フナのヘモグロビンに関する電気泳動的研究

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Starch Gel Electrophoresis of Hemoglobin of "Funa"*

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Hemoglobins (Hb) of five subspecies of "funa", Japanese crucian carp, i. e., "kinbuna", "ginbuna", "gengorobuna", "nigorobuna", and "nagabuna", were analyzed by the starch gel electrophoretic method. Results obtained are summarized as follows: (1) More than two Hb patterns were presented by those subspecies except "nagabuna". Through all the patterns observed, most components showed common migration velocities and the slowest component was almost always predominant. (2) As far as the Hb patterns are concerned, "kinbuna" and "ginbuna" seem to be very closely related to each other. So is the case between "nigorobuna" and "nagabuna". On the other hand, "gengorobuna" appears to be somewhat distantly related to those four subspecies.

In 1903, JORDAN *et al.*[1] gave "funa" a scientific name, *Carassius auratus*. Later, TANAKA[2] advocated that the fish should be synonymized to *C. carassius*, the crucian carp in Europe. In 1963, NAKAMURA[3] raised an objection to those classification systems, proposing to divide the fish, though tentatively, into 5 subspecies: "kinbuna", *C. carassius* subsp.; "ginbuna", *C. carassius langsdorfii*; "gengorobuna", *C. carassius cuvieri*; "nigorobuna", *C. carassius grandoculis*; and "nagabuna", *C. carassius bürgeri*. MATSUBARA *et al.*,[4] on the other hand, suggested to classify the fish into 2 species and 2 subspecies in a slightly different manner. Very recently, NAKAMURA[5] has modified his preceding proposal, replacing the former specific trivial name, *carassius*, by the name, *auratus*, throughout the 5 subspecies he previously classified: e.g., *C. auratus* subsp., and *C. auratus langsdorfii*, were given to "kinbuna", and "ginbuna", respectively. Thus the taxonomy of "funa" seems still somewhat confused and to require further examination.

In the above studies, they used the conventional methods based on morphological and ecological analyses of fish. In the previous paper,[6] the present authors reported the salmonid fish hemoglobin (Hb) patterns as analyzed by the starch gel electrophoretic method, and suggested the usefulness of the method as a supplementary means for classification of the closely related fishes.

The above situation aroused the authors to undertake the present study. This paper communicates electrophoretic patterns of Hb's of "funa".

Experimental

Throughout this study, the classification system of "funa" as proposed by NAKA-

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Table 1. Specifications of the "funa" specimens used.

Name of subspecies	Sign of specimen group	Date of catch	Place of catch**	No. of specimens ♂	♀	Unknown	Body length	Body weight
							cm	g
"Kinbuna", *Carassius auratus* subsp.	*A*	Oct. 23, 1968	L. Kitaura	2	9	0	13.5–22.0	30–170
	B	Dec. 12, 1968	"	0	2	0	17.8–18.0	80– 95
	C	" "	L. Kasumigaura	1	7	0	13.5–22.5	38–185
	D	Feb. 10, 1970	L. Kitaura	6	2	0	12.4–17.0	30– 81
"Ginbuna", *C. auratus langsdorfii*	*E**	1962	R. Oyodo	5	2	0	15.1–17.8	40– 71
	F	Oct. 23, 1968	L. Kitaura	0	3	0	14.4–18.5	45– 90
	G	" "	L. Kasumigaura	0	1	0	21.5	150
	H	Dec. 12, 1968	"	0	8	0	16.7–22.5	70–180
	I	Feb. 20, 1970	L. Kitaura	0	11	0	16.5–24.0	100–305
"Gengorobuna", *C. auratus cuvieri*	*J*	Oct. 23, 1968	L. Kitraura	5	5	1	13.6–28.5	40–380
	K	Dec. 12, 1968	"	1	3	0	30.5–38.0	540–800
"Nigorobuna", *C. auratus grandoculis*	*L**	June, 1962	L. Biwa	5	2	0	12.0–17.2	18– 50
	M	Dec. 12, 1969	"	3	1	3	11.0–18.0	18– 80
"Nagabuna", *C. auratus bürgeri*	*N**	May, 1962	L. Suwa	3	5	0	11.0–16.8	20– 62

* Reared through several generations in isolated ponds of the Research Institure for Natural Resources, Tokyo, and analyzed for Hb in Feb., 1970.

