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# Interspecific and Intraspecific Variations of Muscle Protein in the Japanese Crucian Carp—II

## Starch-gel Electrophoretic Pattern

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**Abstract** Muscle protein patterns of Japanese and European crucian carp were investigated by the starch-gel electrophoretic method. The pattern was affected by the factors such as the quality of starch, the starch-concentration of gel, and pH of both gel- and electrode-buffer. The favorable condition was determined for obtaining the patterns which will clearly demonstrate inter- and intra-specific variations. Under the most favorable condition, the patterns of crucian carp collected from the selected localities from taxonomical point of view were classified into 4 types; Type I represented by four subspecies of *Carassius buergeri*, Type II represented by *C. langsdorfii*, Type III represented by *C. cuvieri*, and Type IV represented by *C. carassius* from Europe. Type I was further divided into three subtypes controlled by two codominant alleles *A* and *B*. The frequency of allele *A* of *Carassius buergeri* varied between 0~1.00. Type II was also divided into four subtypes which were not considered to share the same gene pool. Type II-1 was dominant in the lake Kasumigaura, and Type II-2 was dominant in the waters of western Japan. The pattern of *Carassius carassius* (Type IV) had some similarity with that of *C. buergeri buergeri* (Type I).

### Introduction

Taniguchi and Ishiwatari (1972) reported that the cellulose-acetate electropherogram of muscle proteins soluble in a low ionic strength buffer solution was useful to trace the speciation of the crucian carp. Recently, examining the muscle proteins by the starch-gel electrophoretic method, Taniguchi (1974) found the clearer patterns including inter- and intraspecific variations, and ascertained the existance of a subspecies, *Carassius buergeri buergeri* in the western Japan where this subspecies had been confused with the closely related species, *Carassius langsdorfii*.

In this report, the optimal condition of starch-gel electrophoresis was determined for obtaining the muscle protein patterns which enable to identify the inter- and intra-specific variations of the genus *Carassius*. Under the most favorable condition of electrophoresis obtained here, the authors further investigated the patterns in the Japanese crucian carp collected from selected localities, and also European crucian carp cultured in Japan.

### Materials and methods

The specimens examined here were listed in Table 1. They were collected from 1973 to 1974. The specimens of European crucian carp were offsprings of the specimens imported from Holland and cultured at the pond of National Science Museum in Tokyo by Dr. M. Nakamura.

The storage of samples and the preparation of muscle protein extract were principally the same as those described in Taniguchi and Ishiwatari (1972). The electrophoresis followed mainly the horizontal starch-gel method (Taniguchi, 1974) and partly the acrylamide-gel method (Ogita, 1965). The starch for gel preparation was the hydrolized starch made by Connaught Chemical Lab., Tronto, and Jōko Sangyō Co., Tokyo. The starch-concentration of gel was usually 10%. The starch-gel electrophoresis was carried out usually in discontinuous buffer system; the gel buffer was 0.076 M tris-0.005 M citric acid, pH 8.6 (TC buffer), and the electrode buffer was 0.3 M boric acid-0.5 M NaOH, pH 8.45 (BN

Table 1. Locality, Japanese name, species name, number of specimens, body length, numbers of dorsalfin rays and gillrakers in the Japanese crucian carp and European crucian carp examined in the present study. The number of gillrakers of "nigorobuna" increases with the growth (Taniguchi, 1974), so that the specimens from the river Yasu were identified with "nigorobuna" despite of fewer number of gillrakers.

| Locality | Japanese name | Species | No. of specimens | Standard length (mm) | Dorsal fin rays | Gill-rakers | Composition of muscle protein types |
|---|---|---|---|---|---|---|---|
| Lake Kasumigaura (Ibaraki Pref.) | Kintarōbuna (Kinbuna) | *C. buergeri* subsp-A (c.f. Nakamura, 1963) | 3 | 94～111 | III-13 (13) | 33 (32～34) | I-B: 3 |
| | Ginbuna | *C. langsdorfii* | 3 | 85～113 | III-15.7 (15～16) | 47.3 (46～48) | II-1: 1<br>II-2: 2 |
| | Intermediate form between abve two | | 6 | 106～155 | III-14.8 (14～15) | 40.7 (40～42) | II-3: 5<br>II-4: 1 |
| Lake Suwa (Nagano Pref.) | Nagabuna | *C. buergeri* subsp-B (c.f. Nakamura, 1963) | 25 | 115～201 | III-15.4 (14～17) | 52.5 (48～56) | I-A: 25 |
| | Ginbuna | *C. langsdorfii* | 60 | 80～164 | III-15.1 (14～17) | 43.9 (40～51) | II-2: 60 |
| River Yasu (Shiga Pref.) | Nigorobuna | *C. buergeri grandoculis* | 8 | 53～86 | III-16.5 (16～18) | 43.5 (39～49) | I-A: 4<br>I-AB: 4 |
| | Ginbuna | *C. langsdorfii* | 2 | 65～70 | III-15 (14～16) | 41 (41) | II-2: 2 |
| Lake Kojima (Okayama Pref.) | Kinbuna | *C. buergeri buergeri* | 8 | 135～179 | III-16.5 (15～18) | 44.0 (42～46) | I-A: 6<br>I-AB: 2 |
| | Ginbuna | *C. langsdorfii* | 40 | 101～188 | III-16.9 (16～18) | 46.8 (42～51) | II-1:15, II-3:1<br>II-2:23, II-4:1 |
| River Monobe (Kochi Pref.) | Kinbuna | *C. buergeri buergeri* | 11 | 63～191 | III-16.2 (15～18) | 41.0 (36～50) | I-AB: 1<br>I-B: 10 |
| | Ginbuna | *C. langsdorfii* | 16 | 59～143 | III-17.0 (15～18) | 49.6 (36～50) | II-2: 16 |
| | Gengorōbuna | *C. cuvieri* | 13 | 70～137 | III-17.0 (16～18) | 108.2 (90～124) | III: 13 |
| A pond of Miyazaki city (Miyazaki Pref.) | Kinbuna | *C. buergeri buergeri* | 8 | 170～240 | III-16.3 (16～17) | 45.9 (32～49) | I-A: 8 |
| | Ginbuna | *C. langsdorfii* | 4 | 181～244 | III-17.3 (16～18) | 51.5 (51～53) | II-2: 4 |
| Holland | European crucian carp | *C. carassius* | 5 | 35～75 | III-17.8 (17～19) | 26.0 (24～27) | IV: 5 |

