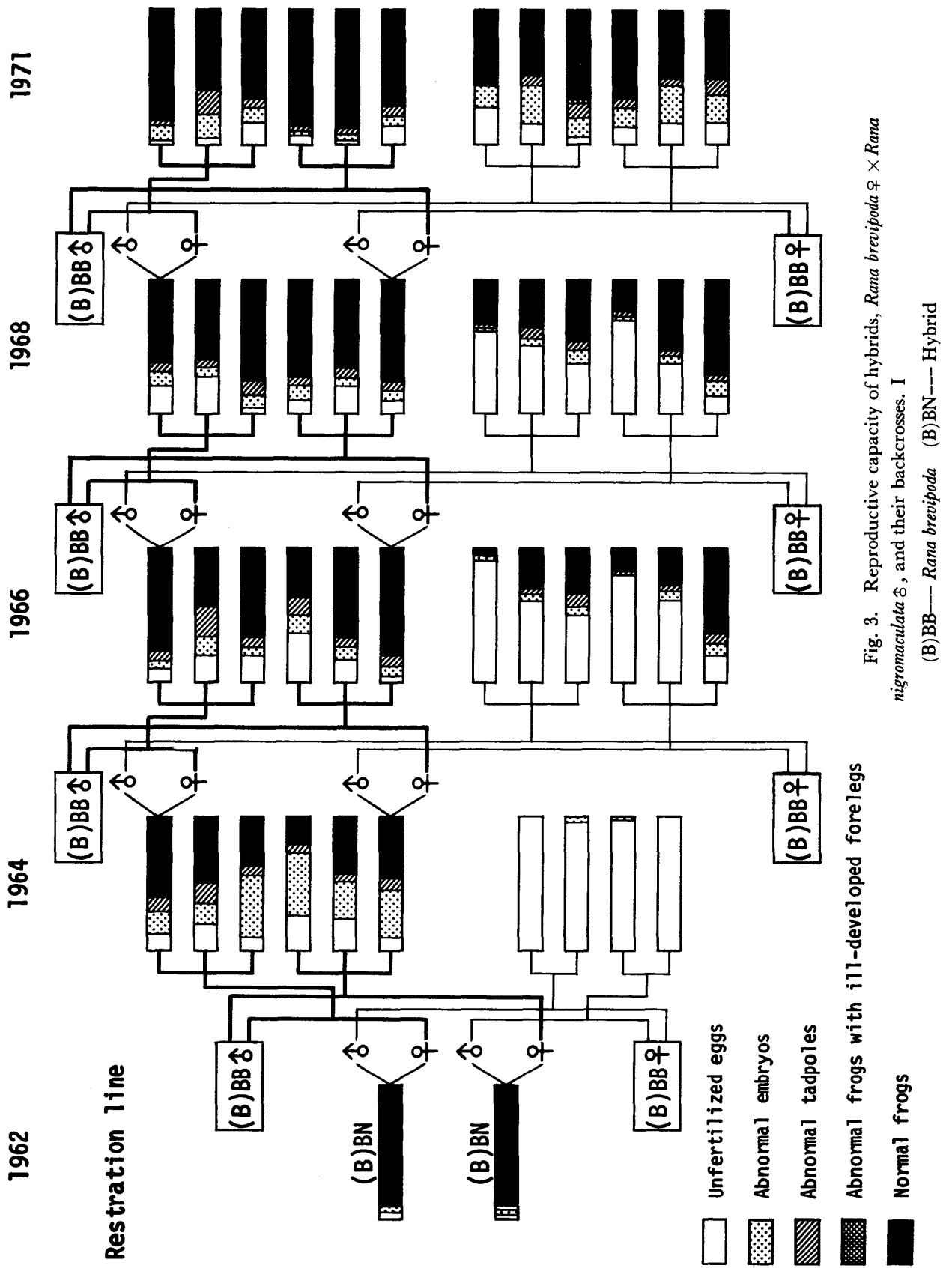
Fig. 3. Reproductive capacity of hybrids, *Rana brevipoda* ♀ × *Rana nigromaculata* ♂, and their backcrosses. I

(B)BB— *Rana brevipoda*   (B)BN— Hybrid

B_3 generation. Only two of these females were the same as their mothers in reproductive capacity, while the other four were more or less inferior to the latter. In the six B_3 series, $73\sim95\%$, 84.7% on the average, of the respective total number of eggs cleaved normally, and $60\sim77\%$, 69.3% on the average, became normal, metamorphosed frogs. The males were still distinctly inferior to the females in reproductive capacity; $30\sim89\%$, 55.3% on the average, cleaved normally, and $23\sim72\%$, 44.0% on the average, became normal, metamorphosed frogs in the six B_3 series. From two B_3 series in which 63% and 77% of the respective total number of eggs became normal, metamorphosed frogs, six females and six males were removed in order to produce the B_4 generation. Four of these females were slightly superior to their mothers in reproductive capacity, while the other two were slightly inferior to the latter. In the six B_4 series, $84\sim98\%$, 92.2% on the average, cleaved normally, and $60\sim88\%$, 76.0% on the average, became normal, metamorphosed frogs. The males were still somewhat inferior to the females in reproductive capacity, although they slightly improved as compared with the B_3 generation; $74\sim94\%$, 84.5% on the average, cleaved normally, and later $48\sim67\%$, 56.5% on the average, became normal, metamorphosed frogs in the six B_4 series.