** L. Kitaura=Lake Kitaura, Ibaragi Pref.; L. Kasumigaura=Lake kasumigaura, Ibaragi Pref.; R. Oyodo=River Oyodo, Miyazaki Pref.; L. Biwa=Lake Biwa, Shiga Pref.; L. Suwa=Lake Suwa, Nagano Pref.

MURA[5] will be followed for convenience.

Materials: Living specimens of the following five subspecies of "funa" were used: "kinbuna", *Carassius auratus* subsp.; "ginbuna", *C. auratus langsdorfii*; "gengorobuna", *C. auratus cuvieri*; "nigorobuna", *C. auratus grandoculis*, and "nagabuna", *C. auratus bürgeri*. Most specimens were wild ones, whereas some were descendants of wild ones as maintained through several generations in isolated ponds of the Research Institute for Natural Resources, Tokyo. Specifications of the fish specimens used are given in Table 1.

Blood was individually collected by heart puncture. Erythrocytes were separated by centrifugation, washed 2–3 times with 5–10-fold volumes of isotonic saline, and hemolyzed with about 2-fold volumes of distilled water. Supernatant hemolyzate solutions obtained by centrifugation were treated with trace amounts each of potassium ferricyanide and potassium cyanide to derive Hb into cyanmet form, and stored at −20°C until analysis.

Methods of analysis: Starch gel electrophoresis was carried out for 18 hrs at a constant voltage of 13 v/cm at about 4°C as reported previously.[7] The 0.033 and 0.33 M tris-borate buffers containing 0.01 % cyanide, pH 8.4, were used for the gel and bridge solution, respectively. At the end of the run, the gel was sliced into two halves and stained with amido black 10B and with benzidine, respectively. The Hb patterns obtained by the two staining methods were practically indistinguishable from each other. Benzidine stained gels were used for immediate observation of the patterns, while amido black stainedgels for densitometric measurement of the percentage distribution of Hb components in each pattern.[8]

Results and Discussion

Hb patterns of five subspecies: Ninty-six specimens of the five subspecies of "funa" were analyzed for Hb pattern to give the results which are shown in Fig. 1 and Table 2. The Hb components detected were numbered from the cathodic side to the anodic for convenience. Within a subspecies, types of pattern were distinguished based on differences in the number and positions of Hb components detected, in other words, on qualitative differences. Even qualitatively indistinguishable patterns were further divided into several types, if those patterns remarkably differed in relative proportions of some corresponding components (e.g., Patterns IV and V of "ginbuna"). As seen in Table 2, ranges of variation of several components were not always reasonably narrow, and hence each of those patterns containing such components might be possible to be distinguished into several patterns. However, no further discrimination was attempted this time considering relatively low accuracy of densitometric measurements.

It will be noticed from Fig. 1 that all those subspecies except "nagabuna" showed

Table 2. Percentage distributions in amount of Hb components of five subspecies of "funa".

Name of subspecies	Type of Hb pattern	Frequency of occurrence	Hb component							
			1	2	3	4	5	6	7	8
			%	%	%	%	%	%	%	%
"Kinbuna"	I	16	81(72–87)*		14(10–21)*	5(2–7)*				
	II	4	61(57–65)		16(15–17)	20(15–24)		3(1–4)		
	III	1	56		31	13	trace			
	IV	8	79(76–85)	9(6–11)	4(2–6)	5(3–6)		3(2–5)		
"Ginbuna"	I	7	80(75–81)	8(6–10)	4(3–5)	8(6–9)		trace		
	II	10	72(62–81)		21(14–29)	7(4–8)				
	III	1	75		18	4		3		
	IV	1	28		13	38		21		trace
	V	11	70(63–80)		8(5–14)	15(9–23)		4(3–5)		3(2–4)
"Gengorobuna"	I	13	69(59–79)	17(12–25)	10(6–14)	4(3–5)				
	II	2	62(56–69)	13(12–14)	11(8–13)	10(8–12)		4(3–5)		
"Nigorobuna"	I	7	81(71–87)	4(3–5)	4(2–6)	9(4–15)		2(1–3)		trace
	II	4	73(72–74)	12(12–12)	6(6–6)	4(4–5)		5(3–6)		
	III	2	70(69–72)	11(10–13)	6(3–8)	7(6–7)		6(5–8)		trace
	IV	1	73	10	5	7		5	trace	
"Nagabuna"	I	8	78(73–84)	3(2–5)	6(4–7)	11(7–13)		2(2–3)		trace

