GENETIC DIFFERENTIATION BETWEEN TWO FORMS OF SHORT-FINNED PILOT WHALES OFF THE PACIFIC COAST OF JAPAN*

SHIRO WADA**

ABSTRACT

The northern and southern forms of the short-finned pilot whale off the Pacific coast of Japan were examined by horizontal starch gel electrophoresis on 36 enzyme loci using 371 specimens. Two and five loci were variable in the northern and southern forms, respectively. The proportion of polymorphic loci under the 0.95 criterion and the value of the average heterozygosity per locus were 0.028 and 0.009 in the northern form and 0.056 and 0.008 in the southern form, respectively. No significant deviation in genotype frequencies from the Hardy-Weinberg expectations was seen in either form. Significant between-form difference in genic proportions revealed that the northern form is genetically isolated from the southern form. From the value of Nei's genetic distance, 0.0004, the degree of genetic differentiation between two forms was considered to be of the local population level.

INTRODUCTION

The short-finned pilot whale, *Globicephala macrorhynchus* Gray, 1846, is one of the important objective species for the coastal whaling in Japan, which has been harvested in two different fisheries. Two forms segregate geographically and thermally off the Pacific coast of Japan (Kasuya, Miyashita and Kasamatsu, 1987). The northern form, *tappanaga*, having been harvested by the small-type whaling (Kasuya and Tai, 1986), distributes off the Pacific coast of northern Japan between 35°N and 42°N in the surface water temperature range of 12–24°C, while the southern form, *magondo*, having been harvested by the small-type whaling and the drive fishery at Taiji (Kasuya, 1986b), distributes in the waters south of the major range of the northern form and waters over 18°C.

Form-identification is easy from the difference of external characteristics. The northern form has a larger body size, a distinct saddle mark and a round contour of the head, while the southern form has a smaller body size, an

---

* *An earlier version of this manuscript was submitted to the Scientific Committee of the International Whaling Commission, Bournemouth, May 1986 (Document No. SC/38/SM18).*

** *Far Seas Fisheries Research Laboratory, 5-7-1, Orido, Shimizu-shi, Shizuoka-ken, 424 Japan*

Sci. Rep. Whales Res. Inst.,
No. 39, 1988, 91–101

indistinct saddle mark and a square contour of adult male head (Kasuya *et al.*, 1987). However, the life history parameters of the two forms differ significantly only in those concerning to body size and to breeding season (Kasuya, 1986a). Both forms share the skull characteristics of *G. macrorhynchus* described by Bree (1971). From these, Kasuya (1986a) suggested that they can be dealt with as separate geographical forms or subspecies.

Electrophoretic survey on a large number of loci for genetic variability or genetic differentiation in cetacea have been reported on rather limited number of species; on minke whale by Wada and Numachi (1979), Simonsen, Kapel and Larsen (1982), and Wada (1982; 1983a; 1984); on fin and sei whales by Daníelsdóttir and Arnason (1987); on fin, sei, Bryde's and minke whales by Wada (1987); on striped dolphin by Wada (1983b); on Dall's porpoise by Winans and Jones (in press); on long-finned pilot whale by Andersen (1987); on 12 species of toothed whale by Shimura and Numachi (1987).

The aim of the present study is to evaluate the magnitude of genetic differentiation between two forms of short-finned pilot whales and to consider their taxonomic relationship from the viewpoint of biochemical genetics.

MATERIALS AND METHODS

Tissue samples, liver and skeletal muscle, of 204 northern forms were collected by Dr T. Kasuya and his co-workers as a part of their biological samplings from the whales caught by the small-type whaling off the Sanriku region and landed at Ayukawa in October and November 1983 (105 specimens) and 1984 (99 specimens).

Tissue samples of 167 southern forms were collected by myself from the whales driven at Taiji and flensed on 7–8 October 1984 (68 specimens from four schools in a single drive of 92 whales) and 11–12 November 1985 (99 specimens from a single drive of about 120 whales which might have been mixed with a few members of a previous drive). Form-identification for the specimens in 1984 was done by Dr N. Miyazaki and for those in 1985 by myself. All tissue samples were stored at −20°C until use.

Methods of sample and gel preparations, and electrophoresis followed the procedures described in Numachi (1974), and Shimura and Numachi (1987), and that of gel preservation in Numachi (1981). Of the 32 enzymes examined, the following three loci and five enzymes showed an indistinct electrophoretic pattern, and therefore I didn't use them in the present study though the last three enzymes were found to be variable; *Adh–2*, *Ak–1*, *Pgm–2*, Aconitase, Fumarase, Peptidases B, C and D. Table 1 shows buffers and tissues used for 36 loci on which reliable genotyping was possible. Recipes for the 100 ml reaction mixture for staining enzymes, prepared according to Harris and Hopkinson (1976) in principle are listed in Table 2.

