Population structure of the humpback whale in the Antarctic feeding ground based on analysis of mitochondrial DNA control region sequences

Luis A. Pastene[1], Mutsuo Goto[1], Toshiyuki Kimura[2] and Shigetoshi Nishiwaki[1]

[1] The Institute of Cetacean Research
Toyomi-cho 4-18, Chuo-ku,
Tokyo 104, Japan

[2]Nagoya University
Department of Earth and Planetary Sciences,
Chikusa-ku
Nagoya 464, Japan

ABSTRACT

Samples from 144 humpback whales, representing three feeding aggregations in the Antarctic and one in the western North Atlantic, were analyzed with respect to the sequence variation in the mitochondrial DNA control region. Antarctic biopsy samples from Areas IV (n=26) and V (n=12) were obtained by the Japanese Whale Research Program under Special Permit in the Antarctic (JARPA) surveys in those Areas. In addition, published sequence data of humpback whale from Area I (n=11) and western North Atlantic (WNA, n=95) were used in the analysis. A consensus 284 base pairs segment of the mtDNA control region was examined in the total sample of 144 individuals. A total of 47 polymorphic sites defined 52 haplotypes. Most of these haplotypes were specific to one of the four geographical localities. The total nucleotide diversity was estimated to be 3.03%. The degree of genetic differentiation among the four localities was estimated using the haplotype (Hst) and sequence (Kst) statistics and tested for statistical significance by Monte Carlo simulations. Significant degree of heterogeneity were found between WNA and all the Antarctic Areas. Within the Antarctic feeding ground, Area IV was significantly different from both Areas V and I. With this sample size, no significant differences were found between Areas I and V. These results provide genetic support for the historical separation of Groups IV and V in the feeding grounds. Nucleotide diversity in humpback whales from Antarctic Area IV was similar to that of their migratory corridor of Western Australia. Nucleotide diversity in humpback whales from Antarctic Area V, however, was higher than that in their migratory corridor of Eastern Australia. The net genetic distance between Groups IV and V were similar in both their feeding grounds and migratory corridors.

INTRODUCTION

Humpback whales were the first species to be caught when Antarctic whaling began in 1904. Heavy commercial exploitation reduced the population to about 2% of the initial population size of about 130,000 individuals (Allen, 1980). The Southern Hemisphere humpback whale has been protected from commercial exploitation by the International Whaling Commission (IWC) since 1963. (Borchers *et al.*, 1990) estimated the actual population of the humpback whale in the Southern Hemisphere south of 30°S in January and February in 12,000

individuals.

The IWC Scientific Committee (SC) has considered the southern humpback whale as one of the priority species for the comprehensive assessment. Since 1993 a considerable amount of time has been spent during the annual SC meetings to deal with various aspects of the assessment. The identification of genetic stocks and their patterns of distribution and mixing has been considered fundamental. With regard the stock identification issue, the SC has identified some topics included within the 'short-term assessment work':

(a) coding of catch and marking data

(b) inventory of biopsy samples

(c) progress in development of photo-identification effort.

DNA analysis is a powerful technique to investigate stock identity, and such analysis can be made using biopsy samples collected from free-ranging humpback whales. The task of an inventory of biopsy samples from Southern Hemisphere humpback whale was undertaken by the SC during the 1994 and 1995 meetings. A total of 463 biopsy samples was listed of which only 66 came from the feeding grounds in the Antarctic. More than half of the biopsy samples from the feeding grounds were from the JARPA surveys. The SC had noted that the analysis of more samples, particularly from the feeding grounds, are necessary before a comprehensive understanding of stock structure could be obtained.

There is a considerable amount of information on the pattern of distribution and seasonal migratory movement of humpback whales in the Southern Hemisphere. Most of that information is derived from the analysis of 'Discovery'-type marks and catch distribution conducted in the past. Mackintosh (1965) showed that humpback whales tend to gather into five or six distinct feeding concentrations in the Antarctic during the austral summer season. He denominated these concentrations as Groups I-VI.

Two of the areas of feeding concentrations in the Antarctic are Areas IV (70°-130°E) and V (130°E-170°W), where JARPA surveys are conducted. The geographical boundaries of these two areas in the Antarctic were defined considering the distribution of catches and the results of mark-recapture analysis in the humpback whale (Omura, 1953; Chittleborough, 1959).

Fig.1 reproduce a figure of Dawbin (1966). This figure summarize the distribution and seasonal migratory movement of humpback whales from Groups IV (W) and V (E) as demonstrated by mark-recapture data. Whales from Group IV move mainly between Antarctic Area IV and Western Australia. Whales from Group V move between Antarctic Area V and Eastern Australia and along the coast of New Zealand and southwest Pacific islands. It should be noted the interchange of a few individuals between Groups IV and V. Also it should be noted that the boundaries of these Groups in the Antarctic do not correspond to the actual boundaries of Areas IV and V.

