Bulletin of the Japanese Society of Scientific Fisheries 45(9) 1169-1178 (1979)

Genetic Variation in Fish Populations*1

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(Received April 20, 1979)

Starch gel electrophoresis was carried out to survey the genetic variation among species of fish as a foundation for more extensive and detail studies. Variation in average level of genetic variation among taxonomic groups of fish was observed. Estimate for the fish as whole was 0.194±0.023 in the proportion of polymorphic loci and 0.059±0.007 in the mean individual heterozygosity.

The correlation of the proportion of polymorphic loci with the mean individual heterozygosity was observed and the relationship was found to be linear. The calculated regression line was near to the theroretical relationship on the basis of the neutral theory of protein polymorphisms. Thus, it may be concluded that variation in average level of heterozygosity among taxonomic groups of fish reflected difference in effective population size.

LEWONTIN and HUBBY[1] have developed and begun to use a method for the basic description of genetic variation in populations, in which they estimated directly genetic heterozygosity from surveys of electrophoretically demonstrable variation in proteins for *Drosophila pseudobscura*. Since then, a large number of electrophoretical surveys on protein polymorphisms have been done in many different species. These results lead to the conclusion that natural populations contain a large amount of genetic variability, though there are some exceptions. Furthermore, it is apparent that the average heterozygosity varies considerably with organisms. Two major hypotheses have been advanced to explain variation in average level of heterozygosity among species of organisms. In neutral theory[2,3], the interpretation is that it reflects differences in effective population size, more variable organisms having larger populations.

On the other hand, SELANDER and KAUFMAN[4] attempted to account for variation in heterozygosity in terms of a strategy for increasing population fitness in a temporally and spatially heterogeneous habitat. According to this hypothesis, heterozygosity is lower in large, mobile animals (most vertebrates) than in small immobile animals (invertebrates).

Marine fishes are well suited for study concerning population structure, ecological interaction, and effects of man-induced alteration of the environment. The purposes in the present work are to survey the variation among species of marine fish as a foundation for more extensive and detail studies, and to explain variation in average level of heterozygosity among taxonomic groups of fish.

Materials and Methods

The 41 species were collected from areas in off Hokkaido, Aomori, and Miyagi as shown in Table 1. The most of species were collected from areas in Sendai Bay. All fish samples were frozen at −20°C until tested. Tissue extraction and starch gel electrophoresis were carried out by the methods previously reported[5]. The 15 enzymes, lactate dehydrogenase (LDH), malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), alpha-glycerophosphate dehydrogenase (αGPD), alcohol dehydrogenase (ADH), sorbitol dehydrogenase (SDH), 6-phosphogluconate dehydrogenase (6PGD), glutamate dehydrogenase (GDH), acid phosphatase (ACP), esterase (EST), phosphoglucomutase (PGM), superoxide dismutase (SOD), asparate aminotransferase (AAT), adenylate kinase (AK), and glucosephosphate isomerase (GPI) were examined.

Results

Fifteen enzymes were studied in the 41 species of marine fish. The electrophoretic patterns of

*1 Supported in part by the Grant from the Ministry of Education, the Goverment of Japan.

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Table 1. Collection information on fish used in this work

