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An update of the mitochondrial DNA and microsatellite analyses in western North Pacific Bryde's whale

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ABSTRACT

On the basis of the JARPNII data presented at the 54SC meeting in Shimonoseki, the Committee agreed that there was no reason to change the stock structure of Bryde's whales inhabiting western North Pacific in the existing RMP trials. Further analyses, however, were also recommended in order to pursue a full and detailed discussion at future meetings. In this paper, we describe the results of mitochondrial DNA control region sequencing and microsatellite analyses of Bryde's whales samples obtained from different localities of western North Pacific: JARPNII samples from 2000 to 2003 and the archived samples of past commercial whaling off Japan (Ogasawara whaling ground and Pelagic samples). A total of 36 haplotypes were detected from sequence variations at 299bp of mtDNA control region analyzed for a total of 401 animals. All of the 17 microsatellite loci analyzed for a total of 385 animals were polymorphic over the samples. Chi-square statistics were used to test genetic heterogeneity between sexes within each of the samples, among the samples collected from a same area in different years (i.e. four JARPNII samples and two Ogasawara area samples) and among all the samples from different localities in the western North Pacific. Both genetic markers showed the same results as none of the tests were statistically significant. In contrast samples from the western North Pacific showed marked mtDNA and microsatellite differences with a sample of Bryde's whale from the eastern Indian Ocean. Our results strongly indicate that the different samples from the western North Pacific came from a single population. These results provide no reason to change the stock structure scenario being considered in the RMP trials.

INTRODUCTION

The comprehensive assessment of North Pacific Bryde's whale was started during the 47th meeting of the IWC Scientific Committee (IWC, 1996). On the basis of the information derived from genetics and non-genetics analyses, the Committee defined five stocks: the East China Sea stock (including inshore waters of Kochi), the western North Pacific stock, the eastern Tropical stock, the Gulf of California stock and the Solomon Island/Southeast Asia dwarf stock.

In 1998, the Committee agreed to place two sub-areas (sub-area 1 and 2 divided at 180°) in the western North Pacific Stock, which created two alternative stock hypotheses for *IST* to be tested. In 1999, however, some members of the Committee concerned that the size of the sub-area 1 was very wide and limited information was available for some parts to make firm conclusion of the structure of the western North Pacific stock. Specifically, they pointed out that the number of sample used for genetic analyses was scarce in some parts of the sub-area 1 as shown in Appendix 14 of Annex D in IWC (1999).

After the first two years of JARPNII surveys, the results of further genetic analyses were presented (Pastene *et al.*, 2002). The analyses utilized both mitochondrial DNA sequencing and microsatellite analyses for new samples from a region of the sub-area 1 where the previous genetic analyses that used only historical samples did not covered. In the analyses, mtDNA control region sequencing analysis involved three localities in the western North Pacific: JARPN II (2000 and 2001), Ogasawara whaling ground and pelagic operation in the western central North Pacific. Overall there were no significant mtDNA haplotype frequencies differences among these localities. Similar results were observed from microsatellite analysis. The authors concluded, therefore, that there was no evidence to support additional stock structure in the western North Pacific.

After discussion of the JARPNII DNA information at the 54SC meeting, the Committee agreed that the results reported provided no reason to change the stock structure in the existing trials, but that as much work as possible should be undertaken before the next meeting to allow a full and detailed discussion at that time. In the

discussion related to the new Implementation Schedule defined at the 54SC meeting it was noted that activities were still within the *Pre-Implementation Review*.

In this document, we conducted mtDNA sequencing and microsatellite analyses to examine genetic stock structure of western North Pacific Bryde's whales. New samples obtained during 2002 and 2003 JARPNII surveys were added to the analyses, and the number of microsatellite loci used was three times more than that of Pastene *et al.* (2002). We believe that these additions should increase the resolution of analyzing stock structure of Bryde's whales.

MATERIALS AND METHODS

Samples

Samples of the ordinary form Bryde's whales were obtained from different localities in the western North Pacific (Table 1). JARPN II samples included the surveys conducted from 2000 to 2003. Archived samples included past coastal commercial whaling off Ogasawara and off Taiji and pelagic commercial whaling (Fig. 1). The samples used for the microsatellite analysis were identical to those used for the mtDNA analysis except some in the Ogasawara and pelagic samples, which were analyzed with either of the two methods. For comparative purpose, we used a sample of Bryde's whale from the eastern Indian Ocean (EIO, n=23) taken in November 1978 during a scientific whaling operation.