Baker *et al.* (1995) presented evidences for genetic differentiation between humpback whales from Western and Eastern Australia, confirming earlier hypotheses that whales feeding in Antarctic Areas IV and V come from separated breeding stocks, to the east and west of

Australia. While genetic analyses demonstrated that whales from Western Australia belong to a different genetic stock than those from Eastern Australia, little is known on the genetic population structure of Groups IV and V in the feeding ground of Areas IV and V. A preliminary mtDNA analysis of humpback whales that used biopsy samples obtained by the JARPA (Pastene *et al.*, 1996a), found no significant differences between these Areas. It should be noted, however, that their analyses was based in small sample sizes and samples from Area IV were mainly from the eastern sector.

Making use of additional biopsy samples collected from Area IV by the JARPA, we presented here a new mtDNA analysis of humpback whales in the Antarctic feeding ground. For comparison, we included in the analysis published mtDNA control region sequences of humpback whales from the Antarctic Peninsula (Area I) and from the western North Atlantic (Palsboll *et al.*, 1995).

MATERIALS AND METHODS

Biopsy samples

Skin biopsy samples were obtained along the sighting surveys of the JARPA, on an opportunistic basis. They have been collected using a biopsy dart shooting gun described by Kasamatsu *et al.* (1991). To avoid re-sampling, ancillary information is obtained for each individual sampled, among them the estimated body length and visual observations of external characters. The same individuals targeted for biopsy sampling are targeted for photo-identification experiments. The relatively large number of biopsy samples of the humpback whale obtained by the JARPA, reflect in part the increasing availability of the species in the research area, and also the non-evasive behavior of the individuals during the biopsy sampling experiments.

Table 1 shows biopsy sampling information of the humpback whale in Areas IV and V. Samples in this table have been ordered by date. There is a total of 42 samples of the humpback whale, 28 from Area IV and 14 from Area V. Samples from Area IV are from two different survey seasons. There is one case of cow-calf pair sampling in Area IV and two cases in Area V. Fig. 2 shows the geographical distribution of the biopsy samples of humpback whales collected by the JARPA surveys in the Antarctic.

After sampling, skin biopsies were stored at -20°C until use.

Biochemical analysis

Extraction of DNA

Genomic DNA (nuclear+mitochondrial DNA) was extracted from approximately 0.05g of the outer epidermal layer of the skin biopsy. For extracting genomic DNA, we used established protocols (Sambrook *et al.*, 1989). The tissue was homogenized in 500ul of TES buffer. Previous addition of 25ul of Sodium Dodecyl Sulfate (20%), 25ul of Proteinase K (20mg/ml) was added and the homogenate was incubated overnight at 37°C. After incubation, the DNA solution was mixed with an equal volume of a 25:24:1 phenol/chloroform/isoamyl alcohol solution, shaken thoroughly and centrifuged to precipitate proteins. Finally DNA was precipitated by adding 1 ml of 99.5% ethanol and incubating at -70°C for 15min. The genomic DNA was then suspended in 500ul of TE buffer and stored at -20°C until use.

Amplification of DNA

We used the polymerase chain reaction (PCR) to amplify a segment of the control region of the mitochondrial genome following instructions given by Hoelzel (1992). For amplification primers MT4-F (Arnason *et al.*, 1993) (5'-CCTCCCTAAGACTCAAGGAAG-3') and P2-R (Hori *et al.*, 1994) (5'-GAAGAGGGATCCCTGCCAAGCGG-3') were used. F and R, respectively, denote a forward- or reverse- oriented primer, with reference to the light strand.

Sequencing analysis

The DNA sequences were determined with an automatic sequencer, the Applied Biosystems 377 (ABI 377), following the protocols of the manufacturer. For each sample both strands were sequenced. Sequences were aligned using the 'Sequence Navigator', a DNA sequence comparison software developed by Applied Biosystems.

Data analysis

Genetic distances among unique sequences (haplotypes) were estimated using the Kimura's two parameters method (Kimura, 1980). The degree of mtDNA diversity within each geographical locality was estimated using the nucleon diversity (Nei and Tajima, 1981) and the nucleotide diversity (Nei and Li, 1979).

The degree of genetic differentiation was estimated using the sequence (Kst) and haplotype (Hst) statistics of Hudson *et al.* (1992). Samples in pairwise comparisons were considered to be significantly heterogeneous when the probability of obtaining the observed, or more extreme, value of either Hst or Kst was less than 0.05 in 1,000 Monte Carlo simulations (Hudson *et al.*, 1992).

RESULTS

Samples and haplotypes

The number of JARPA samples sequenced were 26 (Area IV) and 12 (Area V). Only one of the cow-calf pair sequences was considered for the analysis. In addition 11 published sequences from Area I and 95 from the western North Atlantic (Palsboll *et al.*, 1995) were included in the analysis.

