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Population Genetic Structure of *Carassius auratus* (Pisces: Cypriniformes) in South Korea Inferred from AFLP Markers: Discordance with Mitochondrial Genetic Structure

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ABSTRACT

A recent study on the mitochondrial genetic variation of the *Carassius auratus* population in South Korea suggested that there are 3 distinct mitochondrial lineages in the country, and that they are geographically separated between westward rivers and southward rivers, respectively. In this study, the population genetic structure of amplified fragment length polymorphism (AFLP) of *Carassius auratus* was investigated. The results of analysis of molecular variance (AMOVA) supported the geographic distinction between westward and southward river populations, but only 3.66% of total genetic variance lies among these populations. The panmicticity of the AFLP genetic variation is backed up by the results of the neighbor-joining dendrogram drawn from a linearized pairwise F_{ST} matrix and Bayesian clustering analysis. The discordance of genetic structure between mitochondrial and AFLP genetic variation may come from difference in effective population size between these markers and/or gene flow between westward and southward river populations through river capture events.

Keywords: *Carassius*, amplified fragment length polymorphism, genetic structure, genetic variation, South Korea

INTRODUCTION

Carassius fish species still remain controversial in their species status owing to extreme morphological variation, hybridization with related species (Hanfling et al., 2005; Tóth et al., 2005), and complicated ploidy level (Murakami and Fujitani, 1997). There had been well-established agreement on *Carassius* fish in South Korea until quite recently, as Yang (1985) has investigated genetic and morphological characteristics in South Korean populations, concluding that Korean population of *Carassius* was composed of diploid and triploid individuals of *C. auratus langsdorfi*. Later this view was supported by several studies (e.g., Nam et al., 1989).

However, further information has come to light in a more recent study of mitochondrial genetic variation of *Carassius* populations in South Korea (Jung et al., 2009). Korean populations are composed of 3 mitochondrial genetic lineages, and two of them are geographically structured over westward rivers and southward rivers. The former includes the Han, Geum, and Yeongsan rivers, and the latter includes the Nakdong and Seomjin rivers (see Fig. 1). The northeastern population of China (Weihai, Shantung Province) was found to have a close genetic relationship with westward river populations of Korea. Moreover, all the genetic lineages are not related with *C. auratus langsdorfi*, which had been believed to be the identity of Korean *Carassius* population until that time.

Although mitochondrial genetic studies provide us with valuable information for understanding *Carassius* populations in Korea, they are limited due to their matrilineal nature. Mitochondrial genetic variation, being only one locus, canot provide us with a full account of the *Carassius* population. Multiple loci data, therefore, are required for elucidating the evolution of *Carassius* population. Microsatellite markers for the Korean *Carassius* population have been developed (Jung et al., 2007), and these markers may be useful for enhancing our understanding of genetic structure of *Carassius* fish. Microsatellite, however, also has limitation in its application to *Carassius* populations because reliable analysis methods have not been developed for populations with

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various ploidy individuals.

Amplified fragment length polymorphism (AFLP) may be appropriate for this group of animals. It is easy to implement, doesn't need prior knowledge of genomes, and has good reproducibility. Hence, it has been very popular in its application to population genetics (e.g., Bensch and Åkesson, 2005; Meudt and Clarke, 2007). As AFLP does not consider ploidy level, it is especially useful for organisms with complicated ploidy levels.

In this study, we analyzed the genetic structure of Korean *Carassius* populations using AFLP genetic polymorphism, and compare the results with those of Jung et al. (2009), in which mitochondrial genetic variation was investigated.

MATERIALS AND METHODS

Sampling and DNA extraction

We collected 121 individuals of *Carassius auratus* from 16 locations in South Korea, including the five major river basins (Table 1, Fig. 1), using fishing nets or traps. The fish samples (21 individuals) from Weihai (Shantung Province, China) were bought from the fish market. The samples were fixed using an absolute ethanol solution after capture. A total genomic DNA was extracted by using the standard phenol extraction procedure.

