

Population structure of South Pacific humpback whales and the origin of the eastern Polynesian breeding grounds

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ABSTRACT: Most known concentrations of humpback whales in the southern hemisphere were exploited by commercial whaling operations, first on tropical breeding grounds during the 19th century and then in Antarctic feeding areas and along migratory corridors during the 20th century. However, whaling logbooks of 19th century whalers show almost no records of catches in some regions of current concentration, notably eastern Polynesia, suggesting that humpback whales were formerly absent from these regions or that the locations of their primary concentrations were unknown to early whalers. Here we investigate the population structure of humpback whales across the South Pacific and eastern Indian oceans, with an interest in the origins of whales in eastern Polynesia, using an extensive collection of mitochondrial DNA (mtDNA) sequences obtained from living whales on 6 breeding grounds: New Caledonia, Tonga, Cook Islands, eastern Polynesia (Society Islands of French Polynesia), Colombia and Western Australia. From a total of 1112 samples we sequenced 470 bp of the mtDNA control region, revealing 115 unique haplotypes identified by 71 variable sites. We found significant differentiation, at both the haplotype and nucleotide level ($F_{ST} = 0.033$; $\Phi_{ST} = 0.022$), among the 6 breeding grounds and for most pair-wise comparisons. The differentiation of the eastern Polynesia humpback whales is consistent with the hypothesis of a relic subpopulation, rather than vagrancy or colonization from known neighboring breeding grounds. Regardless of their origin, it seems probable that islands of eastern Polynesia are now the primary breeding grounds for humpback whales feeding in management Area VI (170 to 120° W) of the Antarctic, as defined by the International Whaling Commission.

KEY WORDS: *Megaptera novaeangliae* · mtDNA · Stock structure · Oceania · Indian Ocean · Whaling

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INTRODUCTION

The humpback whale *Megaptera novaeangliae* Borowski, 1781 is found worldwide, with populations in all the major oceans except the Arctic Ocean (Kellogg 1929). During the last 2 centuries, humpback whales have been hunted intensively, especially in the southern hemisphere, where it was estimated that populations were reduced to a few percent of their pre-exploitation abundance (Chapman 1974). Based on catch records corrected for illegal Soviet whaling, a total of more than 200 000 humpback whales were killed from 1904 to 1980 (Clapham & Baker 2002).

From the beginning of this exploitation, it was apparent that humpback whales in the southern hemisphere segregated geographically during their annual migration from winter breeding grounds in tropical waters to summer feeding areas in high latitude waters (Kellogg 1929). Catches during the 19th century by American whalers were made mainly during winter months in 6 tropical breeding grounds. Of these, 3 were in the Pacific Ocean—off Colombia (although located geographically in the northern hemisphere, is considered to be a southern hemisphere population; see below, this section) and Ecuador, around the Tongan archipelago, and northwest of New Caledonia; 2 were in the Atlantic Ocean—off the western coast of Africa and off Brazil; and 1 was in the Indian Ocean—off the northwestern coast of Australia (Townsend 1935, Mackintosh 1942).

During the 20th century, humpback whales were hunted along their migratory corridors and more intensively in their feeding areas in sub-Antarctic and Antarctic waters (Mackintosh 1942, 1965). The distribution of humpback whale catch records led to the identification of 5 main summer feeding areas in the Southern Ocean (see Fig. 1): Area I around the South Shetland Islands (and now considered to extend from 120 to 60° W); Area II in the Weddell Sea and around the Falkland Islands Dependencies (60° W to 0°); Area III between Bouvet and Kerguelen Islands (0 to 70° E); Area IV between Kerguelen Island and Western Australia (70 to 130° E); and Area V between 130° E and 170° W, including the Ross Sea (Mackintosh 1942). A sixth area, 170 to 120° W, was added based on the distribution of blue whales *Balaenoptera musculus*, fin whales *Balaenoptera physalus* or humpback whales, despite little evidence for a concentration of humpback whales (Mackintosh 1942). The 6 feeding areas were later adopted by the International Whaling Commission for purposes of management (Donovan 1991).

'Discovery' marks (stainless steel tags shot into the whale's blubber and later recovered when the whale was killed and flensed) provided the first direct evidence of migratory links between breeding grounds and feeding areas (Mackintosh 1942, Dawbin 1966).

Migratory relationships were established between 3 of the Antarctic Areas—III, IV and V—and the northern breeding grounds closest to them (Mackintosh 1942, Chittleborough 1965, Dawbin 1966). More recently, migratory connections between Colombia/Ecuador and Area I were confirmed through resightings of naturally marked individuals and genetic markers (Stone et al. 1990, Caballero et al. 2001, Stevick et al. 2004). Recently, a migratory connection has been shown between Brazil and Area II by satellite tagging (Zerbini et al. 2006). The putative connections between the western coast of Africa and Areas II and III remain unconfirmed. Thus, it was generally assumed that whales from each feeding area migrated north each year to discrete breeding grounds, forming more or less independent subpopulations or 'stocks' (Mackintosh 1965).

