GENETIC DIVERSITY AND POPULATION STRUCTURE OF HUMPBACK WHALES (*Megaptera novaeangliae*) FROM ECUADOR BASED ON MITOCHONDRIAL DNA ANALYSES. UPDATE OF SC/59/SH11

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ABSTRACT

Information on the genetic characterization of humpback whales (*Megaptera novaeangliae*) breeding off Ecuador (2º10'S, 81º00'W; Breeding Stock G) is presented. Mitochondrial DNA was extracted and sequenced from 103 skin samples collected between 2002 and 2007 to establish the genetic diversity of Ecuadorian humpback whales. Samples were obtained either from beached animals (n=4), biopsies (n=1) or sloughed skin (n=98). Twenty nine different haplotypes were found, five of which were new and unique. Haplotype diversity ($h \pm SD$) was estimated to be 0.893 ± 0.023 and the nucleotide diversity ($\pi \pm SD$) 0.018 ± 0.009. A pair-wise analysis of molecular variance (AMOVA) was used to compare diversity within and between other areas of known distribution for this stock in the Southeast Pacific (Colombia and Magellan Strait) and the Antarctic Peninsula. Significant differentiation at both haplotype and nucleotide levels was found only with Magellan Strait (p<0.0001). When data from 2006 and 2007 were stratified by sex and year, significant differences were found at both haplotype and nucleotide level between females in 2006 and females in 2007 (p<0.01) and between females in 2006 and males in 2007 (p<0.05). Although the pooled dataset analysis suggests panmixia in the Breeding Stock G, stratified data by sex and year indicate some level of heterogeneity, possibly due to differential female migrating behaviour. Such heterogeneity would be also responsible of the skewed sex proportion toward males obtained (2.5:1). Further research and a larger number of samples from different sites are required to assess appropriately the structure of this population.

KEY WORDS: humpback whale, genetics, breeding grounds, South America, Breeding Stock G.

INTRODUCTION

Humpback whales (*Megaptera novaeangliae*) distribute during the austral winter along the northwestern coast of South America, mainly off Colombia and Ecuador, but also further north, off Panama (Kellog, 1929; Townsend, 1935; Mackintosh, 1942). Whales wintering off those neighbouring locations were considered to form a single breeding stock, despite the lack of information supporting that assumption. Only in the last decade the putative single breeding stock hypothesis was addressed, when individually photo-identified whales were compared among those areas (Flórez-González *et al.*, 1998). Despite the reduced number of compared individuals from Ecuador, a few whales were matched, which suggested that interchange among these regions occurred. Recently, humpback whales have been recorded during the austral winter further north, off Costa Rica (Acevedo-Gutiérrez and Smultea, 1995;

Rasmussen *et al.*, 2007), which expands the range of the wintering grounds of this species in the Southeast Pacific population. All those breeding areas have been linked to the feeding areas in the west side of the Antarctic Peninsula (Stone *et al*., 1990; Caballero *et al*., 2001; Garrigue *et al.*, 2002; Stevick *et al.*, 2004; Rasmussen *et al.*, 2007) and the Magellan Strait, in southern Chile (Acevedo *et al.*, 2007; Capella *et al*. 2008).

The discreteness of Southeast Pacific humpback whale population among the Southern Hemisphere metapopulation was also assumed for a long time (Kellog, 1929; Mackintosh, 1942; Omura, 1953), despite, again, any evidence to support this. Only recently, based on photo-identification (Garrigue *et al.*, 2002; Stevick *et al*., 2004) and genetic analyses (Caballero *et al*., 2001; Olavarría *et al*., 2007), the putative discreteness of this breeding stock (Breeding Stock G; IWC, 1998) was confirmed by comparisons with neighbouring Southern Hemisphere breeding stocks.