In case of multi-loci enzymes, a most anodally migrating locus was named as 1, lesser anodal locus as 2, and so on. At a variable locus, alleles

were named alphabetically as *c*, *s*, *n* and *f* from cathode to anode. The *n* allele denotes the most frequent allele in a variable locus, or denotes the single one in a monomorphic locus.

Genotype and allele frequencies were analysed using the *G*-test (Sokal and Rohlf, 1969) for a deviation in genotype frequencies from the Hardy-Weinberg expectations within a form (G_D), and for between-form heterogeneity in gene frequencies (G_H) based on the R (number of alleles) $\times$ C (number of forms) contingency table format. *G*-values were compared with the critical values of Chi-square under (number of genotypes)−(number of alleles) degrees of freedom for G_D, and under $(R-1) \times (C-1)$ degrees of freedom for G_H.

RESULTS

Genetic variability

Genetic variation was found at two loci, *Ada* and *Sod–1*, in the northern form and at five loci, *Ada*, *Mdh–1*, *6Pgd*, *Pgm–3* and *Sod–1*, in the southern form. These loci were all biallelic. The northern form had the same alleles as the southern form at all loci except *Ada*, where a variant allele in the former (*Ada*^{s} gene) was different from that in the latter (*Ada*^{c} gene). Heterozygotes at *Ada* and *Pgm–3* showed a two-banded pattern and those at *Mdh–1*, *6Pgd* and *Sod–1* showed a three-banded pattern, suggesting that the enzymes controlled by the former two and the latter three loci are monomer and dimer, respectively (Fig. 1). The remaining 31 loci were fixed for a single allele in both forms, so that the subunit structure of enzymes controlled by these loci was unknown. These results on the southern form agreed substantially with those in Shimura and Numachi (1987) except a considerable gene frequency difference at *Ldh–A* (their *Ldh–1*) and *Pgm–2*, probably due to difference of sample size between the two studies.

The observed genotype frequencies at five variable loci agreed well with the Hardy-Weinberg expectations. Although the expectations for rare genotypes were less than five, the G_D values for 7 cases, ranged from 0.01 to 0.97, were all less than the critical values of Chi-square at the 5% level of significance (Table 3). This suggests that each sample is homogeneous.

Number of polymorphic loci under the 0.95 criterion ($P_{.95}$: loci on which the most frequent allele is less than or equal to 0.95) was very small, only one (*Ada*) in the northern form and two (*Ada* and *Sod–1*) in the southern form, though *Sod–1* of the former was nearly polymorphic. The $P_{.95}$ values were therefore 0.028 and 0.056 in the northern and southern forms, respectively. The level of average heterozygosity per locus (H) was also low due to low frequencies of variant genes. The estimates of H value calculated in the northern and southern forms were 0.009 ± 0.007 (SE) and 0.008 ± 0.005 (SE), respectively. Standard error (SE) of H was calculated using a formula of Nei and Roychoudhury (1974).

