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Genetic Variation of the MHC *DQB* Locus in the Finless Porpoise (*Neophocaena phocaenoides*)

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The Major Histocompatibility Complex (MHC) is a large multigene coding for glycoproteins that play a key role in the initiation of immune responses in vertebrates. The exon 2 region of the MHC *DQB* locus was analyzed using 160 finless porpoises from 5 populations in Japanese waters. The 5 populations were based on a previous mitochondrial DNA control region analysis, which showed distinct geographical separation. Eight *DQB* alleles were detected, and the geographical distribution of the alleles indicated that most of them are shared among the populations. Heterozygosity of the *DQB* alleles in each population ranged from 0.55 to 0.78, and for all 5 populations was 0.78. Low MHC variability is not a common feature in marine mammals, but the finless porpoise populations inhabiting coastal waters had a relatively high MHC heterozygosity. Balancing selection in the MHC *DQB* alleles of the finless porpoise was indicated by the higher rate of nonsynonymous than synonymous substitutions for PBR; however, an excess of hetrozygotes compared to expectation was not observed. This suggests that the MHC *DQB* locus in the finless porpoise may have been under balancing selection for a long evolutionary time period, and is influenced by genetic drift beyond the effect of balancing selection for short time periods in small local populations.

Key words: balancing selection, cetacean, *DQB* locus, finless porpoise, major histocompatibility complex (MHC)

INTRODUCTION

The Major Histocompatibility Complex (MHC) is a large multigene coding for glycoproteins that play a key role in the initiation of immune responses in vertebrates. In humans, three class II loci (*DP, DQ* and *DR*) are known to code for alpha and beta glycopeptide chains. These peptides form cell-surface heterodimers on antigen-presenting cells, which bind processed antigen peptide fragments (Germain and Margulies, 1993; Harding, 1996).

In most mammals, high levels of genetic variation in MHC classes I and II have been interpreted to be an adaptation for protection against a large diversity of pathogens (Klein and Takahata, 1990). MHC diversity at these immune system loci may reflect the multiplicity of pathogenic mechanisms and various associations between specific infectious diseases. Several MHC genotypes have been identified, including those conferring resistance to malaria (Hill *et al.*, 1991) and HIV-1 (Kaslow *et al.*, 1996). Partial correlation analysis on sticklebacks (fish) revealed the influence of parasite diversity on MHC class IIB variation (Wegner *et al.*, 2003).

Hedrick's (1999) review showed that high polymorphism of the MHC is maintained by balancing selection through heterozygote advantage or frequency-dependent selection, maternal-fetal interaction, and negative-assortative mating (Hughes and Nei, 1989, 1990; Takahata and Nei, 1990; Hill *et al.*, 1992; Edwards and Potts, 1996). The high proportion of nonsynonymous changes in the peptide-binding residues (PBR) (Hughes and Nei, 1988) in the second exon of the beta genes supports the idea of heterozygote advantage. It has been suggested that the allelic variability of the MHC should be taken into account in conservation, such as in the management of captive breeding programs (Hughes, 1991). A deficit in variation at the MHC loci may increase the risk of extinction of isolated populations (Yuhki and O'Brien, 1990).

MHC variation has been analyzed in a few species of cetaceans. Trowsdale (1989) analyzed the MHC Class I and II loci of the fin whale (*Balaenoptera physalus*) and the sei whale (*B. borealis*) using the RFLP method. He found a rel-

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atively low degree of polymorphism for these two cetacean species at these loci. Murray *et al.* (1995, 1998, 1999) used the SSCP method for analysis of genetic variation in the class II *DQB* locus in white whales (*Delphinapterus leucas*) and narwhals (*Monodon monoceros*). These authors also found lower variation in marine mammals than terrestrial mammals. In addition to sequence analyses, the class I gene has been sequenced for the bottlenose dolphin (*Tursiops truncatus*) (Shirai *et al.*, 1998) and the grey whale (*Eschrichtius robustus*) (Flores-Ramirez *et al.*, 2000). Both sequences indicate a structural similarity to those of terrestrial mammals.

