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Analysis of mtDNA sequences in Bryde's whales from the central western North Pacific and Baja California Peninsula

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ABSTRACT

We conducted a mitochondrial DNA control region sequencing analysis using samples of the ordinary or offshore form Bryde's whales from western North Pacific, Hawaiian Islands and Baja California Peninsula. The objectives of this study were a) to study the mtDNA relationship between the western North Pacific stock and whales east of 180° and b) to determine whether or not a local form of Bryde's whale is distributed around the Hawaiian Islands. Twelve biopsy samples from the Baja California Peninsula and six from Hawaiian waters were used in this study. From the western North Pacific (WNP) stock we used JARPN II samples taken from 2000 to 2003 (n=185), historical samples from former Japanese coastal (n=103) and pelagic whaling (n=113). As out-group we used historical samples from past research whaling in eastern Indian Ocean (EIO: n=23) south of Java. A total of 46 haplotypes was defined within a total samples of 442 animals. All the haplotypes in the samples from the Hawaiian Islands were shared with the western North Pacific sample. All the haplotypes in the Baja California Peninsula were specific for that locality. The phylogenetic analyses based on neighbor-joining and parsimony suggested that whales around the Hawaiian Islands are from the ordinary offshore form. Therefore our results do not support the occurrence of a coastal form around the Hawaiian Islands, suggesting that the western North Pacific stock distributes also around these waters. Our result also showed a marked differentiation between Baja California Peninsula and western North Pacific whales. However the small sample size available for the former locality precluded an analysis on differentiation between the IWC-defined Eastern Tropical and Gulf of California stocks .

INTRODUCTION

The Comprehensive Assessment (CA) of the North Pacific Bryde's whale was completed in 1995 and 1996. Four stocks were defined by the Committee in the North Pacific: East China Sea stock (including inshore waters off Kochi); Western North Pacific (WNP) stock, Eastern Tropical stock and Gulf of California stock. The WNP stock is the target of the RMP/*ISTs*. The western North Pacific stock boundaries are supported by a continuous sighting distribution, mark/recapture data and no significant differences found in genetic studies (IWC, 1996). The Committee recommended development of RMP *Implementation Simulation Trials* for the Western North Pacific stock (IWC, 1997).

In 1998 the Committee defined two sub-areas for the purpose of testing two alternative stock structure hypotheses (IWC, 1999) (Fig. 1):

(1) there is only one stock of offshore Bryde's whales in the western stock area;
(2) there are two offshore stocks present in the sub-areas to the east (sub-area 2 in Fig. 1), the western stock and an eastern stock.

In 1998 considerable discussion was carried out by the Committee on whether or not the local form of Bryde's whale is found around oceanic islands (IWC, 1999). As shown in Fig. 1 the definition of the sub-areas specifically excluded the area to the south off Hawaiian Islands and east of 180°, to cover for such a possibility.

At the time of the 1998 meeting there were no genetic data from around the Hawaiian Islands. Recently some few data became available from such locality (LeDuc and Taylor, 2003). These authors examined genetically a few samples from Hawaiian Islands and compared them with samples from the Baja California Peninsula .

Following the new Procedure of Data Availability adopted by the Committee during the 2003 meeting, we requested to and obtained from US scientists the available genetic data from Hawaiian Islands and Baja California Peninsula. The objectives of this study are 1) to compare genetically samples from the WNP stock (making use of the available mtDNA data from whale samples by JARPN II and past commercial whaling) to samples east of 180° to investigate the eastern distribution of the WNP stock, and 2) to investigate whether or not a local form of Bryde's whale is distributed around the Hawaiian Islands.

Based on genetic and morphological evidence Wada *et al*. (2003) proposed a new species of Bryde's whale, Tsunoshima whale (*Balaenoptera omurai*), which distributes in the Sea of Japan, the Solomon Islands and the eastern Indian Ocean. In addition they suggested the separation of the Bryde's whale (*B. brydei*) and the Eden's whale (*Balaenoptera edeni*). The Bryde's whale corresponds to the larger, common offshore Bryde's whale. For comparative purpose we included in our analysis sequence data from Wada *et al*. (2003)'s Eden and Tsunoshima whales.