TABLE 1. LOCI EXAMINED AND ELECTROPHRETIC CONDITIONS FOR THE SHORT-FINNED PILOT WHALES

Enzyme (abbreviation)	Locus	Tissue used	buffer
Adenosine deaminase (ADA)	*Ada*	Liver	AC70
Alcohol dehydrogenase (ADH)	*Adh-1*	Liver	AC70, Mg, NAD
Adenylate kinase (AK)	*Ak-2*	Muscle	AC70
Aldolase (ALD)	*Ald*	Muscle	AC70, Mg
Creatine kinase (CK)	*Ck-1*	Liver	TEB87
	Ck-2	Muscle	TC86
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	*Gapdh*	Liver	TC86, EDTA, NAD
α-Glycerophosphate dehydrogenase (GDH)	*Gdh*	Liver	AC70, Mg, NAD
Glutamate dehydrogenase (GLUDH)	*Gludh*	Liver	TEB87
Glutamate oxaloacetate transaminase (GOT)	*Got-1*	Liver	TC80
	Got-2	Liver	AC70
Glucosephophate isomerase (GPI)	*Gpi*	Liver	TC80
Hexokinase (HK)	*Hk*	Liver	TC86
Isocitrate dehydrogenase (IDH)	*Idh-1*	Liver	AC70, Mg
	Idh-2	Liver	AC70
Lactate dehydrogenase (LDH)	*Ldh-A*	Liver	AC60
	Ldh-B	Liver	AC60
Malate dehydrogenase (MDH)	*Mdh-1*	Liver	AC60
	Mdh-2	Liver	AC60
Malic enzyme (ME)	*Me*	Liver	AC60
Mannosephosphate isomerase (MPI)	*Mpi*	Liver	AC70
NADP-dependent dehydrogenase (NDH)	*Ndh*	Liver	AC70, NADP
Peptidase-A (PEPA)	*PepA*	Liver	AC60
Peptidase-E (PEPE)	*PepE*	Muscle	AC60
Peptidase-S (PEPS)	*PepS*	Liver	TC80, Mg
6-Phophogluconate dehydrogenase (6PGD)	*6Pgd*	Liver	AC70, NADP
Phophoglucomutase (PGM)	*Pgm-1*	Liver	AC70
	Pgm-3	Liver	TC80
Pyruvate kinase (PK)	*Pk*	Muscle	AC60, Mg
Sorbitol dehydrogenase (SDH)	*Sdh*	Liver	AC70, Mg, NAD
Superoxide dismutase (SOD)	*Sod-1*	Liver	AC60
	Sod-2	Liver	AC60
	Sod-3	Liver	AC60
	Sod-4	Muscle	AC60
Xantine oxidase (XOD)	*Xod*	Liver	TEB87
Xylulose reductase (XR)	*Xr*	Liver	AC70, Mg, NADP

AC: amine-citrate buffers originally used in Clayton annd Tretiak (1972), slightly modified by Numachi, Nagahora and Iwata (1979).
Gel: 0.002 M citric acid. Electrode: 0.04 M citric acid.
AC60: adjusted with N-(3-Aminopropyl)-morpholine to pH 6.0.
AC70: adjusted with N-(3-Aminopropyl)-diethanolamine to pH 7.0.
TC: tris-citrate buffers.
TC80: originally used in Clayton and Tretiak (1972). Gel: 0.002 M citric acid. Electrode: 0.04 M citric acid. Both solutions were adjusted with Tris (hydroxymethyl) aminomethane to pH 8.0.
TC86: described in Harris and Hopkinson (1976). Electrode: 0.083 M citric acid adjusted with 0.661 M Tris (hydroxymethyl) amino-methane to pH 8.6. Gel: 27.5 times dilution of electrode buffer.

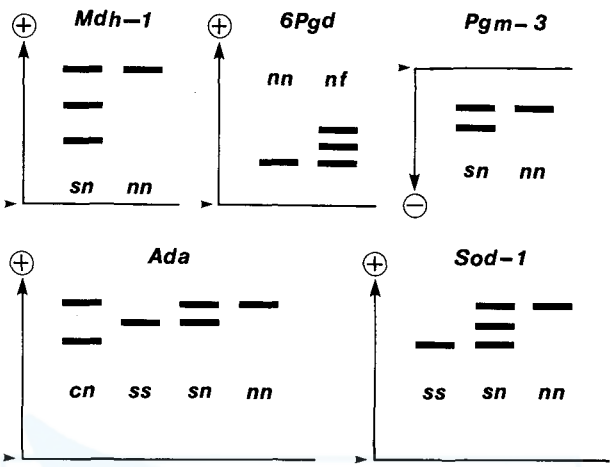

Fig. 1. Electrophoretic patterns at five variable loci in the short-finned pilot whales off the Pacific coast of Japan. The genotype of each phenotype is shown in italic. ▶: sample origin, ⊕: anode, ⊖: cathode

Genetic differentiation

A significant difference in gene frequencies between the two forms was found in the following three loci, *Ada*; G_H = 48.03, d.f. = 2, $P<0.001$, *Mdh–1*; G_H = 10.55, d.f. = 1, $P<0.01$, *6Pgd*; G_H = 4.80, d.f. = 1, $P<0.05$, but the differences at *Pgm–3* and *Sod–1* were not significant. An overall G_H value for the five variable loci was 66.96 (d.f. = 6, $P<0.001$), which indicates that the two forms are genetically isolated stocks.

The extent of genetic differntiation between two forms was measured by Nei's (1972) genetic distance (*D*), which represents the average number of electrophoretically detectable allelic substitutions per locus. It ranges from 0 (all populations share the same alleles in the same frequencies at all loci) to infinite (each population is fixed for an unique allele at all loci). The estimate of *D* value calculated using 36 loci was 0.0004.

