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# Genetic Variation of Five Enzymes in Japanese Pond Frogs

By

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(With 18 Text-figures)

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## INTRODUCTION

Fifty years ago, OKADA (1931) placed all the Japanese pond frogs in a single subspecies *Rana nigromaculata nigromaculata* HALLOWELL (1860), although he recognized six color variations in this subspecies. Ten years later, ITO (1941) happened to observe smaller, short-legged pond frogs which were sympatric with *Rana nigromaculata nigromaculata* in the suburbs of Nagoya. He named them *Rana nigromaculata brevipoda*. MORIYA (1954) observed numerous pond frogs collected from various districts of Japan and classified them into five races. Later, he (1960b) made crossing experiments between different races and clarified that the *nigromaculata* common race was isolated from the other four races by male hybrid sterility. Mainly on the basis of the results obtained by MORIYA (1954, 1960a, b), KAWAMURA (1962) classified the pond frogs distributed in Japan into two species and one subspecies, that is, sympatric *Rana nigromaculata* and *Rana brevipoda brevipoda* and allopatric *Rana brevipoda porosa*. KAWAMURA and NISHIOKA (1977) assumed that *Rana brevipoda porosa* is a stabilized population derived from *Rana brevipoda brevipoda* by introgression of genes from *Rana nigromaculata* since antiquity.

It was considered interesting to the present authors to examine the electro-

phoretic patterns and allele frequencies at each locus of various enzymes in *Rana nigromaculata, Rana brevipoda brevipoda* and *Rana brevipoda porosa* distributed widely in Japan for the purpose of clarifying the relationship between different species, subspecies, races or populations and of confirming the classification proposed by KAWAMURA (1962) and the assumption made by KAWAMURA and NISHIOKA (1977) on the origin of *Rana brevipoda porosa.*

## MATERIALS AND METHODS

Four kinds of Japanese pond frogs, *Rana nigromaculata* HALLOWELL, the typical race and the Nagoya race of *Rana brevipoda brevipoda* ITO and *Rana brevipoda porosa*

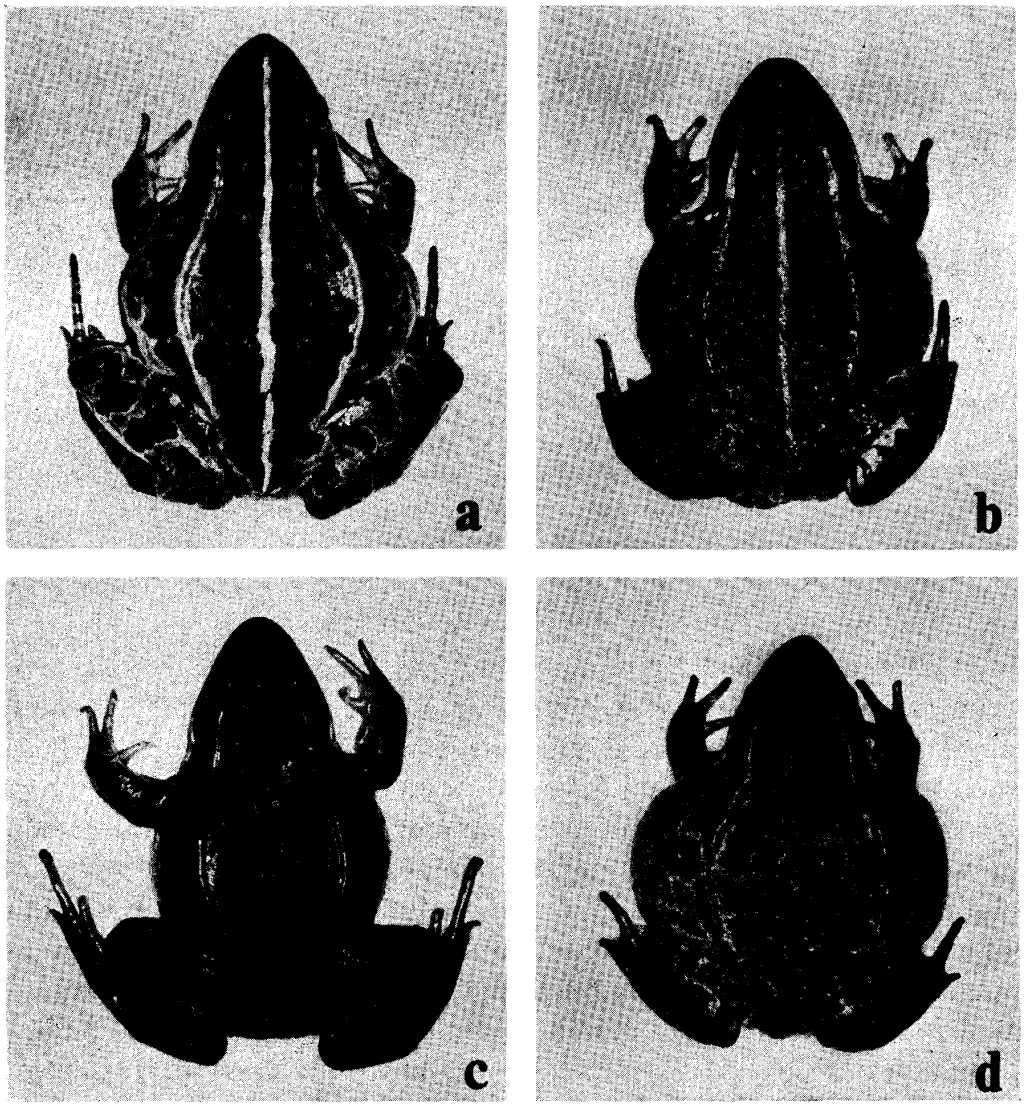


Fig. 1. Four kinds of pond frogs (♀) in Japan.

a. *Rana nigromaculata* from Hiroshima
b. *Rana brevipoda porosa* from Maki
c. *Rana brevipoda brevipoda*, the Nagoya race from Ina
d. *Rana brevipoda brevipoda*, the typical race from Konko

(COPE) have their own range (Fig. 1). While *Rana brevipoda brevipoda* is sympatric with *Rana nigromaculata* in a large part of the districts of San-yo, Kinki and Chubu and the northeastern tip of Shikoku, *Rana brevipoda porosa* is allopatric from *Rana nigromaculata* and distributed in the plains of Kanto, Sendai and Echigo and the basin of the River Shinano (Fig. 2). The areas other than those stated above are generally occupied by *Rana nigromaculata* alone.

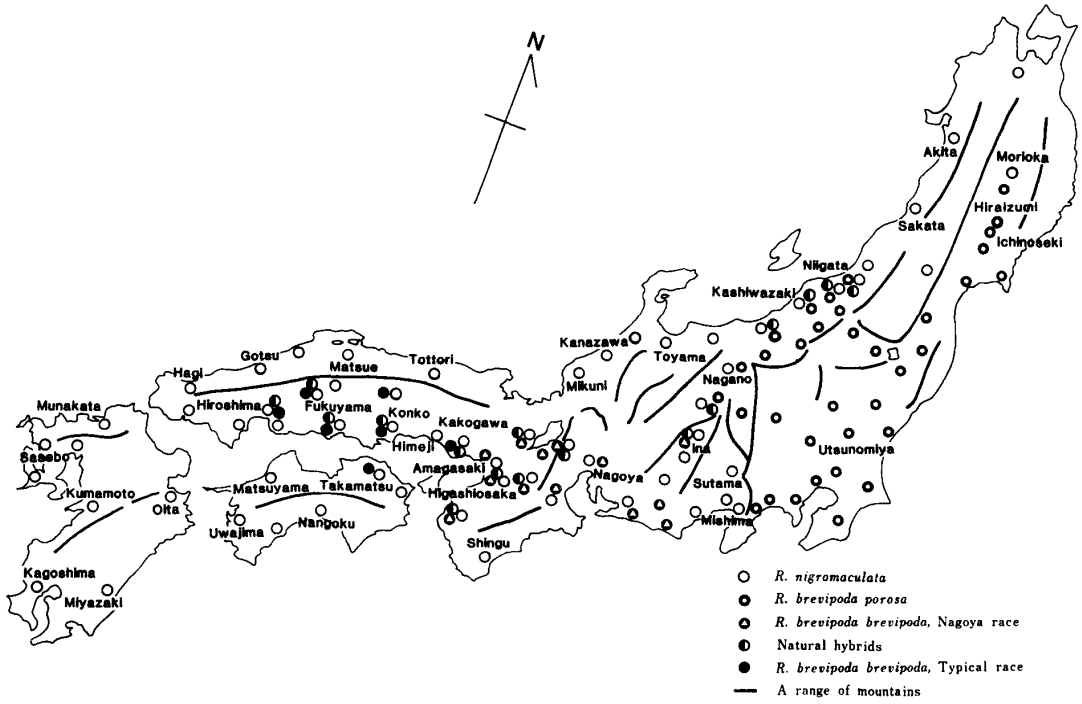


Fig. 2. Distribution of the pond frog group in Japan and the stations where the frogs analyzed were collected.

The frogs used in the present study included all the four kinds of pond frogs stated above. They were collected during five years from 1976 to 1980. A total of 1540 mature *Rana nigromaculata* of both sexes were collected from 39 stations in Honshu, Shikoku and Kyushu and additionally from one station in Korea. A total of 337 mature *Rana brevipoda brevipoda* of both sexes belonging to the typical race and Nagoya race and *Rana brevipoda porosa* were collected from 13 stations in Honshu. The stations and the number of frogs collected from each station were as follows.

1. *Rana nigromaculata*

| District | Prefecture | Station | No. | Station | No. | Station | No. |
|---|---|---|---|---|---|---|---|
| Tohoku | Akita | Akita | 6 | | | | |
| (2 stations) | Yamagata | Sakata | 48 | | | | |
| Hokuriku | Niigata | Shibata | 16 | Niigata | 20 | Kashiwazaki | 26 |
| (7 stations) | | Joetsu* | 36 | | | | |
| | Toyama | Toyama | 37 | | | | |
| | Ishikawa | Kanazawa | 55 | | | | |
| | Fukui | Mikuni | 25 | | | | |
| Chubu | Nagano | Okaya | 64 | Ina | 2 | Iida | 40 |
| (6 stations) | Yamanashi | Sutama | 35 | | | | |
| | Shizuoka | Mishima | 67 | | | | |
| | Aichi | Nagoya | 5 | | | | |
| Kinki | Shiga | Maibara | 13 | | | | |
| (4 stations) | Mie | Igaueno | 48 | | | | |
| | Wakayama | Shingu | 6 | | | | |
| | Osaka | Higashiosaka | 38 | | | | |
| San-in | Tottori | Tottori | 64 | | | | |
| (4 stations) | Shimane | Matsue | 75 | Gotsu | 23 | | |
| | Yamaguchi | Hagi | 66 | | | | |
| San-yo | Hyogo | Himeji | 6 | | | | |
| (6 stations) | Okayama | Konko | 83 | | | | |
| | Hiroshima | Hiroshima | 60 | Kumano | 12 | Hiro | 19 |
| | Yamaguchi | Yamaguchi | 52 | | | | |
| Shikoku | Kagawa | Takamatsu | 42 | | | | |
| (4 stations) | Kochi | Nangoku | 32 | | | | |
| | Ehime | Uwajima | 39 | Matsuyama | 23 | | |
| Kyushu | Fukuoka | Munakata | 58 | | | | |
| (6 stations) | Nagasaki | Sasebo | 58 | | | | |
| | Kumamoto | Kumamoto | 49 | | | | |
| | Kagoshima | Kagoshima | 60 | | | | |
| | Miyazaki | Miyazaki | 56 | | | | |
| | Oita | Oita | 55 | | | | |
| Korea | | Suwon | 21 | | | | |

* Takata in MORIYA's paper (1952)

2. *Rana brevipoda brevipoda* (typical race)

| District | Prefecture | Station | No. |
|---|---|---|---|
| San-yo | Okayama | Konko | 38 |

3. *Rana brevipoda brevipoda* (Nagoya race)

| District | Prefecture | Station | No. |
|---|---|---|---|
| Chubu | Nagano | Ina | 33 |
| Kinki | Shiga | Maibara | 28 |
| (3 stations) | Mie | Igaueno | 4 |
| | Osaka | Higashiosaka | 20 |

4. *Rana brevipoda porosa*

| District | Prefecture | Station | No. | Station | No. | Station | No. |
|---|---|---|---|---|---|---|---|
| Tohoku | Iwate | Morioka | 26 | Hiraizumi | 9 | Ichinoseki | 39 |
| (4 stations) | Fukushima | Sukagawa | 6 | | | | |
| Kanto | Tochigi | Utsunomiya | 56 | | | | |
| Hokuriku | Niigata | Shibata | 31 | Maki | 28 | | |
| Chubu | Nagano | Nagano | 19 | | | | |

Five kinds of enzymes, malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), $\alpha$-glycerophosphate dehydrogenase ($\alpha$-GDH) and superoxide dismutase (SOD), were extracted from skeletal muscles of the hind legs of the above-stated frogs and analyzed by starch-gel electrophoresis.

The method of starch-gel electrophoresis used in the present study has been described previously by NISHIOKA, OHTANI and SUMIDA (1980), except the method for analyzing SOD. In the latter, a tris-borate-EDTA buffer system of pH 8.0 (2.1 M tris, 2.0 M borate and 0.068 M EDTA) was used by diluting to one part in 100 for making starch-gel and to one part in ten for the bridge.

The staining of the electrophoretic pattern from each enzyme was principally made in accordance with BREWER (1970), although the somewhat modified agar overlay method was applied.

## OBSERVATION

### *I. Malate dehydrogenase (MDH)*

The electrophoretic patterns of MDH extracted from skeletal muscles of *Rana nigromaculata* and *Rana brevipoda* consisted of two major and two subordinate bands, which moved toward the anode. No sexual differences were found in these MDH patterns. Of the two major bands, the slowly and fast migrating bands were named MDH-A and MDH-B, respectively. These two bands were controlled by the alleles of two different loci, as previously reported (NISHIOKA, OHTANI and SUMIDA, 1980). It was found that two and six codominant alleles were represented at loci MDH-A and MDH-B, respectively. The former two alleles were named *MDH-A*$^a$ ($A^a$) and *MDH-A*$^b$ ($A^b$), while the latter six were named *MDH-B*$^a$ ($B^a$), *MDH-B*$^b$ ($B^b$), *MDH-B*$^c$ ($B^c$), *MDH-B*$^d$ ($B^d$), *MDH-B*$^e$ ($B^e$) and *MDH-B*$^f$ ($B^f$) in order of mobility of the bands from fast to slow (Fig. 3).

#### 1. MDH-A

a. *Rana nigromaculata* populations

A total of 1540 frogs collected from 39 stations in Honshu, Shikoku and Kyushu and from Suwon, Korea were nearly uniform in MDH-A pattern. They were of monomorphic $A^aA^a$ except one individual found in the Munakata population in the Kyushu district. The MDH-A pattern of this exceptional frog consisted

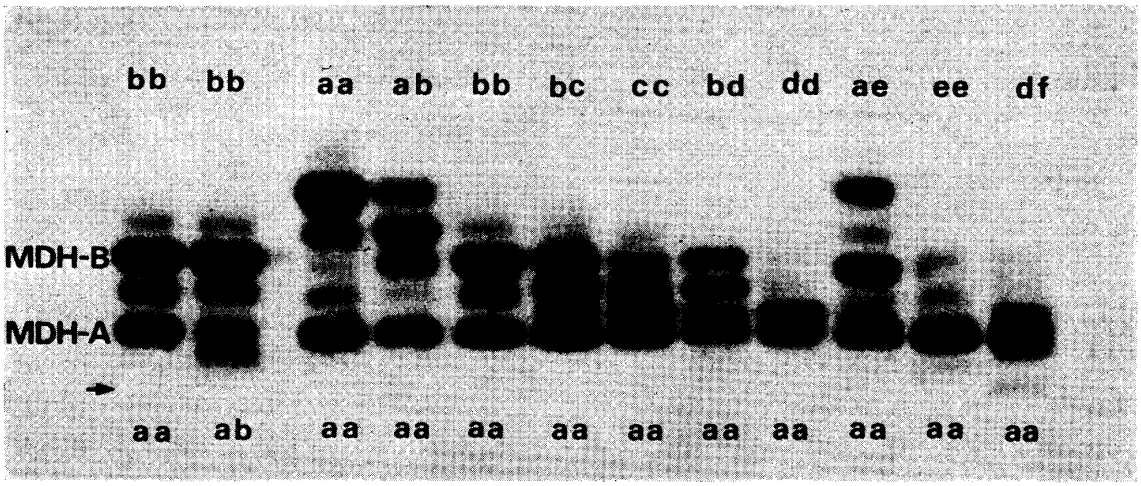


Fig. 3. Ten kinds of phenotypes at the MDH-B locus and two kinds of phenotypes at the MDH-A locus in Japanese and Korean pond frogs.

Upper: MDH-B locus
aa, $B^aB^a$ ab, $B^aB^b$ bb, $B^bB^b$ bc, $B^bB^c$ cc, $B^cB^c$
bd, $B^bB^d$ dd, $B^dB^d$ ae, $B^aB^e$ ee, $B^eB^e$ df, $B^dB^f$
Lower: MDH-A locus
aa, $A^aA^a$ ab, $A^aA^b$

of two $A^aA^b$ bands in phenotype. In the Munakata population, allele $A^a$ was 99.1% in frequency, while it was 100% in the other 39 populations.

b. *Rana brevipoda* populations

A total of 337 frogs belonging to *Rana brevipoda porosa* and the typical race and the Nagoya race of *Rana brevipoda brevipoda* were completely uniform and of monomorphic $A^aA^a$ in MDH-A pattern.

## 2. MDH-B

a. *Rana nigromaculata* populations

The MDH-B patterns of 1540 frogs collected from 39 stations in Japan and one station in Korea revealed 11 kinds of phenotypes; $B^aB^a$ in 192 individuals, $B^bB^b$ in 1135, $B^dB^d$ in 102, $B^eB^e$ in one, $B^aB^b$ in three, $B^aB^c$ in one, $B^aB^e$ in 19, $B^bB^c$ in eight, $B^bB^d$ in 76, $B^bB^f$ in two and $B^dB^f$ in one. These phenotypes were controlled by six codominant alleles, *MDH-B*$^a$, *MDH-B*$^b$, *MDH-B*$^c$, *MDH-B*$^d$, *MDH-B*$^e$ and *MDH-B*$^f$. The number of individuals analyzed and the phenotype and allele frequencies in each population are presented in Table 1. The geographic distribution of MDH-B alleles is shown in Fig. 4.

The populations of Akita and Sakata in the Tohoku district and the populations of Niigata, Kashiwazaki, Joetsu, Kanazawa, Toyama and Mikuni in the Hokuriku district were all of monomorphic $B^bB^b$ in MDH-B pattern. However, the Shibata population in the Hokuriku district was not uniform. As *Rana nigromaculata* were usually mingled with *Rana brevipoda porosa* in distribution and natural hybridization frequently occurred in the area around Shibata, specimens

that closely resembled typical *Rana nigromaculata* in appearance were selected and analyzed in the present study. It was found that eight of them had hybrid phenotype $B^bB^c$ between $B^bB^b$ from *Rana nigromaculata* and $B^cB^c$ from *Rana brevipoda porosa*, while the other eight were all of $B^bB^b$ in MDH-B pattern.

Seventeen populations including the populations of Igaueno, Shingu and Higashiosaka in the Kinki district, the populations of Himeji, Konko, Hiro, Kumano and Hiroshima in the San-yo district, the populations of Takamatsu, Nangoku, Matsuyama and Uwajima in the Shikoku district and the populations of Munakata, Sasebo, Kumamoto, Kagoshima and Oita in the Kyushu district were all of monomorphic $B^bB^b$ in MDH-B pattern. However, in the Miyazaki population, two out of 56 frogs showed hybrid phenotype $B^bB^f$, while the other 54 were of $B^bB^b$ in MDH-B pattern. Allele *MDH-B*$^f$ controlling the enzyme that migrated most slowly was also found in one individual of the Suwon population as hybrid phenotype $B^dB^f$.

The six populations of the Chubu district were characteristic in showing abundant existence of allele *MDH-B*$^a$. The populations of Sutama, Mishima and Ina were all of monomorphic $B^aB^a$ in MDH-B pattern. The bands of this phenotype were the fastest in mobility. Of 64 frogs of the Okaya population, 61 were of $B^aB^a$, while the other three were of hybrid phenotype $B^aB^b$. In the Iida population 24 out of 40 were of $B^aB^a$, while one was of $B^eB^e$ and 15 were of $B^aB^e$ in MDH-B pattern. Of five frogs of the Nagoya population, three were of $B^aB^a$ and the other two of $B^aB^e$. Allele *MDH-B*$^a$ in the populations of Iida and Nagoya were 78.8% and 80.0% in frequency, respectively, although the number of individuals was very small in the latter population. In the Maibara population which was located about 60 km west from the Nagoya population, there were three kinds of phenotypes in MDH-B patterns of 13 frogs. Ten of the latter were of $B^bB^b$, two others of $B^aB^e$ and the remaining was of $B^aB^c$. It was evident that allele *MDH-B*$^c$ was derived from *Rana brevipoda*. Alleles *MDH-B*$^a$, *MDH-B*$^b$, *MDH-B*$^c$ and *MDH-B*$^e$ were 11.5%, 76.9%, 3.8% and 7.7% in frequency, respectively.