Until recently it was thought that Antarctic Area VI did not encompass a population comparable to those of neighboring Areas (V and I), and consequently that there were no major winter breeding grounds in far Polynesia (to the north of Area VI). Despite extensive whaling effort across the central South Pacific during the 19th century (Townsend 1935) and in adjacent Antarctic areas during the 20th century (Mackintosh 1942), no concentrations of humpback whales were identified in these regions. However, in the last decade, evidence has grown in support of a substantial number of humpback whales in Area VI and eastern Polynesia. Following the revelation of extensive illegal whaling by the USSR from 1947 to 1972/73 (Yablokov 1994), revised records showed substantial catches extending east to 135° W during 1959–60 and 1960–61, mostly in Antarctic waters. More recently, sighting surveys have shown relatively high concentrations of humpback whales in Antarctic Area VI (Brown & Burt 1998). Finally, surveys around the Cook Islands and in French Polynesia conducted since the early 1990s have confirmed a significant concentration of humpback whales in these waters during winter months (Hauser et al. 2000, Poole 2002).

Here we present the most comprehensive survey to date of the population structure of mitochondrial DNA (mtDNA) variation among South Pacific humpback whale breeding grounds, including 4 from Oceania (New Caledonia, Tonga, the Cook Islands and French Polynesia), and 1 from the eastern Pacific coast off Colombia. Additionally, we include the breeding ground off Western Australia, which represents the eastern Indian Ocean, to compare with a breeding ground outside of the South Pacific. We extend previous analyses of mtDNA variation (Baker et al. 1993, 1994, 1998, Baker & Medrano-González 2002), using a longer length of control region sequence, much larger sample sizes and a wider geographic coverage. We then consider the genetic evidence in relation to

3 hypotheses regarding the origins of humpback whales in eastern Polynesia: (1) vagrancy; (2) colonization from an adjacent region; and (3) a previously unknown relic population. We conclude that only the hypothesis of a relic population, perhaps having shifted from some unknown location, is concordant with the observed genetic differentiation in relation to neighboring breeding grounds.

MATERIALS AND METHODS

Study area and sampling methods. Skin samples were collected from humpback whales throughout the South Pacific and on the western coast of Australia during the breeding seasons from 1990 to 2002 (Fig. 1, Table 1). Previously analyzed samples (Baker et al. 1993, 1998, Caballero et al. 2001, Baker & Medrano-González 2002) were re-sequenced to allow the analysis of a longer fragment of the mtDNA control region and for confirmation of polymorphic sites using improved automated sequencing technology. Sequences from New Caledonia (Garrigue et al. 2004) were reviewed for inclusion with new sequences from the 2002 season. Most previously published sequences

from Eastern Australia and New Zealand (Baker et al. 1998) were not included in this analysis because of the small sample size from these areas. One sample from Eastern Australia (EA11) was re-sequenced for inclusion because of its unusual position in an earlier phylogenetic analysis (Baker et al. 1998).

Most of the samples were collected as skin biopsies, using darts propelled by either a crossbow or a modified veterinary capture rifle. The other sources of samples were sloughed skin and a small number of beachcast whales. We attempted to avoid biases in sampling of age/sex classes by approaching groups regardless of composition and by attempting to collect samples from all individuals in a group. In New Caledonia and French Polynesia, samples were collected throughout the winter season. In Tonga, samples were collected throughout a 3- to 4-wk period, during the presumed peak of seasonal abundance (August to September). Most of the samples were stored in the field in 70% ethanol at room temperature and transferred to -70°C in the laboratory for long-term storage.

Laboratory analyses. Genomic DNA was extracted using a standard phenol/chloroform extraction protocol modified for small skin samples by Baker et al. (1994). Symmetrical amplification of the mtDNA con-