b) Backcrossing with the father species, *Rana nigromaculata*

While male hybrids produced no fertilized eggs, females were fertile to some extent; $66\sim95\%$, 81.3% on the average, of the respective total number of eggs cleaved normally, and $20\sim70\%$, 50.8% on the average, became normal, metamorphosed frogs (Fig. 4). Six females and six males were removed from two B_1 series which were the best in the production of normal, metamorphosed frogs, and were used for producing the B_2 generation. Two of the six females were slightly superior to or just the same as their mothers in reproductive capacity, while the other four were more or less inferior to the latter. While one of the males was completely sterile, the other five were somewhat fertile, although they were generally low in reproductive capacity. In the five B_2 series derived from these five males, only $10\sim66\%$, 26.7% on the average, cleaved normally, and later $6\sim50\%$, 21.3% on the average, became normal, metamorphosed frogs. In order to produce the B_3 generation, six females and six males were removed from two series which were the best in the production of normal, metamorphosed frogs among the six B_2 series, and were mated with *Rana nigromaculata*. While only one of the six females was superior to her mother in reproductive capacity, the other five were more or less inferior to their mothers. In the six B_3 series, $40\sim86\%$, 62.0% on the average, cleaved normally, and $30\sim80\%$, 52.7% on the average, became normal, metamorphosed frogs. In contrast with the females, one of the males produced no fertilized eggs, while the other five were somewhat fertile. In the B_3 series from these five males, $5\sim80\%$, 28.7% on the average, of the respective number of eggs cleaved normally, and $5\sim63\%$, 21.8% on the average, became normal, metamorphosed frogs.

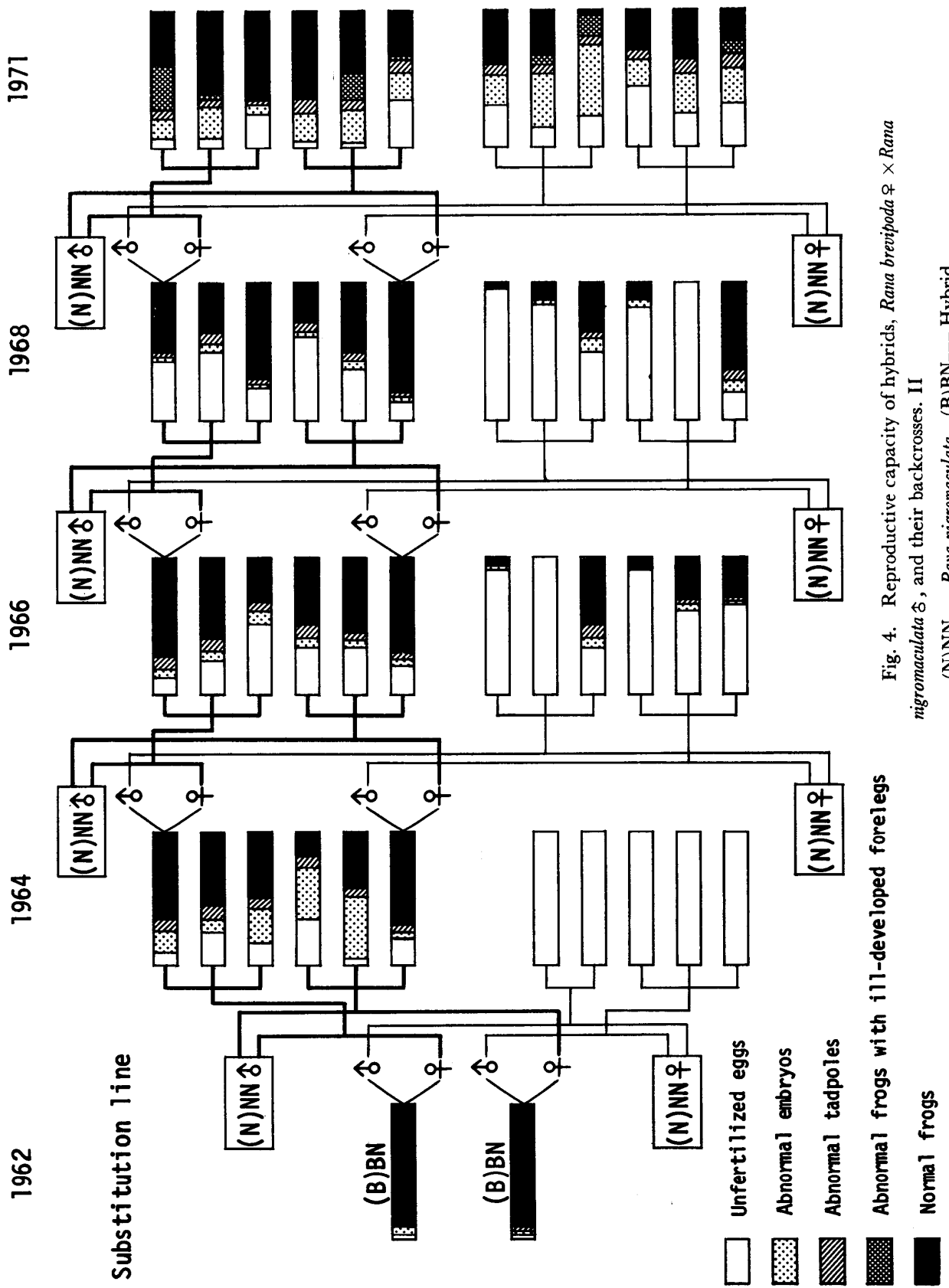For producing the B_4 generation, six females and six males were removed from

