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Genetic Distribution Pattern of Bluegill Sunfish *Lepomis macrochirus* in Freshwater Ecosystems across Korea

Hwee Hui Lau[1], Jingting Huang[1], Ye-Seul Kwan[2], Wan-Ok Lee[3] and Yong-Jin Won[2,*]

[1]School of Life Sciences and Chemical Technology, Ngee Ann Polytechnic, Singapore 599489
[2]Division of EcoScience, Ewha Womans University, Seoul 120-750, Korea
[3]Inland Fisheries Research Institute, Gapyeong, Gyeonggi-do 477-815, Korea

ABSTRACT

Lepomis macrochirus from the family Centrarchidae, commonly known as Bluegill sunfish, is an introduced freshwater fish in Korea that thrives in lakes, ponds, reservoirs and rivers. Since its introduction into Korea in 1969, *Lepomis macrochirus* has rapidly dispersed out and increased in number almost all over the freshwater ecosystems in Korea. Consequently this species causes a severe ecological problem, threatening native fishes due to its omnivorous foraging behaviors upon fish juveniles and many freshwater invertebrates. To address population genetic structure of *L. macrochirus*, 74 fish samples from 10 populations were collected and compared for their mitochondrial D-loop control region. As the result we found that the genetic diversity of *L. macrochirus* is extremely low such as resulting only four haplotypes with a few nucleotide differences among them. Analysis of molecular variance (AMOVA) revealed that the source of population genetic variation is largely retained in the comparisons among individuals within populations, while it is relatively low with slight significance at the highest hierarchical group. This distribution pattern differs from what is expected when biogeography is under the influence of natural geographic barriers such as mountain ranges in Korea. Instead the result is accord with the influential role of random spreading events facilitated by local people for aquaculture and fishing, and subsequent dispersals since its single point of introduction into Korea.

Key words: Bluegill sunfish, *Lepomis macrochirus*, introduced species, AMOVA, mitochondrial D-loop control region

INTRODUCTION

Lepomis macrochirus of the family of Centrarchidae, commonly known as Bluegill sunfish, is a freshwater fish that thrives in lakes, ponds, reservoirs and rivers. It originated from the middle and eastern regions of North America. It has been intentionally introduced to other continents as a game fish. *Lepomis macrochirus* is an omnivore, its food sources range from aquatic plants to small aquatic organisms (Azuma, 1992). This property played a great role in increasing the survival rate of the fish after being exported out of its native continents and into a totally unfamiliar environment.

In the year of 1969, the Fisheries Agency of Korea received a number of 510 Bluegills, *L. macrochirus*, from the Fisheries Agency of Japan. The fishes were then released into the Paldang dam on the Namhan-gang River (Jung, 1977). The 510 fishes were the descendants of 15 *L. macrochirus* which survived out of the 18 that Japan received as a gift from the Mayor of Chicago in 1960 (Maruyama et al., 1987). For the following two decades after 1969, *L. macrochirus* successfully spread through most of the fresh water ecosystems in Korea (Kong et al., 1995). The sudden increase in number of *L. macrochirus* was probably due to ecological adaptation to the new environment where they were introduced to. Absence of natural predator against introduced species allowed them to multiply quickly and threaten the native species in the same ecosystem. It is possible that the introduced species will drive the native species to extinction. In the case of *L. macrochirus*, it has been labeled as notorious exotic species which imposes great negative impacts on the ecosystem if it is introduced (Jun, 1993).

There is a lack of population genetic studies on the introduced *L. macrochirus* in Korea, although a comprehensive investigation on the route of dispersal of the species and population genetic structure was investigated in Japan using mitochondrial D-loop sequences (Kawamura et al., 2006). In the Centrarchid fishes, the D-loop region in the mitochondrial DNA is known to be hypervariable, and it is usual-

*To whom correspondence should be addressed
Tel: 82-2-3277-4471, Fax: 82-2-3277-4514
E-mail: won@ewha.ac.kr

Table 1. *Lepomis macrochirus* samples examined in this study.