Genetic studies on the species have been conducted in recent years in different locations in the Southeast Pacific, including breeding grounds off mainland Ecuador and the Galapagos Islands (Félix *et al.*, 2006a, 2007a), Gorgona Island and Málaga Bay in Colombia (Caballero *et al*., 2000, 2001; Olavarría *et al*., 2007) and feeding areas at the Magellan Strait in southern Chile (Olavarría *et al*., 2006), and along the west coast of the Antarctic Peninsula (Olavarría *et al*., 2000). Such studies have provided an overview of the genetic diversity, which seems to be the lowest among humpback whale stocks in the Southern Hemisphere (Olavarría *et al*., 2007), and have shown a lack of genetic differentiation between the Antarctic Peninsula feeding area and the Colombian breeding ground (Caballero *et al*., 2001; Olavarría *et al*., 2000; 2007). Interestingly, the whales inhabiting the Magellan Strait, represent a separate feeding aggregation (Acevedo *et al.*, 2007), with their own genetic distinctiveness (Olavarría *et al*., 2006).

Despite these recent contributions, there are gaps on our knowledge of this humpback whale stock, particularly about its population structure. Both genetics and photo identification studies (Olavarría *et al*., 2006; Acevedo *et al*., 2007) suggest some level of heterogeneity in the Breeding Stock G, comparable to what occurs in other Southern Stocks (see Rosenbaum *et al*., 2006). Sightings of humpback whales almost year round off Peru (Ramírez, 1988) also suggest that not every animal from this stock complete the annual migration; some animals may stay south of the breeding area, or north of the feeding area if the case, in the high productive waters of the Humboldt Current off Peru and Chile, where they could find food in a predictable manner (Papastavrou and Van Waerebeek, 1997). This migrating behavior would not be exclusive from this stock since it has been reported elsewhere (e.g. Craig and Herman, 1997; Papastavrou and Van Waerebeek, 1997)

In this report we expand our previous analyses on the mitochondrial DNA (mtDNA) control region genetic diversity of humpback whales from Ecuador (see Félix *et al.,* 2006a, 2007a). Our findings show differences at haplotype and nucleotide composition showing a higher level of structure in this population than previously known. We also confirm a lack of differentiation among Ecuador and Colombia breeding grounds and the Antarctic Peninsula feeding area, but significant differences with the Magellan Strait feeding area.

MATERIALS AND METHODS

Sampling

Humpback whale skin samples were obtained between 2002 and 2007 off Ecuador. Four samples were collected from beached animals, 127 from sloughed skin (Amos *et al.*, 1992) and one from biopsing with a Barnett crossbow equipped with a 60cm long arrow and modified tip (Lambertsen, 1987). Three beached animals from mainland Ecuador and the

biopsied whale from Galapagos Islands were reported previously (Félix *et al*., 2006b). Sloughed skin samples were obtained during the breeding seasons 2006-2007 (July-October) onboard whalewatching vessels working at Salinas, Ecuador (2º10'S, 81º00'W) (Figure 1). Sampling was conducted by a research team as part of a long-term research program by the Ecuadorian Foundation for the Study of Marine Mammals – FEMM (see Félix and Haase, 2001, 2005 for additional reference on the study).

When sampling for sloughed skin, the whale-watching boat skippers were asked to approach the site where a whale entered the water after an energetic surface display. Then small pieces of skin were picked up from the upper water column with a net attached to a pole. Pieces of skin were retrieved from the net and stored in plastic containers with either a solution of DMSO saturated NaCl or ethanol 50%. The net was thoroughly washed with sea water until no pieces of skin were visible on its surface, and then the device was considered ready for the next sampling attempt. Once on shore, samples were stored at 4°C for up to six months prior to laboratory analysis.

Sampled whales were photographed for individual identification with the pigmentation pattern on the ventral side of flukes (photo-identification, Katona *et al*., 1979). It was possible to photo-identify, however, only 65% of the sampled whales.