buffer). In some experiments, another system such as 0.083 M tris-0.012 M boric acid-0.0035 M EDTA, pH 8.9 (TBE buffer) for gel and 0.3 M boric acid-0.05 M NaOH, pH 8.6 for electrodes, were employed. In the present report, the names as listed in Table 1 were adopted for material fishes, referring to the classification by Matsubara and Ochiai (1965), by Nakamura (1963, 1969), and by Miyazi et al. (1963).

## Results

### Examination of the electrophoretic condition

The starch-gel electrophoretic patterns were affected by the factors such as the quality of hydrolized starch, the starch-concentration of gel, the kind of buffer system, and pH of both gel- and electrode-buffer.

Figure 1 shows the effect of starch quality on the muscle protein patterns using "ginbuna" (upper three examples) and "gengorōbuna" (lower two) collected from the river Monobe. The electrophoresis was carried out in the hydrolized starch of different lots, 299-1, 314-1, 317-1 of Connaught Chemical Lab. and 363110 of Jōko Sangyō Co. under the same condition: 10% of starch gel, TC buffer pH at 8.6 for gel, and BN buffer pH at 8.45 for electrodes. These patterns were fairly different in separa-

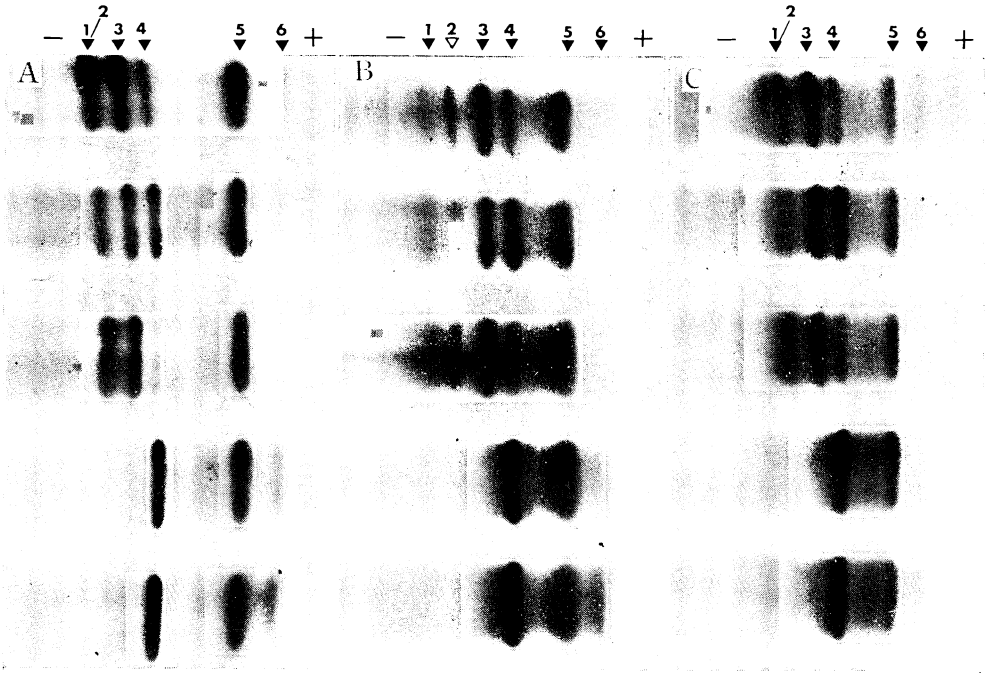


Fig. 1. Effect of starch quality on muscle protein patterns of "ginbuna" (upper three) and "gengorō-buna" (lower two) collected from the river Monobe. Condition of electrophoresis: 10% starch, pH 8.6 TC buffer for gel, pH 8.45 BN buffer for electrodes. A, amylan of lot 363110 (J company); B, starch-hydrolized of lot 299-1 (C lab.); C, lot 314-1 (C lab.). The 2nd band marked by open delta (▽) is specific in Type II-2 of "ginbuna".