MATERIALS AND METHOD

Samples

A total of 12 biopsy samples from Mexican waters around the southern tip of the Baja California Peninsula (BAJA) and six samples from Hawaiian waters (HAW) were used in this study (Table 1 and Fig. 2). Regarding to the BAJA samples, two are from west coast and the others are from within the Gulf of California (Fig.2).

Samples from the Western North Pacific stock (WNP) were from three sources: JARPN II taken during the 2000-2003 surveys (JARPN: n=185), historical samples from former coastal whaling around the Ogasawara Islands in 1983 and 1984 (OGA: n=103) and historical samples from former pelagic whaling in the central western North Pacific in 1979 (PEL: n=113) (Fig. 2). As out-group we used historical samples from past research whaling in eastern Indian Ocean (EIO: n=23) south of Java in 1978.

Molecular Analyses

Sequencing of the mtDNA control region

Details of DNA extraction and mtDNA control region sequencing were reported in Pastene *et al*. (1997, 2002) and LeDuc and Taylor (2003).

Data analysis

Level of polymorphism

Distance between two nucleotide sequence was calculated according to Kimura's two- parameters method (Kimura, 1980). The degree of diversity within each locality was estimated using the nucleotide diversity (Nei, 1987: equation 10.5).

MtDNA Phylogeny

Phylogenetic reconstruction of sequences was made using neighbor-joining method implemented in the computer package PHYLIP version 3.5c (Felsenstein, 1993) and parsimony methods generated using PAUP* v. 4.0b10 (Swofford, 2001). The bootstrap values were obtained by generating 1000 random trees. Finally a majority-rule consensus phylogeny was obtained. These trees were rooted using homologous sequences from a coastal whale form from Kochi, Japan and from a coastal whale form from the Solomon Islands (Yoshida and Kato, 1999; Wada *et al*., 2003). These whales correspond to *B. edeni* and *B. omurai*, respectively, according the classification given by Wada *et al*. (2003).

RESULTS AND DISCUSSION

Variability of mtDNA control region sequences

A consensus 299 base pairs of mtDNA control region (the 5'-end) was analyzed for the total samples of 442 individuals. A total of 39 polymorphic sites defined 46 haplotypes. Except for two in/dels and one transversion, all substitutions were transitions.

The frequencies of haplotypes in the six localities are shown in Table 2. In the western North Pacific samples, a total of 36 haplotypes were identified in 401 individuals whereas only four haplotypes were detected in 23 samples in the eastern Indian Ocean samples. No haplotypes were shared between the western North Pacific and eastern Indian Ocean. On the other hand, five and six haplotypes were detected in the Hawaii samples and Baja samples, respectively. All haplotypes detected in the Hawaiian samples were shared with western North Pacific samples, but no haplotypes were shared between Baja samples and the other localities (Table 2).

Genetic diversity

Nucleotide diversities by locality were 0.0101+/-0.0011, 0.0091+/-0.0010, 0.0107+/-0.0009, 0.0157 +/-0.0019 and 0.0163+/-0.0060 for PEL, OGA, JARPN, BAJA and HAW, respectively. The nucleotide diversity for the samples of the EIO was lower (0.0063+/-0.0029).

MtDNA Phylogeny

The two genealogies estimated using the parsimony and the neighbour-joining method yielded similar topologies. For this reason, only the majority-rule consensus tree of neighbor-joining method with bootstrap values above 50 % is presented (Fig. 3).

None of the 46 haplotypes clustered together with either Wada *et al*. (2003)'s *B. edeni* or *B. omurai*. This means that all samples used in this study are assigned for the ordinary or offshore form of Bryde's whales (Wada *et al*., 2003's *B. brydei*).