Fig. 4. Reproductive capacity of hybrids, Rana brevipoda ♀ × Rana
nigromaculata ♂, and their backcrosses. II

(N)NN—— Rana nigromaculata   (B)BN—— Hybrid

two series which were the best and the third in the production of normal, metamorphosed frogs among the six B_3 series. While only two of the six females were superior to their mother in reproductive capacity, the others were more or less inferior to their mothers; in the six B_4 series, $67 \sim 96\%$, 87.0% on the average, cleaved normally, and $34 \sim 67\%$, 52.3% on the average, became normal, metamorphosed frogs. Besides these normal frogs, there were some abnormal frogs with ill-developed forelegs in four of the six series, as found in the B_4 generation of the reciprocal hybrids. Although the six males were fertile to some extent, they were remarkably inferior to the females in reproductive capacity. In the six B_4 series, $57 \sim 87\%$, 73.2% on the average, cleaved normally, and $4 \sim 40\%$, 27.8% on the average, became normal, metamorphosed frogs. Besides normal frogs, some abnormal ones having ill-developed forelegs were produced in three of the six B_4 series.

3. Comparison among the four lines

The four lines derived from reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda* by repeating backcrosses with the paternal or maternal species were mutually similar in many respects (Figs. 1, 2, 3, 4). In each line, males were distinctly inferior to females in reproductive capacity. This was attributable to the poverty of normally fertilizing spermatozoa in the testes of the males. In each line, there were almost no eggs that cleaved normally in the B_1 generation, while the percentage of normally cleaved eggs increased abruptly in the B_4 generation. On the other hand, a few of the female hybrids in each line were good in reproductive capacity, while the others were more or less poor. The females of each of the B_1, B_2 and B_3 generations in each line were very similar on an average to their mothers of the foregoing generation in the percentages of normally fertilized eggs and normal, metamorphosed frogs produced by them, while they usually differed remarkably from one another in these percentages.

The four lines were similar in sex ratio to one another in that nearly the same number of males and females appeared in interspecific hybrids as well as in their backcrosses of each generation.

The four lines differed from one another in various respects. The frogs of the two restoration lines were generally more fertile than those of the two substitution lines. While abnormal frogs with ill-developed forelegs appeared in the B_4 generations of the substitution lines, there were almost no such frogs in the restoration lines. The substitution line derived from hybrids between female *Rana brevipoda* and male *Rana nigromaculata* was the best among the four lines in the viability of frogs; normal, metamorphosed frogs were active and grew rapidly. In contrast with this line, the substitution line derived from hybrids between female *Rana nigromaculata* and male *Rana brevipoda* was the worst in this respect. Normal, metamorphosed frogs in this line were weak and grew slowly; most of them died without attaining sexual maturity. This fact agrees well with the situation in reciprocal nucleo-cytoplasmic hybrids between *Rana nigromaculata* and *Rana brevipoda* (KAWAMURA and NISHIOKA, 1963). The two restoration lines were inter-

mediate between the two substitution lines in the viability of frogs, although the line derived from hybrids between female *Rana nigromaculata* and male *Rana brevipoda* was somewhat better than that derived from the reciprocal hybrids, as expected from the better growth of *Rana nigromaculata* than that of *Rana brevipoda*.

4. Differences between male and female hybrids in reproductive capacity

The nearly complete sterility of male hybrids was caused from the absence of normally fertilizing spermatozoa in their testes. This has already been reported by MORIYA (1960b) in reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda*. OKUMOTO (1978) has observed in detail that several univalents always appear besides irregular bivalents in the first meiosis of spermatocytes (Figs. 5, 6). Such spermatocytes degenerate without forming spermatozoa or become very abnormal spermatozoa. In contrast with male hybrids, female ones produced mature eggs which were mostly fertilizable. Such a difference in the formation of gametes between male and female hybrids seems to be attributable to a difference in the process of gametogenesis; while the differentiation of spermatozoa starts after the completion of meiosis, the growth of oocytes finishes during the prophase of the first reduction division.

The oocytes of female hybrids were distinctly retarded in growth at the young frog stage as compared with those of the parental species (MORIYA, 1960a, b). However, mature females laid eggs which did not remarkably differ in number from those of the parental species, although many of the fertilized eggs became abnormal and died at the embryonic and tadpole stages. For the purpose of clarifying the cause for abnormalities found in embryos and tadpoles produced from female