In a previous study, Hayashi *et al.* (2003) amplified the *DQB* exon 2 locus using primers reported by Kimura and Sasazuki (1992) and detected several alleles from each of 16 cetacean species. The study found that the frequency of nonsynonymous substitutions was significantly higher than that of synonymous substitutions in the MHC locus analyzed. Amino acid variability in the PBR was notably high. Trans-species polymorphisms (Klein, 1987) were also found. These results imply that positive selection has promoted variability in the cetacean *DQB* gene as for other mammalian MHCs.

For the investigation of MHC genetic variation in local populations of a species, there are primarily two conditions that must be met. First, information on genetic structure from neutral regions, such as the mitochondrial DNA (mtDNA) control region, is important for knowing how the MHC variation is maintained through the history of a wild population. Second, it is necessary to analyse a substantial number of animals in each population to compare the levels of MHC variation within the population or between populations. The finless porpoise (*Neophocaena phocaenoides*) is one of the few cetacean species that can meet these conditions. The finless porpoise populations in Japanese waters have been extensively investigated by Kasuya and Kureha (1979), Shirakihara *et al.* (1992, 1993, 1994), and Yoshida *et al.* (1995, 1997, 1998). In our study, 160 samples were obtained from a previous study by Yoshida *et al.* (2001), who analysed the genetic structure of the finless porpoise using mtDNA sequences.

The finless porpoise is a small member of the Odontoceti (toothed whales) and is distributed throughout tropical and subtropical coastal waters from the Persian Gulf to Japan. According to Shirakihara *et al.* (1994), Japanese finless porpoises are limited to areas less than 50 m in depth and with either a sandy or soft sediment seabed. Such areas are usually also areas with intensive human activity, and the porpoise has been threatened by habitat degradation, water pollution, and bycatch. In Japan, the finless porpoise is mainly distributed in five coastal regions (Shirakihara *et al.*, 1994; Yoshida *et al.*, 1997): Sendai Bay-Tokyo Bay (unknown population size), Ise-Mikawa Bays (about 1900 individuals), Inland Sea-Hibiki Nada (about 4900 individuals), Omura Bay (about 190 individuals), and Ariake Sound-Tachibana Bay (about 3100 individuals). The mtDNA analysis suggested that each population has a distinct haplotype distribution (Yoshida *et al.*, 2001).

The finless porpoise is listed in Appendix I of the Convention on International Trade in Endangered Species (CITES) along with all other cetaceans, and has been identified as 'possibly being at risk' by the International Union for Conservation of Nature and Natural Resources (IUCN) (Reeves and Leatherwood, 1994). The Fisheries Resources Protection Law of Japan prohibits any capture of this species in Japanese waters. Additionally, the animals at Awashima in the Inland Sea were designated a natural treasure of Japan in 1930.

In this study, we conducted an intensive analysis of the MHC class II *DQB* gene exon 2 locus, which has one of the highest levels of variation among MHC genes in the Japanese finless porpoise, with the goal of verifying the degree of MHC variation and examining the geographic distribution of MHC alleles in this species.

MATERIALS AND METHODS

The finless porpoise samples are the same ones used in the mtDNA sequence analysis by Yoshida *et al.* (2001). A total of 160 individuals (Table 1) were originally obtained from the five coastal populations (Fig. 1-A): Sendai Bay-Tokyo Bay (n=18), Ise-Mikawa Bay (n=49), Inland Sea-Hibiki Nada Bay (n=26), Omura Bay (n=6), and Ariake Sound-Tachibana Bay (n=61).

The *DQB* exon 2 region was amplified using primers *DQB*-AMP-A (5'-CATGTGCTACTTCACCAACGG-3' and *DQB*-AMP-B (5'-CTGGTAGTTGTGTCTGCACAC-3') (Kimura and Sasazuki, 1992) and *Ex-Taq* (TaKaRa Bio. Inc.) which has proofreading activity, as in the previous study by Hayashi *et al.* (2003).

