近年の劇的遺伝子変動から推定される,琵琶湖におけるフロリダバスの大規模な侵入

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Mass Introduction of Florida Bass *Micropterus floridanus* into Lake Biwa, Japan, Suggested by Recent Dramatic Genomic Change

Kōji YOKOGAWA[1], Katsuki NAKAI[2] and Kentarou FUJITA[3]

Abstract: The introduction of Florida bass *Micropterus floridanus* into Lake Biwa, where largemouth bass *M. salmoides* from North America had been propagating, was recognized. Isozymes of 194 individuals from four coastal localities in Lake Biwa from 2000 to 2003 were examined for comparison with former samples collected in the 1990s. *M. salmoides* and *M. floridanus* were genetically identified with the diagnostically polymorphic loci of *AAT-1**, *IDHP-1**, *MDH-1**, and *SOD**. Alleles symbolized *M. floridanus* frequently appeared at all of the loci among the individuals examined; in general, about half of the total had those alleles. All of the sample lots fitted the Hardy-Weinberg equilibrium for each polymorphic locus, and most of them showed an excess of heterozygotes. Based on the genotypes at the diagnostic loci, most individuals were identified as F_2 hybrids, backcross or higher-generation offspring, indicating that the hybridization between *M. salmoides* and *M. floridanus* has already progressed considerably in Lake Biwa. Considering the predominance of *M. salmoides* in Lake Biwa in the 1990s, the present results indicate the possibility that mass introduction of *M. floridanus* was carried out on a large scale so as to greatly influence the genetic characteristics of the *M. salmoides* population.

Key words: *Micropterus salmoides*; *Micropterus floridanus*; Lake Biwa; Hybridization

Largemouth bass *Micropterus salmoides* is a species of black bass (Centrarchidae), which originated from North American freshwaters (e.g. Hubbs and Lagler 1970). The species has been introduced into many countries and regions around the world, where it has successfully naturalized and often impacted seriously on indigenous ecosystems under wide climatic conditions (Lever 1996; Nakai 2002).

In Japan, the species was first introduced from the United States in 1925 by an enterpriser, Tetsuma Akaboshi, into Ashi Lake (Ashi-no-ko), Kanagawa Prefecture (Kaneko 1984; Kikkawa 1990; Akaboshi 1996). After a long interval of confinement within restricted water bodies, the largemouth bass started to expand its distribution explosively in the 1970s and has nowadays established nationwide colonization in Japan (Kiryu 1992; Akizuki 1999; Maruyama 2002). Meanwhile, the largemouth bass was also unofficially introduced into Lake Biwa, which is the largest lake in Japan, located in Shiga Prefecture, harboring many endemic species. It was first captured in 1974, explosively proliferated through the 1980s, and declined in the 1990s (Nakai 2001).

The largemouth bass is supposed to comprise two subspecies, that is, the northern largemouth bass *Micropterus salmoides salmoides* which is widely distributed in the northeastern part of North America, and the Florida largemouth bass *Micropterus salmoides floridanus* which

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[1] 13-5 Higashihama, Tadotsu, Nakatado, Kagawa 764-0016, Japan.
[2] Lake Biwa Museum, 1091 Oroshimo, Kusatsu, Shiga 525-0001, Japan.
[3] Division of Ecosystem Management, Environmental Science Graduate School, the University of Shiga Prefecture, 2500 Hassaka, Hikone, Shiga 522-8533, Japan.

has restricted distribution mainly in the Florida Peninsula (Hubbs and Lagler 1970; Ramsey 1975; Tomelleri and Eberle 1990; Jenkins and Burkhead 1993). However, in the review of the genus *Micropterus* based on much morphological and genetic information, Kassler et al. (2002) concluded that the two "subspecies" should be treated as distinct species, being *Micropterus salmoides* and *Micropterus floridanus*, respectively. This study hereafter will follow their taxonomic conclusion provisionally.

Some morphological characteristics are available for the identification of *M. salmoides* and *M. floridanus*. The most distinct diagnostic character is the number of pored scales on the lateral line, that is, the former has 68 or less and the latter has 70 or more (Tomelleri and Eberle 1990). The numbers of vertebrae (ventral and caudal count combination) (Chew 1975; Ramsey 1975) and of pyloric caeca (Chew 1975; Ramsey 1975), otolith morphology (Maceina and Murphy 1989) and body allometry (Bottroff and Lembeck 1978; Maceina and Murphy 1988) show significant differences between the two species.

Parallel with the morphology, there are considerable genetic differences between the two species. Philipp et al. (1981, 1983) detected some significant loci in which the alleles are replaced between the two species, followed by Williamson et al. (1986) reporting several additional isozymic loci available for genetic identification. Also, Carmichael et al. (1986) developed the isozyme analysis technique so as not to endanger live fish by using blood or fin tissues for biopsy. The genetic difference also appeared in characteristics of mitochondrial DNA (hereafter called mtDNA) of the two species (Bremer et al. 1998).

Although it has been reported that there are ecological differences between the two species, such as the spawning season (Isely et al. 1987), their natural hybridization has been observed when inhabiting sympatrically, (Childers 1975; Pelzman 1980; Tomelleri and Eberle 1990; Morizot et al. 1991; Gelwick et al. 1995), thus creating F_1 and F_2 hybrids, backcross and higher-generation offspring (Isely et al. 1987; Maceina et al. 1988). *M. floridanus* is described as growing bigger than *M. salmoides* does (Childers 1975; Week 1984; Tomelleri and Eberle 1990; Jenkins and Burkhead 1993), being preferred by anglers (Chew 1975).