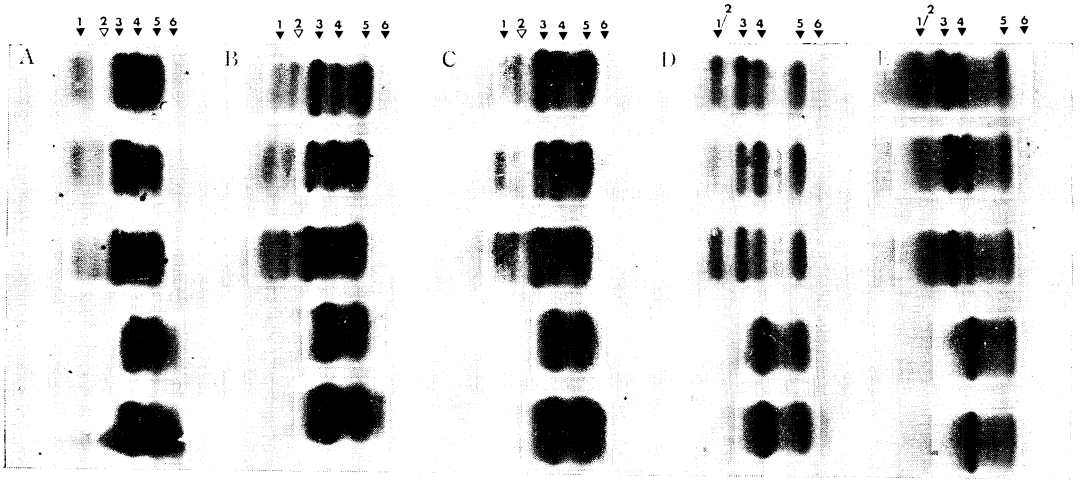


Fig. 2. Effect of starch-concentration of gel on muscle protein patterns of "ginbuna" (upper three) and "gengorōbuna" (lower two) from the river Monobe. Condition of electrophoresis: pH 8.6 TC buffer for gel, pH 8.45 BN buffer for electrodes, starch lot 317 (C lab.). A, 8.0%; B, 8.4%; C, 8.8%; D, 9.2%; E, 10.0%. The 2nd band marked by open delta (▽) is specific in Type II-2 of "ginbuna".

bility of bands. The pattern using lot 299 (Fig. 1, B) was provided with 6 bands including 2nd band marked by open delta which was a characteristic feature in "ginbuna" of western Japan. This 2nd band of "ginbuna" did not appear in the patterns of remaining lots (Fig. 1, A and C, Fig. 2, E). It was an important fact that the ability of separation of the 2nd