AFLP

The AFLP methods used in this study were those of Jung et al. (2006, 2010). Total genomic DNAs were digested using a restriction enzyme mixture for 1 h at 37°C, followed by additional 3 h of digestion at 16°C. After inactivation of restriction enzymes, *Eco*RI adaptor (ECO-F and ECO-R) and the *Mse*I adaptor (MSE-F and MSE-R) were attached to

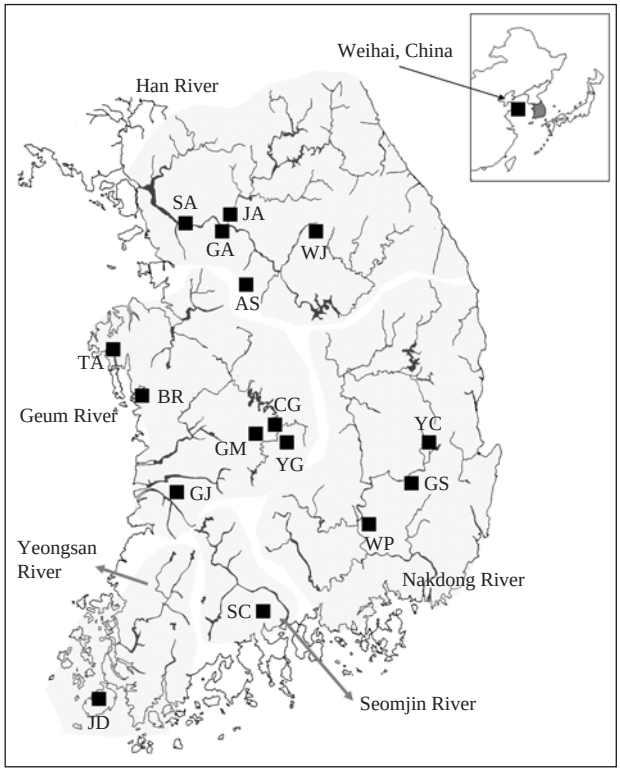

Fig. 1. A map denoting the sampling locations in this study and the major river systems. AS, Anseong; BR, Boryeong; CG, Chogang; GA, Gyeongan; GJ, Gimje; GM, Geumsan; GS, Gyeongsan; JA, Joan; JD, Jindo; SA, Sangam; SC, Sooncheon; TA, Taean; WJ, Wonju; WP, Woopo; YC, Yeongcheon; YG, Yanggang.

Table 1. Information of sampling locations

ID	Location	River system	Coordinate	No. of individuals	Pairwise difference within population
SA	Sangam, Seoul	Han	N37°51′54″, E127°13′16″	7	231.71
GA	Gyeongan, Gyeonggi-do	Han	N37°55′21″, E127°10′15″	15	164.69
JA	Joan, Gyeonggi-do	Han	N37°28′29″, E127°56′02″	3	266.00
AS	Anseong, Gyeonggi-do	Han	N37°27′59″, E127°55′14″	2	60.00
WJ	Wonju, Gangwon-do	Han	N37°28′22″, E127°57′07″	9	168.67
TA	Taean, Chungcheongnam-do	Geum	N36°54′07″, E127°34′06″	5	246.60
BR	Boryeong, Chungcheongnam-do	Geum	N36°53′39″, E127°34′15″	1	0.00
GM	Geumsan, Chungcheongnam-do	Geum	N36°16′16″, E127°34′21″	7	188.10
CG	Chogang, Chungcheongbuk-do	Geum	N36°19′15″, E127°36′55″	3	167.33
YG	Yanggang, Chungcheongbuk-do	Geum	N36°47′45″, E126°51′34″	10	211.42
GJ	Gimje, Jeollabuk-do	Geum	N36°36′35″, E126°45′25″	10	204.76
YC	Yeongcheon, Gyeongsangbuk-do	Nakdong	N35°46′46″, E128°49′27″	4	195.33
GS	Gyeongsan, Gyeongsangbuk-do	Nakdong	N35°46′17″, E128°49′51″	7	194.57
WP	Woopo, Gyeongsangnam-do	Nakdong	N35°48′54″, E128°48′54″	29	307.14
JD	Jindo, Jeollanam-do	Youngsan	N34°58′34″, E126°43′15″	6	200.20
SC	Sooncheon, Jeollanam-do	Seomjin	N35°17′25″, E127°21′35″	3	246.00
China	Weihai, Shantung Province, China			21	265.67
Total				142	252.61