Species	Date	No. of Samples	Location
Order Clupeida			
Clupea pallasi	11 / 6 / 78	50	off Abashiri
Sardinops melanosticta	10 / 18 / 78	30	Sendai Bay
Engraulis japonica	8 / 30 / 78	30	Sendai Bay
Order Belonida			
Cololabis saira	9 / 10 / 78	27	south off Uruppu Is.
Hemiramphus sajori	10 / 25 / 78	12	Obuchinuma
Order Percida			
Scomber japonicus	5 / 27 / 75	39	Sendai Bay
Trachurus japonicus	9 / 29 / 78	15	Sendai Bay
Lateolabrax japonicus	5 / 6 / 77	15	Sendai Bay
Nibea mitsukurii	8 / 13 / 76	15	Sendai Bay
Ammodytes personatus	4 / 27 / 77	30	Sendai Bay
Order Tetraodontida			
Navodon modestus	7 / 20 / 78	30	Sendai Bay
Order Cottida			
Sebastes inermis	6 / 21 / 77	30	Sendai Bay
Sebastes thompsoni	6 / 21 / 77	16	Sendai Bay
Helicolenus hilgendorfi	9 / 24 / 77	15	off kinkazan
Sebastolobus macrochir	5 / 6 / 77	30	Sendai Bay
Agrammus agrammus	5 / 8 / 76	13	Onagawa Bay
Pleurogrammus azonus	11 / 6 / 78	40	off Tomakomai
Hexagrammos otakii	5 / 8 / 76	38	Onagawa Bay
Hemitripterus villosus	5 / 14 / 75	12	Sendai Bay
Chelidonichthys kumu	12 / 17 / 76	28	Sendai Bay
Lepidotrigla microptera	12 / 17 / 76	13	Sendai Bay
Lipalis tanakai	10 / 9 / 76	15	Sendai Bay
Order Pleuronectida			
Paralichthys olivaceus	3 / 12 / 76	12	*Sendai Bay*
Cleisthenes pinetorum herzensteini	5 / 14 / 75	13	Sendai Bay
Eopsetta grigorjewi	6 / 20 / 75	18	Sendai Bay
Verasper variegatus	3 / 12 / 76	15	Sendai Bay
Pleuronichthys cornutus	6 / 4 / 76	19	Sendai Bay
Limanda yokohamae	9 / 26 / 75	21	Sendai Bay
Limanda herzensteini	9 / 26 / 75	27	Sendai Bay
Dexistes rikuzensteini	6 / 29 / 77	30	Sendai Bay
Platichthys stellatus	6 / 12 / 75	48	Sendai Bay
Kareius bicoloratus	10 / 3 / 75	29	Sendai Bay
Clidoderma asperrimum	6 / 20 / 75	12	Sendai Bay
Tanakius kitaharai	5 / 14 / 75	10	Sendai Bay
Microstomus achne	5 / 30 / 75	26	Sendai Bay
Rhinoplagusia japonica	5 / 28 / 76	10	Sendai Bay
Areliscus joyneri	8 / 13 / 76	47	Sendai Bay
Order Gadida			
Lotella maximowiczi	5 / 28 / 76	15	Sendai Bay
Gadus macrocephalus	5 / 28 / 76	5	Sendai Bay
Theragra chalcogramma	6 / 16 / 76	10	off Kinkazan
Order Lophiida			
Lophius litulor	4 / 24 / 76	15	Sendai Bay

all enzymes observed were consistent with patterns and interpretation of the respective enzyme reported in the other species. The results are summarized in Table 2. All enzymes studied showed polymorphism in more than three species.

LDH is a tetrameric molecule and two separate loci which code for A and B-subunits are found in fish. A and B-subunits indiscriminatively as-