The presence of allele *MDH-B*$^d$ was the characteristic feature of the four populations in the San-in district. Of 64 frogs of the Tottori population, 11 were of $B^bB^b$, 27 of $B^dB^d$ and 26 of $B^bB^d$ in MDH-B pattern. Alleles *MDH-B*$^b$ and *MDH-B*$^d$ were 37.5% and 62.5% in frequency, respectively. In the Matsue population, 52 out of 75 frogs were of $B^dB^d$ and the other 23 of $B^bB^d$. No $B^bB^b$ phenotype was found in this population. Alleles *MDH-B*$^b$ and *MDH-B*$^d$ were 15.3% and 84.7% in frequency, respectively. In the Gotsu population, nine were of $B^bB^b$, three of $B^dB^d$ and eleven of $B^bB^d$. Alleles *MDH-B*$^b$ and *MDH-B*$^d$ were 63.0% and 37.0% in frequency, respectively. Of 66 frogs of the Hagi population, 51 were of $B^bB^b$ and 15 of $B^bB^d$ in phenotype. Alleles *MDH-B*$^b$ and *MDH-B*$^d$ were 88.6% and 11.4% in frequency, respectively. In the Yamaguchi population which was located about 25 km south from the Hagi population, one out of 52 frogs was of $B^bB^d$ in phenotype, while the others were of $B^bB^b$. Alleles *MDH-B*$^b$ and *MDH-B*$^d$ were 99.0% and 1.0% in frequency, respectively.

TABLE 1

Frequencies of phenotypes and alleles of

| Station | No. of frogs | Phenotypes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $B^aB^a$ | $B^bB^b$ | $B^cB^c$ | $B^dB^d$ | $B^eB^e$ | $B^fB^f$ | $B^aB^b$ | $B^aB^c$ | $B^aB^e$ |
| 1. Akita | 6 | | 6 | | | | | | | |
| 2. Sakata | 48 | | 48 | | | | | | | |
| 3 Shibata | 16 | | 8 | 0 | | | | | | |
| | | | ( 9.0) | (1.0) | | | | | | |
| 4. Niigata | 20 | | 20 | | | | | | | |
| 5. Kashiwazaki | 26 | | 26 | | | | | | | |
| 6. Joetsu | 36 | | 36 | | | | | | | |
| 7. Toyama | 37 | | 37 | | | | | | | |
| 8. Kanazawa | 55 | | 55 | | | | | | | |
| 9. Mikuni | 25 | | 25 | | | | | | | |
| 10. Okaya | 64 | 61 | 0 | | | | | 3 | | |
| | | (61.1) | ( 0.0) | | | | | (2.9) | | |
| 11. Sutama | 35 | 35 | | | | | | | | |
| 12. Mishima | 67 | 67 | | | | | | | | |
| 13. Ina | 2 | 2 | | | | | | | | |
| 14. Iida | 40 | 24 | | | | 1 | | | | 15 |
| | | (24.8) | | | | (1.8) | | | | (13.4) |
| 15. Nagoya | 5 | 3 | | | | 0 | | | | 2 |
| | | ( 3.2) | | | | (0.2) | | | | ( 1.6) |
| 16. Maibara | 13 | 0 | 10 | 0 | | 0 | | 0 | 1 | 2 |
| | | ( 0.2) | ( 7.7) | (0.1) | | (0.1) | | (2.3) | (0.1) | ( 0.2) |
| 17. Igaueno | 48 | | 48 | | | | | | | |
| 18. Shingu | 6 | | 6 | | | | | | | |
| 19. Higashiosaka | 38 | | 38 | | | | | | | |
| 20. Himeji | 6 | | 6 | | | | | | | |
| 21. Tottori | 64 | | 11 | | 27 | | | | | |
| | | | ( 9.0) | | (25.0) | | | | | |
| 22. Matsue | 75 | | 0 | | 52 | | | | | |
| | | | ( 1.8) | | (53.8) | | | | | |
| 23. Gotsu | 23 | | 9 | | 3 | | | | | |
| | | | ( 9.1) | | ( 3.1) | | | | | |
| 24. Hagi | 66 | | 51 | | 0 | | | | | |
| | | | (51.8) | | ( 0.9) | | | | | |
| 25. Yamaguchi | 52 | | 51 | | 0 | | | | | |
| | | | (51.0) | | ( 0.0) | | | | | |
| 26. Hiroshima | 60 | | 60 | | | | | | | |
| 27. Kumano | 12 | | 12 | | | | | | | |
| 28. Hiro | 19 | | 19 | | | | | | | |
| 29. Konko | 83 | | 83 | | | | | | | |
| 30. Takamatsu | 42 | | 42 | | | | | | | |
| 31. Nangoku | 32 | | 32 | | | | | | | |
| 32. Uwajima | 39 | | 39 | | | | | | | |
| 33. Matsuyama | 23 | | 23 | | | | | | | |
| 34. Munakata | 58 | | 58 | | | | | | | |
| 35. Sasebo | 58 | | 58 | | | | | | | |
| 36. Kumamoto | 49 | | 49 | | | | | | | |
| 37. Kagoshima | 60 | | 60 | | | | | | | |
| 38. Miyazaki | 56 | | 54 | | | | 0 | | | |
| | | | (54.0) | | | | (0.0) | | | |
| 39. Oita | 55 | | 55 | | | | | | | |
| 40. Suwon | 21 | | | | 20 | | 0 | | | |
| | | | | | (20.0) | | (0.0) | | | |
| Total | 1540 | 192 | 1135 | 0 | 102 | 1 | 0 | 3 | 1 | 19 |
| | (%) | (12.5) | (73.7) | | ( 6.6) | (0.1) | | (0.2) | (0.1) | ( 1.2) |

MDH-B in *Rana nigromaculata* populations

| (Expected number) | | | | | | Alleles (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $B^bB^c$ | $B^bB^d$ | $B^bB^e$ | $B^bB^f$ | $B^cB^e$ | $B^dB^f$ | $B^a$ | $B^b$ | $B^c$ | $B^d$ | $B^e$ | $B^f$ |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| 8 | | | | | | | 75.0 | 25.0 | | | |
| (6.0) | | | | | | | | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | 97.7 | 2.3 | | | | |
| | | | | | | 100 | | | | | |
| | | | | | | 100 | | | | | |
| | | | | | | 100 | | | | | |
| | | | | | | 78.8 | | | | 21.2 | |
| | | | | | | 80.0 | | | | 20.0 | |
| 0 | | 0 | | 0 | | 11.5 | 76.9 | 3.8 | | 7.7 | |
| (0.8) | | (1.5) | | (0.1) | | | | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | 26 | | | | | | 37.5 | | 62.5 | | |
| | (30.0) | | | | | | | | | | |
| | 23 | | | | | | 15.3 | | 84.7 | | |
| | (19.4) | | | | | | | | | | |
| | 11 | | | | | | 63.0 | | 37.0 | | |
| | (10.7) | | | | | | | | | | |
| | 15 | | | | | | 88.6 | | 11.4 | | |
| | (13.1) | | | | | | | | | | |
| | 1 | | | | | | 99.0 | | 1.0 | | |
| | ( 1.0) | | | | | | | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | | | | | | | 100 | | | | |
| | | | 2 | | | | 98.2 | | | | 1.8 |
| | | | (1.9) | | | | | | | | |
| | | | | | | | 100 | | | | |
| | | | | | 1 | | | | 97.6 | | 2.4 |
| | | | | | (1.0) | | | | | | |
| 8 | 76 | 0 | 2 | 0 | 1 | 13.2 | 76.6 | 0.3 | 9.1 | 0.7 | 0.1 |
| (0.5) | ( 4.9) | | (0.1) | | (0.1) | | | | | | |

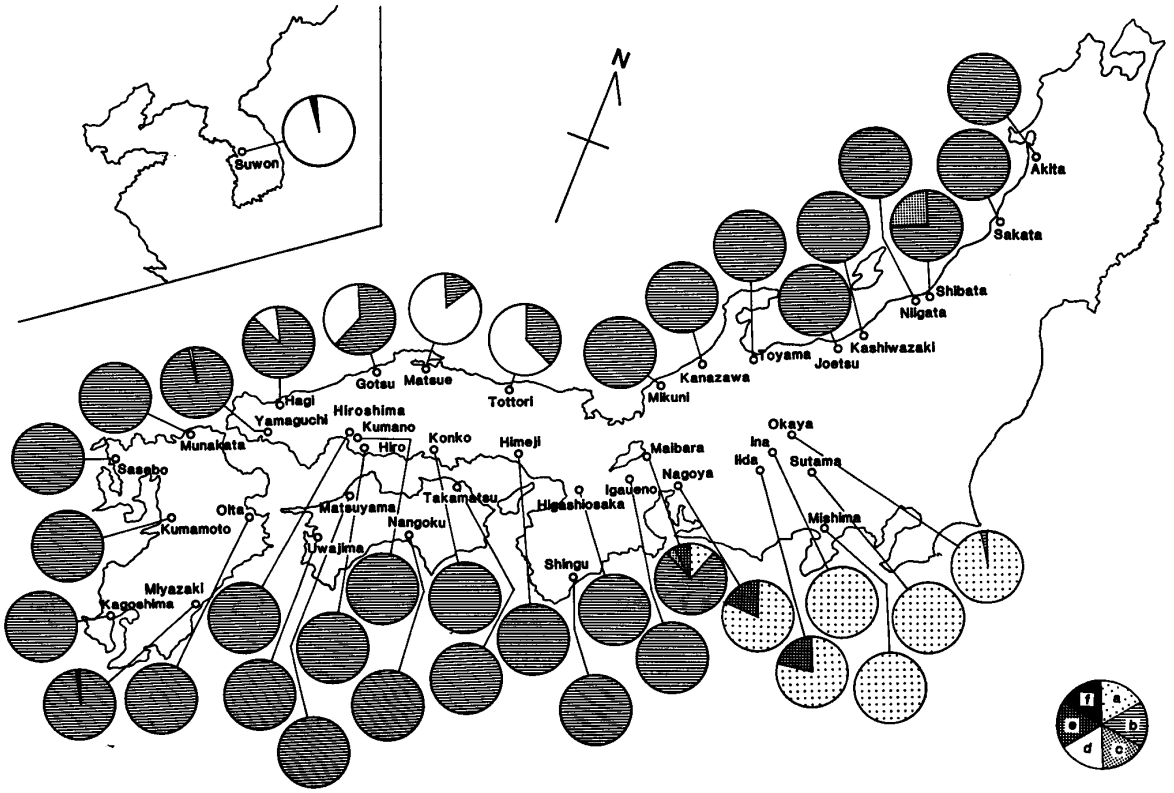


Fig. 4. Geographic distribution of MDH-B alleles among 40 populations of *Rana nigromaculata* in Japan and Korea.

a, Allele *MDH-B*$^a$ b, Allele *MDH-B*$^b$ c, Allele *MDH-B*$^c$
d, Allele *MDH-B*$^d$ e, Allele *MDH-B*$^e$ f, Allele *MDH-B*$^f$

In the Suwon population in Korea, 20 out of 21 frogs were of $B^dB^d$ in MDH-B pattern, while the remaining was of $B^dB^f$. Alleles *MDH-B*$^d$ and *MDH-B*$^f$ were 97.6% and 2.4% in frequency, respectively. It was interesting that there was also a low percentage of allele *MDH-B*$^f$ in the Miyazaki population in the Kyushu district.

b. *Rana brevipoda* populations

Electrophoretic patterns were examined in a total of 337 *Rana brevipoda* from 13 stations. These specimens consisted of 214 *Rana brevipoda porosa* collected from Morioka, Hiraizumi, Ichinoseki, Sukagawa, Utsunomiya, Shibata, Maki and Nagano, 85 *Rana brevipoda brevipoda* of the Nagoya race from Ina, Maibara, Igaueno and Higashiosaka and 38 *Rana brevipoda brevipoda* of the typical race from Konko. It was found that there were six kinds of phenotypes, $B^aB^a$, $B^bB^b$, $B^cB^c$, $B^aB^c$, $B^bB^c$ and triploid $B^bB^cB^c$ in MDH-B pattern of these frogs (Fig. 3). All the individuals in 11 populations other than the populations of Shibata and Ina were of monomorphic $B^cB^c$ in MDH-B pattern. The five kinds of phenotypes other than $B^cB^c$ were all found in the populations of Shibata and Ina (Table 2; Fig. 5).

Of 31 *Rana brevipoda porosa* of the Shibata population in the Hokuriku district, two were of $B^bB^b$, 14 of $B^cB^c$, 13 of $B^bB^c$ and the remaining two of triploid $B^bB^cB^c$

TABLE 2
Frequencies of phenotypes and alleles of MDH-B in *Rana brevipoda* populations

| Station | No. of frogs | Phenotypes (Expected number) | | | | | | Alleles (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $B^aB^a$ | $B^bB^b$ | $B^cB^c$ | $B^aB^c$ | $B^bB^c$ | $B^bB^cB^c$ | $B^a$ | $B^b$ | $B^c$ |
| 1. Morioka | 26 | | | 26 | | | | | | 100 |
| 2. Hiraizumi | 9 | | | 9 | | | | | | 100 |
| 3. Ichinoseki | 39 | | | 39 | | | | | | 100 |
| 4. Sukagawa | 6 | | | 6 | | | | | | 100 |
| 5. Utsunomiya | 56 | | | 56 | | | | | | |
| 6. Shibata | 31 | | 2 | 14 | | 13 | 2 | | 29.7 | 70.3 |
| | | | (2.7) | (15.3) | | (12.9) | | | | |
| 7. Maki | 28 | | | 28 | | | | | | 100 |
| 8. Nagano | 19 | | | 19 | | | | | | 100 |
| 9. Ina | 33 | 1 | | 27 | 5 | | | 10.6 | | 89.4 |
| | | (0.4) | | (26.4) | (6.3) | | | | | |
| 10. Maibara | 28 | | | 28 | | | | | | 100 |
| 11. Igaueno | 4 | | | 4 | | | | | | 100 |
| 12. Higashiosaka | 20 | | | 20 | | | | | | 100 |
| 13. Konko | 38 | | | 38 | | | | | | 100 |
| Total | 337 | 1 | 2 | 314 | 5 | 13 | 2 | 1.0 | 2.8 | 96.2 |
| | (%) | (0.3) | (0.6) | (93.2) | (1.5) | ( 3.9) | (0.6) | | | |

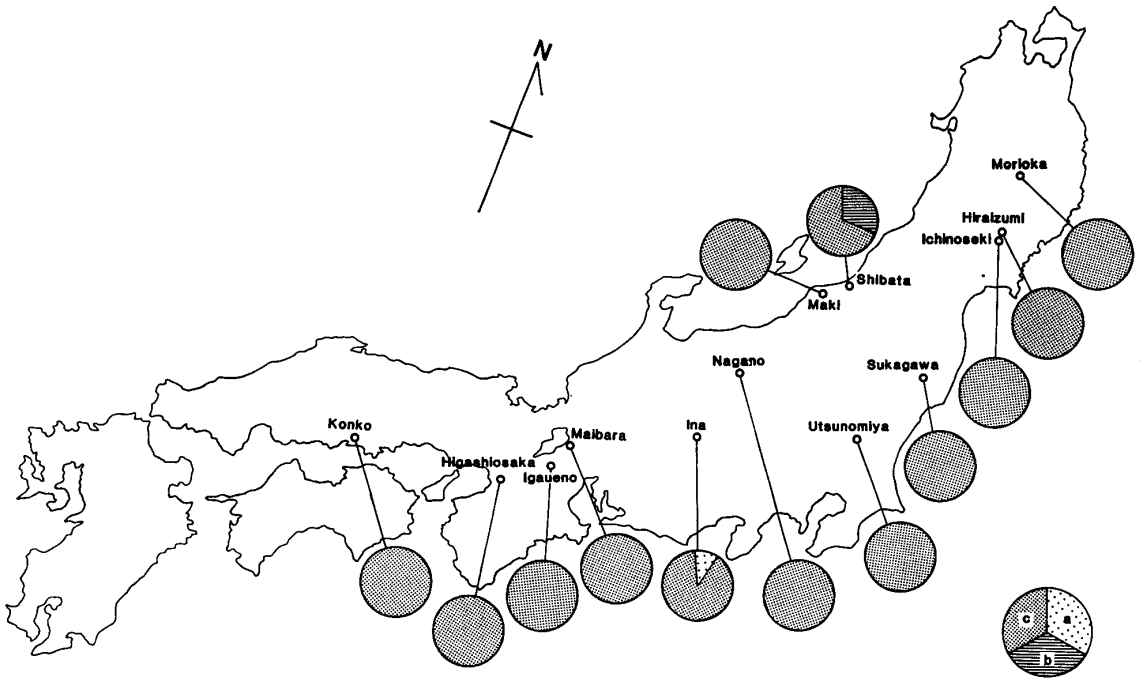


Fig. 5. Geographic distribution of MDH-B alleles among 13 populations of *Rana brevipoda* in Japan.

a, Allele *MDH-B*$^a$ b, Allele *MDH-B*$^b$ c, Allele *MDH-B*$^c$

in MDH-B pattern. Alleles *MDH-B*$^b$ and *MDH-B*$^c$ in this population were 29.7% and 70.3% in frequency, respectively. Of 33 *Rana brevipoda brevipoda* of the Ina population in the Chubu district, 27 were of $B^cB^c$, one was of $B^aB^a$ and the remaining five were of $B^aB^c$ in MDH-B pattern. Alleles *MDH-B*$^a$ and *MDH-B*$^c$ were 10.6% and 89.4% in frequency, respectively (Table 2; Fig. 5).

c. Summary of results from MDH-B

By analyzing MDH extracted from a total of 1540 *Rana nigromaculata* collected from 39 stations in Japan and one station in Korea, it was observed that there were 11 kinds of phenotypes in MDH-B enzyme. These phenotypes were determined by six kinds of codominant alleles, *MDH-B$^a$*, *MDH-B$^b$*, *MDH-B$^c$*, *MDH-B$^d$*, *MDH-B$^e$* and *MDH-B$^f$*, which were 13.2%, 76.6%, 0.3%, 9.1%, 0.7% and 0.1% in frequency, respectively. On the other hand, a total of 337 *Rana brevipoda* including 214 *Rana brevipoda porosa* collected from eight stations, 85 *Rana brevipoda brevipoda* of the Nagoya race collected from four stations and 38 *Rana brevipoda brevipoda* of the typical race from one station were analyzed in terms of enzyme MDH-B. It was found that there were six kinds of phenotypes determined by three kinds of codominant alleles, *MDH-B$^a$*, *MDH-B$^b$* and *MDH-B$^c$*, which were 1.0%, 2.8% and 96.2% in frequency, respectively.

As shown in Fig. 4, the 29 populations of *Rana nigromaculata* distributed in the districts of Tohoku, Hokuriku, Kinki, San-yo, Shikoku and Kyushu were almost completely of monomorphic *B$^b$B$^b$* in MDH-B pattern. The six populations distributed in the Chubu district differed from these populations in that allele *MDH-B$^b$* was very scarce. Three of them were monomorphic and contained allele *MDH-B$^a$* alone. Another population contained a slight amount of *MDH-B$^b$* in addition to *MDH-B$^a$*. In the other two populations, more than three-fourths of allele frequency for enzyme MDH-B were occupied by *MDH-B$^a$*, while the remaining part was by *MDH-B$^e$* which was peculiar to these populations.

The presence of allele *MDH-B$^d$* was also the characteristic feature of the four populations of the San-in district. This allele in the populations of Matsue, Tottori, Gotsu and Hagi was 84.7%, 62.5%, 37.0% and 11.4% in frequency, respectively. The remaining percent in each population was that of allele *MDH-B$^b$*. In the Yamaguchi population located about 25 km south from the Hagi population, allele *MDH-B$^d$* was 1.0% in frequency, while allele *MDH-B$^b$* was 99%. Enzyme MDH-B in the Suwon population of Korea was almost completely controlled by allele *MDH-B$^d$*, although a slight amount of *MDH-B$^f$* was contained in this population. Allele *MDH-B$^f$* was also slightly contained in the Miyazaki population of the Kyushu district in addition to allele *MDH-B$^b$*.