Based on morphological and genetic analyses of largemouth bass from various localities in Japan including Lake Biwa, Yokogawa (1998) concluded that all the examined individuals shared characteristics unique to *M. salmoides*. The only documented introduction of *M. floridanus* was the case of Ikehara Reservoir, Nara Prefecture, which was confirmed by the appearance of haplotypes unique to *M. floridanus* in mtDNA examination (Kitagawa et al. 2000). Other than this exception, there has been no official record either of the introduction or capture of *M. floridanus* in Japan until recently. However, some alleles unique to *M. floridanus* were detected from the samples of largemouth bass collected from Lake Biwa in 2000 by isozyme examination. Therefore, further examinations were carried out to reveal the status of the *M. floridanus* introduction and the process of hybridization with *M. salmoides* previously naturalized in the lake.

Materials and Methods

A total of five sample lots were collected from four coastal localities (Nishiazai, Shiga, Moriyama, and Otsu) in Lake Biwa from 2000 to 2003 (Fig. 1; Table 1). Immediately after collection, they were preserved in a freezer at −20℃ until the genetic examination. Isozymes detected by horizontal starch-gel electrophoresis were used as genetic markers, and the lateral line scales were counted for the Nishiazai 2000 sample after the genetic examination. In order to confirm the alleles unique to *M. floridanus*, some bass individuals from Ikehara Reservoir collected by Kitagawa et al. (2000) were used as control samples, in which some mtDNA haplotypes unique to *M. floridanus* were detected. Extracts from the specimens were arranged with those from Lake Biwa in a single gel and the mobility of electrophoretogram bands was compared.

The method of genetic and morphological examination totally followed Yokogawa (1998). In

addition to enzymes and a protein examined by Yokogawa (1998), the following enzymes were newly examined: aconitate hydratase, E. C. 4. 2. 1. 3 (AH), fructose biphosphate aldolase, E. C. 4. 1. 2. 13 (FBALD), malic enzyme ($NADP^+$), E. C. 1. 1. 1. 40 (MEP). Further, the isozyme data from the Omi-hachiman sample in 1992, examined in a former report (Yokogawa 1998), and those from the Kusatsu sample in 1997 (Yokogawa, unpublished data) were also used for comparison (Fig. 1; Table 1).

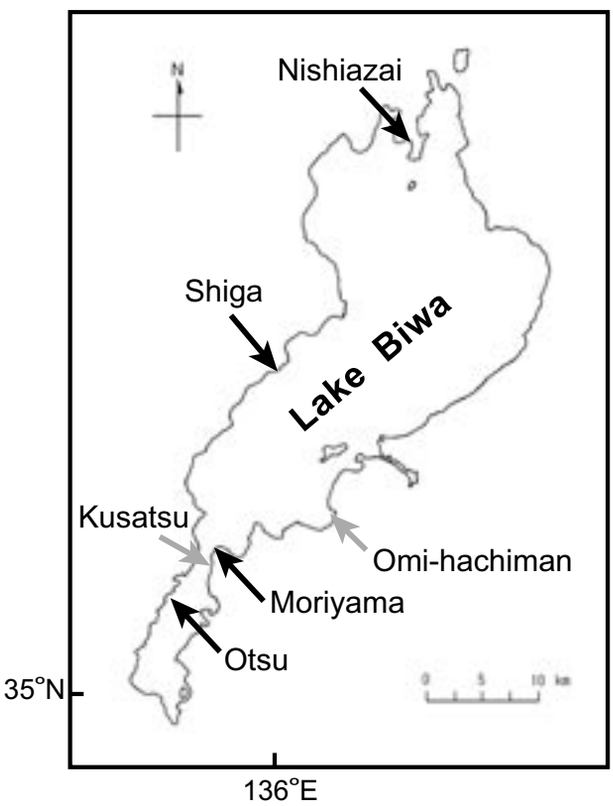

Fig. 1. Collection localities of the samples examined in Lake Biwa.

Results

Table 2 shows the allelic frequencies of the examined 27 loci and the values indicating the genetic features for the five sample lots examined in the present study, together with data of the samples collected in 1992 and 1997.

Philipp et al. (1983) detected four diagnostic isozyme loci to identify *M. salmoides* and *M. floridanus* in which the alleles are replaced or the allelic frequencies significantly differ from each other. Those loci named by them are Aat-B, Idh-B, Mdh-B and Sod-A, corresponding to the *AAT-1**, *IDHP-1**, *MDH-1** and *SOD** loci, respectively, in the present study. At these loci, the isozyme bands uniquely observed in the Ikehara samples were regarded as the alleles specific to *M. floridanus* reported by Philipp et al. (1983), and their mobility was compared with that of the samples from Lake Biwa.

Figure 2 shows an example of such comparison for *IDHP-1**. The mobility of the polymorphic bands in the Ikehara samples which are regarded as the alleles unique to *M. floridanus* corresponded well with the polymorphic bands detected in the present Biwa samples, indicating that the Biwa samples also included the alleles unique to *M. floridanus*. At the other diagnostic loci of *AAT-1**, *MDH-1** and *SOD**, the presence of the alleles unique to *M. floridanus* were similarly confirmed from the Biwa samples.

On the contrary, the alleles unique to *M. floridanus* did not appear in the former samples (Omi-hachiman 1992 and Kusatsu 1997) at any of the diagnostic loci of *AAT-1**, *IDHP-1**, *MDH-1** and *SOD**. However, they appeared in the samples collected in and after 2000, occupying

Table 1. Data from specimens examined, together with those to be referred

Sample name	Date of collection	Locality	Method of sampling	Individuals	Remarks
Nishiazai 2000	Aug. 2, 2000	Sugaura, Nishiazai	Gill net	9	All individuals are fingerlings
Moriyama 2001	March, 2001	Kihama, Moriyama	Fixed shore net	23	
Moriyama 2002	July, 2002	Kihama, Moriyama	Fixed shore net	78	
Otsu 2003	May – July, 2003	Hieitsuji, Otsu	Fixed shore net	3	All individuals are large (57 – 61 cm, TL)
Shiga 2003	Sep. 25, 2003	Kitakomatsu, Shiga	Beach seine	81	
Omi-hachiman 1992	Oct. 6 and Nov. 5, 1992	Omi-hachiman	Beach seine, Fixed shore net	94	Data from Yokogawa (1998)
Kusatsu 1997	Oct., 1997	Oroshimo, Kusatsu	Lure fishing	3	Yokogawa, unpublished data