The phylogenetic tree did not place the 46 haplotypes into geographically unique clades or clusters. No intra-oceanic and oceanic specific clade was found. Even the region-specific haplotypes such as the EIO (Hap37-40) and Baja (Hap 41-46) did not cluster together. This result might suggest little time for ordinary Bryde's whales inhabiting different ocean basin since their divergence to accumulate own unique haplotypes.

Urban and Flores (1996) summarized information on distribution of Bryde's whales in the Gulf of California, Mexico. They suggested that there are differences between the Bryde's whales from the Northern and southern Gulf of California in relation to the seasonal and temporal distribution, calving season and preferences of food items, suggesting the possible presence of two stocks, one restricted to the Gulf of California and the other with a wider distribution on the west of the Baja California Peninsula in the eastern tropical Pacific. That suggestion is confirmed by results of genetic analysis (Dizon *et al*., 1995). The Committee accepted the occurrence of two stocks the Eastern Tropical Stock and the Gulf of California Stock. Due to the small sample size in our analysis (only two individuals come from the west coast of the Baja California Peninsula) we could not examine the genetic differences between these two stocks and further samples are necessary to examine in further details the classification of two stocks in the eastern North Pacific.

Previous phylogenetic analysis separated clearly the coastal form from the ordinary offshore form (Yoshida and Kato, 1999; LeDuc and Dizon, 2002; Wada *et al*., 2003). Such separation was not possible in our phylogenetic analysis. Therefore our results do not support the occurrence of a coastal form around the Hawaiian Islands. All the haplotypes in the samples from the Hawaiian Islands were shared with the western North Pacific sample suggesting that the western North Pacific stock distributes also around these waters. Our result also showed a marked differentiation between Baja California Peninsula and western North Pacific. However the small sample size available for the former locality precluded an analysis on differentiation between the IWC-defined Eastern Tropical and Gulf of California stocks.

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Table 1.Summary of the 18 Bryde's whale samples prepared by American scientists. Shaded individuals are samples from the west coast of Baja California Peninsula.

SWFSC ID	DATE	LATITUDE	LONGITUDE	Haplotype
4110	9-Jun-90	25-29N	111-15W	41
4111	2-Oct-90	24-20N	110-20W	42
4112	11-Apr-89	24-31N	110-29W	43
4113	26-Jun-89	24-29N	110-25W	43
4665	17-Oct-91	25-51N	111-17W	44
4672	17-Oct-91	26-38N	111-28W	45
4952	7-Apr-91	NA	NA	44
15911	10-Aug-95	25-23N	109-21W	45
15912	10-Aug-95	25-23N	109-21W	44
15913	10-Aug-95	25-23N	109-21W	45
17983	31-Jul-96	26-58N	114-21W	45
18003	5-Aug-96	24-4N	111-43W	46
30407	28-Aug-98	27-58N	174-27W	14
30424	20-Sep-98	28-55N	175-33W	14
30430	9-Oct-98	24-49N	157-47W	21
30432	15-Oct-98	25-36N	174-37W	19
30433	15-Oct-98	25-36N	174-37W	5
30451	20-Oct-98	22-26N	161-51W	34

Table 2. Mitochondrial DNA haplotype frequency by geographical locality.

