Molecular Phylogeny and Allozyme Variation of the Five Common Fish Species of the Suborder Percoidei

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Introduction

The fish fauna of Japanese waters is remarkably abundant and about 3,400 species have been ascertained by MASUDA *et al.* (1984). Under such species diversity, the taxonomic and phylogenetic studies have been extensively carried out by many workers from the morphological standpoint. However, many unresolved problems concerning the phylogeny and evolution of fish still remain unclear.

On the other hand, during the last about 20 years, the phylogenetic and evolutionary studies have been revitalized by the application of molecular techniques. Protein electrophoresis, protein sequencing and sequence analysis of mitochondrial DNA or ribosomal RNA are among the molecular methods used in evolutionary studies. Such molecular studies have made it possible for us to estimate the phylogenetic relationships among taxa and their evolutionary processes quantitatively with common parameters such as protein or DNA, and in some cases they have been providing much relevant and critical information about phylogeny in various groups of organisms (FERGUSON, 1980 ; HILLIS *et al.*, 1996). To clarify many unresolved problems about the phylogeny of the fishes, it would be desirable to introduce actively the molecular approaches which can provide the more analytical, quantitative and useful data into the field of the fish phylogeny and taxonomy.

The suborder Percoidei consist of many various families, and therefore it has been considered that the suborder is many phyletic group. However, the phylogenetic relationships among families remained unclear until now. Needless to say, the molecular phylogenetic study has not yet been carried out. In this study, the author adopted five common fish species belonging to the five different families : *Trachurus japonicus* of the Carangidae, *Ditrema temmincki* of the Embiotocidae, *Sillago japonica* of the Sillaginidae, *Arctoscopus japonicus* of the Trichodontidae and *Girella punctata* of the Girellidae belonging to the suborder Percoidei, and attempted to estimate their phylogenetic relationships at allozyme level. Previously, we repoted that allozyme electrophoresis is also effective on the systematics at family level (MATSUOKA, 1987 ; MATSUOKA and INAMORI, 1999 ; ASANUMA and MATSUOKA, 2002). In addition, NEI (1987) stated in his review that protein electrophoresis is almost equivalent to mtDNA analysis in resolving power. Therefore, the author adopted the allozyme analysis. In this paper, the author would like to report about the molecular phylogeny and population genetics of the above five species of the five familes of the suborder Percoidei.

Materials and Methods

Fish

The fishes examined in this study were five species of the suborder Percoidei from Japanes waters : *Trachurus*

japonicus, *Ditrema temmincki*, *Sillago japonica*, *Arctoscopus japonicus* and *Girella punctuata*. The number of individuals examined in each species and localites were as follows : *T. japonicus*, 6, Aomori Pref. ; *D. temmincki*, 6, Aomori Pref. ; *S. japonica*, 6, Shizuoka Pref. ; *A. japonicus*, 6, Aomori Pref. and *G. punctata*, 6, Fukui Pref. After collection, the whole bodies were stored at −45°C. After thawing at 4°C, the three tissues (muscule, liver and intestine) were cut off from these specimens and the crude extracts of these tissues were prepared before allozyme electrophoresis as described below.

Electrophoretic method

Electrophoresis was performed on 7.5% polyacrylamide gels as described previously (MATSUOKA, 1985) : About 0.2—0.3 g of muscle, liver or intestine was individually homogenized in 1 or 5 vols of 20 mM phosphate buffer, pH 7.0, containing 0.1 M KCl and 20 mM EDTA, by using a small polyethylene homogenizer of the Potter-Elvehjem type in an ice water bath. After centrifugation at 10,000 rpm for 5 min, 0.05—0.1 ml of clear supernatant was used for electrophoretic analysis of enzymes. Electrode buffer was 0.38 M Glycine-tris buffer, pH 8.3. After electrophoresis, the gels were stained for the following 11 different enzymes : glucose-6-phosphate dehydrogenase (G6PD), lactate dehydrogense (LDH), malate dehydrogenase (MDH), malic enzyme(ME), nothing dehydrogenase (NDH), superoxide dismutase (SOD), aspartate amino transferase (AAT), peroxidase (PO), alkaline phosaphatase (ALK), esterase (EST) and cytosol aminopeptidase (CAP). Stain recipes for these enzymes have been described previously (MATSUOKA and HATANAKA, 1991).

