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Inter- and Intraspecific Variations of Muscle Proteins in the Japanese Crucian Carp—I. Cellulose-acetate Electrophoretic Pattern

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Abstract Muscle proteins of the Japanese crucian carp were analized by the cellulose-acetate electrophoresis. The muscle protein patterns usually composed of four components were divided into three types by the percentage of each component to total amount of protein. Type I, having more than 20% component 1, is represented by two forms, Kinbuna, *Carassius buergeri buergeri* and Nigorobuna, *Carassius buergeri grandoculis*. This type is also divided into three subtypes, A, AB, and B, by three phenotypic protein patterns controlled by two codominant alleles. A remarkable difference between Kinbuna and Nigorobuna was found in the occurrence of Type 1B, which is dominant in Kinbuna, not in Nigorobuna. Type II characterized by having more than 70% component 2 is represented by "Ginbuna," *Carassius langsdorfii*. Type III possessing components 2 and 3, each of which is nearly equal and about 40%, is represented by "Gengorobuna," *Carassius cuvieri*. The muscle protein electrophoretic patterns are proved to be useful to trace the speciation of the crucian carp.

Introduction

The speciation and evolution of the crucian carp widely distributed in the fresh water of Japan are interesting, since the fish shows marked variation in relation to differences in the environmental conditions, and with geographical isolation.

Temminck and Schlegel (1842) classified the Japanese crucian carp into four species, *Carassius buergeri, Carassius grandoculis, Carassius langsdorfii*, and *Crassius cuvieri*, the common Japanese name of which are Kinbuna, Nigorobuna, Ginbuna, and Gengorobuna respectively. Okada and Nakamura (1948) distinguished the crucian carp collected in Jonuma into three forms, Kinbuna, Ginbuna, and Gengorobuna by the coloration of the body and fins, and some meristic characters such as the dorsal fin rays, gillrakers, lateral line scales, and vertebrae. By examining the differentiation of the pattern of intestine of the Japanese crucian carp, Kafuku (1952) concluded the coiling and size of this organ are relevant to the speciation of this group. Tomoda (1960) also studied the fish from the Lake Biwa, and recognized three forms, Ginbuna(Hiwara), Nigorobuna, and Gengorobuna based on the differences of gill rakers, body depth, mouth form, pharyngeal teeth, airbladder, and pneumatic duct. Those morphological studies of this group clarified the status of the sympatric speciation of the Japanese crucian carp. Recently, Nakamura (1963, 1969) added Kinbuna (emended) *Carassius auratus* subsp., which is mainly distributed in northern Japan including Kanto district. It is said that the form is separable from the allied form, Temminck and Schlegel's *Carassius buergeri* newly named "Nagabuna" in Japanese by Nakamura, and ranged in southwestern Japan in having fewer soft rays and shorter base of dorsal fin.

In order to make up the genetical relationship of those forms, the fish of this genus has also been investigated from karyological and biochemical point of view. Kobayashi (1970) obtained an interesting information that the karyotype of Ginbuna in the Lake Kasumigaura is triploid or tetraploid. Amano et al.

Table 1. Locality, date, number of specimens, body length, number of dorsal fin rays, and gill-rakers of the Japanese crucian carps examined.

Species name	Locality	Date	No. of specimens ♂	♀	unknown	Body length in mm	Dorsal fin rays	Gill-rakers
C. buergeri buergeri (Kinbuna)	Lake Kasumigaura	Dec. '69	7	6	0	65–135	12–14	32–38
	River Monobe	Nov. '69	4	5	1	96–123	14–16	35–43
	River Shimanto	Sep. '70	3	5	5	90–170	14–17	37–50
C. buergeri grandoculis (Nigorobuna)	Lake Biwa	June '70	23	32	0	113–223	14–19	55–75
C. langsdorfii (Ginbuna)	Lake Kasumigaura	Dec. '69	0	8	0	77–168	16–19	45–50
	Lake Biwa	Oct. '69	0	9	0	108–184	16–18	43–51
		Mar. '70	0	9	0	120–139	15–17	43–47
	R. Monobe	Dec. '69	0	24	0	74–161	16–19	42–50
	R. Shimanto	Sept. '70	0	6	3	94–171	16–20	45–54
C. cuvieri (Gengorobuna)	Lake Biwa	Nov. '69	4	3	0	145–195	16–20	106–126
		June '70	2	7	0	139–275	16–17	104–124

(1971) analyzed the hemoglobin of three forms of this group by the starch-gel electrophoretic method, and concluded that Kinbuna and Ginbuna are very closely related to each other, but Gengorobuna is distant from other two forms.