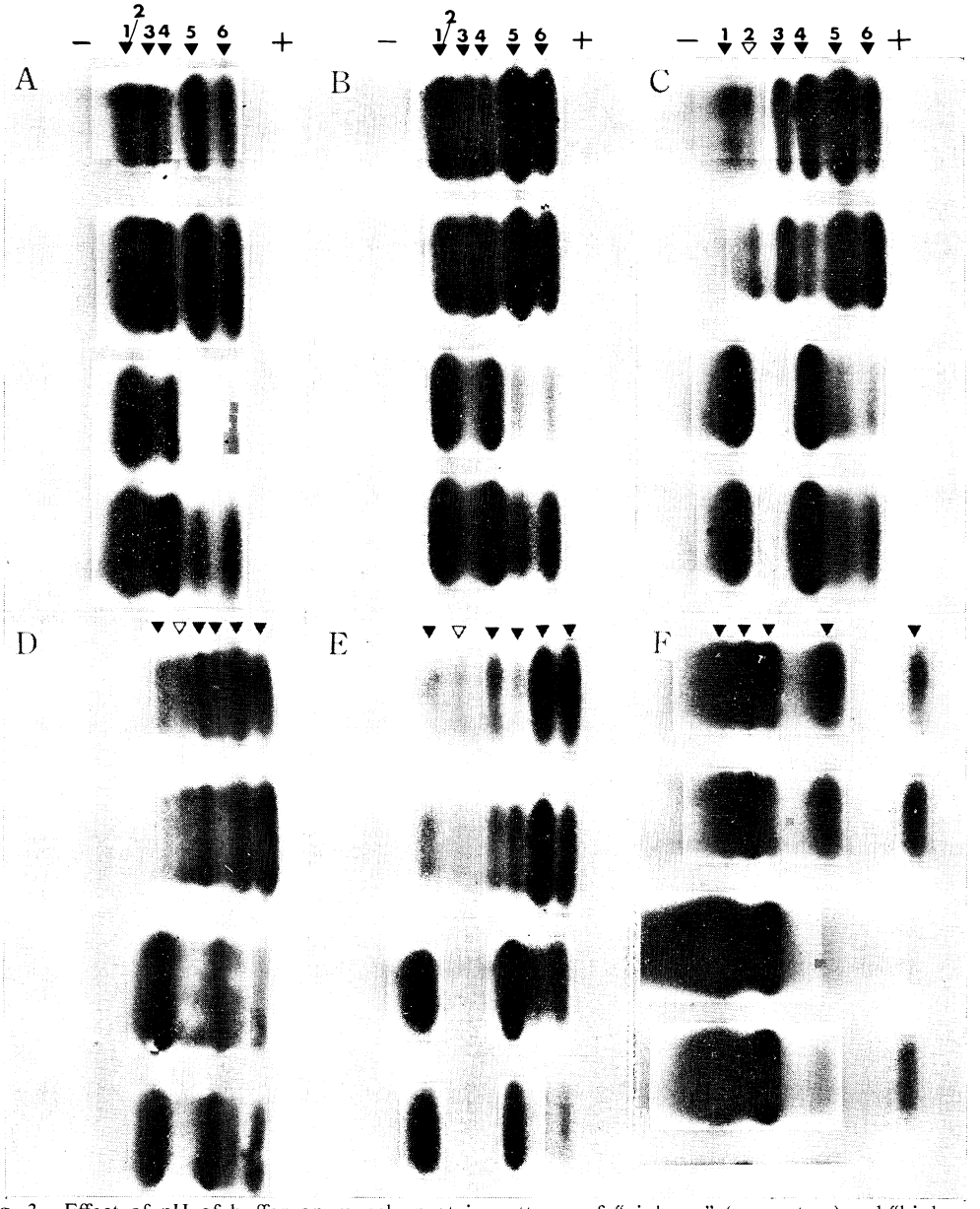


Fig. 3. Effect of pH of buffer on muscle protein patterns of "ginbuna" (upper two) and "kinbuna" (lower two) collected from the river Monobe. Starch used is the one of lot 317 (C lab.). A, pH 8.45 TC buffer for both gel and electrodes, 10% starch; B, pH 8.45 TC for gel, pH 9.0 BN for electrodes, 10% starch; C, pH 8.9 TC for gel, pH 8.6 BN for electrodes, 10% starch; D, pH 8.45 TC for gel, pH 8.6 BN for electrodes, 9% starch; E, pH 8.9 TC for gel, pH 8.6 BN for electrodes, 9% starch; F, pH 8.9 TBE for gel, pH 8.6 BN for electrodes, 10% starch. The 2nd band marked by open delta (▽) is specific in Type II-2 of "ginbuna".

band was different among the lots of starch made by the same factory. The situation of other common bands of two species "ginbuna" and "gengorōbuna" did not vary among the starch lots despite of the difference of separability of the 2nd band.

Figure 2 shows the effect of starch-concentration of gel on the patterns by using the same samples under the same experimental condition as in Fig. 1. The hydrolized starch used in this experiment was the lot 317 which did not appear the 2nd band at 10% starch-concentration of gel. The 2nd band appeared in the starch-concentration of 8.8% or lower. Generally, the separability for the cathodal bands of the 6 bands was clear under the condition of the lower starch-concentration. On the other hand, the separability of the anodal ones was clear under the condition of the higher starch-concentration of gel. There was no variation among the patterns of different starch-concentration in the position of common bands of these two species.

The pH of the gel and electrode buffer solution also affected on the muscle protein pattern (Fig. 3). The samples examined here were "ginbuna" (upper two examples) and "kinbuna" (lower two examples) collected from the river Monobe. The starch used here was lot 317 used in the above experiment. When the pH of gel buffer was identical with or lower than the pH of electrode buffer (Fig. 3, A and B), the 2nd band did not appear in 10% starch. When the pH of gel buffer was higher than the pH of electrode buffer (Fig. 3, C and E), the 2nd band appeared clearly in 9% and 10% starch. In the case of the lower pH of gel buffer than that of electrode, the

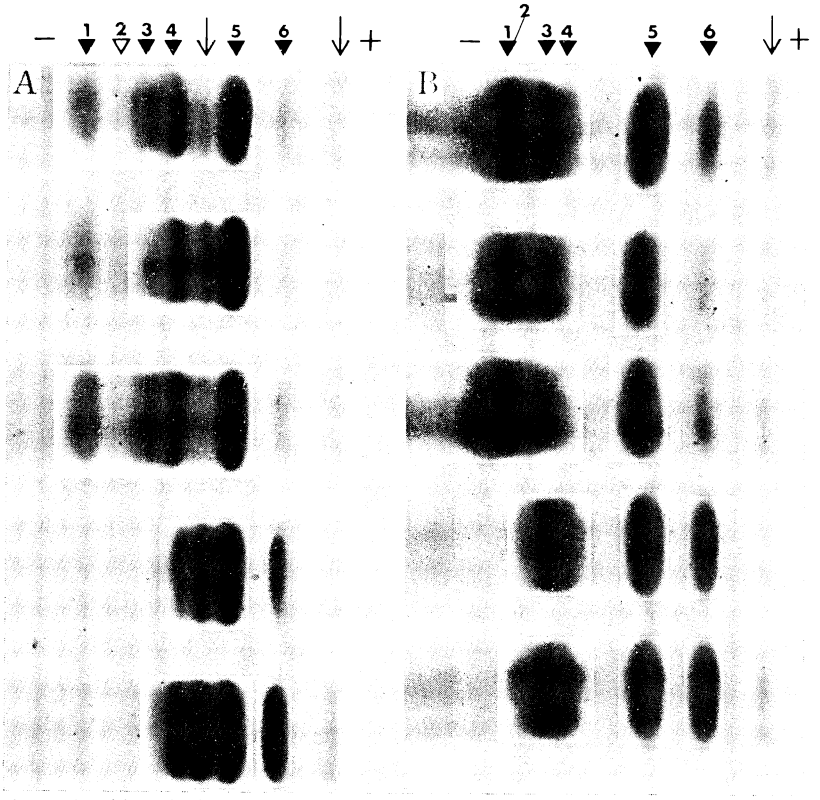


Fig. 4. Polyacrylamide-gel electrophoretic patterns of muscle protein of "ginbuna" (upper three) and "gengorōbuna" (lower two) from the river Monobe. Gel and electrode buffers are the same with Fig. 1. A, 4% polyacrylamide-gel; B, 5% polyacrylamide-gel. The 2nd band marked by open delta (▽) is specific in Type II-2 of "ginbuna" and the bands marked by arrows do not appear in the starch-gel electrophoretic method.

2nd band was observed in the starch concentration, 9% (Fig. 3, D), but separability was not satisfactory. When pH 8.9 TBE buffer for gel and pH 8.6 BN buffer for electrodes were used, the 2nd band was not observed (Fig. 3, F), but the separability of anodal three bands was good enough.