MtDNA

Molecular genetic methods used to obtain mtDNA control region sequences were reported in Pastene *et al.* (1997a). Following recommendations from the Committee, differences in the frequency of haplotypes among the samples were tested by the randomized chi-square test (Roff and Bentzen, 1989) with a total of 10,000 permutations in each test. Heterogeneity tests were conducted to test the differences between sexes within the samples, test the differences among the four JARPNII samples as well as between the two samples from Ogasawara area (Taiji and Ogasawara), and test among the samples from three different localities: JARPNII, Pelagic and Ogasawara.

Microsatellites

Genotypes were scored at 17 microsatellite loci: EV1, EV14, EV21, EV94, EV104 (Valsecchi & Amos 1996), GT011 (Bérubé *et al.* 1998), GT023, GT310, GT575 (Bérubé *et al.* 2000), GATA028, GATA053, GATA098, GATA417, GGAA520, TAA031 (Palsbøll *et al.* 1997), DlrFCB14, and DlrFCB17 (Buchanan *et al.* 1996). Primer sequences and PCR profiles basically follow those of the original authors. All the statistical tests were conducted using the computer program GENEPOP (Raymond and Rousset, 1995). Statistical tests were conducted to test the differences between sexes within the samples, test for the deviation from Hardy-Weinberg genotypic proportion in the samples, test the differences among the four JARPNII samples as well as between two samples from Ogasawara area (Taiji and Ogasawara), and test among the samples from three different localities: JARPNII, Pelagic and Ogasawara. Decision of statistically significance on hypothesis testing was made using the chi-square value obtained from summing the negative logarithm of P-values over the total loci (Fisher, 1950). When needed, sequential Bonferoni correction (Rice 1989) was applied.

Genetic relationship

Cavalli-Sforza's cord measures (Cavalli-Sforza and Edwards, 1967) between all pairs of samples were calculated from mtDNA haplotype frequencies and microsatellite allele frequencies, respectively, using GENDIST of the PHYLIP (Felsenstein, 1993) program after 1000 replicates of bootstrap analysis. These measures were used to create unrooted neighbor-joining trees with NEIGHBOR of the PHYLIP program to analyze the relationships between Bryde's whale samples. The obtained trees were visualized using the TreeView PPC program (Page, 1996). For this analysis, the samples from eastern Indian Ocean (N=23) were used as outgroups.

RESULTS

MtDNA

Sequence variations at 299bp of the mtDNA control region resulted in 36 unique haplotypes. Haplotype diversity ranged from 0.670 in the 00NP to 0.888 in the 01NP with an average of 0.816. Without the 00NP, it was similar among the samples that ranged from 0.800 to 0.816. Percent nucleotide diversity ranged from 0.495 in the Taiji and 1.424 in the 01NP with an average 0.968. Without the Taiji, it was from 0.910 to 1.424. The first three most frequent haplotypes were common among the samples (see Table 2 in SC/56/PFI5).

No significant difference was detected between sexes in each of the samples (Table 2). No significant difference was found among the four JARPNII samples (Table 4) as well as between the Taiji and Ogasawara samples. The former four were combined into one as JARPNII and the latter two as Ogasawara for further analyses. Finally, no significant difference was detected among the samples from the three different localities in the western North Pacific (Table 5). In contrast, statistically significant difference was detected between the samples from western North Pacific and that from eastern Indian Ocean (Table 6).

Microsatellites

All loci were polymorphic at each of the samples analyzed (data not shown). The number of alleles per locus ranged from two at DlrFCB14 to 19 at GATA28 with an average of 8.6. Heterozygosity was quite similar among the samples ranged from 0.656 in the Taiji sample to 0.681 in the Pelagic sample with an average of 0.669.

No significant difference was detected between sexes in each of the samples (Table 2). A few loci with p-value less than 5% could be due to chance effect as the test for all loci combined showed no significance at all of the samples. No significant departure from the Hardy-Weinberg proportions was detected at the 17 loci in each of the samples (Table 3). Again, a few loci with p-value less than 5% could be due to chance effect as the test for all loci combined showed no significance at all of the samples. No significant differences were found among the four JARPNII samples after the correction for the multiple tests (Table 4). Similarly, no significant difference was found between the Taiji and Ogasawara samples (Table 4). The former four were combined into one as JARPNII and the latter two as Ogasawara for further analyses. Finally, no significant difference was detected among the samples from the three different localities after the correction for the multiple tests (Table 5). In contrast, statistically significant difference was detected between the samples from western North Pacific and that from eastern Indian Ocean (Table 6).