A 284 base pairs of the mtDNA control region was analysed for the total sample of 144 individuals. A total of 47 polymorphic sites defined 52 haplotypes (Table 2). Apart one transversion, all substitutions were transitions.

The frequencies of haplotypes in the four geographical localities are shown in Table 2. In the 11 individuals from Area I, seven haplotypes were detected, three of which were found only in single specimens (Palsboll *et al.*, 1995). In the 26 individuals from Area IV, 19 haplotypes were detected, 14 of which were found only in single specimens. In Area V, 9 haplotypes were detected in 12 individuals, 7 of which were found only in single specimens. In the 95 western North Atlantic individuals, 22 haplotypes were detected, 11 of which were detected in single individuals (Palsboll *et al.*, 1995).

Most of the haplotypes were specific to one of the four localities. Haplotypes '2' was shared by Areas I and V. Haplotype '24' was shared by Areas IV and V. Haplotype '4' was shared by Areas I, IV and V. As reported by Palsboll *et al.* (1995), two Area I individuals shared a

haplotype with one western North Atlantic whale (haplotype '3').

Homogeneity test

Table 3 shows the results of the homogeneity test by Hst and Kst. As expected, both statistics separated clearly the three Antarctic Areas from the western North Atlantic population. Within the Antarctic, Area IV was separated from both Areas I and V. However, no significant differences were observed between Areas I and V.

Intrapopulational mtDNA diversity

Table 4 shows the nucleon diversity and the nucleotide diversity for each of the four geographic localities examined. Nucleon diversity for the total sample was 0.9569. This estimate varied from 0.9055 (western North Atlantic) to 0.9692 (Area IV). Nucleotide diversity for the total sample was 0.0303 and this estimate varied from 0.0246 (Area I) and 0.0326 (Area V).

DISCUSSION

Until now, genetic analysis of humpback whales in the Southern Hemisphere were based mainly in biopsy samples obtained in low latitudes areas, involving some known migratory corridors of the species (Baker *et al.* 1995). Biopsy samples provided by the JARPA surveys have made possible the genetic analysis of humpback whales in the feeding grounds.

Samples provided by the JARPA were from Antarctic Areas IV and V. For comparison, our analysis involved a sample of 95 animals from feeding grounds of the the western North Atlantic and 11 samples from the Antarctic Area I (Palsboll *et al.*, 1995). The nucleotide diversity for the total sample (n=144) was 3.03%, which is slightly high than that obtained by Palsboll *et al.* (1995) whom analysed five feeding aggregations in the North Atlantic and one feeding aggregation in the Antarctic using mtDNA control region sequences (2.60%, n=136).

As expected, western North Atlantic humpback whales were significant different from all the Antarctic Areas. This confirm the previous view that different genetic population of the humpback whale occur in different ocean basins. A haplotype was shared between two Area I individuals and one North Atlantic individual, suggesting gene flow between Northern and Southern Hemisphere (Palsboll *et al.*, 1995).

Within the Antarctic feeding ground, analyses using the haplotype (Hst) and the sequence (Kst) statistics revealed significant degree of heterogeneity between Areas IV and both Areas I and V. Given the actual sample size, no significant differences were detected between Areas I and V.

With regard Groups IV and V humpback whale, Baker *et al.* (1995) had provided evidences for the genetic separation of these Groups in their migratory corridors of Western and Eastern Australia. The results of our mtDNA analysis provide support for the genetic separation of Groups IV and V in the feeding ground. Mark-recapture analysis have suggested that Group V could extend its feeding season distribution into the eastern part of Area IV and that there is an overlap in distribution between Groups IV and V in the Antarctic (Fig. 1). However, our sample from Area IV, which involved humpback whales from both sectors, was significantly heterogeneous from that of Area V.

The nucleotide diversity of Group IV in its migratory corridor and in the feeding ground was almost the same. In Group V, however, nucleotide diversity was higher in the feeding ground than in the migratory corridor of Eastern Australia (Table 5). Until now there are no genetic evidences for separating the Eastern Australia samples from the New Zealand samples (Fig. 1). However, the higher diversity observed in Antarctic Area V could be explained by the possible occurrence of two stocks there in the summer season. To clarify this, a direct comparison of mtDNA control region sequences between feeding grounds and migratory corridors areas is necessary. Also the already available JARPA biopsy samples from Area VI should be examined. This work is in progress.

Apart mark-recapture analysis, the movement of individuals between Area IV and Western Australia and between Area V and Eastern Australia have been also documented using photo-identification techniques, for Group IV (Gill and Burton, 1995) and for Group V (Kaufman *et al.* 1990). In the North Pacific, a marked segregation of mtDNA haplotypes between feeding grounds has been reported (Baker *et al.*, 1990). These authors interpreted such segregation as the consequence of maternally directed fidelity to migratory destinations. A significant degree of mtDNA heterogeneity between feeding gounds in the North Atlantic was also reported by Palsboll *et al.* (1995). It is possible that the significant degree of mtDNA heterogeneity found between Antarctic Areas IV and V be the consequence of maternally directed phylopatry in Groups IV and V humpback whales of the Southern Hemisphere. Therefore the population genetic structure of humpback whales in the Antarctic feeding ground could be simpler than that of the minke whale where a complex pattern of temporal and spatial mtDNA variation has been suggested (Pastene *et al.*, 1996b).

