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Are Three Protein Genotypes of a Cyprinid *Gnathopogon* from Lake Ono Morphologically Different from Each Other?

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Abstract : Population of *Gnathopogon* (Cypriniformes, Cyprinidae) from a dammed reservoir Lake Ono in Yamaguchi Prefecture is estimated to be a hybrid swarm between brooklet-dwelling *G. elongatus* and pelagic planktivorous *G. caerulescens* of Lake Biwa. Alleles on a protein-coding locus *Prot-3** are nearly exclusively fixed to **a* and **b* in *G. elongatus* and *G. caerulescens* respectively. *Gnathopogon* from Lake Ono, on the other hand, has both of homozygote and heterozygote genotypes of the alleles in its population, namely *aa*, *ab* and *bb*. Morphological comparison revealed that there are no differences in relative lengths and meristic counts among three genotypes of *Gnathopogon* from Lake Ono, which supports the hybrid swarm theory.

Key words : *Gnathopogon*, Cyprinidae, Lake Ono, hybrid swarm, morphology

Introduction

Lake Ono, a reservoir in Yamaguchi Prefecture, with a surface area of 25 km^2 and a maximum depth of 39 m, appeared in 1950 by construction of the Koto River Dam. A pelagic planktivorous cyprinid *Gnathopogon caerulescens* (Sauvage) was transplanted from Lake Biwa to Lake Ono for propagation purpose in 1953[1]. Today' s *Gnathopogon* fish in Lake Ono, however, are morphologically and genetically intermediate between *G. caerulescens* and a brooklet-dwelling omnivorous *G. elongatus* (Temminck & Schlegel), which strongly suggest that the Lake Ono population is a hybrid swarm[2] between the two species[3].

Gnathopogon elongatus and *G. caerulescens* exhibit different homozygote genotypes on a protein-coding locus *Prot-3**, *aa* and *bb* respectively, with limited exceptions[3]. The Lake Ono population, on the other hand, has homozygote and heterozygote genotypes, namely *aa*, *ab* and *bb*. Morphological comparison among the three genotypes of Gnathopogon from Lake Ono has yet to be made. In the present study, the Lake Ono fish were classified first according to the genotypes of the locus *Prot-3** by the electrophoresis method, following which their morphology was compared in order to confirm whether or not the morphological difference exists among the genotypes.

Materials and Methods

Fish Collection

Two hundred *Gnathopogon* fish were collected from Lake

2011年10月12日受付. Received October 12,2011

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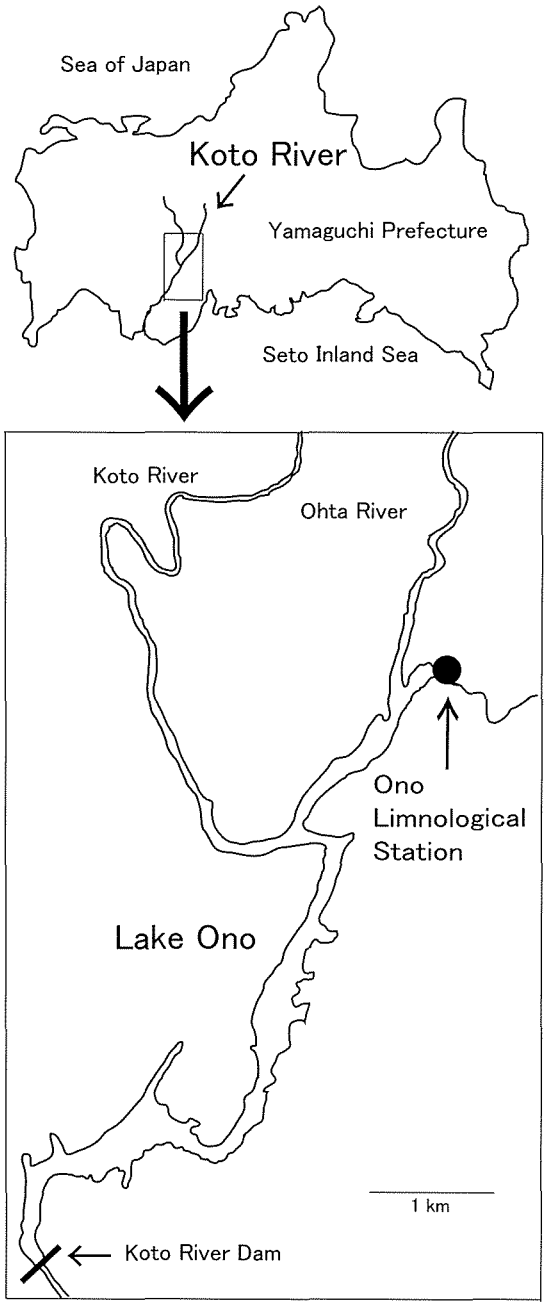

