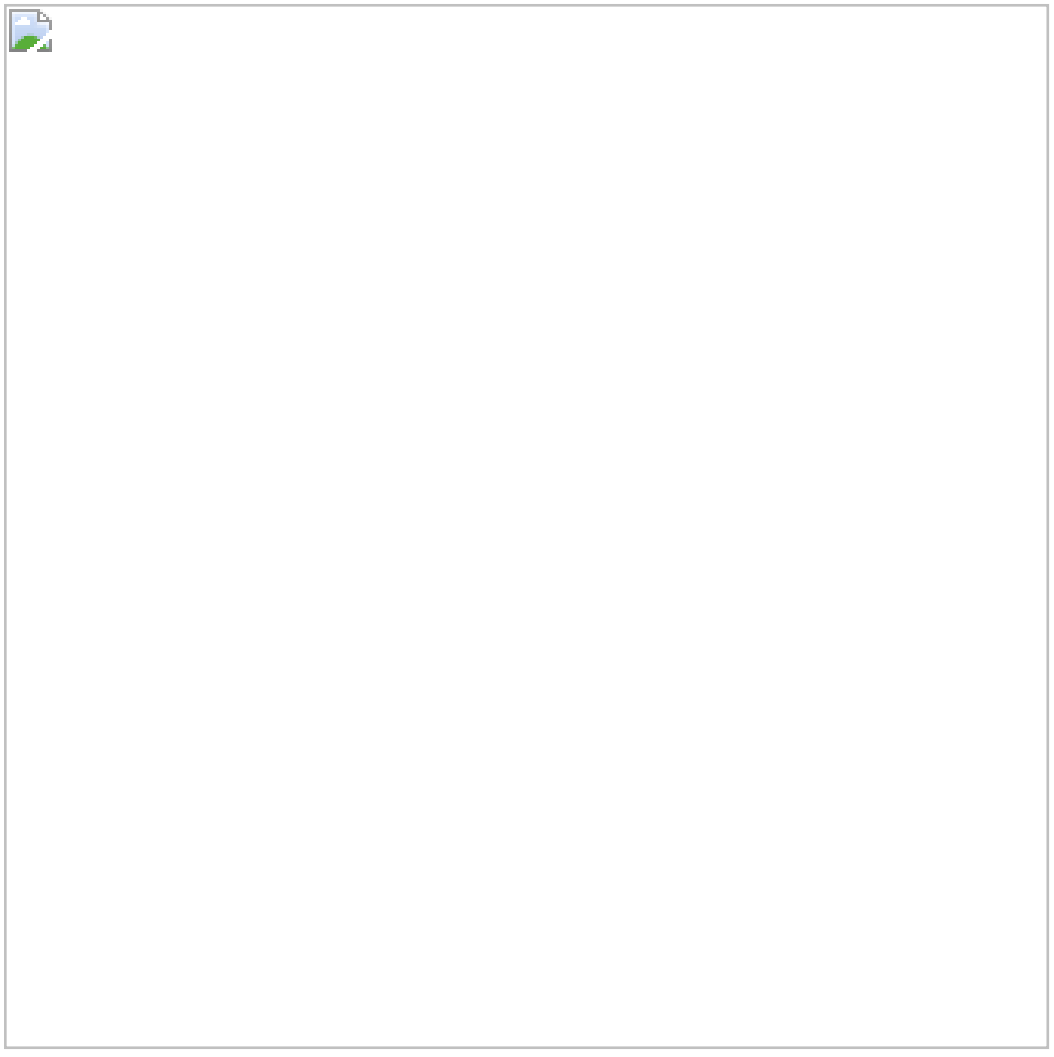

Genetic Structure of Korean Wild Populations of the Medaka Oryzias latipes Inferred from Allozymic Variation

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Genetic Structure of Korean Wild Populations of the Medaka *Oryzias latipes* Inferred from Allozymic Variation

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ABSTRACT—Previous allozymic studies have revealed that Korean wild populations of *Oryzias latipes* have differentiated regionally, and are composed of two distinct groups, the East Korean Population and the China-West Korean Population. Recently, mitochondrial DNA (mtDNA) sequencing and restriction fragment length polymorphism (RFLP) analyses have confirmed these two groups, and shown that the distribution ranges of the two groups overlap in western Korea. In order to describe the detailed distributions of the two groups and the gene flow between them, genotypes of 13 allozymic loci were determined in 444 specimens from 96 localities in Korea. The two major groups were supported by remarkable allele frequency differences at six diagnostic loci: *ACP**, *AMY**, *CK-A**, *LDH-A**, *PGM** and *TF**. Individuals with the typical "eastern" genotype were mainly distributed in eastern and southern areas. In contrast, fish with the "western" genotype were predominant in the western area, and were further divided into two subgroups (the Han River and Geum River Subpopulations) by unique alleles at the *ADH** locus. In the western coast, two distinct (eastern and western) genotypes were distributed in a mosaic fashion. This distribution pattern was identical to those from mtDNA analyses. Although the distribution patterns of the alleles at three loci (*GPI-A**, *LDH-C** and *SOD**) showed introgressive conditions between the two groups, each population was nearly fixed as either the eastern or western genotype at all six diagnostic loci despite the proximity among samples. Therefore, it is suggested that some reproductive isolation mechanisms exist between the two groups in natural habitats.

Key words: phylogeography, electrophoresis, introgression, reproductive isolation

INTRODUCTION

Primary freshwater fishes are particularly suitable for historic biogeographic studies due to their limited dispersal capacity. It is supposed that land is a barrier to their dispersal and thus local populations are confined to their own watershed and isolated from one another. To investigate phylogeographic patterns, genetic studies based on molecular markers such as allozymes and mitochondrial DNA (mtDNA) sequences are effective.

The medaka, *Oryzias latipes*, is an egg-laying freshwater fish native to Japan, Korea and China. During allozymic studies of this species in Korea, two genetically distinct groups were observed (Sakaizumi and Jeon, 1987), the East Korean Population from the coast of the Sea of Japan (East Sea), and the China-West Korean Population in western Korea and China (Sakaizumi, 1986). These two groups can be distinguished by unique alleles at the *ACP**, *AMY**, *CK-A**, *GPI-A**, *LDH-A**, *LDH-C**, *PGM**, *SOD** and *TF** loci, and the Nei's coefficients of genetic distance (*D*) based on 16 loci are no less than 0.7. However, Sakaizumi and Jeon (1987) analyzed only 18 populations (4 populations from northwestern Korea and 14 from eastern Korea), therefore further comprehensive research was required to investigate the detailed distributions of these two groups.

Karyological studies demonstrated that specimens belonging to the China–West Korean Population had 2n, 46 chromosomes including a large metacentric pair, and a karyotype closely related to those from east and southwestern China. In contrast, the specimens identified as the East Korean Population showed 2n, 48 chromosomes without such large chromosomes (Uwa and Jeon, 1987; Uwa *et al*., 1988). The geographic distributions of these two chromosomal forms are consistent with those of the two allozymically distinguished groups, namely, the 2n, 46 form in western Korea (*O. latipes sinensis*), and the 2n, 48 form in eastern and southern Korea (Kim and Moon, 1987; Kim and Lee, 1992).

Furthermore, the authors previously demonstrated the

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same phylogeographic patterns of this species throughout Korea and China based on both restriction fragment polymorphisms (RFLPs) of the entire mitochondrial DNA (mtDNA) (Matsuda *et al.*, 1997) and mtDNA sequences (Takehana *et al.*, 2004). Phylogenetic analyses of mtDNA sequences clearly showed two major clades, D and E, consistent with the distribution ranges of the China-West Korean and East Korean Populations, respectively (Takehana *et al.*, 2004). These results also revealed that in western Korea, where the distributions of these two clades overlapped, a limited extent of gene flow was observed. This limited gene flow plus the large genetic divergence suggests some reproductive isolation mechanisms between the two groups, or introgression between them followed by random drift in each population. However, the mtDNA data alone could not conclude which of these hypotheses is correct.