ACKNOWLEDGMENTS

Skin biopsy samples of humpback whales were obtained during the JARPA surveys of 1993/94, 1994/95 and 1995/96 in Areas IV and V. We would like to acknowledge the researchers and crew members that participated in these surveys. We thanks Per Palsboll, Department of Population Biology, University of Copenhagen, for assistance in the data analysis and for useful comments and suggestions.

REFERENCES

Allen, K.R. 1980. *Conservation and management of whales*. University of Washington Press, Seattle. 107 pp.

Arnason, U., Gullberg, A. and Widegren, B. 1993. Cetacean mitochondrial DNA control region: sequences of all extant baleen whales and two sperm whale species. Mol. Biol. Evol. 10:960-70.

Baker, C.S., Palumbi, S.R., Lambersten, R.H., Weinrich, M.T., Calambokidis, J. and O'Brien, S.J. 1990. Influence of seasonal migration on geographic distribution of mitochondrial DNA haplotypes in humpback whales. *Nature* 344:238-240.

Baker, C.S., Florez-Gonzales, L., Rosenbaum, H.C. and Bannister, J. 1995. Molecular Genetic identification of sex and stock structure among humpback whales of the

Southern Hemisphere. Paper SC/47/SH1 presented to the IWC Scientific Committee, May 1995 (unpublished). 25pp.

Borchers, D.L., Butterworth, D.S. and Kasamatsu, F. 1990. Southern Hemisphere whale abundance estimates south of 30°S derived from IWC/IDCR survey and Japanese scouting vessel data. Paper SC/42/SHMi18 presented to the IWC Scientific Committee, May 1995 (unpublished). 25pp.

Chittleborough, R.G. 1959. Australian marking of humpback whales. Norsk Hvalfangsttid 48: 47-55.

Dawbin, W.H. 1966. The seasonal migratory cycle of humpback whales. In Norris, K.S. (ed.). Whales, dolphins and porpoises. University of California Press, Berkerly. pp. 145-171.

Gill, P.C. and Burton, C.L.K. 1995. Photographic resight of a humpback whale between Western Australia and Antarctic Area IV. Marine Mammal Science 11(1):96-100.

Hoelzel, A.R. (ed.). 1992. Molecular Genetic Analysis of Populations. A practical approach. Oxford University Press. Oxford, New York, Tokyo. 315pp.

Hori, H., Bessho, Y., Kawabata, R., Watanabe, I., Koga, A. and Pastene, L. 1994. World-wide population structure of minke whales deduced from mitochondrial DNA control region sequences. Paper SC/46/SH14 presented to the IWC Scientific Committee, May 1994 (unpublished). 11pp.

Hudson, R.R., Boos, D.D. and Kaplan, N.L. 1992. A statistical test for detecting geographic subdivision. *Mol. Biol. Evol.* 9(1):138-151.

Kasamatsu, F., Iwata, S. and Nishiwaki, S. 1991. Development of biopsy skin sampling system for fast swimming whales in pelagic waters. Rep. int. Whal. Commn 41:555-557.

Kaufman, G.D., Osmond, M.G., Ward, A.J. and Forestell, P.H. 1990. Photographic documentation of the migratory movement of a humpback whale (Megaptera novaeangliae) between east Australia and Antarctic Area V. Rep. int. Whal. Commn (special issue 12):265-267.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111-120.

Mackintosh, N.A. 1965. The stocks of whales. Fishing News (Books) Ltd., London. 232pp.

Nei, M and Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269-5273.

Nei, M. and Tajima, F. 1981. DNA polymorphism detectable by restriction endonucleases. *Genetics* 97:145-163.

Omura, H. 1953. Biological study on humpback whales in the Antarctic whaling areas IV and

V. Scientific Reports of the Whales Research Institute, Tokyo 8:81-102.

Palsboll, P.J., Clapham, P.J., Mattila, D.K., Larsen, F., Sears, R., Siegismund, H.R., Sigurjonsson, J., Vasquez, O. and Arctander, P. 1995. Distribution of mtDNA haplotypes in North Atlantic humpback whales: the influence of behavior on population structure. *Marine Ecology Progress Series* 116:1-10.

Pastene, L.A., Goto, M., Abe, H. and Nishiwaki, S. 1996a. A preliminary analysis of mitochondrial DNA in humpback whales (Megaptera navaeangliae) from Antarctic Areas IV and V. Paper SC/48/SH10 presented to the IWC Scientific Committee, April 1996 (unpublished). 17pp.