Fig. 1. Map showing the sampling location; Ono Limnological Laboratory by Lake Ono, Koto River, Yamaguchi Prefecture, Japan.

Ono near the Ono Limnological Laboratory (Figs. 1 and 2) with a small fish trap in September 1989 and frozen immediately after collection for electrophoretic analysis and morphological observation.

Electrophoresis

Small pieces of muscle were cut off from the frozen samples for electrophoresis, and the remaining bodies were fixed in a formalin solution for morphological observation.

In order to classify the protein genotypes of the Lake Ono fish, general protein electrophoregram was investigated by means of standard horizontal starch gel electrophoresis[3, 4] and the three genotypes on the protein-coding locus *Prot-3** were discriminated (Fig. 3, Table 1).

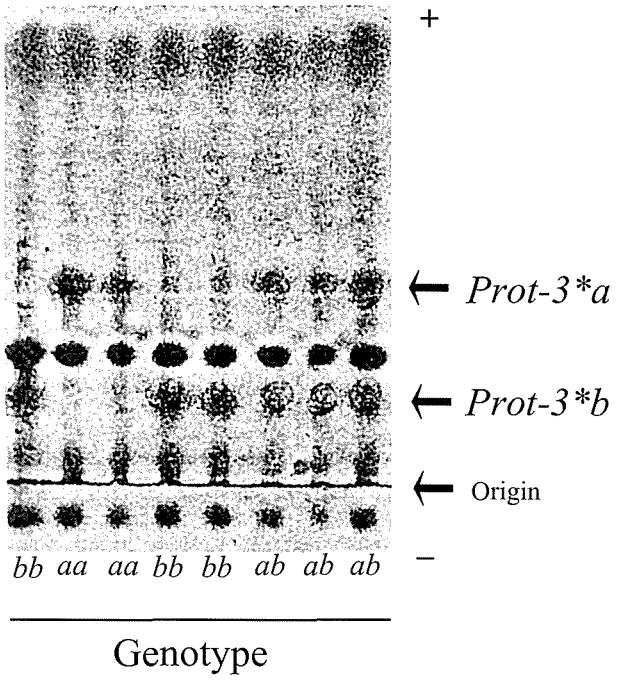

Fig. 3. Electrophoregram of general proteins for eight individuals of *Gnathopogon* from Lake Ono, exhibiting three genotypes, *aa*, *ab* and *bb*, in *Prot-3**

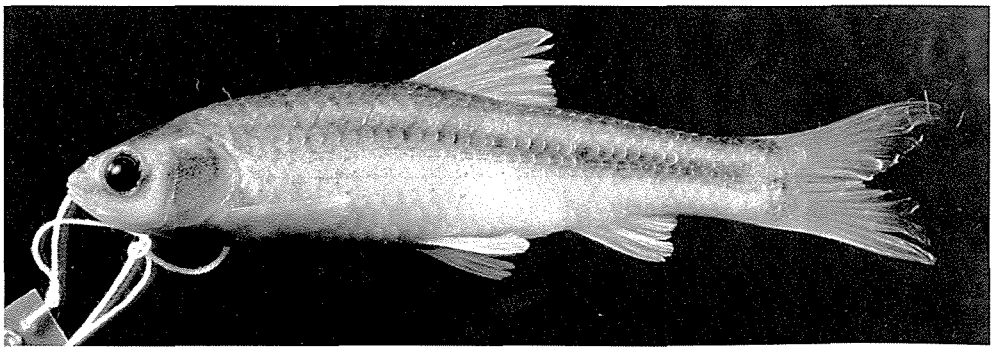

Fig. 2. *Gnathopogon* from Lake Ono, 66.5 mm SL (standard length).