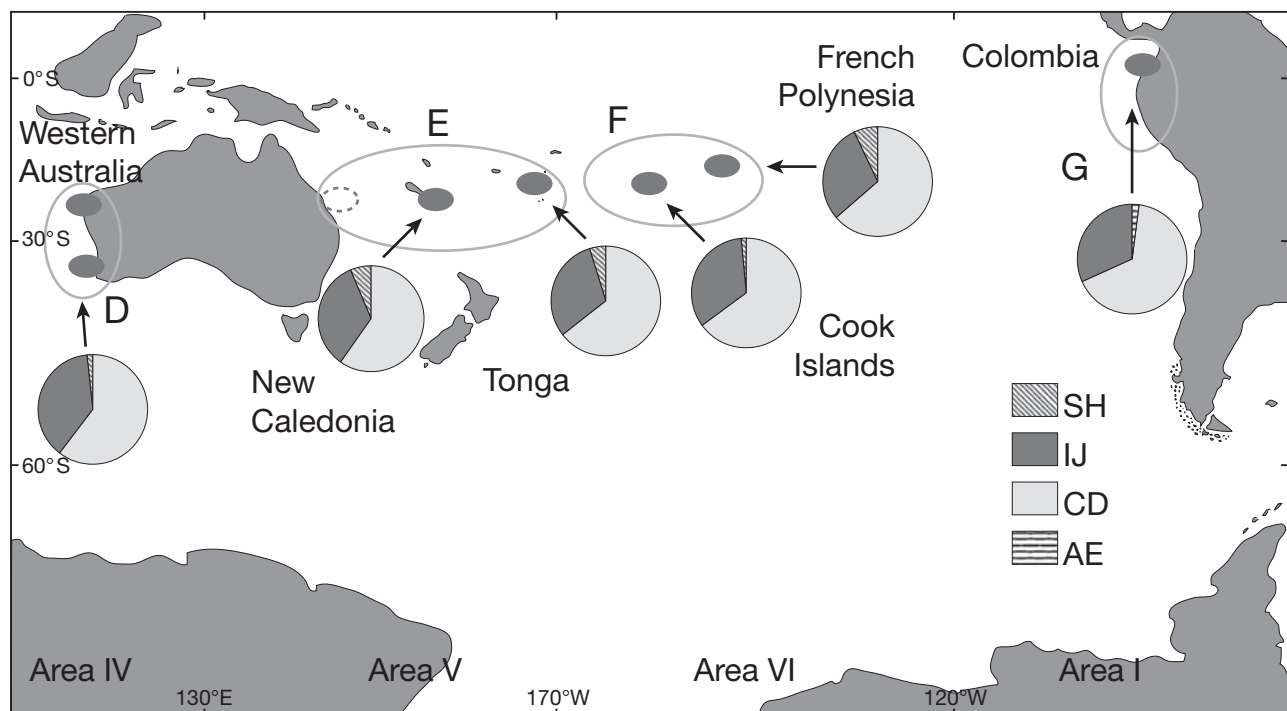


Fig. 1. *Megaptera novaeangliae*. Geographic distribution and proportion of mtDNA control region clades in each breeding ground of the eastern Indian Ocean and South Pacific. Clade denomination (AE, CD and IJ) follows previous publications (e.g. Baker et al. 1993, Caballero et al. 2001, Baker & Medrano-González 2002) and the present study (SH). Division of Antarctic feeding grounds (Areas I and IV to VI) and breeding stocks (D–G) follows the model of stock structure currently used by the Scientific Committee of the International Whaling Commission (IWC 1998)

trol region, proximal to the tPro RNA gene, was performed using PCR following standard protocols. An 800 bp portion of the mtDNA control region was amplified using the primers light-strand tPro-whale Dlp-1.5 and heavy strand Dlp-8G (Garrigue et al. 2004). This region extended across the 2 shorter and partially overlapping fragments used in past analyses, referred to as the 'North Atlantic' and 'Worldwide consensus' regions by Baker & Medrano-González (2002). Amplification and subsequent cycle sequencing were improved by the addition of an M13 forward sequence to the 5' end of the Dlp-1.5 primer. Temperature profiles consisted of a preliminary denaturation period of 2 min at 94°C, followed by 35 cycles of denaturation for 30 s at 94°C, primer annealing for 40 s at 56°C, and polymerase extension for 40 s at 72°C. A final extension period for 10 min at 72°C was included.

Unincorporated primers and nucleotides were removed from PCR products using exonuclease I (Exo I) and shrimp alkaline phosphatase (SAP) and sequenced on an ABI377 or an ABI3100 DNA sequencer (Applied Biosystem) using the primer M13Dlp-1.5. Sequences were aligned and edited using Sequencher (version 4.1.2, Genes Codes). Chromatographs were checked visually for sequencing errors and all variable positions were confirmed by comparison of multiple chromatographs or by reverse sequencing using the Dlp8-G primer (~20% of samples). Comparisons of sequences to identify polymorphic sites and haplotypes were performed using MacClade (version 4.0, Sinauer Associates).

The sex of whales was identified by amplification of sex-specific markers following the protocol of Gilson et al. (1998). This involves a multiplex PCR with primers designed to amplify the male-specific SRY gene and, as positive controls, primers designed to amplify the ZFY/ZFX genes of males and females.

The potential for replicate samples of individual whales was considered for each regional sample set. Replicates were removed in the New Caledonia and Tongan samples, where photographs and microsatellite genotyping allowed for individual identification. Genotypes based on 9 loci were employed in the New Caledonia sample, and between 5 and 9 loci for Tonga (see Garrigue et al. [2004] for details). For other breeding grounds, field notes and individual identification photographs were reviewed to remove replicates, but microsatellite genotypes were not available. The low re-sighting rate of photo-identified whales observed in some of the areas, notably the Cook Islands and French Polynesia (Garrigue et al. 2002), suggests that the number of replicate samples within or between regions was likely to be low.

Data analyses. Genetic diversity was estimated at both the haplotype (without regard to the genetic dis-

tance or number of nucleotide substitutions) and nucleotide level (using unadjusted pair-wise differences between sequences) using the program Arlequin (version 2.0 available from <http://cmpg.unibe.ch/software/arlequin/software/>). The differentiation between breeding grounds was quantified using an Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992) as implemented in Arlequin, calculated for both differences in haplotype frequency (F_{ST}) and nucleotide differentiation (Φ_{ST}). The significance of the observed Φ_{ST} and F_{ST} values was tested using 5000 random permutations of the data matrix. Under the assumption of Wright's Island model of population structure (Takahata & Palumbi 1985), the effective migration rate of females (N_{mf}) was estimated as $(1 - F_{ST}) \times 2F_{ST}^{-1}$ for haplotype and nucleotide indices.