Eleven of the 13 populations of *Rana brevipoda* were of monomorphic *B$^c$B$^c$* in MDH-B pattern, while the remaining two populations, Shibata and Ina, were polymorphic. The area around Shibata is characteristic in that natural hybridization between *Rana nigromaculata* and *Rana brevipoda* has frequently occurred. While about two-thirds of the allele frequency for enzyme MDH-B in the Shibata population of *Rana brevipoda* were occupied by *MDH-B$^c$*, which was proper to this species, the remaining part was occupied by *MDH-B$^b$* derived from *Rana nigromaculata*. On the other hand, there was a similar finding in the Shibata population of *Rana nigromaculata*. While about three-fourths of the allele frequency for enzyme MDH-B were occupied by *MDH-B$^b$*, the other part was occupied by *MDH-B$^c$* derived from *Rana brevipoda*. In the Ina population, a small part of the allele frequency of *Rana brevipoda* was occupied by *MDH-B$^a$*

derived from *Rana nigromaculata,* while the most part was occupied by *MDH-B*$^c$, which was proper to *Rana brevipoda.*

The Maibara population of *Rana nigromaculata* was peculiar in that enzyme MDH-B was polymorphic in electrophoretic pattern. Although about three-fourths of the allele frequency were occupied by *MDH-B*$^b$, the other part was occupied by a mixture of *MDH-B*$^a$, *MDH-B*$^c$ and *MDH-B*$^e$. While alleles *MDH-B*$^a$, *MDH-B*$^b$ and *MDH-B*$^e$ were proper in *Rana nigromaculata,* allele *MDH-B*$^c$ was derived from *Rana brevipoda.*

## *II. Isocitrate dehydrogenase (IDH)*

The electrophoretic patterns of IDH extracted from skeletal muscles of *Rana nigromaculata* and *Rana brevipoda* consisted of two band-groups which migrated toward the anode. When the slowly and fast migrating band-groups were named IDH-A and IDH-B, respectively, the two species differed from each other in IDH-B. The IDH-B patterns of the two species were polymorphic and determined by six codominant alleles which were named *IDH-B*$^b$(*B*$^b$), *IDH-B*$^c$ (*B*$^c$), *IDH-B*$^d$ (*B*$^d$), *IDH-B*$^e$ (*B*$^e$), *IDH-B*$^f$ (*B*$^f$) and *IDH-B*$^g$ (*B*$^g$) in order of mobility of the bands from fast to slow (Fig. 6). There was no sexual difference in the IDH-A or IDH-B patterns of each species.

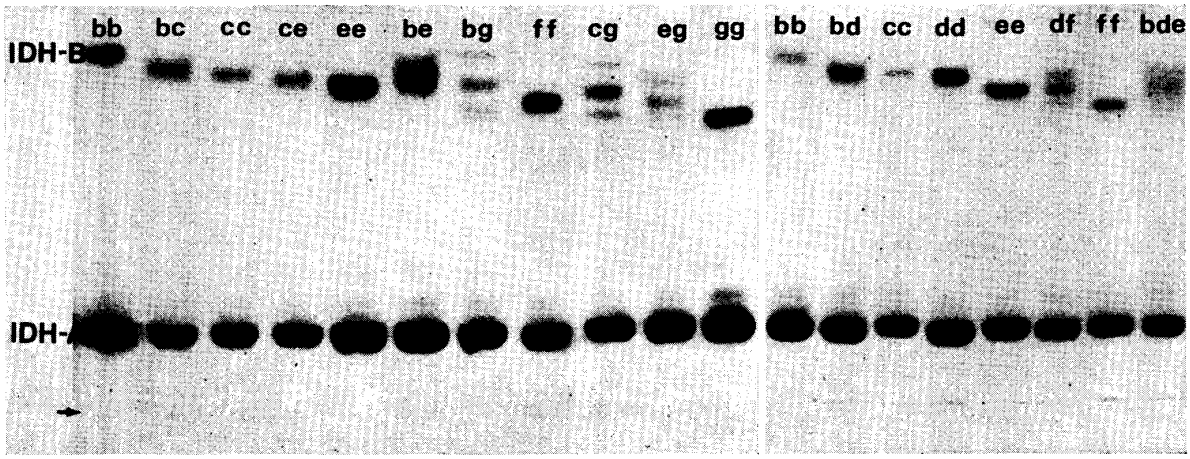


Fig. 6. Fifteen kinds of phenotypes at the IDH-B locus in Japanese and Korean pond frogs.

bb, $B^bB^b$ bc, $B^bB^c$ cc, $B^cB^c$ ce, $B^cB^e$ ee, $B^eB^e$
be, $B^bB^e$ bg, $B^bB^g$ ff, $B^fB^f$ cg, $B^cB^g$ eg, $B^eB^g$
gg, $B^gB^g$ bd, $B^bB^d$ dd, $B^dB^d$ df, $B^dB^f$ bde, $B^bB^dB^e$

### 1. IDH-A

A total of 1540 *Rana nigromaculata* collected from 39 stations in Japan and one station in Korea and a total of 337 *Rana brevipoda* including the typical race and the Nagoya race of *Rana brevipoda brevipoda* from five stations and *Rana brevipoda porosa* from eight stations, were all uniform in IDH-A pattern. All the populations of *Rana nigromaculata* and *Rana brevipoda* were monomorphic in IDH-A pattern.

TABLE 3

Frequencies of phenotypes and alleles of

| Station | No. of frogs | Phenotypes | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $B^bB^b$ | $B^cB^c$ | $B^eB^e$ | $B^fB^f$ | $B^gB^g$ | $B^bB^c$ | $B^bB^e$ |
| 1. Akita | 6 | 6 | | | | | | |
| 2. Sakata | 48 | 43 | | 0 | | | | 5 |
| | | (43.1) | | ( 0.1) | | | | ( 4.7) |
| 3. Shibata | 16 | 9 | | | 0 | | | |
| | | ( 9.8) | | | (0.8) | | | |
| 4. Niigata | 20 | 20 | | | | | | |
| 5. Kashiwazaki | 26 | 26 | | | | | | |
| 6. Joetsu | 36 | 29 | | 0 | | | | 7 |
| | | (29.4) | | ( 0.3) | | | | ( 6.3) |
| 7. Toyama | 37 | 31 | | 2 | | | | 4 |
| | | (29.4) | | ( 0.4) | | | | ( 7.1) |
| 8. Kanazawa | 55 | 17 | | 10 | | | | 28 |
| | | (17.5) | | (10.5) | | | | (27.0) |
| 9. Mikuni | 25 | 9 | | 1 | | | | 15 |
| | | (10.9) | | ( 2.9) | | | | (11.2) |
| 10. Okaya | 64 | 58 | | | 0 | | | |
| | | (58.1) | | | (0.1) | | | |
| 11. Sutama | 35 | 35 | | | | | | |
| 12. Mishima | 67 | 67 | | | | | | |
| 13. Ina | 2 | 2 | | | | | | |
| 14. Iida | 40 | 31 | | 0 | 0 | | | 6 |
| | | (30.6) | | ( 0.3) | (0.1) | | | ( 6.2) |
| 15. Nagoya | 5 | 3 | | 0 | | | | 2 |
| | | ( 3.2) | | ( 0.2) | | | | ( 1.6) |
| 16. Maibara | 13 | 12 | | | 0 | | | |
| | | (12.0) | | | (0.0) | | | |
| 17. Igaueno | 48 | 28 | | 2 | 1 | | | 14 |
| | | (28.2) | | ( 1.8) | (0.0) | | | (14.1) |
| 18. Shingu | 6 | 6 | | | | | | |
| 19. Higashiosaka | 38 | 10 | | 12 | 0 | | | 15 |
| | | ( 8.1) | | (10.5) | (0.0) | | | (18.4) |
| 20. Himeji | 6 | 1 | | 2 | | | | 3 |
| | | ( 1.0) | | ( 2.0) | | | | ( 2.9) |
| 21. Tottori | 64 | 0 | 1 | 53 | | | 1 | 4 |
| | | ( 0.1) | ( 0.3) | (51.6) | | | ( 0.3) | ( 4.5) |
| 22. Matsue | 75 | 0 | 0 | 70 | | | | 1 |
| | | ( 0.0) | ( 0.1) | (70.1) | | | | ( 1.0) |
| 23. Gotsu | 23 | 1 | 13 | 0 | | | 3 | 1 |
| | | ( 0.4) | (12.6) | ( 0.4) | | | ( 4.4) | ( 0.8) |
| 24. Hagi | 66 | 0 | 62 | 0 | | | 2 | 0 |
| | | ( 0.0) | (62.1) | ( 0.0) | | | ( 1.9) | ( 0.0) |
| 25. Yamaguchi | 52 | 5 | 26 | 0 | | | 9 | 6 |
| | | ( 2.9) | (21.6) | ( 0.7) | | | (16.1) | ( 2.9) |
| 26. Hiroshima | 60 | 54 | | 0 | | | | 6 |
| | | (54.2) | | ( 0.2) | | | | ( 5.7) |
| 27. Kumano | 12 | 7 | | 1 | | | | 4 |
| | | ( 6.8) | | ( 0.8) | | | | ( 4.5) |
| 28. Hiro | 19 | 5 | | 6 | | | | 8 |
| | | ( 4.3) | | ( 5.3) | | | | ( 9.5) |
| 29. Konko | 83 | 63 | | 0 | 0 | | | 17 |
| | | (64.3) | | ( 0.9) | (0.0) | | | (14.9) |

IDH-B in *Rana nigromaculata* populations

| (Expected number) | | | | | | Alleles (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $B^bB^f$ | $B^cB^e$ | $B^cB^g$ | $B^dB^e$ | $B^eB^f$ | $B^eB^g$ | $B^b$ | $B^c$ | $B^e$ | $B^f$ | $B^g$ |
| | | | | | | 100 | | | | |
| | | | | | | 94.8 | | 5.2 | | |
| 7 | | | | | | 78.1 | | | 21.9 | |
| (5.5) | | | | | | | | | | |
| | | | | | | 100 | | | | |
| | | | | | | 100 | | | | |
| | | | | | | 90.3 | | 9.7 | | |
| | | | | | | 89.2 | | 10.8 | | |
| | | | | | | 56.4 | | 43.6 | | |
| | | | | | | 66.0 | | 34.0 | | |
| 6 | | | | | | 95.3 | | | 4.7 | |
| (5.7) | | | | | | | | | | |
| | | | | | | 100 | | | | |
| | | | | | | 100 | | | | |
| | | | | | | 100 | | | | |
| 2 | | | | 1 | | 87.5 | | 8.8 | 3.8 | |
| (2.7) | | | | (0.3) | | | | | | |
| | | | | | | 80.0 | | 20.0 | | |
| 1 | | | | | | 96.2 | | | 3.8 | |
| (1.0) | | | | | | | | | | |
| 2 | | | | 1 | | 78.3 | | 19.6 | 2.2 | |
| (1.6) | | | | (0.4) | | | | | | |
| | | | | | | 100 | | | | |
| 0 | | | | 1 | | 46.1 | | 52.6 | 1.3 | |
| (0.5) | | | | (0.5) | | | | | | |
| | | | | | | 41.7 | | 58.3 | | |
| | 5 | | | | | 3.9 | 6.3 | 89.8 | | |
| | ( 7.2) | | | | | | | | | |
| | 4 | | | | | 0.7 | 2.7 | 96.7 | | |
| | ( 3.9) | | | | | | | | | |
| | 5 | | | | | 13.0 | 73.9 | 13.0 | | |
| | ( 4.4) | | | | | | | | | |
| | 2 | | | | | 1.5 | 97.0 | 1.5 | | |
| | ( 1.9) | | | | | | | | | |
| | 6 | | | | | 24.0 | 64.4 | 11.5 | | |
| | ( 7.7) | | | | | | | | | |
| | | | | | | 95.0 | | 5.0 | | |
| | | | | | | 75.0 | | 25.0 | | |
| | | | | | | 47.4 | | 52.6 | | |
| 3 | | | | 0 | | 88.0 | | 10.2 | 1.8 | |
| (2.6) | | | | (0.3) | | | | | | |

Continued

| Station | No. of frogs | Phenotypes | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $B^bB^b$ | $B^cB^c$ | $B^eB^e$ | $B^fB^f$ | $B^gB^g$ | $B^bB^c$ | $B^bB^e$ |
| 30. Takamatsu | 42 | 28 | | 5 | | | | 9 |
| | | (25.2) | | ( 2.1) | | | | (14.7) |
| 31. Nangoku | 32 | 30 | | 1 | | | | 1 |
| | | (29.1) | | ( 0.1) | | | | ( 2.9) |
| 32. Uwajima | 39 | 1 | | 18 | | | | 20 |
| | | ( 3.1) | | (20.1) | | | | (15.8) |
| 33. Matsuyama | 23 | 0 | 3 | 7 | | | 0 | 1 |
| | | ( 0.0) | ( 3.5) | ( 7.9) | | | ( 0.4) | ( 0.6) |
| 34. Munakata | 58 | 0 | 53 | | | | 5 | |
| | | ( 0.1) | (53.1) | | | | ( 4.8) | |
| 35. Sasebo | 58 | | 1 | 38 | | | | |
| | | | ( 1.9) | (38.9) | | | | |
| 36. Kumamoto | 49 | 0 | 3 | 10 | | | 0 | 1 |
| | | ( 0.0) | ( 8.6) | (16.0) | | | ( 0.0) | ( 0.6) |
| 37. Kagoshima | 60 | 0 | 3 | 40 | | | 0 | 4 |
| | | ( 0.1) | ( 1.5) | (39.2) | | | ( 0.6) | ( 3.2) |
| 38. Miyazaki | 56 | | 0 | 52 | | | | |
| | | | ( 0.1) | (52.0) | | | | |
| 39. Oita | 55 | | | 55 | | | | |
| 40. Suwon | 21 | | 0 | 5 | | 1 | | |
| | | | ( 1.4) | ( 4.8) | | (1.4) | | |
| Total | 1540 | 637 | 165 | 390 | 1 | 1 | 20 | 182 |
| | (%) | (41.4) | (10.7) | (25.3) | (0.1) | (0.1) | ( 1.3) | (11.8) |

## 2. IDH-B

a. *Rana nigromaculata* populations

The IDH-B patterns of 1540 frogs of 39 stations in Japan and one station in Korea showed 12 kinds of phenotypes, that is, $B^bB^b$, $B^cB^c$, $B^eB^e$, $B^fB^f$, $B^gB^g$, $B^bB^c$, $B^bB^e$, $B^bB^f$, $B^cB^e$, $B^cB^g$, $B^eB^f$ and $B^eB^g$ (Table 3; Fig. 6). These phenotypes were determined by five kinds of codominant alleles, *IDH-B*$^b$ ($B^b$), *IDH-B*$^c$ ($B^c$), *IDH-B*$^e$ ($B^e$), *IDH-B*$^f$ ($B^f$) and *IDH-B*$^g$ ($B^g$) (NISHIOKA, OHTANI and SUMIDA, 1980; NISHIOKA, UEDA and SUMIDA, 1981). As enzyme IDH-B is a dimer, that controlled by two homozygous or heterozygous alleles reveals a single band or three bands in electrophoretic pattern. The number of individuals analyzed and the phenotype and allele frequencies in each population are presented in Table 3. Geographic distribution of IDH-B alleles is shown in Fig. 7.

Almost all the populations distributed in the districts of Tohoku, Hokuriku, Chubu, Kinki, San-yo and Shikoku were monomorphic ($B^bB^b$), dimorphic ($B^bB^b$ and $B^bB^e$) or polymorphic ($B^bB^b$, $B^eB^e$ and $B^bB^e$) except a few populations. In the populations of the districts of Tohoku and Hokuriku along the Japan Sea, allele *IDH-B*$^e$ gradually increased in frequency from east to west, while the populations of Akita, Niigata and Kashiwazaki were of monomorphic $B^bB^b$ in IDH-B pattern. Five out of 48 frogs in the Sakata population and seven out of 36 frogs in the Joetsu population were both of $B^bB^e$ in IDH-B pattern. Of 37

| (Expected number) | | | | | | Alleles (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $B^bB^f$ | $B^cB^e$ | $B^cB^g$ | $B^dB^e$ | $B^eB^f$ | $B^eB^g$ | $B^b$ | $B^c$ | $B^e$ | $B^f$ | $B^g$ |
| | | | | | | 77.4 | | 22.6 | | |
| | | | | | | 95.3 | | 4.7 | | |
| | | | | | | 28.2 | | 71.8 | | |
| | 12 (10.6) | | | | | 2.2 | 39.1 | 58.7 | | |
| | | | | | | 4.3 | 95.7 | | | |
| | 19 (17.2) | | | | | | 18.1 | 81.9 | | |
| | 35 (23.4) | | | | | 1.0 | 41.8 | 57.1 | | |
| | 13 (15.3) | | | | | 3.3 | 15.8 | 80.8 | | |
| | 4 ( 3.9) | | | | | | 3.6 | 96.4 | | |
| | | | | | | | | 100 | | |
| | 6 ( 5.2) | 5 (2.9) | | | 4 (5.2) | | 26.2 | 47.6 | | 26.2 |
| 21 (1.4) | 111 ( 7.2) | 5 (0.3) | 0 | 3 (0.2) | 4 (0.3) | 48.6 | 15.1 | 35.1 | 0.8 | 0.4 |

frogs in the Toyama population, two and four were of $B^eB^e$ and $B^bB^e$, respectively. Of 55 frogs in the Kanazawa population, 10 and 28 were of $B^eB^e$ and $B^bB^e$, respectively. Of 25 frogs in the Mikuni population, one and 15 were of $B^eB^e$ and $B^bB^e$, respectively. In the populations of Sakata, Joetsu, Toyama, Kanazawa and Mikuni, allele *IDH-*$B^b$ was 94.8%, 90.3%, 89.2%, 56.4%, and 66.0% in frequency, respectively. The Shibata population in the Hokuriku district differed from the other seven populations of this district in that seven out of 16 frogs were of $B^bB^f$ in IDH-B pattern, while the other nine were of $B^bB^b$. It was evident that allele *IDH-*$B^f$ was derived from *Rana brevipoda.*

In the Chubu district, the populations of Sutama, Mishima and Ina were of monomorphic $B^bB^b$. The Okaya population contained 58 frogs of $B^bB^b$ and six frogs of $B^bB^f$. Of 40 frogs of the Iida population, 31 were of $B^bB^b$, six of $B^bB^e$, two of $B^bB^f$ and the remaining was of $B^eB^f$. Of five frogs of the Nagoya population, three were of $B^bB^b$ and two of $B^bB^e$. In the populations of Okaya, Iida and Nagoya, allele *IDH-*$B^b$ was 95.3%, 87.5% and 80.0% in frequency, respectively.

In the Kinki district, six frogs of the Shingu population were all of monomorphic $B^bB^b$ in IDH-B pattern. Of 13 frogs of the Maibara population, 12 were also of $B^bB^b$, while the remaining was of $B^bB^f$. Of 48 frogs of the Igaueno population, 28 were of $B^bB^b$, two of $B^eB^e$, one of $B^fB^f$, 14 of $B^bB^e$, two of $B^bB^f$ and the remaining was of $B^eB^f$. Of 38 frogs of the Higashiosaka population, 10 were of $B^bB^b$, 12 of $B^eB^e$, 15 of $B^bB^e$ and the remaining was of $B^eB^f$ in IDH-B pattern. The frequency of allele *IDH-*$B^b$ in the populations of Maibara, Igaueno

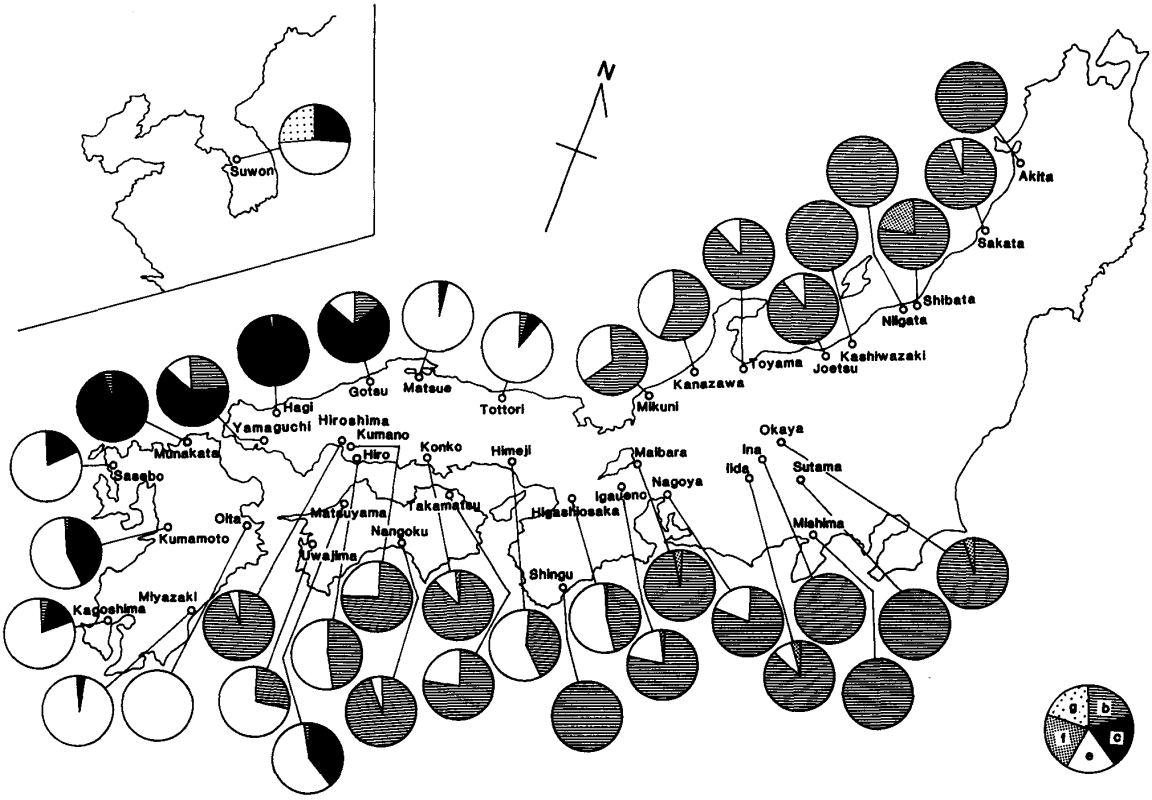


Fig. 7. Geographic distribution of IDH-B alleles among 40 populations of *Rana nigromaculata* in Japan and Korea.

b, Allele *IDH-B*$^{b}$ c, Allele *IDH-B*$^{c}$ e, Allele *IDH-B*$^{e}$
f, Allele *IDH-B*$^{f}$ g, Allele *IDH-B*$^{g}$

and Higashiosaka was 96.2%, 78.3% and 46.1%, respectively. In the Higashiosaka population, allele *IDH-B*$^{e}$ was 52.6% in frequency.