Population	Location	Sample size	Year	Number of haplotypes	Haplotype diversity
Northwestern group					
1	Hantan-gang River	8	2008	3	0.4643±0.2000
2	Asan-ho Lake	6	2008	2	0.3333±0.2152
Southwestern group					
3	Yongdam-ho Lake	8	2008	1	0.0000±0.0000
4	Jangseong-ho Lake	5	2008	2	0.6000±0.1753
5	Naju-ho Lake	8	2008	2	0.2500±0.1802
6	Shinpyeong-cheon River	7	2008	1	0.0000±0.0000
Southeastern group					
7	Junam reservoir	8	2008	3	0.4643±0.2000
8	Samnyangjin River	8	2008	3	0.6071±0.1640
9	Taehwa-gang River	8	2008	3	0.7143±0.1227
10	Songjeon reservoir	8	2008	3	0.6786±0.1220
Total		74			

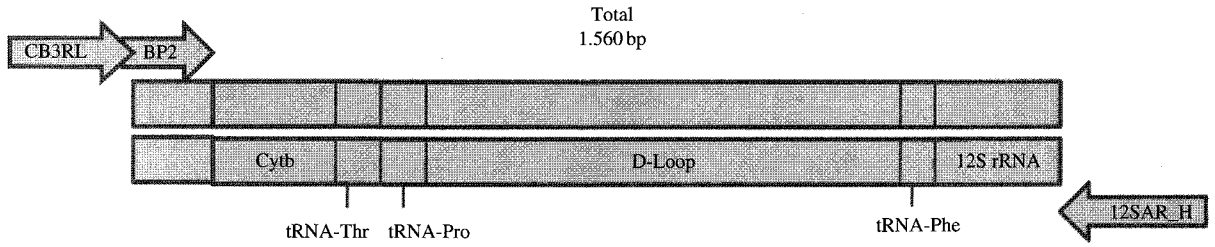

Fig. 1. Sequences of primers used in this study and each length of genes encompassed by PCR. CB3RL: 5′-CAYATYMARCCMGAA TGRTATTT-3′, 12SAR_H: 5′-AGCCATATTTACATGTATG-3′, BP2: 5′-ATARTRGGGTATCTAATCCYAGTT-3′, Cytochrome b gene (Cyt b, 239 bp), tRNA-Thr (72 bp), tRNA-Pro (69 bp), D-loop (828 bp), tRNA-Phe (68 bp), 12S ribosomal RNA (12S rRNA, 284 bp).

ly used to evaluate population structure and relationships (Kitagawa et al., 2000, Quattro et al., 2001).

The objectives of this study were to find out the (a) diversity of mitochondrial haplotype among the introduced *L. macrochirus* populations, and also (b) to map the distributional pattern of *L. macrochirus* across South Korea. For this purpose, we compared the mitochondrial D-loop noncoding control region of *L. macrochirus* samples collected across ten populations in South Korea.

MATERIALS AND METHODS

Seventy four *Lepomis macrochirus* samples used in the study were collected from ten different locations around South Korea: the Hantan-gang River, Shinpyeong-cheon, Naju-ho, Jangseong-ho, Yongdam-ho, Songjeon reservoir, Asan-ho, Taehwa-gang River, Junam reservoir and Samnyangjin River (Table 1). Fin samples were collected by cutting the caudal and/or dorsal fin of individual fishes, and then preserved in absolute ethanol until delivered to laboratory. These specimens were stored at −20°C in freezer until eventual extraction of DNA was carried out.

Mitochondrial DNA analysis

Genomic DNA was extracted using QIAGEN DNasy Blood and Tissue Kit (*QIAGEN, US*). Presence and quality of DNA in samples was checked using 1% agarose gel electrophoresis. PCRs were then performed to amplify the D-loop region in the mitochondrial DNA.

A total of three different primers were used in the study. For the first PCR, the primer pair used was CB3R-L and 12SAR-H (Palumbi et al., 2001). Then we applied a nested PCR method to the first PCR products; the second PCR was done using the primer pair BP2, an inner primer introduced, and the same 12SAR-H (Fig. 1). PCR condition was as followed: beginning with initiation denaturation at 94°C for 2 min, following that, a series of 40 cycles of denaturing step at 94°C for 30 s, annealing of primers to DNA at 55°C for 30 s, and elongation of sequences at 72°C for 2 min were performed. Final extension at 72°C for 5 min marked the end of PCR reaction. Samples were stored at 4°C after final extension. Profiles of PCR generated fragments were confirmed by 1% agarose gel electrophoresis. PCR products were