Pastene, L.A., Goto, M., Itoh, S. and Numachi, K. 1996b. Spatial and temporal patterns of mitochondrial DNA variation in minke whales from Antarctic Areas IV and V. *Rep. int. Whal. Commn* 46:305-314.

Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual. Second Edition. Cold Spring Harbor Laboratory, New York.

Table 1: Ancillary biopsy sampling information for the humpback whale *= same school as individual above; **=cow-calf pair.

Sample code	Sampling date	Sampling position	Estimated body length	School size
93IVH001	07 Dec.1993	63°00'S, 123°34E	13.1m	1
93IVH002	18 Dec.1993	61°18'S, 115°15E	11.4m	1
93IVH003	13 Jan.1994	60°43'S, 114°35E	12.2m	2
93IVH004	17 Jan.1994	61°31'S, 108°10E	14.3m	2
93IVH005	17 Jan.1994	61°31'S, 108°10E	13.1m	2*
93IVH006	18 Jan.1994	60°25'S, 106°57E	11.6m	1
93IVH007	19 Jan.1994	61°17'S, 101°39E	11.0m	2
93IVH008	19 Jan.1994	61°17'S, 101°39E	11.6m	2*
93IVH009	20 Jan.1994	60°51'S, 100°50E	11.0m	4
93IVH010	20 Jan.1994	60°51'S, 100°50E	11.6m	4*
93IVH011	20 Jan.1994	61°20'S, 101°45E	13.1m	4
93IVH012	24 Jan.1994	64°20'S, 092°51E	12.2m	2
93IVH013	12 Feb.1994	66°54'S, 070°07E	9.1m	2**
93IVH014	12 Feb.1994	66°54'S, 070°07E	14.3m	2**
93IVH015	09 Mar.1994	64°44'S, 115°41E	12.2m	2
93IVH016	09 Mar.1994	64°44'S, 115°41E	13.1m	2*
93IVH017	10 Mar.1994	65°16'S, 120°28E	13.1m	2
93IVH018	10 Mar.1994	65°18'S, 120°50E	12.8m	2
93IVH019	12 Mar.1994	65°34'S, 120°20E	12.8m	2
93IVH020	12 Mar.1994	65°34'S, 120°20E	12.2m	2*
94VH101	08 Dec.1994	65°06'S, 176°42W	11.0m	2
94VH102	16 Dec.1994	62°01'S, 169°38E	10.4m	1
94VH103	29 Dec.1994	64°09'S, 138°33E	11.6m	2
94VH104	29 Dec.1994	64°09'S, 138°33E	13.1m	2*
94VH105	15 Jan.1995	63°07'S, 153°38E	11.6m	3
94VH106	15 Jan.1995	63°07'S, 153°38E	12.8m	3*
94VH107	15 Jan.1995	63°07'S, 153°38E	11.3m	3*
94VH108	25 Jan.1995	64°52'S, 173°55E	12.2m	2**
94VH109	25 Jan.1995	64°52'S, 173°55E	8.0m	2**
94VH110	30 Jan.1995	65°48'S, 177°21W	13.4m	2**
94VH111	30 Jan.1995	65°48'S, 177°21W	6.7m	2**
94VH112	02 Feb.1995	65°32'S, 172°14W	13.1m	2
94VH113	13 Feb.1995	65°40'S, 170°26W	13.1m	2
94VH114	13 Feb.1995	65°40'S, 170°26W	10.7m	2*
95IVH021	31 Dec.1995	62°57'S, 082°47E	10.0-10.5m	2
95IVH022	01 Jan.1996	62°25'S, 088°06E	13.0m	2
95IVH023	06 Jan.1996	63°46'S, 092°45E	10.5-11.0m	3
95IVH024	07 Jan.1996	63°47'S, 094°41E	-----	2
95IVH025	17 Jan.1996	62°09'S, 083°46E	10.0-11.0m	2
95IVH026	13 Feb.1996	62°19'S, 111°33E	12.0m	2
95IVH027	26 Feb.1996	64°38'S, 114°01E	8.0-11.5m	3
95IVH028	29 Feb.1996	65°01'S, 107°09E	11.0-13.0m	2

Table 2: Variable sites defining 52 humpback whale unique sequences in the mtDNA control region. The column on the left are haplotype ID. The numbers above list the nucleotide position of the polymorphic sites starting from the 5' end of the mtDNA control region. haplotype 2 through 52 are listed with reference to haplotype 1. A dot indicate an identical nucleotide at the position relative to haplotype 1. A hyphen indicate a deletion. On the right side of the table are the frequencies of the 52 haplotypes in the three Antarctic Areas and in the western North Atlantic.