In the San-yo district, there were no monomorphic populations in IDH-B pattern. Of six frogs of the Himeji population, one was of $B^bB^b$, two were of $B^eB^e$ and three of $B^bB^e$. Of 83 frogs of the Konko population, 63 were of $B^bB^b$, 17 of $B^bB^e$ and the remaining three of $B^bB^f$. The Hiro population contained five frogs of $B^bB^b$, six frogs of $B^eB^e$ and eight frogs of $B^bB^e$, while the Kumano population contained seven frogs of $B^bB^b$, one frog of $B^eB^e$ and four frogs of $B^bB^e$, and the Hiroshima population contained 54 frogs of $B^bB^b$ and six frogs of $B^bB^e$. The Yamaguchi population differed from the other populations of the San-yo district in the possession of allele *IDH-B*$^{c}$ like those of the San-in district. Of 52 frogs, five were of $B^bB^b$, 26 of $B^cB^c$, nine of $B^bB^c$, six of $B^bB^e$ and the remaining six of $B^cB^e$ in IDH-B pattern. In the populations of Himeji, Konko, Hiro, Kumano, Hiroshima and Yamaguchi, allele *IDH-B*$^{b}$ was 41.7%, 88.0%, 47.4%, 75.0%, 95.0% and 24.0% in frequency, respectively. Alleles *IDH-B*$^{c}$ and *IDH-B*$^{e}$ contained in the Yamaguchi population were 64.4% and 11.5% in frequency, respectively.

In the Shikoku district, the Takamatsu population contained 28 frogs of $B^bB^b$, five frogs of $B^eB^e$ and nine frogs of $B^bB^e$. Of 32 frogs of the Nangoku population,

30 were of $B^bB^b$, one was of $B^eB^e$ and the remaining of $B^bB^e$. Of 39 frogs of the Uwajima population, one was of $B^bB^b$, 18 were of $B^eB^e$ and 20 of $B^bB^e$. The Matsuyama population differed from the other populations in the Shikoku district in the possession of allele *IDH-B*$^c$, as in the Yamaguchi population in the San-yo district. Of 23 frogs, three were of $B^cB^c$, seven of $B^eB^e$, one was of $B^bB^e$ and the remaining 12 were of $B^cB^e$ in IDH-B pattern. The frequency of allele *IDH-B*$^b$ in the populations of Takamatsu, Nangoku, Uwajima and Matsuyama was 77.4%, 95.3%, 28.2% and 2.2%, respectively. Alleles *IDH-B*$^c$ and *IDH-B*$^e$ contained in the Matsuyama population were 39.1% and 58.7% in frequency, respectively.

The populations in the districts of San-in and Kyushu remarkably differed from those of the other districts in the scarcity of allele *IDH-B*$^b$. Of 64 frogs of the Tottori population in the San-in district, one was of $B^cB^c$, 53 of $B^eB^e$, one of $B^bB^c$, four of $B^bB^e$ and five of $B^cB^e$ in IDH-B pattern. Of 75 frogs of the Matsue population, 70 were of $B^eB^e$, one of $B^bB^e$ and the remaining four of $B^cB^e$. In the San-in district, the populations of Gotsu and Hagi distinctively differed from the populations of Tottori and Matsue in the abundance of allele *IDH-B*$^c$. The Gotsu population contained one frog of $B^bB^b$, 13 frogs of $B^cB^c$, three frogs of $B^bB^c$, one frog of $B^bB^e$ and five frogs of $B^cB^e$. The Hagi population contained 62 frogs of $B^cB^c$, two frogs of $B^bB^c$ and two frogs of $B^cB^e$. The Munakata population of the Kyushu district was very similar to the populations of Gotsu and Hagi in the San-in district in the abundance of allele *IDH-B*$^c$. Of 58 frogs of this population, 53 were of $B^cB^c$ and five of $B^bB^c$. The other populations in the Kyushu district were very abundant in allele *IDH-B*$^e$ in place of *IDH-B*$^c$. The Sasebo population contained one frog of $B^cB^c$, 38 frogs of $B^eB^e$ and 19 frogs of $B^cB^e$. Of 49 frogs of the Kumamoto population, three were of $B^cB^c$, ten of $B^eB^e$, one of $B^bB^e$ and the remaining 35 of $B^cB^e$. Of 60 frogs of the Kagoshima population, three were of $B^cB^c$, 40 of $B^eB^e$, four of $B^bB^e$ and 13 of $B^cB^e$. While the Miyazaki population contained 52 frogs of $B^eB^e$ and four frogs of $B^cB^e$, the Oita population contained 55 frogs of $B^eB^e$ alone.

Allele *IDH-B*$^c$ in the populations of Tottori, Matsue, Gotsu, Hagi, Munakata, Sasebo, Kumamoto, Kagoshima and Miyazaki was 6.3%, 2.7%, 73.9%, 97.0%, 95.7%, 18.1%, 41.8%, 15.8% and 3.6% in frequency, respectively. While allele *IDH-B*$^e$ in these populations was 89.8%, 96.7%, 13.0%, 1.5%, 0%, 81.9%, 57.1%, 80.8% and 96.4% in frequency, respectively.

The Suwon population in Korea was similar to the Sasebo population in the Kyushu district in possession of alleles *IDH-B*$^c$ and *IDH-B*$^e$ as well as lack of allele *IDH-B*$^b$. However, this population had the characteristic possession of allele *IDH-B*$^g$, which was completely absent in all the populations distributed in Japan. In the Suwon population, alleles *IDH-B*$^c$, *IDH-B*$^e$ and *IDH-B*$^g$ were 26.2%, 47.6% and 26.2% in frequency, respectively.

Allele *IDH-B*$^f$ was found in the populations of Shibata, Okaya, Iida, Maibara, Igaueno, Higashiosaka and Konko. While this allele was 21.9% in frequency in the Shibata population, it was 1.3~4.7% in the other populations.

b. *Rana brevipoda* populations

Electrophoretic patterns of IDH-B examined in 214 *Rana brevipoda porosa* from eight stations, 85 *Rana brevipoda brevipoda* of the Nagoya race from four stations and 38 *Rana brevipoda brevipoda* of the typical race from Konko indicated that there were eight kinds of phenotypes, that is, $B^bB^b$, $B^dB^d$, $B^fB^f$, $B^bB^d$, $B^bB^f$, $B^dB^f$, $B^bB^dB^f$ and $B^bB^fB^f$. These phenotypes of enzyme IDH-B were determined by three kinds of codominant alleles, *IDH-*$B^b$, *IDH-*$B^d$ and *IDH-*$B^f$ (Table 4; Fig. 8).

TABLE 4

Frequencies of phenotypes and alleles of IDH-B in *Rana brevipoda* populations

| Station | No. of frogs | Phenotypes (Expected number) | | | | | | | | Alleles (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $B^bB^b$ | $B^dB^d$ | $B^fB^f$ | $B^bB^d$ | $B^bB^f$ | $B^dB^f$ | $B^bB^dB^f$ | $B^bB^fB^f$ | $B^b$ | $B^d$ | $B^f$ |
| 1. Morioka | 26 | | 6 (3.1) | 14 (11.1) | | | 6 (11.8) | | | | 34.6 | 65.4 |
| 2. Hiraizumi | 9 | | 2 (2.3) | 2 ( 2.3) | | | 5 ( 4.5) | | | | 50.0 | 50.0 |
| 3. Ichinoseki | 39 | | 6 (6.7) | 13 (13.6) | | | 20 (18.9) | | | | 41.0 | 59.0 |
| 4. Sukagawa | 6 | | 1 (0.7) | 3 ( 2.7) | | | 2 ( 2.7) | | | | 33.3 | 66.7 |
| 5. Utsunomiya | 56 | | | 56 | | | | | | | | 100 |
| 6. Shibata | 31 | 3 (1.7) | 0 (0.1) | 17 (15.3) | 1 (0.9) | 6 (10.2) | 2 ( 2.7) | 1 | 1 | 23.4 | 6.3 | 70.3 |
| 7. Maki | 28 | | 0 (1.1) | 17 (18.1) | | | 11 ( 8.8) | | | | 19.6 | 80.4 |
| 8. Nagano | 19 | | | 19 | | | | | | | | 100 |
| 9. Ina | 33 | 0 (0.0) | | 31 (31.0) | | 2 ( 1.9) | | | | 3.0 | | 97.0 |
| 10. Maibara | 28 | | | 28 | | | | | | | | 100 |
| 11. Igaueno | 4 | | | 4 | | | | | | | | 100 |
| 12. Higashiosaka | 20 | | | 20 | | | | | | | | 100 |
| 13. Konko | 38 | | | 38 | | | | | | | | 100 |
| Total | 337 (%) | 3 (0.9) | 15 (4.5) | 262 (77.7) | 1 (0.3) | 8 ( 2.4) | 46 (13.6) | 1 (0.3) | 1 (0.3) | 2.5 | 11.5 | 85.9 |

The populations of Morioka, Hiraizumi, Ichinoseki and Sukagawa of *Rana brevipoda porosa* in the Tohoku district showed three kinds of phenotypes in IDH-B pattern. Of 26 frogs of the Morioka population, six were of $B^dB^d$, 14 of $B^fB^f$ and six of $B^dB^f$. Of nine frogs of the Hiraizumi population, two were of $B^dB^d$, two of $B^fB^f$ and five of $B^dB^f$. The Ichinoseki population contained six frogs of $B^dB^d$, 13 frogs of $B^fB^f$ and 20 frogs of $B^dB^f$, while the Sukagawa population contained one frog of $B^dB^d$, three frogs of $B^fB^f$ and two frogs of $B^dB^f$. The frequencies of allele *IDH-*$B^f$ in the populations of Morioka, Hiraizumi, Ichinoseki and Sukagawa were 65.4%, 50.0%, 59.0% and 66.7%, respectively (Table 4; Fig. 8).

The Utsunomiya population in the Kanto district and the Nagano population in the Chubu district included 56 and 19 *Rana brevipoda porosa,* respectively, and were of monomorphic $B^fB^f$ in IDH-B pattern, while the Ina population in the

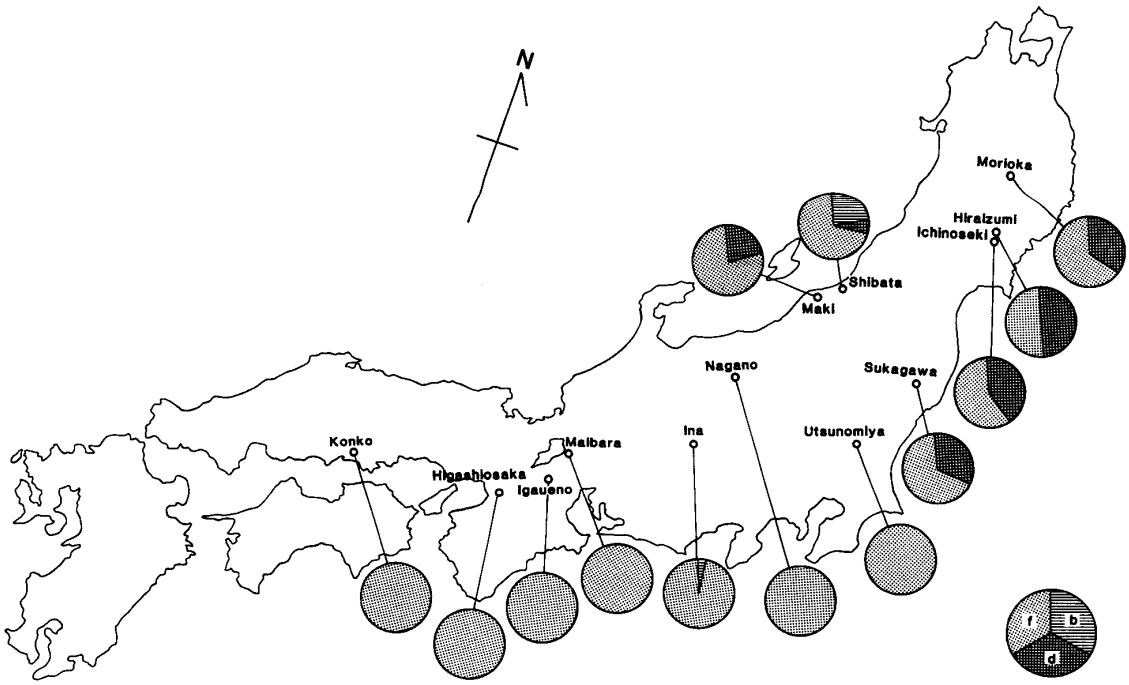


Fig. 8. Geographic distribution of IDH-B alleles among 13 populations of *Rana brevipoda* in Japan. b, Allele *IDH-B*$^b$ d, Allele *IDH-B*$^d$ f, Allele *IDH-B*$^f$

Chubu district contained 33 *Rana brevipoda brevipoda* of the Nagoya race, of which 31 were of $B^fB^f$ and two of $B^bB^f$.

The populations of Maibara, Igaueno and Higashiosaka in the Kinki district included 28, 4 and 20 *Rana brevipoda brevipoda* of the Nagoya race, while the Konko population included 38 *Rana brevipoda brevipoda* of the typical race. All these populations of *Rana brevipoda brevipoda* were of monomorphic $B^fB^f$ in IDH-B pattern.

The Maki population in the Hokuriku district contained 28 *Rana brevipoda porosa* which were similar to those of the populations in the Tohoku district in that there were three kinds of phenotypes. Of these frogs, 17 were of $B^fB^f$ and the other 11 were of $B^dB^f$ in IDH-B pattern. It was found that allele *IDH-B*$^f$ was 80.4% in frequency. In contrast, the Shibata population showed seven kinds of phenotypes in IDH-B pattern. Of 31 *Rana brevipoda porosa,* three were of $B^bB^b$, 17 of $B^fB^f$, one of $B^bB^d$, six of $B^bB^f$, two of $B^dB^f$, one of triploid $B^bB^dB^f$ and one of triploid $B^bB^fB^f$. In this population, alleles *IDH-B*$^b$, *IDH-B*$^d$ and *IDH-B*$^f$ were 23.4%, 6.3% and 70.3% in frequency, respectively. It was evident that allele *IDH-B*$^b$ had been derived from *Rana nigromaculata* by introgressive hybridization.

c. Summary of results from IDH-B

The results of analyses of enzyme IDH extracted from skeletal muscles of a total of 1540 *Rana nigromaculata* from 39 stations in Japan and one station in Korea showed that IDH-B had 12 kinds of phenotypes determined by five kinds of codominant alleles, *IDH-B*$^b$, *IDH-B*$^c$, *IDH-B*$^e$, *IDH-B*$^f$ and *IDH-B*$^g$. These

alleles were 48.6%, 15.1%, 35.1%, 0.8% and 0.4% in frequency, respectively. On the other hand, by analyzing IDH extracted from a total of 337 *Rana brevipoda* including 214 *Rana brevipoda porosa* from eight stations, 85 *Rana brevipoda brevipoda* of the Nagoya race from four stations and 38 *Rana brevipoda brevipoda* of the typical race from Konko, it was found that IDH-B had eight phenotypes determined by three kinds of codominant alleles, *IDH-B*$^b$, *IDH-B*$^d$ and *IDH-B*$^f$. These alleles were 2.5%, 11.5% and 85.9% in frequency, respectively.

Allele *IDH-B*$^b$ was abundant in *Rana nigromaculata* distributed in the eastern half of Japan as well as in the districts of San-yo and Shikoku (Fig. 7). In the districts of Tohoku and Hokuriku, three populations contained allele *IDH-B*$^b$ alone, while this allele was 56.4~94.8% in frequency in the other six populations. In the districts of Chubu and Kinki, four populations contained allele *IDH-B*$^b$ alone, while this allele was 78.3~96.2% in frequency in five other populations and 46.1% in the Higashiosaka population. In the districts of San-yo and Shikoku, allele *IDH-B*$^b$ was 75.0~95.3% in frequency in five populations of Konko, Takamatsu, Nangoku, Kumano and Hiroshima, while it was 47.4% in the Hiro population and 41.7% in the Himeji population. In the Yamaguchi population which was located in the western extremity of the San-yo district, allele *IDH-B*$^b$ was 24.0% in frequency, while in the populations of Uwajima and Matsuyama located in the western area of Shikoku, it was 28.2% and 2.2%, respectively. The populations of Yamaguchi and Matsuyama contained allele *IDH-B*$^c$ which was 64.4% and 39.1% in frequency, respectively.

The eastern half of the San-in district as well as the Kyushu district except the northern extremity were most abundant in allele *IDH-B*$^e$, although the adjacent areas were also considerably abundant in this allele. The populations of Tottori, Matsue, Sasebo, Kagoshima, Miyazaki and Oita were 80.8~100% in frequency of allele *IDH-B*$^e$, while the Kumamoto population was 57.1%. The populations of Himeji, Higashiosaka, Mikuni and Kanazawa located near the Tottori population were 58.3%, 52.6%, 34.0% and 43.6%, respectively, in frequency of *IDH-B*$^e$. The populations of Uwajima, Matsuyama and Hiro located near the Oita population were 71.8%, 58.7% and 52.6%, respectively.

The distribution of allele *IDH-B*$^c$ was limited within the western half of Japan. The western half of the San-in district and the adjacent areas were most abundant in this allele. The populations of Gotsu, Hagi, Yamaguchi and Munakata were 64.4~97.0% in frequency. The populations of Sasebo, Kumamoto, Kagoshima and Miyazaki located along the western coast of Kyushu were 3.6~41.8% in frequency, while the populations of Tottori and Matsue located in the eastern half of the San-in district were 6.3% and 2.7%, respectively. The Matsuyama population was the only one containing allele *IDH-B*$^c$ among the four populations in Shikoku; it was 39.1% in frequency. The Suwon population in Korea was remarkable in containing allele *IDH-B*$^g$ in addition to *IDH-B*$^c$ and *IDH-B*$^e$. Alleles *IDH-B*$^c$, *IDH-B*$^e$ and *IDH-B*$^g$ were 26.2%, 47.6% and 26.2% in frequency, respectively.

Alleles *IDH-B*$^d$ and *IDH-B*$^f$ were peculiar to *Rana brevipoda*. *Rana brevipoda*

*brevipoda* of the typical race and the Nagoya race and *Rana brevipoda porosa* in the Kanto district contained almost exclusively allele *IDH-B*$^{f}$. The six populations of *Rana brevipoda porosa* in the districts of Tohoku and Hokuriku were 50.0~80.4% in frequency of *IDH-B*$^{f}$, while 6.3~50.0% in frequency of *IDH-B*$^{d}$. In the Shibata population of the Hokuriku district and the Ina population of the Chubu district, allele *IDH-B*$^{b}$ derived from *Rana nigromaculata* was contained in frequency of 23.4% and 3.0%, respectively. On the other hand, seven populations of *Rana nigromaculata*, that is, the populations of Shibata, Okaya, Iida, Maibara, Igaueno, Higashiosaka and Konko, contained allele *IDH-B*$^{f}$ derived from *Rana brevipoda* in frequency of 1.3~21.9%.

