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RESEARCH ARTICLE

Mitochondrial DNA diversity of the Southwestern Atlantic humpback whale (*Megaptera novaeangliae*) breeding area off Brazil, and the potential connections to Antarctic feeding areas

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Abstract In the Southwestern Atlantic Ocean, humpback whales migrate every winter to the Brazilian coast for breeding and calving in the Abrolhos Bank. This breeding stock represents the remnants of a larger population heavily exploited during the beginning of the 20th century. Despite its relevance to conservation efforts, the degree of current genetic variation and the migratory relationship with Antarctic feeding areas for this population are still largely unknown. To examine these questions, we sequenced ~400 bp of the mitochondrial DNA control region from samples taken off the Brazilian coast ($n = 171$) and near the Antarctic Peninsula ($n = 77$). The genetic variability of the Brazilian humpback whale breeding population was high and similar to that found in other Southern Hemisphere breeding grounds. Phylogenetic analysis suggested the existence of a new mitochondrial clade that exists at low frequency among Southern Hemisphere populations. Direct comparison between the Brazilian and the Colombia breeding populations and the Antarctic Peninsula feeding population showed no genetic differentiation between this feeding region and the Colombian breeding area or between feeding Areas I and II near the Antarctic Peninsula. In contrast, these populations were genetically distinct from the Brazilian population. Two humpback whales sampled off South Georgia Islands, in the Scotia Sea, shared identical haplotypes to whales from Brazil. Our results, supported by photo-identification and satellite telemetry data, suggest that the main feeding area of the Southern Hemisphere humpback whale population is likely to be located near the South Georgia/South Sandwich Islands area and not in the Antarctic Peninsula.

Keywords Humpback whale · Mitochondrial DNA · Abrolhos Bank · Antarctic Peninsula · Genetic diversity · *Megaptera novaeangliae*

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Introduction

In the Southern Hemisphere, humpback whales (*Megaptera novaeangliae*) typically migrate from summer feeding areas in Antarctica to winter breeding grounds in tropical and subtropical regions (Omura 1953; Chittleborough 1965; Mackintosh 1965; Dawbin 1966). The International Whaling Commission (IWC) currently recognizes eight humpback whale breeding grounds (termed A–G, plus X to refer to the Arabian Sea humpback whales) in tropical waters of the Southern Hemisphere (IWC 2005) (Dawbin 1966; Clapham and Mead 1999). In Antarctic waters, the IWC recognizes six major feeding areas (I–VI) originally defined based on whale catch records and since then used to establish management units for commercial whaling in the region (Tonnessen and Johnsen 1982). Migratory links between feeding and breeding areas have been observed for some winter grounds (e.g. Mackintosh 1942; Chittleborough 1965; Dawbin 1966; Caballero et al. 2001; Stevick et al. 2004), but connections remain uncertain for most breeding populations.

Humpback whales wintering off the Brazilian coast are considered part of the breeding stock A (IWC 2005). Previous studies (Engel 1996; Siciliano 1997; Martins et al. 2001; Freitas et al. 2004) suggested the Abrolhos Bank in Brazil (16°40′–19°30′S and 37°25′–39°45′W; Fig. 1a) as the main mating and calving ground of this species in the Southwestern Atlantic Ocean. This was corroborated by aerial surveys along coastal waters between 12°10′S and 20°42′S (Andriolo et al. 2006). It has been suggested that the Antarctic feeding area associated with this breeding ground includes the Antarctic Peninsula, on the boundary between Areas I and II, which are currently divided by meridian 60°W (Donovan 1991) and/or the Scotia Sea, off South Georgia/South Sandwich Islands, in Area II (see Zerbini et al. 2006) (Fig. 1a). Comparisons based in photo-identification catalogs (e.g. Stevick et al. 2004) did not result in any match between the Abrolhos Bank and Antarctic Area I populations. These data, associated with mitochondrial DNA (mtDNA) analyses (Olavarría et al. 2000; Caballero et al. 2001), have demonstrated a migratory link between the population that feeds around the Antarctic Peninsula and that which breeds along the Pacific coast of Colombia and Ecuador (stock G) (Fig. 1a). Although studies in the Antarctic Area II have been less extensive, a recent comparison with humpback whales from Shag Rocks, off South Georgia, revealed the first photo-identification match with the Abrolhos Bank breeding ground (Stevick et al. 2006). This connection has been recently supported by additional photographic matches between Abrolhos Bank and the South Sandwich Islands (Engel et al. unpublished results). Furthermore, another study has shown that two humpback whales tagged with satellite transmitters off the Brazilian coast migrated to feeding areas close to South Georgia and South Sandwich Islands (Zerbini et al. 2006). However, no genetic study comparing the Southwestern Atlantic Ocean breeding stock with the Antarctic feeding grounds has so far been published.