Table 2 (1). Summary of the results of electrophoretic analysis of fish

Species	No. of tested	Enzyme														
		LDH	MDH	IDH	αGPD	ADH	SDH	6PGD	GDH	ACP	EST	SOD	PGM	AAT	AK	GPI
Order Clupeida																
Clupea pallasi	50	2M	P*+M	P+M	2M	P	2M	M	—	M	—	M	P	M	—	P+V
		(2)	(2)	(2)	(2)	(1)	(2)	(1)		(1)		(1)	(1)	(1)		(1)
Sardinops melanosticta	30	2M	2M	2M	2M	V	2M	—	—	P*	3P	2M	M	P+2M	—	2M
		(2)	(2)	(2)	(2)		(2)			(1)	(3)	(2)	(1)	(3)		(2)
Engraulis japonica	30	P*+M	2M	P*+M	P*+M	M	NA	P	P	M	3P+M	M	M	2M	M	M+V
		(2)	(2)	(2)	(2)	(1)		(1)	(1)	(1)	(4)	(1)	(1)	(2)	(1)	(1)
Oder Belonida																
Cololabis saira	27	2M	P+M	2P	P+M	P	NA	P	M	P	P	2M	M	2M	M	P
		(2)	(2)	(2)	(2)	(1)		(1)	(1)	(1)	(1)	(2)	(1)	(2)	(1)	(1)
Hemiramphus sajori	12	P+M	2M	2M	2M	M	4M	P*	—	P	P	M	M	P	—	2M
		(2)	(2)	(2)	(2)	(1)	(4)	(1)		(1)	(1)	(1)	(1)	(1)		(2)
Oder Percida																
Scomber japonicus	39	2M	2M	P*+M	M+NA	—	M	P	—	P	P+M	P	P*	M	M	P
		(2)	(2)	(2)	(1)		(1)	(1)		(1)	(2)	(1)	(1)	(1)	(1)	(1)
Trachurus japonicus	15	2M	2M	P*+M	2M	V	P*+M	P	V	P	P+M	M	M	P*	M	P+M
		(2)	(2)	(2)	(2)		(2)	(1)		(1)	(2)	(1)	(1)	(1)	(1)	(2)
Lateolabrax japonicus	15	P+M	P+M	2M	P+M	—	2P+M	—	M	—	3m	M	P	—	—	P+M
		(2)	(2)	(2)	(2)		(3)		(1)		(3)	(1)	(1)			(2)
Nibea mitsukurii	15	2M	2M	P*+M	M+V	V	2M	—	V	—	2M	M	P*	—	—	P+M
		(2)	(2)	(2)	(1)		(2)				(2)	(1)	(1)			(2)
Ammodytes personatus	30	2M	2M	P+M	P+M	—	—	—	M	P*	P	P	P*	—	—	P*+M
		(2)	(2)	(2)	(2)				(1)	(1)	(1)	(1)	(1)			(2)
Order Tetraodontida																
Navodon modestus	30	M	2M	2M	P*+M	M	2M	P*	M	M	P+P*+4M	P*+M	M	—	M	M
		(1)	(2)	(2)	(2)	(1)	(2)	(1)	(1)	(1)	(6)	(2)	(1)		(1)	(1)

M=monomorphic; P=polymorphic; P*=variant allele less than 0.05; V=variable, no single genetic explanation; NA=no activity detected; —=not tested; ( )=number of loci.

Table 2 (2). Summary of the results of electrophoretic analysis of fish

Species	No. of tested	Enzyme														
		LDH	MDH	IDH	αGPD	ADH	SDH	6PGD	GDH	ACP	EST	SOD	PGM	AAT	AK	GPI
Order Cottida																
Sebastes inermis	30	M	2M	P*+M	2M	M	P*+M	—	—	M	M	M	M	—	—	P+M
		(1)	(2)	(2)	(2)	(1)	(2)			(1)	(1)	(1)	(1)			(2)
Sebastes thomsoni	16	M	2M	2M	2M	M	3M	—	M	M	M	M	M	—	—	P*+M
		(1)	(2)	(2)	(2)	(1)	(3)		(1)	(1)	(1)	(1)	(1)			(2)
Helicolenus hilgendorfi	15	M	2M	P+M	P+M	—	M	—	—	M	3M	M	P	—	—	2M
		(1)	(2)	(2)	(2)		(1)			(1)	(3)	(1)	(1)			(2)
Sebastolobus macrochir	30	M	P+M	P+M	P*+NA	P*	M	—	—	M	3M	M	P	—	—	2M
		(1)	(2)	(2)	(1)	(1)	(1)			(1)	(3)	(1)	(1)			(2)
Agrammus agrammus	13	M	2M	2M	P+NA	M	M	M	M	M	V	M	P	—	—	M
		(1)	(2)	(2)	(1)	(1)	(1)	(1)	(1)	(1)		(1)	(1)			(1)
Pleurogrammus azonus	40	P	P*+M	2M	2M	2M	2M	M	—	M	V	M	P	P*	—	2M
		(1)	(2)	(2)	(2)	(2)	(2)	(1)		(1)		(1)	(1)	(1)		(2)
Hexagrammos otakii	38	M	2M	2M	2M	P*	3M	M	V	M	3M	M	M	—	M	2M
		(1)	(2)	(2)	(2)	(1)	(3)	(1)		(1)	(3)	(1)	(1)		(1)	(2)
Hemitripterus villosus	12	M	2M	2M	2M	—	2M	—	M	M	P+3M	M	M	M	—	M
		(1)	(2)	(2)	(2)		(2)		(1)	(1)	(4)	(1)	(1)	(1)		(1)
Chelidonichthys kumu	28	M	P*+M	2M	P*+M	—	M	M	—	—	2M	P	M	—	—	P*
		(1)	(2)	(2)	(2)		(1)	(1)			(2)	(1)	(1)			(1)
Lepidotrigla microptera	13	M	P*+M	P*+M	P*+M	P	2M	—	—	—	P+2M	M	P*	—	—	P*+M
		(1)	(2)	(2)	(2)	(1)	(2)				(3)	(1)	(1)			(2)
Liparis tanakai	15	M	2M	2M	2M	2M	3M	—	—	—	5M	M	2M	—	—	2M
		(1)	(2)	(2)	(2)	(2)	(3)				(5)	(1)	(2)			(2)