A phylogeny of the humpback whale haplotypes was constructed using the Neighbor-Joining method and maximum parsimony as implemented in PAUP* (version 4.b10, Sinauer Associates). For the Neighbor-Joining method, minimum evolution was used as the default optimality criterion. For parsimony, heuristic search conditions were starting trees obtained by stepwise addition with 10 random sequence addition replicates and tree bisection reconnection (TBR) branch swapping, with searches limited to 100 rearrangements for each replicate. For Neighbor-Joining, the sequences were adjusted for multiple substitutions using the Kimura 2-parameter model. Bootstrap support for Neighbor-Joining reconstruction was calculated after 1000 simulations. The large number of sequences precluded the use of maximum likelihood for phylogenetic reconstruction. Phylogenies were rooted using a blue whale (GenBank accession number X72204) and a fin whale (GenBank accession number X61145) as outgroups, because of their close taxonomic relationship (Sasaki et al. 2005).

RESULTS

Genetic diversity

A total of 1112 humpback whale skin samples collected in 6 discrete regional wintering breeding grounds were used in our analyses (Fig. 1, Table 1). A 470 bp consensus region of the mtDNA control region was defined and used in all subsequent analyses. This consensus region begins at Position 6 of the reference humpback whale control region sequence (GenBank accession number X72202), and is considered to include more than 85% of the variation in the entire control region (Baker & Medrano-González 2002). Seventy-one variable sites defined 115 unique haplotypes among the 1112 samples (GenBank numbers

Table 1. *Megaptera novaeangliae*. Summary of sampling period (years), number of samples (known or assumed to represent individual whales) and haplotypes of mtDNA control region from 6 breeding grounds of the southern hemisphere, including haplotype diversity (h), percentage of nucleotide diversity (π), number of individuals in each clade (AE, CD, IJ or SH) and of each sex (M = male, F = female, U = unknown). For details of stocks, regions and clades see Fig.1

Stock	Region M/F/U	Years	Samples	No. of haplotypes	h	π (%)	AE/CD/IJ/SH
D	Western Australia (WA) 127/43/4	1990, 1993 1994, 2002	174	53	0.970 ± 0.004	2.04 ± 1.04	0/105/66/3
E	New Caledonia (NC) 147/102/1	1995–2002	250	61	0.974 ± 0.003	2.12 ± 1.08	0/149/85/16
E	Tonga (Tg) 216/86/8	1991, 1994–2002	310	48	0.962 ± 0.004	2.01 ± 1.02	0/196/99/15
F	Cook Islands (CI) 70/56/5	1998–2002	131	23	0.923 ± 0.010	1.94 ± 1.00	0/85/44/2
F	French Polynesia (FP) 55/31/13	1997–2002	99	21	0.913 ± 0.012	1.94 ± 1.00	0/63/29/7
G	Colombia (Col) 90/43/15	1991–1999	148	27	0.900 ± 0.016	1.88 ± 0.96	3/98/47/0
	Total 705/361/46		1112	115	0.975 ± 0.001	2.04 ± 1.03	3/696/370/43

DQ768307 to DQ768421, Fig. 2). The variable nucleotides included 3 insertions/deletions, 3 transversions and 65 transitions. The overall haplotype diversity (h) was 0.975 ± 0.001 , and ranged between 0.900 and 0.974 in the regional samples. Nucleotide diversity (π) was $2.04 \pm 1.03\%$, and ranged between 1.88 and 2.12% (Table 1). These were similar to those previously reported and high in comparison to populations in other oceans (Baker & Medrano-González 2002). New Caledonia showed the highest haplotype and nucleotide diversity across the entire study area and Colombia the lowest, suggesting an increase in both measures of diversities from east to west across the study area. A modified t -test (Nei 1987) revealed no significant difference in diversity among breeding grounds at the nucleotide level. However, haplotype diversity was significantly higher in the western region breeding grounds (Western Australia, New Caledonia and Tonga) when compared with those of the eastern region (Cook Islands, French Polynesia and Colombia).

Of the 115 haplotypes found in this study, 2 occurred in all 6 sampled regions, 3 in all 5 South Pacific regions and 60 in only 1 breeding ground (Fig. 2). Western Australia showed the highest proportion of unique haplotypes (50.9%), perhaps reflecting some isolation from the South Pacific or greater interchange with other regions of the Indian and South Atlantic oceans. The Cook Islands sample showed the lowest propor-

tion of unique haplotypes (4.4%); Tonga (8.3%) and French Polynesia (9.5%) had similar proportions of unique haplotypes, and New Caledonia (27.9%) and Colombia (33.3%) had intermediate proportions.

The sex of most whales ($n = 1066$) was identified using molecular methods; however, a small number of sloughed skin samples ($n = 21$) and biopsy samples ($n = 25$) failed to amplify for sex markers. A significant bias towards males was observed (705 males, 361 females, $\chi^2 = 111.09$, $p < 0.001$, Table 1). The male bias was significant at each breeding ground except the Cook Islands ($\chi^2 = 1.56$, $p = 0.21$). A similar bias towards males in tropical catches was reported during commercial whaling (Mackintosh 1942, Chittleborough 1965) and in other more contemporary studies from breeding areas (e.g. Baker et al. 1994, 1998, Brown et al. 1995).