Haplotype	PELAGIC	OGA	JARPNII	HAWAII	BAJA	EIO	Total
1	13	17	20	0	0	0	50
2	2	0	0	0	0	0	2
3	10	9	16	0	0	0	35
4	1	2	0	0	0	0	3
5	46	36	74	1	0	0	157
6	5	3	0	0	0	0	8
7	2	5	7	0	0	0	14
8	4	3	7	0	0	0	14
9	1	2	0	0	0	0	3
10	3	2	1	0	0	0	6
11	1	4	0	0	0	0	5
12	1	0	3	0	0	0	4
13	3	0	2	0	0	0	5
14	1	0	0	2	0	0	3
15	1	1	6	0	0	0	8
16	2	1	3	0	0	0	6
17	1	1	0	0	0	0	2
18	4	3	7	0	0	0	14
19	2	1	5	1	0	0	9
20	1	1	2	0	0	0	4
21	1	0	2	1	0	0	4
22	1	1	3	0	0	0	5
23	2	0	0	0	0	0	2
24	1	0	1	0	0	0	2
25	1	1	5	0	0	0	7
26	1	3	3	0	0	0	7
27	1	0	0	0	0	0	1
28	1	3	2	0	0	0	6
29	0	3	5	0	0	0	8
30	0	1	1	0	0	0	2
31	0	0	1	0	0	0	1
32	0	0	1	0	0	0	1
33	0	0	3	0	0	0	3
34	0	0	2	1	0	0	3
35	0	0	1	0	0	0	1
36	0	0	2	0	0	0	2
37	0	0	0	0	0	19	19
38	0	0	0	0	0	2	2
39	0	0	0	0	0	1	1
40	0	0	0	0	0	1	1
41	0	0	0	0	1	0	1
42	0	0	0	0	1	0	1
43	0	0	0	0	2	0	2
44	0	0	0	0	3	0	3
45	0	0	0	0	4	0	4
46	0	0	0	0	1	0	1
Total	113	103	185	6	12	23	442

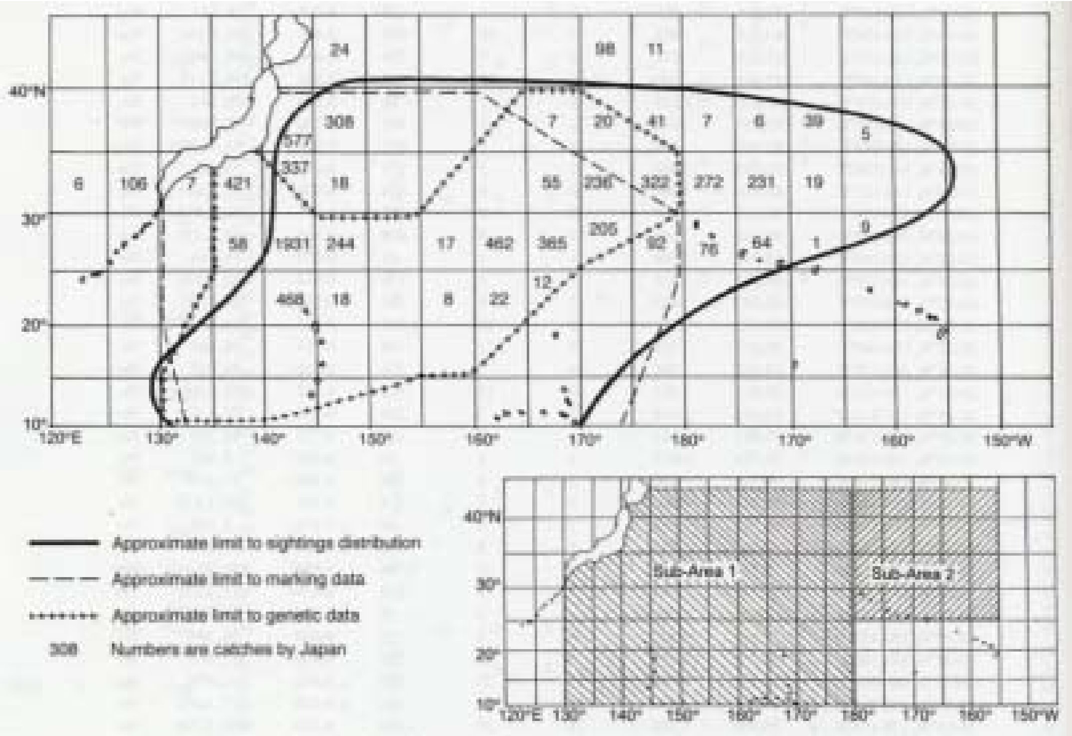

Fig. 1. Summary of information for determining stock boundaries for *Implementation Simulation Trials* for western North Pacific Bryde’s whales and agreed boundaries for the western stock and two sub-areas.(After IWC 1999)