Table 2. Allelic frequencies with values indicating genetic features of bass samples

Locus	Allele	Omi-hachiman 1992	Kusatsu 1997	Nishiazai 2000	Moriyama 2001	Moriyama 2002	Otsu 2003	Shiga 2003
*AAT-1**	**200* C[1]	0.000	0.000	0.444	0.250	0.625	0.500	0.389
	**100* A	1.000	1.000	0.556	0.750	0.375	0.500	0.611
*AAT-2**	**−100* A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*ADH**	**−100* A	1.000	1.000	0.944	0.957	0.987	1.000	0.994
	**−105* B	0.000	0.000	0.056	0.043	0.013	0.000	0.006
*AH**	**100* A		1.000	0.889	0.978	0.962	1.000	0.956
	**85* B		0.000	0.111	0.022	0.038	0.000	0.044
*FBALD-2**	**−100* A		1.000	1.000	1.000	1.000	1.000	1.000
*FBALD-3**	**−100* A		1.000	1.000	1.000	1.000	1.000	1.000
*GAPDH-1**	**100* A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*GAPDH-2**	**100* A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*GAPDH-3**	**130* B	0.000	0.000	0.000	0.000	0.019	0.000	0.000
	**100* A	1.000	1.000	1.000	1.000	0.981	1.000	1.000
*GPI-1**	**100* A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*GPI-2**	**100* A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*IDDH**	**−100* A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*IDHP-1**	**150* C	0.000	0.000	0.000	0.000	0.006	0.000	0.000
	**120* B[1]	0.000	0.000	0.556	0.413	0.596	0.500	0.475
	**100* A	1.000	1.000	0.444	0.587	0.397	0.500	0.525
*IDHP-2**	**100* A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*LDH-1**	**100* A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*LDH-2**	**0* A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*MDH-1**	**145* C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	**140* B	0.005	0.000	0.444	0.239	0.558	0.500	0.469
	**100* A	0.995	1.000	0.556	0.761	0.442	0.500	0.531
*MDH-2**	**150* B[1]	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	**125* C	0.000	0.000	0.222	0.043	0.006	0.000	0.037
	**100* A	1.000	1.000	0.778	0.957	0.994	1.000	0.963
*MDH-3**	**−100* A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*MEP**	**110* B		0.000	0.000	0.000	0.000	0.000	0.006
	**100* A		1.000	1.000	1.000	1.000	1.000	0.994
*PGDH**	**110* B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	**100* A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*PGM-1**	**100* A	1.000	1.000	1.000	1.000	0.994	1.000	1.000
	**55* B	0.000	0.000	0.000	0.000	0.006	0.000	0.000
*PGM-2**	**−100* A		1.000	1.000	1.000	0.994	1.000	1.000
	**−200* B		0.000	0.000	0.000	0.006	0.000	0.000
*PROT-1**	**100* A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*PROT-2**	**100* A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*PROT-3**	**0* A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*SOD**	**100* A	1.000	1.000	0.889	0.826	0.870	1.000	0.889
	**45* B[1]	0.000	0.000	0.111	0.174	0.130	0.000	0.111
Alleles/Locus		1.050	1.000	1.320	1.360	1.440	1.208	1.308
P*		0.000	0.000	0.360	0.200	0.200	0.125	0.192
P		0.050	0.000	0.000	0.120	0.240	0.000	0.154
P+P*		0.050	0.000	0.360	0.320	0.440	0.125	0.346
Average	Ho	0.001	0.000	0.111	0.077	0.091	0.125	0.062
Heterozygosity	He	0.001	0.000	0.093	0.069	0.074	0.063	0.071
	Ho/He	1.005	–	1.194	1.125	1.225	2.000	0.875

P* : Polymorphism less than 0.95%, Ho : Observed heterozygosity, P : Polymorphism over 0.95%, He : Expected heterozygosity.
[1] Allele unique to *Micropterus floridanus*.

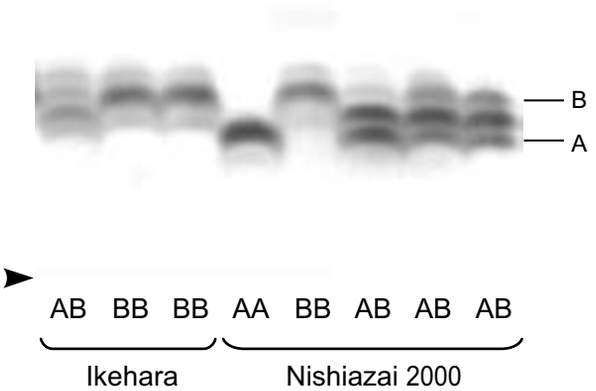

Fig. 2. Electrophoretogram indicating *IDHP-1**. Upper case letters represent genotypes.

considerable proportions of each locus (Table 2).

At the *AAT-1** locus, the alleles were completely replaced between the two species, that is, *M. salmoides* has A (named B_1 by Philipp et al. 1983) or B (named B_2 by Philipp et al. 1983) alleles, while *M. floridanus* has C (named B_3 by Philipp et al. 1983) or D (named B_4 by Philipp et al. 1983) alleles (Philipp et al. 1981, 1983). In Lake Biwa, the C allele, a marker for *M. floridanus*, appeared in all of the samples collected in and after 2000 with frequencies of 0.250 − 0.625 − i.e., about half of the loci having the C allele except for the Moriyama (2001) sample (Table 2).