Morphological Observation

Seven relative lengths in % of standard length, three relative lengths in % of head length, and six meristic counts were measured (see Table 2 for measured characteristics), all of which are significantly different between *G. caerulescens* and *G. elongatus*[3]. Barbel length that is characteristically different between *G. caerulescens* and *G. elongatus*[3] was not able to measure due to fragility of barbels of the frozen samples. Methods for measuring and counting followed Nakamura[5]. The number of vertebrae was counted with soft X-ray projection, the Weberian apparatus being counted to be four.

Differences among the three genotypes in mean values of 17 characters including standard length were tested by ANOVA (Analysis of Variance). To make an overall morphological comparison, a principal component analysis of 17 characters in log-standardized values was carried out.

Table 1. Number of individuals of three genotypes on general protein locus *Prot-3** of *Gnathopogon* fish from Lake Ono, and the result of chi-square test

	Number of individuals	
	observed	expected
Genotype		
aa	106	102.2
ab	74	81.5
bb	20	16.3
Total	200	200
Chi-square value	1.698	
Probability	$p > 0.3$	

Results

The number of individuals of each genotype is shown in Table 1, exhibiting no significant departure from the Hardy-Weinberg expectation (chi-square test, $p>0.3$). Allelic frequencies of alleles **a* and **b* on the locus *Prot-3** were 0.715 and 0.285 respectively. Standard lengths and all the characteristics of the three genotypes exhibited almost no significant difference (ANOVA, $p>0.1 - 0.9$, Table 2).

Table 3. Eigenvectors, eigenvalues and their cumulative contribution for PC 1, PC 2 and PC 3 in standard length, relative lengths and counts

	Eigenvectors		
	PC 1	PC 2	PC 3
Standard length	0.337	0.371	0.063
Head length	-0.348	0.184	-0.355
Body depth	-0.127	0.485	0.025
Caudal depth	-0.226	0.311	-0.017
Pectoral fin length	-0.348	0.136	-0.057
Pelvic fin lenrth	-0.416	0.028	0.147
Dorsal fin length	-0.432	-0.092	0.111
Anal fin length	-0.345	-0.019	0.215
Snout length	-0.032	-0.085	0.410
Eye diameter	-0.047	-0.341	0.392
Interorbital width	0.080	-0.049	0.454
Lateral line scales	0.183	0.192	0.003
Predorsal scales	0.063	0.197	0.368
Upper transverse scales	-0.043	0.356	0.265
Lower transverse scales	0.132	0.350	0.161
Vertebrae	0.135	-0.120	-0.160
Gill rakers	0.114	0.059	0.010
Eigenvalue	3.514	2.026	1.556
Cumulative contribution (%)	20.7	32.6	41.7

Table 2. Standard lengths, mean relative lengths and counts with standard deviation in parentheses in three genotypes on general protein locus *Prot-3** of *Gnathopogon* fish from Lake Ono with comparative data of *G. caerulescence* and *G. elongatus* appeared in Sakai et al. (2011)