Results and Discussion

Protein polymorphism

Nineteen loci were inferred from the allozyme variation in the 11 enzymes. Of 19 genetic loci, eight loci (*MDH-1*, *MDH-2*, *EST-1*, *EST-4*, *EST-5*, *CAP-1*, CAP-2 and *CAP-3*) were polymorphic. In general, the enzymes such as EST and CAP were also highly polymorhic in echinoderms or insects studied in our laboratory (*eg.*, MATSUOKA and INAMORI, 1996 ; MATSUOKA *et al.*, 1998 ; MATSUOKA and Asano, 1998 ; MATSUOKA and HATANAKA, 1991, 1998). Other 11 loci were monophoric. Table 1 summarizes the degree of genetic variation in five species. The number of alleles per locus was in the range of 1.06—1.11, with a mean of 1.11. The proportion of polymorphic loci (P) is in the range of 5.9—15.8%, with a mean of 10.5%. The expected average heterozygosity per locus (H) is in the range of 1.6—7.9%, with a mean of 4.5%. Gyllensten (1985) reported that the mean H value in various fish species was about 5%. More recently, we also reported that the mean H value was 6.1% in seven species of the order Clupeiformes (ASANUMA and MATSUOKA, 2002), and it was 4.3% in four species of the family Hexagrammidae (MATSUOKA *et al.*, 2000). The present H values (4.5%) were comparable to those in many other fish species.

With respect to the relationship between enzyme function and heterozygosity, Yamazaki (1977) and Gojobori (1982) showed that the substrate-specific enzymes with functional constraints have lower heterozygosity than the nonspecific enzymes. These hold true for the fish enzymes here studied ; the non-glucose metabolizing enzymes (the mean H=5.5%) including nonspecific enzymes were more variable than the glucose-metabolizing enzymes (the mean H=3.1%) with functional constraints. Previously, similar result was observed in seven fish species of the order Clupeiformes (ASANUMA and MATSUOKA, 2002). Furthermore, the similar phenomena were also found in our previous allozyme studies of echinoderms (*eg.*, MATSUOKA and HATANAKA, 1991). These results can be explained by the neutral theory of Kimura (1983) : The glucose-metabolizing enzymes have functional importance, and therefore functional constraint of these enzymes is much stronger than that of other non-glucose metabolizing enzymes. The more strictly functional constraint decrease neutral regions of the molecules, and thus the probability of neutral mutation would be smaller for the glucose metabolizing-enzymes than other non glucose-metabolizing enzymes.

Table 1. Genetic variation in the five fish species of the suborder Percoidei

Parameter	*Aj*	*Sj*	*Tj*	*Dt*	*Gp*
1. No. of alleles per locus : *A*	1.06	1.16	1.09	1.11	1.11
2. Proportion of polymorphic loci : *P* (%)	5.9	15.8	9.1	10.5	11.1
3. Expected average heterozygosity per locus : *H* (%)	1.6	7.9	3.7	5.3	4.0

Aj=*Arctoscopus japonicus*, *Sj*=*Sillago japonica*, *Tj*=*Trachurus japonicus*, *Dt*=*Ditrema temmincki*, *Gp*=*Girella punctata*.

Table 2. Genetic identities (above diagonal) and genetic distances (below diagonal) between five fish species of the suborder Percoidei

Species	1	2	3	4	5
1. *Arctoscopus japonicus*	—	0.445	0.224	0.412	0.316
2. *Sillago japonica*	0.810	—	0.259	0.360	0.442
3. *Trachurus japonicus*	1.496	1.352	—	0.523	0.230
4. *Ditrema temmincki*	0.888	1.021	0.648	—	0.264
5. *Girella punctata*	1.153	0.816	1.469	1.334	—

Genetic identity (*I*) and genetic distance (*D*) were calculated by the method of NEI (1972).

