GENETIC VARIABILITY AND DIFFERENTIATION IN THE TOOTHED WHALES

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ABSTRACT

Genetic variability and differentiation of three families containing 12 species of toothed whales mainly from the sea around Japan have been examined by starch-gel electrophoresis at 19 genetic loci encoding enzymes. The amount of genic variations presented by proportion of polymorphic loci and average heterozygosity of whole species studied were 0.207 ± 0.151 and 0.063 ± 0.051, respectively, and were found to be similar to the average of other vertebrates. The genetic distances and genetic identities among the species, genera and families of the toothed whales studied were discussed, and the degree of genetic divergence of given taxonomic levels were shown to be low in comparison with corresponding taxa of other organisms. The phylogenetic relationships among the species studied were estimated by the dendrogram of genetic distances.

INTRODUCTION

Electrophoretic data consisting of allele frequencies have been widely accumulated in a large variety of organisms, to estimate the amount of genetic variation in natural populations and the degree of genetic differentiation at various levels of taxonomic groups. In general, vertebrates including mammals are known to have lower levels of genetic variability than invertebrates. Selander and Kaufman (1973) and Valentine (1976) supposed that large, mobile animals, especially vertebrates had lower levels of variability than small, relatively immobile animals of most invertebrates from the viewpoint of adaptive strategy.

The cetacean is an unique group of mammals distinctly different from the other mammals in many aspects of their biology by perfectly adapting to an aquatic form of life, and is possibly a representative of large, active vertebrates. However, electrophoretic survey of protein molecules in the cetaceans is relatively few. It is probably because of some difficulties in sample collection. Several preliminary studies of electrophoretic examination of proteins have dealt with hemoglobin (Horvath, Chiodi, Ridgeway and Azar, Jr., 1968; Baluda, Kulu and Sparkes, 1972; Border, 1975), some blood proteins (Sharp, 1981) and lactate dehydrogenase (Numachi, 1970). Recently, studies of en-

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TABLE 1. MATERIALS USED IN THIS STUDY

Name of species	Locality	No. of samples Liver	Muscle
Ziphiidae			
North pacific giant bottlenose whale (*Berardius bairdii*)	Wadaura, Bōsō Pen.	9	12
Delphinidae			
Short-finned pilot whale (*Globicephala macrorhynchus*)	Taiji, Kii Pen.	39	39
Many-toothed blackfish (*Peponocephala electra*)	Taiji	6	1
False killer whale (*Pseudorca crassidens*)	Iki Island	31	31
Striped dolphin (*Stenella coeruleoalba*)	Kawana, Izu Pen.	370	204
Bridled dolphin (*Stenella attenuata*)	Kawana	183	90
Bottlenose dolphin (*Tursiops truncatus*)	Iki Island	35	35
Pacific whiteside dolphin (*Lagenorhynchus obliquidens*)	Iki Island	30	27
Routh-toothed dolphin (*Steno bredanensis*)	Taiji	10	29
Phocoenidae			
Harbor porpoise (*Phocoena phocoena*)	The east coast of Hokkaido	3	3
Finless porpoise (*Neophocaena phocaenoides*)	The coast of Kii Pen.	3	5
Dall porpoise (*Phocoenoides dalli dalli*-type)	North Pacific & the east coast of Hokkaido	483	61
(*P. dalli truei*-type)	Off Sanriku region	54	400

zyme polymorphisms and genetic structure of population in the species of cetacean were made by several authors (Wada and Numachi, 1979; Simonsen, Kapel and Larsen, 1982; Wada, 1982, 1983a, 1983b, 1984, 1986; Numachi and Shimura, 1984; Winans and Jones, 1986). Nevertheless, genetic variability and the degree of genetic differentiation in the cetaceans are still unclear.

In the present study, we examine enzyme polymorphism in 12 species of toothed whales mainly found off the coast of Japan. On the basis of the data obtained for 19 genetic loci encoding 12 enzymes, estimates of the proportion of polymorphic loci, average heterozygosity, genetic identity and distance among the various taxa of different taxonomic levels are presented. These values obtained here are discussed in comparison with those in other organisms. The dendrogram of genetic distance are presented, and the genetic relationships and the time of divergence of the species are discussed.

MATERIALS AND METHODS

Liver and/or skeletal muscle were obtained from the specimens caught either by harpoon, gill net or drive during the course of commercial fishing or research expedition off the coast of Japan and the North Pacific, between winter 1978 and autumn 1981. Twelve species, sampling locations and number of samples are shown in Table 1. The locations are also shown in Fig. 1.

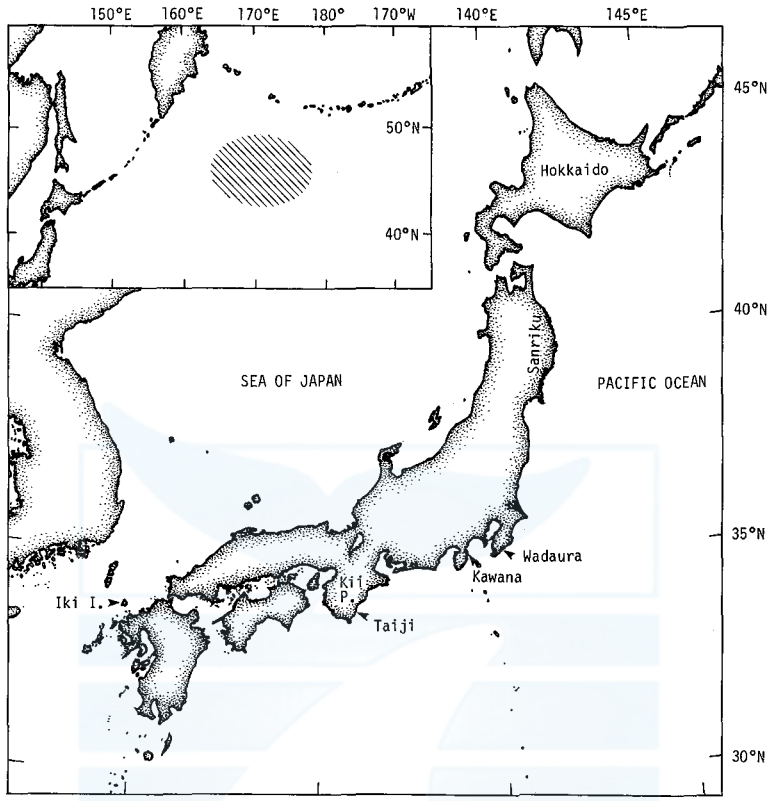

Fig. 1. Map showing the positions of collection. Shaded area represents the range of catch of *Phocoenoides dalli* (*dalli*-type) in the North Pacific.

Tissue samples were frozen in a container with dry ice, or in a freezing room on board immediately after the catch and thereafter stored below −20°C until use. For the electrophoretic run, after thawing tissues, cell lysates were collected by soaking with a small piece of filter paper, and then the filter paper was inserted in 12.5% starch gel (Electrostarch; Electrostarch Inc., Madison, Wisconsin, U.S.A.).

Electrophoresis was conducted horizontally at a high voltage of a 20 V/cm and stopped when the marker of Amide black 10B migrated 7 cm from the origin. During the electrophoresis, gels were set at 5°C and a glass-plate pan filled with ice water was placed on it for cooling the gel. Following two buffer systems were used by modifying the method of Clayton and Tretiak (1972): (1) C-AEA, pH 7.0 consisting of gel buffer, 2 mM citric acid adjusted to pH 7.0 with N-(3-aminopropyl)-diethanolamine (AEA), and electrode buffer of 40 mM citric acid adjusted to pH 7.0 with the same amine, and (2) C-T, pH 8.0 consisting gel buffer 3 mM citric acid-10 mM Tris, and electrode buffer of 16 mM citric acid-62 mM Tris. $MgCl_2$ (10 mM) was added to all the gels.

