Genetic Divergence of Fishes in Genus
_Lateolabrax_ (Perciformes: Percichthyidae)

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Abstract: Genetic divergence of three species of genus _Lateolabrax_ (L. japonicus, L. sp. and L. latus), Percichthyidae, was examined by isozyme analysis. _L. latus_ exhibited complete replacement of alleles both with _L. japonicus_ and _L. sp._, at the _ADH*_, _EST-2*_, _GPI-1*_, _PGM*_, _PROT-1*_, _PROT-2*_, and _SOD-1*_ loci, plus a considerable difference in allelic frequencies at the _MEP*_, indicating a greater divergence than the interspecific level appeared between _L. japonicus_, and _L. sp._ _L. latus_ was somewhat closer to _L. japonicus_ rather than _L. sp._ in genetic distance, because the common major alleles occurred only with _L. japonicus_ at two loci (_FBALD-1* and _FBALD-2*_), compared with a single major allele common with _L. sp._ at just one loci (_LDH*). The genetic information suggested that _L. latus_ represented an early offspring from the common ancestor of _Lateolabrax_, _L. japonicus_ and _L. sp._ diverged considerably later.

Key words: Sea bass; _Lateolabrax_; Genetic divergence; Isozyme

Bleeker1) established genus _Lateolabrax_ (Percichthyidae, Perciformes), for a single species, _Lateolabrax japonicus_ (Cuvier). Thereafter, Katayama2) described a second species (_L. latus_), and more recently, Yokogawa and Seki3) concluded that differences between the Japanese and Chinese forms of _L. japonicus_ were sufficient for the Chinese form to be recognized as a distinct species (referred to as _Lateolabrax_ sp.). Subsequently, Yokogawa and Tajima4) reported the Taiwanese form of _L. japonicus_ to be specifically referable to _L. sp._, although it differed somewhat both morphologically and genetically, they named it “spotted sea bass”. At this point, three valid species of _Lateolabrax_ are recognized. In this study, genetic characteristics of the three species were examined using isozyme analysis and their pattern of divergence were discussed.

Materials and Methods

_Lateolabrax japonicus_ specimens used in this study comprised of 37 individuals, 21 caught in December, 1995, and 16 caught in December, 1996, both by trawling in the southern Harima Sea, Seto Inland Sea. Forty examples of _L. sp._ were obtained in November, 1995, from cultured fish transported from Yentai, Shandong Province, China, to Hiketa nursery, Kagawa, in spring 1995. _L. latus_ specimens comprised of 10 individuals, 1 caught in November, 1993 at off Kamaguchi, Awaji Island, Seto Inland Sea, by angling, the others caught in coastal waters off Tsubakidomari, Tokushima, by fixed shore net (3 in January-March, 1993, and the other 6 in January-March, 1995, respectively).

The specimens were maintained at either -30°C or -80°C. Electrophoresis was performed as Yokogawa and Seki5) described. In this study, in addition to the 20 loci identified by Yokogawa and Seki5), the following 8 loci were detected; adenylate kinase, E. C. 2. 7. 4. 3 (AK*, from liver tissue), esterase, E. C. 3. 1. 1. - (EST-1*, EST-2*, from liver tissue), fructose biphosphate aldolase, E. C. 4. 11. 2. 3 (FBALD-1*, FBALD-2*, from muscle tissue), isocitrate dehydrogenase (NADP*), E. C. 1. 1. 1. 42 (IDHP-2*),

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from muscle tissue \( [*] \) in the former reports was renamed \( IDHP-1 \) \( ^* \)). General protein \( (PROT-3 \) \( ^* \), from muscle tissue), superoxide dismutase, E. C. 1. 15. 1. 1 \( (SOD-2 \) \( ^* \), from liver tissue \( [ \) in the former reports was renamed \( SOD-1 \) \( ^* \)).

Genetic distances (\( D \) values) after Nei\(^{[5]} \) were calculated from the allelic frequencies of the three species. Unbiased values for each \( D \) value were calculated after Nei\(^{[5]} \) and 95% confidence intervals according to each unbiased \( D \) value were calculated after Shinjo\(^{[7]} \). In addition, years of divergence according to the \( D \) values were given after Nei\(^{[6]} \).