Bivalents   Bivalents
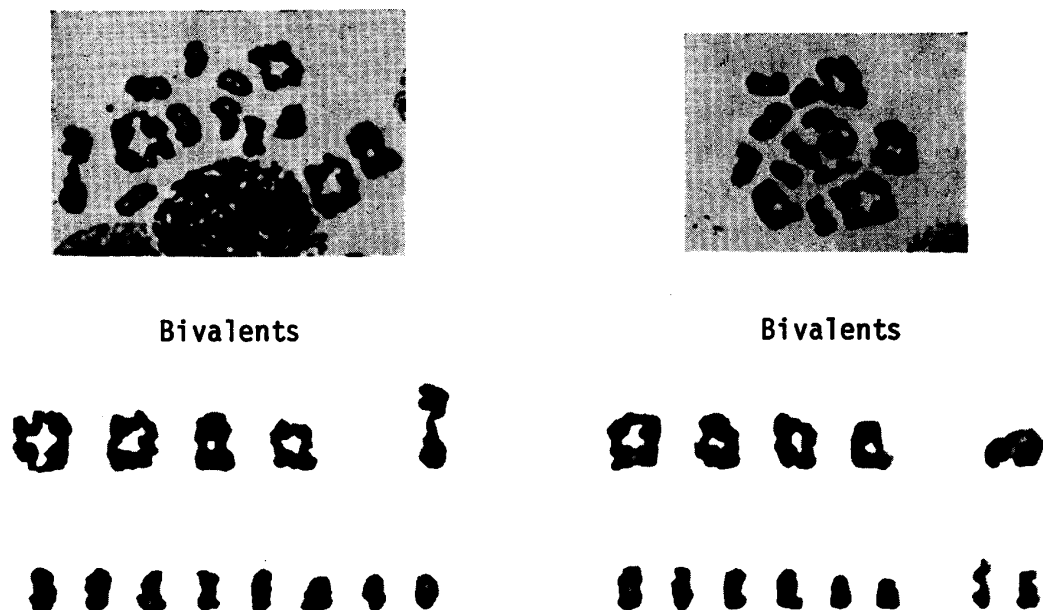
Fig. 5. Meiotic chromosomes of *Rana nigromaculata* at the first metaphase. Each of two spreads contains 13 bivalents (from OKUMOTO, 1978).   × 960
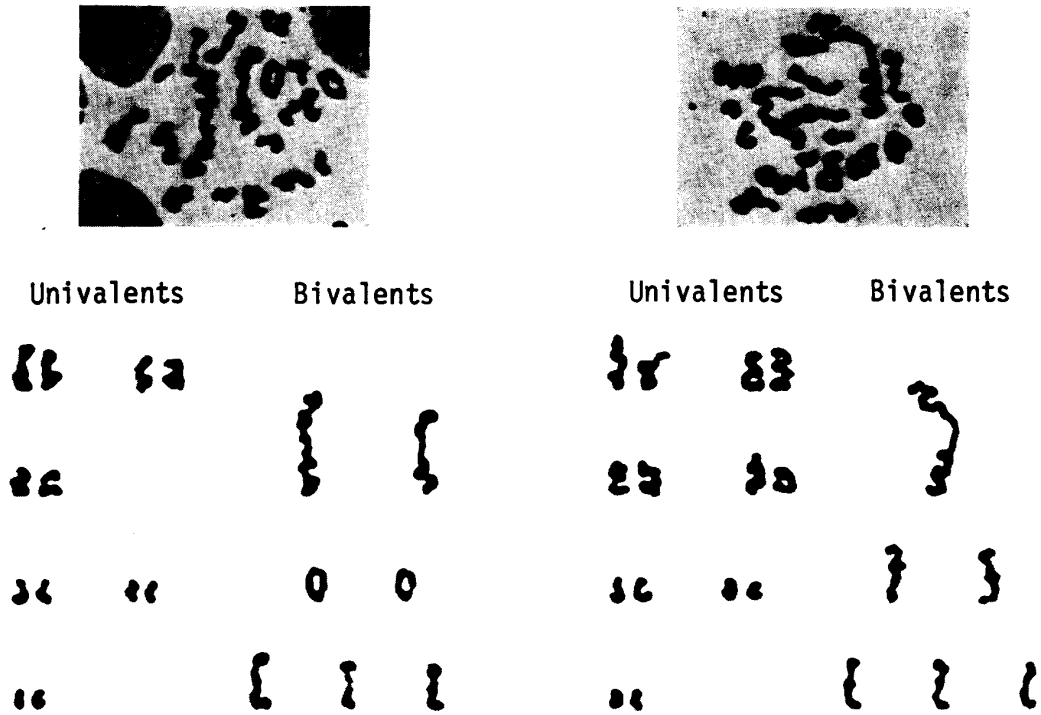
Fig. 6. Meiotic chromosomes of a hybrid, *Rana brevipoda* ♀ × *Rana nigromaculata* ♂, at the first metaphase. One spread contains 7 bivalents and 12 univalents; the other 6 bivalents and 14 univalents (from OKUMOTO, 1978). ×960

hybrids by backcrossing, 991 mitotic figures were analyzed in 200 normally shaped tadpoles removed from among B_1 tadpoles belonging to the four lines. As a result, it was found that there were numerous chromosome aberrations in many tadpoles; 112 (11.3%), 201 (20.3%) and 67 (6.8%) of the mitotic figures were monosomic, trisomic and approximately triploid, respectively, while the remaining 661 mitotic figures were normally or abnormally diploid (Figs. 7~14). In addition, 58 minute chromosomes and 13 fragments in total were found in the whole mitotic figures. While most of the tadpoles were each uniform in karyotype, the others were mosaics consisting of two kinds of karyotypes. Although the karyotypes of abnormally shaped embryos and tadpoles were not examined in the present study, there was no doubt that they were much more abnormal than those of normally shaped tadpoles, as found in the second-, third- and fourth-generation offspring of nucleo-cytoplasmic hybrids between *Rana japonica* and *Rana ornativentris* (KAWAMURA and NISHIOKA, 1972). Thus, it was presumed that chromosome aberrations should be the cause for morphological abnormalities found in many embryos and tadpoles as well as for the production of abnormal offspring.