Phylogenetic and evolutionary relationships

In order to quantify the degree of genetic differentiation between five species, we calculated the genetic identity (*I*) and genetic distance (*D*) between each species by the method of NEI (1972). Table 2 shows the matrices of *I* and *D* values between all pairs of five species. The lowest *D* value (*D*=0.648) was found between *T. japonicus* of the family Carangidae and *D. temmincki* of the family Embiotocidae. On the other hand, the highest *D* value (*D*=1.496) was found between *A. japonicus* and *T. japonicus*. These values are comparable to those observed in different families of many other animal groups (Thorpe, 1982). Namely, the present molecular data are well consistent with their taxonomic position based on morphological criteria. To clarify their phylogenetic relationships, the molecular phylogenetic tree was constructed from the NEI's genetic distance matrix of Table 2 by using the UPGMA clustering method of Sneath and Sokal (1973). The divergence time inferred from the NEI's equation (NEI, 1975) using genetic distance is also given in the molecular phylogenetic tree. The phylogenetic tree (Fig. 1) indicated the followings : (1) The five fish species of five different families are divided in two large clusters : one consists of *T. japonicus* and *D. temmincki*, and the other *A. japonicus*, *S. japonica* and *G. punctata*. (2) *T. japonicus* and *D. temmincki* are most closely related to each other among the five species and diverged later (about 3 million years ago : 3MY)). (3) In second cluster, *A. japonicus* and *S. japonica* are much closely related to each other and diverged in about 4MY. (4) *G. punctata* is more genetically differentiated from other four species. It suggests that *G. punctata* might be one of the primitive fish.

The morphological data for speculating their phylogenetic relationships is much scanty, since they belong to the different families in taxonomy, and thus they have not almost common characters. In such cases, the molecular approach would provide useful information for their phylogeny as shown in ASANUMA and MATSUOKA (2002). The molecular phylogenetic tree demonstrated the affinity between *T. japonica* and *D. temmincki*, and that among the other three species. This is well consistent with the morphological similarity of the tail fin described in MASUDA *et al.* (1988). Further, the close relation between *A. japonicus* and *S. japonica* agrees with their similar body shape of slender type. Of many members of the suborder Percoidei, *D. temmincki* is much specialized fish : the species is ovoviviparity and produces about 10—15 young fish in each year. This suggests that the species is highly evolved fish and would be differentiated from other species in more recent times as shown by the present molecular study. In future, this study would provide some useful data for the systematics of the suborder Percoidei.

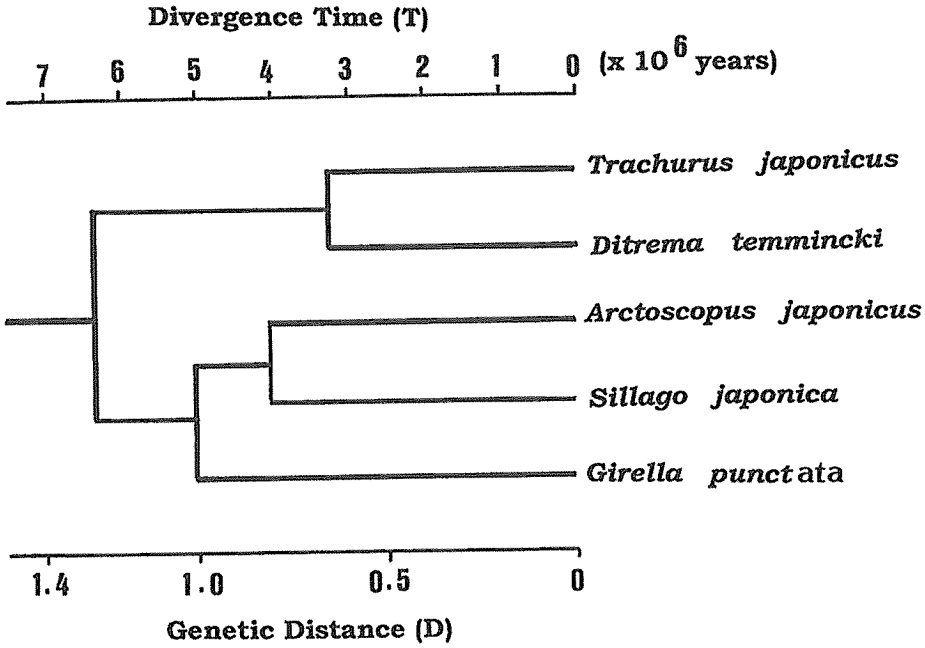

Fig. 1. Molecular phylogenetic tree for the five common species of the suborder Percoidei. It was constructed from the NEI's genetic distance by using the UPGMA clustering method. The divergence time estimated from the NEI's equation using the genetic distance is also given in the phylogenetic tree.