* In this table, average values and ranges of variation (parenthesized) of respective components are given for each type of Hb pattern.

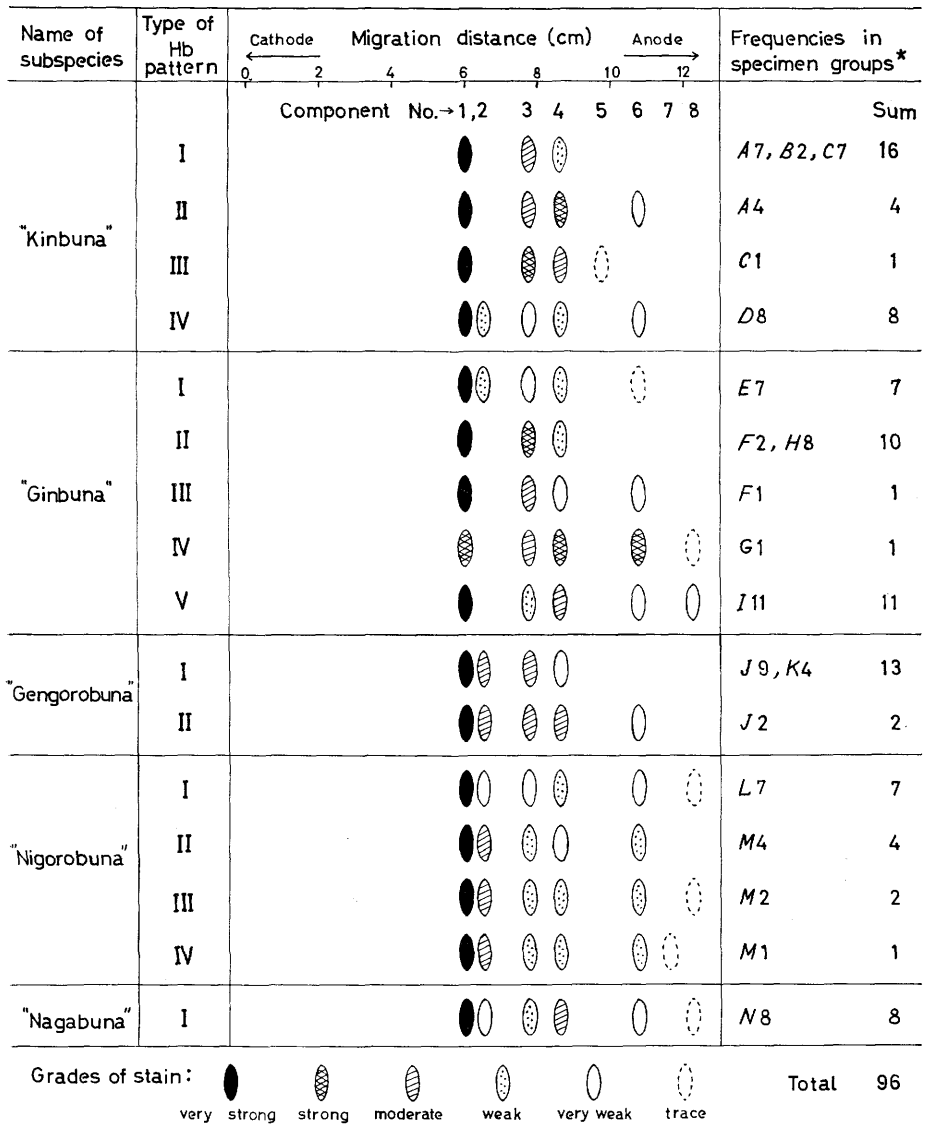

Fig. 1. Starch gel electrophoretic patterns of Hb of five subspecies of "funa", and their frequencies of occurrence in Specimen Groups, *A* ~ *N*.