Table 2 (3). Summary of the results of electrophoretic analysis of fish

Species	No. of tested	Enzyme														
		LDH	MDH	IDH	αGPH	ADH	SDH	6PGD	GDH	ACP	EST	SOD	PGM	AAT	AK	GPI
Order Pleuronectida																
Paralichthys olivaceus	12	M	2M	P+M	2M	M	P+M	M	P	M	2M	2M	P	—	M	M
		(1)	(2)	(2)	(2)	(1)	(2)	(1)	(1)	(1)	(2)	(2)	(1)		(1)	(1)
Cleisthenes pinetorum herzensteini	13	M	2M	P+M	P+P*	M	2M	M	P	M	P+2M	2M	P	P+M	M	M
		(1)	(2)	(2)	(2)	(1)	(2)	(1)	(1)	(1)	(3)	(1)	(1)	(2)	(1)	(1)
Eopsetta grigorjewi	18	M	P*+M	2M	2M	P	2M	P*	M	M	3M	2M	M	M	M	M
		(1)	(2)	(2)	(2)	(1)	(2)	(1)	(1)	(1)	(3)	(2)	(1)	(1)	(1)	(1)
Verasper variegatus	15	M	2M	2M	2M	M	2M	M	P	P	P+2M	2M	M	2M	M	P*
		(1)	(2)	(2)	(2)	(1)	(2)	(1)	(1)	(1)	(3)	(2)	(1)	(2)	(1)	(1)
Pleuronichthys cornutus	19	M	P+M	P*+M	2P	P	P+M	P*	P	M	2P+M	2M	P	P+M	P*	P
		(1)	(2)	(2)	(2)	(1)	(2)	(1)	(1)	(1)	(3)	(2)	(1)	(2)	(1)	(1)
Limanda yokohamae	21	M	P*+M	P+M	2M	P	2M	M	P	P	3M	2M	M	2M	M	P
		(1)	(2)	(2)	(2)	(1)	(1)	(1)	(1)	(1)	(3)	(2)	(1)	(2)	(1)	(1)
Limanda herzensteini	27	M	P*+M	P+P*	P+P*	P	2M	M	P	P	P+2M	2M	P	2M	M	P
		(1)	(2)	(2)	(2)	(1)	(2)	(1)	(1)	(1)	(3)	(2)	(1)	(2)	(1)	(1)
Dexistes rikuzenius	30	M	2M	2P*	2M	—	P*M	—	M	M	MW	M	P	—	—	M
		(1)	(2)	(2)	(2)		(2)		(1)	(1)	(1)	(1)	(1)			(1)
Platichthys stellatus	48	M	P+M	P+M	2P	P*	P+M	P	P	P	2P+M	P+M	P	P	P	M
		(1)	(2)	(2)	(2)	(1)	(2)	(1)	(1)	(1)	(3)	(2)	(1)	(1)	(1)	(1)
Kareius bicoloratus	29	M	2M	P+M	2M	P	2M	M	P	P	P+2M	2M	P	P	M	M
		(1)	(2)	(2)	(2)	(1)	(2)	(1)	(1)	(1)	(3)	(2)	(1)	(1)	(1)	(1)
Clidoderma asperrimum	12	M	2M	2P	2M	P	2M	P	P	M	3M	2M	M	P+M	M	M
		(1)	(2)	(2)	(2)	(1)	(2)	(1)	(1)	(1)	(3)	(2)	(1)	(2)	(1)	(1)
Tanakius kitaharai	10	M	2M	2M	P+M	M	2M	M	P	M	3M	2M	P	P+M	M	M
		(1)	(2)	(2)	(2)	(1)	(2)	(1)	(1)	(1)	(3)	(2)	(1)	(2)	(1)	(1)
Microstomus achne	26	M	2M	2P	2P*	P	2M	M	P	P	2M	2M	P	M	P	M
		(1)	(2)	(2)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(2)	(1)	(1)	(1)	(1)
Rhinoplagusia japonica	10	P	2M	2M	2P	M	P+M	P	P	M	3M	P+M	2P	—	M	—
		(1)	(2)	(2)	(2)	(1)	(2)	(1)	(1)	(1)	(3)	(2)	(2)		(1)	
Areliscus joyneri	47	P*	2M	P*+M	2P	P	2M	P	M	M	2P	2M	P	—	P	P
		(1)	(2)	(2)	(2)	(1)	(2)	(1)	(1)	(1)	(2)	(2)	(1)		(1)	(1)