**Results**

Allelic frequencies of the 28 loci in the three species with values to indicate their genetic characteristics are shown in Table 1, and electrophoretograms of some significant isozymes were illustrated in Fig. 1. *Lateolabrax latus* showed complete replacement of alleles, compared with *L. japonicus* and *L. sp., at the ADH* \( ^* \), EST-2* \( ^* \), GPI-1* \( ^* \), PGK* \( ^* \), PROT-1* \( ^* \), PROT-2* \( ^* \) and SOD-1* \( ^* \) loci, and replacement of a major allele at the MEP* \( ^* \) locus (Fig. 1, Table 1). The genetic status of *L. japonicus* and *L. sp. reported by Yokogawa and Seki\(^{[3]} \) was confirmed by the 8 newly-detected loci in this study, which exhibited replacement of major alleles between the two species at the FBALD-1* and FBALD-2* loci and common major alleles at other loci (Fig. 1, Table 1).

The genetic distances (\( D \) values) between the three species and a dendrogram based on unbiased genetic distances plus a time scale\(^{[6]} \) are given in Table 2 and Fig. 2, respectively. *L. latus* was found to be somewhat closer to *L. japonicus* rather than to *L. sp. in genetic distance (Table 2), owing to the existence of alleles common only to the two former species at two loci (FBALD-1* and FBALD-2*), compared with a single major allele common only to *L. latus* and *L. sp. at just one loci (Ldh* \( ^* \)) (Table 1). The dendrogram shows that *L. japonicus* and *L. sp. form a single cluster, of genetic distance about 0.18 (0.9 Ma). The genetic distance of

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**Table 1. Allelic frequencies with values to indicate genetic characteristics of three species of *Lateolabrax***

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAT-1*</td>
<td>*120</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
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<td>ADH*</td>
<td>*-35</td>
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<td>*-30</td>
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<td></td>
<td>*-70</td>
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<tr>
<td></td>
<td>*100</td>
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</tr>
<tr>
<td>AK*</td>
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<td>EST-1*</td>
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<tr>
<td></td>
<td>*250</td>
<td>0.000</td>
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<tr>
<td>FBALD-1*</td>
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</tr>
<tr>
<td></td>
<td>*100</td>
<td>0.000</td>
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<tr>
<td>GAPDH-1*</td>
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<td>GAPDH-2*</td>
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<td>GPI-1*</td>
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<td>*100</td>
<td>1.000</td>
</tr>
<tr>
<td>Alleles/Locus</td>
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<td>1.853</td>
</tr>
</tbody>
</table>

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\( P^* \): Polymorphism less than 0.25.

\( P \): Polymorphism over 0.95.

\( Ho \): Observed heterozygosity.

\( He \): Expected heterozygosity.
Fig. 1. Selected electrophoretograms of some isozymes in three species of *Lateolabrax*.

Table 2. Interspecific genetic distances (D values) in *Lateolabrax*

<table>
<thead>
<tr>
<th></th>
<th><em>L. japonicus</em></th>
<th><em>L. sp.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. japonicus</em></td>
<td>0.1754</td>
<td></td>
</tr>
<tr>
<td><em>L. sp.</em></td>
<td>0.3864</td>
<td>0.4304</td>
</tr>
</tbody>
</table>

Fig. 2. Dendrogram based on unbiased genetic distances between three species of *Lateolabrax*. Open bars indicate 95% confidence intervals.

that cluster from *L. latus* was about 0.44 (2.2 Ma) (Fig. 2).

**Discussion**

The *Lateolabrax* sp. population from Yentai examined in this study showed a different genetic composition in allelic frequencies from the *L. sp.* population examined by Yokogawa and Seki\(^3\), at the *GPI-2*\(^*\), *IDHP-1*\(^*\), *MPI-1*\(^*\) and *PGM*\(^*\) loci. The genetic distance between the two *L. sp.* populations, calculated from the 20 loci used by Yokogawa and Seki\(^3\), was 0.0233, a figure significant at the subspecific divergence level\(^6\).