Allozymic analysis is a powerful tool not only in the delimitation of a boundary between reproductively independent populations but also in the detection of natural hybridization (Duggins *et al.*, 1995; Trenham *et al.*, 1998; Toda *et al.*, 2001). Furthermore, when natural hybridization occurs, this approach, by targeting variation in the nuclear gene system, is also useful in analyzing the detailed structures of the hybridizing populations (Arnold, 1992). This paper describes the detailed distributions of the East Korean and China–West Korean Populations through an extensive allozymic survey of the wild populations in Korea. The purpose was to determine the extent and nature of gene flow between the two groups, especially in western Korea. The discussion focuses on the reproductive isolation between the groups in Korea on the basis of the distribution patterns of allozymic variation.

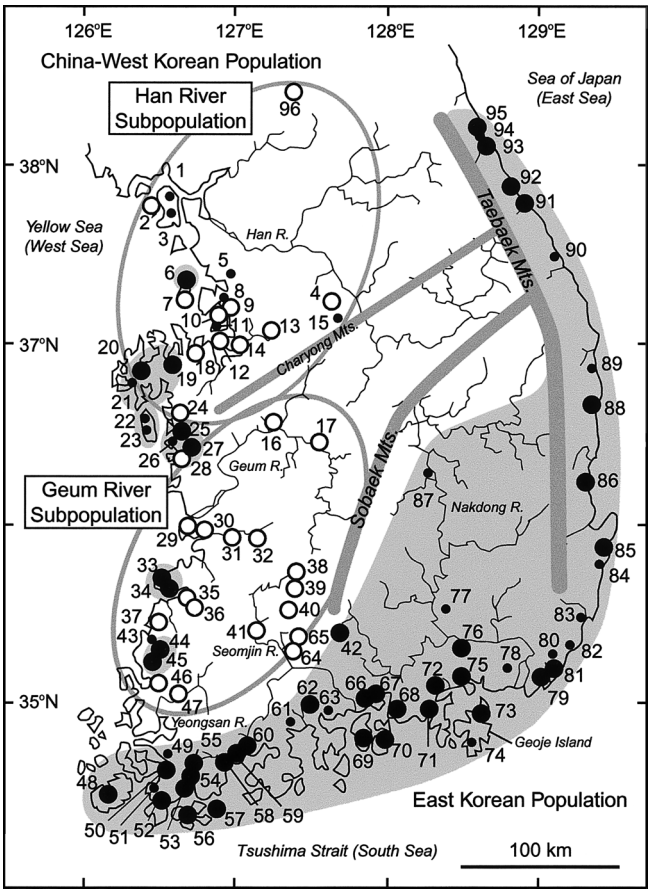

Fig. 1. Collection sites of *Oryzias latipes* in Korea. Site numbers refer to Appendix 1. Shaded and circled areas show the distribution ranges of the East Korean Population and the two subgroups in the China-West Korean Population, respectively. Solid circles and open circles indicate eastern and western mtDNA types, respectively. Dots indicate samples that were not studied in previous mtDNA analyses. The mtDNA data were taken from Takehana *et al.* (2004).

MATERIALS AND METHODS

Fish specimens

Fishes were collected during 1986–89 from 96 localities in Korea (Fig. 1), and transferred to the laboratory alive. The collection sites and dates are listed in Appendix 1.

Electrophoresis and staining

The liver, eyes, intestines and skeletal muscle of each individual were separated and homogenized with 10 mM Tris-HCl (pH 7.0). Each homogenate was centrifuged at 15,000 *g* for 10 min and the resultant supernatant was used for electrophoretic analysis. In this study, we analyzed 11 enzymes and 1 serum protein, presumably encoded by 13 loci including the 9 loci that showed remarkable allele frequency differences between the East Korean Population and the China–West Korean Population in Sakaizumi and Jeon (1987). The enzymes and protein examined and their Enzyme Commission (E.C.) numbers, presumptive loci and tissues used are shown in Table 1. The genetic nomenclature and inscriptions followed Shaklee *et al.* (1990). Amylase (AMY), creatine kinase (CK-A), esterase (EST), muscle- and eye-lactate dehydrogenases (LDH-A and -C), superoxide dismutase (SOD) and transferrin (TF) were analyzed on native, discontinuous, vertical polyacrylamide slab gels using the buffer system of Davis (1964). Each gel sheet of 140×100×1 mm was composed of a 10% acrylamide (6% for muscle-lactate dehydrogenase), 0.125% bisacrylamide separation gel and a 3% acrylamide, 0.8% bisacrylamide stacking gel. Aspartic aminotransferase (AAT), acid phosphatase (ACP), alcohol dehydrogenase (ADH), glucose phosphate isomerase (GPI-A), L-iditol dehydrogenase (IDDH) and phosphoglucomutase (PGM) were separated by electrophoresis in horizontal agarose gels. Each gel sheet of 140×180×1 mm was composed of 0.9% agarose and 2% polyvinylpyrrolidone (K-90; NACALAI TESQUE, Japan). The buffer system for AAT was 90 mM Tris-hydroxymethylaminomethane, 50 mM boric acid, 2 mM EDTA (pH 8.7) for the electrodes, and 27 mM Tris, 15 mM boric acid, 0.6 mM EDTA (pH 7.0) for the gel. The buffer system for ACP and PGM was 135 mM Tris-hydroxymethylaminomethane, 43 mM citric acid (pH 7.0) for the electrodes, and 9 mM Tris, 3 mM citric acid (pH 7.0) for the gel. Phosphate buffer (25 mM, pH 7.0) was used for both the electrode buffer and the gel buffer for the separation of ADH, IDDH and GPI. Potentials of 400 V for the acrylamide were applied for two hours at 4°C. For agarose-gel electrophoresis, 300 V was applied for 1.5 hours. The staining procedures used were those described by Shaw and Prasad (1970). For staining of amylase, the gel was soaked in a solution of soluble starch (1% w/v) in 20 mM phosphate buffer (pH 7.0) for 15 min at 37°C. White bands appeared against a brown background after staining with 100 mM I –180 mM KI solution.

Table 1. Enzymes and protein, presumptive loci and tissues used in the analyses of variations among populations of *Oryzias latipes* from Korea.

Enzyme or protein	E.C. number	Locus	Tissue
Aspartic aminotransferase	2.6.1.1	*AAT**	Muscle
Acid phosphatase	3.1.3.2	*ACP**	Liver
Alcohol dehydrogenase	1.1.1.1.	*ADH**	Liver
Amylase	3.2.1.1	*AMY**	Intestine
Creatine kinase	2.7.3.2	*CK-A**	Muscle
Esterase	3.1.1.–	*EST**	Liver
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-A**	Muscle
L-Iditol dehydrogenase	1.1.1.14	*IDDH**	Liver
L-Lactate dehydrogenase	1.1.1.27	*LDH-A**	Muscle
		*LDH-C**	Eye
Phosphoglucomutase	5.4.2.2	*PGM**	Liver
Superoxide dismutase	1.15.1.1	*SOD**	Liver
Transferrin	–	*TF**	Muscle

RESULTS

Of the 13 presumptive loci examined, all were polymorphic. The Korean wild population of *Oryzias latipes* was divided into two major groups of the eastern and western parts by means of the phenotypic patterns of six diagnostic loci: *ACP**, *AMY**, *CK-A**, *LDH-A**, *PGM** and *TF**. Regarding the *ACP** locus, most fish caught in eastern and southern Korea (47 collection sites) had the anodally slower allele *ACP*d*, whereas the faster allele, *ACP*b,* was predominant in the western inland area. In the western area (49 collection sites), 27 populations were fixed for *ACP*b*, 14 along the coastal region were fixed for *ACP*d*, and the remaining 8 populations had both alleles (Fig. 2a). Similar conditions were observed for the five other loci, *AMY** (Fig. 2b), *CK-A**, *LDH-A**, *PGM** and *TF** (see Appendix 2).