On the other hand, about one- or two-tenths of the females of reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda* laid a considerable number of large eggs together with normal size eggs. The karyotypes of 145 normally shaped tadpoles which had been raised from both large and normal size eggs of a female hybrid between a female *Rana brevipoda* and a male *Rana nigromaculata* were
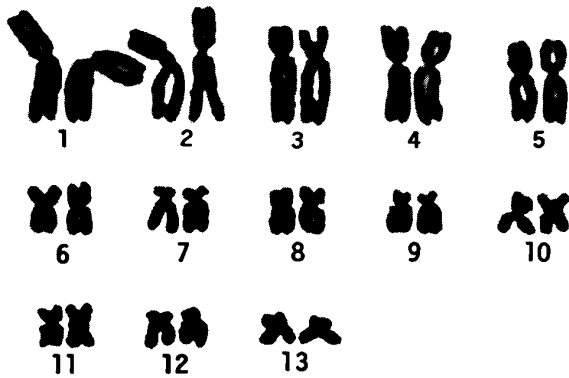
Fig. 7.   Karyotype of *Rana nigromaculata*, obtained from the tail tip of a normal tadpole.
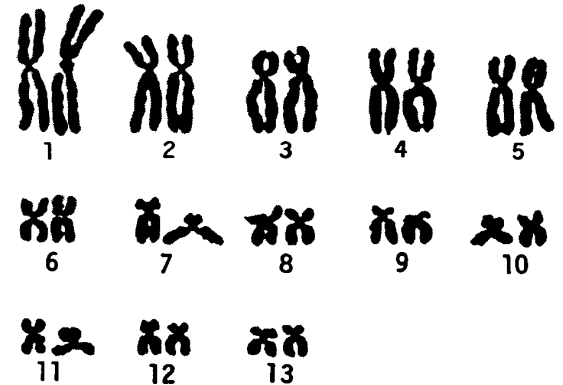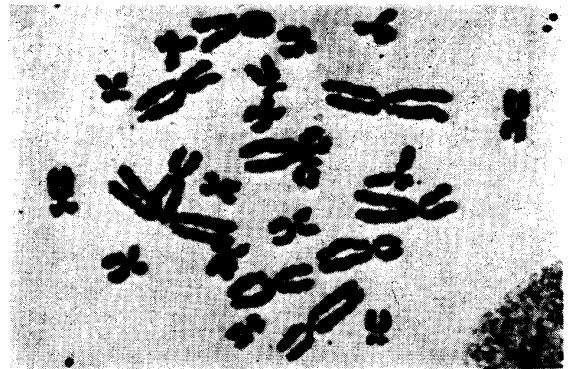× 1500

Fig. 8.   Karyotype of *Rana brevipoda*, obtained from the tail tip of a normal tadpole.
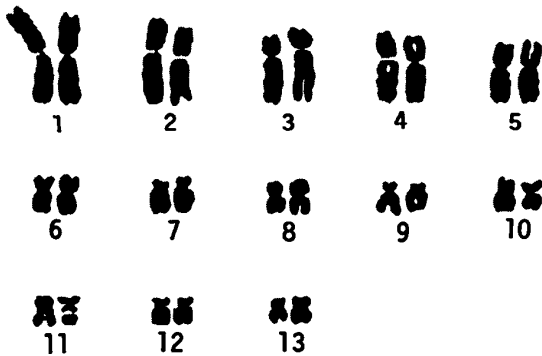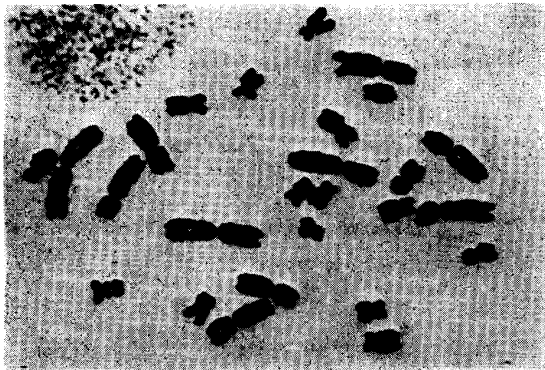× 1500

Fig. 9.   Karyotype of a hybrid, *Rana nigromaculata* ♀ × *Rana brevipoda* ♂, obtained from the tail tip at the tadpole stage.   × 1500
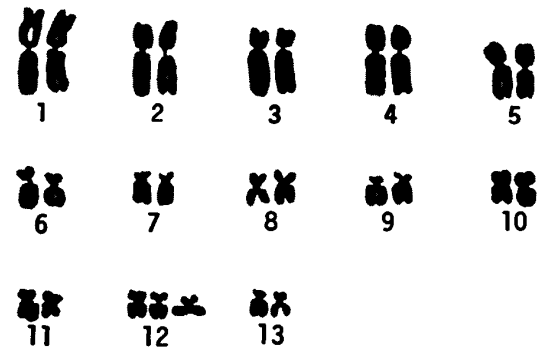