Initially, 50 individuals were randomly chosen from among all populations to determine heterozygotes and homozygotes by means of TA cloning. PCR products were cloned into pCR 2.1 vector using TOPO TA cloning kits (Invitrogen Corp.). Ten to 15 colonies were checked for the correct sized PCR product of 172 bp. After the insert check, at least 5 correctly sized products were

Table 1. Sample sizes and the number of alleles in each of the 5 populations of finless porpoises in Japanese waters. The frequencies of the 8 MHC *DQB* exon 2 region alleles are indicated for each allele, *Neph-a* through *Neph-h*, in each of the populations. H_E is expected heterozygosity, H_O is the observed heterozygosity, and the nucleotide diversity is shown in % for each population.

Areas	No. of samples	No. of alleles	Frequency in each region								Heterozigosity		Nucleotide diversity(π)
			Neph-a	*Neph-b*	*Neph-c*	*Neph-d*	*Neph-e*	*Neph-f*	*Neph-g*	*Neph-h*	H_E	H_O	
Sendai Bay-Tokyo Bay	18	5	0.194	0.361	0.056	0.028	0.361	0.000	0.000	0.000	0.72	0.50*	1.27
Ise-Mikawa Bays	49	5	0.011	0.245	0.000	0.112	0.622	0.010	0.000	0.000	0.55	0.47	1.15
Inland Sea-Hibiki Nada	26	8	0.115	0.500	0.135	0.096	0.077	0.039	0.019	0.019	0.71	0.77	0.64
Omura Bay	6	4	0.000	0.083	0.000	0.250	0.333	0.333	0.000	0.000	0.78	0.67	1.10
Ariake Sound-Tachibana Bay	61	6	0.000	0.066	0.008	0.508	0.156	0.172	0.091	0.000	0.68	0.57*	0.91
Total	160	8	0.044	0.225	0.031	0.256	0.316	0.088	0.038	0.003	0.78	0.57	1.21

* Significant difference (p<0.05) between H_O and H_E

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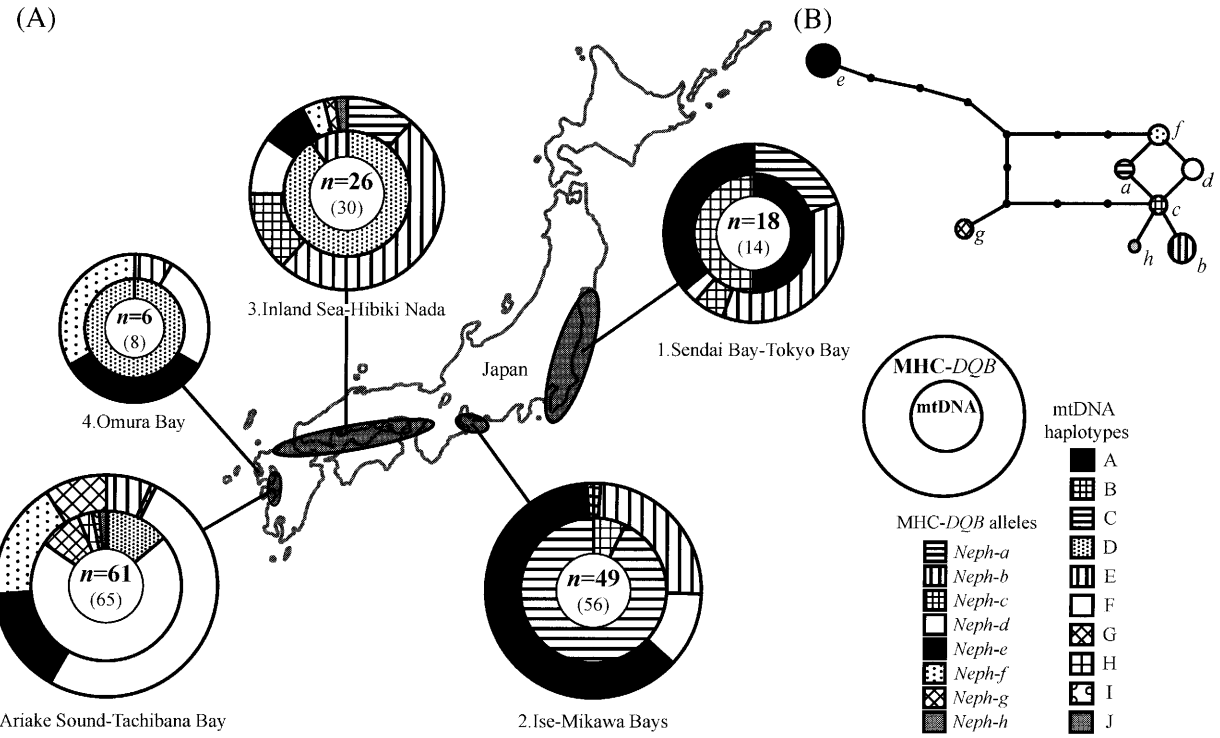