Genetic relationships

Genetic relationships of the samples were clearly depicted in unrooted neighbor-joining trees obtained from the frequencies of mtDNA haplotypes and microsatellite alleles, respectively (Fig. 2). The samples from the western North Pacific were clustered together very closely while the outgroup EIO sample was quite distant.

DISCUSSION

Pastene *et al*. (1997b) examined the intra- and inter-oceanic patterns of genetic differences in the Bryde's whales, and revealed striking genetic differences among the samples from the different oceanic regions but no significant differences within the WNP. For that study only two archived samples of past commercial whaling, however, were available for the western North Pacific Bryde's whales: Ogasawara and Pelagic.

Pastene *et al*. (2002) further analyzed stock structure of Bryde's whales with additional samples from the western North Pacific and additional genetic markers. They utilized two hyper-variable genetic markers, namely, mtDNA control region sequencing and microsatellites to increase resolution and added two JARPNII samples (2000 and 2001 surveys) to the analyses that allowed them to cover wider area in the western North Pacific. JARPN II surveys obtained the samples from higher latitudes within sub-area 1 approximately between 35° and 45°N compared to the archived samples between 20°N and 30°N, yet different longitudinal localities within sub-area 1 (Fig. 1). In Pastene *et al*. (2002), both mtDNA and microsatellite results showed similar pattern of stock structure to Pastene *et al*. (1997b). That is, striking genetic differences were observed at the inter-oceanic level, but not at intra-oceanic level.

The present study included two additional samples from JARPNII obtained in 2002 and 2003, increasing sample size for the analysis in the intra-oceanic level. In addition, the number of microsatellite loci analyzed in the present study (17 loci) was much greater than that in the previous study (5 loci). These improvements should have increased the power of detecting population differentiation.

Genetic similarity of the samples from the western North Pacific was observed from all of the analyses performed in this study using both of the genetic markers, indicating that these samples came from a genetically same group of Bryde's whales. If multiple breeding populations exist, we think we should have detected temporal genetic differences among samples from multiple-year samplings in feeding grounds. In contrast, even though the sample size of the EIO sample was small, substantial genetic difference was detected against the samples from the western North Pacific. Therefore, our analysis strongly supports the single population inhabiting western North Pacific.

The most effective way to address questions on stock identity is to consider results from several techniques, genetics and non-genetics (Donovan, 1991; Pastene *et al*., *2000*; Perrin, 2001; Rugh *et al*., 2003). That is the way our group has used to address stock identity questions in the North Pacific minke whale, Antarctic minke whale and bowhead whale. The genetic results of the present study do not support additional stock structure in the western North Pacific Bryde's whale and these results are supported by the analysis of other non-genetic approaches conducted before or during the CA.

Nemoto (1959) examined mark-recapture data from the Pacific side of Japan and demonstrated movement of whales between Ogasawara and Sanriku (northern part of Honshu Island). Ohsumi (1978) studied the movement of Bryde's whale using mark-recapture data. He found that some whales marked near the Equator were recaptured in both the pelagic grounds of the western North Pacific and in coastal areas of Japan. He used this to suggest that Bryde's whales found in pelagic waters belong to the same stock as those found near the coast of Japan. In a more recent analysis of mark-recapture data, Kishiro (1996) suggested that Bryde's whales summering in the whaling grounds (coastal and offshore), winter over a wide latitudinal range, between 1°S and 25°N. The author could not find evidence of more than one stock in the western North Pacific whaling grounds. Kato and Yoshioka (1993) examined the areal and temporal variations of biological parameters in the western North Pacific Bryde's whale with the objective of identifying populations. Mean body length, mean body length at maturity, pregnancy rate and seasonality of breeding were found to vary on a temporal and spatial basis. However these authors considered that the variations in these parameters were due to differences in whaling regulations between coastal and pelagic whaling and under-representation of pregnant females in coastal samples. They also concluded that there was no evidence supporting the occurrence of different stocks in the western North Pacific.

We conclude therefore that available genetic and non-genetic evidences support the single stock scenario for western North Pacific Bryde's whale.