At the *IDHP-1** locus, alleles were also completely replaced between the two species, that is, *M. salmoides* has the A (named B_1 by Philipp et al. 1983) allele, while *M. floridanus* has the B (named B_3 by Philipp et al. 1983) allele (Philipp et al. 1981, 1983). In Lake Biwa, the B allele appeared in all of the recent samples in and after 2000, with frequencies of 0.413 − 0.596, about half of the loci having the B allele (Table 2).

The *MDH-1** locus of *M. floridanus* is occupied by the B (named B_2 by Philipp et al. 1983) allele, whereas that of *M. salmoides* is occupied by the A (named B_1 by Philipp et al. 1983) allele in a few northern populations, but includes the B allele in some frequencies of other populations in the original distribution range in North America (Philipp et al. 1981, 1983). The frequencies of the A (B_1) allele are highly correlated with physical and environmental variables, such as latitude and temperature (Philipp et al. 1985). In the case of the bass in Lake Biwa, the B allele had been detected in the Omi-hachiman (1992) sample (Table 2), which was regarded to represent the original population. However, as its frequency had a very low value of 0.005 (Table 2), the appearance of the B allele in Lake Biwa can be regarded as coming from the introduction of *M. floridanus*. The B allele appeared in all of the recent samples with frequencies of 0.239 − 0.558, about half of the loci being occupied with the B allele except for the Moriyama 2001 sample, suggesting a recent introduction of *M. floridanus*. The frequencies of the B allele at the *MDH-1** locus were similar to those at the *AAT-1** locus in each sample (Table 2).

At the *SOD** locus, *M. salmoides* has the A (named A_2 by Philipp et al. 1983) allele, while *M. floridanus* includes the B (named A_1 by Philipp et al. 1983) allele in some frequencies with the A allele (Philipp et al. 1981, 1983). Therefore, the appearance of the B allele, which is unique to *M. floridanus*, can be regarded as evidence of the introduction of *M. floridanus*. In Lake Biwa, the B allele appeared in all of the recent samples except the Otsu 2003 sample with frequencies of 0.111 − 0.174 (Table 2), which were lower than those of the alleles unique to *M. floridanus* at the other diagnostic loci. The absence of the B allele in the Otsu 2003 sample would not negate a genetic inflow of *M. floridanus* because the

Table 3. Classification of individuals by diagnostic genetic markers

Type 1	*M. salmoides* pure individual (corresponding to all of the conditions of 1 − 4 listed below) 1 AA genotype at the *AAT-1** locus 2 AA genotype at the *IDHP-1** locus 3 AA genotype at the *MDH-1** locus 4 AA genotype at the *SOD** locus
Type 2	*M. floridanus* pure individual (corresponding to all of the conditions of 1 − 3 listed below) 1 CC genotype at the *AAT-1** locus 2 BB genotype at the *IDHP-1** locus 3 BB genotype at the *MDH-1** locus
Type 3	F_1 hybrid (corresponding to all of the conditions of 1 − 4 listed below) 1 AC genotype at the *AAT-1** locus 2 AB genotype at the *IDHP-1** locus 3 AB genotype at the *MDH-1** locus 4 AA or AB genotype at the *SOD** locus
Type 4	F_2 hybrid, backcross or higher-generation offspring (not corresponding to the categories of type 1 − type 3)

Table 4. Individual genotypes and type classification for Nishiazai 2000 sample

No.	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8	B-9
*AAT-1**	CC	AA	AC	AC	AC	AC	AC	AC	AC
*IDHP-1**	BB	AA	AB	BB	AA	BB	AB	AB	AB
*MDH-1**	AB	AA	AB	AB	BB	AB	AB	AA	AB
*SOD**	AA	AA	AA	AA	AA	AA	AA	AB	AB
Type[1]	4	1	3	4	4	4	3	4	3
LLS[2]	71	63	69	66	68	67	69	64	69

[1] Refer to Table 3. [2] Lateral line scale counts.

frequency of the B allele is low and the sample size is small ($n = 3$) (Table 1).

The values of the allele numbers per locus, the proportions of polymorphic loci (P, P*) and the average heterozygosity (Ho, He) were significantly higher in the recent samples than in the former samples (Table 2). A recent increase of genetic variability indicated by these indices is ascribed to the introduction of *M. floridanus* having genetic characteristics different from *M. salmoides*. The Ho/He ratio exceeded 1 in all the recent samples except the Shiga 2003 ones, indicating an excess of heterozygotes (Table 2).

An examination of fitness for the Hardy-Weinberg law by chi-square tests on the polymorphic loci resulted in no significance (5%) at any locus of all the samples, corresponding with the Hardy-Weinberg equilibrium.

All the individuals examined were genetically classified into four types based on the genotypes at the diagnostic four loci in order to identify *M. salmoides* and *M. floridanus*. Table 3 shows the keys to classification into the four genetic types. An example of the classification is given for the nine individuals of the Nishiazai 2000 sample with the data on the lateral line scale counts (LLS), which can be a morphological key to identify the two species (Table 4). All of the three individuals classified into type 3 (F_1 hybrid) had an LLS of 69, which is exactly intermediate between *M. salmoides* (68 or less) and *M. floridanus* (70 or more) (Table 4). One of the five individuals of type 4 (B-1) had an LLS of 71, showing the characteristic of *M. floridanus*, whereas the remaining four individuals of type 4 showed an LLS value within the range of *M. salmoides* (Table 4). The continuity of the LLS values shown in the present study suggests the impossibility of correct identification of the two species and hybrids based only on the LLS.