Commercial whaling was estimated to have reduced humpback whale populations to a small fraction of their pre-exploitation size especially in the Southern Hemisphere, where more than 200,000 humpback whales were caught in the past century (Gambell 1973; Clapham 2002). The Brazilian breeding stock was exploited from the 17th century onwards. Before the 1900s whaling operations were coastal and of small scale, on the order of a few dozen whales per year (Ellis 1969). However, its coastal habitat has rendered the humpback whale especially vulnerable to modern whaling methods that, associated with the expansion of the whaling activities in the feeding grounds in Antarctic and Sub-Antarctic waters since 1904, increased the annual catch to several thousand whales, inducing the collapse of the population in about a decade (Tonnessen and Johnsen 1982; Findlay 2001). For example, from 1904 to 1913, 19,000 humpback whales were caught in South Georgia surroundings (Headland 1984). The catch records off Brazil are incomplete but the available data suggests a crash in the population that coincides with a similar crash in South Georgia, although it is not possible yet to establish a causal relationship (Engel et al. unpublished results). Since the worldwide protection of humpback whales from whaling established in 1966 (Rice 1978) most populations have shown signs of recovery, including the Brazilian breeding stock (Engel et al. unpublished results).

It has been argued that for most populations of whales the size reduction during the intense commercial whaling period was probably not severe or long enough to significantly reduce their genetic diversity (Amos 1996). For most humpback whale breeding populations studied so far for mtDNA diversity this prediction seems to hold true (e.g. Baker et al. 1993; Palsboll et al. 1995; Olavarría et al. 2007). Despite the practical importance of genetic diversity parameters for understanding the historical demography of the populations (Baker and Clapham 2004), the current levels of genetic variation of the Southwestern Atlantic stock are still unknown.

In this study, we used mtDNA control-region variation to investigate the genetic diversity and the putative association between the Southwestern Atlantic Ocean humpback whale breeding stock with the Antarctic Peninsula feeding area in order to (1) clarify the migratory links between the functionally different locations and (2) apply genetic information to aid in conservation management issues.