TABLE 2. LIST OF ENZYME AND LOCI EXAMINED AND TISSUE AND BUFFER USED FOR ELECTROPHORESIS

Enzyme	Loci encoding	Tissue used	Buffer
Sorbitol dehydrogenase (SDH)	*Sdh*	liver	C–T
Lactate dehydrogenase (LDH)	*Ldh–1*	liver, muscle	C–AEA
	Ldh–2	liver, muscle	C–AEA
Malate dehydrogenase (MDH)	*Mdh–1*	liver, muscle	C–AEA
	Mdh–2	liver, muscle	C–AEA
Malic enzyme (ME)	*Me*	liver, muscle	C–AEA
Isocitrate dehydrogenase (IDH)	*Idh–1*	liver, muscle	C–AEA
	Idh–2	liver, muscle	C–AEA
6-Phosphogluconate dehydrogenase (6-PGD)	*6–Pgd*	liver, muscle	C–AEA
Superoxide dismutase (SOD)	*Sod*	liver, muscle	C–T
Esterase	*Est–1*	liver	C–AEA
	Est–2	liver	C–AEA
Glutamate oxaloacetate transaminase (GOT)	*Got–1*	liver, muscle	C–T
	Got–2	liver, muscle	C–T
Phosphoglucomutase (PGM)	*Pgm–1*	liver, muscle	C–AEA
	Pgm–2	liver	C–AEA
	Pgm–3	liver	C–AEA
Mannose phosphate isomerase (MPI)	*Mpi*	liver, muscle	C–AEA
Phosphohexose isomerase (PHI)	*Phi*	liver	C–AEA

The buffer systems and tissues used for detecting each enzyme are shown in Table 2. For IDH, ME and 6-PGD, 30 mM NADP was added to the gel and cathodal electrode buffer, and for SDH 30 mM NAD to the both of them.

Details of reaction mixture for staining enzymes are; (1) SDH: 500 mg sorbitol, 50 mg sodium pyruvate, 20 mg NAD, 20 mg nitro blue tetrazolium (NBT), 5 mg phenazine methosulphate (PMS) or 1-methoxy-PMS, in 100 ml 0.1 M Tris-HCl buffer, pH 8.0, (2) LDH: 0.625 ml sodium lactate (50%), 20 mg NAD, 20 mg NBT, 5 mg PMS or 1-methoxy-PMS, in 100 ml 0.1 M Tris-HCl buffer, pH 8.7, (3) MDH: 250 mg sodium malate in the same buffer and reagent mixture as (2), (4) ME: 250 mg sodium malate, 1 ml 1M $MgCl_2$, 20 mg NADP, 20 mg NBT, 5 mg PMS or 1-methoxy-PMS, in 100 ml 0.1 M Tris-HCl buffer, pH 8.0, (5) IDH: 37 mg isocitric acid as substrate in the same mixture as (4), (6) 6-PGD: 100 mg 6-phosphogluconic acid in the same mixture, (7) SOD: 400 mg EDTA·2Na, 2 g β-D-glucose, 40 mg NBT, 50 mg PMS, in 100 ml 0.1 M Tris-HCl buffer, pH 9.5, (8) Esterase: detected by negative staining method of Harris and Hopkinson (1976), (9) GOT: 15 ml of L-aspartic acid solution (2.7 g / 100 ml D.W.) and 15 ml of 2-oxoglutaric acid solution (1.5 g / 100 ml D.W.), both adjusted pH 7.5 with 2 M KOH, 500 mg Fast blue BB, in 70 ml 0.2 M phosphate buffer, pH 7.5, (10) PGM: 150 mg D-glucose-1-phosphate containing D-glucose-1,6-diphosphate, 80 unit glucose-6-phosphate dehydrogenase (G-6-PDH), 1 ml 1 M $MgCl_2$, 20 mg NADP, 20 mg NBT, 5 mg PMS, in 100 ml 0.1 M Tris-HCl buffer, pH 8.0,

(11) MPI: 25 mg D-mannose-6-phosphate, 50 unit glucose phosphate isomerase, 80 unit G-6-PDH, 1 ml 1 M $MgCl_2$, 20 mg NADP, 20 mg NBT, 10 mg PMS, in 100 ml 0.1 M Tris-HCl buffer, pH 8.0, (12) PHI: 150 mg fructose-6-phosphate, 80 unit G-6-PDH, 20 mg NADP, 20 mg NBT, 5 mg PMS, in 100 ml 0.1 M Tris-HCl buffer, pH 8.0.

All the gels after electrophoresis and the staining were dried (Numachi, 1981) and then preserved for the future analysis of isozyme pattern.

RESULTS

Genetic variability in the toothed whales

A total of 19 genetic loci encoding 12 enzymes was surveyed in 12 species of toothed whales. Electrophoretic patterns of enzymes, the number of isozymes and tissue distribution showed that all the 12 species essentially have the same isozyme systems, each under the control of corresponding locus which is homologous phylogenetically throughout all the species studied. Electrophoretic patterns and schematic drawings of each enzyme assayed are shown in Plates I-VI. Alleles at the 19 loci are listed in Table 3. The frequencies of phenotypes on electrophoretic patterns were in good agreement with the Hardy-Weinberg proportion, except for two cases of *Idh-1* locus in *Pseudorca crassidens* ($\chi^2=6.758$, $0.01>p>0.005$, d.f.$=1$) and of the *Sdh* locus in *truei*-type of *Phocoenoides dalli* ($\chi^2=7.746$, $0.01>p>0.005$, d.f.$=1$). In both cases, the deviation was observed in heterozygotes excess.

The incidence of polymorphic loci (P) and average heterozygosity (H) is summarized in Table 4. P and H values of 12 species of the toothed whales showed a wide range of 0-0.474, and 0-0.154, respectively. In the two types of *P. dalli*, the highest values of P and H were obtained. By average of all the 12 species, proportion of polymorphic loci (P) in the definition of 5% level was 20.7%, and 16.4% excluding *P. dalli*. The average heterozygosity (H) was 0.063 in average of the 12 species, and 0.047 excluding *P. dalli*. Recently, Wada (1983b, 1986) showed that the proportion of polymorphic loci and/or the average heterozygosity were $P=0.13$ and $H=0.021$ in the striped dolphin, and $H=0.008$ in the short-finned pilot whale. Winans and Jones (1986) also showed that the average heterozygosity of *P. dalli* is 0.058. These values were something different from those obtained here, probably caused by the difference in the sample size and choice of loci examined. However, all the values obtained by other authors should be considered to be in the range of the values shown in Table 4.

Genetic differentiation between taxa

Genetic identity and genetic distance by Nei (1972) were calculated from the data in Table 3. Genetic distance (D) and genetic identity (I) between the populations, X and Y, are given by

TABLE 3. ALLELE FREQUENCIES AT THE 19 HOMOLOGOUS LOCI IN THE
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	Ldh–1	*Ldh–2*	*Mdh–1*	*Mdh–2*	*Idh–1*	*Idh–2*	*Me*	*Sdh*	*6–Pgd*	*Got–1*	*Got–2*
1. *Berardius bairdii*	c	a	a	b	b	b	c	b	j	c	c
2. *Globicephala macrorhynchus*	a(.051) d(.949)	a	a(.990) b(.010)	b	c	b	b	b	b(.013) f(.013) g(.974)	b	b
3. *Peponocephala electra*	d	a	a	b	c	b(.750) d(.250)	b	b	g	b	b
4. *Pseudorca crassidens*	d	a	a	c	b(.100) c(.900)	b	b	b	g	b	b
5. *Stenella coeruleoalba*	d	a	a	b(.948) d(.052)	c	b	b	b	c(.013) g(.972) k(.015)	a(.020) b(.980)	b
6. *Stenella attenuata*	d	a	a	a(.022) b(.978)	c	b	b	b	f(.010) g(.990)	b	b
7. *Tursiops truncatus*	d	a	a	b	c	b	b	b	g	b	b
8. *Lagenorhynchus obliquidens*	d	a	a	b	a(.036) c(.946) e(.018)	b	b	b	g	b	a(.075) b(.925)
9. *Steno bredanensis*	d	a	a	b	b	b	c	b	e(.020) h(.980)	b	a(.050) b(.950)
10. *Phocoena phocoena*	e	a	a	b	d	a	a	c	i	c	c
11. *Phocoenoides dalli* (*dalli*-type)	e	a	a(.987) b(.013)	b(.983) e(.017)	b(.305) d(.695)	a(.906) c(.094)	a(.074) c(.916) d(.010)	a(.475) c(.525)	a(.967) b(.010) i(.023)	b(.013) c(.987)	b(.025) c(.975)
12. *Phocoenoides dalli* (*truei*-type)	b(.011) e(.989)	a	a(.987) b(.013)	b(.979) e(.021)	b(.280) d(.720)	a(.904) c(.096)	a(.083) c(.903) d(.014)	a(.441) c(.559)	a(.965) d(.016) i(.019)	b(.075) c(.925)	c
13. *Neophocaena phocaenoides*	e	b	a	b	d	a	c	c	i	c	c