Fig. 1. (A) Sampling locations and geographical distribution of MHC *DQB* alleles (outer circle) and haplotypes of the mtDNA control region (inner circle: Yoshida *et al.*, 2001) for 5 populations of finless porpoise (*Neophocaena phocaenoides*) in Japanese waters. (B) Network tree based on MHC *DQB* alleles of the finless porpoise.

sequenced for each individual. Heterozygote and homozygote identifications for the remaining 110 individuals were determined by direct sequencing.

PCR products used for sequencing were purified with a PCR Product Pre-sequencing kit (USB Corp.), and cycle sequencing was performed with a DTCS Quick Start kit containing Dye Terminators (Beckman Coulter Inc.). Sequencing was conducted with a CEQ2000XL DNA auto-sequencer (Beckman Coulter Inc.).

Identified *DQB* allele sequences were aligned using GENETYX-MAC software, Ver. 11.1 (Software Development Co. Ltd.) and translated into amino acid sequences using the HLA-*DQB*1 amino acid sequence as a reference (Brown *et al.*, 1993). MEGA version 2.1 (Kumar *et al.*, 2001) software was used to determine the ratio of synonymous substitutions per synonymous site (d_S) and nonsynonymous substitution per nonsynonymous site (d_N) within and between species, as calculated by the Nei-Gojobori method (Nei and Gojobori, 1986) with the Jukes-Cantor correction and 500 bootstrap replications.

As an indicator for genetic diversity, the Hardy-Weinberg test for expected heterozygosity (h_e) (Nei and Tajima, 1981) and the test of statistical significance of heterozygote deficit were conducted using Genepop web version 3.4 (Raymond and Rousset 1995; http://wbiomed.curtin.edu.au/genepop/).

Nucleotide diversities (π) were calculated using the formula $\pi=\Sigma x_i x_j d_{ij}\ n\ /\ (n\text{-}1)$, where x_i is the frequency of genotype i in the population x, x_j is the frequency of type j in the same population x, and d_{ij} is the nucleotide substitution distance between genotypes i and j (Nei and Jin, 1989).

Using the Arlequin 2.0 program (Schneider *et al.*, 2000), the level of genetic differentiation between population pairs was estimated as Wright's *F*-statistics (F_{ST}: Wright 1951), and statistical significance of F_{ST} was tested by permutation of the alleles between the populations. The Ewens-Watterson test (Ewens 1972; Watterson, 1978) was applied to determine whether the distribution of allele frequencies was different from that expected by neutrality. Geographic heterogeneity of the frequency distribution of alleles was tested using the exact test. A network tree was constructed using the TCS network method (Clement *et al.*, 2000) with a 90% connection limit.