Regarding the esterase systems, all individuals from eastern Korea showed the same pattern with the cathodally slowest band, while many variations without the cathodal band were found in specimens from western Korea. The distribution pattern of this dimorphism was similar to those of the above mentioned loci. The polymorphic patterns were so complicated that they cannot yet be interpreted. However, this result supported the existence of two major groups.

The alleles at the *GPI-A**, *LDH-C** and *SOD** loci showed distribution patterns that were similar to, but slightly different from, those of the six diagnostic loci mentioned above. The frequency of the “eastern” allele, *GPI-A*b*, was higher than those of *ACP**, *AMY** and others in western Korea (Fig. 3b). In contrast, the frequencies of the “eastern” alleles, *LDH-C*a* and *SOD*g*, were lower than those of the diagnostic loci in the coastal region along the Yellow Sea

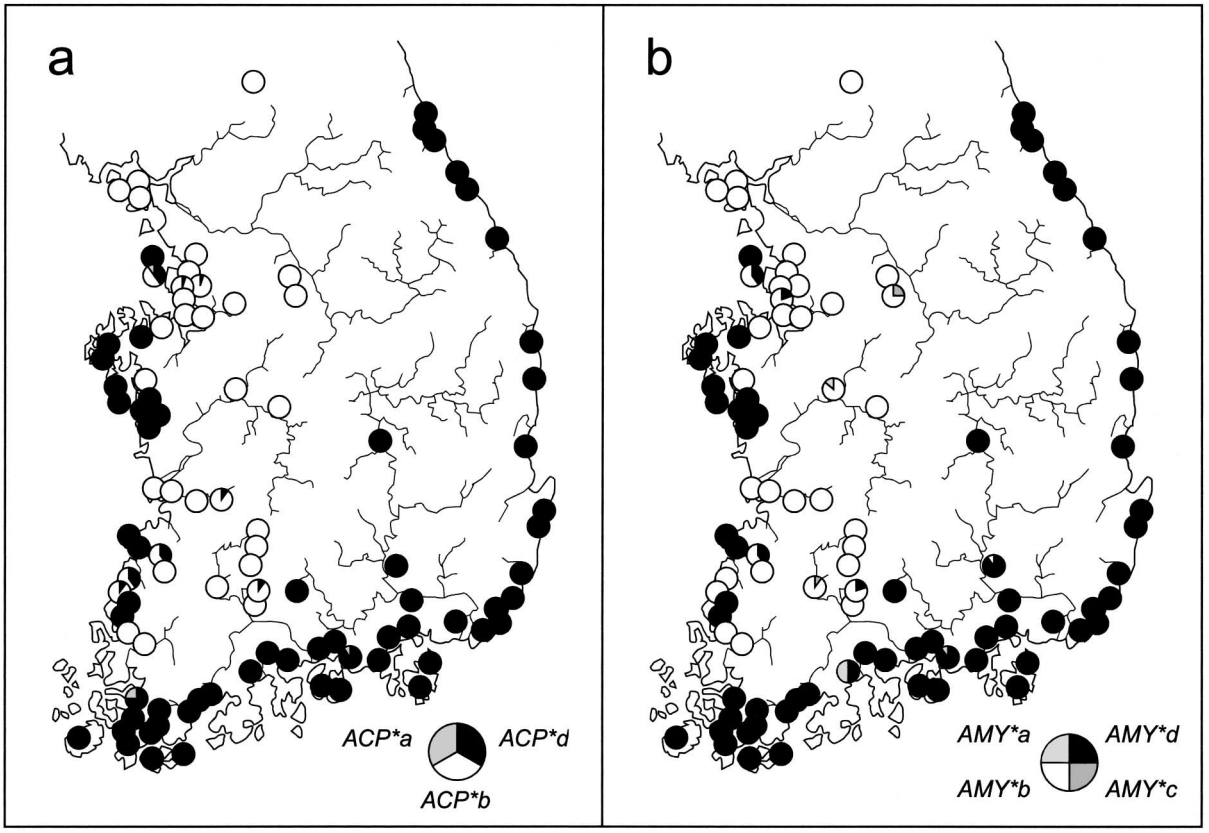

Fig. 2. Allelic distribution of the *ACP** (a) and *AMY** (b) loci.

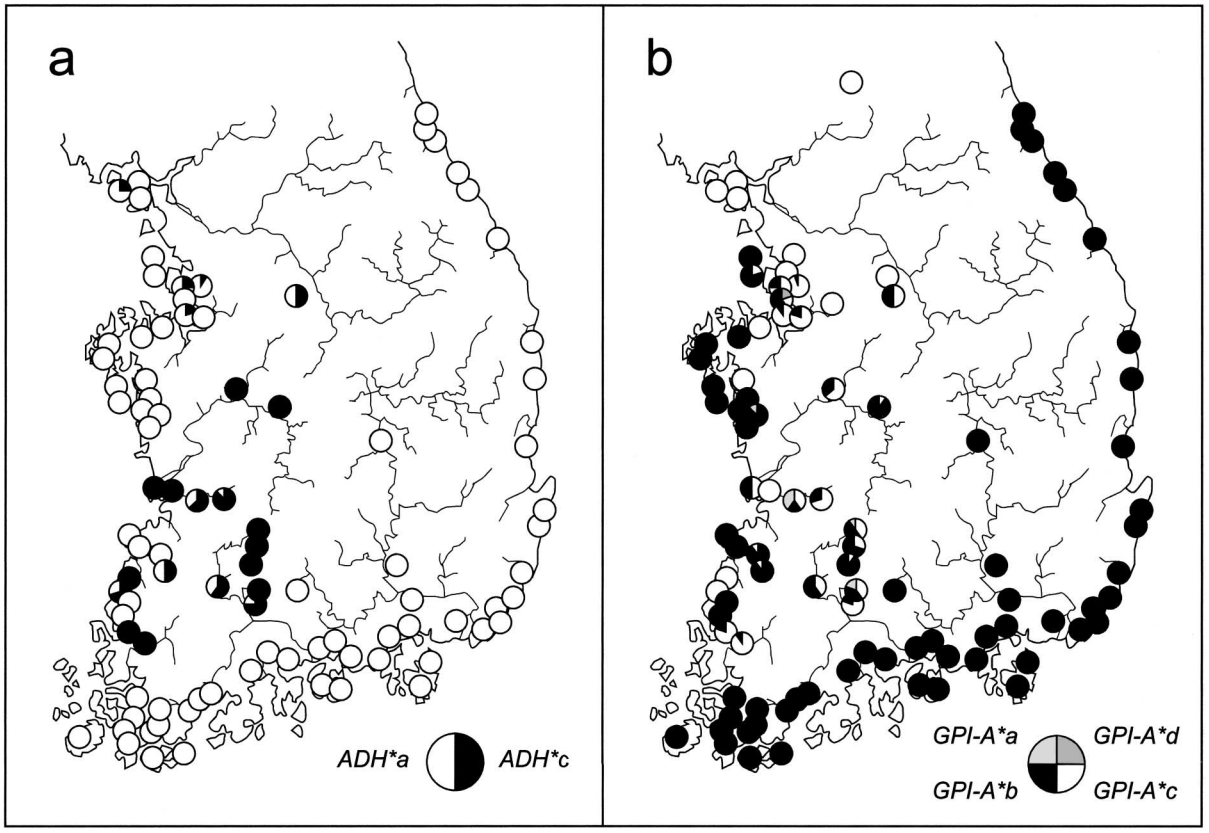

Fig. 3. Allelic distribution of the *ADH** (a) and *GPI-A** (b) loci.