Phylogenetic reconstruction

The Neighbor-Joining and parsimony reconstructions of haplotypes recovered 2 clades (Fig. 3), referred to in previous phylogenetic analyses as the CD and IJ clades (Baker et al. 1993), even though bootstrap support for these was weak ($< 50\%$, Fig. 3). Two basal haplotypes (SP8 and SP9) did not form a clear clade in the Neighbor-Joining reconstruction but corresponded to the previously described AE clade, which is otherwise

SP2	..A..T..T..T...-C...-GT.....T..G.....CT..C..A.....T...C.	SH	6	1					7
SP3	..A..T..T..C...-C...-GT.....T..G.....CT..C..A.....T...C.	SH	6	12	2	2			22
SP4CT..C...-C...-GT.....T..G.....CT..C..A.....T...C.	SH	4	1					5
SP5CT..C...-C...-GT.....C.....T..G.....CT..C..AC.....C.	SH		1					1
SP6C...CG...-C...-GT.....C.....T..G.....CT..C..A.....A.C.	SH					5		5
SP7C...CG...-C...-GT.....C.....T..G.....CT..C..AC.....A.C.	SH	3						3
SP8T..C...-T.C...-GGT.....A..T.....C.G.C.A.....AC...C..	AE						2	2
SP9C...T..C...-T.C...-GGT.....T.....C...C.A...TC..T....	AE						1	1
SP10	...C...T.CC...-T.C...-G...T.....T.....T...A.....	IJ	1	6	2			4	13
SP11	...C...T.CC...-T.C...-GGT...T.....T.....T...AC.....	IJ		4					4
SP12	...C...T.CC...-T.C...-GG...T.....T.....T...AC.....	IJ		2	3	14	3	1	23
SP13	...C...T.CC...-T.C...-G...T.....T.....T...AC.....	IJ		8	10	2			20
SP14	...C...T.CC...-T.C...-G...T.....T.....T...AC.....	IJ	6	17	17	8	1	4	53
SP15	...C...T.CC...-T.C...-G...T.....T.....T...AC.....C	IJ	2	2					4
SP16	...C...T.CC...-T.C.C.-G...T.....T.....T...AC.....????	IJ	1						1
SP17	...C...CT.CC...-T.C...-G...T.....T.....T...AC.....	IJ		11	2				13
SP18	...C...CT.CC...-T.C...-G...T.....T.....T...AC.....	IJ		2					2
SP19	...C...CT.CC...-T.C...-G...T.....T.....T...AC.C.....	IJ	3		9	4	14		30
SP20	...C...CT.CC...-T.C...-G...T.....T.....T...AC.C.....C	IJ	1						1
SP21	??..C...T.CC...-T.C...-G...T.....T.....T...AC.C.....	IJ	1						1
SP22	...C...T.CC...-T.C...-G...T.....T.....T...AC..G.....	IJ	3		10				13
SP23	...C...T.CC...-T.C...-G...T.....T.....T...AC..G.....	IJ	4						4
SP24	...C...CC...-T.C...-G...T.....T.....T...AC.....	IJ			1	3	1		5
SP25	...C...T.CC...-T.C...-G...T.....T.....T...A.....	IJ	1				1		2
SP26	...C...T.CC...-T.C...-G...T.....T.....T...A.....	IJ		2	4				6
SP27	...C...T..C...-C...-G..C.T.....T..G...T...ACT.....	IJ	6	4	8				18
SP28	...C...T..C...-C...-G..C.T.....T..G...T...ACT.....T....	IJ		5					5
SP29	...C...T..C...-C...-G...T.....T.....T...AC.....	IJ		3	4		6	1	14
SP30	...C...T..C...-C...-G...T.....C...T.....T...AC.....	IJ	5						5
SP31	...C...T..C...-C...-G..G..T.....T.....T...AC.....	IJ					1		1
SP32	...C...T..C...-C...-G...T.....T.....T...AC.....T....	IJ						17	17
SP33	.A..C...T..C...-C...-G...T.....T.....T...AC.....C.....	IJ		2	3			6	11
SP34	.A..C...T..C...-C...-GT...T.....T.....T...AC.....	IJ	1						1
SP35	...C...T..C...-C...-G...T.....T.....CT..AC.....	IJ	1						1
SP36	...C...T..C...-C...-GGT...T.....T.....C...A.....	IJ	3	5					8
SP37	...C...T..C...-C...-GGT...T.....T.....CT..A.....	IJ			6	2			8
SP38	...C...CT..C...-C...-GGT...TGT.....T.....A..T...A.....	IJ		4	4	2			10
SP39	...C...T..C...-C...-GGT...TGT.....T.....A..T...A.....	IJ			3				3
SP40	...C...T..C...-C...-GGT...T.....T.....C..A..T...A.....T....	IJ	1						1
SP41	...C...CT..C...-C...-GGT...T.....T.....T...A..C...C.....	IJ	2	2					4
SP42	...C...CT..C...-C...-GGT...T.....T.....T...A...C.....	IJ	12	2	5	6			25
SP43	...C...CT..C...-C...-GGT...T.....TT.....T...A...C.....	IJ			3	3		2	8
SP44	...C...CT..C...-C...-GGT...T.....TT.....CT..A...C.....	IJ		1					1
SP45	...C...CT..C...-C...-GGT...T.....T..G...T...A...C.....	IJ	7						7
SP46	...C...CT..C...-C...-GGT...T.....TT..G...T...A...C.....	IJ		1					1
SP47	??..C...T..C...-C...-GGT...T.....TT..G...T...A...C.....	IJ	1						1
SP48	...C...T..C...-C...-GGT...T.....TT.....T...A...C.....	IJ	3						3
SP49	...C...T..C...-C...-GGT...T.....T.....T...A...C.....	IJ	1	1					2
SP50	...C...T..C...-C...-GGT...T.....T.....T...A..C...C.....	IJ			5		3	11	19
SP51	...C...T..C...-C...-GGT...T.....T.....T...A...C.....	IJ	1						1

Fig. 2 (continued)

characteristic of humpback whales in the North Pacific (Baker et al. 1993, Caballero et al. 2001, Baker & Medrano-González 2002). A fourth clade referred to here as SH (for southern hemisphere) had not been described previously¹, but included the sample EA11 from Eastern Australia (Haplotype SP2), previously noted as unusual in its phylogenetic placement (Baker et al. 1998).