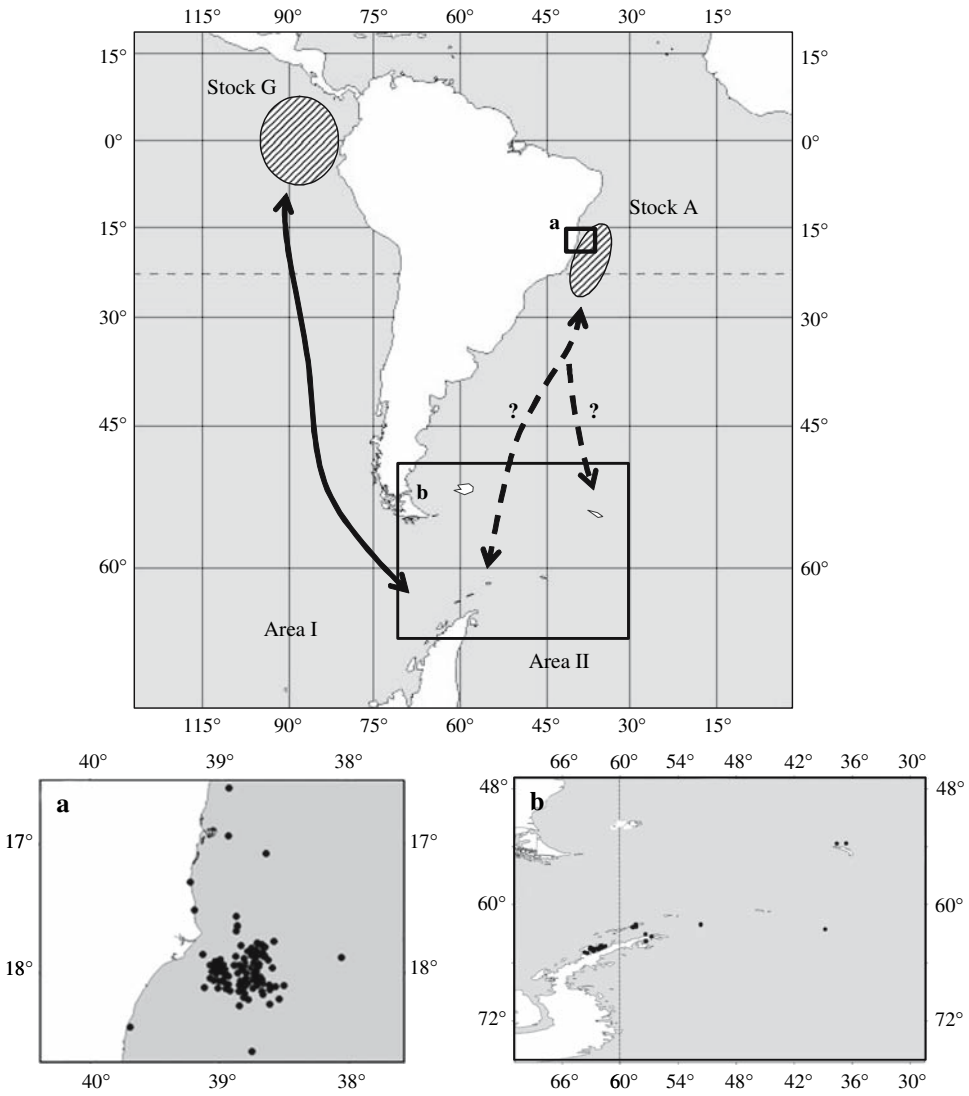

Fig. 1 Map of the studied populations, showing the breeding stocks that occur in South America and their migratory links to the Antarctic Areas I and II, which are limited by the meridian 60°W. Sampling sites comprise the breeding ground off the Brazilian coast and the Antarctic feeding areas, including the Antarctic Peninsula and off South Georgia, in the Scotia Sea. Details on the location of specimens sampled in the Abrolhos bank and in the Antarctic feeding areas are provided in panels (**a**) and (**b**), respectively. For scale reasons, samples resulting from strandings are not represented

Materials and methods

Sampling and mtDNA sequencing

Skin samples of humpback whales, mostly from the Abrolhos Bank, were periodically taken during the breeding season (July–November), from 1997 through 2001 (1997 = 11, 1998 = 38, 1999 = 79, 2000 = 8, 2001 = 35) (Fig. 1a). Some samples were collected from individuals stranded in Bahia and Espírito Santo States or from other locations on the Brazilian coast. Free-ranging whales were sampled using a Barnett Wildcat XL crossbow with stainless steel biopsy darts (8 mm diameter, 15 mm length sampling tip). Samples were kept in 70% ethanol or DMSO (Amos and Hoelzel 1990). Adult animals were sampled randomly among social groups; while sampling of calves was not conducted as part of this study. Whenever possible, multiple sampling of individuals was avoided by using morphological characteristics to identify them on the field. Additionally, a set of 10 microsatellite loci was studied for most samples, and individuals showing the same STR profile were removed from analysis (Cypriano-Souza, Lima-Rosa, Fernández-Stolz, Engel and Bonatto unpublished data). For each sampled whale, date, GPS coordinates and group composition were recorded.

Skin samples were also obtained in the Gerlache and Bransfield Straits and in the Weddell Sea, near the Antarctic Peninsula, using similar sampling procedures as described above (Fig. 1b). These samples were obtained during the expeditions by the Brazilian Antarctic Program (PROANTAR) in the austral summers of the years 1999 and 2000 (see Secchi et al. 2001). Finally, two skin samples obtained four miles off South Georgia, Scotia Sea in 2006 were also compared.