PECIES OF TOOTHED WHALES. ALLELES AT EACH LOCUS WERE DESIGNATED
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OLYMORPHISMS ARE ALSO SHOWN IN PARENTHESES

Sod	*Mpi*	*Phi*	*Pgm-1*	*Pgm-2*	*Pgm-3*	*Est-1*	*Est-2*
e	f(.830) g(.170)	d	a	c	c	b	not assayed
a(.850) b(.150)	a	b	b	e(.750) f(.250)	f	a(.050) b(.950)	b(.075) c(.925)
	a	c	b	e	f	b	a(.170) b(.830)
a(.048) b(.952)	a	b(.975) e(.025)	a(.025) b(.975)	c(.025) e(.800) f(.175)	f(.950) g(.050)	a(.100) b(.900)	b
b	d(.030) f(.050) e(.030) g(.890)	d(.970) f(.030)	a(.023) b(.977)	d(.105) f(.711) e(.131) h(.053)	f(.974) i(.026)	a(.050) b(.925) e(.025)	a(.132) c(.210) b(.632) d(.026)
b(.920) c(.080)	d(.025) e(.025) g(.950)	d(.975) f(.025)	b	b(.025) f(.400) e(.550) g(.025)	c(.025) f(.975)	b(.425) c(.550) f(.025)	b(.075) c(.875) d(.050)
b	d(.275) g(.725)	d	b	e(.825) f(.175)	f	b	a(.974) b(.026)
b(.480) c(.520)	b(.525) g(.475)	b	b	e(.325) f(.675)	h	a(.050) b(.950)	a
d	a	b	b	e	f	b	c
a	f(.830) h(.170)	b	b(.830) d(.170)	c	c	b(.170) d(.830)	not assayed
a	f(.725) i(.275)	a(.025) b(.975)	a(.025) b(.975)	a(.025) d(.100) c(.800) f(.075)	a(.075) d(.100) b(.700) e(.075) c(.025) f(.025)	a(.050) b(.950)	e(.029) f(.971)
a	c(.025) f(.775) d(.025) i(.175)	a(.025) b(.975)	b(.977) c(.023)	b(.175) c(.725) f(.100)	b(.850) d(.025) c(.100) e(.025)	b	e(.050) f(.950)
a	f	b	b	c	b	b	not assayed

$$D = -\log_e I,$$

$$I = J_{XY}/\sqrt{J_X \cdot J_Y}$$

J_{XY}, J_X and J_Y are defined as

$$J_X = \sum_j \sum_i (x_{ij})^2/r$$

$$J_Y = \sum_j \sum_i (y_{ij})^2/r$$

$$J_{XY} = \sum_j \sum_i x_{ij} \cdot y_{ij}/r$$

(r: number of examined loci)

where x_{ij} and y_{ij} are the frequencies of the i^{th} allele at the j^{th} locus in X and Y populations. When X and Y populations share the same allele frequencies at all loci, genetic distance and identity are given by $D = 0$ and $I = 1$. Conversely when they do not share the common allele over all loci, the values become $D = \infty$ and $I = 0$. Estimates of D and I values among 12 species of the toothed whales based on allele frequencies at 18 loci are shown in Table 5. Allele frequencies of *Est-2* were excluded in calculation because of insufficiency of data. The frequency distribution of genetic identity was obtained in

TABLE 4. SUMMARY OF GENETIC VARIABILITY IN THE TOOTHED WHALES

Species	No. of loci	Proportion of polymorphic loci (P^*)	Average heterozygosity ($H^{**} \pm$ S.E.)
1. *Berardius bairdii*	18	0.056	0.016 ± 0.069
2. *Globicephala macrorhynchus*	19	0.263	0.054 ± 0.106
3. *Peponocephala electra*	19	0.105	0.035 ± 0.108
4. *Pseudorca crassidens*	19	0.211	0.051 ± 0.092
5. *Stenella coeruleoalba*	19	0.263	0.089 ± 0.160
6. *S. attenuata*	19	0.263	0.089 ± 0.170
7. *Tursiops truncatus*	19	0.105	0.039 ± 0.113
8. *Lagenorhynchus obliquidens*	19	0.316	0.093 ± 0.182
9. *Steno bredanensis*	19	0.053	0.007 ± 0.024
10. *Phocoena phocoena*	18	0.167	0.047 ± 0.111
11. *Phocoenoides dalli* (*dalli*-type)	19	0.421	0.154 ± 0.184
12. *P. dalli* (*truei*-type)	19	0.474	0.147 ± 0.170
13. *Neophocaena phocaenoides*	18	0.000	0.000
Average(1)***		0.207 ± 0.151	0.063 ± 0.051
Average(2)***		0.164 ± 0.112	0.047 ± 0.035

* P values were calculated in the level which included the loci where frequencies of variant alleles were found at more than 5%.

** H is calculated by averaging the value of heterozygosity of each locus (h) over all loci. The heterozygosity (h) is defined as $1 - \sum_{i=1}^{n} x_i^2$, where x_i is the frequency of the i th allele and n is the number of alleles at the locus.

*** Average(1) means all the 12 species, and (2) excluding values of 2 types of *P. dalli*.

TABLE 5. COEFFICIENTS OF GENETIC IDENTITY (ABOVE THE DIAGONAL) AND GENETIC DISTANCE (BELOW THE DIAGONAL) BETWEEN SPECIES OF THE TOOTHED WHALES

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1. *Berardius bairdii*		.341	.325	.291	.409	.383	.407	.356	.450	.451	.522	.519	.438
2. *Globicephala macrorhynchus*	1.075		.893	.902	.829	.827	.853	.851	.777	.340	.419	.421	.330
3. *Peponocephala electra*	1.123	.113		.879	.851	.854	.892	.791	.716	.230	.304	.306	.225
4. *Pseudorca crassidens*	1.235	.103	.129		.812	.808	.837	.807	.725	.235	.307	.308	.224
5. *Stenella coeruleoalba*	.895	.188	.162	.208		.974	.974	.850	.627	.233	.308	.310	.224
6. *S. attenuata*	.959	.190	.157	.213	.026		.974	.832	.633	.232	.279	.281	.197
7. *Tursiops truncatus*	.900	.159	.115	.178	.026	.026		.837	.670	.232	.307	.310	.227
8. *Lagenorhychus obliquidens*	1.034	.162	.235	.214	.162	.184	.178		.655	.299	.377	.382	.290
9. *Steno bredanensis*	.799	.252	.335	.321	.466	.458	.401	.423		.286	.435	.434	.335
10. *Phocoena phocoena*	.797	1.078	1.469	1.450	1.457	1.462	1.461	1.207	1.253		.778	.777	.787
11. *Phocoenoides dalli* (*dalli*-type)	.649	.869	1.192	1.182	1.178	1.275	1.180	.975	.832	.252		.996	.856
12. *P. dalli* (*truei*-type)	.656	.865	1.185	1.176	1.171	1.270	1.172	.964	.835	.252	.004		.861
13. *Neophocaena phocaenoides*	.825	1.109	1.494	1.496	1.496	1.624	1.485	1.239	1.095	.239	.156	.150	

all possible pairwise comparisons at every locus between species, genera and families (Fig. 2).