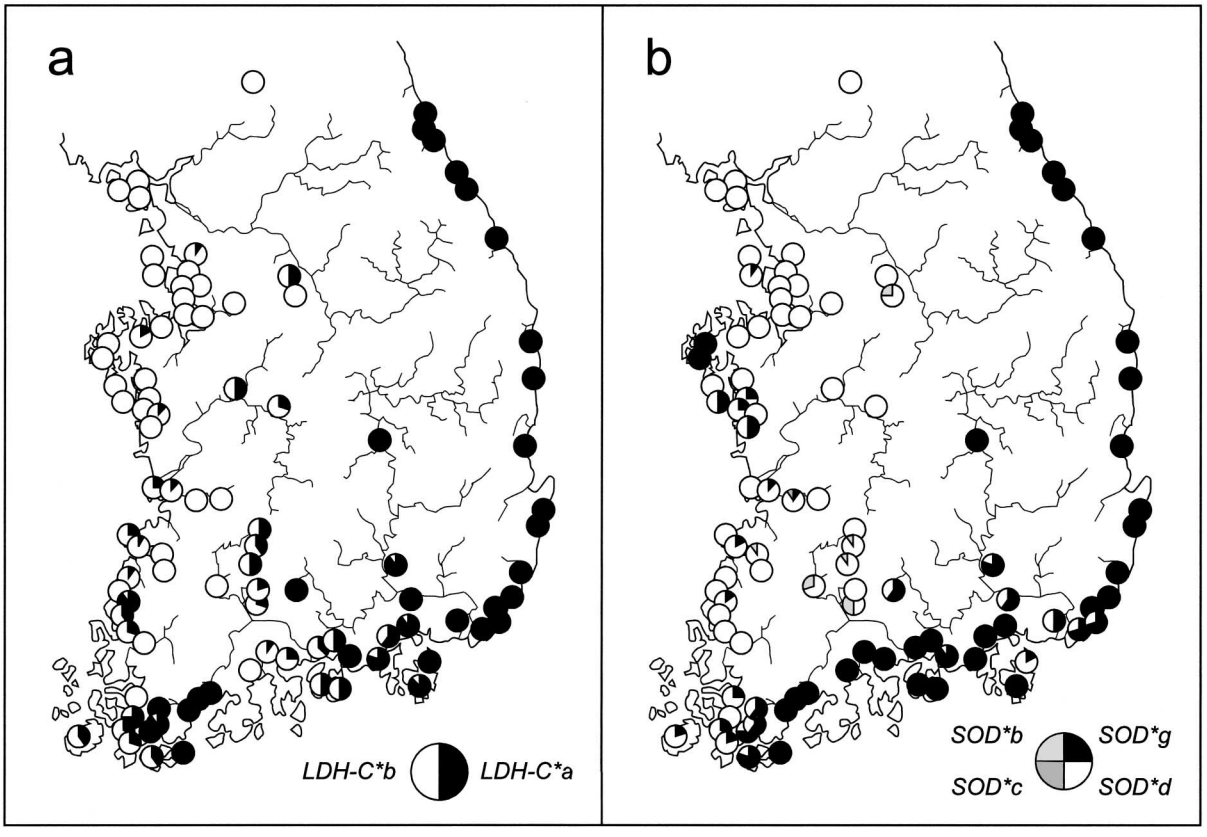

Fig. 4. Allelic distribution of the *LDH-C** (a) and *SOD** (b) loci.

(West Sea) (Fig. 4). The "western" alleles, *LDH-C*b* and *SOD*d*, were also frequently observed along the coast of the Tsushima Strait (South Sea). Furthermore, *LDH-C*a* was distributed in the inland area of western Korea.

In addition to these loci, the alleles at the *ADH** locus showed a geographically specific distribution. This locus was essentially monomorphic where *ADH*a* was observed. However, the specimens from the water basins of the Geum River and the Yeongsan River, and the inland area of the Seomjin River system had the cathodally slower allele, *ADH*c*, at high frequency (Fig. 3a). According to the genotypes at this locus, the West Korean Population could be divided into two subgroups, the "Han River Subpopulation" from northwestern Korea and the "Geum River Subpopulation" from southwestern Korea. The Han River Subpopulation had the *IDDH*c* allele at high frequency. At the *AAT** locus, *AAT*a* was found in the Han River Subpopulation, and the anodally slowest allele, *AAT*c*, was observed for Geoje Island (sites #73 and #74) (Appendix 2).

DISCUSSION

Regional differentiation

According to the genotypes of the six diagnostic protein loci, *O. latipes* distributions in Korea could be classified into two distinct groups, the China-West Korean Population and East Korean Population. The China-West Korea Population, characterized by high frequencies of *ACP*b*, *AMY*b*, *CK-A*b*, *LDH-A*d*, *PGM*b* and *TF*b,* is distributed only on the western side of the Korean Peninsula. This group could be further divided into two subgroups by means of unique allele distributions at the *ADH** locus. In contrast, the East Korean Population, with high frequencies of *ACP*d*, *AMY*d*, *CK-A*a*, *LDH-A*c*, *PGM*e* and *TF*c*, was not only found along the Sea of Japan (East Sea) and Tsushima Strait (South Sea), but also along the Yellow Sea (West Sea) coast. The boundary primarily separating the East Korean and China-West Korean Populations corresponds to the backbone mountains of the Korean Peninsula, for example, the Taebaek Mountains and Sobaek Mountains, which might have been a barrier between the two groups.

Consistency among karyological, mtDNA and allozymic variations

Karyological studies also indicated differences between the East Korean Population and China–West Korean Population (Kim and Lee, 1992; Kim and Moon, 1987; Uwa and Jeon, 1987; Uwa *et al*., 1988), and RFLP analysis of the entire mtDNA and mitochondrial cytochrome *b* sequencing in the Korean wild populations confirmed these two groups (Matsuda *et al*., 1997; Takehana *et al*., 2004). This consistency among the different analyses suggests the existence of a long-term isolation event between the two groups.

The results of this study indicated that the China–West Korean Population could be divided further into two subgroups, the Han River Subpopulation and Geum River Subpopulation. The mtDNA studies also demonstrated identical distribution patterns of the two subgroups separated by the Charyong Mountains (Matsuda *et al*., 1997; Takehana *et al*., 2004). Phylogenetic analyses of the mitochondrial cytochrome *b* sequences showed that local populations from southwestern Korea, that is, the Geum River Subpopulation, were closely related to those from eastern China, suggesting a recent dispersal event from eastern China to southwestern Korea (Takehana *et al*., 2004). However, the *ADH*c* allele, specific to the Geum River Subpopulation, has not been observed in samples from Beijing or Shanghai (Sakaizumi, 1986). This inconsistency can be explained by the founder effect, since the mtDNA variations were extremely low within the Korean subgroup (Takehana *et al*., 2004). It is likely that a small number of populations with *ADH*c* in eastern China migrated into southwestern Korea, and expanded in distribution range.

Reproductive isolation and introgression between the two major groups

Previous mtDNA analyses showed that in the western region of Korea, where the distributions of the two major mtDNA groups overlap, a limited extent of gene flow was observed (Takehana *et al*., 2004). This limited gene flow among local populations along with the large genetic divergence suggested reproductive isolation mechanisms between the two groups, or introgression followed by random drift in each local population. In this study, two distinct genotypes (eastern and western) at six diagnostic loci (*ACP**, *AMY**, *CK-A**, *LDH-A**, *PGM** and *TF**) were distributed in a mosaic fashion along the western coast (Fig. 2). Each population was nearly fixed as having either eastern or western alleles at all six diagnostic loci, despite the proximity of the samples. This distribution pattern of the East Korean and China-West Korean Populations in western Korea was identical to that of the two distinct mtDNA groups. On the other hand, two populations (sites #7 and #35) in the western coast of Korea showed a hybrid condition, sharing eastern and western alleles at all six diagnostic loci. Both populations were fixed for the western mtDNA type. No other populations with the same allelic pattern were observed. These results suggest low current gene exchange between the two groups. This limited gene flow, observed in both the allozymic and mtDNA analyses, suggests that some reproductive isolation mechanisms exist between the two groups.