All breeding grounds included haplotypes of the CD, IJ and SH clades except Colombia, which lacked SH haplotypes (Fig. 1, Table 1). Colombia was unique in

that it included haplotypes of the AE clade. The CD clade was the most common across all the regions, followed by the IJ clade and, to a lesser extent, by SH and AE clades. Two clades were recognized by fixed characters; SH was distinguished by 2 transitions at positions 254 (G from A) and 269 (C from T), and clade IJ was distinguished by 2 transitions at positions 62 (C from T) and 168 (T from C).

Differentiation and gene flow among breeding grounds

The AMOVA showed significant overall differentiation among the 6 breeding grounds at the haplotype and nucleotide level ($F_{ST} = 0.033$; $\Phi_{ST} = 0.022$). All pairwise comparisons showed significant differences ex-

¹The SH clade was discovered simultaneously by M. H. Engel and colleagues working on samples from humpback whales on the Brazilian breeding grounds (pers. comm.). The clade denomination was a joint proposal by this group and ourselves

cept that between Tonga and the Cook Islands at the nucleotide level (Table 2). Colombia showed the greatest differentiation from all the other grounds, both at the haplotype and nucleotide levels, most notably with the 2 geographically closest breeding grounds of eastern Polynesia (French Polynesia and Cook Islands). For the rest of the pair-wise comparisons, the observed tendency was towards smaller genetic differentiation values among geographically closer regions.

Assuming Wright's Island model, the overall effective female migration rate per generation (N_{mi}) was estimated to be 14.6 (based on F_{ST}) or 22.9 (based on Φ_{ST}). Assuming a generation time of 18 yr (Roman & Palumbi 2003), this suggested an exchange of around 1 female per year among each breeding ground. However, pair-wise comparisons suggested that migration was more likely between some neighboring breeding grounds (Table 2). The highest estimated gene flow was between Tongan and New Caledonian humpback whales at the haplotype level ($N_{mi} = 57$ females per generation, or interchange of 3 females per year) and between the Cook Islands and Tonga at the nucleotide level ($N_{mi} = 130$ females per generation, or interchange of 7 females per year). The lowest gene flow occurred between Colombia and all the other breeding grounds, estimated to be less than 1 female every 2 yr using either F_{ST} or Φ_{ST} .

DISCUSSION

Humpback whale population structure in the southern hemisphere

The significant geographic differentiation of mtDNA variation among this extensive set of samples supports the recognition of 4 or 5 subpopulations of humpback whales across the South Pacific, each corresponding to a specific winter breeding ground. This differentiation suggests that dispersal of females across the South