	Genotype			Difference	*G. caerulescence*	*G. elongatus*
	aa	*ab*	*bb*	ANOVA, $p<0.05$	N = 91	N = 64
Standard length in mm (SL)	34.9 - 65.0	39.4 - 67.5	38.0 - 55.0	insignificant	60.1 - 93.1	22.1 - 68.8
In % of SL						
Head length (HL)	28.3 (1.1)	28.2 (1.1)	28.3 (1.1)	insignificant	25.4 (1.1)	30.3 (1.4)
Body depth	22.5 (1.5)	22.8 (1.9)	22.3 (1.6)	insignificant	19.4 (1.4)	24.6 (1.3)
Caudal depth	11.4 (0.8)	11.3 (0.5)	11.3 (0.5)	insignificant	9.3 (0.3)	12.2 (0.7)
Pectoral fin length	17.7 (1.0)	17.9 (1.0)	17.8 (1.1)	insignificant	15.7 (0.9)	17.3 (1.6)
Pelvic fin lenrth	15.6 (0.9)	15.7 (0.8)	15.8 (1.0)	insignificant	13.7 (0.7)	15.2 (1.3)
Dorsal fin length	21.7 (1.2)	22.0 (1.0)	21.8 (1.1)	insignificant	18.4 (1.0)	21.8 (1.5)
Anal fin length	15.1 (1.0)	15.3 (0.9)	15.3 (1.0)	insignificant	11.8 (0.9)	16.3 (1.2)
In % of HL						
Snout length	25.7 (1.7)	26.0 (1.6)	25.8 (1.2)	insignificant	26.6 (2.0)	27.3 (2.8)
Eye diameter	27.0 (1.3)	27.0 (1.5)	27.4 (1.4)	insignificant	19.6 (1.7)	20.3 (1.6)
Interorbital width	30.2 (1.9)	30.3 (1.9)	29.7 (2.0)	insignificant	28.5 (2.3)	33.5 (3.2)
Lateral line scales	36.6 (0.8)	36.6 (0.8)	36.3 (0.8)	insignificant	39.8 (1.3)	35.5 (1.3)
Predorsal scales	13.5 (0.8)	13.6 (0.8)	13.4 (0.6)	insignificant	14.7 (0.8)	12.8 (0.8)
Upper transverse scales	5.2 (0.4)	5.3 (0.4)	5.3 (0.5)	insignificant	6.1 (0.3)	5.1 (0.3)
Lower transverse scales	4.2 (0.4)	4.2 (0.4)	4.3 (0.4)	insignificant	4.9 (0.4)	4.0 (0)
Vertebrae	36.3 (0.6)	36.3 (0.6)	36.1 (0.8)	insignificant	39.2 (1.6)	36.0 (0.9)
Gill rakers	9.8 (0.9)	9.7 (1.0)	9.8 (1.2)	insignificant	16.8 (1.3)	8.4 (1.0)

Eigenvectors, eigenvalues and their cumulative contribution to principal components (PC 1, PC 2, and PC 3) in 17 characteristics are indicated in Tables 3. Among the characters whose absolute eigenvector value were more than 0.4 were pelvic fin length and dorsal fin length in PC 1, body depth in PC 2, and snout length and interorbital width in PC 3, which indicates their comparatively large contribution to the variation of respective principal component scores.

Mean principal component scores of the three genotypes were not different in all the three components (PC 1, PC 2, and PC 3) (ANOVA, p>0.1 – 0.8). Individuals of the three genotypes were scattered widely on three PC planes, namely PC 1 – PC 2, PC 1 – PC 3, and PC 2 – PC 3, overlapping largely with each other (Fig. 4).