Usually only one animal was sampled by group but occasionally it was possible to collect two or three samples, presumably from different individuals. When more than one sample was taken from the same group the results of the genetic analyses were necessary to define its distinctiveness. If it resulted in the same sex and haplotype, then duplicity was presumed and the sample was not included in the statistical analysis. This criterion was not applied to a mother with female calf. The bias introduced by duplicity would be in the same order of the within year resighting rate obtained simultaneously with photo-ID, this was 6.4% in the season 2006 (Félix *et al*., 2007b). However, from photo-ID records it was established that only one whale was sampled twice (1.5% of the identified whales).

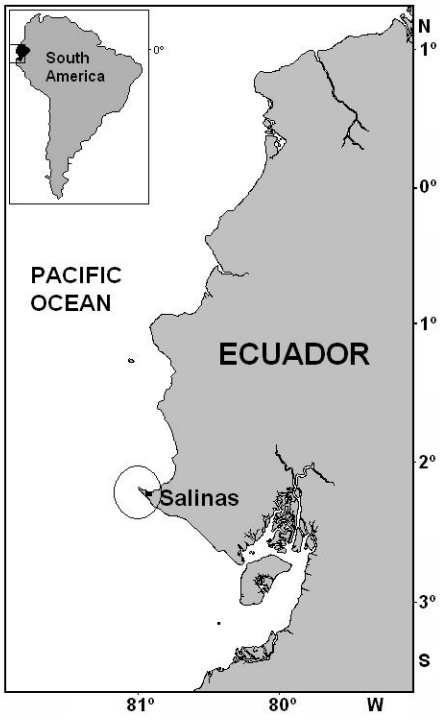

Figure 1. The study area on the coast of Ecuador.

Molecular analyses

A fragment of approximate 800bp of the mitochondrial DNA control region (CR) was amplified via the Polymerase Chain Reaction (Saiki *et al*., 1988) using standard reaction conditions (Palumbi, 1996). For the PCR, we used the primer combination t-Pro-whale Dlp1.5 (5'-TCACCCAAAGCTGRARTTCTA-3') and Dlp8 (5'CCATCGWGATGTCTTATTTAAGRGGAA-3') (Baker *et al.*, 1998; Olavarría *et al.*, 2007). The PCR profile was as follows: an initial denaturation at 95°C for 2 minutes, 36 cycles of 94°C for 30 seconds, 55°C for 1 minute and 72°C for 1 minute and 30 seconds, and a final extension at 72°C for 5 minutes. Free nucleotides and primers were removed from the PCR products using the PCR Cleaning kit (Invitrogen). PCR products were sequenced in both directions using the standard protocols of Big Dye™ terminator sequencing chemistry on an ABI 3100 automated capillary sequencer (Perkin Elmer), using the same PCR primers.

All sequences were manually edited and aligned using Sequencher 4.1 software (Gene Codes Corporation). Sequences were trimmed to 469bp to match a consensus region analysed previously (Olavarría *et al*., 2006; 2007). Control region sequences were compared using MacClade (Maddison and Maddison, 2000) to identify haplotypes. Ecuador haplotypes were compared with haplotypes previously identified in other humpback whale populations in the South Pacific (Olavarría *et al*., 2006, 2007).

Specific sex markers for gender determination followed the methodology of Gilson *et al*. (1998), which amplify a fragment (224 bp) of the *SRY* gene located on the Y chromosome. As internal positive control against PCR amplification failure, homologous ZFY/ZFX region (445bp) were amplified. Thus, in the electrophoresis analysis two bands of 224 and 445 bp were be present in males and only one in females (445bp).

Data analyses

Genetic diversity at haplotype (H) and nucleotide (π) levels were computed with the software Arlequin Ver 3.1 (Schneither *et al*., 2006). Within population differentiation in haplotype frequencies (F_{ST}) and nucleotide (Φ_{ST}) composition as well as at geographic level between Ecuador and Colombia, Antarctic Peninsula and Magellan Strait was quantified using an Analysis of Molecular Variance (AMOVA) (Excoffier, 1995). Stratified data from 2006 and 2007 by sex and year were additionally tested with an exact test of sample differentiation based on haplotype frequencies (Raymond and Rousset, 1995). Both AMOVA and exact test are implemented in the Arlequin software.