RESULTS

Detection of *DQB* alleles

Sequences of the *DQB* exon 2 alleles for 50 individuals representing 5 populations of finless porpoise were determined following TA cloning. The analysis of multiple clones from each individual did not reveal more than two different alleles. Alignment of the 172 bp nucleotide sequences of the *DQB* alleles revealed 10 variable sites (Fig. 2), defining 8 alleles (*Neph-a* to *Neph-h*: GenBank accession numbers AB164212–164219). The allelic identifications of the remaining 110 individuals were determined by direct sequencing. Directly sequenced heterozygotes were determined by clear double-peak nucleotides in the chromatogram. No new substitution sites or alleles were found beyond the 10 sites and 8 alleles detected through TA cloning. All the mutations were base substitutions; no indels were found. The nucleotide substitution rate among the 8 alleles of the finless porpoise indicated a maximum value for transitions per site of 3.06% between *Neph-e* and *Neph-h*, and a minimum value for transversions per site of 2.99% between *Neph-e* and *Neph-a, -c, -d, -f*, and *-h*.

The network tree generated using the 8 *DQB* alleles (Fig. 1B) showed that 4 alleles (*Neph-a, -c, -d* and *-f*) formed a linked network, while *Neph-b* and *-h* radiated from *Neph-c*. Alleles *Neph-e* and *Neph-g* branched off with 4 and 7 sub-

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Neph-a  C ACG GAG CGG GTG CGG TTC GTG GAA AGA TAC ATC TAT AAC CGG GAG GAG TAC GTG CGC TTC GAC AGC GAC GTG GGC GAG TAC CGG GCG
Neph-b  . ... ... ... ... ... C.. ... ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ... ... ... ... ... ... ... ...
Neph-c  . ... ... ... ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Neph-d  . ... ... ... ... ... C.. ... ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Neph-e  . ... ... ... ... ... ... ... ... ... C.. ... ... ... ... ... ... .T. C.. ... ... ... ... ... ... ... ... ... ... ...
Neph-f  . ... ... ... ... ... ... ... ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Neph-g  . ... ... ... ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Neph-h  . ... ... ... ... ... C.. ... ... ... ... ... C.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Neph-a    GTG ACC GAG CTG GGC CGG CCG GAC GCC AAG TAC TGG AAC GGC CAG AAG GAC ATC CTG GAG CAG AAA CGG GCC GAG CTG GAC ACG 172
Neph-b    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 172
Neph-c    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 172
Neph-d    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 172
Neph-e    ... ... ... ... ... ... .A. AT. ... G.. A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 172
Neph-f    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 172
Neph-g    ... ... ... ... ... ... .G. AC. ... G.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 172
Neph-h    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 172
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Fig. 2. Alignment of the MHC *DQB* allele sequences for finless porpoises (*Neophocaena phocaenoides*). *Neph-a* is the reference sequence, with a dot indicating the same nucleotide as in the reference and the differing nucleotides displayed.

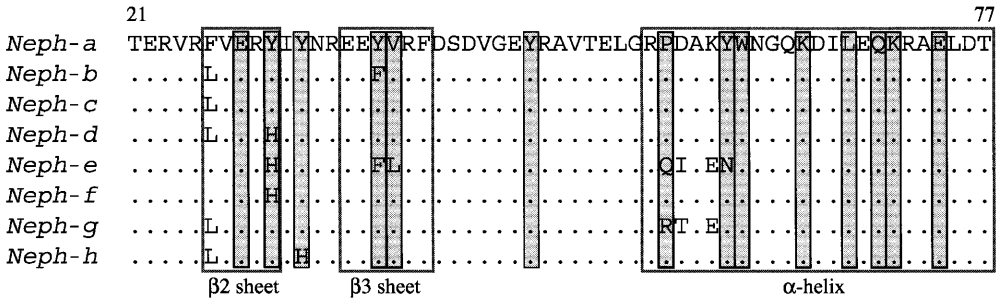

Fig. 3. Putative amino acid sequences translated from 172 bp sequences of MHC *DQB* alleles for the finless porpoise (*Neophocaena phocaenoides*). Amino acids are shown by the single-letter code. A dot indicates the same amino acid as the *Neph-a* allele. The putative positions corresponding to the protein binding region are shaded. Boxes show ,2 and ,3 sheets, and the α-helix. The numbers across the sequence correspond to the amino acid position based on the β, chain of the *DR* structure (Brown *et al.*, 1993).

stitution differences, respectively, from the closest allele in the linked network.