P. dalli consisted of two types, *dalli*-type and *truei*-type. These two were recently considered as the geographic variation of the color patterns of the single species (Houck, 1976; Kasuya, 1978, 1982). These two types were highly polymorphic, but alleles at these loci were common between them. In terms of genetic identity, almost 100% of the loci were very same ($I > 0.95$). Genetic identity and genetic distance between the two types were 0.996 and 0.004, respectively.

Stenella coeruleoalba and *S. attenuata* were compared for genetic divergency at specific level. Between the two species, eight of the 19 genetic loci were monomorphic for the same genes, and common or some species specific genes were shared at the other 11 polymorphic loci. Thus, about 90% of the loci were nearly identical ($I > 0.95$), as shown in Fig. 2a. Genetic identity and genetic distance between the two species were 0.974 and 0.026, respectively.

The 12 species of toothed whales examined here represent 11 genera. More than 70% of loci were nearly identical ($I > 0.95$) between these genera (Fig. 2b), but 15% of them were quite different ($I < 0.05$). Mean genetic identity and distance between the genera were 0.812 and 0.213, respectively.

The 12 species of toothed whales belong to three families; Ziphiidae (*Berardius*), Delphinidae (*Globicephala*, *Peponocephala*, *Pseudorca*, *Stenella*, *Tursiops*, *Lagenorhynchus* and *Steno*), and Phocoenidae (*Phocoena*, *Phocoenoides* and *Neophocaena*). Levels of genetic identity between the families covered a broad range, and the distribution of the values had a strong U-shaped pattern (Fig. 2c). The identical loci ($I > 0.95$) in genic compositions were only 30%, and 65% of loci were replaced by different genes. The frequency distribution of genetic distance (D) ranged widely from 0.6 to 1.65 (Fig. 3). The genetic

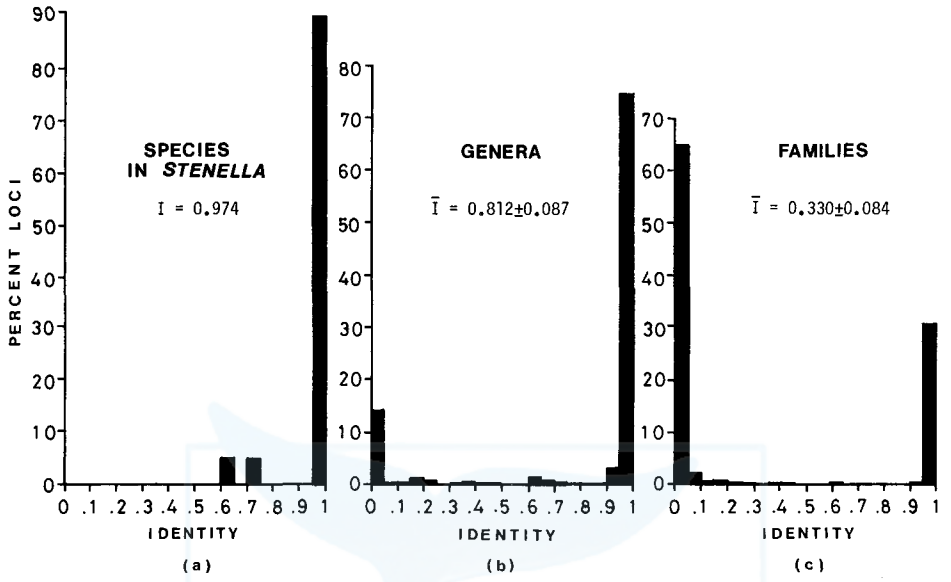

Fig. 2. Frequency distribution of loci with respect to genetic identity when comparing the different species of the same genus (a), between the different genera (b) and between the families (c). $\bar{I}$ is the mean genetic identity with its standard error.

distances between the families were much larger than those between the genera. Mean values of the genetic identity and distance between the families were 0.330 and 1.140, respectively.

Table 6 shows the values of mean genetic distance (D) of 4 taxonomic levels of the toothed whales and other animals. Higher D values were obtained in the higher taxonomic levels, but D values showed a wide range of variation among the taxa. D values of the toothed whales were generally small, and thus the degree of genetic divergency of the toothed whales seemed to be lower than the other animals. D value of the toothed whales at the family level was similar to those of *Drosophila* at the specific level ($D=1.056$) and of sunfish and salamanders at the generic level ($D=1.170-1.340$), indicating lower genetic divergency between the toothed whale families.

Genetic relationships of the toothed whales

Unweighted pair-group method of cluster analysis (Sokal and Sneath, 1963) were applied to the matrix of genetic distance (Table 5), to elucidate genetic relationships among the species studied. The dendrogram is shown in Fig. 4. In general, the dendrogram agreed with currently accepted opinions on phylogenetic relationship in toothed whales deduced from morphology (kasuya, 1973; Mead, 1975; Gaskin, 1982).

A crude estimate of divergence time (t) can be obtained by an equation, $t = 5 \times 10^6 D$, provided by Nei (1975). The time scale of divergence was also

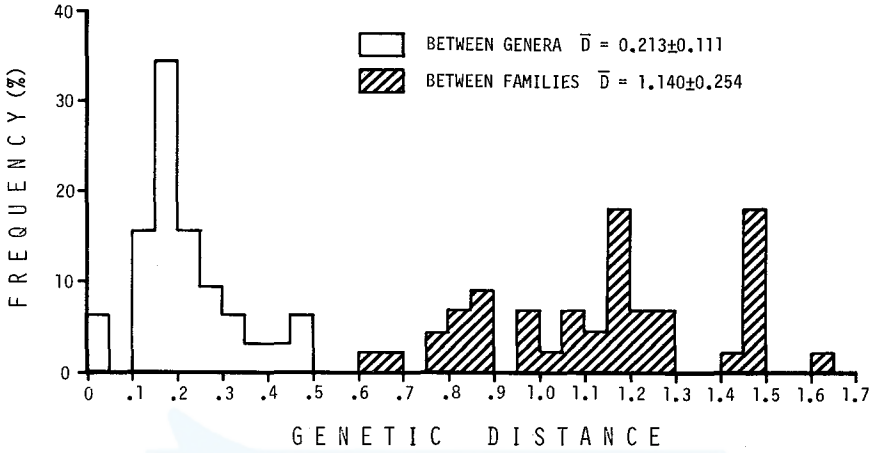

Fig. 3. Distribution of genetic distances among toothed whales between species in different genera and families. Mean distance and its standard error are indicated in each case.

shown in Fig. 4. The result represented that the families diverged 3.5–5.5 million years ago, and that speciation of the species within the same family occurred two million years ago.

DISCUSSION

Selander and Kaufman (1973), Powell (1975), Selander (1976) and Nevo (1978) made reviews of allozymic variations in natural populations of plants and animals so far studied. In three major taxa (plants, invertebrates and vertebrates), both P and H values increased in the following order: vertebrates, plants and invertebrates. Within vertebrates consisting of following five groups, fishes, amphibians, reptiles, birds and mammals, no significant difference was found (Nevo, 1978). Nevo (1978) reported $P=0.173 \pm 0.119$ (in 5% level) and $H=0.0494 \pm 0.0365$ for 135 species of vertebrates, and $P=0.147 \pm 0.098$ and $H=0.0359 \pm 0.0245$ for 46 species of mammals. P and H values of the toothed whales in the present study were $P=0.207$ and $H=0.047$. These values were slightly larger than those of mammals but similar to those of the vertebrates. Accordingly, the genetic variation level of the toothed whales is considered to be in the range of vertebrates in spite of their ecological specialization.