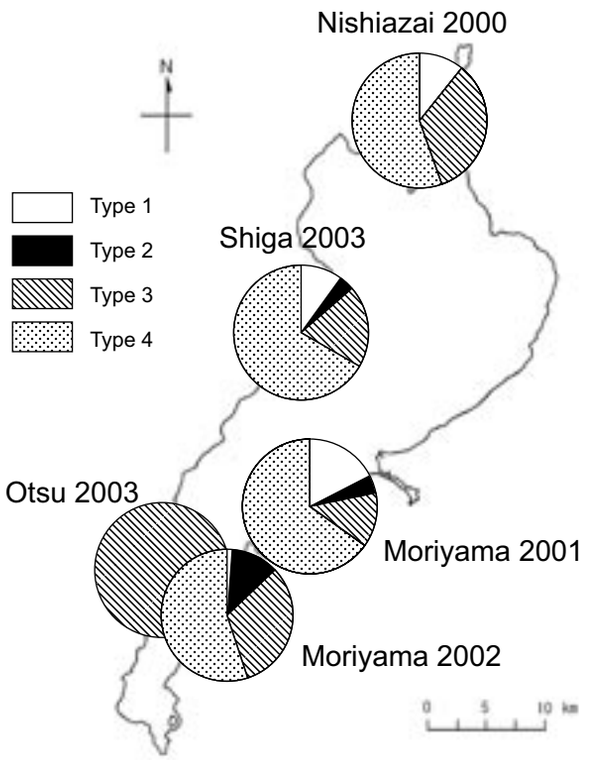

Fig. 3. Type compositions of examined samples geographically plotted (Refer to Table 3 for classification of the types).

Compositions of the types with pie graphs for the five recent samples were geographically plotted (Fig. 3). All the individuals of the Otsu 2003 sample which comprises only three large fish were type 3 (F_1 hybrid), while in the other samples, type 4 occupied the biggest proportion, followed by type 3. All the samples were characterized by the predominance of hybrid individuals (Fig. 3). Pure individuals, types 1 (*M. salmoides*) and 2 (*M. floridanus*), were also found in the sample lots other than Otsu 2003 at lower frequencies; the highest proportion of type 1 was found in Moriyama 2001 and that of type 2 was

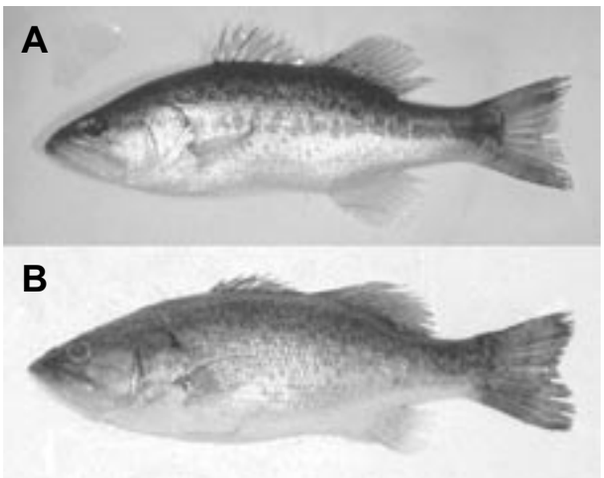

Fig. 4. Appearance of typical individuals of black bass in Lake Biwa. A, an individual from Omi-hachiman 1992 sample (130.6 mm TL); B, an individual from Nishiazai 2000 sample (No. B-6, type 4, 170.0 mm TL).

also in Moriyama (Fig. 3).

Figure 4 compares the appearance of typical bass individuals which inhabited Lake Biwa formerly and recently; the former pure *M. salmoides* (type 1 in the Omi-hachiman 1992 sample; Fig. 4-A) and the hybrid (type 4 in the Nishiazai 2000 sample; Fig. 4-B). It appears to be significant that body shape and coloration have considerably "changed" in the bass inhabiting Lake Biwa.

Discussion

The present study revealed the recent genomic inflow of *M. floridanus* on quite a large scale into the pre-existing *M. salmoides* population. The fact that the alleles unique to *M. floridanus* were absent in the former samples but appeared in unexpectedly great proportion in the recent samples suggests that an intentional introduction of a considerable number of *M. floridanus* into Lake Biwa was recently carried out in spite of prohibition by the local fishing regulations in Shiga Prefecture. Such a dramatic genetic change never occurs as a natural process. Those responsible for the introduction might have aimed to propagate larger bass with *M. floridanus* for lure fishing. As *M. floridanus* has been actively stocked in southern waters in the United States, it was introduced into Ikehara Reservoir in order to create the "monster" bass that would attract many anglers to an underpopulated mountainous region (Nishiyama 1988).

It is widely accepted that *M. floridanus* grows bigger than *M. salmoides* does (Childers 1975; Week 1984; Tomelleri and Eberle 1990; Jenkins and Burkhead 1993). However, this seems to be related not to inherited factors but to the conditions of the environment which it inhabits (Chew 1975).

Experimental culture of the two species and their F_1 hybrids resulted in a growth rate which was highest for *M. salmoides* under the same rearing conditions (Zolczynski Jr. and Davis 1976; Isely et al. 1987; Kleinsasser et al. 1990; Williamson and Carmichael 1990; Philipp and Whitt 1991). Similarly, *M. salmoides* showed greater growth than the F_1 hybrid in the same experiment (Buck and Hooe 1986), and it grew bigger than coexisting *M. floridanus* in a single natural lake (Maceina et al. 1988). Such information would suggest that the growth rate of *M. floridanus* is essentially inferior to that of *M. salmoides*.

McCauley and Kilgour (1990) statistically evaluated the growth of the largemouth bass, including *M. floridanus*, and the geographic air temperature over North America, concluding that the growth rate of the bass was highly correlated with the temperature. In conclusion, the larger size achieved by *M. floridanus* is due to differences in longevity rather than a difference in the growth rate, (Childers 1975) attributed to the warm Florida climate, a longer growing season and other favorable environmental factors (Chew 1975).