The present study aimed to analized genetically both intra- and interspecific speciation of the Japanese crucian carp, sympatric or allopatric, by using the cellulose-acetate electrophoresis of muscle proteins.

Materials and method

The data of samples examined in this study, localities, dates of capture, body length, the number of dorsal fin rays and gillrakers were shown in Table 1. Even now, there are some confusions among taxonomists in identifying the species of the Japanese crucian carp. The present report followed the classification made by Matsubara and Ochiai (1965 : 531–536) as follows: Kinbuna, *Carassius buergeri burgeri* Temminck and Schlegel; Nigorobuna, *Carassius buergeri grandoculis* Temminck and Schlegel; Ginbuna, *Carassius langsdorfii* Temminck and Schlegel; Gengorobuna, *Carassius cuvieri* Temminck and Schlegel. The samples of Kinbuna collected from the Rivers of Monobe and Shimanto, Kochi Pref., seem possibly to be Nagabuna reported by Nakamura (1963). As already pointed out by him, the morphological and ecological characteristics of Nagabuna are still far from being satisfactorily understood. The specimens from Pref. Kochi here treated as Kinbuna along with those from the Lake Kasumigaura.

The samples were frozen immediately after catch, carried to the laboratory by packing with dry ice in ice box, and stored in a deep freezer at −20°C for the test.

The procedures of extraction of muscle proteins and cellulose-acetate electrophoresis were principally the same as those described in the previous report (Taniguchi, 1969). A small quantity of muscle, usually 3 g, cut off from a part of the trunk below the dorsal fin was homogenized by a glass mortar at the room temperature, with twice amount of Veronal buffer solution of pH 8.6 with μ=0.05 containing 50% of glycerol. The homogenates were centrifuged for 15 min. at 12,000 G. shortely after homogenization. The electrophoresis was carried out for 1.5 hours with current of 0.5 mA per 1 cm width of cellulose acetate sheet (Ceparax) made by Fuji Shashin

Co. Ltd. The muscle protein was stained with Ponceau 3R. After destaining and drying, the sheet of cellulose-acetate was made transparent with the liquid paraffin and scanned with a densitometer. The densitometric patterns were analyzed for the quantitative and qualitative comparison of muscle protein components.

Results

The patterns of muscle proteins of 157 specimens of crucian carp were classified into three groups, types I, II, and III on the basis of the percentage and mobility of those components. Three subgroups were discriminated in Type I, based on the differences of mobility and percentage in amount of each component in electrophoretic patterns of muscle proteins (Figs. 1, 2). There are four components here designated 1, 2, 3, and 4 by their positions from the cathodal side to anodal side.

Type I has component 1, amount of which is much higher than that of other types, and ranged from 20 to 51% (Table 2). The percentage of two components 2+3, is somewhat lower than that of other types, usually less than 70%. All of the specimens belonging to Kinbuna and Nigorobuna are included in Type I. This type is further divided into three subtypes, types IA, IAB, and IB by the percentages of component 2 and 3. In Type IA, component 2 is higher than 50%, but component 3 is not present entirely. Type IB, however, is characterized by possessing component 2 usually less than 20% and component 3 more than 35%. In Type IAB, the percentages of both components are intermediate between those two subtypes. All specimens of Kinbuna taken from the Lake Kasumigaura and River Monobe belong to Type IB. Of the 13 specimens of Kinbuna from the River Shimanto, three subtypes, A, AB, and B were found in the ratio 1: 6: 6. As the Kinbuna is a diploid species (Kobayashi, 1970), the expected ratio of those three subtypes controlled by two codominant alleles on a gene is calculated as 1.23: 5.54: 6.23. The difference in observed and expected values is not significant. Nigorobuna examined in this study has three subtypes, the ratio of which is as follows: A: AB: B=19: 22: 14. The difference between observed and expected values (16.33: 27.28: 11.36) is not significant statistically ($\chi^2=2.05$, $0.25>P>0.10$).