* For example, *A* 7 and *B* 2 represent seven specimens of Group *A* and two specimens of Group *B*, respectively. Refer to Table 1 for the detail of specimen groups.

more than two types of pattern. The highest number of the types was five in "ginguna , followed by four in "kinbuna" and "nigorobuna". However, the number of specimens analyzed was generally small, and was only thirty even in "ginbuna", the number of which was greatest here. With any of the five subspecies, types of Hb pattern other than those here obtained may be found if the same electrophoretic analysis is extended to many more specimens. This seems to be true especially for "nagabuna" in which the

number of specimens analyzed was least here. It was also noteworthy that migration velocities of most components were common through all the patterns of a given subspecies, and that Component 1, the slowest one, was almost always most dominant, accounting for about 60–80% of the total. These features were recognized also throughout all the patterns of five subspecies. As a whole, there was a tendency that the faster the migration velocity of component, the lower its relative proportion. Component 8 was of trace amounts in most patterns where it appeared.

Correlations were examined between the type of pattern and the place of catch, the season of catch, sex, and size of fish. As seen in Fig. 1 frequencies of occurrence of a given pattern in a subspecies do not depend on specimen groups (refer to Table 1 for the detail of specimen groups), and so no clear correlations appear to exist between Hb pattern and the sex and size of fish. Some correlation may exist between the pattern and the place of catch in the case of "ginbuna": Group *E* specimens which were from Miyazaki Pref., exclusively showed a single Hb pattern (Pattern I) which was easily distinguished from the other "ginbuna" patterns (Patterns II–IV) of the specimens collected in Ibaragi Pref., suggesting some correlation of Hb pattern with the place of catch. On the other hand, specimens of several groups in "kinbuna", "binbuna", and "nigorobuna" presented Hb patterns which seemed to suggest the presence of correlation with the season of catch: e.g., in the case of "kinbuna", Group *D* specimens collected in Feb. showed only Pattern IV, in contrast to the other specimens caught in Oct. to Dec. showing Patterns I–III at random. In this respect, however, any clear conclusion was not drawn from those limited data.

In this connection, the specimens of each of Group *E* ("ginbuna"), *L* ("nigorobuna"), and *N* ("nagabuna") showed a single pattern respectively. However, it is not certain whether specimens of each group had had originally the same patterns at those observed here, or they were changed to present those patterns as a result of the rearing through several generations as mentioned above.

Comparison of Hb patterns of five subspecies: As Fig. 1 and Table 2 show, some pairs of practically indistinguishable patterns were recognized between two subspecies: Pattern I of "kinbuna" and Pattern II of "ginbuna", Pattern IV of "kinbuna" and Pattern I of "ginbuna", and Pattern I of "nigorobuna" and Pattern I of "nagabuna". In these pairs, the ranges of variation of relative proportion of corresponding components overlapped to considerable extent. In addition, those six patterns showed relatively high frequencies of occurrence. Therefore, in most cases, even if not all, "kinbuna" and "ginbuna" should be hardly distinguishable from each other based on electrophoretic patterns. So should be the case between "nigorobuna" and "nagabuna". On the other hand, Hb patterns, especially the dominant one (Pattern I), of "gengorobuna" were easily distinguishable from any pattern of the other four subspecies.

As far as the Hb patterns are concerned, it might be concluded that "kinbuna" and "ginbuna", or "nigorobuna" and "nagabuna" are much more closely related to each other among the five subspecies than are any two subspecies of the other combinations, while "gengorobuna" is somewhat distantly related to the other four subspecies.

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