Genomic DNA extraction was carried out following a method based on phenol/chloroform precipitation (Palsboll

et al. 1995). Approximately 450 bp from the most variable portion of the mtDNA control region were amplified using primers Dlp-1.5 and Dlp-5 (Baker et al. 1993). PCR reactions contained approximately 100 ng of genomic DNA, 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 units of Taq DNA polymerase, 0.2 μM of each primer, and 0.2 mM dNTPs in 25 μl of reaction volume. The amplified material was purified with shrimp alkaline phosphatase and exonuclease I. Sequencing reactions were performed with the ET terminator kit (GE Healthcare) and read in the MegaBACE 1000 system (GE Healthcare) using standard electrophorectic conditions. Part of the sequences was obtained using the Applied Biosystems (ABI) 377 DNA Sequencer. Sequences were checked by visual inspection of the resulting chromatogram with Chromas v.2.0 (available at http://www.technelysium.com.au) and with the Phred/Phrap/Consed package (available at http://bozeman.mbt.washington.edu/phredphrapconsed.html).

Statistical methods

Sequence alignment was performed using the program Clustal X under default parameters (Thompson et al. 1997) and corrected by hand in the BioEdit program (Hall 1999). In order to classify the sampled haplotypes according to the three previously described humpback whale mtDNA clades (AE, CD, and IJ) (Baker et al. 1993), the sequences from other populations obtained from GenBank (Baker et al. 1993; Olavarría et al. 2007) were also used. Phylogenies were estimated using the maximum likelihood (ML) method (PhyML program, Guindon and Gascuel 2003) and the neighbor-joining method (Saitou and Nei 1987). The evolutionary model TrN + I + G (Tamura–Nei with invariants and gamma) was selected in the ModelTest program (Posada and Crandall 1998). Support for the groupings was estimated with 100 bootstrap replications. A median-joining haplotypic tree was also estimated using standard parameters with program Network 4.2 (available at http://fluxus-engineering.com) and the sequences obtained here.

Nucleotide and haplotype diversity, and the genetic structure (AMOVA) (Excoffier et al. 1992), were calculated with the ARLEQUIN 3.1 software (Excoffier et al. 2005). For the AMOVA, samples from Brazil, Antarctic Area I and Antarctic Area II were considered as three independent populations. The effect of grouping any two populations was also studied so that all three possible pairs were tested. An AMOVA was performed using conventional haplotype frequencies only and statistical significance was tested using 1,000 permutations. The significance of pairwise F_{ST}s and Φ_{ST}s was assessed using 1,000 permutations.

The sequences from Colombia published by Olavarría et al. (2007) were used to compare the differences between the Brazilian breeding ground and Antarctic feeding areas to those found between the Colombian breeding ground and Antarctic feeding areas. The different sample sizes of these populations made statistical comparisons difficult; therefore jackknife replications were performed to generate 100 datasets with 77 individuals (the size of the smallest sample, Antarctic Peninsula) for each population (Brazil, Antarctic Peninsula, and Colombia) and the distribution of F_{ST} and Φ_{ST} values computed using ARLEQUIN.

Results

Variability of mtDNA control region sequences

About 400 bp of the first segment of the mtDNA control region were sequenced from 171 Brazilian and 77 Antarctic feeding ground samples (46 from Area I and 31 from Area II). Sequences were deposited in GenBank (accession numbers: AY329844–AY330096). For the Brazilian sample, 59 polymorphic sites were identified defining 61 haplotypes. For the Antarctic samples, 33 and 30 segregation sites were detected defining 17 and 14 haplotypes for Areas I and II respectively (Table 1).

The nucleotide and haplotype diversities of these populations were compared to those reported for other breeding grounds within three ocean basins (North Atlantic, North Pacific and Southern Hemisphere) and Antarctic feeding areas (Table 1). The Brazilian haplotype diversity ($h = 0.972$) was high and similar to that found in the majority of the Southern Hemisphere breeding grounds, but it was statistically higher than Colombia and the two Antarctic feeding areas analyzed in the present study. The very similar values for Antarctic Areas I and II ($h = 0.913$, and $h = 0.916$, respectively) were not statistically different, and very close to another estimate available for Area I (Table 1). The nucleotide diversity value found for the Brazilian population ($\pi = 0.025$) was not statistically different from any other population sampled on both breeding and feeding grounds.

mtDNA phylogeny and clade distribution

Sequences from Baker et al. (1993) and Olavarría et al. (2007) were used to reconstruct the mtDNA phylogeny of humpback whales together with the newly detected haplotypes (results not shown). As with these previous studies, the AE, CD, and IJ clades were clearly recovered, despite the low number of common sites (250 bp). However, five haplotypes found in nine individuals from Brazil did not cluster in any of the three previously described clades, grouping with one previously unassigned Eastern Australia