The starch-gel patterns of muscle protein were compared with the polyacrylamide-gel patterns (Fig. 4). The experiment condition of electrophoresis was the same as in Fig. 1. The bands were recognized more numerous in the 4% acrylamide concentration (Fig. 4, A) than in 5% of gel (Fig. 4, B). In the former, two bands marked by arrows newly appeared in addition to the 2nd band in the starch-gel pattern. The ability of separation was excellent in the acrylamide-gel method as to the three bands which are fastly migrating toward the anode. The 2nd band did not appear in the gel of 5%, and was faint in the gel of 4%, so that the acrylamide-gel method was not always good in the development of the 2nd band which was important to distinguish two species, "kinbuna" and "ginbuna" of western Japan.

**The classification of muscle protein patterns (Fig. 5)**

The starch-gel patterns of muscle protein of crucian carp were classified into four basic types. Type I was provided with the wide and deep-stained 1st band, the deep-stained 3rd and, or 4th bands, and the light-stained 5th and 6th bands, but lacking the 2nd band. In regard to the 3rd and 4th bands, this type was further divided into three subtypes I-A with the 3rd band, I-AB with both 3rd and 4th bands, and I-B with the 4th band. It was proved statistically in the previous paper that these three subtypes were controlled by two codominant alleles *A* and *B* (Taniguchi and Ishiwatari, 1972). Type I was recognized in "kinbuna", "kintarōbuna", "nagabuna", and "nigorobuna", the latter two showing somewhat lighter 1st band and thicker 5th band.

Type II was characterized by the deep-stained 5th band. This type was further divided into four subtypes, II-1 with the thick 3rd band, and lacking 1st, 2nd, and 4th bands,

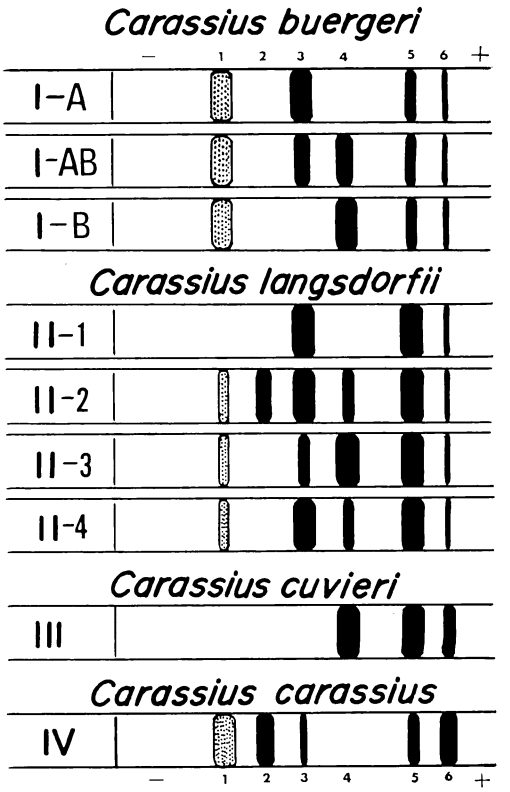


Fig. 5. The schematic figure of muscle protein patterns of Japanese and European crucian carps under the experiment condition, pH 8.6 TC buffer for gel, pH 8.45 BN buffer for electrodes, and 10% starch of lot 299. The dotted band (1st band) is characteristic in unsharp-convergency of the band.

II-2 with the light-stained 1st, 2nd, and 4th bands and the deep-stained 3rd band, II-3 with the thick 4th band and the light-stained 1st and 3rd bands, and II-4 with the thick 3rd band and the light-stained 1st and 4th bands. The Types II-1 and II-3 were usually observed in crucian carp from the lake Kasumigaura, II-2 was usually observed in those of waters of western Japan, and II-4 was very rarely observed. The pattern of "ginbuna" belonged to these types.

Type III was provided with the deep-stained 4th and 5th bands, the light stained 6th band, and lacking the 1st, 2nd, and 3rd bands. This type was observed in the pattern of "gengorō-buna".

Type IV was provided with the wide and deep-stained 1st band, the deep-stained 2nd