Table 2 (4). Summary of the results of electrophoretic analysis of fish

Species	No. of tested	Enzyme														
		LDH	MDH	IDH	αGPD	ADH	SDH	6PGD	GDH	ACP	EST	SOD	PGM	AAT	AK	GPI
Order Gadida																
Lotella maximowiczi	15	2M	2M	2M	2M	V	2M	—	V	M	M	M	M	—	—	P*
		(2)	(2)	(2)	(2)		(2)			(1)	(1)	(1)	(1)			(1)
Gadus macrocephalus	5	2M	2M	2M	2M	P	2M	—	M	—	—	M	M	—	—	P
		(2)	(2)	(2)	(2)	(1)	(2)		(1)			(1)	(1)			(1)
Theragra chalcogramma	10	2M	2M	2M	P+M	M	2M	—	M	—	M	M	M	—	—	2M
		(2)	(2)	(2)	(2)	(1)	(2)		(1)		(1)	(1)	(1)			(2)
Order Lophiida																
Lophius litulor	15	2M	2M	M+NA	M+NA	M	2M	—	—	M	2M	M	M	—	—	M
		(2)	(2)	(1)	(1)	(1)	(2)			(1)	(2)	(1)	(1)			(1)
Total number of loci		55	82	81	77	34	77	25	25	35	94	58	43	31	20	55
Number of polymorphic loci		4	5	16	17	12	7	8	14	8	24	5	18	8	3	12
Proportion of polymorphic loci		0.073	0.061	0.196	0.221	0.382	0.091	0.320	0.560	0.229	0.255	0.086	0.419	0.258	0.150	0.218

sociate and form five tetrameric molecule forms (A_4, A_3B_1, A_2B_2, A_1B_3, and B_4), which can be visualized as five distinct bands by electrophoresis. This typical pattern was observed in members of the orders, Clupeida, Belonida, Percida, Gadida, and Lophiida. More activity of A-subunits than B-subunits was observed in skeletal muscles and more activity of B-subunits than A-subunits was observed in heart muscles. Among fish studied, members of the orders, Tetraodonida, Cottida, and Pleuronectida showed that there was only a single gene locus which coded for LDH subunits in somatic tissue. It revealed that duplication of a gene locus for LDH subunits had apparently not occurred in this groups, though in most vertebrates with two separate gene loci for LDH the gene duplication was considered to occur at the very beginning of vertebrate evolution.