Karyological studies demonstrated that the East Korean Population had 2n, 48 chromosomes, while the China-West Korean Population had 2n, 46 chromosomes including a large metacentric pair (Uwa and Jeon, 1987; Uwa *et al*., 1988). The heterozygotes disadvantage has been proposed as a possible selective force in chromosomal hybrid zones because of the exception of reduced fertility in karyotypic heterozygotes arising from meiotic aberrations (see King, 1993). However, male and female progeny from hybrids of the two groups were fully fertile when bred in a laboratory

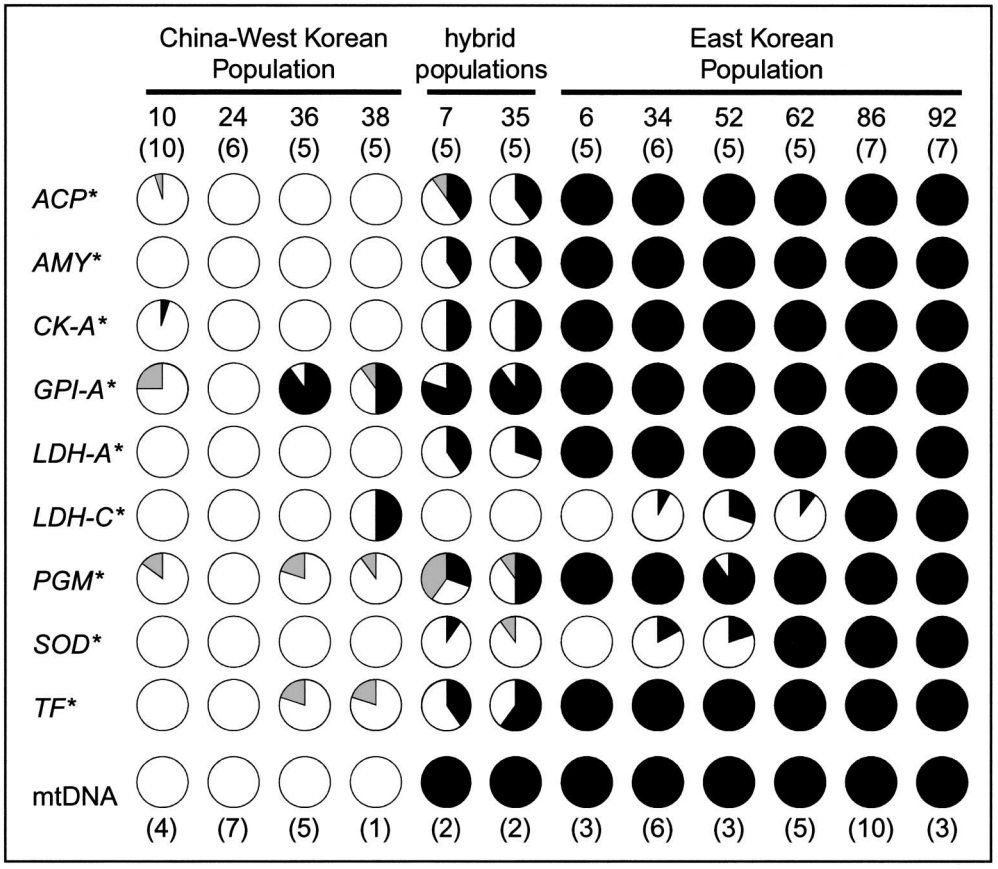

Fig. 5. Pie diagrams constructed from 12 representative populations of *Oryzias latipes* showing the allele frequencies at 9 loci and the frequencies of the mtDNA types that distinguish the two major groups. Sample numbers correspond to those in Fig. 1 and Appendix 1. Numbers in parentheses represent the sample sizes. The mtDNA data were taken from Takehana *et al.* (2004). Black, "eastern type" alleles (or eastern mtDNA type); white and gray, "western type" alleles (or western mtDNA type).

(Sakaizumi *et al.*, 1992). Therefore, it is unlikely that the difference in chromosome composition between the two groups strongly affects the natural selection of hybrids.

Figure 5 indicates the allele frequencies at 9 polymorphic loci and the frequencies of the mtDNA types in representative populations. As previously mentioned, the two major groups showed nearly fixed frequency differences at the six diagnostic loci and mtDNA. However, the distribution of alleles at three loci, *GPI-A**, *LDH-C** and *SOD**, showed introgressive conditions. Some populations of the China-West Korean Population (for example, at sites #36 and #38) had a high frequency of the "eastern" *GPI-A*b* and *LDH-C*a* alleles (Fig. 5). Furthermore, the East Korean Population along the coast of the Yellow Sea (West Sea) and Tsushima Strait (South Sea) (for example, sites #6, #34, #52 and #62) had a high frequency of the "western" *LDH-C*b* and *SOD*d* alleles. These introgressive populations might have been formed through repeated generations of backcross after past hybridization of the two groups. The different levels of introgression among the loci found in this study suggest that selective filters are acting against the foreign alleles. It is possible that much of the genome is protected by selection from inter-group gene flow, while several genomic regions appear to accept foreign alleles or chromosomal segments. Such differential selection is likely to depend on the location of the loci on the genome rather than the functional or structural properties of the enzymes. Introgression of the loci (and linked markers) contributing to isolation is expected to be retarded, whereas neutral or positively selected chromosomal regions (and linked markers) should introgress at higher frequencies. Differential selective filters were also seen from genetic mapping of introgression (Riseberg *et al.*, 1999; Martinsen *et al.*, 2001). There are now more than 800 DNA markers mapped to the medaka genome (Naruse *et al.*, 2004). A detailed genome survey of wild populations based on such DNA markers should make it possible to locate the chromosomal blocks contributing to isolation.

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REFERENCES

Arnold LM (1992) Natural hybridization as an evolutionary process. Annu Rev Ecol Syst 23: 237–261

Davis DA (1964) Disk electrophoresis-II. Method and application to human serum proteins. Ann NY Acad Sci 121: 404–427

Duggins CF Jr, Karlin AA, Mousseau TA, Relyea KG (1995) Analysis of a hybrid zone in *Fundulus majalis* in a northeastern Florida ecotone. Heredity 74: 117–128

Kim IS, Lee EH (1992) New record of ricefish, *Oryzias latipes sinensis* (Pisces, Oryzidae) from Korea. Korean J Syst Zool 8: 177–182

Kim IS, Moon KC (1987) The karyotype of a ricefish, *Oryzias latipes* from southern Korea. Korean J Zool 30: 379–386 (In Korean with English abstract)

King M (1993) Species evolution. The role of chromosome change. Cambridge Univ Press, New York

Martinsen GD, Whitham TG, Turek RJ, Keim P (2001) Hybrid populations selectively filter gene introgression between species. Evolution 55: 1325–1335

Matsuda M, Yamagishi T, Sakaizumi M, Jeon SR (1997) Mitochondrial DNA variation in the Korean wild population of medaka, *Oryzias latipes*. Korean J Limnol 30: 119–128

Naruse K, Tanaka M, Mita K, Shima A, Postlethwait J, Mitani H (2004) A medaka gene map: the trace of ancestral vertebrate proto-chromosomes revealed by comparative gene mapping. Genome Res 14: 820–828

Rieseberg LH, Whitton J, Gardner K (1999) Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. Genetics 152: 713–727

Sakaizumi M (1986) Genetic divergence in wild populations of the Medaka *Oryzias latipes* (Pisces: Oryziatidae) from Japan and China. Genetica 69: 119–125

Sakaizumi M, Shimizu Y, Hamaguchi S (1992) Electrophoretic studies of meiotic segregation in inter- and intraspecific hybrids among East Asian species of the genus *Oryzias* (Pisces: Oryziatidae). J Exp Zool 264: 85–92

Sakaizumi M, Jeon SR (1987) Two divergent groups in the wild populations of medaka *Oryzias latipes* (Pisces: Oryziatidae) in Korea. Korean J Limnol 20: 13–20 (in Korean with English abstract)