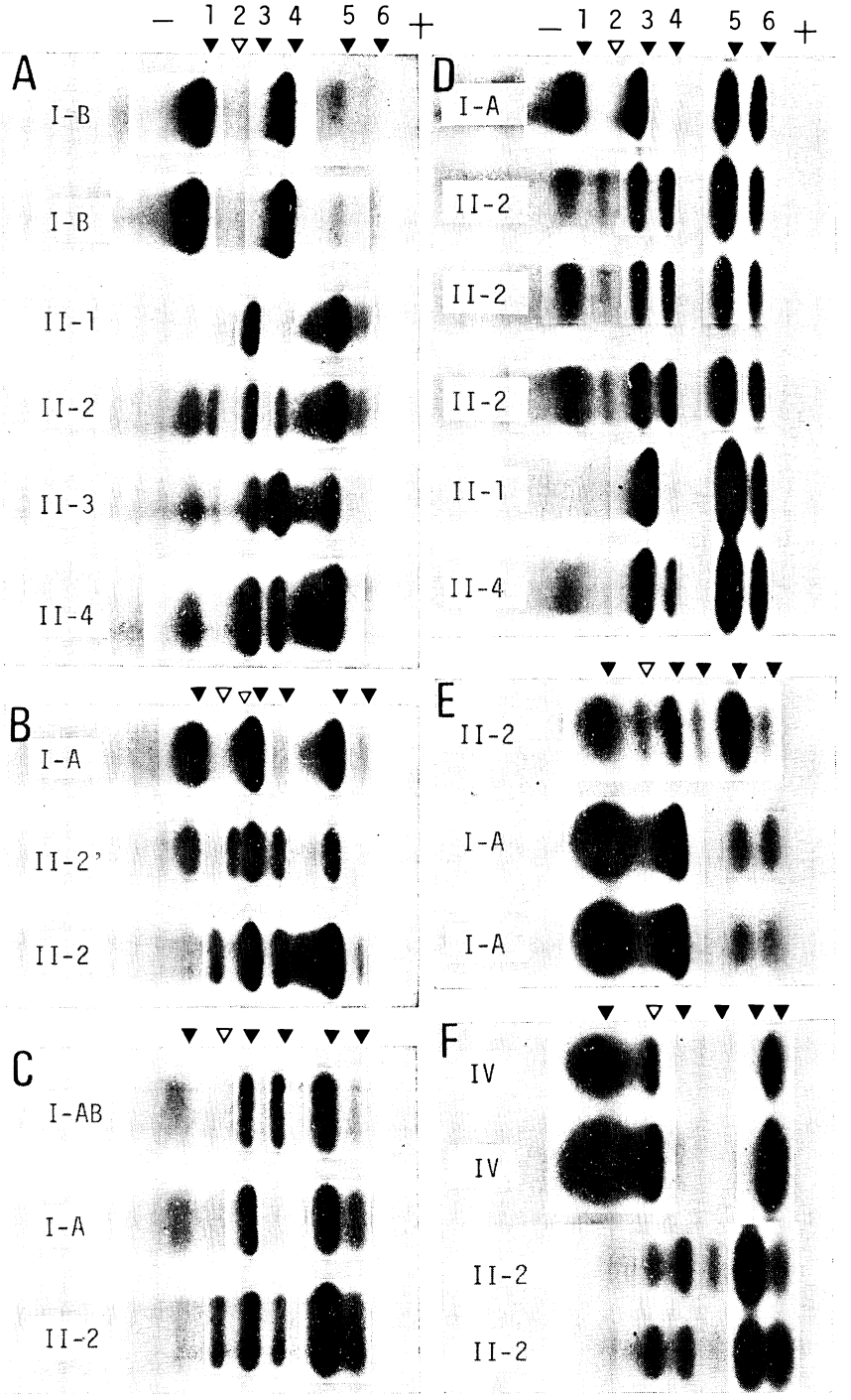


Fig. 6. Examples of starch-gel electrophoretic patterns of Japanese and European crucian carps. A, the lake Kasumigaura; B, the lake Suwa; C, the river Yasu flowing into the lake Biwa; D, the lake Kojima; E, a pond of Miyazaki city; F, European crucian carp (Type IV) and Japanese ones (Type II-2). The codition of electrophoresis is the same with Fig. 5.

and 6th bands, the light-stained 3rd and 5th bands, but laking 4th band. The 1st band of this type was similar to that of Type I. The pattern of European crucian carp belonged to this type.

**Comparison of the muscle protein patterns between *Carassius buergeri* and *Carassius langsdorfii* from the selected localities**

The patterns of the crucian carp from the lake Kasumigaura included Types I-B, II-1, II-2, II-3, and II-4 (Fig. 6, A). All the samples of "kintarōbuna" *Carassius buergeri* subsp-A, examined in this study and previous report (48 individuals) belonged Type I-B. The typical "ginbuna" *Carassius langsdorfii* (Nakamura, 1963) belonged to Type II-1 (73 individuals) or Type II-2 (4 individuals). As described before (Taniguchi, 1974), the samples belonging to Type II-3 (40 individuals) were intermediate between "kintarōbuna" and "ginbuna" morphologically and electrophoretically. Type II-4 was also intermediate between these two forms morphologically, but very few in number.

Figure 6B shows the patterns of crucian carp collected from the lake Suwa. Two patterns were observed, I-A in "nagabuna" and II-2 in "ginbuna". Concerning to the deep-stained 5th band, the pattern of "nagabuna" was not similar to Type I-A of the western Japan (Fig. 6, E), but similar to the pattern of "nigorobuna" (Fig. 6, C). A few individuals of "ginbuna" from the lake Suwa had a band which appeared between the 2nd and 3rd bands. These individuals, Type II-2′, might be mutant of Type II-2 of "ginbuna".

Figure 6C shows the patterns of crucian carp collected from the river Yasu flowing into the lake Biwa where "nigorobuna" is dwelling restrictedly. In this river, Types I-A, I-AB, and II-2 were observed. Types I-A and I-AB were similar to those of the "nigorobuna" (Taniguchi, 1974), concerning to the deep-stained 5th band. The individuals belonging to Type I-A and I-AB were identified with "nigorobuna" tentatively, in spite of comparatively fewer number of gillrakers (39-49) than in "nigorobuna" (50-70) because of the fact that the gillrakers of this subspecies increase with growth (Taniguchi, 1974).

Figure 6D shows the patterns of crucian carp collected from the lake Kojima. In this lake, the patterns were divided into 6 types, I-A, I-AB, II-1, II-2, II-3, and II-4. The individuals belonging to Type I were identified with "kinbuna" tentatively, and the individuals of Type II with "ginbuna". It was noticiable fact that Type II-1, which was rarely recorded in western Japan by this time, was observed plentifully in this lake.