*L. sp.* is widely distributed along the Chinese coast including Taiwan, reaching south to the borders of Vietnam, north to Korea\(^8-14\). Although the collecting sites of the specimens...
used by Yokogawa and Seki\(^3\) was unknown, it is reasonable to conclude that it was geographically distinct from that of the L. sp. examined here. Thus, some genetically-independent populations of L. sp. may exist along the Chinese coast. In fact, Yokogawa and Tajima\(^4\) reported that the Taiwanese form of L. sp. was somewhat divergent both morphologically and genetically from the Chinese form, which supports the above suggestion.

The *Lateolabrax japonicus* specimens examined in this study showed a similar genetic composition to those studied by Yokogawa and Seki\(^3\), the specimens in both studies having been obtained from the same area of the Harima Sea. The genetic distance between the two *L. japonicus* samples was 0.0027, a value slightly larger than the inter-population level of *L. japonicus* in the Harima Sea, according to Ohtani et al.\(^{15}\), but here regarded as being within the local population level for *L. japonicus*, except the Ariake Sea population, as summarized by Yokogawa et al.\(^{16}\).

The genetic relationships among the three *Lateolabrax* species obtained in this study suggested a specific divergent process as follows: The common ancestral species of the three present species existed before 3 Ma. *L. latus* diverged initially, at about 2.2 Ma (middle Pliocene). Thereafter, *L. japonicus* and L. sp. diverged at about 0.9 Ma (early Pleistocene) (Fig. 2).

The fact that *L. latus* is distributed only in coastal waters around southern Japan\(^{8,9,17}\) suggests that initial divergence occurred in Japanese waters. At that time (mid-Pliocene), it has been hypothesized that the Japan Sea was a semi-closed area, being strongly influenced by a cold current\(^{18,19}\), with, concurrently, a huge enclosed sea existing in the region of the present East China Sea\(^{20,21}\). The specific divergence of *L. latus* may have resulted from reproductive isolation in such enclosed waters.

Thereafter, in the early Pleistocene, the Korea Channel formed, subsequently closing and opening repeatedly in step with the eustacies by cyclic occurrence of glacial and inter-glacial epochs\(^{19,22,25}\). During this period, *L. japonicus* and L. sp. may have diverged owing to repeated isolation of populations. Nishimura\(^{23}\) hypothesized that some present marine species in the Japan Sea were established in this period following reproductive isolation caused by the closing of the Korea Channel.

During repeated diastrophisms, newly-divergent *L. japonicus* and L. sp. may have remained genetically mixed in some waters, now being represented by a relictual hybrid population. The Ariake form of *L. japonicus*, a specialized population\(^{16,26}\), can be regarded as an example of such. Further, relictual gene flow following hybridization may be evidenced by the presence in some young *L. japonicus* of black dots, being a typical morphological characteristic of L. sp.\(^{27}\).

Yokogawa\(^{28}\) summarized some ecological characteristics of the three *Lateolabrax* species, showing the general tendencies in each. *L. sp.* has a rather strong tolerance of fresh water, often entering rivers, sometimes as far as the upper zone. *L. latus*, on the other hand prefers coastal rocky areas bordering the open sea, and hardly enters rivers. *L. japonicus* appeared to be an intermediate between *L. latus* and L. sp.\.

A proposal that L. sp. is systematically closest to the common ancestral *Lateolabrax*, on the basis of its having the widest distribution of the present three species, leads to the suggestion that an ancestral population of L. sp. become removed from a less saline habitat to the open sea, subsequently giving rise to *L. latus*. Habitat changes can be postulated, since the fresh waters which were initially utilized diminished in area. In addition, during colder periods, fresh water avoidance, owing to the lower temperatures than in sea water, could also have been a factor. The earlier-mentioned hypothesis that *L. latus* was established in the Japan Sea, during colder Pliocene climates\(^{18,19}\) may be significant. A second specific divergence, that of *L. japonicus*, might be explained on the similar grounds.

On the other hand, if *L. latus* is the closest to the common ancestral species, divergence of populations, that had become isolated in fresh of estuarine waters may have resulted in subsequent speciation. At this time, however, in the absence of, for example, fossil evidence, further
speculation is unwarranted.

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References


スズキ属魚類の遺伝的分化

横川浩治