### *III. Lactate dehydrogenase (LDH)*

The electrophoretic patterns of LDH extracted from skeletal muscles of *Rana nigromaculata* and *Rana brevipoda* consisted of two band-groups which migrated toward the anode. There was no sexual difference in these LDH patterns. The two band-groups were controlled by alleles at two different loci. The slowly and fast migrating band-groups were named LDH-A and LDH-B, respectively (Nishioka, Ohtani and Sumida, 1980; Nishioka, Ueda and Sumida, 1981). Each band-group was controlled by alleles at its own locus. As LDH is a tetramer enzyme, two different alleles always formed five bands arranged at almost equal intervals. As there were various phenotypes in each of the LDH-A and LDH-B patterns, the alleles determining these phenotypes were named *LDH-A*$^{a}$ (*A*$^{a}$), *LDH-A*$^{b}$ (*A*$^{b}$), *LDH-A*$^{c}$ (*A*$^{c}$) and *LDH-A*$^{d}$ (*A*$^{d}$), and *LDH-B*$^{a}$ (*B*$^{a}$), *LDH-B*$^{b}$ (*B*$^{b}$),

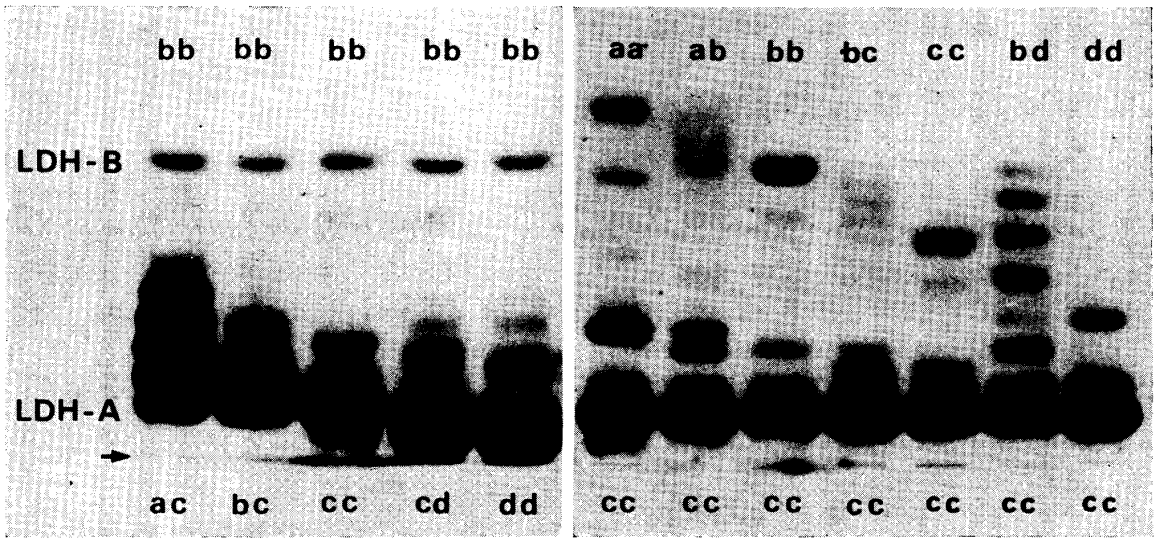


Fig. 9. Seven kinds of phenotypes at the LDH-B locus and five kinds of phenotypes at the LDH-A locus in Japanese and Korean pond frogs.

Upper: LDH-B locus
aa, $B^{a}B^{a}$ ab, $B^{a}B^{b}$ bb, $B^{b}B^{b}$ bc, $B^{b}B^{c}$
cc, $B^{c}B^{c}$ bd, $B^{b}B^{d}$ dd, $B^{d}B^{d}$
Lower: LDH-A locus
ac, $A^{a}A^{c}$ bc, $A^{b}A^{c}$ cc, $A^{c}A^{c}$ cd, $A^{c}A^{d}$ dd, $A^{d}A^{d}$

*LDH-B*$^c$ ($B^c$) and *LDH-B*$^d$ ($B^d$) in order of mobility from fast to slow (Fig. 9). These alleles were codominant over each other.

## 1. LDH-A

a. *Rana nigromaculata* populations

A total of 1540 mature frogs of both sexes collected from 39 stations in Japan and one station in Korea were examined in terms of LDH-A pattern. The results showed that there were five kinds of phenotypes; 1510 (98.1%), 7 (0.5%), 14 (0.9%), 5 (0.3%) and 4 (0.3%) frogs were of $A^cA^c$, $A^dA^d$, $A^aA^c$, $A^bA^c$ and $A^cA^d$, respectively. The four kinds of alleles, *LDH-A*$^a$, *LDH-A*$^b$, *LDH-A*$^c$ and *LDH-A*$^d$, were 0.5%, 0.2%, 98.8% and 0.6% in frequency, respectively. Of the 40 populations examined, 35 were of monomorphic $A^cA^c$ in LDH-A pattern. Even in the other five populations, the individuals showing $A^cA^c$ were overwhelmingly abundant (Table 5).

Of 25 frogs in the Mikuni population of the Hokuriku district, five were of $A^bA^c$, while the other 20 were of $A^cA^c$ (Fig. 10). Alleles *LDH-A*$^c$ and *LDH-A*$^b$ in this population were 90.0% and 10.0% in frequency, respectively. In the Matsue population of the San-in district, one of 75 frogs was of $A^cA^d$ in LDH-A pattern, while the others were of $A^cA^c$. Alleles *LDH-A*$^c$ and *LDH-A*$^d$ were 99.3% and 0.7% in frequency, respectively. Of 23 frogs in the Matsuyama population of the Shikoku district, 17 were of $A^cA^c$, five of $A^dA^d$ and the remaining of $A^cA^d$. Alleles *LDH-A*$^c$ and *LDH-A*$^d$ were 76.1% and 23.9% in frequency, respectively. In the Kyushu district, 54 of 58 frogs in the Munakata population were of $A^cA^c$, while two others were of $A^dA^d$ and the remaining two were of $A^cA^d$. Alleles *LDH-A*$^c$ and *LDH-A*$^d$ were 94.8% and 5.2% in frequency, respectively. On the other hand, in the Sasebo population of the same district, 44 of 58 frogs were of $A^cA^c$ in LDH-A pattern, while the other 14 were of $A^aA^c$. Alleles *LDH-A*$^a$ and *LDH-A*$^c$ were 12.1% and 87.9% in frequency, respectively.

b. *Rana brevipoda* populations

A total of 337 mature male and female frogs including 214 *Rana brevipoda porosa* from eight stations, 85 *Rana brevipoda brevipoda* of the Nagoya race from four stations and 38 *Rana brevipoda brevipoda* of the typical race from Konko were examined. It was found that all the populations were of monomorphic $A^cA^c$ in LDH-A pattern, that is, the enzyme was determined by allele *LDH-A*$^c$ alone.

## 2. LDH-B

a. *Rana nigromaculata* populations

Electrophoretic examination of a total of 1540 frogs collected from 39 stations in Japan and one station in Korea showed that 1524 (99.0%) frogs were of $B^bB^b$ in LDH-B pattern, while one was of $B^cB^c$, one of $B^aB^b$, ten were of $B^bB^c$ and the remaining four of $B^bB^d$. The four kinds of alleles, *LDH-B*$^a$, *LDH-B*$^b$, *LDH-B*$^c$ and *LDH-B*$^d$, which determined these phenotypes were 0.03%, 99.4%, 0.4% and 0.1% in frequency, respectively.

TABLE 5
Frequencies of phenotypes and alleles of LDH-A in *Rana nigromaculata* populations

| Station | No. of frogs | Phenotypes (Expected number) | | | | | | | Alleles (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $A^aA^a$ | $A^bA^b$ | $A^cA^c$ | $A^dA^d$ | $A^aA^c$ | $A^bA^c$ | $A^cA^d$ | $A^a$ | $A^b$ | $A^c$ | $A^d$ |
| 1. Akita | 6 | | | 6 | | | | | | | 100 | |
| 2. Sakata | 48 | | | 48 | | | | | | | 100 | |
| 3. Shibata | 16 | | | 16 | | | | | | | 100 | |
| 4. Niigata | 20 | | | 20 | | | | | | | 100 | |
| 5. Kashiwazaki | 26 | | | 26 | | | | | | | 100 | |
| 6. Joetsu | 36 | | | 36 | | | | | | | 100 | |
| 7. Toyama | 37 | | | 37 | | | | | | | 100 | |
| 8. Kanazawa | 55 | | | 55 | | | | | | | 100 | |
| 9. Mikuni | 25 | | 0 (0.3) | 20 (20.3) | | | 5 (4.5) | | | 10.0 | 90.0 | |
| 10. Okaya | 64 | | | 64 | | | | | | | 100 | |
| 11. Sutama | 35 | | | 35 | | | | | | | 100 | |
| 12. Mishima | 67 | | | 67 | | | | | | | 100 | |
| 13. Ina | 2 | | | 2 | | | | | | | 100 | |
| 14. Iida | 40 | | | 40 | | | | | | | 100 | |
| 15. Nagoya | 5 | | | 5 | | | | | | | 100 | |
| 16. Maibara | 13 | | | 13 | | | | | | | 100 | |
| 17. Igaueno | 48 | | | 48 | | | | | | | 100 | |
| 18. Shingu | 6 | | | 6 | | | | | | | 100 | |
| 19. Higashiosaka | 38 | | | 38 | | | | | | | 100 | |
| 20. Himeji | 6 | | | 6 | | | | | | | 100 | |
| 21. Tottori | 64 | | | 64 | | | | | | | 100 | |
| 22. Matsue | 75 | | | 74 (74.0) | 0 (0.0) | | | 1 (1.0) | | | 99.3 | 0.7 |
| 23. Gotsu | 23 | | | 23 | | | | | | | 100 | |
| 24. Hagi | 66 | | | 66 | | | | | | | 100 | |
| 25. Yamaguchi | 52 | | | 52 | | | | | | | 100 | |
| 26. Hiroshima | 60 | | | 60 | | | | | | | 100 | |
| 27. Kumano | 12 | | | 12 | | | | | | | 100 | |
| 28. Hiro | 19 | | | 19 | | | | | | | 100 | |
| 29. Konko | 83 | | | 83 | | | | | | | 100 | |
| 30. Takamatsu | 42 | | | 42 | | | | | | | 100 | |
| 31. Nangoku | 32 | | | 32 | | | | | | | 100 | |
| 32. Uwajima | 39 | | | 39 | | | | | | | 100 | |
| 33. Matsuyama | 23 | | | 17 (13.3) | 5 (1.3) | | | 1 (8.4) | | | 76.1 | 23.9 |
| 34. Munakata | 58 | | | 54 (52.1) | 2 (0.2) | | | 2 (5.7) | | | 94.8 | 5.2 |
| 35. Sasebo | 58 | 0 (0.8) | | 44 (44.8) | | 14 (12.3) | | | 12.1 | | 87.9 | |
| 36. Kumamoto | 49 | | | 49 | | | | | | | 100 | |
| 37. Kagoshima | 60 | | | 60 | | | | | | | 100 | |
| 38. Miyazaki | 56 | | | 56 | | | | | | | 100 | |
| 39. Oita | 55 | | | 55 | | | | | | | 100 | |
| 40. Suwon | 21 | | | 21 | | | | | | | 100 | |
| Total | 1540 (%) | 0 | 0 | 1510 (98.1) | 7 (0.5) | 14 (0.9) | 5 (0.3) | 4 (0.3) | 0.5 | 0.2 | 98.8 | 0.6 |

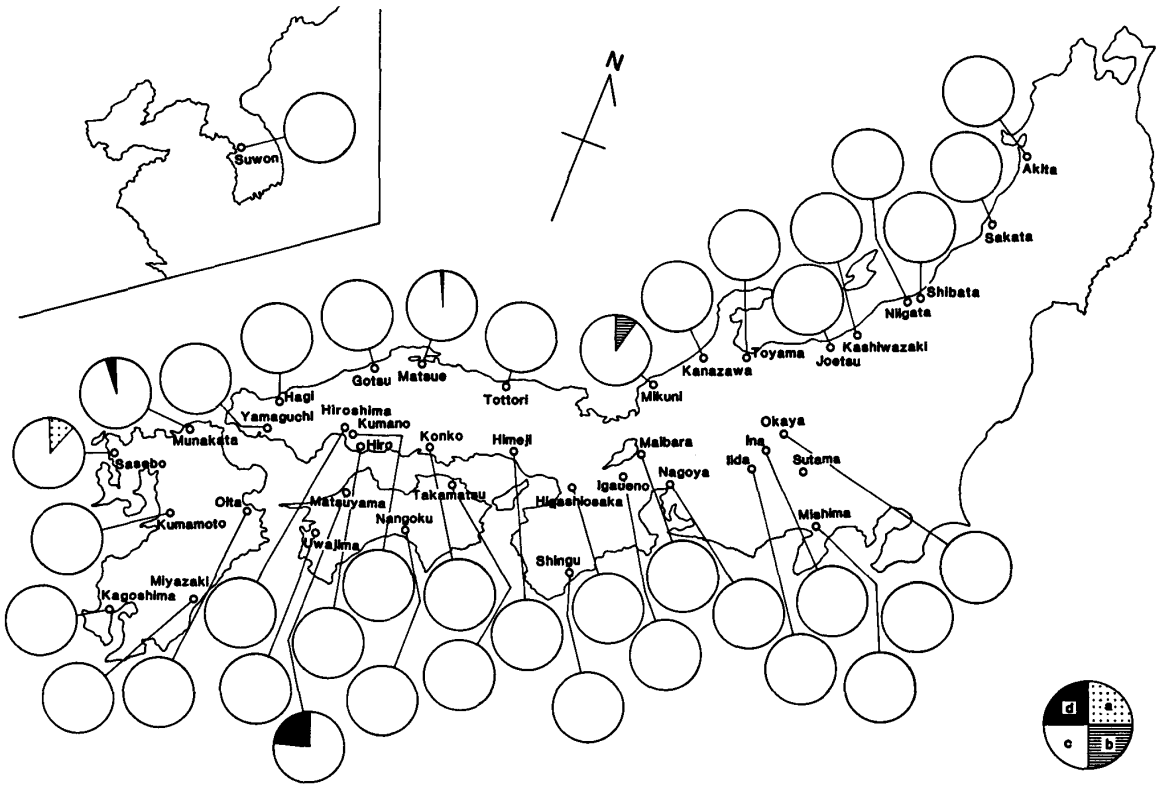


Fig. 10. Geographic distribution of LDH-A alleles among 40 populations of *Rana nigromaculata* in Japan and Korea.

a, Allele *LDH-A*$^a$ b, Allele *LDH-A*$^b$ c, Allele *LDH-A*$^c$ d, Allele *LDH-A*$^d$

Of the 39 populations in Japan, 24 in Honshu, four in Shikoku and six in Kyushu were of monomorphic $B^bB^b$ in LDH-B pattern. The remaining five populations of Shibata, Maibara, Igaueno, Higashiosaka and Konko contained allele *LDH-B*$^c$ or *LDH-B*$^d$ derived from *Rana brevipoda* (Table 6; Fig. 11). Of 16 frogs in the Shibata population, six were of $B^bB^b$, one was of $B^cB^c$ and the remaining nine were of $B^bB^c$ in phenotype. Alleles *LDH-B*$^b$ and *LDH-B*$^c$ were 65.6% and 34.4% in frequency, respectively. In the populations of Maibara, Igaueno and Higashiosaka in the Kinki district, one out of 13, two out of 48 and one out of 38 frogs were of $B^bB^d$ in LDH-B pattern, respectively, while the others were of $B^bB^b$. Allele *LDH-B*$^d$ in these populations was 3.8%, 2.1% and 1.3% in frequency, respectively. It was evident that this allele had been derived from *Rana brevipoda*. Of 83 frogs in the Konko population, 82 were of $B^bB^b$ and the remaining was of $B^bB^c$ in LDH-B pattern. Alleles *LDH-B*$^b$ and *LDH-B*$^c$ were 99.4% and 0.6% in frequency, respectively. It was also evident that allele *LDH-B*$^c$ had been derived from *Rana brevipoda*.

Of 21 *Rana nigromaculata* in the Suwon population of Korea, 20 were of $B^bB^b$ and one was of $B^aB^b$. Alleles *LDH-B*$^a$ and *LDH-B*$^b$ were 2.4% and 97.6% in frequency, respectively. It was evident from unpublished data obtained by the present authors that allele *LDH-B*$^a$ had been derived from *Rana plancyi chosenica*.

TABLE 6
Frequencies of phenotypes and alleles of LDH-B in *Rana nigromaculata* populations

| Station | No. of frogs | Phenotypes (Expected number) | | | | | | | Alleles (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $B^aB^a$ | $B^bB^b$ | $B^cB^c$ | $B^dB^d$ | $B^aB^b$ | $B^bB^c$ | $B^bB^d$ | $B^a$ | $B^b$ | $B^c$ | $B^d$ |
| 1. Akita | 6 | | 6 | | | | | | | 100 | | |
| 2. Sakata | 48 | | 48 | | | | | | | 100 | | |
| 3. Shibata | 16 | | 6 | 1 | | | 9 | | | 65.6 | 34.4 | |
| | | | ( 6.9) | (1.9) | | | (7.2) | | | | | |
| 4. Niigata | 20 | | 20 | | | | | | | 100 | | |
| 5. Kashiwazaki | 26 | | 26 | | | | | | | 100 | | |
| 6. Joetsu | 36 | | 36 | | | | | | | 100 | | |
| 7. Toyama | 37 | | 37 | | | | | | | 100 | | |
| 8. Kanazawa | 55 | | 55 | | | | | | | 100 | | |
| 9. Mikuni | 25 | | 25 | | | | | | | 100 | | |
| 10. Okaya | 64 | | 64 | | | | | | | 100 | | |
| 11. Sutama | 35 | | 35 | | | | | | | 100 | | |
| 12. Mishima | 67 | | 67 | | | | | | | 100 | | |
| 13. Ina | 2 | | 2 | | | | | | | 100 | | |
| 14. Iida | 40 | | 40 | | | | | | | 100 | | |
| 15. Nagoya | 5 | | 5 | | | | | | | 100 | | |
| 16. Maibara | 13 | | 12 | | 0 | | | 1 | | 96.2 | | 3.8 |
| | | | (12.0) | | (0.0) | | | (1.0) | | | | |
| 17. Igaueno | 48 | | 46 | | 0 | | | 2 | | 97.9 | | 2.1 |
| | | | (46.0) | | (0.0) | | | (2.0) | | | | |
| 18. Shingu | 6 | | 6 | | | | | | | 100 | | |
| 19. Higashiosaka | 38 | | 37 | | 0 | | | 1 | | 98.7 | | 1.3 |
| | | | (37.0) | | (0.0) | | | (1.0) | | | | |
| 20. Himeji | 6 | | 6 | | | | | | | 100 | | |
| 21. Tottori | 64 | | 64 | | | | | | | 100 | | |
| 22. Matsue | 75 | | 75 | | | | | | | 100 | | |
| 23. Gotsu | 23 | | 23 | | | | | | | 100 | | |
| 24. Hagi | 66 | | 66 | | | | | | | 100 | | |
| 25. Yamaguchi | 52 | | 52 | | | | | | | 100 | | |
| 26. Hiroshima | 60 | | 60 | | | | | | | 100 | | |
| 27. Kumano | 12 | | 12 | | | | | | | 100 | | |
| 28. Hiro | 19 | | 19 | | | | | | | 100 | | |
| 29. Konko | 83 | | 82 | 0 | | | 1 | | | 99.4 | 0.6 | |
| | | | (82.0) | (0.0) | | | (1.0) | | | | | |
| 30. Takamatsu | 42 | | 42 | | | | | | | 100 | | |
| 31. Nangoku | 32 | | 32 | | | | | | | 100 | | |
| 32. Uwajima | 39 | | 39 | | | | | | | 100 | | |
| 33. Matsuyama | 23 | | 23 | | | | | | | 100 | | |
| 34. Munakata | 58 | | 58 | | | | | | | 100 | | |
| 35. Sasebo | 58 | | 58 | | | | | | | 100 | | |
| 36. Kumamoto | 49 | | 49 | | | | | | | 100 | | |
| 37. Kagoshima | 60 | | 60 | | | | | | | 100 | | |
| 38. Miyazaki | 56 | | 56 | | | | | | | 100 | | |
| 39. Oita | 55 | | 55 | | | | | | | 100 | | |
| 40. Suwon | 21 | 0 | 20 | | | 1 | | | 2.4 | 97.6 | | |
| | | (0.0) | (20.0) | | | (1.0) | | | | | | |
| Total | 1540 | 0 | 1524 | 1 | 0 | 1 | 10 | 4 | 0.03 | 99.4 | 0.4 | 0.1 |
| | (%) | | (99.0) | (0.1) | | (0.1) | (0.6) | (0.3) | | | | |

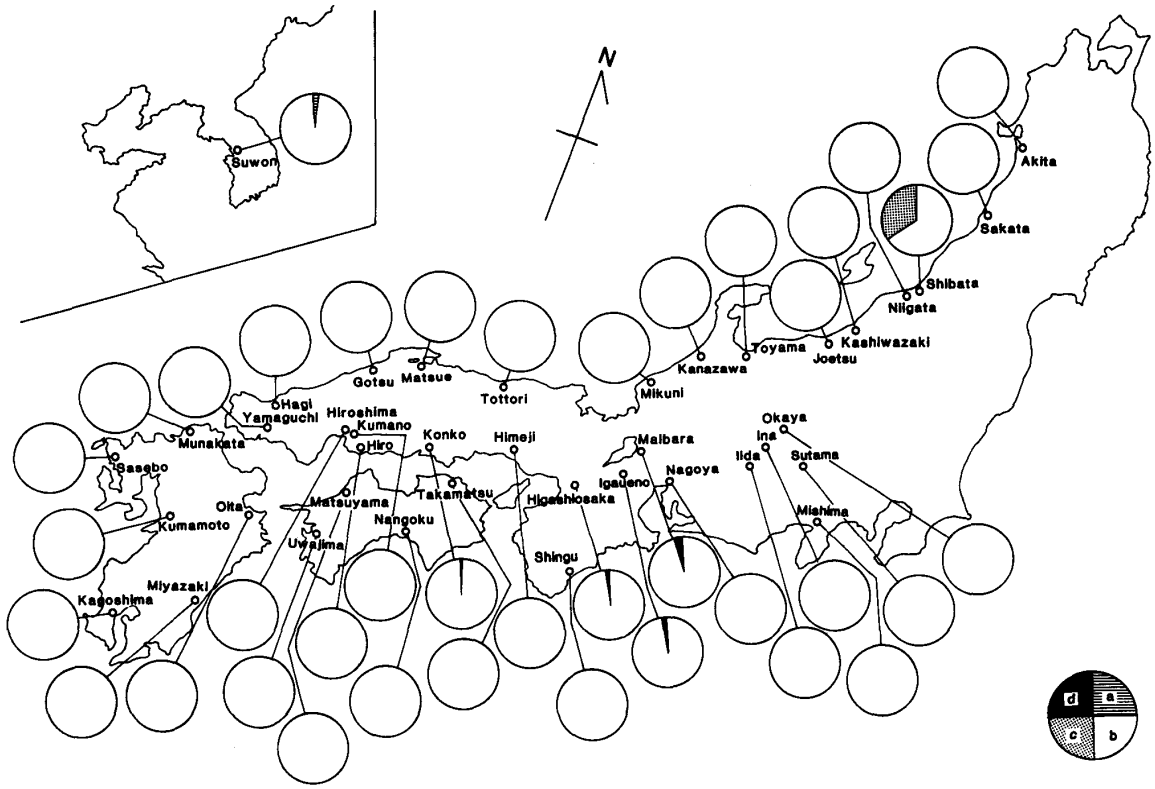


Fig. 11. Geographic distribution of LDH-B alleles among 40 populations of *Rana nigromaculata* in Japan and Korea.

a, Allele *LDH-B*$^a$ b, Allele *LDH-B*$^b$ c, Allele *LDH-B*$^c$ d, Allele *LDH-B*$^d$

b. *Rana brevipoda* populations

Electrophoretic examination of a total of 337 frogs in 13 populations including eight of *Rana brevipoda porosa* and five of *Rana brevipoda brevipoda* of the Nagoya race and the typical race indicated that there were six kinds of phenotypes, $B^bB^b$, $B^cB^c$, $B^dB^d$, $B^bB^c$, $B^bB^d$ and triploid $B^bB^cB^c$, which were determined by three kinds of codominant alleles, *LDH-B*$^b$, *LDH-B*$^c$ and *LDH-B*$^d$.