Figure 6E shows the patterns of crucian carp collected from a pond of Miyazaki city. Eight of twelve individuals belonged to Type I-A,

Table 2. Comparison of types of muscle protein patterns for *Carassius buergeri* and *C. langsdorfii* examined in the present study and previous report (Taniguchi, 1974).

| Locality (Prefecture) | *Carassius buergeri* | | | | *Carassius langsdorfii* | | | |
|---|---|---|---|---|---|---|---|---|
| | I-A | I-AB | I-B | Frequency of gene A | II-1 | II-2 | II-3 | II-4 |
| Lake Kasumigaura (Ibaraki) | 0 | 0 | 48 | 0 | 73 | 4 | 40 | 1 |
| Lake Suwa (Nagano) | 25 | 0 | 0 | 1.00 | 0 | 60 | 0 | 0 |
| Lake Biwa (Shiga) | 103 | 178 | 69 | 0.55 | 0 | 13 | 0 | 0 |
| River Yasu (Shiga) | 4 | 4 | 0 | 0.75 | 0 | 2 | 0 | 0 |
| Lake Kojima (Okayama) | 6 | 2 | 0 | 0.88 | 15 | 23 | 1 | 1 |
| Lake Shinji (Shimane) | 0 | 6 | 23 | 0.10 | 0 | 68 | 0 | 0 |
| Rivers of eastern part of Kochi (Kochi) | 0 | 2 | 104 | 0.01 | 0 | 146 | 0 | 0 |
| River Shimanto (Kochi) | 6 | 63 | 57 | 0.30 | 0 | 135 | 0 | 0 |
| River Rokkaku (Saga) | 1 | 3 | 7 | 0.23 | 0 | 13 | 0 | 0 |
| A pond of Miyazaki city (Miyazaki) | 8 | 0 | 0 | 1.00 | 0 | 4 | 0 | 0 |

and four individuals to Type II-2. The external features of these two types were different with each other morphologically, so that the individuals of Type I-A having fewer number of gillrakers and slenderer body were identified with "kinbuna" and these of Type II-2 having numerous gillrakers and more compressed body with "ginbuna".

Figure 6F shows the patterns of European crucian carp compared with those of Japanese form in order to clarify the position of each band of the European crucian carp. The pattern of European crucian carp did not coincide with any type of the Japanese fishes.

## Discussion

The composition of muscle protein types of crucian carp from each locality examined in the present study and previous report (Taniguchi, 1974) was summalized in Table 2. The "gengorōbuna" was excluded from this table, for all of the specimens taken from various localities belonged to Type III. In almost all the localities studied here, the samples were divided into two groups, one with the pattern of Type I and the other with Type II. On the basis of the pattern, these two groups were believed to be not sharing the common gene pool, but maintained themselves respectively. Four subspecies of *Carassius buergeri* were similar with each other on account of belonging to Type I, but they had the difference in the frequency of allele *A* and density of 5th band. It was significant to see that these four subspecies distributed allopatrically and the respective subspecies of *Carassius buergeri* was living with "ginbuna" *Carassius langsdorfii* sympatrically. Among the waters of western Japan, the waters of Kochi included "kinbuna" *Carassius buergeri buergeri* and "ginbuna" *C. langsdorfii* which were well

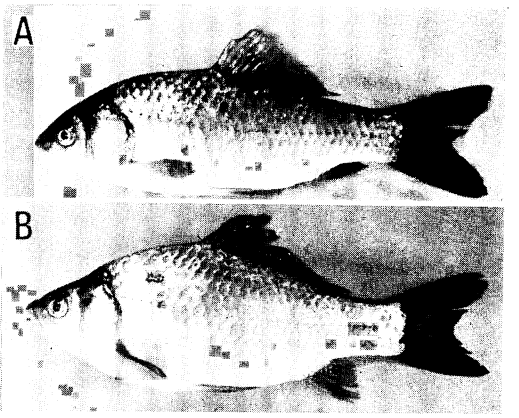


Fig. 7. Examples of crucian carp from the river Monobe. A, "kinbuna" *Carassius buergeri buergeri* showing the pattern of Type I-B; B, "ginbuna" *Carassius langsdorfii* showing the pattern of Type II-2.

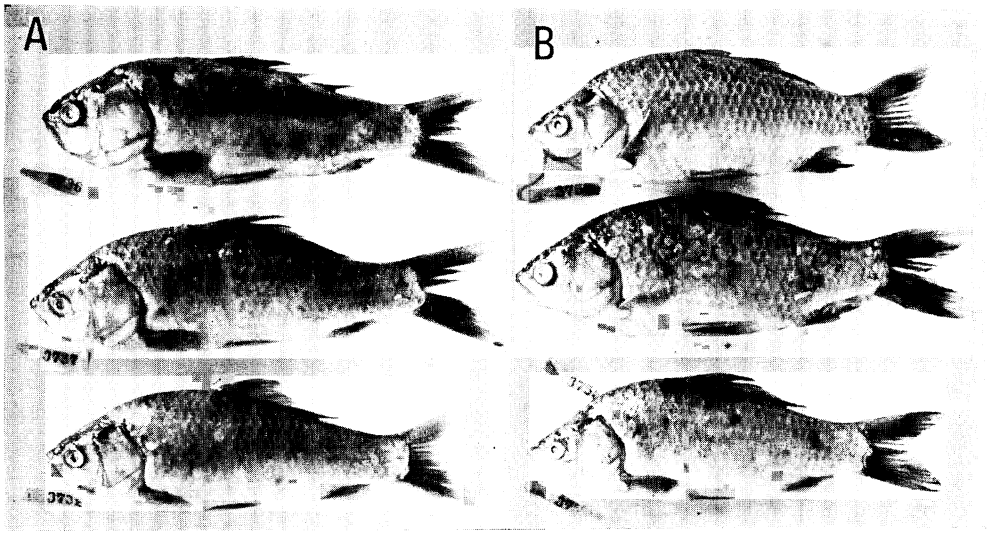


Fig. 8. Examples of crucian carp from the lake Suwa. A, "nagabuna" *Carassius buergeri* subsp-B showing muscle protein pattern of Type I-A; B, "ginbuna" *Carassius langsdorfii* showing the pattern of Type II-2.

discriminated morphologically (Fig. 7).