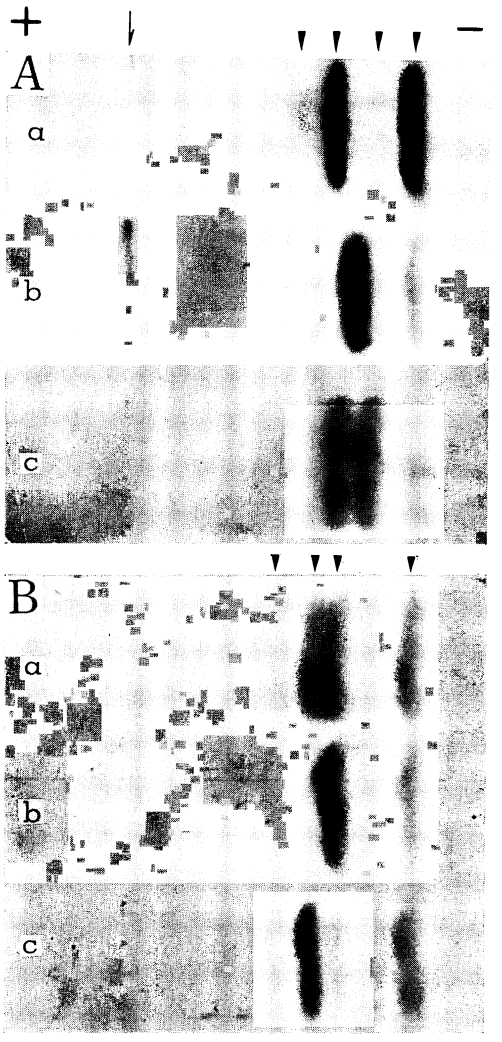

Fig. 1. The cellulose acetate electrophoretic pattern of muscle proteins of the crucian carp. The arrow shows the origin. A: the patterns of three forms. a. *Carassius buergeri buergeri* (Kinbuna), b. *Carassius langsdorfii* (Ginbuna), c. *Carassius cuvieri* (Gengorobuna). B: the variation found in *Carassius buergeri grandoculis* (Nigorobuna). a. Type I-AB, b. Type I-A, c. type I-B.

Table 2. Percentage distributions in amount of muscle protein components of the Japanese crucian carp. The mean value is followed by range in parantheses.

Name of species	Locality	Number of specimens	Type of pattern	Muscle protein component(%) 1	2	3	4	2+3
Kinbuna	Lake Kasumigaura	13	I-B	40.5(32–51)	8.5(5–12)	42.0(32–48)	8.9(6–12)	50.4(42–57)
	River Monobe	10	I-B	32.1(29–35)	9.8(6–14)	47.7(44–51)	10.3(9–13)	57.4(55–62)
	River Shimanto	1	I-A	23.2	67.2		9.6	67.2
		6	I-AB	30.8(28–33)	40.9(35–49)	23.3(14–26)	5.1(3–7)	64.1(49–75)
		6	I-B	37.7(20–46)	7.7(3–19)	47.6(44–51)	6.3(3–10)	55.2(47–70)
Nigorobuna	Lake Biwa	19	I-A	29.0(27–33)	57.1(52–65)		13.9(8–20)	57.1(52–65)
		22	I-AB	30.2(22–36)	34.9(31–38)	25.6(21–32)	9.4(6–13)	60.4(54–66)
		14	I-B	27.3(21–40)	19.1(10–23)	41.2(35–48)	12.4(9–18)	60.3(50–63)
Ginbuna	Lake Kasumigaura	8	II	10.4(12–15)	78.2(74–83)		11.4(5–20)	78.2(74–83)
	Lake Biwa	9	II	14.0(7–20)	72.3(70–78)		13.7(10–16)	72.3(70–78)
	River Monobe	24	II	10.3(8–15)	74.0(71–76)		15.7(10–21)	74.0(71–76)
	River Shimanto	9	II	9.8(7–14)	79.8(77–82)		10.4(6–16)	79.8(77–82)
Gengorobuna	Lake Biwa	16	III	10.7(6–14)	37.8(30–49)	38.9(25–48)	12.6(7–16)	76.7(71–83)