						AI	AIV	AV	NA
	1111111	1111111112	2222222222	22222222					
Hap.	225566788	990122233	3456666893	3445556666	66667788				
ID	7134617423	684504534	5500146258	9561260234	56782323				
1	TCGGTTCTTT	TCCG-CCGG	TTATTACTTA	TTCTATAGTC	CGTCATCT	1			
2	C.....C	.....TA.	....C....G	.....T	.A.TG...	1		1	
3	....C.....	.....A.	.C....T..G	.....T	TA.T...C	2			1
4	C	.A....TAA	.C.....G	.C.....T	.A.TG...	2	2	2	
5	..A.....C	.A....TA.	.C..C....G	.C.....T	.A.TG...	2			
6	C	.A....TAA	.C.....G	.C.....T	TA.TG...	1			
7	.....	.....TAA	.C.....G	.C.....	TA.TG...	2			
8	.....	.....TA.	....C....G	.....T	.A.TG...		1		
9	.....	.....TA.	.....C.G	.C.....CT	.A..G...		1		
10	C	.A.A..TAA	.C.....G	.C.....T	TA.T....		1		
11	C	.A....TAA	.C.....G	.C...C...T	.A.TG...		1		
12	.....C..C.	.....T..	.....T..G	.....T	TA.T....		2		
13	.....C.	.A....TA.	.C..C....G	.C...C...T	.A.TG...		1		
14	.....C..C.	.....T..	.....T..G	..T.....T	TA.T....		2		
15	.....	.....TA.	....C....G	.....T	.A..G...		2		
16	.....	.....TA.	....C..C.G	.C.....CT	.A..G...		4		
17	C	.A....TA.	.C..C....G	.....	TA.TG...		1		
18	.....C....	C...T..A.	.C....T..G	.C.....T	TA.T...C		1		
19	..A.....	.....TA.	.C.....CG	C.T....A..	.A.TG...		1		
20	.....C....	C...T..A.	.C....T..G	.....T	.A.T....		1		
21	.....C.	.A....TAA	.C..C....G	.C.....T	TA.TG...		1		
22	C	.....TA.	....C....G	.....T	.A.TG...		1		
23	.....C....	.....T..	.....T..G	..T.....T	TA.T....		1		
24	.....	.....TA.	....C....G	.C...C..C.	.A..G...		1	1	
25	.....C....	.....T..	.....T..G	.....T	TA.T....		1		
26	C	.T....TAA	.C.....G	.C.....T	.A.TG...			1	
27	..A..C....	.....A.	.C....T..G	.....T	TA.T...C			1	
28	C	.A.A..TAA	.C.C....G	.C.....T	TA.T....			1	
29	....C..C..	.A....TA.	.C..C....G	.C.....T	TA.TG...			1	
30	...A..T...	.T.....A.	.....G	....G.....	TACT....			1	
31	...A..T...	.....A.	.....G	....G.....	TACT....			3	
32	.....C.	.....TA.	....C..C.G	.....G.CT	.A..G...				11
33	.....C....	.....A.	.C.C..T..G	.....T	TA.TG..C				18
34	.....C....	.T...T.A.	.C....T..G	.....T	TA.T..TC				9
35	.....C....	.T...T.A.	.....T..G	.....T	TA.T...C				1
36	.....	.....TA.	....C..C.G	.....CT	.A..G...				5
37	.....C.	.....TA.	....C..C.G	.....CT	.A..G...				16
38	.....C....	....T.A.	.C....T..G	.....T	TA.T....				1
39	.....C....	.T...T.A.	.CG...T..G	.....T	TA.T..TC				1
40	.....C....	.....A.	.CC.C..T..G	.....T	TA.TG..C				1
41	.....C.	.....TA.	....C....G	.....CT	.A..G...				2
42	.....C..C.	.....A.	.C....T..G	.....T	TA.T...C				6
43	.....C..C.	.....A.	.C....TC.G	.....T	TA.T...C				1
44	.....C....	.T...T.A.	.C....T..G	...C.....T	TA.T..TC				4
45	.T.....C.	.....TA.	....C..C.G	.....CT	.A..G...				1
46	.....C....	.....T.A.	.C....T..G	.....T	TA.T...C				5
47	.....C....	.T...T.A.	.C....T..G	.....T	TA.T...C				6
48	.....C....	.....T.A.	.C....T..G	.....A.T	TA.T...C				1
49	.....C....	.....A.	.C.C.GT..G	.....T	TA.TG..C				2
50	.....C.	.....TA.	.C..C..C.G	.....G.CT	.A..G...				1
51	.....C....	..T....A.	.C.C..T..G	.....T	TA.TG..C				1
52	.....C....	.....A.	.C.C..T..G	.....T	TA.TGA.C				1
					Total	11	26	12	95

Table 3: Hst (above diagonal) and Kst (below diagonal) in pairwise comparisons and the significance level (P) of these values. The overall Hst and Kst were 0.0359 (P=0.0000) and 0.0834 (P=0.0000), respectively.