Six of the eight *Rana brevipoda porosa* populations and the Konko population consisting of *Rana brevipoda brevipoda* of the typical race were of monomorphic $B^cB^c$ in LDH-B pattern (Table 7; Fig. 12).

The populations of Shibata and Maki in the Hokuriku district contained allele *LDH-B*$^b$ derived from *Rana nigromaculata* in addition to allele *LDH-B*$^c$. Of 31 frogs of the Shibata population, three were of $B^bB^b$, 17 of $B^cB^c$, nine of $B^bB^c$ and the remaining two of triploid $B^bB^cB^c$ in LDH-B pattern. Of 28 frogs of the Maki population, 27 were of $B^cB^c$ and the remaining was of $B^bB^c$. Alleles *LDH-B*$^b$ and *LDH-B*$^c$ were 26.6% and 73.4% in frequency, respectively, in the Shibata population and 1.8% and 98.2%, respectively, in the Maki population.

The four populations of *Rana brevipoda brevipoda* of the Nagoya race in the Chubu district remarkably differed from those of *Rana brevipoda porosa* and *Rana brevipoda brevipoda* of the typical race in that they contained allele *LDH-B*$^d$ in place of *LDH-B*$^b$. The populations of Maibara, Igaueno and Higashiosaka were of

monomorphic $B^dB^d$ in LDH-B pattern. In the Ina population, 29 out of 33 frogs were of $B^dB^d$ and the other four of $B^bB^d$ in phenotype. While allele *LDH-B*$^d$ was 93.9% in frequency, allele *LDH-B*$^b$ derived from *Rana nigromaculata* was 6.1%.

TABLE 7
Frequencies of phenotypes and alleles of LDH-B in *Rana brevipoda* populations

| Station | No. of frogs | Phenotypes (Expected number) | | | | | | Alleles (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $B^bB^b$ | $B^cB^c$ | $B^dB^d$ | $B^bB^c$ | $B^bB^d$ | $B^bB^cB^c$ | $B^b$ | $B^c$ | $B^d$ |
| 1. Morioka | 26 | | 26 | | | | | | 100 | |
| 2. Hiraizumi | 9 | | 9 | | | | | | 100 | |
| 3. Ichinoseki | 39 | | 39 | | | | | | 100 | |
| 4. Sukagawa | 6 | | 6 | | | | | | 100 | |
| 5. Utsunomiya | 56 | | 56 | | | | | | 100 | |
| 6. Shibata | 31 | 3 | 17 | | 9 | | 2 | 26.6 | 73.4 | |
| | | (2.2) | (16.7) | | (12.1) | | | | | |
| 7. Maki | 28 | 0 | 27 | | 1 | | | 1.8 | 98.2 | |
| | | (0.0) | (27.0) | | ( 1.0) | | | | | |
| 8. Nagano | 19 | | 19 | | | | | | 100 | |
| 9. Ina | 33 | 0 | | 29 | | 4 | | 6.1 | | 93.9 |
| | | (0.1) | | (29.1) | | (3.8) | | | | |
| 10. Maibara | 28 | | | 28 | | | | | | 100 |
| 11. Igaueno | 4 | | | 4 | | | | | | 100 |
| 12. Higashiosaka | 20 | | | 20 | | | | | | 100 |
| 13. Konko | 38 | | 38 | | | | | | 100 | |
| Total | 337 | 3 | 237 | 81 | 10 | 4 | 2 | 3.3 | 72.2 | 24.6 |
| | (%) | (0.9) | (70.3) | (24.0) | ( 3.0) | (1.2) | (0.6) | | | |

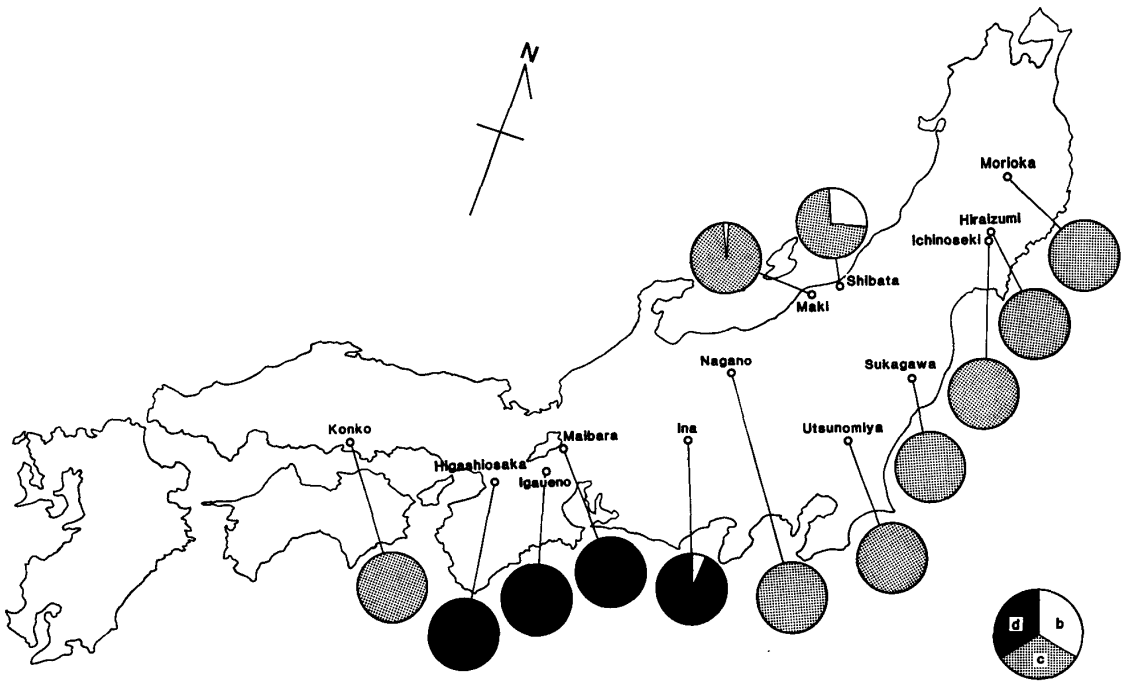


Fig. 12. Geographic distribution of LDH-B alleles among 13 populations of *Rana brevipoda* in Japan.

b, Allele *LDH-B*$^b$ c, Allele *LDH-B*$^c$ d, Allele *LDH-B*$^d$

### *IV. α-Glycerophosphate dehydrogenase (α-GDH)*

The electrophoretic patterns of α-GDH extracted from skeletal muscles of *Rana nigromaculata* and *Rana brevipoda* consisted of five bands that principally migrated toward the anode. There was no sexual difference in α-GDH pattern. The patterns of *Rana nigromaculata* remarkably differed from those of *Rana brevipoda*. The α-GDH patterns of each species were slightly polymorphic. There were six kinds of alleles which determined various kinds of α-GDH phenotypes in the two species. These alleles were named *α-GDH$^a$* ($G^a$), *α-GDH$^b$* ($G^b$), *α-GDH$^c$* ($G^c$), *α-GDH$^d$* ($G^d$), *α-GDH$^e$* ($G^e$) and *α-GDH$^f$* ($G^f$) in order of mobility from fast to slow (Fig. 13).

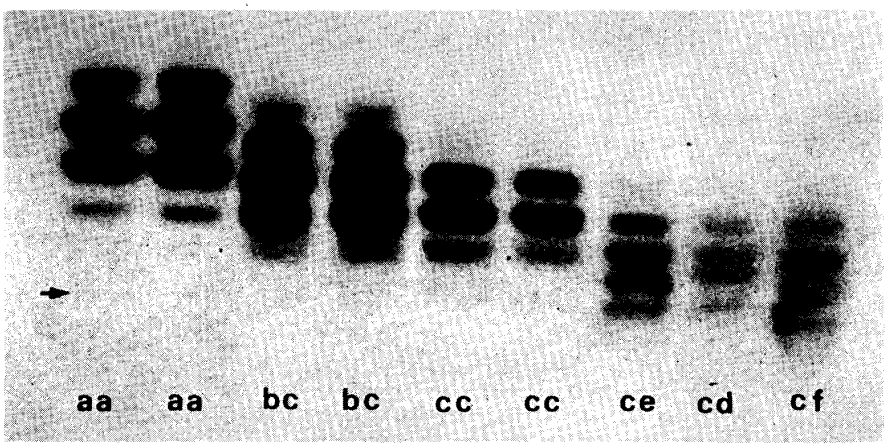


Fig. 13. Six kinds of phenotypes at the α-GDH locus in Japanese and Korean pond frogs.

aa, $G^aG^a$ bc, $G^bG^c$ cc, $G^cG^c$ ce, $G^cG^e$ cd, $G^cG^d$ cf, $G^cG^f$

a. *Rana nigromaculata* populations

By examining α-GDH patterns in a total of 1540 mature male and female frogs from 39 stations in Japan and one station in Korea, it was found that there were seven phenotypes, $G^aG^a$, $G^cG^c$, $G^aG^c$, $G^bG^c$, $G^cG^d$, $G^cG^e$ and $G^cG^f$, of which $G^cG^c$ occupied 1517 (98.5%) individuals (Table 8; Fig. 14). Of the remaining frogs, ten were of $G^aG^c$, four of $G^bG^c$, four of $G^aG^a$, two of $G^cG^d$, two of $G^cG^e$ and one was of $G^cG^f$. These phenotypes were determined by six kinds of codominant alleles, *α-GDH$^a$*, *α-GHD$^b$*, *α-GDH$^c$*, *α-GDH$^d$*, *α-GDH$^e$*, and *α-GDH$^f$*. Allele *α-GDH$^c$* was overwhelmingly high in frequency, that is, 99.1% in frequency, while allele *α-GDH$^a$* derived from *Rana brevipoda* was 0.6% (Table 8). The other alleles *α-GDH$^b$*, *α-GDH$^d$*, *α-GDH$^e$* and *α-GDH$^f$* were 0.1%, 0.1%, 0.1% and 0.03% in frequency, respectively.

Of the 40 populations, 33 were of monomorphic $G^cG^c$ in α-GDH pattern. In the other seven populations, one or two phenotypes were found in addition to phenotype $G^cG^c$. In the Sakata population, one out of 48 frogs was of phenotype

$G^cG^d$, while the others were of $G^cG^c$. In the Shibata population, four, six and six out of 16 frogs were of phenotypes $G^aG^a$, $G^cG^c$ and $G^aG^c$, respectively. Alleles $\alpha$-$GDH^a$ and $\alpha$-$GDH^c$ in this population were 43.8% and 56.3% in frequency, respectively. It was evident that allele $\alpha$-$GDH^a$ had been derived from *Rana brevipoda*. Allele $\alpha$-$GDH^a$ was also contained in the populations of Ina, Igaueno and Higashiosaka. Of two frogs of the Ina population, one was of phenotype $G^cG^c$ and the other was of $G^aG^c$. In the Igaueno population, two out of 48 frogs were of $G^aG^c$, while the others were of $G^cG^c$. Allele $\alpha$-$GDH^a$ in this population was 2.1% in frequency. In the Higashiosaka population, one and one out of 38 frogs were of phenotypes $G^aG^c$ and $G^cG^d$, respectively, while the others were of $G^cG^c$. Alleles $\alpha$-$GDH^a$, $\alpha$-$GDH^c$ and $\alpha$-$GDH^d$ were 1.3%, 97.4% and 1.3% in frequency, respectively.

In the Munakata population of the Kyushu district, four out of 58 frogs were of phenotype $G^bG^c$ and the others were of $G^cG^c$. Allele $\alpha$-$GDH^b$ was 3.4% in frequency. In the Suwon population of Korea, 18, two and one out of 21 frogs were of phenotypes $G^cG^c$, $G^cG^e$ and $G^cG^f$, respectively. In this population, alleles $\alpha$-$GDH^c$, $\alpha$-$GDH^e$ and $\alpha$-$GDH^f$ were 92.9%, 4.8% and 2.3% in frequency, respectively.

b. *Rana brevipoda* populations

A total of 337 frogs collected from 13 stations were examined in terms of $\alpha$-GDH patterns. The results showed that there were four phenotypes determined by two kinds of codominant alleles, $\alpha$-$GDH^a$ and $\alpha$-$GDH^c$, which were 96.2% and 3.8% in frequency, respectively. While nine of these 13 populations were of monomorphic $G^aG^a$ determined by allele $\alpha$-$GDH^a$, the other four populations contained allele $\alpha$-$GDH^c$ derived from *Rana nigromaculata* in addition to $\alpha$-$GDH^a$ (Table 9; Fig. 15).

In the Shibata population of *Rana brevipoda porosa*, four and ten out of 31 frogs were of phenotypes $G^cG^c$ and $G^aG^c$, respectively, while fifteen others were of $G^aG^a$ and the remaining two were of triploid $G^aG^aG^c$. In this population, alleles $\alpha$-$GDH^a$ and $\alpha$-$GDH^c$ were 68.8% and 31.2% in frequency, respectively. In the Maki population located near the Shibata population, one out of 28 frogs was of $G^aG^c$, while the others were of $G^aG^a$. In this population allele $\alpha$-$GDH^c$ was 1.8% in frequency. Four out of 33 *Rana brevipoda brevipoda* of the Nagoya race in the Ina population of the Chubu district and one out of 28 *Rana brevipoda brevipoda* of the same race in the Maibara population of the Kinki district were of $G^aG^c$, while the remaining frogs were of $G^aG^a$. In these populations, allele $\alpha$-$GDH^c$ was 6.1% and 1.8% in frequency, respectively.

TABLE 8

Frequencies of phenotypes and alleles of

| Station | No. of frogs | Phenotypes | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $G^aG^a$ | $G^bG^b$ | $G^cG^c$ | $G^dG^d$ | $G^eG^e$ | $G^fG^f$ | $G^aG^c$ |
| 1. Akita | 6 | | | 6 | | | | |
| 2. Sakata | 48 | | | 47 | 0 | | | |
| | | | | (47.0) | (0.0) | | | |
| 3. Shibata | 16 | 4 | | 6 | | | | 6 |
| | | (3.1) | | ( 5.1) | | | | ( 7.9) |
| 4. Niigata | 20 | | | 20 | | | | |
| 5. Kashiwazaki | 26 | | | 26 | | | | |
| 6. Joetsu | 36 | | | 36 | | | | |
| 7. Toyama | 37 | | | 37 | | | | |
| 8. Kanazawa | 55 | | | 55 | | | | |
| 9. Mikuni | 25 | | | 25 | | | | |
| 10. Okaya | 64 | | | 64 | | | | |
| 11. Sutama | 35 | | | 35 | | | | |
| 12. Mishima | 67 | | | 67 | | | | |
| 13. Ina | 2 | 0 | | 1 | | | | 1 |
| | | (0.1) | | ( 1.1) | | | | ( 0.8) |
| 14. Iida | 40 | | | 40 | | | | |
| 15. Nagoya | 5 | | | 5 | | | | |
| 16. Maibara | 13 | | | 13 | | | | |
| 17. Igaueno | 48 | 0 | | 46 | | | | 2 |
| | | (0.0) | | (46.1) | | | | ( 2.0) |
| 18. Shingu | 6 | | | 6 | | | | |
| 19. Higashiosaka | 38 | 0 | | 36 | 0 | | | 1 |
| | | (0.0) | | (36.1) | (0.0) | | | ( 1.0) |
| 20. Himeji | 6 | | | 6 | | | | |
| 21. Tottori | 64 | | | 64 | | | | |
| 22. Matsue | 75 | | | 75 | | | | |
| 23. Gotsu | 23 | | | 23 | | | | |
| 24. Hagi | 66 | | | 66 | | | | |
| 25. Yamaguchi | 52 | | | 52 | | | | |
| 26. Hiroshima | 60 | | | 60 | | | | |
| 27. Kumano | 12 | | | 12 | | | | |
| 28. Hiro | 19 | | | 19 | | | | |
| 29. Konko | 83 | | | 83 | | | | |
| 30. Takamatsu | 42 | | | 42 | | | | |
| 31. Nangoku | 32 | | | 32 | | | | |
| 32. Uwajima | 39 | | | 39 | | | | |
| 33. Matsuyama | 23 | | | 23 | | | | |
| 34. Munakata | 58 | | 0 | 54 | | | | |
| | | | (0.1) | (54.1) | | | | |
| 35. Sasebo | 58 | | | 58 | | | | |
| 36. Kumamoto | 49 | | | 49 | | | | |
| 37. Kagoshima | 60 | | | 60 | | | | |
| 38. Miyazaki | 56 | | | 56 | | | | |
| 39. Oita | 55 | | | 55 | | | | |
| 40. Suwon | 21 | | | 18 | | 0 | 0 | |
| | | | | (18.1) | | (0.0) | (0.0) | |
| Total | 1540 | 4 | 0 | 1517 | 0 | 0 | 0 | 10 |
| | (%) | (0.3) | | (98.5) | | | | (11.7) |

$\alpha$-GDH in *Rana nigromaculata* populations

| (Expected number) | | | | | | Alleles (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $G^aG^d$ | $G^bG^c$ | $G^cG^d$ | $G^cG^e$ | $G^cG^f$ | $G^eG^f$ | $G^a$ | $G^b$ | $G^c$ | $G^d$ | $G^e$ | $G^f$ |
| | | | | | | | | 100 | | | |
| | | 1 | | | | | | 99.0 | 1.0 | | |
| | | (1.0) | | | | | | | | | |
| | | | | | | 43.8 | | 56.3 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | 25.0 | | 75.0 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | 2.1 | | 97.9 | | | |
| | | | | | | | | 100 | | | |
| 0 | | 1 | | | | 1.3 | | 97.4 | 1.3 | | |
| (0.0) | | (1.0) | | | | | | | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
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| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | 4 | | | | | | 3.4 | 96.6 | | | |
| | (3.8) | | | | | | | | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | | | | | | 100 | | | |
| | | | 2 | 1 | 0 | | | 92.9 | | 4.8 | 2.3 |
| | | | (1.9) | (1.0) | (0.0) | | | | | | |
| 0 | 4 | 2 | 2 | 1 | 0 | 0.6 | 0.1 | 99.1 | 0.1 | 0.1 | 0.03 |
| | (3.8) | (0.1) | (0.1) | (0.1) | | | | | | | |