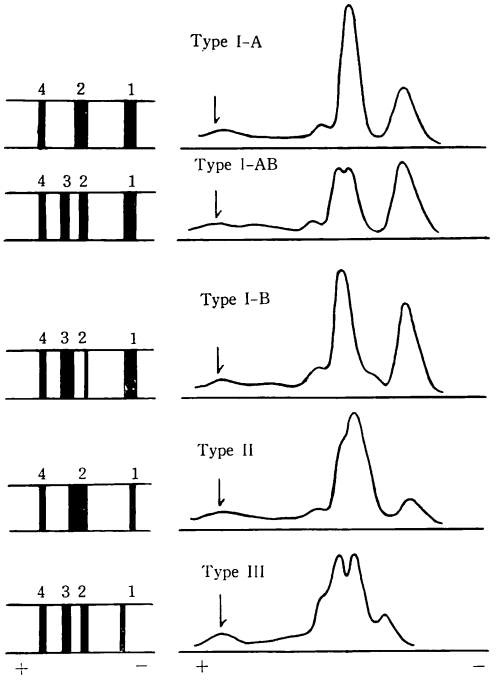

Fig. 2. The densitometric pattern (right side) and the schematic figure (left side) of the cellulose-acetate electrophoresis of muscle proteins for the crucian carp. The arrow shows the origin. The type I including three subtypes is represented by *Carassius buergeri buergeri* (Kinbuna) and *Carassius buergeri grandoculis* (Nigorobuna), the type II by *Carassius langsdorfii* (Ginbuna), and the type III by *Carassius cuvieri* (Gengorobuna).

Type II is distinguishable from Type I in component 1, the percentage of which is less than 20. The large amounts of component 2 in type II (70 to 80% in amount) may suggest that the component consists of two component 2 and 3. All of specimens of Ginbuna collected from Lake Kasumigaura, Lake Biwa, the Rivers of Monobe, and Shimanto were classified into type II. There are no significant differences on the percentages of each component among four different localities as given in Table 2.

Type III possesses components 2 and 3, each of which about 40 in percentage. This type resembles to Type IAB, in general, except for lower percentage of component 1. All examples of Gengorobuna from Lake Biwa belonged to this type.

Discussion

Four forms of the Japanese crucian carp, Kinbuna, Ginbuna, Nigorobuna and Gengorobuna, inhabiting the Lake Kasumigaura and Lake Biwa were identified in this study by the number of gillrakers and dorsal fin-rays as given Table 1. Those diagnoses were not adequate for the identification of Kinbuna and

Ginbuna from two rivers of Kochi Pref. The numbers of fin-rays and gillrakers of these two were more or less overlapped each other (Table 1). Coloration of the body, pectoral and pelvic fins is only useful character to separate them, though it is variable correlating to the environmental differences. The results obtained here, therefore, give important instrument for the taxonomy, genetics, and speciation of this group. The muscle protein pattern ought to be used for the identification for prevention of error.

Tomoda (1962) stated that Nigorobuna living endemically in the Lake Biwa alone is nearer in relationship to Kinbuna than Ginbuna or Gengorobuna, deriving from Kinbuna widely distributed in Japan. This opinion obtained from morphological analysis is well supported by the present results that both forms belong to Type I of muscle protein pattern.

Of the fishes of Type I, Kinbuna from the Lake Kasumigaura is slightly different from Kinbuna of the Rivers Monobe and Shimanto showing a higher percentage of component I. This minor difference suggests the intraspecific divergence of Kinbuna as already stated by Nakamura (1963, 1969).

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日本産フナの筋肉蛋白の電気泳動像にみられる種間および種内変異　　谷口 順彦・石渡 卓

日本産フナの有効な分類基準を得るため，それらの水溶性筋肉蛋白をセルロースアセテート電気泳動法により分析した．筋肉蛋白の電気泳動像は通常出現する四つの組成の濃度比により三つの型に分けられた．キンブナ (*Carassius buergeri buergeri*) およびニゴロブナ (*Carassius buergeri grandoculis*) は組成1の濃度が20%以上を示す (タイプI)．このタイプはさらにA, ABおよびBの三つのサブタイプに分けられる．これらの変異の出現度数はHardy-Weinbergの法則で期待される比とよく一致し，一つの遺伝子座の二種類の対立遺伝子によって支配されていると推定された．また，この変異の出

現率はキンブナとニゴロブナで著しく異っていた．ギンブナはタイプIIを示し，著しく高い濃度の組成 2(70%以上）により特徴づけられる．

ゲンゴロウブナでは組成2と組成3の濃度がほぼ等しく，組成1と組成4の濃度が著しく低い（タイプ III)．

このように筋肉蛋白の電気泳動像は日本産フナの分類基準として有効であり，フナ類の種分化に関する研究に役立つと思われる．

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