Four species of fish were polymorphic for LDH; *Hemiramphus sajori*, *Lateolabrax japonicus*, *Pleurogrammus azonus*, and *Rhinoplagusia japonica*, and two species showed a few variants; *Engraulis japonica* and *Areliscus joyneri*.

MDH showed monomorphic three-banded phenotypes in 29 species, suggesting that two fixed loci coding for MDH were present and that random association of subunits was occurring. Considerable variation was seen in five species, *Cololabis saira*, *Lateolabrax japonicus*, *Sebastolobus macrochir*, *Pleuronichthys cornutus*, and *Platichthys stellatus*, and a few variants were observed in 7 species.

IDH appeared as two bands on gels in monomorphic 17 species, suggesting that two fixed loci coding for IDH were present and that one of them acted mainly in liver and the other in muscle. *Lophius litulor* showed a single band in liver but not the activity in muscle. Heterozygous individuals showed three banded phenotype and homozygotes single banded phenotype, indicating a probable dimeric structure of the enzyme. Polymorphism was seen at one or two loci in 13 species and a few variants at one or two loci were found in 10 species.

αGPD showed a similar expression to IDH. However, no or less activity was observed in liver of *Scomber japonica*, *Sebastolobus macrochir*, and *Lophius litulor*, and in muscle of *Agrammus agrammus*. Thirteen species of fish were polymorphic for αGPD and 6 species showed a few variants.

ADH was examined in liver and observed as a single band in monomorphic fish. Heterozygous individuals had three bands in most of fish, indi-

cating dimeric structure of the enzyme, but two bands in *Hexagrammos otakii* and *Gadus macrocephalus*, indicating monomeric structure of the enzyme. Polymorphism was observed in 12 of the 34 species examined and a few variants in 3 species. However, 4 species showed the ADH pattern which varied between individuals. The pattern was difficult to interpret because of inconsistency between the observed patterns and the expected pattern from theory such as monomer and dimer.

SDH in liver and muscle was observed as a single band, respectively, in most of monomorphic fish. Heterozygous individuals had three or five bands and homozygotes had one band, indicating dimeric or tetrameric structure of the enzyme. Five species were polymorphic for SDH. A monomorphic two-banded phenotypes were found in liver and muscle of *Hemiramphus sajori*, respectively. It suggests that gene duplication has occurred in this species at the two loci. Similar gene duplication was found in the locus coded for liver specific SDH in *Lateolabrax japonicus*.

6PGD was expressed in liver. The typical pattern of a dimer was observed. It was polymorphic in 8 of the 25 species examined and 3 species showed a few variants.

GDH was examined in liver. Polymorphism was shown in 14 of the 28 species examined and the varied pattern observed in 2 species was difficult to interpret.

ACP was examined in blood and showed the typical pattern of a monomer. Heterozygous individuals expressed two-banded phenotypes, while homozygotes showed single-banded phenotypes. Polymorphism was shown in 10 of the 34 species examined and a few variants was found in a species.

EST was detected by α-naphthyl acetate in blood and liver. EST showed the activity in several zones. Some of the patterns varied between individuals was difficult to interpret and the others showed the typical pattern of a monomer. It was polymorphic for EST in 16 of the 39 species examined.

SOD was expressed as the typical pattern of a dimer in liver. Polymorphism was shown in five species; *Scomber japonicus* with three alleles, and *Ammodytes personatus, Chelidonichthys kumu, Platichthys stellatus*, and *Rhinoplagusia japonica* with two alleles.

PGM was examined in muscle and showed the typical pattern of a monomer. It was polymorphic in 6 species and a few variants was shown in 4 species. A monomorphic two-banded pattern was found in *Liparis tanakai*, which suggests that gene duplication has occurred in this species at this locus. Similar gene duplication was revealed by the existence of variants in *Rhinoplagusia japonica*.

AAT was examined in both liver and muscle, and the typical patterns of a dimer. Two loci coding for AAT were suggested; one of them acted mainly in liver and the other in muscle. Some species were examined in muscle but not in liver. Polymorphism was shown in 8 of the 20 species examined. *Sardinops melanostica* exhibited three-banded monomorphic patterns in liver, suggesting that gene duplication has occurred at the locus coded for liver specific AAT.