In recent years, many workers have adopted mitochondrial DNA (mtDNA) as the molecular marker for investigating the evolutionary and phylogenetic study. The molecular marker of mtDNA was developed by A. C. Willson at California University. However, NEI (1987) showed that the resolving power of mtDNA is not necessarily higher than that of allozyme study. According to the estimation of NEI (1987), allozyme study is expected to survey about 100 nucleotides per one genetic locus. As we usually examined about 30 loci in allozyme study, it is equivalent to studing 3,000 base pairs at mtDNA level. Therefore, the resoloving power of allozyme study is not lower than mtDNA analysis. Murphy *et al.* (1996) claimed that in phylogenetic and evolutionary studies many molecular characters should be used and that the enzyme loci are the important molecular characters in allozyme study. Needless to say, the number of molecular characters adopted in protein electrophoresis is more enough than that of mtDNA study. In future, the author is planning to study the phylogenetic relationships among many and various families of the suborder Percoidei at molecular level.

Summary

The phylogenetic relationships and allozyme variation were studied in the five common fish species (*Trachurus japonicus*, *Ditrema temmincki*, *Sillago japonica*, *Arctoscopus japonicus* and *Girella punctata*) belonging to the five different families of the suborder Percoidei from Japanese waters by allozyme study of 11 enzymes. From the allozyme variation observed in 29 genetic loci scored, it was calculated that average heterozygosities (H values) are in the range of 1.6—7.9%, with a mean of 4.5%. The H values were comparable to those observed in other fish species. The NEI's genetic distances (mean $D=1.10$) between five species of the suborder Percoidei were comparable to those observed between different families in other animal groups. The molecular phylogenetic tree for the five species of the five different families which was constructed from NEI's genetic distances indicated the followings : The five species of the suborder Percoidei were divided into two large clusters : one consists of *T. japonicus* and *D. temmincki*, and the other *A. japonicus*, *S. japonica* and *G. punctata*. Among five species, *T. japonicus* and *D. temmincki* are most closely related to each other and diverged later (about 3MY). In second cluster, *A. japonicus* and *S. japonica* are closely related to each other and diverged in about 4MY. *Girella punctata*

is more genetically differentiated from other four species and might be primitive type. These molecular results were compared with non-molecular evidence.

References

ASANUMA, T. and MATSUOKA, N. (2002) Phylogenetic relationships among seven fish species of the order Clupeiformes inferred from allozyme variation. *Bull. Fac. Agric. & Life Sci., Hirosaki Univ.*, 4 : 1-15 (In Japanese).

FERGUSON, A. (1980) *Biochemical Systematics and Evolution*. Blackie, Glasgow.

GOJOBORI, T. (1982) Means and variances of heterozygosity and protein function. In : *Molecular Evolution, Protein Polymorphism and the Neutral Theory* (Edited by Kimura, M), pp. 137-148. Japan Scientific Societies Press, Springer-Verlag, Berlin.

GYLLENSTEN, U. (1985) The genetic structure of fish : Differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous, and freshwater species. *J. Fish Biol.*, 26 : 691-699.

HILLIS, D. M., MORITZ, C. and MABLE, B. K. (1996) *Molecular Systematics*. Sinauer, MA.

KIMURA, M. (1983) *The Neutral Theory of Molecular Evolution*. Cambridge University Press, Cambridge.

MASUDA, H., AMAOKA, K., ARAGA, C., UENO, T. and YOSHINO, T. (1984) *The Fishes of the Japanese Archipelago*. Tokai University Press, Tokyo (In Japanese).

MATSUOKA, N. (1985) Biochemical phylogeny of the sea-urchins of the family Toxopneustidae. *Comp. Biochem. Physiol.*, 80B : 767-771.

MATSUOKA, N. (1987) Biochemical study on the taxonomic situation of the sea-urchin, *Pseudocentrotus depressus*. *Zool. Sci.*, 4 : 339-347.

MATSUOKA, N. and ASANO, F. (1998) Molecular taxonomic study on the variation of body color in the starfish *Asterina pectinifera*. *Bull. Fac. Agric. & Life Sci., Hirosaki Univ.*, 1 : 1-8.