The values of the mean genetic distances of the toothed whales at the four taxonomic levels (Table 6) were distinctly smaller than those of other animals except the birds. The distribution patterns of the genetic identity as shown in Fig. 2 were also shown in *Drosophila* (Ayala, Tracey, Barr, McDonald and Pérez-sales, 1974), sunfish (Avise and Smith, 1977) and rodents (Zimmerman, Kilpatrick and Hart, 1978). Compared with these patterns, the genetic

TABLE 6. MEAN GENETIC DISTANCES (NEI'S *D*) AT FOUR TAXONOMIC LEVELS

Taxa	Local Populations	Species	Genera	Families	References
Drosophila	0.028	1.056	-------	-------	Ayala, 1975
Sunfish	0.024	0.626	1.340	-------	Avise & Smith, 1977
Salamanders	0.051	0.462	1.170	-------	Hedgecock & Ayala, 1974
Rodents	0.030	0.323	-------	-------	Zimmerman *et al.*, 1978
Birds					
Passerines	0.003	0.100	0.214	-------	Barrowclough *et al.*, 1981
Procellariiformes	-------	-------	0.435	0.683	Barrowclough *et al.*, 1981
Parulidae	-------	0.100	0.179	-------	Barrowclough & Corbin, 1978
Toothed whales	(0.004)*	0.026	0.213	1.140	Present study

* Two types of *Phocoenoides dalli*

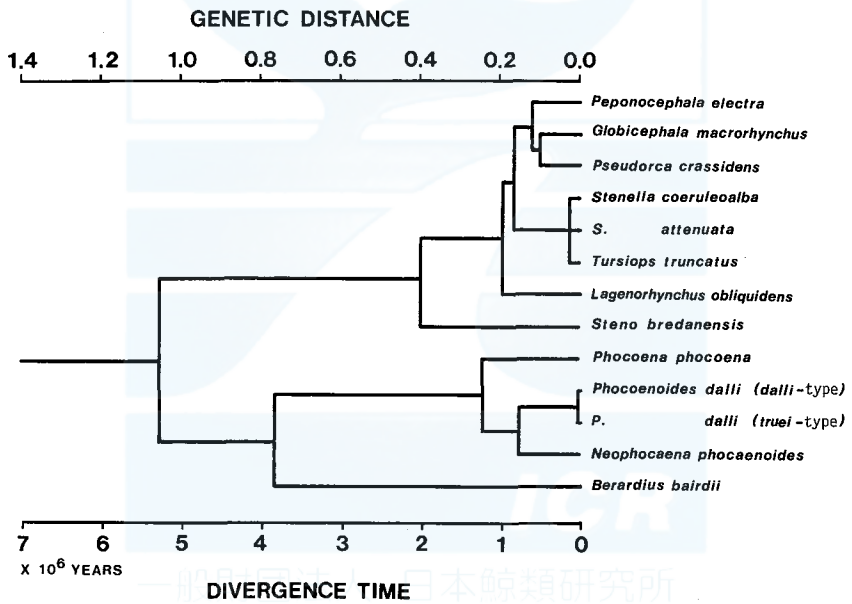

Fig. 4. Biochemical similarity dendrogram of toothed whales based on genetic distance. Divergence time by calibration of Nei (1975) is also shown.

divergence of the toothed whales was much smaller than those of the other species. The pattern obtained for the species of the toothed whales (Fig. 2a) was the very same as the populations of the other organisms, and the genus level (Fig. 2b) was much similar to the species level of the other organisms.

Most of cetologists have considered that Phocoenidae is fully distinguished from Delphinidae. However, Rice (1977) classified true porpoises into Delphinidae. Our result showed that *D* value between Phocoenidae and

Delphinidae were considerably high (D=1.051). Therefore, Phocoenidae and Delphinidae may be differentiated in the family level judging from the allozymic comparison.

The dendrogram showed that three species of globicephalids were not so different from other delphinids genetically. The D value btween *Steno* and other species of Delphinidae was slightly large compared with other genera, but considerably small when compared at the family level of Phocoenidae and Delphinidae. Therefore, the genetic difference of *Steno* is considered to be at the generic level in Delphinidae. In regard to the genetic relationship of Delphinidae, the dendrogram seems to be in good agreement with morphological relationships among the groups in this family.

Berardius bairdii is a species in a well-distinguished family, Ziphiidae. Gaskin (1982) suggested that Ziphiidae diverged from other families of Odontoceti in early evolutionary time deduced from the karyological characteristic of Ziphiids species. Based on our electrophoretic study, the relationship between Ziphiidae and Phocoenidae was relatively closer than that between Delphinidae and Phocoenidae.

Time of divergence was estimated from D values using the equation of Nei (1975). Our estimation of divergence time is different from that shown by the geological study. It is supposed that the major families of the toothed whales were established in Miocene (ca. 10–25 million years ago) (Kellog, 1928), and that morphologically "modern" delphinids became abundant in the following Pliocene (ca. 1–10 million years ago) (Gaskin, 1982). Our result by Nei's conversion represented that three families diverged 3.5–5.5 million years ago, and that the speciation within Delphinidae started from two million years ago. Accordingly, the time of divergence based on these calculation was much shorter than that of geological one. Possible reasons for the differences may be (1) the rate of genetic changes presumed by Nei may not fit to that of the toothed whales, and (2) the time of divergence tends to give underestimate when D is larger than 1 (Nei, 1975). Carlson, Wilson and Maxon (1978) found that 1 albumin immunological distance (*AID*) unit was equivalent to 0.54 million years (r=0.97) through the comparison between paleontological records and *AID*. Further, Wyles and Gorman (1980) reported a D of 1.0 corresponded to 35.6 *AID* units on the average. Thus, a D of 1.0 represents about 19 million years of continuous separation (Grant, Teel, Kobayashi and Schmitt, 1984). We estimated the time of divergence of toothed whale families using this method as about 13.3–20.0 million years, and for genera in Delphinidae 7.6 million years. These estimates properly agree with the paleontological time of divergence.

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EXPLANATION OF PLATES

PLATE I

Figs 1a and 1b. Electrophoretic patterns of tetrameric SDH isozymes from liver extracts in *Phocoenoides dalli* (*dalli*-type) (1a). Intraspecific variations appeared on the zymogram. Schematic drawings show the patterns detected through the species studied (1b). Designations of alleles (*a, b,* etc.) correspond to those of Table 3. Genotypes postulated and positions of homopolymeric isozymes of subunits produced by each allele are shown on the schematic drawings.

Figs 2a to 2d. Electrophoretic patterns of MDH and ME isozymes from muscle extracts in *Phocoenoides dalli* (*dalli*-type) (2a). Dimeric MDH isozymes appeared on both sides of the gel, and tetrameric ME isozymes only anodal side. Three schematic drawings show the patterns detected in each isozyme system through the species studied (2b, 2c and 2d). For designation see Plate I, Fig. 1.

PLATE II

Figs 1a and 1b. Electrophoretic patterns of LDH isozymes from liver extracts of all the species studied (1a). The numbers correspond to those of the species in Table 3. The positions of homopolymeric isozymes of subunits produced by each allele of two LDH loci are shown on the patterns. *Neophocaena phocaenoides* (no. 13) had specific variation in LDH-2. For LDH-1, three variations corresponded to the interfamilial differences. Schematic drawings (1b) show the patterns of LDH-1 detected through the species studied. Rare variations (*ad* and *be*) were observed in *Globicephala macrorhynchus* and *Phocoenoides dalli* (*truei*-type).

Fisg 2a to 2c. Electrophoretic patterns of dimeric IDH isozymes from liver extracts in *Phocoenoides dalli* (dalli-type) (2a). IDH-1 and -2 appeared separately on both sides of the gel. One or two conformeric bands appeared on the anodal side of the major bands of IDH-2. Two schematic drawings show the patterns detected in each isozyme system through the species studied (2b and 2c). For designation see Plate I, Fig. 1.