RESULTS

Genetic diversity

From the 132 samples obtained, 14 failed sequencing and 15 were considered as duplicates. Thus 103 samples were considered in subsequent analyses, which revealed twenty-nine haplotypes (Table 1). The variable nucleotides include two insertion/deletions, 32 transitions and 1 transversion. Haplotype diversity ($h \pm SD$) was estimated to be 0.893 ± 0.023 and the nucleotide diversity (π) 0.018 ± 0.009. The mean of pair-wise differences was 8.44 ±3.93.

When compared with a total of 120 haplotypes from other Southern Hemisphere breeding grounds and feeding areas (Olavarría *et al*., 2006, 2007), 24 Ecuador haplotypes matched. The remaining five haplotypes were new and corresponded to single Ecuadorian individual whales (Table 2). Five of the previously reported haplotypes (SP16, SP19, SP26, SP41 and SP42) had been not found in either a breeding ground or a feeding area of the Stock G.

Interestingly SP16 haplotype had been only reported in the Western Australian population (see Olavarría *et al*., 2007).

Table 1. Variable sites of 5 new haplotypes from Ecuador when compared with 120 humpback whales mtDNA control region sequences (Olavarría *et al*., 2006, 2007). Alignment in relation to SP1 haplotype.

Haplotype	Variable sites														
	24	62	84	97	99	136	163	168	247	264	267	271	285	381	385
SP1	G	T	C	T	A	A	T	C	C	G	C	T	T	C	C
Mno25Ec06	.	C	T	.	C	G	.	T	T	.	T	.	.	T	.
Mno32Ec06	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
Mno35Ec06	.	.	.	.	C	G	.	.	T	A	.	C	.	.	T
Mno52Ec06	.	C	T	.	C	G	.	.	T	.	T	.	C	T	.
Mno37Ec07	A	.	.	.	.	G	C	.	.	.	.	.	.	T	.

Table 2. Ecuador humpback whale haplotype diversity and frequency of mtDNA control region sequences and proportion of haplotypes by sex and year (2006 and 2007). Haplotype nomenclature follows Olavarría *et al.* (2006, 2007).

Haplotype	**Females**				**Males**				**Unknown**	**Overall**	
(469 pb)	**2006**	**2007**	**Total**	**%**	**2006**	**2007**	Total	**%**	**sex**	**n**	**%**
SP1		1	1	4,0	4	1	5	7,6		6	5,8
SP10						1	1	1,5		1	1,0
SP14					1		1	1,5		1	1,0
SP16	1		1	4,0						1	1,0
SP19						3	3	4,5		3	2,9
SP25	1		1	4,0	2	2	4	6,1	1	6	5,8
SP26	1		1	4,0						1	1,0
SP32		2	2	8,0	3	3	6	9,1	1	9	8,7
SP33	1		1	4,0	2		2	3,0		3	2,9
SP41		1	1	4,0						1	1,0
SP42	1		1	4,0	1		1	1,5		2	1,9
SP50		1	1	4,0	2		2	3,0		3	2,9
SP52					1		1	1,5		1	1,0
SP60	1		1	4,0	3	1	4	6,1		5	4,9
SP61	1		1	4,0	1		1	1,5	1	3	2,9
SP62					2	1	3	4,5		3	2,9
SP68		2	2	8,0		1	1	1,5		3	2,9
SP72						1	1	1,5		1	1,0
SP73	1		1	4,0		1	1	1,5		2	1,9
SP90		4	4	16,0	9	10	19	28,8	7	30	29,1
SP98		3	3	12,0	1	3	4	6,1	1	8	7,8
SP100						1	1	1,5		1	1,0
SP101						1	1	1,5		1	1,0
Mno03MA02						2	2	3,0	1	3	2,9
MnoEc2506					1		1	1,5		1	1,0
MnoEc3206					1		1	1,5		1	1,0
MnoEc3506	1		1	4,0						1	1,0
MnoEc3707		1	1	4,0						1	1,0
MnoEc5206	1		1	4,0						1	1,0
Total	10	15	25	100,0	34	32	66	100,0	12	103	100,0

Sex identification

The sex identification analysis revealed a significant sex bias towards males (68 males, 27 females; χ^2=17.7, p<0.01). The same proportion was also found when only calves were considered (from seven sampled calves, five resulted males and two females).