the sticky ends of the restriction fragments using T4 DNA ligase. Pre-amplification was conducted using two AFLP primers (ECO-A and MSE-A) (see Jung et al. [2006, 2010] for detailed information of reaction mixtures and experimental conditions). In selective amplification, ECO-AGG and MSE-AXX primers were used. ECO-AGG was labeled with fluorescent dye, 6FAM, and three types of MSE-AXX−MSE-ACC, MSE-ACG, and MSE-AGG−were prepared. The composition of the PCR reaction mixture was identical to that employed in pre-amplification, except that a 20-fold diluted pre-amplification PCR product was utilized as the template. All the PCR amplifications were performed on a GeneAmp 9700 machine (Applied Biosystems, Foster City, CA, USA). The selective amplification products were determined using a Genetic Analyzer 3730 (Applied Biosystems), and the band size and genotype were determined using the GENEMAPPER 4.0 (Applied Biosystems).

Data analysis

We evaluated independence among the polymorphic loci and then eliminated redundant loci by using AFLPOP (Duchesne and Bernatchez, 2002). Pairwise difference among individuals within population, implicating genetic variation within population, was determined by using ARLEQUIN 3.11 (Excoffier et al., 2005). With the same software, pairwise F_{ST} values between local populations were calculated. Then these values were linearized with Slatkin's transformation ($F_{ST}/(1-F_{ST})$). A neighbor-joining dendrogram denoting the genetic relationship among populations was determined from a matrix of linearized pairwise F_{ST} values by using MEGA 5.0 (Tamura et al., 2011). Analysis of molecular variance (AMOVA) (Weir and Cockerham, 1984) was performed by using ARLEQUIN 3.11 (Excoffier et al., 2005) with various combinations of river systems (see Table 2). Bayesian clustering of individuals based on their AFLP genotypes was determined by using STRUCTURAMA (Huelsenbeck et al., 2011). The number of populations and the expected prior number of populations were randomly drawn from gamma distribution (parameter setting: shape, 1; scale, 1). Markov chain Monte Carlo (MCMC) simulation was executed for 30,000 steps of Markov chain, and simulated parameters were sampled every 100 steps. After MCMC simulation, sampled parameters were summarized and individuals were assigned to populations.

RESULTS

AFLP analysis on 142 individuals of *Carassius auratus* revealed 1,218 loci ranging from 40 to 999 bp in size. The mean number of pairwise difference among all individuals in our study was 252.61 ± 108.66. Individuals from the Woopo represented the highest pairwise difference among individuals (Table 1). AMOVA revealed that there was a small (3.66%) but significant ($p < 0.05$) genetic variance between westward river systems (Han, Geum, and Yeongsan rivers, and the Weihai population of China) and southward river systems (Nakdong and Seomjin rivers) (Table 2). However, the results of the neighbor-joining dendrogram (Fig. 2), drawn from a linearized pairwise F_{ST} matrix, told a different story, where local populations were not organized with respect to the river systems from which they had originated. Bayesian clustering

Table 2. Results of analysis of molecular variance (AMOVA)

Group assignment	Source of variation	F-value	Variation (%)
(H+G+N+Y+S+C)	Among populations	0.08965**	8.97
	Within populations		91.03
(H+G+C), (N+Y+S)	Among groups	0.02340	2.34
	Among populations within groups	0.07868**	7.68
	Within populations	0.10024**	89.98
(H+G+Y+C), (N+S)	Among groups	0.03661*	3.66
	Among populations within groups	0.07340**	7.07
	Within populations	0.10732**	89.21
(H+C), (N+S), (G+Y)	Among groups	0.02169	2.17
	Among populations within groups	0.07484**	7.32
	Within populations	0.09490**	90.51
(H+C), (G), (N), (Y), (S)	Among groups	0.01450	1.45
	Among populations within groups	0.07933**	7.82
	Within populations	0.09367**	90.73
(H), (G), (N), (Y), (S), (C)	Among groups	0.01348	1.35
	Among populations within groups	0.07902**	7.80
	Within populations	0.09144**	90.86

H, Han River system; G, Geum River system; N, Nakdong River system; Y, Yeongsan River system; S, Seomjin River system; C, Weihai, China.
*$0.01 < p < 0.05$, **$p < 0.01$.