PLATE III

Figs 1a to 1c. Electrophoretic patterns of 6-PGD isozymes from liver extracts of all the species studied (1a). The numbers correspond to those of species in Table 3. Black points indicate the differences of the mobility of the patterns. Each band shows the most common one of each species. Electrophoretic patterns of *Stenella coeruleoalba* are also shown (1b). Two dimeric variations appeared. Schematic drawings show the patterns detected through the species studied (1c). For designation see Plate I, Fig. 1.

Figs 2a and 2b. Electrophoretic patterns of dimeric SOD isozymes from muscle extracts in *Phocoenoides dalli* (*dalli*-type) (2a). SOD isozymes appeared on the anodal side of the gel. Very rare variation in this species was detected here. Schematic drawings (2b) show the patterns detected through the species studied. For designation see Plate I, Fig. 1.

PLATE IV

Figs 1a and 1c. Electrophoretic patterns of Esterase isozymes from liver extracts in *Stenella coeruleoalba* (1a). Two Esterases appeared on the anodal side of the gel. Esterase-1 had dimeric isozymes and Esterase-2 monomeric. Two schematic drawings show the patterns detected in each isozyme system through the species studied (1b and 1c). For designation see Plate I, Fig. 1.

Figs 2a and 2b. Electrophoretic patterns of dimeric GOT isozymes from muscle extracts in *Phocoenoides dalli* (*dalli*-type) (2a). GOT-1 and -2 appeared separately on both sides of the gel. In GOT-1 patterns, three individuals were heterozygous. Schematic drawings (2b) show the patterns detected both in GOT-1 and -2 isozyme systems through the species studied. For designation see Plate I, Fig. 1.

PLATE V

Figs a to d. Electrophoretic patterns of monomeric PGM isozymes from liver extracts in *Phocoenoides dalli* (*truei*-type) (a). Heavily stained PGM-1 isozymes moved to cathodal side of the gel, and other two PGM appeared on anodal side. Three schematic drawings for each PGM isozyme system show the patterns detected through the species studied (b, c and d). For designation see Plate I, Fig. 1.

PLATE VI

Figs 1a and 1b. Electrophoretic patterns of monomeric MPI isozymes from muscle extracts in *Phocoenoides dalli* (*truei*-type) (1a). MPI was highly polymorphic in this species. Postulated genotypes of homozygotes in this zymogram are *cc* and *ff*, and heterozygotes *cf*, *df* and *fi* as shown in schematic drawings (1b). Arrow indicates a heterozygote of predominant allele *f* and a rare allele which is not named in this study. The schematic drawings show the patterns detected through the species studied. For designation see Plate I, Fig. 1.

Figs 2a and 2b. Electrophoretic patterns of dimeric PHI isozymes from liver extracts in *Phocoenoides dalli* (*dalli*-type) (2a). A heterozygote appeared. Schematic drawings (2b) show the patterns detected through the species studied. For designation see Plate I, Fig. 1.

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PLATE I

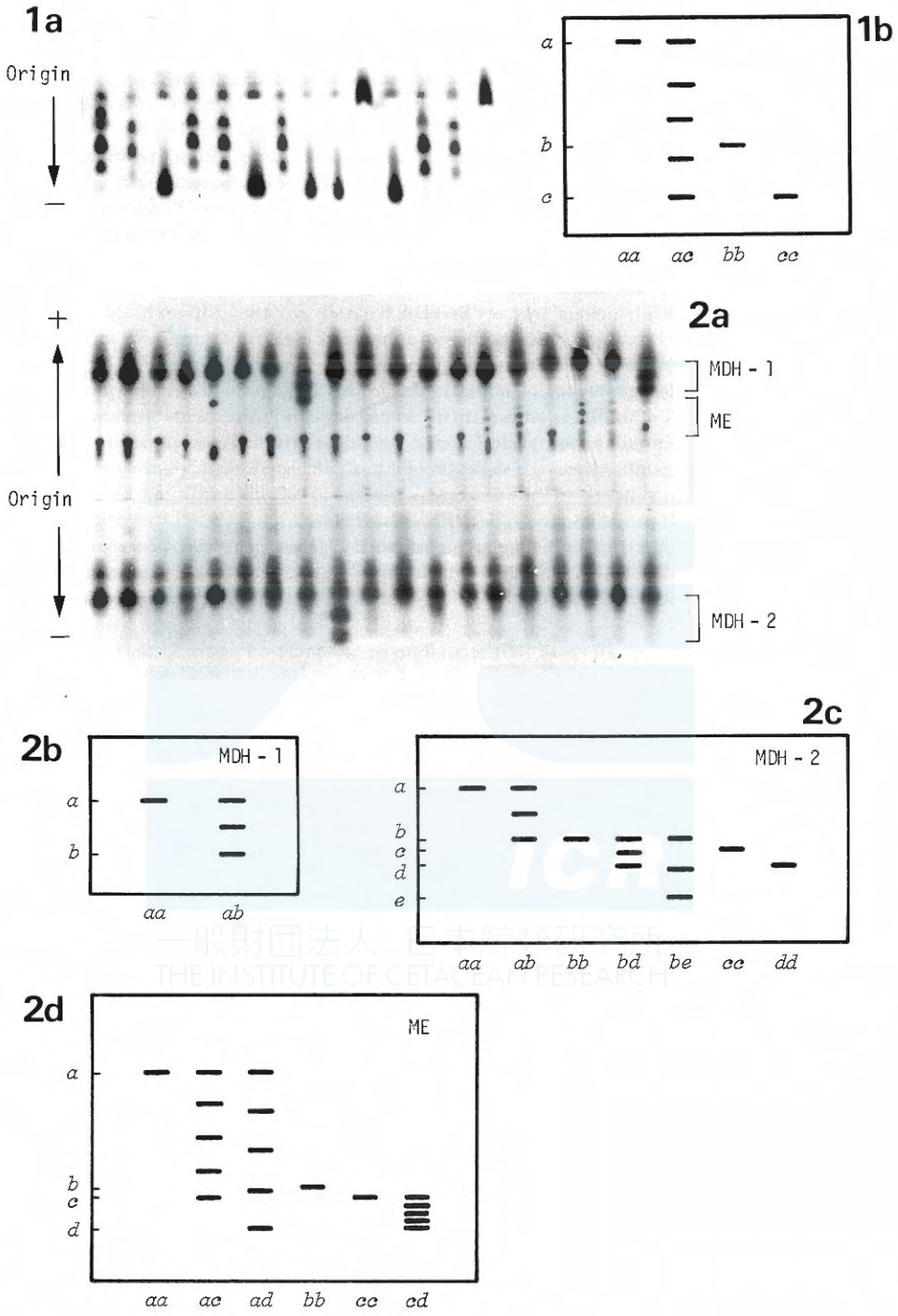

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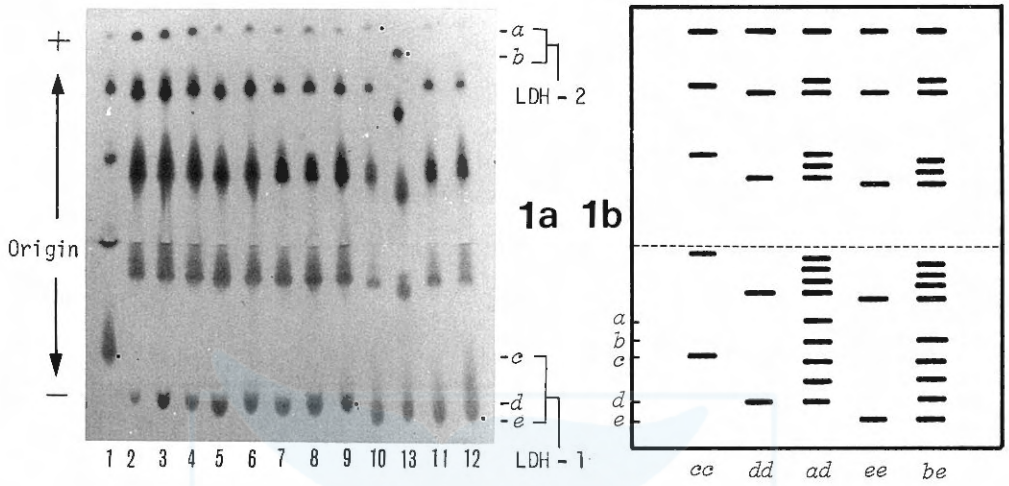
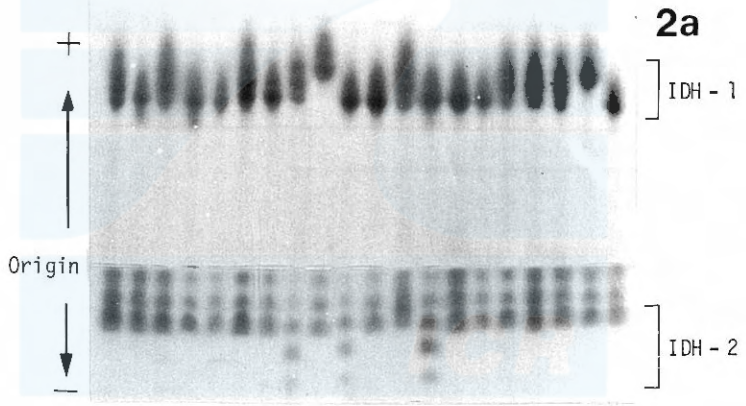
PLATE II