Shaklee JB, Allendorf FW, Morizot DC, Whitt GS (1990) Gene nomenclature for protein-cording loci in fish. Trans Am Fish Soc 119: 2–15

Shaw CR Prasad R (1970) Starch gel electrophoresis of enzymes. A compilation of recipes. Biochem Genet 4: 297–320

Takehana Y, Uchiyama S, Matsuda M, Jeon SR, Sakaizumi M (2004) Geographic variation and diversity of the cytochrome *b* gene in wild populations of medaka (*Oryzias latipes*) from Korea and China. Zool Sci 21: 483–491

Toda M, Okada S, Ota H, Hikida T (2001) Biochemical assessment of evolution and taxonomy of the morphologically poorly diverged geckos, *Gekko yakuensis* and *G. hokouensis* (Reptilia: Squamata) in Japan, with special reference to their occasional hybridization. Biol J Linne Soc 73: 153–165

Trenham PC, Shaffer HB, Moyle PB (1998) Biochemical identification and assessment of population subdivision in morphologically similar native and invading smelt species (*Hypomesus*) in the Sacramento-San Joaquin Estuary, California. Trans Amer Fish Soc 127: 417–424

Uwa H, Jeon SR (1987) Karyotypes in two divergent groups of a ricefish, *Oryzias latipes*, from Korea. Korean J Limnol 20: 139–147 (in Korean with English abstract)

Uwa H, Wang RF, Chen YR (1988) Karyotypes and geographical distribution of ricefishes from Yunnan, southwestern China. Jpn J Ichthyol 35: 332–340

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Appendix 1. Collection sites and dates of *Oryzias latipes* in Korea. The numbers of electrophoretically examined specimens are shown in parentheses.

1. Cheolsan, Yangsa, Ganghwa, Incheon, 2 July '87 (2), 2. Seokmo, Samsan, Ganghwa, Incheon, 30 July '87 (5), 3. Oepo, Naega, Ganghwa, Incheon, 30 July '87 (1), 4. Daeso, Janghowon, Icheon, Gyeonggi, 31 July '88 (2), 5. Palgok, Banwol, Shiheung, Gyeonggi, 20 Sept. '86 (5), 6. Buk, Daebu, Ongjin, Gyeonggi, 24 Feb. '89 (5), 7. Seongam, Daebu, Ongjin, Gyeonggi, 24 Feb. '89 (5), 8. Yulam, Paltan, Hwaseong, Gyeonggi, 24 Feb. '89 (1), 9. Haechang, Paltan, Hwaseong, Gyeonggi, 8 July '86 (9), 11 Mar. '89 (10), 10. Seokcheon, Ujong, Hwaseong, Gyeonggi, 1 Mar. '89 (10), 11. Hwasan, Ujeong, Hwaseong, Gyeonggi, 1 Mar. '89 (5), 12. Maehyang, Ujeong, Hwaseong, Gyeonggi, 1 Mar. '89 (5), 13. Nae, Daedok, Anseong, Gyeonggi, 30 Nov. '86 (7), 14. Sukseong, Oseong, Pyeongtaik, Gyeonggi, 7 Nov. '87 (5), 15. Byeongam, Sainggeuk, Eumseong, Chungcheongbuk, 19 Mar. '89 (2), 16. Bugang, Buyong, Cheongwon, Chungcheongbuk, 7 July '86 (7), 17. Godang, Shimcheon, Yeongdong, Chungcheongbuk, 18 Oct. '87 (5), 18. Haingjeong, Dangjin, Dangjin, Chungcheongnam, 22 Nov. '87 (5), 19. Gonam, Seongyeon, Seosan, Chungcheongnam, 5 May '88 (3), 20. Daegi, Wonbuk, Seosan, Chungcheongnam, 5 May '88 (2), 21. Yongshin, Keunheung, Seosan, Chungcheongnam, 16 Nov. '88 (2), 22. Changgi, Anmyon, Seosan, Chungcheongnam, 17 Aug. '88 (1), 23. Seungeon, Anmyon, Seosan, Chungcheongnam, 17 Aug. '88 (2), 24. Shingok, Guhang, Hongseong, Chungcheongnam, 29 May '88 (6), 25. Daecheon, Daecheon, Boryeong, Chungcheongnam, 29 May '88 (4), 26. Gungchon, Taechon, Boryeong, Chungcheongnam, 18 Aug. 88 (2), 27. Jinjuk, Cheongso, Boryeong, Chungcheongnam, 27 June '88 (4), 28. Gyoseong, Ocheon, Boryeong, Jeollabuk, 17 Aug. '88 (1), 29. Shingwan, Miseong, Okgu, Jeollabuk, 7 Oct. '88 (2), 30. Dangbuk, Oksan, Okgu, Jeollabuk, 18 Aug. '88 (4), 31. Baikgu, Baikgu, Gimje, Jeollabuk, 11 Apr. '89 (5), 32. Dongsan, Jochon, Wanju, Jeollabuk, 16 Aug. '87 (5), 33. Daehang, Byonsan, Buan, Jeollabuk, 21 May '88 (5), 34. Seokpo, Jinseo, Buan, Jeollabuk, 21 May '88 (6), 35. Seokgyo, Heungdeok, Gochang, Jeollabuk, 9 Apr. '89 (5), 36. Jodong, Seongnae, Gochang, Jeollabuk, 16 Aug. '87 (5), 37. Galma, Daesan, Gochang, Jeollabuk, 9 Apr. '89 (5), 38. Yongam, Shinpyeong, Imshil, Jeollabuk, 21 Apr. '89 (5), 39. Dugok, Imshil, Imshil, Jeollabuk, 21 Apr. '89 (5), 40. Gunpyeong, Dunnam, Imshil, Jeollabuk, 21 Apr. '89 (5), 41. Jecheon, Jusaing, Namwon, Jeollabuk, 21 Apr. '89 (5), 42. Hwasu, Unbong, Namwon, Jeollabuk, 21 Apr. '89 (5), 43. Ipseok, Yeonggwang, Yeonggwang Jeollanam, 9 Apr. '89 (5), 44. Keumgye, Bulgap, Yeonggwang, Jeollanam, 8 Apr. '89 (10), 45. Noksan, Bulgap, Yeonggwang, Jeollanam, 16 Aug. '87 (5), 46. Hampyeong, Hampyeong, Hampyeong, Jeollanam, 7 Apr. '89 (5), 47. Yeongdong, Dashi, Naju, Jeollanam, 7 Apr. '89 (5), 48. Bojeon, Jisan, Jindo, Jeollanam, 18 Mar. '88 (5), 49. Deokjeong, Gyegok, Haenam, Jeollanam, 29 July '88 (2), 50. Bopyeong, Haenam, Haenam, Jeollanam, 29 July '88 (2), 51. Haechang, Hwasan, Haenam, Jeollanam, 17 Aug. '87 (3), 52. Eupho, Hyeonsan, Haenam, Jeollanam, 17 Aug. '87 (5), 53. Shinwol, Bugil, Haenam, Jeollanam, 17 May '89 (2), 54. Yonghwa, Shinjeon, Gangjin, Jeollanam, 17 Aug. '87 (5), 55. Mandeok, Doam, Gangjin, Jeollanam, 17 May '89 (5), 56. Jeongdo, Wando, Wando, Jeollanam, 17 May '89 (5), 57. Gwansan, Yaksan, Wando, Jeollanam, 12 Jan. '88 (8), 58. Suyang, Anryang, Jangheung, Jeollanam, 17 Aug. '87 (3), 59. Dangam, Anryang, Jangheung, Jeollanam, 17 Aug. '87 (5), 60. Byeokgyo, Hoecheon, Boseong, Jeollanam, 17 Aug. '87 (5), 61. Guryong, Byeollyang, Sungju, Jeollanam, 17 Aug. '87 (1), 62. Deogam, Suncheon, Jeollanam, 18 Aug. '87 (5), 63. Yonggang, Kwangyang, Kwangyang, Jeollanam, 18 Aug. '87 (2), 64. Naingcheon, Masan, Gurye, Jeollanam, 21 Apr. '89 (5), 65. Jicheon, Gwangeui, Gurye, Jeollanam, 21 Apr. '89 (5), 66. Gyecheon, Geumnam, Hadong, Gyeongsangnam, 18 Aug. '87 (4), 67. Seongnae, Gonyang, Sacheon, Gyeongsangnam, 18 May '89 (5), 68. Bangji, Sanam, Sacheon, Gyeongsangnam, 18 Aug. '87 (5), 69. Murim, Idong, Namhae, Gyeongsangnam, 18 May '89 (5), 70. Naneum, Samdong, Namhae, Gyeongsangnam, 18 May. '89 (4), 71. Daedok, Goseong, Goseong, Gyeongsangnam, 19 May '89 (5), 72. Bojeon, Maam, Goseong, Gyeongsangnam, 18 Aug. '87 (5), 73. Yeonsa, Yeoncho, Geoje, Gyeongsangnam, 8 Oct. '87 (9), 74. Sanyang, Tongbu, Geoje, Gyeongsangnam, 19 May '89 (4), 75. Imyeong, Jinjeon, Changwon, Gyeongsangnam, 18 Aug. '87 (5), 76. Gangju, Beobsu, Haman, Gyeongsangnam, 17 Oct. '87 (5), 77. Topyeong, Ibang, Changnyeong, Gyeongsangnam, 18 Feb. '87 (5), 78. Sainggok, Noksan, Gimhae, Gyeongsangnam, 18 Aug. '87 (4), 79. Hwajeon, Ilgwang, Gijang, Busan, 29 Nov. '86 (5), 80. Hyoam, Jangan, Gijang, Busan, 19 Aug. '87 (5), 81. Myeongsan, Seosaing, Ulsan, 29 Nov. '86 (5), 82. Bal, Onyang, Ulsan, 29 Nov. '86 (5), 83. Seonam, Nam, Ulsan, 29 Nov. '86 (5) , 84. Naa, Yangnam, Gyeongju, Gyeongsangbuk, 29 Nov. '86 (5), 85. Gugil, Yangbuk, Gyeongju, Gyeongsangbuk, 29 Nov. '86 (5), 86. Heungan, Heunghae, Pohang, Gyeongsangbuk, 29 Nov. '86 (7), 87. Nongso, Okseong, Seonsan, Gyeongsangbuk, 13 Nov. '88 (1), 88. Shinpyeong, Yeonghae, Yeongdeok, Gyeongsangbuk, 12 Oct. '86 (5), 89. Samdal, Pyonghae, Uljin, Gyeongsangbuk, 12 Oct. '86 (5), 90. Naan, Donghae, Gangweon, 8 Nov. '86 (5) , 91. Gangmun, Gangneung, Gangweon, 8 Nov. '86 (5), 92. Sacheonjin, Sacheon, Gangneung, Gangweon, 8 Nov. '86 (5), 30 Apr. '89 (2), 93. Cheonghak, Sokcho, Gangweon, 15 Apr. '89 (3) , 94. Yeongnang, Sokcho, Gangweon, 15 Aug. '86 (4), 95. Yongchon, Toseong, Goseong, Gangweon, 15 Aug. '86 (5), 96. Daema, Cheolwon, Cheolwon, Gangweon, 17 Aug. '89 (4)