(Footnote continued)

TEB: described in Harris and Hopkinson (1976). Stock: 0.9 M Tris (hydroxymethyl) aminomethane-0.02 M ethylenediamine tetraacetic acid disodium salt (EDTA-2Na)-0.5 M boric acid, pH 8.7. Anodal electrode: 7 times dilution of stock; cathodal electrode: 5 times dilution of stock. Gel: 20 times dilution of stock.
EDTA: gel contains 0.242 mM or 9 mg/100 ml EDTA-2Na.
Mg: gel contains 10 mM magnesium chloride.
NAD: gel and electrode buffers contain 4 mg/200 ml nicotinamide adenine dinucleotide. For GAPDH only, 15 mg/200 ml was used.
NADP: gel and electrode buffers contain 4 mg/200 ml nicotinamide adenine dinucleotide phosphate.

TABLE 2. STAIN RECIPIES FOR THE 100ml REACTION MIXTURE

Enzyme[6]	Substrate(s) 1)	Buffer	Coenzyme and coupling dye 2)	Iron 3)	Linking enzyme 4)	Other components 5)
ADA	40mg Adenosine	0.05M Phosphate, pH7.4	NBT, PMS	−	1u XOD 1u NP	2g Agar
ADH	1ml l-octanol 9ml 95% ethanol	0.1M Tris-HCl, pH8.7	NAD*, NBT, PMS	−	—	—
AK	500mg D-glucose 100mg ADP	0.1M Tris-HCl, pH8.0	NADP, NBT, PMS	+	100u HK 60u G6PD	2g Agar
ALD	200mg Fructose-1,6-diphosphate	0.1M Tris-HCl, pH8.0	NAD, NBT, PMS	−	100u GAPDH	500mg Na arsenate
CK	100mg Creatine phosphate 500mg D-glucose 100mg ADP	0.1M Tris-HCl, pH8.0	NADP, NBT, PMS	+	100u HK 60u G6PD	2g Agar
GAPDH	400mg Fructose-1,6-diphosphate	0.1M Tris-HCl, pH8.0	NAD*, NBT, PMS	−	25u ALD	500mg Na arsenate
GDH	2g α-glycerophosphate	0.1M Tris-HCl, pH8.7	NAD, NBT, PMS	−	—	1g EDTA-2Na
GLUDH	2g L-glutamate	0.1M Tris-HCl, pH8.0	NAD, NBT, PMS	−	—	—
GOT	75mg 2-oxoglutaric acid 225mg L-aspartic acid	adjusted to pH7.5 with 2M KOH	500mg Fast blue BB	−	—	100mg EDTA-2Na 3g Na phosphate monobasic
GPI	100mg Fructose-6-phosphate	0.1M Tris-HCl, pH7.4	NADP, NBT, PMS	−	60u G6PD	2g Agar
HK	2g D-glucose 200mg ATP	0.1M Tris-HCl, pH8.0	NADP, NBT, PMS	+	60u G6PD	2g Agar
IDH	200mg DL-isocitrate	0.1M Tris-HCl, pH8.0	NADP, NBT, PMS	+	—	—
LDH	2ml 50% DL-lactate	0.1M Tris-HCl, pH8.7	NAD, NBT, PMS	−	—	—
MDH	2g DL-malate	0.1M Tris-HCl, pH8.7	NAD, NBT, PMS	−	—	—
ME	2g DL-malate	0.1M Tris-HCl, pH8.0	NADP, NBT, PMS	+	—	—
MPI	100mg D-mannose-6-phosphate	0.1M Tris-HCl, pH7.4	NADP, NBT, PMS	−	60u G6PD 50u GPI	—
NDH	—	0.1M Tris-HCl, pH8.0	NADP, NBT, PMS	+	—	—
PEPA	100mg Glycyl-DL-glycine	0.05M Phosphate, pH7.4	75mg Dianisidine-HCl	+	10u AAO 100u POD	2g Agar
PEPE	100mg L-leucyl-β-naphtyl-amide as substrate in the same buffer and reagent mixture as PEPA					
PEPS	100mg L-leucyl-l-leucine as substrate in the same buffer and reagent mixture as PEPA					
6PGD	100mg 6-phospho-gluconate	0.1M Tris-HCl, pH8.0	NADP, NBT, PMS	+	—	—
PGM	200mg D-glucose-1-phosphate*	0.1M Tris-HCl, pH8.0	NADP, NBT, PMS	+	60u G6PD	—
PK	100mg Phosphoenol pyruvate 500mg D-glucose 100mg ADP	0.1M Tris-HCl, pH8.0	NADP, NBT, PMS	++	100u HK 60u G6PD	2g Agar
SDH	3g D-sorbitol	0.1M Tris-HCl, pH8.0	NAD, NBT, PMS	−	—	—
SOD	—	0.1M Tris-HCl, pH8.7	NBT, PMS	−	—	500mg EDTA-2Na
XOD	250mg Hypoxanthine	0.1M Tris-HCl, pH8.0	NBT, PMS	−	—	—
XR	3g D-xylitol	0.1M Tris-HCl, pH8.0	NADP, NBT, PMS	+	—	—