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REFERENCES

Bérubé, M., Aguilar, A., Dendanto, D., Larsen, F., Notarbartolo-di-Sciara, G., Sears, R., Sigurjónsson, J., Urban-Ramirez, J. and Palsbøll, P.J. 1998. Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus, 1758); analysis of mitochondrial and nuclear loci. *Mol. Ecol.* 7:585-599.

Bérubé, M., Jørgensen, H., Mcewing, R. and Palsbøll, P.J. 2000. Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale, *Megaptera novaeanglliae*. *Mol. Ecol.* 9:2181-2183.

Buchanan, F.C., Friesen, M.K., Littlejohn, R.P. and Clayton, J.A. 1996. Microsatellites from beluga whale *Delphinapterus leucas*. *Mol. Ecol.* 5:571-575.

Cavalli-Sforza, L.L. and Edwards, A.W. 1967. Phylogenetic analysis: models and estimation procedures. *Amer. J. Human Genet.* 19:233-257.

Donovan, G. 1991. A review of IWC stock boundaries. *Rep. int. Whal. Commn* (special issue 13): 39-68.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) 3.5c. University of Washington, WA, Seattle.

Fisher, R.A. 1950. Statistical Methods for Research Workers. 11th ed. Oliver and Boy, London.

International Whaling Commission. 1996. Report of the Scientific Committee, Annex G. Report of the sub-committee on North Pacific Bryde's whales. *Rep. int. Whal. Commn.* 46:147-59.

International Whaling Commission. 1999. Report of the Scientific Committee, Annex D. Report of the sub-committee on the Revised Management Procedure. *J Cetacean Res. Manage.* 1 (Suppl.):61-116.

Kato, H. and Yoshioka, M. 1993. Biological parameters and their areal and temporal variations of Bryde's whales in the North Pacific, with special reference to stock identification. Paper SC/45/O8 presented to the IWC Scientific Committee, April 1993 (unpublished). 18pp.

Kishiro, T. 1996. Movements of marked Bryde's whales in the western North Pacific. *Rep. int. Whal. Commn* 46:421-8.

Nemoto, T. 1959. Food of baleen whales with reference to whale movements. *Sci. Rep. Whales Res. Inst.,* Tokyo 14:149-290.

Ohsumi, S. 1978. Bryde's whales in the North Pacific in 1976. *Rep. int. Whal. Commn* 28:277-80.

Page, R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Comput. Appli. Biosci.* 12:357-358.

Palsbøll, P.J., Bérubé, M., Larsen, A.H. and Jørgensen, H. 1997. Primers for the amplication of tri-and tetramer microsatellite loci in baleen whales. *Mol. Ecol.* 6:893-895.

Pastene, L.A., Goto, M., Palsboll, P.J. and Kato, H. 1997a. Population structure of the Bryde's whale (*Balaenoptera edeni*) in the North Pacific based on analysis of mitochondrial DNA control region sequences. Document SC/49/NP5 presented to the IWC Scientific Committee, September 1997 (unpublished). 12pp.

Pastene, L.A., Goto, M., Itoh, S., Wada, S. and Kato, H. 1997b. Intra- and inter-oceanic patterns of mitochondrial DNA variation in the Bryde's whale, *Balaenoptera edeni*. *Rep. int. Whal Commn.* 47:569-574.

Pastene, L. A., Goto, M. and Fujise, Y. 2000. Review of the studies on stock identity in the minke whale *Balaenoptera acutorostrata* from the North Pacific. Paper SC/F2K/J1 presented to IWC Scientific Committee Workshop to review JARPN, February 2000(unpublished).

Pastene, L.A., Goto, M. and Kanda, N. 2002. Mitochondrial DNA and microsatellite analyses in the western North Pacific Bryde's whale, including samples from JARPNII 2000 and 2001 surveys. Appendix 10, pp146-155. In: Fujise, Y., Kawahara, S., Pastene, L.A. and Hatanaka, H. (eds.) Report of 2000 and 2001 feasibility study of the Japanese Whale Research Program under special permit in the western North Pacific-Phase II (JARPNII). Paper SC/54/O17 presented to the IWC Scientific Committee, 2002 (unpublished).

Perrin, W. 2001. Draft review of the utility of non-genetic data for differentiating stocks of whales. Paper SC/53/SD2 presented to the IWC Scientific Committee, July 2001 (unpublished).