Appendix 2. Allele frequencies at 12 polymorphic loci in 96 populations of *Oryzias latipes* from Korea. Site numbers correspond to those in Fig. 1 and Appendix 1. Numbers in parentheses represent the sample sizes. ND, not detected.

Locus		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
(N)		(2)	(5)	(1)	(2)	(5)	(5)	(5)	(1)	(19)	(10)	(5)	(5)	(7)	(5)	(2)	(7)	(5)	(5)	(3)	(2)	(2)	(1)	(2)	(6)
*AAT**	**a*	.25	.20						ND	.04	.17	.17			.30	ND									.08
	**b*	.75	.80	1.0	1.0	1.0	1.0	1.0		.96	.83	.83	1.0	.93	.70		1.0	1.0	1.0	1,0	1.0	1.0	1.0	1.0	.92
	**c*													.07											
*ACP**	**a*							.10		.05	.05														
	**b*	1.0	1.0	1.0	1.0	1.0		.50	1.0	.95	.95	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0						1.0
	**d*						1.0	.40												1.0	1.0	1.0	1.0	1.0	
*ADH**	**a*	1.0	.75	1.0	ND	ND	1.0	1.0	ND	.91	.75	1.0	.80	ND	1.0	.50			1.0	1.0	1.0	1.0	1.0	1.0	1.0
	**c*		.25							.09	.25		.20			.50	1.0	1.0							
AMY	**a*																.14								
	**b*	1.0	1.0	1.0	1.0	1.0		.60	1.0	1.0	1.0	.80	1.0	1.0	1.0	.75	.86	1.0	1.0						1.0
	**c*															.25									
	**d*						1.0	.40				.20								1.0	1.0	1.0	1.0	1.0	
*CK-A**	**a*						1.0	.50		.03	.05	.10							.20	.50		1.0		1.0	
	**b*	1.0	1.0	1.0	1.0	1.0		.50	1.0	.97	.95	.90	1.0	1.0	1.0	1.0	1.0	1.0	.80	.50	1.0		1.0		1.0
*GPI-A**	**a*																								
	**b*						1.0	.80		.05	.25	.60	.10		.20	.50	.36	.90		1.0	1.0	1.0	1.0	1.0	
	**c*	1.0	1.0	1.0	1.0	1.0		.20	1.0	.95	.75	.20	.90	1.0	.80	.50	.64	.10	1.0						1.0
	**d*								1.0			.20													
*IDDH**	**a*	.25	.20		1.0	1.0	1.0	.90	1.0	.92	1.0	.40	.60	.93	.50	1.0	1.0	.80	.80	1.0	1.0	1.0	1.0	1.0	1.0
	**c*	.75	.80	1.0				.10		.08		.60	.40	.07	.50				.20						
	**d*																	.20							
*LDH-A**	**c*						1.0	.40												1.0	1.0	1.0	1.0	1.0	
	**d*	1.0	1.0	1.0	1.0	1.0		.60	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0						1.0
*LDH-C**	**a*				.50	.10											.50	.30		.17					
	**b*	1.0	1.0	1.0	.50	.90	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.50	.70	1.0	.83	1.0	1.0	1.0	1.0	1.0
*PGM**	**a*	.50	.50			.30		.40		.34	.15	.50	.20	.07	.10										
	**b*	.50	.30	1.0	1.0	.70		.30	1.0	.66	.85	.50	.80	.93	.90	1.0	1.0	1.0	.90						1.0
	**e*		.20				1.0	.30											.10	1.0	1.0	1.0	1.0	1.0	
*SOD**	**b*															.25									
	**c*																								
	**d*	1.0	1.0	1.0	1.0	1.0	1.0	.90	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.75	1.0	1.0	1.0	1.0			1.0	.50	1.0
	**g*							.10													1.0	1.0		.50	
*TF**	**a*													.21											
	**b*	1.0	1.0	1.0	1.0	1.0		.60	1.0	1.0	1.0	1.0	1.0	.79	1.0	.50	1.0	1.0	1.0						1.0
	**c*						1.0	.40												1.0	1.0	1.0	1.0	1.0	
	**d*															.50									

Appendix 2. Continued.