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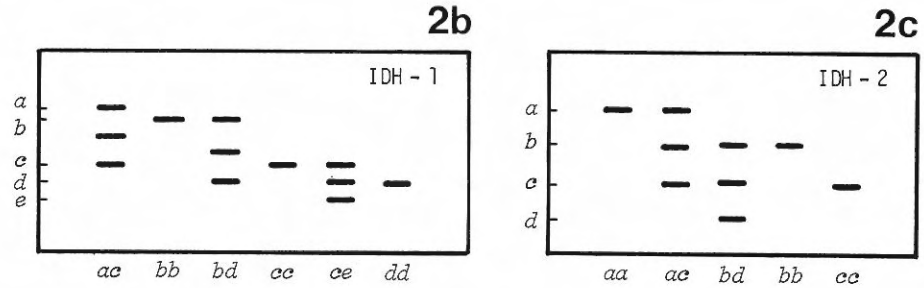

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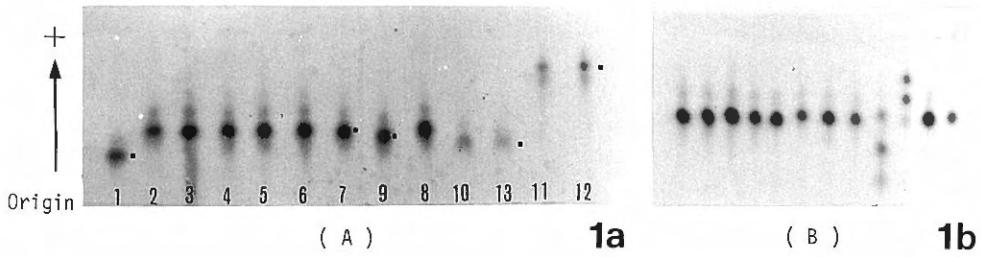