Amino acid alignments derived from the *DQB* nucleotide sequences (Fig. 3), showed that all the substitutions were nonsynonymous. The nonsynonymous substitutions per nonsynonymous site (d_N) percentage in all regions analyzed was 3.25 (Table 2). The d_N percentages in PBR and non-PBR were 7.13 and 1.99, respectively. Thus, the d_N percentage in PBR was 3.55 times higher than that in non-PBR.

Table 2. The number of synonymous substitutions per synonymous site (d_S), and the number of nonsynonymous substitutions per nonsubstitution site (d_N) at the peptide binding region (PBR), non-PBR, and across all the sequences of MHC *DQB* exon 2 region analysed for the finless porpoise (*Neophocaena phocaenoides*).

PBR		Non-PBR		All region	
d_S	d_N	d_S	d_N	d_S	d_N
0.00±0.00	7.13±2.78	0.00±0.00	1.99±1.18	0.00±0.00	3.25±1.15

d_S and d_N are indicated as percentages.
The Standerd errors were caluculated using 500 bootstrap replications.

The *DQB* allele frequencies in the 5 finless porpoise populations (Table 1) showed that the most common alleles in each population were *Neph-b* (36%) in Sendai Bay-Tokyo Bay, *Neph-e* (62%) in Ise-Mikawa Bays, *Neph-b* (50%) in Inland Sea-Hibiki Nada, *Neph-d* (50%) in Ariake Sound-Tachibana Bay, and *Neph-e* and *-f* (both 33%) in Omura Bay.

Heterozygosity and nucleotide diversity

The expected heterozygosity (H_E) ranged from 0.55 to 0.78 among the various finless porpoise populations, with all but one value ≥0.68. The overall heterozygosity was high, with a value of 0.78 (Table 1).

Expected heterozygosity (H_E) for each of the 5 populations, except Inland Sea-Hibiki Nada, exceeded the observed heterozygosity (H_O). H_O of the Sendai Bay-Tokyo Bay and the Ariake Sound-Tachibana Bay populations were significantly different from H_E according to the Hardy-Weinberg test. The distribution of allele frequencies in each local population was not significantly different from that expected by neutrality.

Calculated nucleotide diversity (π) (Table 1) ranged from 1.27% in the Sendai Bay-Tokyo Bay population, to 0.64% in the Inland Sea-Hibiki Nada population. Nucleotide diversity values for the *DQB* alleles were also notably high.

Geographical distribution of *DQB* alleles in the finless porpoise

The geographical distribution of the *DQB* alleles (Fig. 1A) indicates that a variety of alleles are maintained in each population, and that most alleles are shared among the populations. Three alleles (*Neph-b*, *-d* and *-e*) were found in all 5 populations, and *Neph-f* was found in 4 populations, while *Neph-a* and *-c* were found in 3 populations. *Neph-h* was found only in the Inland Sea-Hibiki Nada population. The exact test of independence (Table 3) was used to compare the allele frequencies among populations, and each popula-

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Table 3. F_{ST} values (lower matrix) and probability values of exact test (upper matrix) using the MHC *DQB* alleles from 5 populations of the finless porpoise (*Neophocaena phocaenoides*) in Japanese waters.