Population structure by sex

A comparison of haplotype composition by sex was made in order to establish possible variability within the population. For this purpose information from 91 individuals with known haplotype and sex (25 females and 66 males) sampled in 2006 and 2007 was used. Through AMOVA tests the haplotype composition of females and males separately (two groups) as well as between sexes and years (four groups) were compared.

For females there were ten different haplotypes in 2006 and eight in 2007, whereas in males there were 15 different haplotypes in both years (Table 2). Most haplotypes were shared by both sexes and the proportion in most cases was similar, although the haplotype SP90 was almost 50% less represented in females. There was not a significant difference in haplotype frequency and nucleotide composition between sexes (F_{ST} = -0.003, p = 0.535 and Φ_{ST} = -0.0036, p = 0.578).

When comparison included sex and year some differences arose, particularly in females. Haplotypes found in females were different between 2006 and 2007. It is surprising that the most common haplotype in the Breeding Stock G (SP90) was not present in females in 2006, although in the overall result this and other common haplotypes (e.g. SP32, SP25 and SP98) were well represented. In contrast, males showed a fairly regular haplotype frequency in both years. Significant differences in both haplotype frequency and nucleotide composition were found between females in 2006 and females in 2007 (p < 0.01 in both cases), as well as between females in 2006 and males in 2007 (p < 0.05 in both cases) (Table 3).

Table 3. Pair-wise test of differentiation for mtDNA control region sequence by sex and year based on the F_{ST} and Φ_{ST} indices. F = females, M = males, periods 2006 and 2007. The significance was analyzed using 5,000 non-parametric permutations of the data matrix.

	F 2006		F 2007		M 2006	
	Fst	*p*-value	*Fst*	*p*-value	*Fst*	*p*-value
F 2007	0.1700	0.0083 ± 0.0014				
M 2006	0.0758	0.0506 ± 0.0030	-0.0072	0.4886 ± 0.0075		
M 2007	0.1126	0.0157 ± 0.0018	-0.0062	0.4650 ± 0.0073	-0.0089	0.6175 ± 0.006

	F 2006		F 2007		M 2006	
	Φ_{ST}	*p*-value	Φ_{ST}	*p*-value	Φ_{ST}	*p*-value
F 2007	1.6229	0.0085				
M 2006	0.6752	0.0591	-0.0329	0.4393		
M 2007	1.0756	0.0183	-0.0253	0.4165	-0.0749	0.6175

A global exact test of differentiation gave a p-value = 0.07960 ± 0.0344 (20,000 Markov chain steps). However, when the differentiation test included samples by sex and year results were similar to those found based on the F_{ST} and Φ_{ST} indices (a highly significant difference between females in 2006 and females in 2007 (p < 0.01) and a significant difference between

females in 2006 and both males in 2006 and males in 2007 ($p < 0.05$); 100,000 Markov chain steps).

Population differentiation analysis with other Southeast Pacific areas

Ecuadorian haplotype frequencies were compared with other locations in the Southeast Pacific in both breeding (Colombia) and feeding areas (Magellan Strait and the Antarctic Peninsula) as reported by Olavarría *et al*., (2006, 2007). The most common haplotype reported in Ecuador, SP90 (29.1%), is the most common haplotype in the Southeastern Pacific. The second and third most common haplotypes found in Ecuador SP32 (8.7%) and SP98 (7.8%) were also among the most common haplotypes in Colombia and Antarctic Peninsula, but were absent from Magellan Strait. The second most common haplotype from Magellan Strait was recorded in three whales from Ecuador, but was absent in Colombia and Antarctic Peninsula. Two relatively common haplotypes in Colombia SP52 y SP10 were recorded once in Ecuador; the former was found with similar frequency in Colombia and Antarctic Peninsula. Haplotype SP25 was recorded in 6 whales in Ecuador, but only once in Colombia and was absent in Antarctic Peninsula and Magellan Strait. The haplotype found in Galapagos (SP61), was recorded twice off mainland Ecuador, once off Colombia and two at Antarctic Peninsula. Overall, Ecuador humpback whales shared 17 haplotypes of 27 previously reported in Colombia (63%), 15 of 25 in Antarctic Peninsula (60%) and three of four from Magellan Strait (75%).