Locus		25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
(N)		(4)	(2)	(4)	(1)	(2)	(4)	(5)	(5)	(5)	(6)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(10)	(5)	(5)	(5)	(5)
*AAT**	**a*											ND		ND		ND				ND	ND				
	**b*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0		1.0		1.0		1.0	1.0	1.0			1.0	1.0	1.0	1.0
	**c*																								
*ACP**	**a*																								
	**b*					1.0	1.0	1.0	.90			.60	1.0	.60	1.0	1.0	1.0	1.0		.90			1.0	1.0	
	**d*	1.0	1.0	1.0	1.0				.10	1.0	1.0	.40		.40					1.0	.10	1.0	1.0			1.0
*ADH**	**a*	1.0	1.0	1.0	1.0			.37	.12	1.0	1.0	1.0	.50					.40	1.0	.30	1.0	1.0			1.0
	**c*					1.0	1.0	.63	.88				.50	1.0	1.0	1.0	1.0	.60		.70			1.0	1.0	
AMY	**a*																								
	**b*					1.0	1.0	1.0	1.0			.60	1.0	1.0	1.0	1.0	1.0	.90		1.0			1.0	1.0	
	**c*																	.10							
	**d*	1.0	1.0	1.0	1.0					1.0	1.0	.40							1.0		1.0	1.0			1.0
*CK-A**	**a*	.75	1.0		1.0		.12			.90	1.0	.50					.20		1.0		1.0	1.0	.10		1.0
	**b*	.25		1.0		1.0	.88	1.0	1.0	.10		.50	1.0	1.0	1.0	1.0	.80	1.0		1.0			.90	1.0	
*GPI-A**	**a*			.12				.40							.10										
	**b*	1.0	1.0	.88	1.0	.50		.20	.30	1.0	1,0	.90	.90		.50	.70	.90	.60	1.0		1.0	1.0	.20	.10	1.0
	**c*					.50	1.0	.40	.70			.10	.10	1.0	.40	.30	.10	.40		1.0			.80	.90	
	**d*																								
*IDDH**	**a*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.70	1.0	.90	1.0	1.0	1.0	1.0	1.0	.30	1.0	1.0	.90	.90	1.0
	**c*											.30		.10						.70			.10	.10	
	**d*																								
*LDH-A**	**c*	.88	1.0	.88	1.0					1.0	1.0	.30							.70		1.0	1.0	.10		1.0
	**d*	.12		.12		1.0	1.0	1.0	1.0			.70	1.0	1.0	1.0	1.0	1.0	1.0	.30	1.0			.90	1.0	
*LDH-C**	**a*			.12		.25	.12			.25	.08			.10	.50	.40	.50		1.0		.90	.40	.30		.40
	**b*	1.0	1.0	.88	1.0	.75	.88	1.0	1.0	.75	.92	1.0	1.0	.90	.50	.60	.50	1.0		1.0	.10	.60	.70	1.0	.60
*PGM**	**a*							.10	.30			.10	.20	.40	.10	.30	.10			.80			.20	.40	
	**b*				.75	1.0	.90	.70			.40	.80	.60	.90	.70	.90	1.0		.20			.80	.60		
	**e*	1.0	1.0	1.0	1.0	.25				1.0	1.0	.50							1.0		1.0	1.0			1.0
*SOD**	**b*											.10				.10	.10	.30							
	**c*							.10																	
	**d*	.75	.75	1.0	.50	1.0	.88	.80	1.0	1.0	.83	.90	1.0	1.0	1.0	.90	.90	.70	.40	1.0	.85	1.0	1.0	1.0	.80
	**g*	.25	.25		.50		.12	.10			.17								.60		.15				.20
*TF**	**a*																								
	**b*					1.0	1.0	1.0	1.0			.40	.80	1.0	.80	.80	.80	.60		1.0			.80	.70	
	**c*	1.0	1.0	1.0	1.0					1.0	1.0	.60							1.0		1.0	1.0			1.0
	**d*												.20		.20	.20	.20	.40					.20	.30	

Appendix 2. Continued.

Locus		49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
(N)		(2)	(2)	(3)	(5)	(2)	(5)	(5)	(5)	(8)	(3)	(5)	(5)	(1)	(5)	(2)	(5)	(5)	(4)	(5)	(5)	(5)	(4)	(5)	(5)
*AAT**	**a*																								.10
	**b*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.90
	**c*																								
*ACP**	**a*	.25																			.10				
	**b*																1.0	.90							
	**d*	.75	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0		.10	1.0	1.0	.90	1.0	1.0	1.0	1.0
*ADH**	**a*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.25		1.0	1.0	1.0	1.0	1.0	1.0	1.0
	**c*																.75	1.0							
AMY	**a*													.50											
	**b*																1.0	.80							
	**c*																								
	**d*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.50	1.0	1.0		.20	1.0	1.0	1.0	1.0	1.0	1.0	1.0
*CK-A**	**a*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.20		1.0	1.0	1.0	1.0	1.0	1.0	1.0
	**b*																.80	1.0							
*GPI-A**	**a*																	.20							
	**b*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1,0	1.0	1.0	1.0	1.0	1.0	.20	.40	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	**c*																.80	.40							
	**d*																								
*IDDH**	**a*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.90	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	**c*																.10								
	**d*																								
*LDH-A**	**c*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0		.20	1.0	1.0	.90	1.0	1.0	1.0	1.0
	**d*																1.0	.80			.10				
*LDH-C**	**a*		.75	.50	.30	1.0	.90	1.0	.40	1.0	1.0	1.0	1.0		.10	.25	.30	.20	.37	.50	1.0	.50	.50	.80	.60
	**b*	1.0	.25	.50	.70		.10		.60					1.0	.90	.75	.70	.80	.63	.50		.50	.50	.20	.40
*PGM**	**a*																	.10							
	**b*				.10				.10	.12							1.0	.90							
	**e*	1.0	1.0	1.0	.90	1.0	1.0	1.0	.90	.88	1.0	1.0	1.0	1.0	1.0	1.0			1.0	1.0	1.0	1.0	1.0	1.0	1.0
*SOD**	**b*																.50								
	**c*																								
	**d*	.75	1.0	.67	.80	.25	.40	.60	.20								.50	1.0			.10				
	**g*	.25		.33	.20	.75	.60	.40	.80	1.0	1.0	1.0	1.0	1.0	1.0	1.0			1.0	1.0	.90	1.0	1.0	1.0	1.0
*TF**	**a*																								
	**b*																.50	1.0							
	**c*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0			1.0	1.0	1.0	1.0	1.0	1.0	1.0
	**d*																.50								

Appendix 2. Continued.

Locus		73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
(N)		(9)	(4)	(5)	(5)	(5)	(4)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(7)	(1)	(5)	(5)	(5)	(5)	(7)	(3)	(4)	(5)	(4)
*AAT**	**a*																								
	**b*	.17	.88	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	**c*	.83	.12																						
*ACP**	**a*																								
	**b*																								1.0
	**d*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
*ADH**	**a*	1.0	1.0	1.0	1.0	.90	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	ND
	**c*					.10																			
AMY	**a*																								
	**b*					.10																			1.0
	**c*																								
	**d*	1.0	1.0	1.0	1.0	.90	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
*CK-A**	**a*	1.0	1.0	1.0	1.0	.80	1.0	1.0	.90	1.0	1.0	1.0	1.0	1.0	1.0		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
	**b*					.20			.10							1.0									1.0
*GPI-A**	**a*																								
	**b*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1,0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
	**c*																								1.0
	**d*																								
*IDDH**	**a*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.88
	**c*																								.12
	**d*																								
*LDH-A**	**c*	1.0	1.0	1.0	1.0	.90	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.12
	**d*					.10																			.88
*LDH-C**	**a*	1.0	.88	.90	1.0	.90	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
	**b*		.12	.10		.10																			1.0
*PGM**	**a*																								.88
	**b*												.30	.40											.12
	**e*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.70	.60	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
*SOD**	**b*																								
	**c*																								
	**d*	.83			.40	.20	.50	.30		.30															1.0
	**g*	.17	1.0	1.0	.60	.80	.50	.70	1.0	.70	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
*TF**	**a*																								
	**b*																								1.0
	**c*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.70	.80	.90	.93	1.0	1.0	.60	
	**d*																	.30	.20	.10	.07			.40	