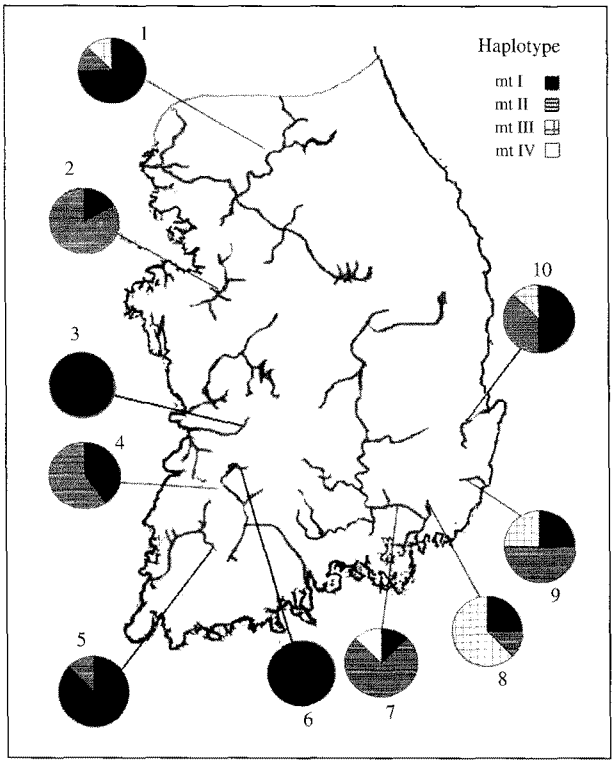

Fig. 2. Map of distribution of the four haplotypes of mitochondrial D-loop control region among ten *Lepomis macrochirus* populations. The pie chart proportionally illustrates the frequency of each mtDNA haplotype. For code and sample size of population, refer to Table 1.

purified using Labopass PCR Purification Kit (*COSMO Genetech, Seoul, South Korea*). PCR products were sequenced using ABI 3100 automatic sequencer (*Applied Biosystems, US*).

DNA alignment and population genetic analysis

Sequence alignment was done with computer software AlignIR ver. 2.0.48 (*LI-COR Inc, US*). The diversity of haplotype in each population and total samples were calculated using the computer software, DnaSP ver. 5.00 (Librado and Rozas, 2009) (Table 1). With the values computed using the software, the percentage of each haplotypes found in each population of *L. macrochirus* was calculated (Fig. 2).

AMOVA (Analysis of molecular variance framework) test was conducted using the computer software, Arlequin ver. 3.11 (Excoffier et al., 1992) to define the sources influencing genetic variations in *L. macrochirus*. In order to examine population genetic structure, if any, and compare it with those of other native fish species, we adopted the hypothetical and general biogeographical boundaries for freshwater fish which correspond to the 'West-South-Eastnorth Korea Sub-district' in Korea (Kim and Park, 2002). We applied this hypothetical biogeography to our data set for AMOVA. Accordingly, the Bluegill samples were divided into three groups, namely Group I, Group II and Group III (Tables 1, 3).

Table 2. Variable nucleotide positions in different haplotypes of D-loop region of *Lepomis macrochirus* mtDNA.

Haplotypes	Nucleotide sites (1,560 bp)			
	303	383	848	884
mt I*	T	–	A	T
mt II	.	T	.	.
mt III	C	T	.	.
mt IV	.	T	G	–

*DNA sequence of mt I haplotype was deposited to GenBank (Accession No. GQ254270).
"." indicates identical base with mt I, "–" indicates deletion of a single nucleotide in the sequence.

RESULTS

Upon completion of DNA alignment and population genetic analysis, a total of four haplotypes, designated as mt I, mt II, mt III and mt IV (Table 2), were identified among the 74 samples from the 10 populations. The D-loop region (1,560 bp) sequenced in this study had two transitions and two deletions. The results revealed that the four haplotypes were highly similar, differing by only a few bases (Table 2).

For the Shinpyeong-cheon River and Yongdam-ho Lake populations, only haplotype mt I was found among the samples. Three haplotypes (mt I, mt II, mt III) were all present in populations from Hantan-gang River, Songjeon reservoir, Taehwa-gang River and Samnyangjin River. For the three populations of *L. macrochirus* collected from the Lake of Naju-ho, Jangseong-ho and Asan-ho, two haplotypes (mt I, mt II) were found. Finally, in the Junam reservoir population, three haplotypes (mt I, mt II, mt IV) were found. In all the *L. macrochirus* samples used for this study, only one individual sample had the haplotype mt IV, and this individual was collected from Junam reservoir (Fig. 2).