It is still uncertain whether the thermal condition in Lake Biwa should be a limiting factor for *M. floridanus*, which is believed to have speciated under a subtropical climate. The minimum water temperature (about 5－6℃) of the lake surface (Lake Biwa Research Institute and National Institute for Research Advancement 1984) might be so low as to restrict its survival in the northern region of the United States, as suggested by Childers (1975). Nevertheless, the present results show that *M. floridanus*, which is expected not to adapt to cold climates, actually survives in Lake Biwa.

In Lake Biwa, largemouth bass larger than 60 cm in total length have occasionally been reported in the last several years in magazines specializing in black bass fishing, inferring the possible occurrence of *M. floridanus* (Nakai, unpublished). Warm water temperatures higher than 15℃ from late April to November with a maximum (ca 27℃) in August (Lake Biwa Research Institute and National Institute for Research Advancement 1984) may be appropriate for growth and survival of the newly introduced *M. floridanus*, and might result in the occurrence of the large individuals.

Although a dramatic genomic inflow of *M. floridanus* between 1992 and 2000 has been demonstrated, it is difficult to determine the exact timing when *M. floridanus* was introduced. However, it is very likely that it took place illegally during the period between those years. Following a dramatic decline in the largemouth bass population in the earlier 1990s, the estimated population size in Lake Biwa was stable in the later 1990s (Nakai 2001). Even after the decline, largemouth bass were still predominant in the littoral fish community, ranking first and sixth in biomass and abundance, respectively (Shiga Prefectural Fisheries Experimental Station 1996; Nakai 2001), inferring some effects of the introduction. The three large F_1 individuals of the Otsu 2003 sample (57 – 61 cm, TL), which are presumed to be over 5 years old from their body sizes (Webb and Reeves 1975; Yoshizawa 1992), might be the initial hybrids between the released *M. floridanus* and the original *M. salmoides*.

In spite of the difficulty of estimating the exact number of *M. floridanus* individuals introduced, the scale of introduction was inevitably so great as to dramatically affect the genetic properties of the extant huge population of *M. salmoides*, as long as the mechanism by which the genes of either species are selectively retained is not present. If some portion of the *M. floridanus* initially introduced could not have survived because of maladaptation to the winter water temperature of Lake Biwa, the number of individuals introduced should have been more than that estimated from the proportions of *M. floridanus* alleles observed in the present study. In addition, the fact that the alleles unique to *M. floridanus* appeared in similar frequencies in all the localities may suggest the possibility that *M. floridanus* were successively introduced in plural localities.

In Japan, until this study, the genes of *M. floridanus* have been recognized only from the bass inhabiting Ikehara Reservoir. It can thus be speculated that the introduced *M. floridanus* were imported from Ikehara Reservoir, but this hypothesis can not be positively supported for the following reasons:

1: Because the proportion of the *M. floridanus* haplotype in Ikehara Reservoir is 0.568 (Kitagawa et al. 2000), about twice the individual number of original *M. salmoides* is required for the present allelic composition of *M. salmoides* and *M. floridanus* in Lake Biwa. Such a great deal of bass stock is not available in Ikehara Reservoir.

2: *M. floridanus* pure individuals occasionally occur in Lake Biwa (Fig. 5). Even though they may be F_1 hybrids between pure *M. floridanus* parents, it is apparent that the released bass included at least some *M. floridanus* pure individuals. However, most of the bass inhabiting Ikehara Reservoir were genetically and morphologically presumed to be hybrids between *M. floridanus* and *M. salmoides* (Kitagawa et al. 2000; Senou 2002).

3: Minor isozymic alleles which appear in the bass from Ikehara Reservoir or downstream Nanairo Reservoir have not been detected in the bass from Lake Biwa (Yokogawa, unpublished data).

Thus, most of the bass released into Lake Biwa might be *M. floridanus* pure individuals which were not from Ikehara Reservoir but through another route such as direct mass import from North America.

Since all the examined samples included many hybrid individuals, most of them showed an excess of heterozygotes (Fig. 3; Table 2). The present results showed that hybridization has so progressed in Lake Biwa that all of the samples corresponded with the Hardy-Weinberg equilibrium. As hybridization is still presumed to be progressing, it can be predicted that the

bass inhabiting Lake Biwa will totally be type 4 hybrids in the near future. Such potential can also be inferred from reports in North America that bass proportions rapidly assumed Florida-like characteristics after introgression of *M. floridanus* (Bottroff and Lembeck 1978), and that the genomic inflow from *M. floridanus* into *M. salmoides* is rapid (Maceina et al. 1988).

Hereafter, it is necessary to monitor the hybridizing process of the bass in Lake Biwa, including expansion of the *M. floridanus* gene into other freshwater bodies in Japan, such as Kawaguchi Lake (Kawaguchi-ko), Yamanashi Prefecture, where local fisheries cooperatives grant fishing rights for largemouth bass and translocate them from Lake Biwa to satisfy anglers' demands (Takahashi 1999). Genetic examination is indispensable for such monitoring, because it is difficult to identify hybrids with morphological characters such as LLS when type 4 individuals are generated in the process of hybridization (Table 4). Pelzman (1980) also maintained that meristic values were not reliable for classifying individuals from a mixed population composed of *M. salmoides*, *M. floridanus* and their hybrids. mtDNA, used for detecting the haplotypes unique to *M. floridanus*, is not available for identification of hybrids. Instead, information on the nuclear genome and isozyme analyses are helpful in monitoring such things as AFLP and microsatellite DNA.

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References

Akaboshi, T. (1996) *Black Bass*. Ihatov Pupl., Tokyo, 189 pp. (in Japanese).

Akizuki, I. (1999) *Black Bass Eats Medaka*. Takarajima Publ., Tokyo, 222 pp. (in Japanese).

Bottroff, L. J. and M. E. Lembeck (1978) Fishery trends in reservoirs of San Diego County, California, following the introduction of Florida largemouth bass *Micropterus salmoides floridanus*. *Calif. Fish and Game*, **64**, 4-23.