Raymond, M. and Rousset, F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism . *J. Heredity* 86:248-249.

Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223-225.

Roff, D.A. and Bentzen, P. 1989. The statistical analysis of mtDNA polymorphisms: chi-square and the problem of small samples. *Mol. Biol. Evol.* 6:539-45.

Rugh, D., DeMaster, D., Rooney, A., Breiwick, J., Shelden, K. and Moore, S. 2003. A review of bowhead whale (*Balaena mysticetus*) stock identity. *J. Cetacean Res. Manage* 5(3):267-279.

Valsecchi, E. and Amos, W. 1996. Microsatellite markers for the study of cetacean populations. *Mol. Ecol.* 5:151-156.

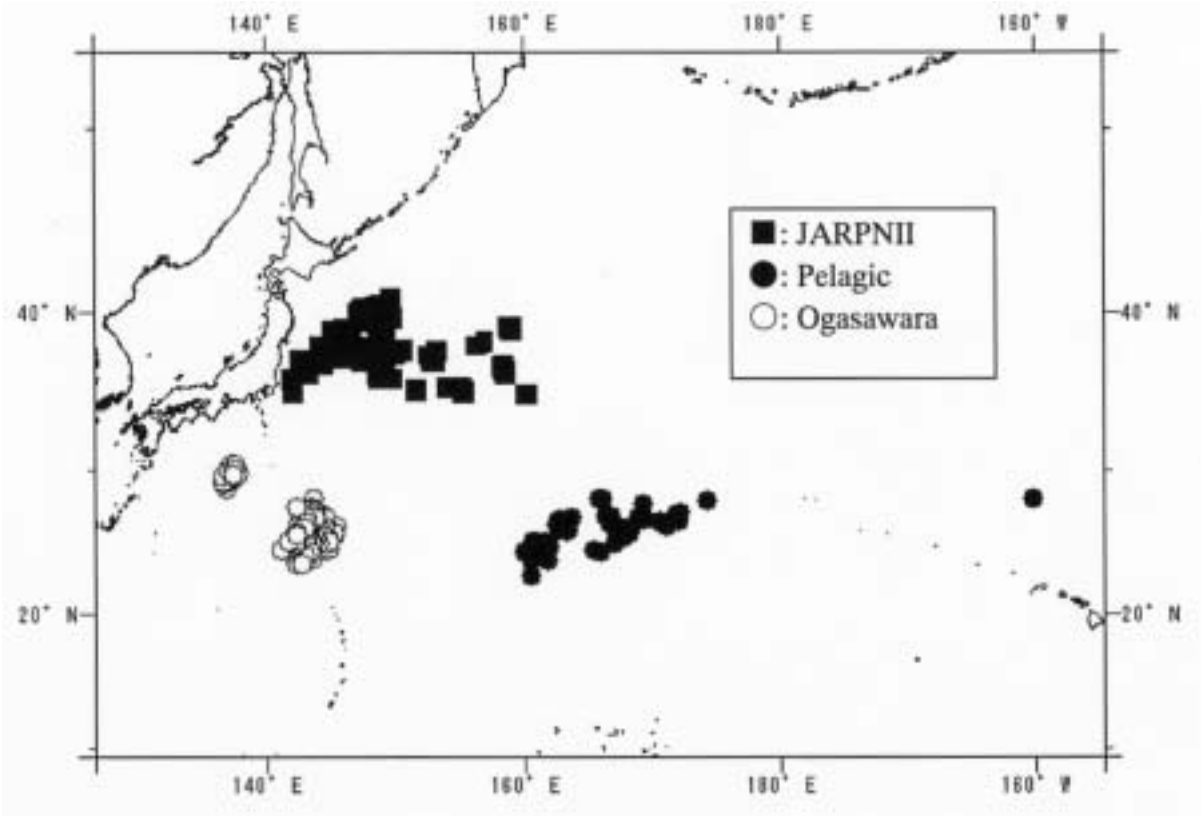

Fig. 1: Sampling localities in the western North Pacific Bryde's whales used in this study.