Haplotype mt I was found in nine of the ten populations sampled, which was the most dominant haplotype among the *L. macrochirus* samples with the occurrence rate of 54.1%. Following haplotype mt I, mt II had an occurrence rate of 32.4%, and mt III was not as common in the populations, with occurrence rate of 12.1%. Haplotype mt IV had the lowest occurrence rate of 1.4%; merely one fish sample had this haplotype among the 74 samples of *L. macrochirus* in ten populations sampled.

AMOVA revealed that the source of variation from the

Table 3. AMOVA result according to the hierarchical grouping method shown in Table 1.

Source of Variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indices (p-value)
Among groups*	2	5.993	0.09657 Va	22.61	0.22606 (0.03030)
Among populations within groups	7	5.105	0.06251 Vb	18.68	0.18908 (0.00587)
Within populations	64	17.158	0.26810 Vc	62.76	0.37240 (0.00000)

*According to the mountain ranges, three groups were erected: Group I (1, 2), Group II (3, 4, 5, 6), Group III (7, 8, 9, 10) (Numbers in parenthesis represent population ID).

comparisons among individuals within populations had the largest proportion in the overall, contributing to 62.76% of variation in the mitochondrial DNA of *L. macrochirus* (Table 3). On the other hand, the source of variation among groups which were hypothesized by the typical natural mountain ranges in Korea showed relatively a lower value (merely 23%) with slight significance (P=0.03).

DISCUSSION

In South Korea, *Lepomis macrochirus* is an introduced species. This species is distributed in most of the freshwater ecosystems around South Korea. In the present study of population genetics of *Lepomis macrochirus*, we can conclude that the genetic diversity of this species is extremely low (Table 1, Fig. 2) as expected by the nature of small number of founders, 510 fishes, resulting in just four haplotypes with a few nucleotide differences among them (Table 2).

Similarly Kawamura et al. (2006) identified only five haplotypes in *L. macrochirus* collected from three locations in Korea (Jecheon, Shingal and Jinyanag Reservoir) as well as entire Japan. Such a low genetic diversity suggested that *L. macrochirus* both in Korea and Japan was originated from an extremely small founder population. For instance *L. macrochirus* in Korea originated from only 510 fishes from the Fisheries Agency of Japan. These 510 fishes were the offspring of the 15 *L. macrochirus* presented to Japan from the Mayor of Chicago in 1960 (Maruyama et al., 1987). Therefore it is not likely to find another hyplotypes in Korea compared to those of Japan.

For AMOVA, we divided *L. macrochirus* samples into three groups, Group I, Group II and Group III (Tables 1, 3), which accords well with the conventional geographical subdivisions. These subdivisions are well known as 'West-South-Eastnorth Korea Subdistrict' (Kim and Park, 2002) that generally define the boundaries of freshwater fish species in Korea. As the result of AMOVA, we found that the source of variation within populations, that is among individuals, had the most influential on the overall mitochondrial DNA variation (Table 3). This result differs from an expectation by the hypothetical biogeographical boundaries, suggesting the distribution of *L. macrochirus* in Korea is not relevant to long and natural processes which govern differentiation of populations and species observed in other native fish species. On the contrary the result is quite consistent with the natural history of *L. macrochirus* in Korea, that is, small founder size of introduction and reintroduction in random by local people within country for aquaculture and fishing purposes. It is not likely that highly polymorphic microsatellite markers will reveal another unseen population structure of *L. macrochirus* shaped by mountain ranges and river tributary systems in Korea, because the natural history of the fish completely differs from concordant evolutionary histories of the native Korean fishes which have been commonly affected by unique geographical topology of mountains and rivers in Korea.

As suggested (Sakai et al., 2003), an introduced species usually experiences founder effect and genetic drift, resulting in declining of genetic diversity during its colonization process into a new environment. Usually, the decline of genetic diversity will probably lead to depletion of genetic variation, which might in turn greatly deprive the species of viability in the long run. However, it is not yet observed in the case of *L. macrochirus*. Absence of natural enemies and suitable environments made Bulegill sunfish expand ranges and become the dominant species in the freshwater ecosystem in Korea (Kong et al., 1995). Nevertheless for a sound ecological conservation and management of native freshwater fishes, it is needed to continuously monitor population changes in distribution and size of *L. macrochirus* in Korea. For the long them monitoring, the present study of genetic distribution pattern of *L. macrochirus* may provide basic information on this introduced and problem species.

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