MATSUOKA, N. and HATANAKA, T. (1991) Molecular evidence for the existence of four sibling species within the sea-urchin, *Echinometra mathaei* in Japanese waters and their evolutionary relationships. *Zool. Sci.*, 8 : 121-133.

MATSUOKA, N. and HATANAKA, T. (1998) Genetic differentiation among local Japanese populations of the starfish *Asterias amurensis* inferred from allozyme variation. *Genes Genet. Syst.*, 73 : 59-64.

MATSUOKA, N. and INAMORI, M. (1996) Phylogenetic relationships of echinoids of the family Temnopleuridae inferred from allozyme variation. *Genes Genet. Syst.*, 71 : 203-209.

MATSUOKA, N. and INAMORI, M. (1999) Phylogenetic relationships among four echinoids of the family Cidaridae (Cidaroida) based on allozymes. *Zool. Sci.*, 16 : 529-534.

MATSUOKA, N., HOSOYA, T., HAMAYA, T. and ABE, A. (1998) Phylogenetic relationships among four species of stag-beetles (Coleoptera : Lucanidae) based on allozymes. *Comp. Biochem. Physiol.*, 119B : 401-406.

MATSUOKA, N. and ASANUMA, T. (2000) Phylogenetic relationships among four fish species of the family Hexagrammidae from Japanese waters based on allozymes. *Bull. Fac. Agric. & Life Sci., Hirosaki Univ.*, 3 : 5-13.

MURPHY, R. W., SITES, J. W., BUTH, D. G. and HAUFLER, C. H. (1996) Proteins : Isozyme Electrophoresis. In *Molecular Systematics* (Edited by Hillis, D. M., Moritz, C. and Mable, B. K.), pp. 51-120. Sinaur Assoc. MA.

NEI, M. (1972) Genetic distance between populations. *Am. Nat.*, 106 : 283-292.

NEI, M. (1975) *Molecular Population Genetics and Evolution*. North-Holland, Amsterdam.

NEI, M. (1987) *Molecular Evolutionary Genetics*. Columbia University Press, NY.

SNEATH, P. H. A. and SOKAL, P. R. (1973) *Numerical Taxonomy*. Freeman, San Francisco, CA.

THORPE, J. P. (1982) The molecular clock hypothesis : Biochemical evolution, genetic differentiation, and systematics. *Ann. Rev. Ecol. Syst.*, 13 : 139-168.

YAMAZAKI, T. (1977) Enzyme polymorphism and functional difference : mean, variance, and distribution of heterozygosity. In : *Molecular Evolution and Polymorphism* (Edited by Kimura, M.), pp. 127-147, Mishima : National Institute of Genetics, Mishima.

日本産スズキ亜目魚類5種の分子系統学的研究

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硬骨魚類の中で，最も大きく多様な分類群であるスズキ亜目の科の間の系統進化学的関係は未だ不明のままである。それは，科が異なるため，分類群間で共有する形態形質が極めて乏しいからである。そのような場合には，分子的研究が有力である。本研究では，日本近海のスズキ亜目の普通種である5科5種(マアジ，ウミタナゴ，シロギス，ハタハタ，メジナ)の系統類縁関係と，それらの集団内に存在するタンパク多型現象をアロザイム分析により調査した。その結果，11酵素29遺伝子座が検出され，1遺伝子座あたりの対立遺伝子数は，1.0から1.1，多型的遺伝子座の割合は，5.9%から15.8%，平均ヘテロ接合体率は，1.6%から7.9%を示した。これらの値は，他の硬骨魚類で報告されている値と同程度のものであった。Nei(1972)の遺伝的距離からスズキ亜目5科5種の分子系統樹をUPGMA法により作成した。その結果，5種は大きく2つのクラスターに分かれた。1つのクラスターは，マアジとウミタナゴで，他のクタスターは，シロギス，ハタハタ，メジナであった。5種の中で，最も近縁関係にあるのは，マアジとウミタナゴであり，次に近縁なのはシロギスとハタハタであった。一方，5種の中で最も遺伝的に分化した種はメジナであり，この種は祖先タイプの種であると推察された。これら5種の系統類縁関係は，鰭の形態とよく一致し，また，スズキ亜目では極めて稀な卵胎生であるウミタナゴが，比較的近年に種分化したことも分子系統樹から示唆された。

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