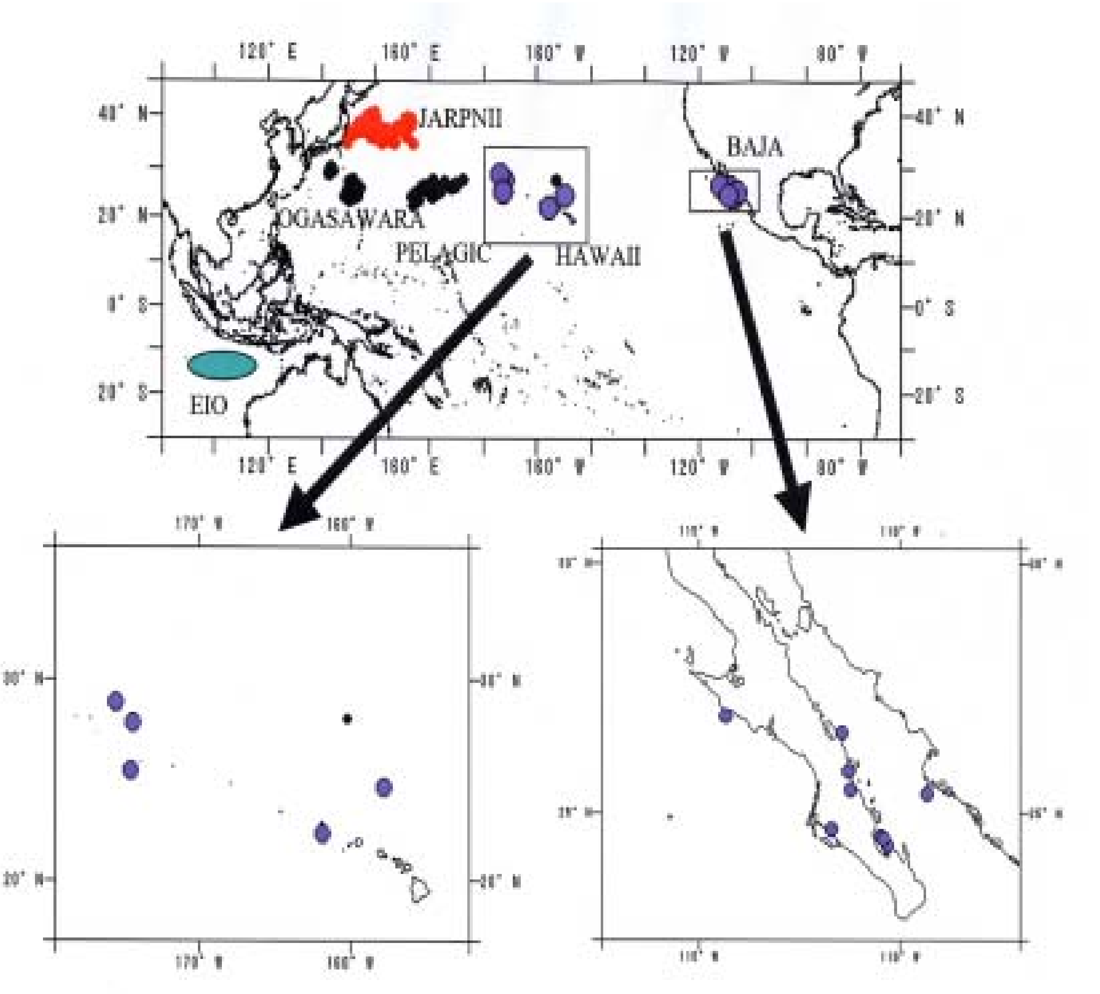

Fig. 2 Sampling localities of Bryde’s whales in the western North Pacific, Baja California Peninsula and eastern Indian Ocean (EIO)