Fig. 10.   Karyotype of a trisomic backcross, (*Rana brevipoda* ♀ × *Rana nigromaculata* ♂) ♀ × *Rana nigromaculata* ♂, obtained from the tail tip at the tadpole stage.   × 1500
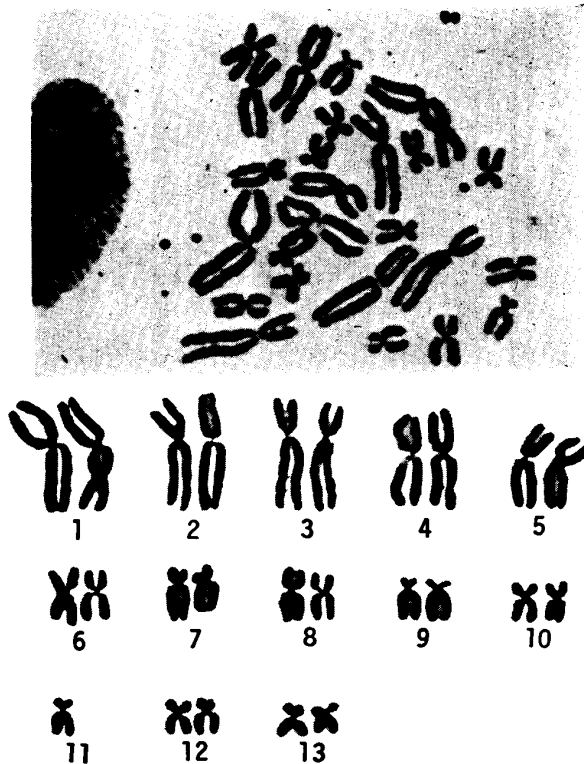
Fig. 11.   Karyotype of a monosomic backcross, (*Rana brevipoda* ♀ × *Rana nigromaculata* ♂) ♀ × *Rana nigromaculata* ♂, obtained from the tail tip at the tadpole stage.   One of No. 11 chromosomes is lost. A chromosome No. 7 has a deletion in the long arm.
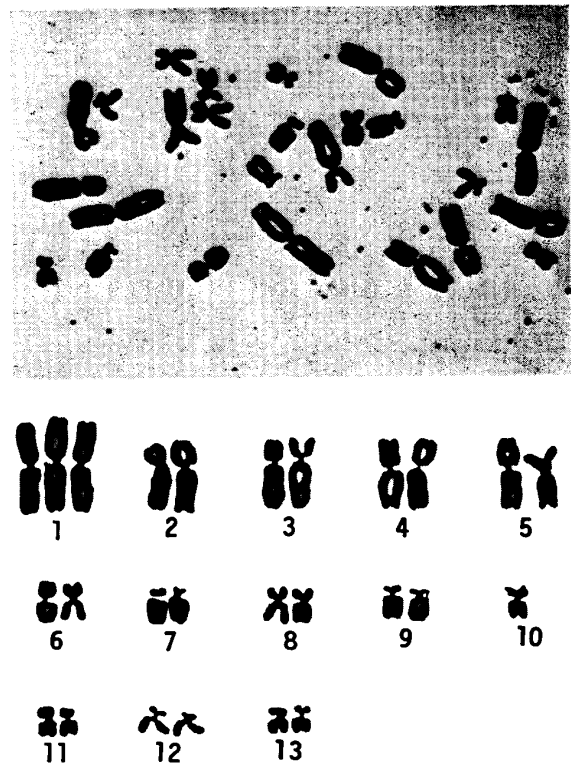
× 1500

Fig. 12.   Karyotype of an abnormally diploid backcross, (*Rana nigromaculata* ♀ × *Rana brevipoda* ♂) ♀ × *Rana nigromaculata* ♂, obtained from the tail tip at the tadpole stage.   An additional chromosome No. 1 and a single chromosome No. 10 are observed.   × 1500

analyzed.   As a result, it was found that 42 and 3 of 45 tadpoles raised from large eggs were triploids and mosaics of hyper- and hypotriploid cells, respectively, while 92 and 8 of 100 tadpoles raised from normal size eggs were diploids and triploids, respectively.   Of 413 mitotic figures from the latter 100 tadpoles, 200, 151, 22 and 40 contained 26 (diploid), 25 (monosomic), 27 (trisomic) and about 39 (triploid) chromosomes, respectively.   Ten minute chromosomes and three fragments were also found in these mitotic figures.   All 181 mitotic figures of the former 45 tadpoles raised from large eggs had approximately triploid chromosomes, and they also contained ten chromosomal fragments in all.   A similar phenomenon concerning the production of triploids from large and normal size eggs was observed in two of ten other hybrids between female *Rana nigromaculata* and male *Rana brevipoda* as well as in two of ten hybrids obtained from the reciprocal crosses. The large eggs were probably diploid ones derived from tetraploid oogonia. However, it was not clear whether the normal size eggs that produced triploids were of the same origin as that of the large eggs or not.   The mosaics constructed of two kinds of karyotypes seemed to have been established at an early cleavage stage by non-disjunction of a chromosome.
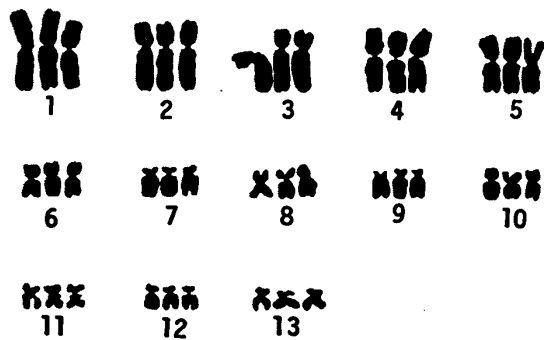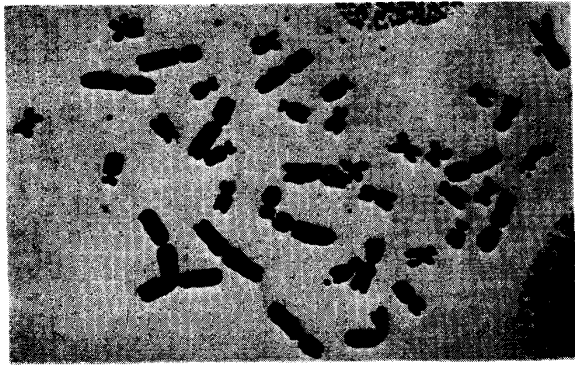
Fig. 13. Karyotype of a triploid backcross, (*Rana nigromaculata* ♀ × *Rana brevipoda* ♂) ♀ × *Rana nigromaculata* ♂, obtained from the tail tip at the tadpole stage.   × 1500
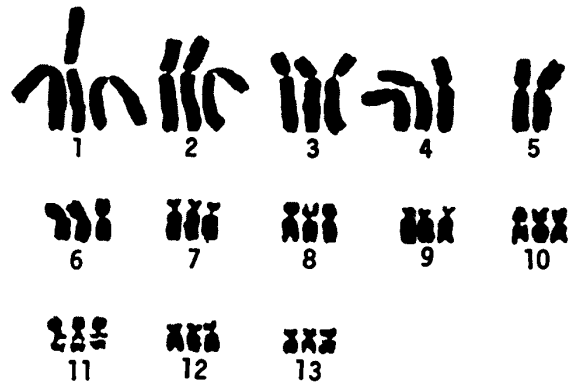
Fig. 14. Karyotype of a hypotriploid backcross, (*Rana nigromaculata* ♀ × *Rana brevipoda* ♂) ♀ × *Rana nigromaculata* ♂, obtained from the tail tip at the tadpole stage. No. 5 alone consists of two chromosomes.   × 1500