AK was examined in muscle and shown as the typical pattern of a dimer. Polymorphism was observed in 3 of the 20 species examined and a few variants were found in only a species.

GPI was examined in muscle and shown as the typical pattern of a dimer which was encoded by two loci. A and B subunits were expressed in three forms (A_2, AB, B_2). Only a locus coded for A-subunit was recorded in some species, because it was smear or not readible for the B-subunit band. Polymorphism was shown in 12 species and a few variants were found in 6 species.

The genetic variant described in this survey should provide a useful basis for further studies of the species examined. For examples, it can be used for comparisons with these species in other localities or with those in the same localities at future dates.

The proportion of polymorphic loci was calculated on the assumption that a locus was polymorphic when the frequency of the most common allele was no greater than 0.95, and the mean individual heterozygosity was calculated by direct counts of the heterozygotes observed. The results are given in Table 3. A variation was observed in average level of genetic variation among taxonomic groups of fish. Higher levels of genetic variation were found in members of the orders, Belonida and Pleuronectida than in most other fish species. The lowest degrees of genetic variation among fish species were found in members of the orders, Tetraodntida, Cottida, Gaddida, and Lophiida. Members of the orders, Clupeida anc Percida, showed a median level of genetic variation. Average for the fish species examined as whole was 0.194 ± 0.023 in the proportion of polymorphic loci and 0.059 ± 0.007 in the mean individual heterozygosity.

Table 3. Genetic variation in some groups of fish

Group	Total No. of loci	No. of polymorphic loci	Proportion of polymorphic loci	Heterozygosity
Oder Clupeida				
Clupea pallasi	17	4	0.235	0.058
Sardinops melanostica	22	4	0.182	0.064
Engraulis japonica	22	5	0.227	0.067
Mean			0.215	0.068
Oder Belonida				
Cololabis saira	20	9	0.450	0.174
Hemiramphus sajori	21	4	0.190	0.063
Mean			0.320	0.119
Order Percida				
Scomber japonicus	19	5	0.263	0.093
Trachurus japonicus	21	4	0.190	0.048
Lateolabrax japonicus	19	6	0.316	0.095
Nibea mitsukurii	15	1	0.067	0.018
Ammodytes personatus	15	4	0.267	0.067
Mean			0.221	0.064
Order Tetraodontida				
Navodon modestus	24	1	0.042	0.013
Order Cottida				
Sebastes inermis	16	1	0.063	0.013
Sebastes thomsoni	18	0	0.000	0.004
Helicolenus hilgendorfi	16	3	0.188	0.083
Sebastolobus macrochir	16	3	0.188	0.063
Agrammus agrammus	14	2	0.143	0.060
Pleurogrammus azonus	18	2	0.111	0.035
Hexagrammos otakii	21	0	0.000	0.003
Hemitripterus villosus	19	1	0.053	0.022
Chelidonichthys kumu	14	1	0.071	0.041
Lepidotrigla microptera	18	2	0.111	0.042
Liparis tanakai	22	0	0.000	0.000
Mean			0.084	0.034
Order Pleuronectida				
Paralichthys olivaceus	20	4	0.200	0.050
Cleisthenes pinetorum herzensteini	23	6	0.261	0.107
Eopsetta grigorjewi	22	2	0.091	0.015
Verasper variegatus	23	2	0.087	0.032
Pleuronichthys cornutus	23	11	0.478	0.087
Limanda yokohamae	23	5	0.217	0.064
Limanda herzensteini	23	8	0.348	0.121
Dexistes rikuzenius	15	2	0.133	0.058
Platichthys stellatus	22	14	0.636	0.120
Kareius bicoloratus	22	7	0.318	0.099
Clidoderma asperrimum	23	6	0.261	0.062
Tanakius kitaharai	23	4	0.174	0.030
Microstomus achne	21	7	0.333	0.108
Rhinoplagusia japonica	21	9	0.429	0.124
Areliscus joyneri	20	9	0.450	0.146
Mean			0.294	0.082
Order Gadida				
Lotella maximowiczi	15	0	0.000	0.004
Gadus macrocephalus	15	2	0.133	0.067
Theragra chalcogramma	17	1	0.059	0.006
Mean			0.064	0.026
Order Lophiida				
Lophius litulor	15	0	0.000	0.000