Table 1 Summary of mtDNA diversity statistics from humpback whale populations sampled worldwide, with emphasis on the Southern Hemisphere

Region	*n*	Ha	*L*	*h* (SD)	π (SD)
Breeding (winter) grounds					
Colombia[a]	148	27	470	0.900 (0.016)	0.019 (0.010)
Abrolhos, Brazil[b]	171	61	360	0.972 (0.004)	0.025 (0.013)
Western Australia[a]	174	53	470	0.970 (0.004)	0.020 (0.010)
New Caledonia[a]	250	61	470	0.974 (0.003)	0.021 (0.011)
Tonga[a]	310	48	470	0.962 (0.004)	0.020 (0.010)
Cook Islands[a]	131	23	470	0.923 (0.010)	0.019 (0.010)
French Polynesia[a]	99	21	470	0.913 (0.012)	0.019 (0.010)
Feeding (summer) areas					
Antarctic Area I[c]	11	7	288	0.927 (0.054)	0.026 (0.015)
Antarctic Area I[b]	46	17	360	0.913 (0.021)	0.023 (0.012)
Antarctic Area II[b]	31	14	360	0.916 (0.029)	0.025 (0.013)
Other oceanic basins					
North Atlantic[d]	246	NA[e]	283	0.881 (0.015)	0.024 (0.001)
North Pacific[d]	109	NA[e]	283	0.772 (0.024)	0.046 (0.008)

n, sample size; Ha, number of haplotypes; *L*, sequence length; *S*, number of singletons; *h* (SD), haplotype diversity and standard deviation; and π (SD), nucleotide diversities and standard deviation

[a] Olavarría et al. 2007

[b] This study

[c] Palsboll et al. 1995

[d] Baker and Medrano-Gonzales 2002

[e] Not available

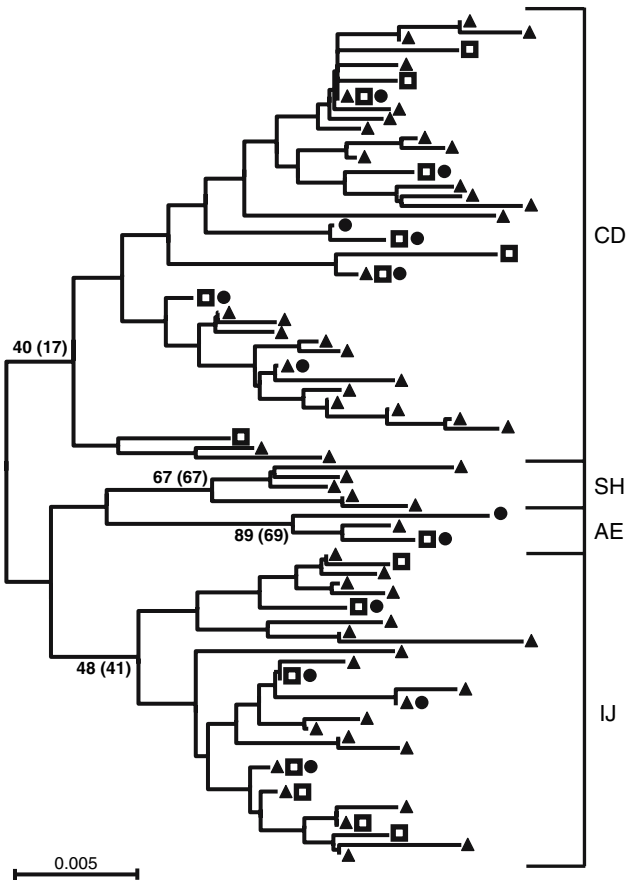

Fig. 2 Maximum-likelihood tree obtained under Tamura–Nei substitution model. The use of alternative models had little impact over the resulting topology. Statistical support for the clades (% bootstrap) are shown in the branches leading to each clade and in parenthesis is the support obtained with neighbor-joining using Tamura–Nei distance. Triangles, open squares, and circles represent haplotypes found in Brazil, Antarctic Area I, and Antarctic Area II, respectively

haplotype (EA11, Baker et al. 1993). This clade was very divergent from its sister clade AE and was found, in some analyses, in a basal position in the trees, in closer proximity to the outgroups.