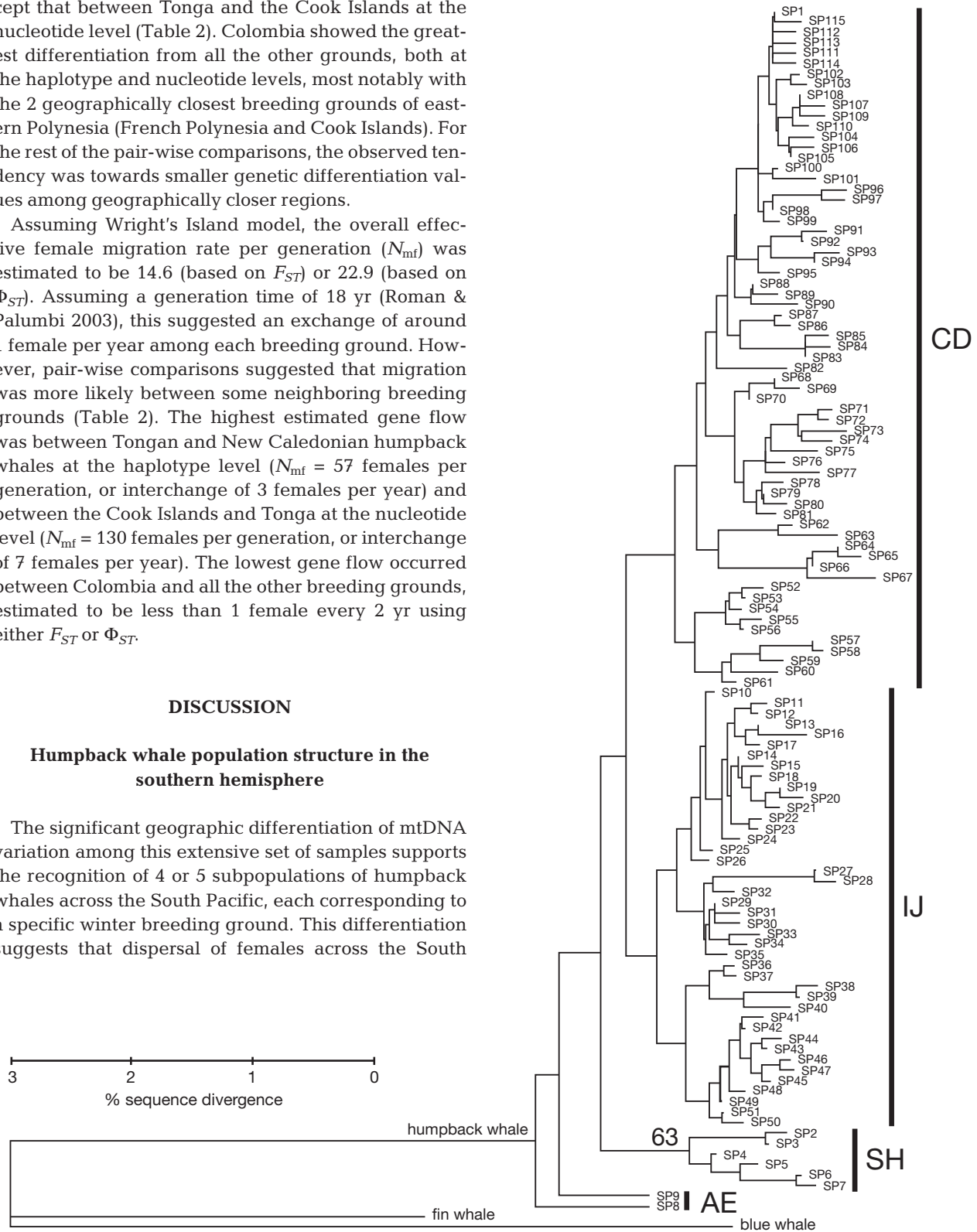


Fig. 3. *Megaptera novaeangliae*. Phylogenetic reconstruction of 115 humpback whales mtDNA control region haplotypes (470 bp in length) using Neighbor-Joining and Kimura 2-parameter distances. Bootstrap support (after 1000 simulations) for major clades (CD, IJ, SH) indicated above branch when > 50%; fin and blue whale sequences included as outgroups

Pacific is limited, despite the absence of geographic barriers, probably as a result of strong maternal fidelity to migratory destinations (Baker et al. 1990). The differentiation of mtDNA is consistent with concurrent studies of individually identified whales (comparable to the number of whale samples in this study for each region) showing regional fidelity and relatively low rates of demographic interchange between adjacent regions, e.g. New Caledonia and Tonga, Tonga and the Cook Islands, and the Cook Islands and French Polynesia (Garrigue et al. 2002).

Analyses of molecular variance and phylogenetic reconstructions of the mtDNA control region show that, in terms of maternal gene flow, the greatest isolation within the South Pacific is between Colombia and Oceania. Whales from Colombia show the highest Φ_{ST} and F_{ST} values in all the pair-wise comparisons with other regions (Table 2). In the phylogenetic reconstruction, Colombia is the only breeding ground represented in the AE clade (Fig. 2) which is otherwise characteristic of the North Pacific population (Baker & Medrano-González 2002), and is the only region not represented in the SH clade (Table 2). The relative isolation of the Colombian breeding ground is consistent with the lack of observed individual interchange with Oceania and western South Atlantic, based on com-

parison of photo-identified whales (Garrigue et al. 2002, Stevick et al. 2004). The genetic distinctiveness of the Colombian subpopulation could be related, in part, to the influence of historic or ongoing trans-equatorial gene flow thought to occur along the Pacific coast of central America (Baker et al. 1990, Caballero et al. 2001, Medrano-González et al. 2001).

The differentiation between Colombia and Oceania is low compared with the differentiation between the Indian Ocean breeding ground (Western Australia) and Oceania, considering the geographic separation (5000 nautical miles from New Caledonia) and the barrier of the Australian mainland that separates the closest breeding ground considered in this study. Given the estimated migration rate of 2 and 4 females per year, it is likely that large-scale comparison of photographic catalogues will reveal individual movements between Western Australia and Eastern Australia or breeding grounds of Oceania. Alternatively, it is possible that such exchange is episodic, as suggested previously by Chittleborough (1965) and Dawbin (1966), based on shifts in humpback whale distribution in feeding areas and recovery of Discovery marks, and more recently by Noad et al. (2000) based on a sudden intrusion of song from the Western Australian into the Eastern Australian population.

Table 2. *Megaptera novaeangliae*. Pair-wise test of differentiation for mtDNA control region sequence between 6 breeding grounds or stocks/sub-stocks of humpback whales in the southern hemisphere showing nucleotide, Φ_{ST} (below diagonal) and haplotype frequency, F_{ST} (above diagonal) differentiation values. Values in **bold** are significantly greater than those found in 5% of 5000 random permutations of the data matrix ($p < 0.05$), after adjustment for multiple comparison with the sequential Bonferroni correction test (Rice 1989). Probability (p) of obtaining greater values by chance alone is given in *italics*. Underlined values show estimated female effective migration rate (N_{mf}). See Table 1 for region abbreviations; see Fig. 1 for location of stocks; stocks defined in 'Introduction'