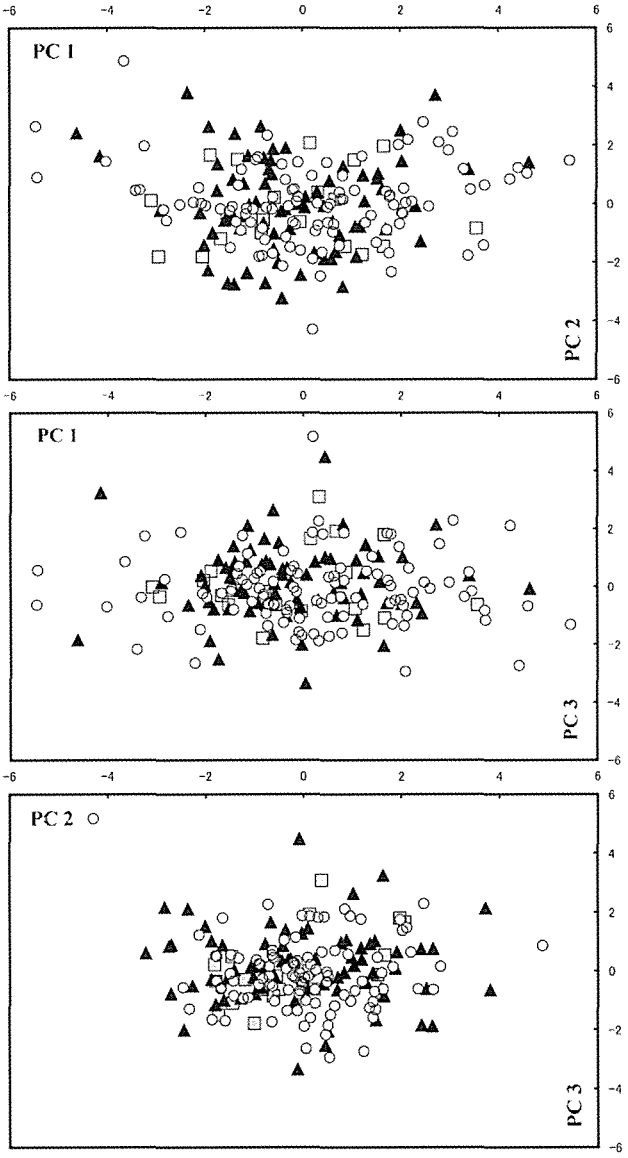

Fig. 4. Scattered diagrams of principal component scores PC 1 – PC 2, PC 1 – PC 3, and PC 2 – PC 3 based on 17 characters including standard length of three genotypes, *aa* (open circles), *ab* (closed triangles) and *bb* (gray squares) of *Gnathopogon* from Lake Ono.

Discussion

The population genetic data of the protein-coding locus *Prot-3** has reconfirmed that the Lake Ono population of *Gnathopogon* is a Mendelian population, having both of respective alleles peculiar to *G. caerulescens* and *G. elongatus*[3].

Individuals of *Gnathopogon* from Lake Ono were clearly divided into three *Prot-3** genotypes, *aa*, *ab* and *bb*. Relative lengths and meristic counts, however, were not different among these genotypes. The overall morphological comparison made no difference among these genotypes in any planes of three principal components, either.

As a result, the Lake Ono population of *Gnathopogon* has been confirmed again as a hybrid swarm[2] between introduced *G. caerulescens* and *G. elongatus*[3], with the any genotypes of the population crossing freely and exhibiting no morphological difference from each other.

One of other big issues concerned would be to monitor whether or not the Lake Ono population of *Gnathopogon* fish continues to be a stable hybrid swarm hereafter.

Acknowledgment

We are indebted to the staff of the Ono Limnological Laboratory for their cooperation in collecting materials and to K. Sakai for English correction.

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山口県小野湖産コイ科魚類モロコ *Gnathopogon* の筋肉タンパク質遺伝子型によって分けられる3型間に形態的差異はあるか

酒井治己，米花正三

山口県の小野湖（厚東川ダム湖）に生息するコイ科魚類のモロコ *Gnathopogon* 集団は，西日本の小川に生息するタモロコ *G. elongatus* と琵琶湖から移植された沖合性でプランクトン食性のホンモロコ *G. caerulescens* との雑種群と考えられている。筋肉タンパクをコードする遺伝子座 *Prot-3** において，タモロコは対立遺伝子 **a* に，ホンモロコは **b* にほぼ固定されているが，小野湖産のモロコ集団の遺伝子型には，それらのホモ接合体 *aa* および *bb* のほかにヘテロ接合体 *ab* が存在することが分かっている。このたび，小野湖産モロコ集団の各遺伝子型間で形態学的比較を行ったところ，計測形質にも係数形質にも差異がなく，本集団が両種の雑種群であることを裏付けた。