DISCUSSION

The finding that the females of reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda* are fertile to a large extent in contrast with the males was reported by MORIYA (1951, 1960a, b). The present study further clarified that they were more or less inferior to their parents in reproductive capacity; $17 \sim 77\%$ of the eggs of each female hybrid became normal, metamorphosed frogs, while $90 \sim 93\%$ of the eggs laid by each of their mothers did so. It was remarkable that the female hybrids differed distinctly from one another in reproductive capacity. Similar deficiency and diversity in reproductive capacity were also found in the females of the B_1, B_2 or B_3 generation in each of the four lines, consisting of two restoration and two substitution lines. The percentage of normal, metamorphosed frogs produced by the three sisters of an experimental series in each line was nearly the same on the average to the percentage of such frogs produced by their mother. There was scarcely any improvement of females on an average in reproductive capacity with the progress of generations, although the best grown females were always removed from an experimental series and used in producing their next generations (Figs. 1, 2, 3, 4).

In each line, males raised from female parents were distinctly inferior to their

sisters in reproductive capacity. On the other hand, males and females of the substitution lines were also remarkably inferior to those of the restoration lines in this capacity. Moreover, in the B_4 generations of the substitution lines, a considerably large number of abnormal frogs with ill-developed forelegs were produced by many male and female parents. Consequently, the substitution line derived from hybrids between female *Rana brevipoda* and male *Rana nigromaculata* does not seem to produce their descendants for a long time, although it was the best in viability of frogs among the four lines. However, if backcrosses with normal reproductive capacity were produced from this substitution line, they should be indistinguishable from *Rana nigromaculata*. In contrast, the substitution line derived from female hybrids between female *Rana nigromaculata* and male *Rana brevipoda* by backcrossing with the male species seems incompetent for producing descendants with normal reproductive capacity, as this line was the worst in viability of frogs among the four lines. Thus, it is most probable that backcrosses with normal reproductive capacity are derived from female hybrids, passing over one or both of the two restoration lines.

It may be worthy here to note that the inheritance of external characters between the two species occurs in the same way as in each species. The dorsomedian stripe and the white color of the ventral surfaces of *Rana nigromaculata* are dominant characters. Each of these characters is controlled by a single gene. In contrast with these characters, the size, shape and number of dark spots or small dermal ridges on the back and many other quantitative characters seem to be controlled by polygenes; in interspecific hybrids or backcrosses, they are almost intermediate between those of their parents. Accordingly, the frogs of the restoration line derived from hybrids between female *Rana nigromaculata* and male *Rana brevipoda* by backcrossing with *Rana nigromaculata* can be hardly distinguished from *Rana nigromaculata* by appearance in the B_2 and later generations. While most of the frogs of the restoration line derived from hybrids between female *Rana brevipoda* and male *Rana nigromaculata* by backcrossing with *Rana brevipoda* are also almost indistinguishable from *Rana brevipoda*, some frogs disclose their hybrid origin by expressing the dominant characters of *Rana nigromaculata*.

Although the crosses between females of the small short-legged frog species (*Rana brevipoda*) and males of the large long-legged frog species (*Rana nigromaculata*) seem more likely to occur than the reciprocal crosses, it is so far unknown whether both kinds of crosses between these two species really occur or not in the field. When they occur, it is possible that *Rana nigromaculata* or *Rana brevipoda* with normal reproductive capacity are regained from female hybrids by passing over the restoration lines. The subspecies *Rana brevipoda porosa* is believed to be a product of the restoration line backcrossed with *Rana brevipoda*. During a very long period of time, the dominant as well as the recessive genes of *Rana nigromaculata* entered abundantly into the gene pool of *Rana brevipoda brevipoda* and consequently formed a new population, *Rana brevipoda porosa*, which is intermediate in various characters between *Rana nigromaculata* and *Rana brevipoda brevipoda*. Although *Rana*

brevipoda porosa is very similar to the hybrids of *Rana nigromaculata* and *Rana brevipoda brevipoda* in external characters, it is completely normal in reproductive capacity and scarcely isolated from the original *Rana brevipoda brevipoda* by hybrid inviability or sterility.