Bremer, J. S. A., L. Zhang, J. S. Bulak and B. Ely (1998) A polymerase chain reaction-restriction fragement length polymorphism (PCR-RFLP) assay for the discrimination of mitochondrial DNA from Florida and northern subspecies of largemouth bass. *Trans. Am. Fish. Soc.*, **127**, 507-511.

Buck, D. H. and M. L. Hooe (1986) Comparative growth of northern largemouth bass and F_1 hybrid largemouth bass through three growing seasons. *Trans. Am. Fish. Soc.*, **115**, 296-304.

Carmichael, G. J., J. H. Williamson, M. E. Schmidt and D. C. Morizot (1986) Genetic marker identification in largemouth bass with electrophoresis of low-risk tissues. *Trans. Am. Fish. Soc.*, **115**, 455-459.

Chew, R. L. (1975) The Florida largemouth bass. In "*Black Bass Biology and Management*" (ed. by R. H. Stroud and H. Clepper), Sport Fish. Inst., Washington, pp. 450-458.

Childers, W. F. (1975) Bass genetics as applied to culture and management. In "*Black Bass Biology and Management*" (ed. by R. H. Stroud and H. Clepper), Sport Fish. Inst., Washington, pp. 362-372.

Gelwick, F. P., E. R. Gilliand and W. J. Matthews (1995) Introgression of the Florida largemouth bass genome in Oklahoma. *Trans. Am. Fish. Soc.*, **124**, 550-562.

Hubbs, C. L. and K. F. Lagler (1970) *Fishes of the Great Lakes Region*. 3rd ed., Univ. Michigan Press, Ann

Arbor, xv+213 pp., 44 pls.

Isely, J. J., R. L. Noble, J. B. Koppelman and D. P. Philipp (1987) Spawning period and first-year growth of northern, Florida, and intergrade stocks of largemouth bass. *Trans. Am. Fish. Soc.*, **116**, 757-762.

Jenkins, R. E. and N. M. Burkhead (1993) *Freshwater Fishes of Virginia*, Amer. Fish. Soc., Bethesda, xxiii+1079 pp., 40 pls.

Lever, C. (1996) *Naturalized Fishes of the World*. Academic Press, London, xxiv+408 pp.

Kaneko, Y. (1984) *Black Bass*. Anglers Co., Tokyo, 167 pp. (in Japanese).

Kassler, T. W., J. B. Koppelman, T. J. Near, C. B. Dillman, J. M. Levengood, D. L. Swofford, J. L. VanOrman, J. E. Claussen and D. P. Philipp (2002) Molecular and morphological analyses of the black basses: implications for taxonomy and conservation. In "*Black Bass: Ecology, Conservation, and Management*" (ed. by D. P. Philipp and M. S. Ridgway), Am. Fish. Soc., Bethesda, pp. 291-322.

Kikkawa, M. (1990) On black bass in Ashi Lake. *Tansuigyohogo*, **3**, 129-134 (in Japanese).

Kiryu, T. (1992) Distribution and habitats. In "*All about Black Bass and Bluegill*" (ed. by the Union of Inland Fisheries Cooperatives in Japan), The Union of Inland Fisheries Cooperatives in Japan, Tokyo, pp. 20-27.

Kitagawa, T., T. Okita, Y. Banno, S. Sugiyama, T. Okazaki, M. Yoshioka and M. Kashiwagi (2000) Mitochondrial DNA of the Florida subspecies of largemouth bass *Micropterus salmoides floridanus* detected in Ikahara Reservoir, Nara Prefecture, Japan. *Nippon Suisan Gakkaishi*, **66**, 805-811 (in Japanese).

Kleinsasser, L. J., J. H. Williamson and B. G. Whiteside (1990) Growth and catchability of northern, Florida, and F_1 hybrid largemouth bass in Texas ponds. *North Am. Jour. Fish. Manag.*, **10**, 462-468.

Lake Biwa Research Institute and National Institute for Research Advancement (eds.) (1984) *Data Book of World Lakes – A Compilation of Physiographic and Socio-Economic Data with Emphasis on Eutrophication*. Secretariat of the Shiga Conference '84 on Conservation and Management of World Lake Environment, Otsu, ix+513 pp. (in Japanese).

Maceina, M. J., B. R. Murphy and J. J. Isely (1988) Factors regulating Florida largemouth bass stocking success and hybridization with northern largemouth bass in Aquilla Lake, Texas. *Trans. Am. Fish. Soc.*, **117**, 221-231.

Maceina, M. J. and B. R. Murphy (1988) Variation in the weight-to-length relationship among Florida and northern largemouth bass and their intraspecific F_1 hybrid. *Trans. Am. Fish. Soc.*, **117**, 232-237.

Maceina, M. J. and B. R. Murphy (1989) Differences in otolith morphology among the two subspecies of largemouth bass and their F_1 hybrid. *Trans. Am. Fish. Soc.*, **118**, 573-575.

Maruyama, T. (2002) Favorable National Policy for Bass Fishing. In "*Black Basses, Invaders in Rivers and Lakes*" (ed. by the Committee to Preserve Nature, the Ichthyological Society of Japan), Koseishakoseikaku, Tokyo, pp. 99-125 (in Japanese).

McCauley, R. W. and D. M. Kilgour (1990) Effect of air temperature on growth of largemouth bass in North America. *Trans. Am. Fish. Soc.*, **119**, 276-281.

Morizot, D. C., S. W. Calhoun, L. L. Clepper and M. E. Schmidt (1991) Multispecies hybridization among native and introduced centrarchid basses in central Texas. *Trans. Am. Fish. Soc.*, **120**, 283-289.

Nakai, K. (2001) History and the present situation of the alien fish problem in Lake Biwa. *The Heredity*, **56**(6): 35-41 (in Japanese).