	Area I (n=11)	Area IV (n=26)	Area V (n=12)	Western NA (n=95)
Area I		0.0167 **(P=0.0170)**	0.0158 (P=0.1530)	0.0166 **(P=0.0020)**
Area IV	0.0255 **(P=0.0200)**		0.0135 **(P=0.0320)**	0.0227 **(P=0.0000)**
Area V	0.0155 (P=0.1720)	0.0310 **(P=0.0130)**		0.0169 **(P=0.0000)**
Western NA	0.0428 **(P=0.0000)**	0.0550 **(P=0.0000)**	0.0473 **(P=0.0000)**	

Table 4: Estimates of the nucleon diversity and nucleotide diversity within each geographical locality.

	Nucleon diversity	Nucleotide diversity
Area I (n=11)	0.9273	0.0246 (SE=0.0038)
Area IV (n=26)	0.9692	0.0275 (SE=0.0017)
Area V (n=12)	0.9394	0.0326 (SE= 0.0026)
Western NA (n=95)	0.9055	0.0267 (SE=0.0008)
Total (n=144)	0.9569	0.0303 (SE=0.0007)

Table 5: Average % divergence (based on the Kimura 2-parameters) within and between Groups IV and V, in both, migratory corridors of Western and Eastern Australia (Baker *et al.*, 1995) and feeding grounds of Areas IV and V.

	Migratory corridor	Feeding ground
Within Group IV	2.80% (n=22)	2.84% (n=26)
Within Group V	2.20% (n=14)	3.39% (n=12)
Between Groups IV and V*	0.30%	0.26%

*= adjusted for within-region variation.