Region-Stock	WA-D	NC-E	Tg-E	CI-F	FP-F	Col-G
WA-D		0.014 <i><0.0002</i>	0.016 <i><0.0002</i>	0.028 <i><0.0002</i>	0.039 <i><0.0002</i>	0.058 <i><0.0002</i>
		<u>36</u>	<u>32</u>	<u>18</u>	<u>12</u>	<u>8</u>
NC-E	0.007 <i>0.019</i> <u>68</u>		0.009 <i><0.0002</i>	0.032 <i><0.0002</i>	0.046 <i><0.0002</i>	0.055 <i><0.0002</i>
			<u>57</u>	<u>15</u>	<u>10</u>	<u>9</u>
Tg-E	0.012 <i>0.003</i> <u>42</u>	0.004 <i>0.045</i>		0.022 <i><0.0002</i>	0.031 <i><0.0002</i>	0.058 <i><0.0002</i>
		<u>125</u>		<u>22</u>	<u>16</u>	<u>8</u>
CI-F	0.014 <i>0.009</i> <u>36</u>	0.013 <i>0.007</i> <u>39</u>	0.004 <i>0.109</i> <u>130</u>		0.025 <i><0.0002</i> <u>20</u>	0.073 <i><0.0002</i> <u>6</u>
FP-F	0.032 <i><0.0002</i> <u>15</u>	0.028 <i>0.001</i> <u>18</u>	0.025 <i>0.001</i> <u>20</u>	0.020 <i>0.008</i> <u>24</u>		0.079 <i><0.0002</i> <u>6</u>
Col-G	0.038 <i><0.0002</i> <u>13</u>	0.041 <i><0.0002</i> <u>12</u>	0.044 <i><0.0002</i> <u>11</u>	0.054 <i><0.0002</i> <u>9</u>	0.045 <i>0.0002</i> <u>11</u>	

Humpback whale stock definition and implications for management

The degree of isolation among breeding stocks observed in this study should be considered in stock identity models used by the Scientific Committee of the International Whaling Commission (IWC) for management of humpback whales in the southern hemisphere. The IWC currently recognizes 3 breeding stocks in the South Pacific based on the location of breeding grounds: 1 north of Area V (referred to as Stock E, IWC 1998), 1 north of Area VI (Stock F) and 1 north of Area I (Stock G). Our results confirm the differences between these 3 stocks, but indicate that breeding Stock E should be further divided into 2 units, representing the difference between New Caledonia and Tonga. In the absence of available genetic material from the east coast of Australia, it remains unknown whether whales from the larger breeding ground along the

Great Barrier Reef (Chittleborough 1965, Dawbin 1966) differ significantly from those found around these 2 island breeding grounds. A similar but more marked division is supported within Stock F, between Cook Islands and the French Polynesia breeding grounds; however, the hypothesized migratory link between those breeding grounds and the adjacent Antarctic Area VI remains unconfirmed. Although whales from around the Cook Islands also showed low but significant mtDNA differentiation, photo-identification comparisons suggest a relatively high demographic interchange with the adjacent breeding ground of Tonga (Garrigue et al. 2002).

The importance of adopting smaller stock subdivisions as described here is emphasized by the variable levels of recovery among humpback whale populations in the South Pacific. Although populations along the east and west coasts of Australia have shown recent increases in abundance (Bannister & Hedley 2001, Paterson et al. 2001), other stocks in the South Pacific seem to have lagged far behind in recovery. Some, such as those formerly found around Fiji and New Zealand, were extirpated as the result of extensive whaling until the late 1950s, and currently comprise only low numbers of whales (Gibbs et al. 2006).

Origin of breeding grounds in eastern Polynesia

The genetic differentiation observed in this study, together with available demographic evidence demonstrating only limited movement of individuals among breeding grounds (Garrigue et al. 2002), is most consistent with the hypothesis of a historically unrecognized (relic) breeding stock in far Polynesia, rather than with alternate hypotheses of recent colonization or vagrancy from neighboring breeding grounds. If the latter 2 hypotheses were to be supported, both an absence of differentiation and evidence of greater interchange by individual whales from other breeding grounds should have been observed.

However, if the relic breeding stock hypothesis is correct, the lack of historical accounts of humpback whales in eastern Polynesia is puzzling. Tahiti (in the Society Islands, French Polynesia) was a popular port of provisioning for whaling vessels in the South Pacific during the 19th century, as chronicled in several documents (Beale [1839] among others). A general lack of interest by whalers in the presence of humpback whales seems unlikely, as humpback whales were an economic, although less desirable, alternative to more valuable sperm whales (Bannister & Hedley 2001). In other ports of the South Pacific, detailed descriptions exist of whalers engaged in hunting for sperm whales and subsequently turning their attention to humpback

whales in winter (Reeves 2002), as depicted by Bullen (1902) in his account of whaling near the Tongan islands of Vava'u.

It is possible that the current distribution represents a relocation into eastern Polynesia by humpback whales from a more remote and unknown area. Although this hypothesis is inconsistent with the general observation that humpback whales show strong fidelity to breeding grounds and feeding areas (Chittleborough 1965, Dawbin 1966), a similar case for recent relocation or colonization of breeding grounds was made for both Hawai'i (Herman 1979) and the northern West Indies (Reeves et al. 2001), neither of which appeared to host large concentrations of humpback whales until recently. Thus, the origin—although not the existence—of the breeding grounds in eastern Polynesia must remain, for now, an open question.

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