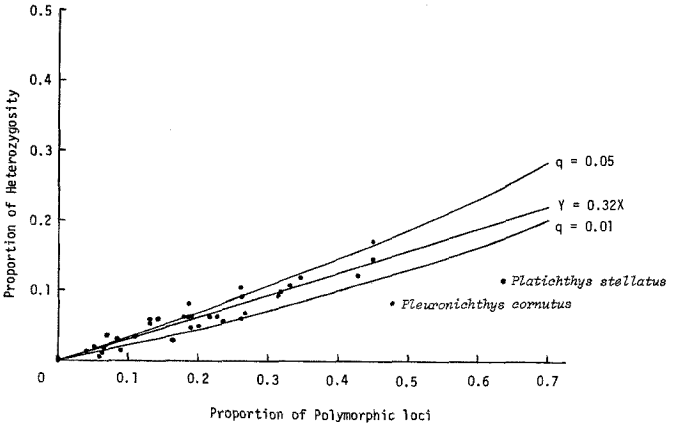

Fig. 1. Relationship between the proportion of polymorphic loci and the mean individual heterozygosity. The line represents the regression based on the observed values. The two curves represent the theoretical relationship based on the neutral polymorphism theory. The dotes represent observed values.

The correlation of the proportion of polymorphic loci with the mean individual heterozygosity was observed, and the relationship was linear as shown Fig. 1. Regression of the mean individual heterozygosity on the proportion of polymorphic loci was calculated as $Y=0.32X$. Most of the species were plotted on the regression line, but only two species, *Pleuronichthys cornutus* and *Platichthys stellatus*, were far from the regression line. Their mean individual heterozygosities were low as compared with their high proportion of polymorphic loci. The regression coefficient for the equation means that the mean individual heterozygosity per polymorphic locus is constant.

Discussion

The estimates obtained in this work for the proportion of polymorphic loci and the mean individual heterozygosity in a given species were agreed on those reported in other studies for fishes[6,7]. The estimate for the fish seems to be equal to those values reported for other vertebrates and to be less than those values reported for invertebrates.[4]

KIMURA and OHTA[2] proposed that the relationship between the probability of polymorphism (P_{poly}) and the average heterozygosity ($\bar{H}$) can be expressed by the following equation in the neutral theory of protein polymorphism

$$P_{poly}=1-q^{\bar{H}/(1-\bar{H})},$$

where the q is the sum of the frequencies of variant allele in monomorphic population. The regression line of the mean individual heterozygosity on the proportion of polymorphic loci was near to their theoretical curve at q (0.05) within variation of the proportion of polymorphic loci obtained in this work as shown in Fig. 1. Two exceptions, *Pleuronichthys cornutus* and *Platichthys stellatus* population could be interpreted by the admixture of other species. The mixture of the sibling species in *P. cornutus* population was elucidated by electrophoretic patterns of 8 enzymes, MDH, IDH, SDH, ACP, EST, PGM, AAT, and GPI, though the sibling species was resemble to *P. cornutus* in morphological characters[8]. The admixture of *Kareius bicoloratus* in *P. stellatus* population by natural hybridization between them was known[9]. The both species were plotted on the regression line by removing the other species from the population.

Thus, it might be concluded that variation in average level of heterozygosity among taxonomic groups of fish reflected difference in effective population size on the basis of neutral interpretation. However, it is not easy to determine population size. The population size of a species is a complex concept. In fact, a species does not always have just one population size but has several in time. A variety of effective population size depends on population structure, that is, the amount of inbreeding and the amount of isolation between subpopulations.

Acknowledgements

The authors wish to thank Dr. K. Nozawa of

Primate Research Institute, Kyoto University for reading the manuscript and for his valuable suggestions.

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