1) ADP: Adenosine-5′-diphosphate; ATP: Adenosine-5′-triphosphate; *: containing D-glucose-1,6-diphosphate. Ca. 1%.
2) NAD: 30mg Nicotinamide adenine dinucleotide; NAD*: 90mg; NADP: Nicotinamide adenine dinucleotide phosphate; NBT: 20mg Nitroblue tetrazolium; PMS: 5mg Phenazine methosulfate.
3) +: 10mM $MgCl_2$; ++: 10mM $MgCl_2$, 150mg KCl.
4) NP: Nucleoside phospholylase; G6PD: Glucose-6-phosphate dehydrogenase; AAO: Amino acid oxidase; POD: Peroxidase.
5) EDTA: Ethylenediaminetetraacetic acid.
6) see Table 1

TABLE 3. GENOTYPE AND ALLELE FREQUENCIES FOR FIVE VARIABLE LOCI IN THE NORTHERN AND SOUTHERN FORMS OF THE SHORT-FINNED PILOT WHALE OFF THE PACIFIC COAST OF JAPAN

Locus	Form	Genotypes					Allele frequencies			GD value
		cn	*ss*	*sn*	*nn*	Total	*c*	*s*	*n*	
Ada	N	0	1	17	51	69	.000	.138	.862	0.64
	S	13	0	0	86	99	.066	.000	.934	0.91
			ss	*sn*	*nn*	Total		*s*	*n*	
Mdh-1	N		0	0	154	154		.000	1.000	
	S		0	8	159	167		.024	.976	0.20
			nn	*nf*	*ff*	Total		*n*	*f*	
6Pgd	N		204	0	0	204		1.000	.000	
	S		164	3	0	167		.991	.009	0.03
			ss	*sn*	*nn*	Total		*s*	*n*	
Pgm-3	N		0	0	204	204		.000	1.000	
	S		0	2	165	167		.006	.994	0.01
			ss	*sn*	*nn*	Total		*s*	*n*	
Sod-1	N		0	18	186	204		.044	.956	0.83
	S		1	16	150	167		.054	.946	0.97

S: southern form. N: northern form. GD: G-value for deviation in genotype frequencies from the Hardy-Weinberg expectations within a form.

DISCUSSION

Reliable estimates of average heterozygosity for marine mammals have been reported from nine species of pinniped, four species of baleen whale and 12 species of toothed whale. Table 4 summarises the level of their genetic variabilities. Mean H value for pinnipeds is 0.019, ranging from 0 in northern elephant seal, *Mirounga angustirostris* (Bonnell and Selander, 1974) to 0.047 in ribbon seal, *Phoca fasciata* (Fujio and Saito, 1986). Wada (1987) calculated H values for three stocks of fin whale, *Balaenoptera physalus*, two stocks of sei whale, *B. borealis*, five stocks of Bryde's whale, *B. edeni*, and three stocks of minke whale, *B. acutorostrata*, which allowed to calculate the mean values of H for fin, sei, Bryde's and minke whales as 0.017, 0.021, 0.022 and 0.051, respectively. Simonsen *et al.* (1982) reported $H = 0.046$ for the Atlantic minke whales. Using these values, the mean H values for four stocks of minke whales and four baleen whale species have been calculated as 0.050 and 0.028, respectively.

On toothed whales, Wada (1983b) reported the H value in striped dolphin, *Stenella coeruleoalba*, off the Pacific coast of Japan as 0.021. Shimura and Numachi (1987) calculated H values on 12 species in 11 genera from 4 families, which ranged from 0 in finless porpoise, *Neophocaena phocaenoides*, to 0.154 in Dall's porpoise, *Phocoenoides dalli* (*dalli*-type). Winans and Jones (in press) also