	Sendai Bay-Tokyo Bay	Ise-Mikawa Bays	Inland Sea-Hibiki Nada	Omura Bay	Ariake Sound-Tachibana Bay
Sendai Bay-Tokyo Bay	–	0.0000	0.0111	0.0001	0.0000
Ise-Mikawa Bays	0.053*	–	0.0000	0.0007	0.0000
Inland Sea-Hibiki Nada	0.031*	0.236*	–	0.0003	0.0000
Omura Bay	0.101*	0.152*	0.207*	–	0.1933
Ariake Sound-Tachibana Bay	0.195*	0.253*	0.235*	–0.018	–

* Significance level of <0.05 were generated using Arlequin (Schneider *et al.*, 2000)

tion comparison was found to be significant (P=0.005 in 5 comparisons), except for that between the Omura Bay and Ariake Sound-Tachibana Bay populations.

The F_{ST} values for *DQB* alleles (Table 3) were positive, with statistical significance except for the comparison between the Omura Bay and Ariake Sound-Tachibana Bay populations. Amongst all comparisons, the highest F_{ST} value was 0.25 between the Ise-Mikawa Bay and Ariake Sound-Tachibana Bay populations. The lowest value was 0.03 between the Sendai Bay-Tokyo Bay and Inland Sea-Hibiki Nada populations.

DISCUSSION

Sequences of the *DQB* exon 2 alleles for 50 individuals were determined by TA cloning for the first step. At least 2 identical sequences from each PCR amplification pool are required for the identification of an allele (Gyllensten *et al.*, 1990; Murray *et al.*, 1995), due to the necessity to distinguish point mutations from PCR artefacts. Murray *et al.* (1995) suggested that if every allele were amplified at an equal frequency, then sequencing 5 clones would present a low probability (P=0.0625) of not detecting both alleles in the case of heterozygotes. Sequencing more than 5 clones did not detect any individuals with more than 2 alleles, indicating that a single *DQB* locus was being amplified. Allelic identification of the remaining 110 individuals was determined by direct sequencing. No new substitution sites were found by the direct sequencing. These results indicate that direct sequencing was sufficient to identify all the alleles that were detected by the cloning method.

Hayashi *et al.*, (2003) showed that all alleles detected from 16 cetacean species, including the 8 alleles from the finless porpoise, were monophyletic within a HLA-*DQB*1 lineage, and were separated from the lineages of HLA-*DQB*2 and *DQB*3. The 8 alleles from the finless porpoise formed a clade which separated into 2 subclades, one containing *Neph-a*, *b*, *c*, *d*, *f*, and *h*, and the other having *Neph-e* and *g*, and *Phph-a* from the harbour porpoise. This shows trans-species polymorphism between the finless porpoise and the harbour porpoise.

Each population of finless porpoises had 4 to 8 alleles, and most alleles were shared among populations. Heterozygosities in each population ranged from 0.55 to 0.78, and for all 5 populations it was 0.78. The High Arctic population of white whales (Murray *et al.*, 1995) showed 5 *DQB* alleles among 43 individuals. We calculated the heterozygosity at 0.69, using their data (Murray *et al.*, 1995). The southern population of white whales in Canada had 4 *DQB* alleles among 190 individuals, with a heterozygosity of 0.38. Hoelzel *et al.* (1999) found 8 alleles from 109 southern elephant seals with a heterozygosity of 0.84, and 2 *DQB* alleles (122 bp overlap with our sequence) from 69 northern elephant seals with a heterozygosity of 0.41. These comparisons with other marine mammals show the existence of populations with both high and low MHC variability.

One explanation is that the differences in MHC variability may be due to different exposures to pathogens, as Slade (1992) suggested. Murray *et al.* (1995) also suggested that the differences between High Arctic and Southern populations of white whales may correspond to differences in pathogens encountered. Finless porpoises inhabit shallow waters on the coast, where they may be influenced by terrestrial runoff to the sea. Since the finless porpoise inhabits coastal areas, relatively higher exposure to pathogens in the coastal waters would have resulted in maintaining higher MHC variability in this species than in other cetacean species in the oceanic environment.