A pair-wise AMOVA between Ecuador and the other Stock G locations calculated a between variance of 5.97% and a within variance of 94.03%. The high proportion of the within variance indicates a high genetic similarity between the compared sites, as expected for a panmictic population. However, a significant difference was found between Ecuadorian and Magellan Strait whales in both haplotype frequency and nucleotide composition ($p<0.001$ in both cases) (Table 4).

Table 4. Pair-wise test of differentiation for mtDNA control region sequence between Ecuador and other Stock G humpback whales based on the F_{ST} and Φ_{ST} indeces. The significance was analyzed using 5,000 non-parametric permutations of the data matrix.

	Colombia	**Magellan Strait**	**Antarctic Peninsula**
F_{ST}	0.0023	0.1670	0.00206
p-value	0.1979	< 0.0001	0.2416
Φ_{ST}	0.00210	0.14464	0.0018
p-value	0.1966	<0.0001	0.2430

DISCUSSION

The analysis presented here is the first attempt to assess the putative panmixia within the breeding Stock G, including for first time in the analyses samples from Ecuador. Ecuadorian genetic diversity was similar to those from Colombia and Antarctic Peninsula areas as reported by Olavarría *et al*. (2006, 2007), supporting that the Stock G has the lowest genetic diversity among humpback whales in the South Pacific. In fact, the mtDNA diversity of the Breeding Stock G would be the lowest in the entire Southern Hemisphere with the exception the North Indian Ocean stock (see Rosenbaum *et al*., 2006b), perhaps as a result of whaling activities during the XIX and XX centuries and/or a lower gene flow with other southern

stocks. The latter explanation is concordant with the lower number of shared haplotypes found in this population respect to other South Pacific stocks (Olavarría *et al*., 2007).

The results of the AMOVA analysis suggesting panmixia within the breeding grounds of the Breeding Stock G (Ecuador and Colombia) is supported at the demographic level by analyses of photo-identified individuals (Flórez-González *et al*., 1998; Castro *et al*, 2008), although a more thorough comparison is needed, including larger sample size catalogues from these breeding areas and others further north (Costa Rica and Panama). However, when the haplotype dataset was stratified by sex and year the AMOVA analysis showed significant differences at haplotype and nucleotide composition, which suggest that the population would not have a complete panmictic distribution. Differences in sampling timing between females in 2006 and 2007 were responsible of such heterogeneity. Heterogeneity within humpback whale Southern Breeding Stocks was reported by Rosenbaum *et al.* (2006) when data were stratified by sex, year and substocks, although contrarily to our findings the authors reported a higher level of structure in males.

While heterogeneity in Ecuadorian whales could be the result of bias caused by a small sample size, variability in the migrating behaviour cannot be excluded. Evidence of such variability exists for both Northern and Southern populations (see Dawbin, 1966; Craig and Herman, 1997). It has been found, for example, that the migration regime in Hawaiian humpback whales is different for each sex, with males undertaking or completing the winter migration more often than females (Craig and Herman, 1997). Our results support this belief, with males arriving to the breeding area off Ecuador in an annual basis and females with a periodicity still to be defined. Even more, females would not be arriving in a random manner but with a year to year variation as showed by the different haplotypes found in each of two compared years. Such level of structure has not been reported in humpback whales before and may be related with a coordinated migration of whales from the same feeding areas. Influence of maternal lineages in feeding aggregations has been found in humpback whales in the North Atlantic (Weinrich *et al.* 1996) and also in the Southeast Pacific at Magellan Strait (Olavarría *et al*., 2006). It is possible that such influence maintains during the breeding migration and may last up to an early stage at breeding grounds. After their arrival, whales would distribute differently, since no relatedness has been found in breeding groups (Pomilla and Rosenbaum, 2006). Although the breeding migration is in function of the hormonal state, other aspects may influence females' choice to not complete the annual migration or not to undertake it at all, including the energetic costs involved in gestation and lactation and the return to feeding grounds before reaching the breeding area if pregnancy occurs during the migration (Craig and Herman, 1997). The issue requires further research because its potential implications in population assessments and modeling.