As already mentioned, "nagabuna" *Carassius buergeri* subsp-B was similar to "nigorobuna" *C. b. grandoculis* electrophoretically, and "nagabuna" was also provided with morphological characters similar to "nigorobuna" such as slenderer body, angular shape of mouth (Fig. 8), and comparatively numerous gillrakers, 52 (45～56). As shown in Fig. 8, "nagabuna" was easily distinguishable from "ginbuna" of the same water.

The frequency of allele *A* of *Carassius buergeri* varied between 0～1.00 and was rather high in samples from the lake Suwa, the river Yasu, the lake Kojima, and a pond of Miyazaki, and relatively low in samples from the lake Kasumigaura, the lake Shinji, the rivers of Kochi, and the river Rokkaku, and nearly intermediate in sample from the lake Biwa. The frequencies were different significantly even between closely located two waters, for instance, the lake Biwa—the river Yasu, and the rivers of eastern part of Kochi—the river of western part of Kochi (the river Shimanto).

The Type II was divided into four subtypes. But judging from these patterns, the relation among these subtypes could not be explained in the basis of Mendelian population concept, so that these four groups should not be considered to share the same gene pool. The typical "ginbuna" of the lake Kasumigaura included Type II-1 dominantly and Type II-2 rarely, and almost all of the "ginbuna" of western Japan except the lake Kojima belonged to Type II-2. Though the difference was observed in the composition of the pattern between the lake Kasumigaura and the waters of western Japan, these "ginbuna" were similar in morphological character with each other. As already mentioned, "ginbuna" of western Japan was featured by the 2nd band which was not recognized in other types of Japanese crucian carp but found in Type IV of European crucian carp.

In the samples of Type II of "ginbuna", males were rarely recognized (2 males out of 598 individuals). It was reported that "ginbuna" consisted of female only in the lake Kasumigaura, and the chromosomes was 3n (Kobayasi et al., 1970). The present authors also found that the number of chromosomes of "ginbuna" of western Japan was 3n (unpublished). Therefore, the gynogenetic reproduction of "ginbuna" suggested by Kobayasi (1971) was assumed to be performed in the same species of western Japan.

In the lake Kasumigaura, the intermediate form (Type II-3) between "kintarōbuna" and "ginbuna" was observed in the present study (Table 1). The specimens falling on this form showed the same pattern which was obtained by mixing the protein extracts of "kintarōbuna" (I-B) and "ginbuna" (II-1) at the ratio of 2 : 1, and showed the intermediate morphological characters between these two, suggesting the form sharing the genomes of these two species. Such instance of biochemical evidence for heterogenome-ploidy was already found in Poeciliidae (Balsano et al., 1972). As the male individual was not found in the samples of Type II-3, this form also seemed to be stable and maintaining themselves.

It was suggested that "gengorōbuna" was distantly related to "kintarōbuna" and "ginbuna", but the latter two were very closely affiliated to each other in hemoglobin pattern (Amano et al., 1971). Our study also suggested that "gengorōbuna" was remotely related to the other two species, "kinbuna" and "ginbuna", because of the clear difference in the muscle protein pattern.

The Type IV of European crucian carp examined here was similar to Type I of Japanese crucian carp in sharing the deep-stained 1st band and light-stained 5th band. Truweller et al. (1973) showed the muscle protein patterns of two crucian carps of U.S.S.R., *Carassius carassius* and *Carassius auratus gibelio*, in their study on the variability in disc-electrophoretic patterns of muscle myogens in carp. Our pattern of European crucian carp seemed to be similar to that of *Carassius carassius* by Truweller and others, but their pattern of *Carassius auratus gibelio* did not match any pattern of Japanese forms, although precise comparison in the same electrophoretic condition may be needed for confirmation.

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## 日本産フナの筋漿蛋白の電気泳動像にみられる種間および種内変異—II. デンプンゲル電気泳動像

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日本産フナの有効な分類基準を得るため，それらの筋漿蛋白像をデンプンゲル電気泳動法により分析した．デンプンゲル泳動像はデンプンの質，ゲルのデンプン濃度，ゲルと電極槽の緩衝液の pH などの要因の影響を受け，若干変化した．そこで種内および種間変異を最も明瞭に示す実験条件下で，分類学上重要ないくつかの地点から採集したフナ類の筋漿蛋白像を比較したところ，それらは基本的4型に類別された．第I型は *Carassius buergeri* の4亜種；キンタロウブナ，キンブナ，ニゴロブナ，ナガブナを含む．第1型は1遺伝子座の2対立遺伝子に支配される3変異型，I-A, I-AB, I-B 型に細分される．キンブナ類のA遺伝子頻度は地域によって異なり，0～1.00 の範囲で変動した．

第 II 型にはギンブナが含まれる．第 II 型もまた4変異型に細分されるが，バンドの数とそれらの濃度などから判断して，これらの変異が単に有性生殖集団内における共通の座の対立遺伝子による変異とは考え難い．これら4変異型のうち II-1 および II-3 型は霞ヶ浦に多く，II-2 型は西日本に多く，II-4 型は霞ヶ浦でわずかに認められた．ゲンゴロウブナは例外なく第 III 型に含まれた．ヨーロッパブナは日本産フナのどの泳動像にもあてはまらないが，キンブナの像にいくぶん似たところが認められた．

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