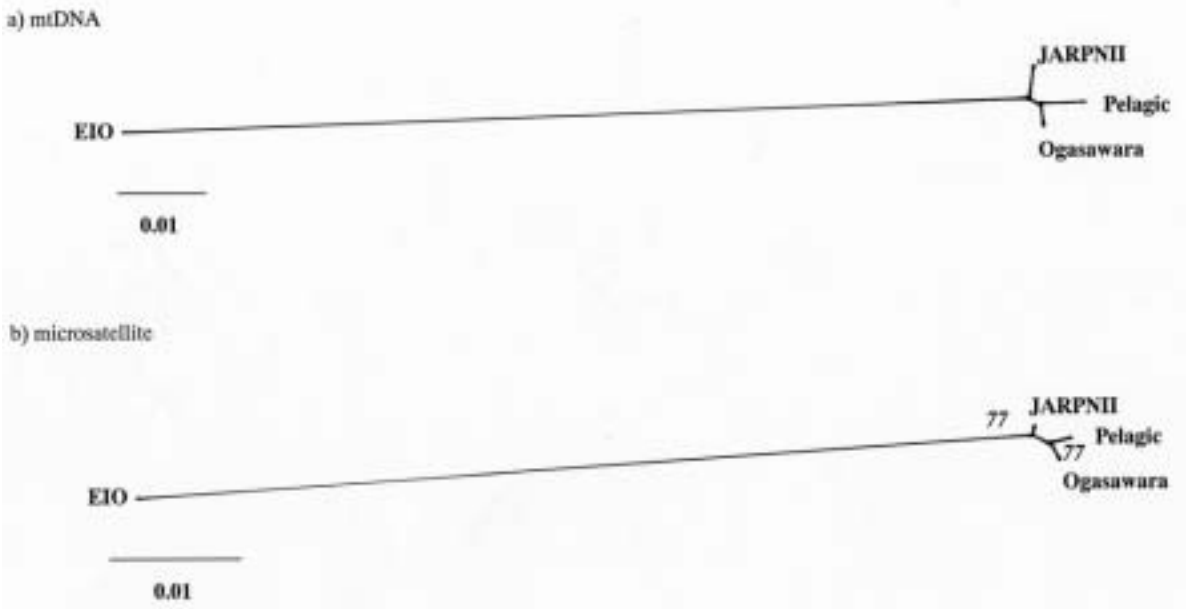

Fig. 2 Unrooted neighbor-joining trees based on Cavalli-Sforza's cord measure (Cavalli-Sforza and Edwards 1967) from frequencies of a) mtDNA haplotypes and b) microsatellite alleles that portray the genetic relationships among the Bryde's whales samples collected from the western North Pacific (JARPNII, Pelagic, Ogasawara) and the eastern Indian Ocean (EIO). Bootstrap values (%) from 1,000 replicates are given for b). No bootstrap value was smaller than 100% from 1000 replicates in a).

Table 1: Bryde's whales samples used in this study

Sample name	Source	Year	Period	MtDNA			Microsatellite		
				Female	Male	Total	Female	Male	Total
00NP	JARPNII	2000	Aug.-Sep.	21	21	42	21	21	42
01NP	JARPNII	2001	May-July	30	13	43	30	13	43
02NP	JARPNII	2002	July-Sep.	25	25	50	25	25	50
03NP	JARPNII	2003	May-Aug.	31	19	50	31	19	50
Pelagic	Past commercial	1979	Apr.-May	43	70	113	37	61	98
Off Taiji	Past commercial	1983	Apr.-June	11	9	20	12	9	21
Ogasawara	Past commercial	1984	Apr.-June	31	52	83	30	51	81

Table 2. Heterogeneity tests between sexes within each of the samples.

Locus	00NP	01NP	02NP	03NP	Pelagic	Off Taiji	Ogasawara
mtDNA	0.758	0.351	0.828	0.457	0.288	0.900	0.234
GATA98	0.044	0.991	0.515	0.803	0.566	0.586	0.322
EV104	0.635	0.721	0.479	0.102	0.754	0.070	0.737
GT011	0.249	0.747	0.698	0.710	0.030	0.755	0.322
GA53	0.386	0.009	0.168	0.609	0.367	0.529	0.257
GATA417	0.255	0.600	0.912	0.962	0.035	0.777	0.482
DlrFCB14	0.272	1.000	0.549	1.000	0.557	0.356	0.737
DlrFCB17	0.567	0.084	0.100	0.589	0.223	0.440	0.072
GT23	0.872	0.350	0.165	0.865	0.531	0.584	0.119
EV14	0.648	0.117	0.670	0.264	0.208	0.769	0.662
GT310	0.235	0.395	0.133	0.203	0.955	0.582	0.308
EV1	0.379	0.652	0.156	0.315	0.663	0.653	0.106
EV94	0.438	0.747	0.178	0.233	0.925	0.640	0.737
GGAA520	0.552	0.931	0.839	0.646	0.123	0.902	0.994
EV21	0.635	0.363	0.848	0.905	0.030	0.440	0.864
GT575	0.785	0.374	0.524	0.537	0.647	0.563	0.074
GATA28	0.803	0.741	0.327	0.728	0.975	0.263	0.986
TAA31	0.458	0.741	1.000	0.478	0.311	0.176	0.274
Total loci	0.638	0.587	0.522	0.909	0.177	0.835	0.382