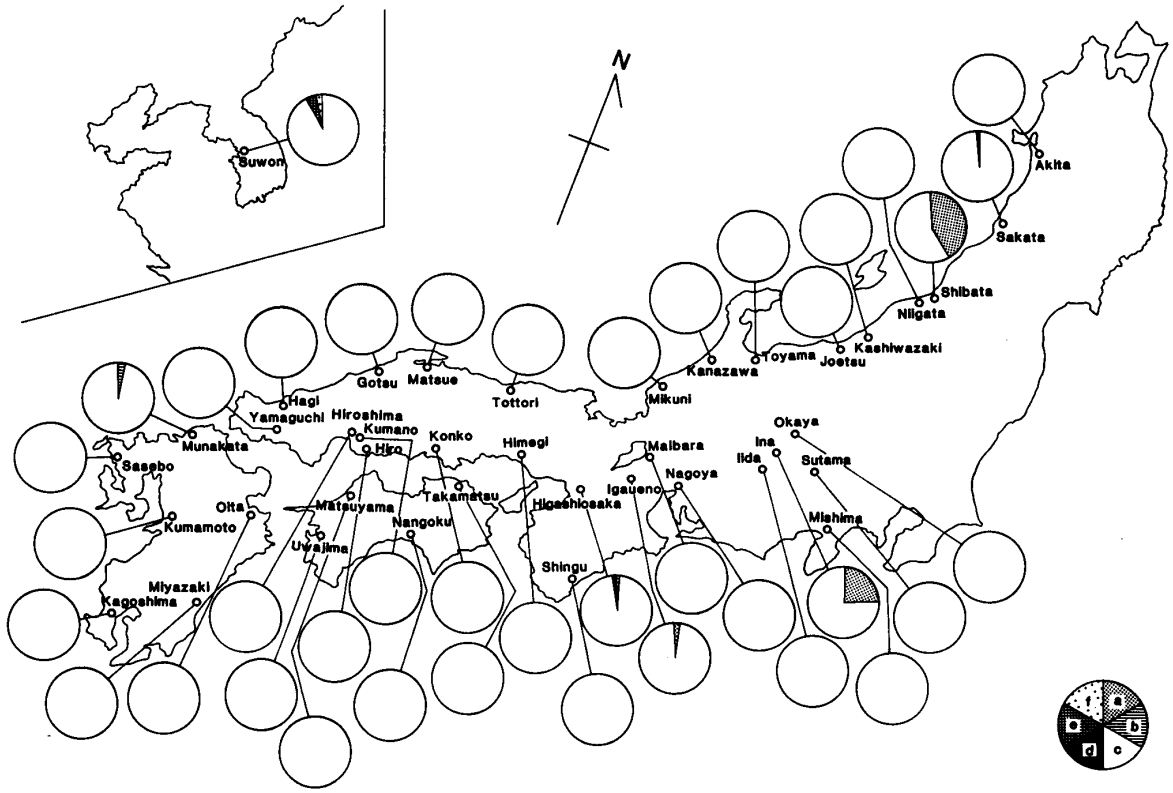


Fig. 14. Geographic distribution of $\alpha$-GDH alleles among 40 populations of *Rana nigromaculata* in Japan and Korea.

a, Allele $\alpha$-$GDH^a$ b, Allele $\alpha$-$GDH^b$ c, Allele $\alpha$-$GDH^c$
d, Allele $\alpha$-$GDH^d$ e, Allele $\alpha$-$GDH^e$ f, Allele $\alpha$-$GDH^f$

TABLE 9

Frequencies of phenotypes and alleles of $\alpha$-GDH in *Rana brevipoda* populations

| Station | No. of frogs | Phenotypes (Expected number) | | | | Alleles (%) | |
|---|---|---|---|---|---|---|---|
| | | $G^aG^a$ | $G^aG^c$ | $G^cG^c$ | $G^aG^aG^c$ | $G^a$ | $G^c$ |
| 1. Morioka | 26 | 26 | | | | 100 | |
| 2. Hiraizumi | 9 | 9 | | | | 100 | |
| 3. Ichinoseki | 39 | 39 | | | | 100 | |
| 4. Sukagawa | 6 | 6 | | | | 100 | |
| 5. Utsunomiya | 56 | 56 | | | | 100 | |
| 6. Shibata | 31 | 15 | 10 | 4 | 2 | 68.8 | 31.2 |
| | | (14.7) | (13.3) | (3.0) | | | |
| 7. Maki | 28 | 27 | 1 | 0 | | 98.2 | 1.8 |
| | | (27.0) | ( 1.0) | (0.0) | | | |
| 8. Nagano | 19 | 19 | | | | 100 | |
| 9. Ina | 33 | 29 | 4 | 0 | | 93.9 | 6.1 |
| | | (29.1) | ( 3.8) | (0.0) | | | |
| 10. Maibara | 28 | 27 | 1 | 0 | | 98.2 | 1.8 |
| | | (27.0) | ( 1.0) | (0.0) | | | |
| 11. Igaueno | 4 | 4 | | | | 100 | |
| 12. Higashiosaka | 20 | 20 | | | | 100 | |
| 13. Konko | 38 | 38 | | | | 100 | |
| Total | 337 | 315 | 16 | 4 | 2 | 96.2 | 3.8 |
| | (%) | (93.5) | ( 4.7) | (1.2) | (0.6) | | |

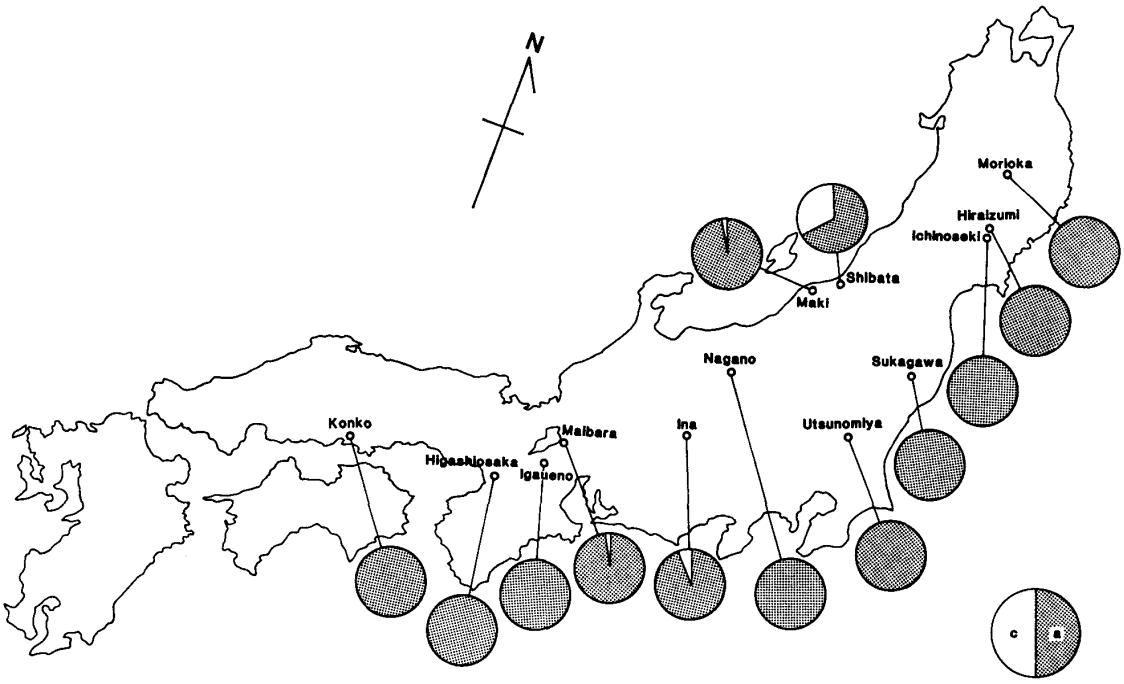


Fig. 15. Geographic distribution of $\alpha$-GDH alleles among 13 populations of *Rana brevipoda* in Japan.
a, Allele $\alpha$-$GDH^a$ c, Allele $\alpha$-$GDH^c$

## *V. Superoxide dismutase (SOD)*

The electrophoretic patterns of SOD extracted from skeletal muscles of *Rana nigromaculata* and *Rana brevipoda* consisted of two bands and four or six bands migrating toward the anode in the existence of homo- and heterozygous alleles, respectively. They were controlled by three kinds of codominant alleles which were named $SOD^a$ ($S^a$), $SOD^b$ ($S^b$) and $SOD^c$ ($S^c$) in order of mobility from fast to slow (Fig. 16).

a. *Rana nigromaculata* populations

The SOD patterns examined in a total of 1540 mature male and female frogs belonging to 40 populations showed three phenotypes controlled by two kinds of codominant alleles, $SOD^a$ and $SOD^b$. While the SOD patterns of two frogs consisted of only two fast migrating bands, those of 1520 others consisted of two slowly migrating bands. The former were of $S^aS^a$ determined by homozygous $SOD^a$ alleles, while the latter were of $S^bS^b$ determined by homozygous $SOD^b$ alleles. The remaining 18 frogs were of $S^aS^b$ which consisted of four bands. Alleles $SOD^a$ and $SOD^b$ in the 40 populations were 0.7% and 99.3% in frequency (Table 10).

Of the 40 populations, 35 were of monomorphic $S^bS^b$ in SOD pattern and determined by allele $SOD^b$ alone (Fig. 17). The other five populations contained allele $SOD^a$ in addition to $SOD^b$. In the Toyama population of the Hokuriku district, 34 and 3 out of 37 frogs were of $S^bS^b$ and $S^aS^b$, respectively. Alleles $SOD^b$ and $SOD^a$ in this population were 95.9% and 4.1% in frequency,

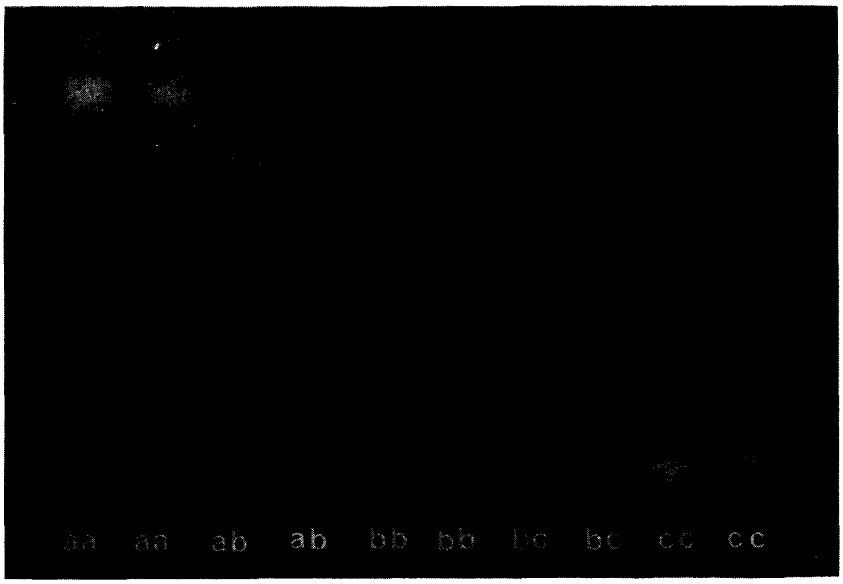


Fig. 16. Five kinds of phenotypes at the SOD locus in Japanese and Korean pond frogs.
aa, $S^aS^a$ ab, $S^aS^b$ bb, $S^bS^b$ bc, $S^bS^c$ cc, $S^cS^c$

respectively. Of 83 frogs in the Konko population of the San-yo district, two were of $S^aS^a$, 69 of $S^bS^b$ and 12 of $S^aS^b$ in SOD pattern. In this population, alleles $SOD^a$ and $SOD^b$ were 9.6% and 90.4% in frequency, respectively. In the Kumano population of the San-yo district, eleven and one out of 12 frogs were of $S^bS^b$ and $S^aS^b$, respectively; alleles $SOD^a$ and $SOD^b$ were 4.2% and 95.8% in frequency, respectively. Of 42 frogs in the Takamatsu population in Shikoku, 41 were of $S^bS^b$ and one was of $S^aS^b$ in SOD pattern; alleles $SOD^a$ and $SOD^b$ were 1.2% and 98.8% in frequency, respectively. The Suwon population in Korea contained 20 frogs of $S^bS^b$ and one frog of $S^aS^b$ in SOD pattern. Alleles $SOD^a$ and $SOD^b$ in this population were 2.4% and 97.6% in frequency, respectively.

b. *Rana brevipoda* populations

Electrophoretic patterns of SOD examined in a total of 337 mature male and female frogs including *Rana brevipoda porosa* from eight stations and *Rana brevipoda brevipoda* of the Nagoya race and the typical race from five stations showed five phenotypes determined by three kinds of codominant alleles, $SOD^a$, $SOD^b$ and $SOD^c$. Of these alleles, $SOD^b$ was 93.2% in frequency. While 11 of the 13 populations were monomorphic $S^bS^b$, the other two were polymorphic. Of 28 *Rana brevipoda brevipoda* of the Nagoya race in the Maibara population, 21 were of $S^bS^b$, two of $S^cS^c$ and the remaining five of $S^bS^c$ in SOD pattern. While allele $SOD^b$ was 83.9% in frequency, allele $SOD^c$ whose bands migrated most slowly among those controlled by three kinds of alleles, $SOD^a$, $SOD^b$ and $SOD^c$, was 16.1% in frequency. Of 38 *Rana brevipoda brevipoda* of the typical race in the Konko population, nine were of $S^aS^a$, 10 were of $S^bS^b$ and the remaining 19 were of $S^aS^b$ in SOD pattern. Alleles $SOD^a$ and $SOD^b$ in this population were 48.7% and 51.3% in frequency, respectively (Table 11, Fig. 18).

TABLE 10
Frequencies of phenotypes and alleles of SOD in *Rana nigromaculata* populations

| Station | No. of frogs | Phenotypes (Expected number) | | | Alleles (%) | |
|---|---|---|---|---|---|---|
| | | $S^aS^a$ | $S^bS^b$ | $S^aS^b$ | $S^a$ | $S^b$ |
| 1. Akita | 6 | | 6 | | | 100 |
| 2. Sakata | 48 | | 48 | | | 100 |
| 3. Shibata | 16 | | 16 | | | 100 |
| 4. Niigata | 20 | | 20 | | | 100 |
| 5. Kashiwazaki | 26 | | 26 | | | 100 |
| 6. Joetsu | 36 | | 36 | | | 100 |
| 7. Toyama | 37 | 0 | 34 | 3 | 4.1 | 95.9 |
| | | (0.1) | (34.0) | ( 2.9) | | |
| 8. Kanazawa | 55 | | 55 | | | 100 |
| 9. Mikuni | 25 | | 25 | | | 100 |
| 10. Okaya | 64 | | 64 | | | 100 |
| 11. Sutama | 35 | | 35 | | | 100 |
| 12. Mishima | 67 | | 67 | | | 100 |
| 13. Ina | 2 | | 2 | | | 100 |
| 14. Iida | 40 | | 40 | | | 100 |
| 15. Nagoya | 5 | | 5 | | | 100 |
| 16. Maibara | 13 | | 13 | | | 100 |
| 17. Igaueno | 48 | | 48 | | | 100 |
| 18. Shingu | 6 | | 6 | | | 100 |
| 19. Higashiosaka | 38 | | 38 | | | 100 |
| 20. Himeji | 6 | | 6 | | | 100 |
| 21. Tottori | 64 | | 64 | | | 100 |
| 22. Matsue | 75 | | 75 | | | 100 |
| 23. Gotsu | 23 | | 23 | | | 100 |
| 24. Hagi | 66 | | 66 | | | 100 |
| 25. Yamaguchi | 52 | | 52 | | | 100 |
| 26. Hiroshima | 60 | | 60 | | | 100 |
| 27. Kumano | 12 | 0 | 11 | 1 | 4.2 | 95.8 |
| | | (0.0) | (11.0) | ( 1.0) | | |
| 28. Hiro | 19 | | 19 | | | 100 |
| 29. Konko | 83 | 2 | 69 | 12 | 9.6 | 90.4 |
| | | (0.8) | (67.8) | (14.4) | | |
| 30. Takamatsu | 42 | 0 | 41 | 1 | 1.2 | 98.8 |
| | | (0.0) | (41.0) | ( 1.0) | | |
| 31. Nangoku | 32 | | 32 | | | 100 |
| 32. Uwajima | 39 | | 39 | | | 100 |
| 33. Matsuyama | 23 | | 23 | | | 100 |
| 34. Munakata | 58 | | 58 | | | 100 |
| 35. Sasebo | 58 | | 58 | | | 100 |
| 36. Kumamoto | 49 | | 49 | | | 100 |
| 37. Kagoshima | 60 | | 60 | | | 100 |
| 38. Miyazaki | 56 | | 56 | | | 100 |
| 39. Oita | 55 | | 55 | | | 100 |
| 40. Suwon | 21 | 0 | 20 | 1 | 2.4 | 97.6 |
| | | (0.0) | (20.0) | ( 1.0) | | |
| Total | 1540 | 2 | 1520 | 18 | 0.7 | 99.3 |
| | (%) | (0.1) | (98.7) | ( 1.2) | | |

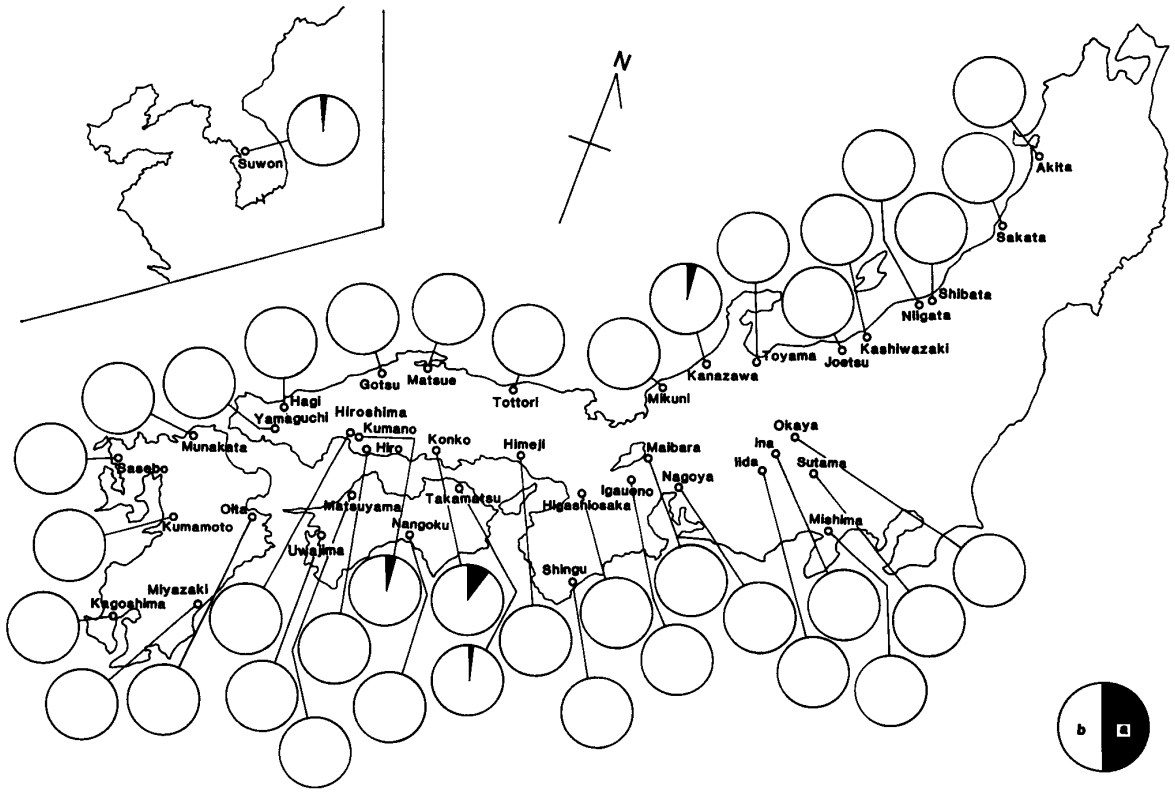


Fig. 17. Geographic distribution of SOD alleles among 40 populations of *Rana nigromaculata* in Japan and Korea.