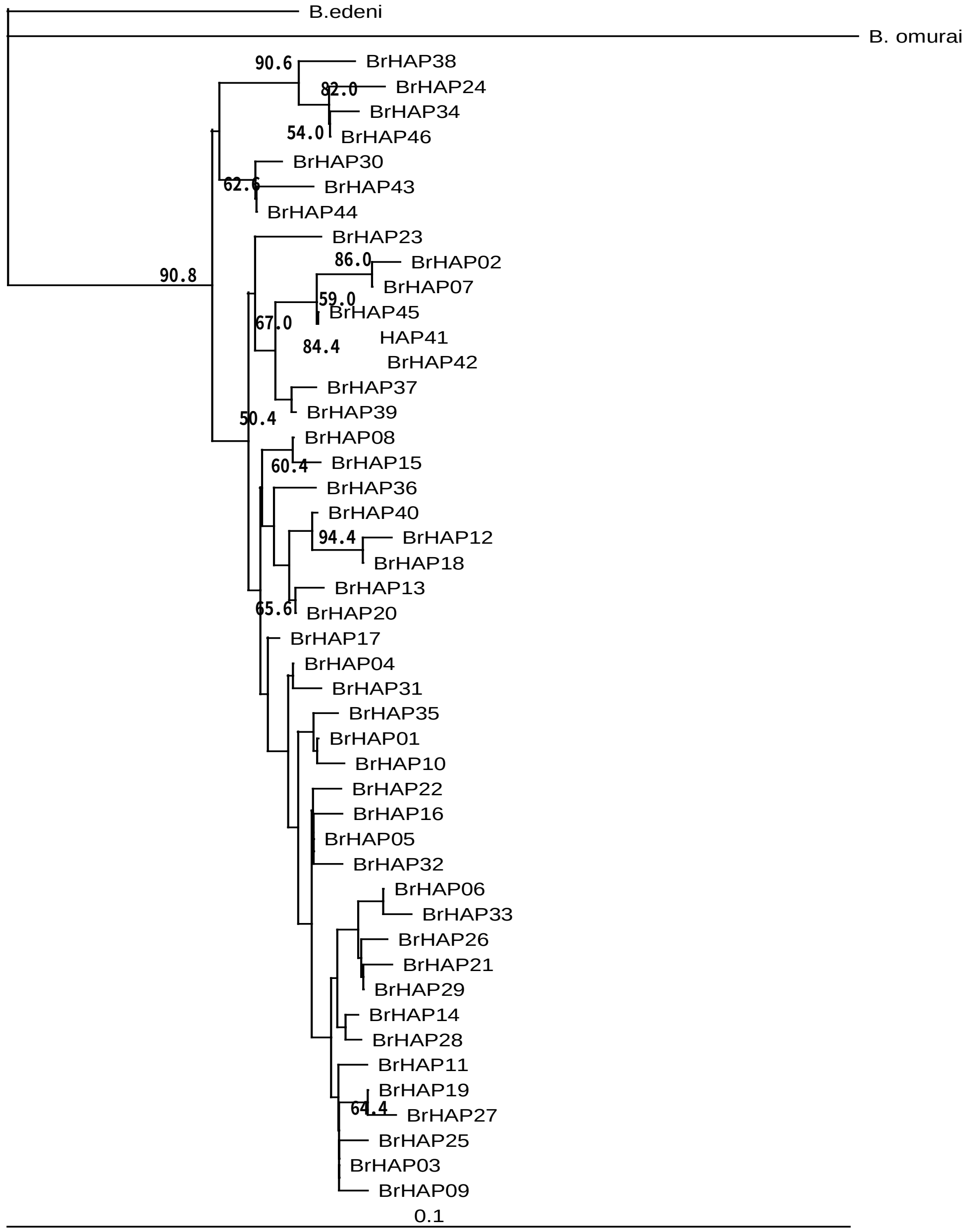

Fig. 3. Majority rule consensus tree estimated from mtDNA haplotypes of Bryde's whales using a neighbor-joining method. Only the bootstrap values above 50 % are shown in this figure.