The population history of each species could be another explanation for the differences in MHC variability. In the Australian bush rat, a substantial lack of MHC variation was observed in small island populations despite high diversity in mainland populations (Seddon and Baverstock, 1999). The endemic South African antelope, *Damaliscus pygargus*, which has two subspecies, showed that erosion of allelic diversity in *D.p. pygargus* was most likely the result of severe bottleneck events caused by hunting pressure and parasitic infection (Van der Walt *et al.*, 2001). In marine mammals, the northern elephant seal is known to have experienced a severe population bottleneck (<30) (Hoelzel *et al.*, 1993), and the low level of MHC variation in this species is more a reflection of the demographic history of the species than a consequence of living in a marine environment (Hoelzel *et al.*, 1999).

The mtDNA analysis of the finless porpoise in Japanese waters (Yoshida *et al.*, 2001), suggested that it was divided into distinct local populations. Differentiation of local populations of the finless porpoise is also supported by ecological information, including field observations. F_{ST} values for the mtDNA haplotypes were remarkably high, ranging from 0.56 to 0.89. This may be caused by genetic drift, since the population size might not be large enough to maintain local populations. In contrast, F_{ST} values for the MHC *DQB* alleles were relatively low, but most of them were positive and statistically significant. This may suggest that genetic drift also influenced MHC *DQB* variability in the local populations of the finless porpoise. The differences in F_{ST} between mtDNA

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and the MHC locus might be caused by a difference in effective population size, because the value for mtDNA is 1/4 that for nuclear DNA.

Genetic drift might have been high because in Japan the finless porpoise is located at the northern fringe of its range, having only recently migrated into Japanese waters since the last glaciation. The mtDNA haplotype network, which shows a relatively recent demograph, suggests that the southern (Ariake) population, with 6 haplotypes, might be ancestral to the other Japanese populations. Climate warming was so rapid that so-called pioneer effects and/or strong genetic drift could have occurred during range expansion into northern areas, resulting in differentiation of the northern populations.

Hedrick *et al.* (2002) suggested that balancing selection occurs in the *DRB1* locus of red wolves. The supporting evidence includes (i) an excess of heterozygotes compared to expectations, when corrected using an inbreeding coefficient, (ii) a higher rate of nonsynonymous than synonymous substitutions for PBR, (iii) higher average heterozygosity of individual amino acids at PBR than at non-PBR, (iv) amino acid divergence that is greater than expected from a simulation of genetic drift, and (v) a more even distribution of alleles than expected under neutrality.

In the present study, all substitutions were nonsynonymous, and the d_N percentages in PBR and non-PBR were 7.13 and 1.99, respectively, with the d_N percentage in PBR thus 3.55 times higher than that in non-PBR, supporting Hedrick's *et al.* (2002) second line of evidence. Relative to the amino acid divergence, trans-species polymorphisms were found between the finless porpoise and the harbour porpoise (Hayashi *et al.*, 2003). Moreover, the geographical distribution of *DQB* alleles showed that most alleles were shared among populations. These results suggest a role of balancing selection in the MHC *DQB* alleles of the finless porpoise, especially over a long evolutionary time scale.

Referring to the first and last items from Hedrick *et al.* (2002), H_O of all finless porpoise populations was less than *He*, and the distribution of alleles did not differ from that expected under neutrality for the *DQB* alleles. This also indicates that genetic drift affected the *DRB* locus for local populations in this species. Interbreeding within the local populations might also cause a decrease in H_O values. The MHC *DQB* locus in the finless porpoise may be under balancing selection for long evolutionary time periods, but significantly influenced by genetic drift, beyond the effect of balancing selection, for short time periods in small local populations.

In this study, low heterozygosities in the Ise-Mikawa Bays and Ariake Sound-Tachibana Bay populations may point to the need for conservation management for this species. To assist in conservation plans and to better understand the dynamics of MHC genes in local populations, continuous monitoring of target genes will be needed. Future MHC analyses, especially at other MHC loci, will be required to accurately quantify the degree of variability of this complex.

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