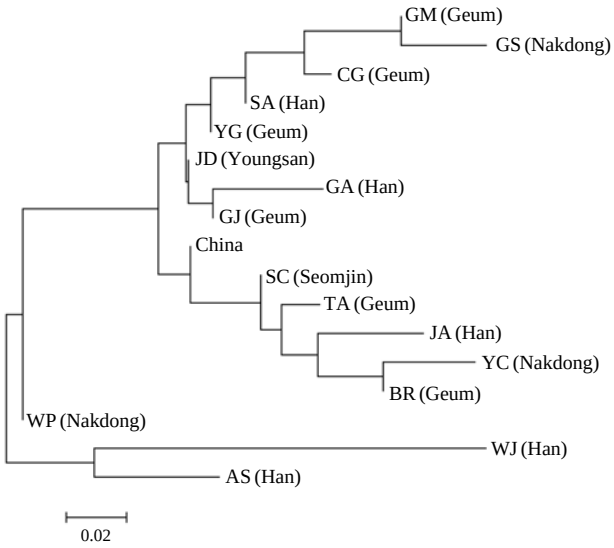

Fig. 2. A neighbor-joining dendrogram depicting the genetic relationship among local populations in this study. The major river system including each local population is denoted in parenthesis. AS, Anseong; BR, Boryeong; CG, Chogang; GA, Gyeongan; GJ, Gimje; GM, Geumsan; GS, Gyeongsan; JA, Joan; JD, Jindo; SA, Sangam; SC, Sooncheon; TA, Taean; WJ, Wonju; WP, Woopo; YC, Yeongcheon; YG, Yanggang.

analysis supported that there is one population of *Carassius auratus* in South Korea.

DISCUSSION

A recent study on the mitochondrial genetic variation of the Korean *Carassius* population revealed that there are 3 mitochondrial genetic lineages (pgA, pgB, and pgC) (Jung et al., 2009). Of these, pgA and pgB are distributed over westward rivers and southward rivers, respectively, indicating that mitochondrial genetic variation is geographically structured in South Korea. The results of AMOVA in this study are very similar to those of mitochondrial genetic variation. That is, significant genetic variance has been determined between westward rivers and southward rivers, and the Chinese population is closely related to the westward river population of Korea.

However, other results, such as those of the genetic relationship among populations and Bayesian clustering analysis implicate that the Korean *Carassius* population is panmictic in view of AFLP genetic variation. Although genetic variance between the westward and southward river populations is statistically significant, only 3.66% of total genetic variance is allocated between these groups. This means that most of genomic variations don't contribute to the genetic structure of the *Carassius* population between two large river systems in South Korea.

Considering that almost AFLP loci are located in the nuclear region, it is probable that mitochondrial genetic structure discords with genetic structure inferred from AFLP data. This discordance may be explained by two points. First, differences in the rate of lineage sorting can make this pattern. Lineage sorting must have worked on *Carassius* populations separated by river systems. The rate of lineage sorting is primarily dependent on the effective population size of the genetic markers concerned. As mitochondrial DNAs are theoretically one-fourth of nuclear DNAs in terms of their effective population size, mitochondrial DNAs shows faster lineage sorting. Therefore, AFLP genetic variations are not fully differentiated between westward and southward river populations, despite the fact that their mitochondrial genetic variations are. Second, there has possibly been gene flow between populations of westward rivers and southward rivers, which may keep AFLP genetic variations from being sorted between these groups. It is suggested that river capture between river systems has taken place, from the result of mitochondrial genetic variation (Jung et al., 2009). As these two factors are not mutually exclusive, both may have influenced the shaping of these patterns.

Compared to the results of mitochondrial genetic data, those of AFLP seem to complicate our understanding of the history and taxonomy of Korean *Carassius* fish populations. The evolution and taxonomy of *Carassius* fish in Korea could be clarified only after grasping diverse phenomena such as polyploidization and extreme morphological variation etc., observed in this group of fish. Hereafter, approaches that integrate diverse evidences such as chromosomal, morphological and genetic data are required.

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