Most sampled individuals in Ecuador share haplotypes with whales from Colombia, Magellan Strait and the Antarctic Peninsula. As it was expected, the most common haplotype found in the samples from Ecuador (SP90) is the most common haplotype found in those sampled areas, likewise occurred with the second and third most common haplotypes (SP32 and SP98). These haplotypes have not been recorded in other stocks of the South Pacific basin (see Olavarría *et al*., 2007) and therefore could be used to characterize this humpback whale stock. Interestingly, Ecuador and Colombia breeding grounds parallel their genetic relationship with the Antarctic Peninsula, confirming this last as the main feeding area of this stock. Ecuadorian whales, however, differentiated from the Magellan Strait, supporting that heterogeneity would occur in the migratory pattern of the Stock G (Olavarría *et al*., 2006). This is also supported by photo-identified individuals that show a significant higher correspondence between whales feeding at Magellan Strait with whales breeding at Panama than with Ecuador and Colombia (Acevedo *et al*., 2007).

The presence of haplotype SP16 in the sample from Ecuador, which has been found only in the Western Australian population, is puzzling. It has been demonstrated that extensive movement across humpback whale stocks occur, as shown by the movement of marked whales from Western Australia into de South Pacific (Chittleborough, 1965) and of individually identified whales across the South Pacific (SPWRC, 2006; Robbins *et al*., 2008; Steel *et al.*, *In prep*). Although the absence of haplotype SP16 in the samples from the Eastern and Central Pacific cannot be ruled out due to sampling, an alternative explanation would be a genetic flow from far distant Western Australian whales. Pomilla and Rosenbaum (2005) demonstrated that humpback whales move between the Indian and Atlantic Ocean basins and therefore such a move could also happen between Ecuador and Western Australia. No matches have been found using photo-ID whales from the Southeast Pacific and the Western Atlantic (Stevick *et al*., 2004), however, a Southeast Pacific characteristic haplotype has been reported in Brazilian breeding ground (Engel *et al.*, 2008). Collaboration between research groups working with this species in the Southern Hemisphere (e.g. Rosenbaum *et al*., 2006a) will provide a better understanding of the genetic flow of the species at hemispheric scale.

The sex bias found in this study with males outnumbering females (2.5:1) is similar to that reported in other studies carried out at breeding areas (1.7:1 in the North Atlantic, Palsbøll *et al*. (1997); 1.86:1 in Hawaii, Craig and Herman (1997); 2.4:1 in Eastern Australia, Brown *et al*. (1995); 1.95:1 in South Pacific Olavarría *et al*. (2007)). It has been postulated that the sex bias observed at breeding grounds could be related to migration behavior and/or females heterogeneous distribution (Félix and Haase, 2005), given that such a difference does not occur at feeding grounds (Clapham, *et al*., 1995), neither in the unique population of the Arabian Sea (Mikhalev, 1997). It is unknown how such a disproportion could affect genetic diversity studies based on mtDNA which is maternally transmitted, although Rosenbaum *et al*. (2006b) did not find a measurable effect regarding sex proportion when examined the population structure of several whale stocks in the Southern Hemisphere. The results of our analysis by sex and haplotype composition support the hypothesis of differences in the migrating behavior in this species as a valid explanation for the skewed proportion toward males in this species found in breeding grounds.

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