TABLE 4. SUMMARY OF THE GENETIC VARIABILITY IN MARINE MAMMALS

Species	No. loci examined	$P_{.95}$	H	Reference
Seals				
Mirounga angustirostris	24	0.000	0.000	Bonnell and Selander (1974)
M. leonina	18	0.278	0.030	McDermid, Ananthakrishnan and Agar (1972)
Odobenus rosmarus rosmarus	32	0.094*	0.026	Simonsen, Born and Kristensen (1982)
Pagophilus groenlandicus	21	0.048*	0.007	Simonsen, Allendorf, Eanes and Kapel (1982)
Cystophora cristata	21	0.048*	0.009	Simonsen, *et al.* (1982)
Pusa hispida	21	0.095*	0.009	Simonsen, *et al.* (1982)
Phoca fasciata	34	0.147	0.047	Fujio and Saito (1986)
P. viturina stejnegeri	34	0.059	0.023	Fujio and Saito (1986)
P. largha	34	0.059	0.017	Fujio and Saito (1986)
Baleen whales				
Balaenoptera physalus[1)]	45	0.138*	0.017	Wada (1987)
B. borealis[2)]	45	0.158*	0.021	Wada (1987)
B. edeni[3)]	45	0.114*	0.022	Wada (1987)
B. acutorostrata[1)]	45	0.207*	0.051	Wada (1987)
B. acutorostrata	21	0.095*	0.046	Simonsen, *et al.* (1982)
Toothed whales				
Berardius bairdii	18	0.056	0.016	Shimura and Numachi (1987)
Globicephala macrorhynchus (N.F.)	36	0.028	0.009	Present study
G. macrorhynchus (S.F.)	36	0.056	0.008	Present study
G. macrorhynchus (S.F.)	19	0.263	0.054	Shimura and Numachi (1987)
Peponocephala electra	19	0.105	0.035	Shimura and Numachi (1987)
Pseudorca crassidens	19	0.211	0.051	Shimura and Numachi (1987)
Stenella coeruleoalba	15	0.130	0.021	Wada (1983b)
S. coeruleoalba	19	0.263	0.089	Shimura and Numachi (1987)
S. attenuata	19	0.263	0.089	Shimura and Numachi (1987)
Tursiops truncatus	19	0.105	0.039	Shimura and Numachi (1987)
Lagenorhynchus obliquidens	19	0.316	0.093	Shimura and Numachi (1987)
Steno bredanensis	19	0.053	0.007	Shimura and Numachi (1987)
Phocoena phocoena	18	0.167	0.047	Shimura and Numachi (1987)
Phocoenoides dalli (*dalli*-type)	26	0.231	0.058	Winans and Jones (in press)
P. dalli (*dalli*-type)	19	0.421	0.154	Shimura and Numachi (1987)
P. dalli (*truei*-type)	19	0.474	0.147	Shimura and Numachi (1987)
Neophocaena phocaenoides	18	0.000	0.000	Shimura and Numachi (1987)

* under the 0.99 criterion
1) mean value for three stocks. 2) mean value for two stocks. 3) mean value for five stocks.
N.F. = the northern form. S.F. = the southern form.

examined other specimens of the latter species from three areas in the North Pacific and Bering Sea, and calculated H value as 0.058. However, there are large differences in the reported values of $P_{.95}$ and H for *S. coeruleoalba*, *P. dalli* (*dalli*-type) and *G. macrorhynchus* between authors (Table 4). However, comparing the common loci only, these studies show very similar results apart from a few loci where sample size is small. The mean of the above 17 H values for 13 toothed whale species (Table 4) is 0.054, which is higher than that for

pinnipeds ($\bar{H}$ = 0.019), baleen whales ($\bar{H}$ = 0.028) and 184 mammalian species ($\bar{H}$ = 0.041; Nevo, Beiles and Ben-Shlomo, 1984).

Selander and Kaufman (1973) suggested that large, mobile animals generally have lower levels of genetic variability than smaller, less mobile ones. However, both forms of the short-finned pilot whale showed almost the lowest levels of genetic variability among cetacean species, which were lower than Baird's beaked (*Berardius bairdii*), Bryde's, sei and fin whales that have a larger body size thereby a lower level of genetic variability than the pilot whales is predicted.

Nei (1975) showed the *D* values to correlate with the systematic distance, i.e., 0–0.058 of interpopulation level, 0.004–0.351 (mainly 0.1–0.2) of intersubspecies level and 0.05–2.73 (mainly 0.5–2.0) of interspecies level. However, Avise and Aquadro (1982) showed that the degree of genetic differentiation differ considerably from species to species, and that the mean *D* value between species was 1.75 in amphibia, 0.67 in reptilia, 0.41 in mammalia and 0.08 in Aves.