a, Allele $SOD^a$ b, Allele $SOD^b$

TABLE 11

Frequencies of phenotypes and alleles of SOD in *Rana brevipoda* populations

| Station | No. of frogs | Phenotypes (Expected number) | | | | | Alleles (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $S^aS^a$ | $S^bS^b$ | $S^cS^c$ | $S^aS^b$ | $S^bS^c$ | $S^a$ | $S^b$ | $S^c$ |
| 1. Morioka | 26 | | 26 | | | | | 100 | |
| 2. Hiraizumi | 9 | | 9 | | | | | 100 | |
| 3. Ichinoseki | 39 | | 39 | | | | | 100 | |
| 4. Sukagawa | 6 | | 6 | | | | | 100 | |
| 5. Utsunomiya | 56 | | 56 | | | | | 100 | |
| 6. Shibata | 31 | | 31 | | | | | 100 | |
| 7. Maki | 28 | | 28 | | | | | 100 | |
| 8. Nagano | 19 | | 19 | | | | | 100 | |
| 9. Ina | 33 | | 33 | | | | | 100 | |
| 10. Maibara | 28 | | 21 (19.7) | 2 (0.7) | | 5 (7.6) | | 83.9 | 16.1 |
| 11. Igaueno | 4 | | 4 | | | | | 100 | |
| 12. Higashiosaka | 20 | | 20 | | | | | 100 | |
| 13. Konko | 38 | 9 (9.0) | 10 (10.0) | | 19 (19.0) | | 48.7 | 51.3 | |
| Total | 337 (%) | 9 (2.7) | 302 (89.6) | 2 (0.6) | 19 ( 5.6) | 5 (1.5) | 5.5 | 93.2 | 1.3 |

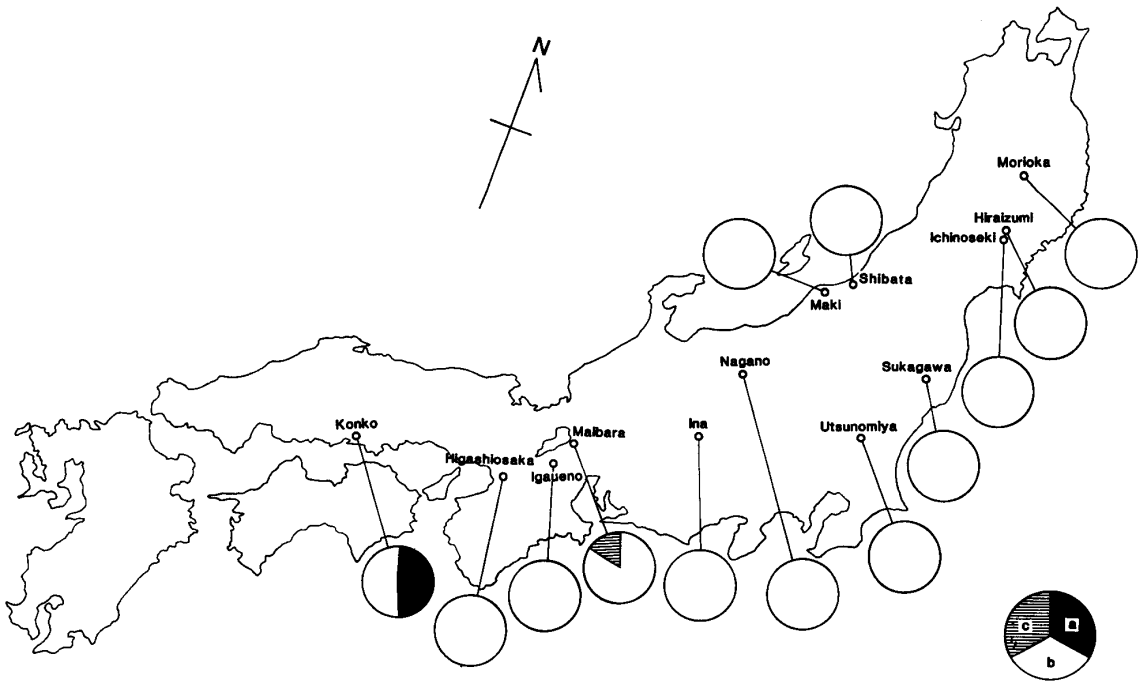


Fig. 18. Geographic distribution of SOD alleles among 13 populations of *Rana brevipoda* in Japan. a, Allele $SOD^a$ b, Allele $SOD^b$ c, Allele $SOD^c$

## DISCUSSION

Japanese pond frogs belonging to the *Rana nigromaculata* group are the most popular in Japan and distributed abundantly in Honshu, Shikoku and Kyushu. They were classified as one subspecies, *Rana nigromaculata nigromaculata* HALLOWELL (1860) by OKADA (1931) in his monograph "The tailless batrachians of the Japanese Empire". In pond frogs distributed in Korea, OKADA (1926) described a new subspecies, *Rana nigromaculata chosenica*, in addition to *Rana nigromaculata nigromaculata*. This classification was generally followed for about ten years until ITO (1941) noticed two types of pond frogs in the vicinity of Nagoya. These differed from each other in size and shape. ITO gave the smaller short-legged frogs a new subspecific name, *Rana nigromaculata brevipoda*.

MORIYA (1951) found that the two subspecies distributed around Nagoya were also sympatric in the area around Okayama. They were reproductively isolated from each other by various isolating mechanisms (MORIYA, 1951). In order to clarify the ranges of the two subspecies, he (1954) collected numerous pond frogs from many different localities of Japan and compared them with each other in morphological characters. The results showed that the Japanese pond frogs including the two subspecies were divided into five races, that is, the *nigromaculata* common, Niigata intermediate, Tokyo intermediate, Nagoya *brevipoda* and Okayama *brevipoda* race. The five races differed from each other in morphological characters of eggs and tadpoles as well as adult frogs. In these characters, the *nigromaculata* common race most remarkably differed from the Okayama *brevipoda* race, while the other three races, Niigata intermediate, Tokyo

intermediate and Nagoya *brevipoda*, were arranged in order of similarity between these two. MORIYA (1960b) reported that the five races were sorted into two types in terms of hybrid sterility. While the *nigromaculata* common race was isolated from the other four races by almost complete sterility of male hybrids, these four races were scarcely isolated from each other. Soon afterward, KAWAMURA, MORIYA, NISHIKAWA and KOIZUMI (unpublished) confirmed that the Niigata intermediate race consisted of a mixture of the Tokyo intermediate race, natural hybrids and their backcrosses.

In accordance with MOORE (1955, 1960) who repeatedly expressed his view that the two subspecies, *nigromaculata* and *brevipoda*, are different species, KAWAMURA (1962) proposed that the Japanese pond frogs should be divided into *Rana nigromaculata* HALLOWELL, *Rana brevipoda brevipoda* ITO and *Rana brevipoda porosa* (COPE) on the basis of the observations performed mostly by MORIYA (1954, 1960a, b). In *Rana brevipoda brevipoda*, the Nagoya race was distinguished from the typical (Okayama) race by morphological and distributional differences, as described by MORIYA (1954). The distribution of these four forms of Japanese pond frogs and the occurrence of natural hybridization between the two species were continuously pursued by the members of the Laboratory for Amphibian Biology, Hiroshima University (KAWAMURA and NISHIOKA, 1977). It was found that natural hybridization was rather frequent in the areas near the lower and upper Shinano River. As a few natural hybrids were found by MORIYA (1959) in the suburbs of Okayama where *Rana nigromaculata* and *Rana brevipoda brevipoda* were sympatric and nearly completely isolated from each other by various isolating mechanisms, it was not unlikely that natural hybridization rarely occurred in any other areas.

According to KAWAMURA and NISHIOKA (1977), *Rana brevipoda porosa* is probably a stabilized population in which introgression of genes from *Rana nigromaculata* has occurred since antiquity and reached such an extent that we can not distinguish the population from interspecific hybrids by outward appearance. This newly established population, that is, *Rana brevipoda porosa* dislodged the *Rana nigromaculata* from the wide plains in the eastern Japan. The introgression has slightly extended to the population of *Rana brevipoda brevipoda* distributed sympatrically with *Rana nigromaculata* and produced the Nagoya race.

In the *Rana esculenta* group distributed in Europe, BERGER (1968) has maintained that *Rana esculenta* are the hybrids between *Rana ridibunda* and *Rana lessonae* on the basis of the results obtained from his field work (1966) and crossing experiments (1967). This finding was repeatedly confirmed by himself (1971, 1976, 1977), GÜNTHER (1973), BLANKENHORN, HEUSSER and VOGEL (1971) and BLANKENHORN (1977). The hybridity of *Rana esculenta* was verified by electrophoretic analysis of their serum protein, albumin, LDH or various kinds of enzymes (TUNNER, 1970, 1972, 1973; ENGELMANN, 1972, 1973; HEMMER, 1973; VOGEL, 1973, 1977; TUNNER and UZZELL, 1974; UZZELL and BERGER, 1975; VOGEL and CHEN, 1976a, b, 1977; TUNNER and DOBROWSKY, 1976). In contrast with morphological characters, electrophoretic patterns of these proteins gave an indubitable

evidence on the hybrid origin of *Rana esculenta* which were very similar to the parental species in appearance.

Genetic variation was similarly clarified by examining the electrophoretic patterns of transferrin, hemoglobin, albumin or various kinds of enzymes in some anurans such as *Rana pipiens* and allied species (WRIGHT and MOYER, 1966, 1968; SALTHE, 1969; WRIGHT and SUBTELNY, 1971, 1973; PLATZ, 1972, 1976; PLATZ and PLATZ, 1973; SUBTELNY, 1974; WRIGHT, 1975, 1978; WRIGHT, HUANG and CHUOKE, 1976), genus *Bufo* (FOX, DESSAUER and MAUMUS, 1961; GUTTMANN, 1967, 1969, 1972, 1975; GUTTMAN and WILSON, 1973; ROGERS, 1973), *Acris crepitans* (DESSAUER and NEVO, 1969; SALTHE and NEVO, 1969) and genus *Hyla* (GERHARDT, GUTTMAN and KARLIN, 1980).

The results of the present electrophoretic studies on the pond frogs distributed widely in Japan completely support the classification proposed by KAWAMURA (1962). If the populations collected from Shibata and a few other stations are excluded, *Rana nigromaculata* and *Rana brevipoda* evidently differ from each other in the alleles controlling various kinds of enzymes. While MDH-B of *Rana nigromaculata* is almost completely determined by alleles $MDH\text{-}B^a$, $MDH\text{-}B^b$, $MDH\text{-}B^d$, and $MDH\text{-}B^e$, that of *Rana brevipoda* is determined by allele $MDH\text{-}B^c$ alone. While IDH-B of *Rana nigromaculata* is determined by alleles $IDH\text{-}B^b$, $IDH\text{-}B^c$ and $IDH\text{-}B^e$, that of *Rana brevipoda* is determined by alleles $IDH\text{-}B^d$ and $IDH\text{-}B^f$. While LDH-B of *Rana nigromaculata* is determined by allele $LDH\text{-}B^b$ alone, that of *Rana brevipoda* is determined by alleles $LDH\text{-}B^c$ and $LDH\text{-}B^d$. While $\alpha$-GDH of *Rana nigromaculata* is almost completely determined by allele $\alpha\text{-}GDH^c$ alone, that of *Rana brevipoda* is determined by allele $\alpha\text{-}GDH^a$ alone. Although SOD of *Rana nigromaculata* was the same as that of *Rana brevipoda* in that it was generally determined by allele $SOD^b$ alone, allele $SOD^c$ was contained in a low frequency in a single population of *Rana brevipoda*. In another population of *Rana brevipoda*, allele $SOD^a$ occupied nearly half of the allele frequency.

Introgression of alleles at the loci of MDH-B, LDH-B and $\alpha$-GDH from *Rana nigromaculata* into *Rana brevipoda* or *vice versa* was remarkably observed in the population from Shibata, around which natural hybridization has frequently occurred. Similar introgression was also observed at a less extent in a few populations collected from stations where the two species were sympatric and natural hybridization seemed likely to occur. Various kinds of phenotypes determined by heterozygous alleles from the two species were always found in some frogs of these populations.

Distinct local variation in allele frequency as found in *Rana pipiens* and *Rana palustris* (SALTHE, 1969), *Acris crepitans* (DESSAUER and NEVO, 1969; SALTHE and NEVO, 1969) and *Bufo americanus* (GUTTMAN, 1969, 1975; GUTTMAN and WILSON, 1973) was observed at the loci of MDH-B and IDH-B of *Rana nigromaculata*. The populations of the Chubu district differed from those of the other districts in kind of alleles at the MDH-B locus. Those of the San-in district had also allele $MDH\text{-}B^d$ in a high frequency, while this allele was completely lacking in the other populations. As the Suwon population in Korea almost exclusively con-

tained allele *MDH-B*$^d$, this allele contained in the populations of the San-in district seemed to have been derived from Korea. A somewhat similar status was found in the IDH-B locus. The populations of the San-in district contained alleles *IDH-B*$^c$ and *IDH-B*$^e$ in fairly high frequencies as the Suwon population did, although the latter had allele *IDH-B*$^g$ that was not found in Japanese populations. The populations along the western coast of Kyushu also contained alleles *IDH-B*$^c$ and *IDH-B*$^e$ in fairly high frequencies.

*Rana brevipoda brevipoda* and *Rana brevipoda porosa* were similar to each other in having allele *MDH-B*$^c$ at the MDH-B locus, *IDH-B*$^f$ at the IDH-B locus and *α-GDH*$^a$ at the α-GDH locus. All the populations of the two subspecies were monomorphic in MDH-B and α-GDH pattern. On the other hand, the *Rana brevipoda porosa* populations of the districts of Tohoku and Hokuriku were polymorphic in IDH-B pattern, while the *Rana brevipoda porosa* populations of the Kanto district and a part of the Chubu district and the *Rana brevipoda brevipoda* populations of the remaining part of the Chubu district and the districts of Kinki and San-yo were monomorphic. Thus, there was no noticeable difference between the two subspecies in terms of alleles at each of the three loci.

The findings of enzyme LDH-B somewhat differed from those of the above three kinds of enzymes. While all the populations of *Rana brevipoda porosa* and the typical (Okayama) race of *Rana brevipoda* contained allele *LDH-B*$^c$ alone, those of the Nagoya race of *Rana brevipoda brevipoda* contained allele *LDH-B*$^d$ alone. It was noteworthy that the differences in the kind and distribution of alleles at the LDH-B locus exactly corresponded with the division of the three kinds of *Rana brevipoda*, the typical race and Nagoya race of *Rana brevipoda brevipoda* and *Rana brevipoda porosa*. It was also interesting that the introgression of alleles from *Rana nigromaculata* into *Rana brevipoda* or *vice versa* was found only in the populations collected from a few stations where natural hybridization has frequently, rarely or probably occurred, and that such introgression was scarcely found in the other populations, especially in those of *Rana brevipoda porosa*.

## SUMMARY

1. Five kinds of enzymes, malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), α-glycerophosphate dehydrogenase (α-GDH) and superoxide dismutase (SOD), extracted from skeletal muscles of 1540 *Rana nigromaculata* from 39 populations in Japan and one population in Korea and 337 *Rana brevipoda* including *Rana brevipoda porosa* from eight stations and *Rana brevipoda brevipoda* of the Nagoya race and the typical race from five stations were analyzed by starch-gel electrophoresis in order to clarify the biochemical relationships between different species, subspecies, races or populations of the Japanese pond frogs.

2. MDH-B of *Rana nigromaculata* had eleven phenotypes determined by six kinds of codominant alleles, *MDH-B*$^a$, *MDH-B*$^b$, *MDH-B*$^c$, *MDH-B*$^d$, *MDH-B*$^e$ and *MDH-B*$^f$. These alleles were 13.2%, 76.6%, 0.3%, 9.1%, 0.7% and 0.1%

in frequency, respectively. MDH-B of *Rana brevipoda* had six phenotypes determined by three kinds of codominant alleles, *MDH-B*$^a$, *MDH-B*$^b$ and *MDH-B*$^c$. These alleles were 1.0%, 2.8% and 96.2% in frequency, respectively.

*Rana nigromaculata* distributed in the districts of Tohoku, Hokuriku, Kinki, San-yo, Shikoku and Kyushu almost contained *MDH-B*$^b$ exclusively, while those distributed in the Chubu district scarcely contained other than *MDH-B*$^a$ or a mixture of alleles *MDH-B*$^a$ and *MDH-B*$^e$. *Rana nigromaculata* distributed in the San-in district contained a mixture of alleles *MDH-B*$^b$ and *MDH-B*$^d$, while those from Suwon, Korea, contained *MDH-B*$^d$ and a very low frequency of *MDH-B*$^f$. There was introgression of *MDH-B*$^c$ from *Rana brevipoda* into the Shibata population and another population. *Rana brevipoda* contained only *MDH-B*$^c$ except the populations of Shibata and Ina, which had *MDH-B*$^b$ and *MDH-B*$^a$ derived from *Rana nigromaculata*.

3. IDH-B of *Rana nigromaculata* had 12 phenotypes determined by five kinds of codominant alleles, *IDH-B*$^b$, *IDH-B*$^c$, *IDH-B*$^e$, *IDH-B*$^f$ and *IDH-B*$^g$. These alleles were 48.6%, 15.1%, 35.1%, 0.8% and 0.4% in frequency, respectively. IDH-B of *Rana brevipoda* had eight phenotypes determined by three kinds of codominant alleles, *IDH-B*$^b$, *IDH-B*$^d$ and *IDH-B*$^f$. These alleles were 2.5%, 11.5% and 85.9% in frequency, respectively.

*Rana nigromaculata* distributed in the eastern half of Japan as well as in the districts of San-yo and Shikoku scarcely contained other than *IDH-B*$^b$ or a mixture of *IDH-B*$^b$ and *IDH-B*$^e$ except the populations of Shibata and Matsuyama. In the Shibata population and some other populations, there was introgression of *IDH-B*$^f$ from *Rana brevipoda*. *Rana nigromaculata* distributed in the eastern half of the San-in district and adjacent areas, Kyushu except the northern extremity, the west of Shikoku, a part of the San-yo district and Suwon, Korea, were very abundant in *IDH-B*$^e$, while those distributed in the western half of the San-in district and adjacent areas were very abundant in *IDH-B*$^c$. *Rana brevipoda* distributed in the districts of Tohoku and Hokuriku contained a mixture of alleles *IDH-B*$^d$ and *IDH-B*$^f$, and those distributed in the other districts contained only *IDH-B*$^f$, although there was introgression of *IDH-B*$^b$ from *Rana nigromaculata* into the populations of Shibata and Ina.

4. LDH-A of *Rana nigromaculata* had five phenotypes determined by four kinds of codominant alleles, *LDH-A*$^a$, *LDH-A*$^b$, *LDH-A*$^c$ and *LDH-A*$^d$, of which *LDH-A*$^c$ was 98.8% in frequency. LDH-A of *Rana brevipoda* was monomorphic and determined by allele *LDH-A*$^c$ alone.

LDH-B of *Rana nigromaculata* had five phenotypes determined by four kinds of codominant alleles, *LDH-B*$^a$, *LDH-B*$^b$, *LDH-B*$^c$ and *LDH-B*$^d$, of which *LDH-B*$^b$ was 99.4% in frequency. LDH-B of *Rana brevipoda* had six kinds of phenotypes determined by three kinds of codominant alleles, *LDH-B*$^b$, *LDH-B*$^c$ and *LDH-B*$^d$. The eight populations of *Rana brevipoda porosa* and the Konko population consisting of *Rana brevipoda brevipoda* of the typical race contained only *LDH-B*$^c$, and the four populations of *Rana brevipoda brevipoda* of the Nagoya race contained only *LDH-B*$^d$, except that there was introgression of *LDH-B*$^b$ from *Rana*

*nigromaculata* into the populations of Shibata, Maki and Ina.

5. α-GDH of *Rana nigromaculata* had seven phenotypes determined by six kinds of codominant alleles, *α-GDH*$^a$, *α-GDH*$^b$, *α-GDH*$^c$, *α-GDH*$^d$, *α-GDH*$^e$ and *α-GDH*$^f$, of which *α-GDH*$^c$ was 99.1% in frequency. α-GDH of *Rana brevipoda* had four phenotypes determined by two kinds of codominant alleles, *α-GDH*$^a$ and *α-GDH*$^c$, which were 96.2% and 3.8% in frequency, respectively. Introgression of *α-GDH*$^a$ or *α-GDH*$^c$ was found in the Shibata population and some other populations of *Rana nigromaculata* or *Rana brevipoda*.

6. SOD of *Rana nigromaculata* had three phenotypes determined by two kinds of codominant alleles, *SOD*$^a$ and *SOD*$^b$, of which *SOD*$^b$ was 99.3% in frequency. Allele *SOD*$^a$ was contained in a low frequency in the frogs collected from Konko and adjacent areas. SOD of *Rana brevipoda* had five phenotypes determined by three kinds of codominant alleles, *SOD*$^a$, *SOD*$^b$, *SOD*$^c$, of which *SOD*$^b$ was 93.2% in frequency. Alleles *SOD*$^a$ and *SOD*$^c$ were contained in considerable frequencies in the populations of Konko and Maibara, respectively.

7. Introgression of alleles *MDH-B*$^c$, *IDH-B*$^f$, *LDH-B*$^c$ and *α-GDH*$^a$ from *Rana brevipoda* into *Rana nigromaculata* and of alleles *MDH-B*$^b$, *IDH-B*$^b$, *LDH-B*$^b$ and *α-GDH*$^c$ from *Rana nigromaculata* into *Rana brevipoda* were most distinctly found in the Shibata population of *Rana nigromaculata* and *Rana brevipoda porosa*. The same kind of introgression in some degree was also found in some other populations of the two species.

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