Nakai, K. (2002) The black bass problem in Japan: present status and future perspectives. In "*Black Basses, Invaders in Rivers and Lakes*" (ed. by the Nature Conservation Committee of the Ichthyological Society of Japan), Koseishakoseikaku, Tokyo, pp. 127-147 (in Japanese).

Nishiyama, T. (1988) Here comes the Frolida bass!. *Basser*, (9), 132-133 (in Japanese).

Pelzman, R. J. (1980) Impact of Florida largemouth bass, *Micropterus salmoides floridanus*, introductions at selected northern California waters with a discussion of the use of meristics for detecting introgression and for classifying individual fish of intergraded populations. *Calif. Fish and Game*, **66**, 133-162.

Philipp, D. P., W. F. Childers and G. S. Whitt (1981) Management implications for different genetic stocks of largemouth bass (*Micropterus salmoides*) in the United States. *Can. J. Fish. Aquat. Sci.*, **38**, 1715-1723.

Philipp, D. P., W. F. Childers and G. S. Whitt (1983) A biochemical genetic evaluation of the northern and Florida subspecies of largemouth bass. *Trans. Am. Fish. Soc.*, **112**, 1-20.

Philipp, D. P., W. F. Childers and G. S. Whitt (1985) Correlations of allele frequencies with physical and environmental variables for populations of largemouth bass *Micropterus salmoides* (Lacepede). *J. Fish. Biol.*, **27**, 347-365.

Philipp, D. P. and G. S. Whitt (1991) Survival and growth of northern, Florida, and reciprocal F_1 hybrid largemouth bass in central Illinois. *Trans. Am. Fish. Soc.*, **120**, 58-64.

Ramsey, J. S. (1975) Taxonomic history and systematic relationship among species of *Micropterus*. In "*Black Bass Biology and Management*" (ed. by R. H. Stroud and H. Clepper), Sport Fish. Inst., Washington, pp. 67-75.

Senou, H. (2002) Taxonomy of fishes in Genus *Micropterus* introduced into Japan. In "*Black Basses, Invaders in Rivers and Lakes*" (ed. by the Committee to Preserve Nature, the Ichthyological Society of Japan), Koseishakoseikaku, Tokyo, pp. 11-30 (in Japanese).

Shiga Prefectural Fisheries Experimental Station (1996) *Faunal Survey on Fish and Other Organisms in Lake Biwa and Rivers in the Fiscal Years of 1994 and 1995*. Hikone, 177 pp. (in Japanese).

Takahashi, K. (1999) Change in fish fauna in five lakes around Mt. Fuji and Shibire Lake. *Bull. Yamanashi*

Pref. Fish. Tech. Center, 1999 (Heisei 11), 57-80 (in Japanese).

Tomelleri, J. R. and M. E. Eberle (1990) *Fishes of the Central United States*. Univ. Press Kansas, Lawrence, xv+226 pp., 52 pls.

Webb, F. W. and W. C. Reeves (1975) Age and growth of Alabama spotted bass and northern largemouth bass. In "*Black Bass Biology and Management*" (ed. by R. H. Stroud and H. Clepper), Sport Fish. Inst., Washington, pp. 204-215.

Week, L. E. (1984) Age and growth of Florida largemouth bass, *Micropterus salmoides floridanus*, in Hidden Valley Reservoir, Lake County, California. *Calif. Fish and Game*, **70**, 59-60.

Williamson, J. H., G. J. Carmichael, M. E. Schmidt and D. C. Morizot (1986) New biochemical genetic markers for largemouth bass. *Trans. Am. Fish. Soc.*, **115**, 460-465.

Williamson, J. H. and G. J. Carmichael (1990) An aquacultural evaluation of Florida, northern, and hybrid largemouth bass, *Micropterus salmoides*. *Aquaculture*, **85**, 247-257.

Yokogawa, K. (1998) Morphological and genetic structures of largemouth bass *Micropterus salmoides* in Japanese fresh waters. *Suisanzoshoku*, **46**, 321-332.

Yoshizawa, K. (1992) Largemouth bass, growth. In "*All about Largemouth Bass and Bluegill*" (ed. by All Japan Inland-water United Fisheries Union), All Japan Inland-water United Fisheries Union, Tokyo, pp. 39-55 (in Japanese).

Zolczynski, S. J. Jr. and W. D. Davis (1976) Growth characteristics of the northern and Florida subspecies of largemouth bass and their hybrid, and a comparison of catchability between the subspecies. *Trans. Am. Fish. Soc.*, **105**, 240-243.

近年の劇的遺伝子変動から推定される，琵琶湖におけるフロリダバスの大規模な侵入

横川浩治・中井克樹・藤田建太郎

北米原産のオオクチバス *Micropterus salmoides* が沿岸域で優占していた琵琶湖に，近年，近縁種のフロリダバス *M. floridanus* の侵入が確認された。琵琶湖沿岸の4地域から2000～2003年に得られた合計194個体のアイソザイムを調べた。オオクチバスとフロリダバスは *AAT-1**，*IDHP-1**，*MDH-1**，*SOD** の4遺伝子座において識別されるが，調べたすべての標本群のこれら遺伝子座においてフロリダバスを特徴づける遺伝子が頻繁に出現し，概略，全体の半分がフロリダバスの遺伝子で占められていた。すべての標本群において Hardy-Weinberg の遺伝平衡からのずれは認められず，大部分はヘテロ過剰傾向を示した。マーカー座における個体ごとの遺伝子型から判断して，雑種 F_2 以降あるいは戻し交雑個体が大半であると推定され，琵琶湖内ではオオクチバスとフロリダバスの交雑が既にかなり進行していることが示唆された。以上の結果は，従来生息していたオオクチバスに匹敵する数量のフロリダバスが琵琶湖に侵入したことを示し，人為的な大規模放流があったものと推定される。