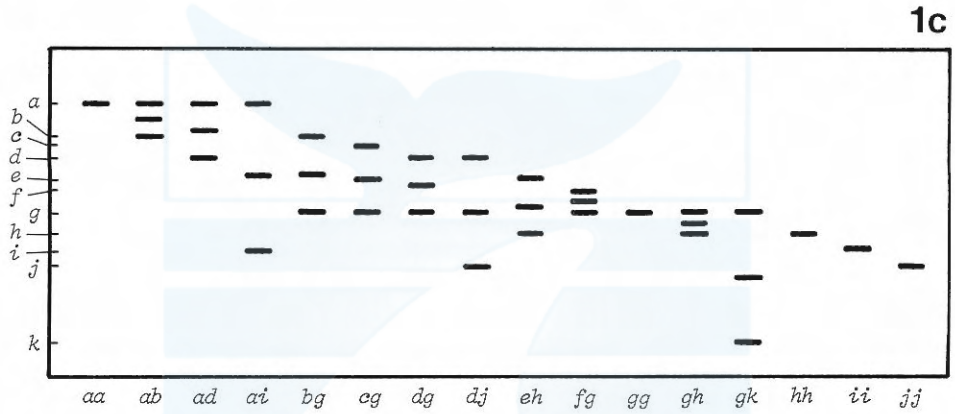

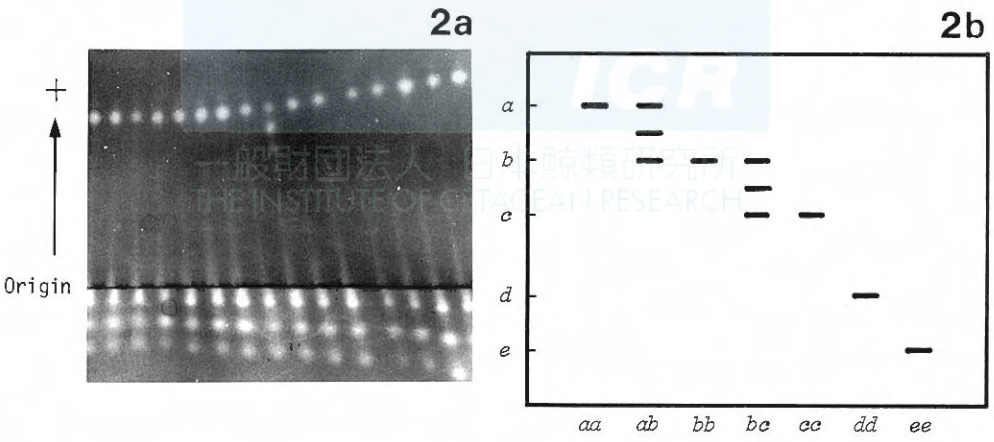

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PLLATE IV

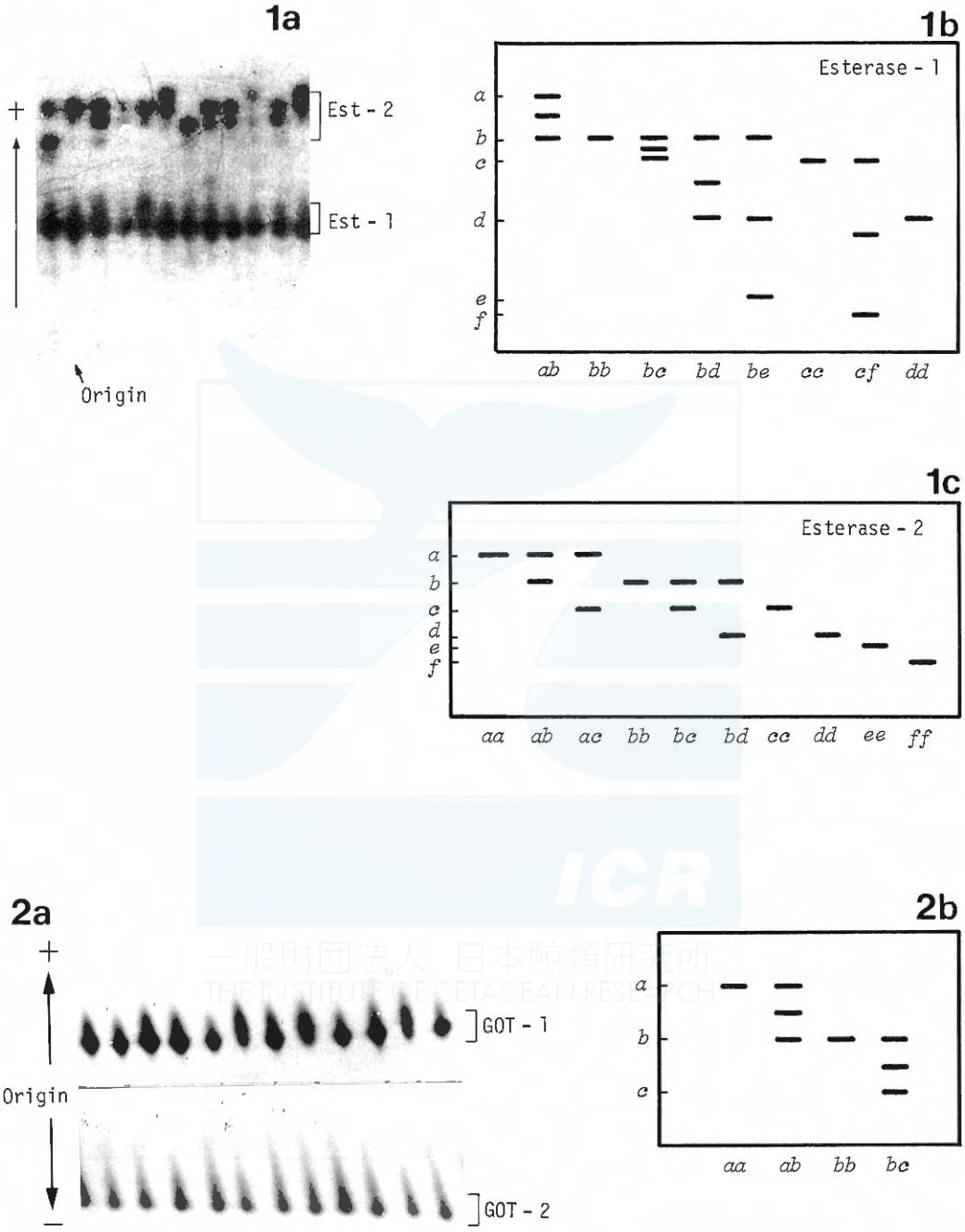

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PLATE V

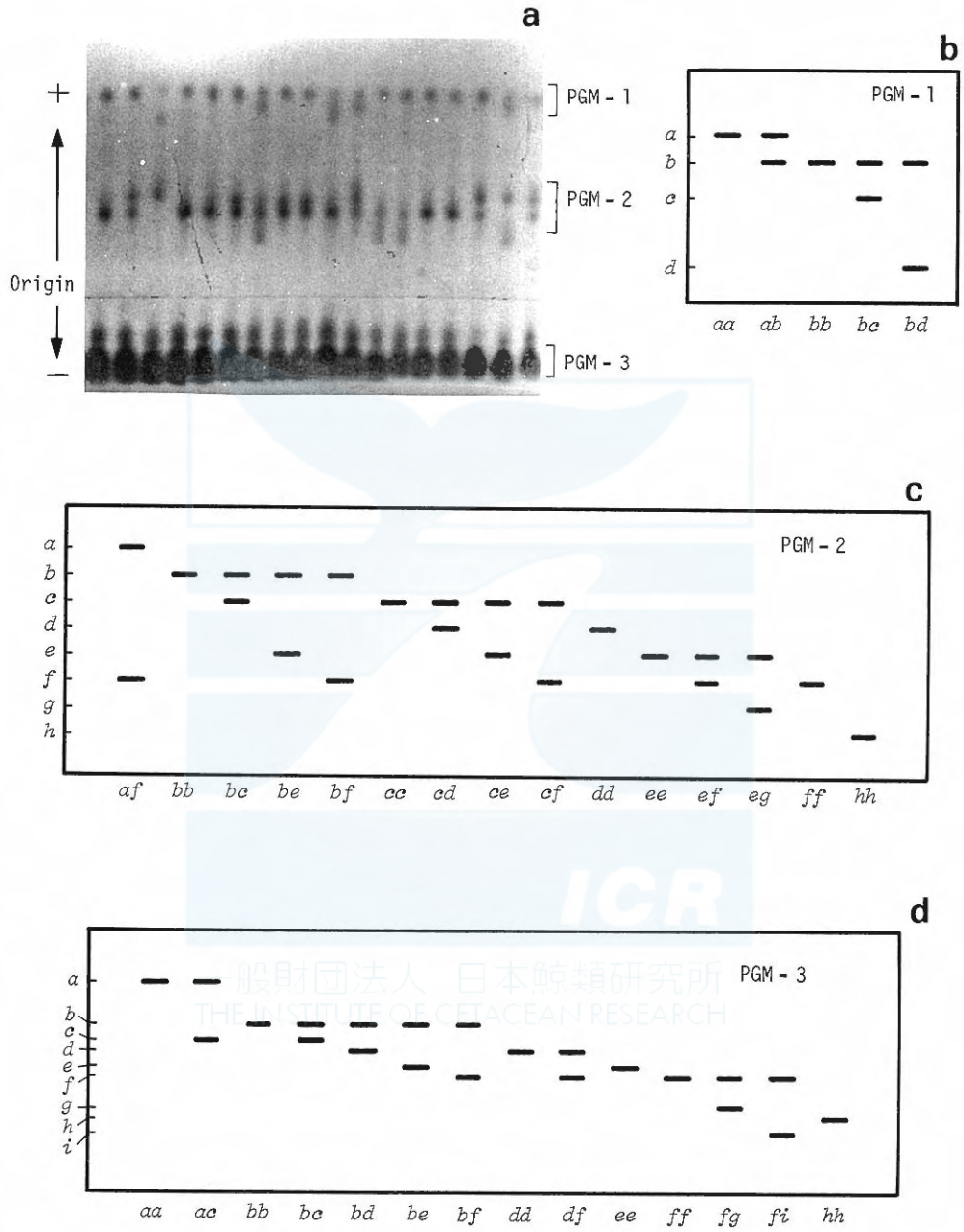

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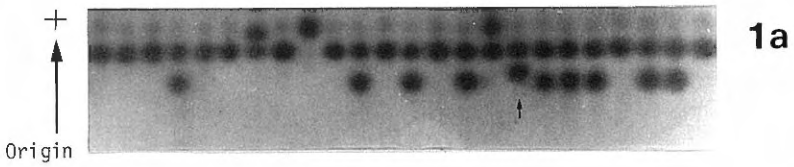
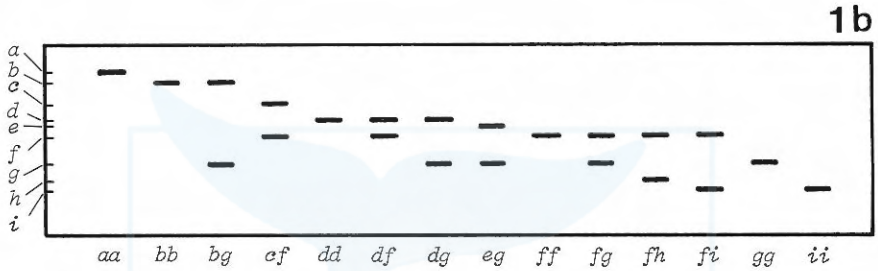
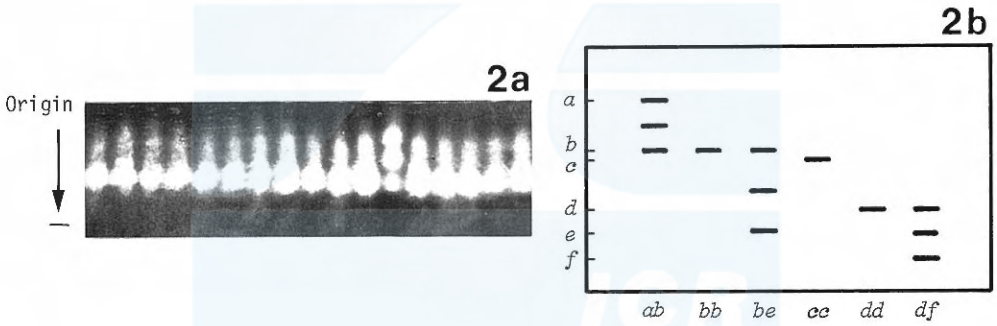
PLATE VI







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