Table 3. Test for the deviation from Hardy-Weinberg genotypic proportion at microsatellite loci in each of the samples.

Locus	00NP	01NP	02NP	03NP	Pelagic	Off Taiji	Ogasawara
GATA98	0.781	0.786	0.551	0.309	0.386	0.333	0.912
EV104	0.268	0.846	0.540	0.983	0.662	0.595	0.333
GT011	1.000	0.878	0.653	0.285	0.839	0.404	0.822
GA53	0.499	0.989	0.714	0.100	0.370	0.893	0.665
GATA417	0.703	0.994	0.753	0.008	0.445	0.289	0.895
DlrFCB14	0.532	0.750	0.768	0.774	0.533	0.377	0.263
DlrFCB17	0.972	0.999	0.013	0.976	0.948	0.264	0.440
GT23	0.860	0.596	0.753	0.307	0.406	0.001	0.075
EV14	0.046	0.393	0.991	0.124	0.372	0.151	0.061
GT310	0.378	0.131	0.085	0.946	0.443	0.178	0.610
EV1	0.194	0.501	0.709	0.019	0.454	0.114	0.259
EV94	0.784	0.424	0.840	0.230	0.007	0.410	0.057
GGAA520	0.488	0.279	0.556	0.639	0.668	0.180	0.598
EV21	0.967	0.622	0.840	0.662	0.061	0.423	0.236
GT575	0.351	0.690	0.794	0.759	0.709	0.841	0.362
GATA28	0.303	0.792	0.201	0.817	0.325	0.760	0.674
TAA31	1.000	1.000	0.592	0.608	0.223	0.214	0.645
Total	0.878	0.995	0.813	0.192	0.318	0.052	0.377

Table 4. Heterogenity tests among the four JARPNII samples and between the Taiji and Ogasawara samples.

	Test results	
Locus	JARPNII	Taiji x Ogasawara
mtDNA	0.245	0.192
GATA98	0.620	0.433
EV104	0.100	0.552
GT011	0.515	0.604
GA53	0.173	0.913
GATA417	0.359	0.131
DlrFCB14	0.010	1.000
DlrFCB17	0.290	0.628
GT23	0.515	0.977
EV14	0.033	0.853
GT310	0.493	0.622
EV1	0.454	0.983
EV94	0.967	0.226
GGAA520	0.173	0.356
EV21	0.361	0.660
GT575	0.749	0.790
GATA28	0.707	0.741
TAA31	0.254	0.092
Total micro.	0.101	0.942

Table 5. Heterogeneity tests among the samples from the different localities: JARPNII, Pelagic, and Ogasawara.

Locus	Test results
mtDNA	0.201
GATA98	0.034
EV104	0.969
GT011	0.165
GA53	0.539
GATA417	0.620
DlrFCB14	0.465
DlrFCB17	0.932
GT23	0.073
EV14	0.778
GT310	0.760
EV1	0.278
EV94	0.016
GGAA520	0.133
EV21	0.624
GT575	0.224
GATA28	0.512
TAA31	0.538
Total micro.	0.165

Table 6. Heterogeneity tests between the samples from the western North Pacific and that from the eastern Indian Ocean.

Locus	Test results
mtDNA	0.000
EV104	0.000
GT011	0.000
GA53	0.576
GATA417	0.557
DlrFCB14	0.115
DlrFCB17	0.010
GT23	0.000
EV14	0.005
GT310	0.000
EV1	0.052
EV94	0.000
GGAA520	0.000
EV21	0.000
GT575	0.011
GATA28	0.000
TAA31	0.000
Total micro.	Highly signif.