In cetacea, however, much smaller *D* values have been reported between populations. Among five stocks of Bryde's whale in the Indian and Pacific Oceans, Wada (1987) obtained $\bar{D}$ = 0.0029, ranging from 0.0003 to 0.0050. The *D* value between two color types of the Dall's porpoise was 0.004 (Shimura and Numachi, 1987). Andersen (1987) calculated the genetic distance among nine schools of the long-finned pilot whale, *Globicephala melaena*, around the Faroe Islands. The largest *D* value estimated from her gene frequency data is 0.0032. The *D* value of 0.0004 calculated in the present study was in the lower range of above values.

Nei and Roychoudhury (1974) and Nei (1978) studied on the sampling variance of *D*, and decomposed a total variance into the intralocus and interlocus variances. The former is due to a restricted number of genes sampled for each locus, and is almost negligible when the sample size is more than 50. The latter is due to a restricted number of loci sampled from the genome. Therefore, the reliability of the estimate of *D* mainly depends on the number of loci or chance of sampling particular loci.

In cetacea examined to date, the most frequent allele at each locus was common in all populations within a species except in minke whales. This yielded a small value of a single-locus distance; the largest ones between populations were 0.0034 at *Me* in fin whale, 0.0150 at *Ada* in short-finned pilot whale, 0.0211 at *Pgm–3* in Dall's porpoise, 0.1164 at *Sdh* in sei whale and 0.1316 at *Pgm–1* in Bryde's whale. If a hypothetical *D* is calculated between the two forms of the short-finned pilot whales off Japan assuming an additional 37th locus with a single-locus distance value of 0.15, tenfold of the actually observed value, the estimate of *D* over all loci increases from 0.0004 to 0.0040. Even under this extreme assumption, the *D* value is still smaller than the smallest ones obtained from species comparison; *D* = 0.026 between striped dolphin, *Stenella coeruleoalba* and spotted dolphin, *S. attenuata*, and *D*

= 0.047 between sei and Bryde's whales. From this, I conclude that the extent of genetic differentiation between the two forms of the short-finned pilot whale is nothing more than the level of interpopulation. It is quite reasonable to say that their current taxonomic relationship is the two local forms within a species. This conclusion coincides with the results of the comparative studies on life history parameters (Kasuya, 1986a) and on morphology and segregation (Kasuya *et al.*, 1987) between the two forms.

ACKNOWLEDGEMENTS

I wish to thank the following three persons: Dr T. Kasuya, who identified the whales at Ayukawa as northern form, collected tissue samples from them, and read the manuscript critically. Dr N. Miyazaki, who identified the whales at Taiji in 1984 as southern form and helped me in collecting tissue samples from them. Miss T. Kohno, who helped me in eletrophoresis.

REFERENCES

ANDERSEN, L.W. 1987. An investigation of the population structure of the long-finned pilot whales, *Globicephala melaena*, around the Faroe Islands on basis of isozymes. IWC/SC/39/SM13. 14pp.

AVISE, J.C. and C.F. AQUADRO, 1982. A comparative summary of genetic distances in the vertebrates. *Evol. Biol.*, 15: 151–185.

BONNELL, M.L. and R.K. SELANDER, 1974. Elephant seals: genetic variation and near extinction. *Science*, 184: 908–909.

BREE, P.J.H. VAN, 1971. On *Globicephala sieboldii* Gray, 1946, and other species of pilot whales. *Beaufortia*, 19(247): 79–87.

CLAYTON, J.W. and D.N. TRETIAK, 1972. Amine-citrate buffers for pH controls in starch gel electrophoresis. *J. Fish. Res. Bd. Can.*, 29: 1169–1172.

DANIELSDOTTIR, A.K. and A. ARNASON, 1987. Progress report on the study of genetic variation at enzyme loci in fin whales (*Balaenoptera physalus*) and sei whales (*Balaenoptera borealis*). IWC/SC/39/Ba8. 15 pp.

FUJIO, Y. and Y. SAITO, 1986. Population genetics in harbor seal, *Phoca vitulina*, spotted seal, *Phoca largha* and ribbon seal, *Phoca fasciata*. pp. 195–213. *In*: K. WADA, T. ITO, A. NIIZUMA, S. HAYAMA, and M. SUZUKI, (eds.) *Ecology and Conservation for Harbor Seals*. Tokai Univ. Press, Tokyo. 418 pp. (in Japanese)

HARRIS, H. and D.A. HOPKINSON, 1976. *Handbook of Enzyme Electrophoresis in Human Genetics*. North-Holland, Amsterdam.