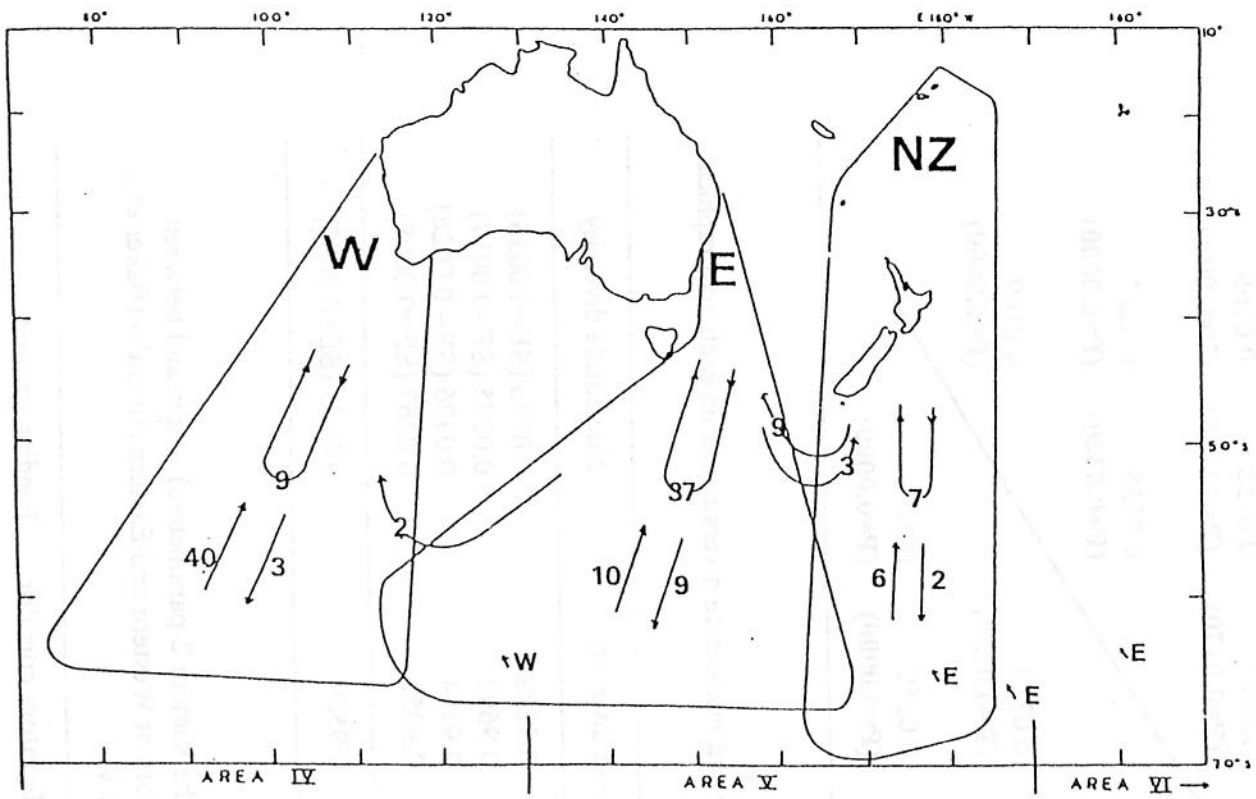

Fig. 1: A modified version of Fig. 2 of Dawbin (1966) showing the movement of humpback whales between low-latitude localities and the Antarctic Areas IV and V, and between low-latitude localities. Information is based in the results of the analysis of mark-recapture data between 70°E and 150°W. W= Western Australian group, E= Eastern Australian group, NZ= New Zealand group. The geographical range of the three groups were defined by the positions of whales marked in the Antarctic and recovered in low-latitude localities (arrows pointing north), the position of whales marked in low-latitude localities and recovered in the Antarctic (arrows pointing south), the position of whales marked and recovered in the same low-latitude locality, in one or more season later (looped arrows within each locality). Interchange between low-latitude localities is shown by curved arrows crossing boundaries between W, E and NZ (see more details in Dawbin, 1966). Note that the boundaries in the Antarctic do not correspond with the actual boundaries of Areas IV and V and that there is an overlap at the boundaries between groups.

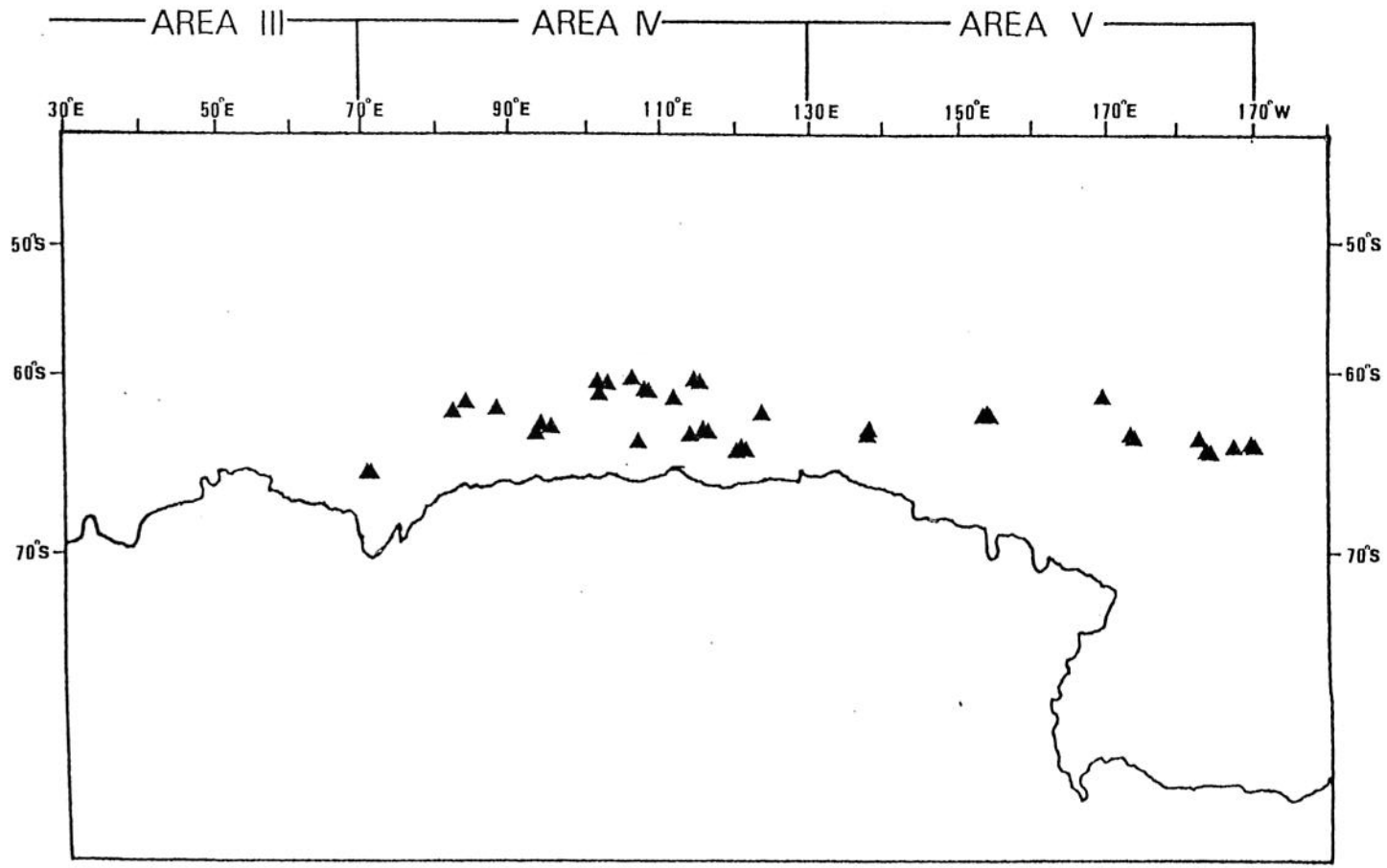

Fig. 2: Geographic distribution of biopsy samples of the humpback whales obtained by the JARPA surveys in Antarctic Areas IV and V.