In the two areas where natural hybridization occurs frequently (KAWAMURA and NISHIOKA, 1977), *Rana nigromaculata* and *Rana brevipoda porosa* are sympatrically distributed together with interspecific hybrids and backcrosses. While the interspecific hybrids are somewhat distinguishable from each of the parents, as their quantitative characters are intermediate between the two parents, the offspring produced by backcrossing with *Rana brevipoda porosa* are very similar to the latter in external characters, although most of them are remarkably abnormal in reproductive capacity. As a population of *Rana brevipoda porosa* in these areas is usually surrounded by a larger population of *Rana nigromaculata,* the following two kinds of circumstances may result from different degrees in the frequency of natural hybridization between *Rana nigromaculata* and *Rana brevipoda porosa.* When the hybridization occurred very frequently, two types of frogs, *Rana nigromaculata* and a group of backcrosses, would exist. When it did not frequently occur, three types of frogs, *Rana nigromaculata, Rana brevipoda porosa* and a group of interspecific hybrids and backcrosses, would be in existence, as usually found in the two areas of Japan where natural hybridization has occurred. When a population of *Rana nigromaculata* is smaller than that of *Rana brevipoda porosa* in a narrow isolated area, two types of frogs, that is, *Rana brevipoda porosa* and a group of backcrosses, or only descendants of backcrosses would remain in this area.

In the central Europe, four kinds of circumstances have been reported regarding the distribution of *Rana esculenta*; there are co-existence of *Rana lessonae* and *Rana esculenta* (BLANKENHORN, HEUSSER and VOGEL, 1971; BLANKENHORN, 1973, 1977; BLANKENHORN, HEUSSER and NOTTER, 1973; HALFMANN and MÜLLER, 1972; TUNNER, 1974; TUNNER and DOBROWSKY, 1976; UZZEL and BERGER, 1975; GÜNTHER and HÄHNEL, 1976), co-existence of *Rana lessonae, Rana ridibunda* and *Rana esculenta* (BERGER, 1964, 1966, 1968, 1970; BLANKENHORN, HEUSSER and VOGEL, 1971; BLANKENHORN, 1973; GÜNTHER, 1973; UZZEL and BERGER, 1975), sole existence of *Rana esculenta* (GÜNTHER, 1973, 1974; UZZEL and BERGER, 1975; EBENDAL, 1978) and co-existence of *Rana esculenta* and *Rana ridibunda* (GÜNTHER, 1968, 1973, 1974; GÜNTHER and HÄHNEL, 1976; BLANKENHORN, 1973; UZZEL and BERGER, 1975). It would be interesting to consider the European "*esculenta*" problem by replacing *Rana lessonae, Rana ridibunda* and *Rana esculenta* with *Rana nigromaculata, Rana brevipoda porosa* and a group of interspecific hybrids and backcrosses, respectively, although *Rana nigromaculata* is larger than *Rana brevipoda porosa.* It seems also probable that a population of *Rana ridibunda* corresponding to *Rana brevipoda brevipoda* are distributed in the estern Europe. The present authors are now carrying out their researches on European water frogs collected from various districts in order to examine the presence of such a parallelism.

SUMMARY

1. Reciprocal hybrids between two Japanese pond-frog species, *Rana nigromaculata* and *Rana brevipoda*, were repeatedly backcrossed by the paternal and maternal species until B_4 offspring were produced.

2. The four lines derived from the reciprocal hybrids by backcrossing with the parental species were similar to one another in many respects. In each line, males produced from female parents by backcrossing were distinctly inferior to their sisters in reproductive capacity, except those in the B_3 generation of the restoration line derived from a female hybrid, *Rana nigromaculata* ♀ × *Rana brevipoda* ♂. The defect in reproductive capacity of males was attributable to the poverty of normal spermatozoa in their testes. A few males as well as more numerous females in each line were somewhat good in reproductive capacity. There was scarcely any improvement of females of the B_1, B_2 or B_3 generation on an average in reproductive capacity as compared with their female parents.

3. The four lines differed from one another in various respects. The frogs of the two restoration lines were generally much more fertile than those of the two substitution lines. Abnormal frogs with ill-developed forelegs appeared in considerably large numbers in the B_4 generations of the substitution lines, while they are very scarce in the restoration lines.

4. The substitution line derived from hybrids between female *Rana brevipoda* and male *Rana nigromaculata* was the best in viability of frogs among the four lines, while that derived from the reciprocal hybrids was the worst. The restoration line derived from hybrids between female *Rana nigromaculata* and male *Rana brevipoda* was better in this respect than that derived from the reciprocal hybrids.

5. Numerous chromosome aberrations were found in normally shaped tadpoles produced from female hybrids. They were assumed to be the cause for morphological abnormalities of embryos and tadpoles as well as for the production of abnormal offspring.

6. About one- or two-tenths of the females of reciprocal hybrids laid a considerable number of large eggs together with normal size eggs. All these large eggs developed into triploid tadpoles by fertilization with normal spermatozoa. Triploid tadpoles were also raised from a few normal size eggs.

ACKNOWLEDGMENTS

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