KASUYA, T. 1986a. Comparison of life history parameters between two stock of short-finned pilot whales off the Pacific coast of Japan. IWC/SC/38/SM10. 32 pp.

KASUYA, T. 1986b. Japanese fisheries exploiting southern form short-finned pilot whales. IWC/SC/38/SM19. 12 pp.

KASUYA, T., T. MIYASHITA and F. KASAMATSU, 1988. Segregation of two forms of short-finned pilot whales off the Pacific coast of Japan. *Sci. Rep. Whales Res. Inst.*, 39: 77–90.

KASUYA, T. and S. TAI, 1986. Operation of Japanese small-type whaling on the short-finned pilot whale off the northeastern Japan. IWC/SC/38/SM16. 16 pp.

MCDERMID, E.M., R. ANANTHAKRISHNAN and N.S. AGAR, 1972. Electrophoretic investigation of plasma and red cell proteins and enzymes of Macquarie Island elephant seals. *Anim. Blood Grps. Biochem. Genet.*, 3: 85–94.

NEI, M. 1972. Genetic distance between populations. *Amer. Natur.*, 106: 282–292.

NEI, M. 1975. *Molecular Population Genetics and Evolution*. North-Holland, Amsterdam, 288 pp.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89: 583–590.

NEI, M. and A.K. ROYCHOUDHURY, 1974. Sampling variances of heterozygosity and genetic distance. *Genetics*, 76: 379–390.

NEVO, E., A. BEILES and R. BEN-SHLOMO, 1984. The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. pp. 13–213. *In*: G.S. MANI (ed.) *Lecture Notes in Biomathematics*, 53. Springer-Verlag, Berlin. 312 pp.

NUMACHI, K. 1974. Genetic characteristics of fish population. pp. 5–36. *In*: M. NISHIWAKI (ed.) *Shigen-seibutsuron*. Tokyo University Press, Tokyo, 214 pp. (in Japanese).

NUMACHI, K. 1981. A simple method for preservation and scanning of starch gel. *Biochem. Genet.*, 19: 233–236.

NUMACHI, K., S. NAGAHORA and M. IWATA, 1979. Genetic demonstration of hybrids between chum and pink salmon in the northwest Pacific. *Otsuchi Mar. Res. Cent. Rep.*, 5: 87–102.

SELANDER, R.K. and D.W. KAUFMAN, 1973. Genetic variability and strategies of adaptation in animals. *Proc. Nat. Acad. Sci. USA*, 70: 1875–1877.

SIMONSEN, V., F.W. ALLENDORF, W.F. EANES and F.O. KAPEL, 1982. Electrophoretic variation in large mammals. III. The ringed seal, *Pusa hispida*, the harp seal, *Pagophilus groenlandicus*, and the hooded seal, *Cystophora cristata*. *Hereditas*, 97: 87–90.

SIMONSEN, V., E.W. BORN and T. KRISTENSEN, 1982. Electrophoretic variation in large mammals. IV. The Atlantic walrus, *Odobenus rosmarus rosmarus* (L.). *Hereditas*, 97: 91–94.

SIMONSEN, V., F. KAPEL and F. LARSEN, 1982. Electrophoretic variation in the minke whale, *Balaenoptera acutorostrata* Lacépède. *Rep. int. Whal. Commn*, 32: 275–278.

SHIMURA, E. and K. NUMACHI, 1987. Genetic variability and differentiation in the toothed whales. *Sci. Rep. Whales Res. Inst.*, 38: 141–163.

SOKAL, R.R. and F.J. ROHLF, 1969. *Biometry*. W.H. FREEMAN and Co., San Francisco. 859 pp.

WADA, S. 1983a. Genetic structure and taxonomic status of minke whales in the coastal waters of Japan. *Rep. int. Whal. Commn*, 33: 361–363.

WADA, S. 1983b. Genetic heterozygosity in the striped dolphin off Japan. *Rep. int. Whal. Commn*, 33: 617–619.

WADA, S. 1987. biochemical analyses of genetic differentiation among the populations and species in *Balaenoptera*. *Doctoral thesis submitted to Faculty of Agriculture, University of Tokyo*, 145 pp. (in Japanese).

WADA, S. and K. NUMACHI, 1979. External and biochemical characters as an approach to stock identification for the Antarctic minke whale. *Rep. int. Whal. Commn*, 29: 421–432.

WINANS, G.A. and L. JONES (in press). Electrophoretic variability in the Dall's porpoise (*Phocoenoides dalli*) in the North Pacific Ocean and Bering Sea. *J. Mamm.*