The phylogenetic tree estimated by the ML method using exclusively haplotypes reported here (~400 bp) showed similar topologies to the reconstructions using shorter sequences, supporting the existence of the three previously known clades and of the divergent clade formed by the five distinct haplotypes from the Brazilian population (Fig. 2). Interestingly, this same clade was found in low frequency in South Pacific populations (Olavarría et al. 2007). Due to its distinctiveness and structured distribution, it was considered as a new mtDNA clade, named SH (jointly discovered by Olavarría et al. 2007). As expected in the relationship between low divergent intraspecific haplotypes (Posada and Crandall 2001) and observed in other studies (e.g. Palsboll et al. 1995; Olavarría et al. 2007), the bootstrap support values for some clades were low. In this context, it is noteworthy that the bootstrap value for the SH clade was relatively high (Fig. 2). The median-joining network also recovered these main clades (results not shown).

Populational comparisons

Although the haplotype clades did not show strong phylogeographic structure with respect to humpback whale distribution, the global frequency distribution of haplotypes among the three studied areas was significantly different (Table 2, $\chi^2 = 20.953$; $P = 0.002$). These data show an excess of the SH clade in Brazil, and of the AE clade in the Antarctic Area II, and a deficit of the AE clade in Brazil.

Table 2 Frequency (%) of each clade in the three areas analyzed

Groups/clades	CD	IJ	AE	SH
Brazil	108 (63.1)	53 (31.0)	1 (0.6)*	9 (5.3)*
Antarctic Area I	31 (67.4)	14 (30.4)	1 (2.2)	0 (0)
Antarctic Area II	18 (58.1)	8 (25.8)	5 (16.1)*	0 (0)

The global frequency distribution among the three populations was significantly not homogeneous ($\chi^2 = 20.953$; $P = 0.002$)

*Significant residuals ($P < 0.05$)

Table 3 Private and shared haplotypes between Brazil (BR), Antarctic Area I (A1) and Antarctic Area II (A2) populations

Haplotypes	Number (% in parenthesis) of haplotypes		
	Population		
	BR	A1	A2
Private	54 (88.5)	6 (35.3)	2 (14.3)
Shared with BR	–	4 (23.5)	5 (35.7)
Shared with A1	4 (6.6)	–	9 (64.3)
Shared with A2	5 (8.2)	9 (52.9)	–
Total[a]	61	17	14

[a] Two haplotypes were common to all populations

An adjusted pairwise comparison further revealed that while the Brazilian and Antarctic Area II populations remain statistically different ($\chi^2 = 17.023$; $P = 0.001$), neither differs from the Antarctic Area I population ($\chi^2 = 5.198$; $P = 0.316$, and $\chi^2 = 5.118$; $P = 0.327$, respectively).

The proportion of private haplotypes differed greatly among the three populations (Table 3). While in the Brazilian sample 88.5% of population specific haplotypes were observed, in the Antarctic samples this proportion was 35.3% in Area I and 14.3% in Area II. The analysis of shared haplotypes was also suggestive of a higher distinctiveness of the Brazilian population, since it revealed nine common haplotypes shared between Antarctic Areas I and II, but only four between Brazil and Area I, and five between Brazil and Area II, despite the much higher sample size of the Brazilian population. Two haplotypes were shared among all populations.

The AMOVA showed that when each area (Brazil, AI, and AII) was considered a separate group, 95.7% of the mtDNA variability was found within the areas (Table 4). Comparing the Brazilian population with the two Antarctic areas considered as a group resulted in the highest value for the among groups component of total variation (4.2%), higher than that found for any other alternative grouping

Table 4 AMOVA results for the pairwise comparisons between Brazil and Antarctic Areas I (AI) and Area II (AII) using mtDNA control region data

Breeding and feeding grounds	Source of variation			F_{ST}*
	Among groups	Among populations within groups	Within populations	
Brazil × AI × AII	–	4.58	95.42	0.04579
Brazil × (AI + AII)	4.41	0.58	95.01	0.04991
AI × (Brazil + AII)	0.32	4.37	95.31	0.04690
AII × (Brazil + AI)	−1.89	5.50	96.39	0.03607

*$P < 0.05$ for all analyses

Table 5 Pairwise F_{ST} (lower triangle) and Φ_{ST} (upper triangle) values between populations

	Antarctic Area I	Antarctic Area II	Brazil
Antarctic Area I	–	0.00085	0.03207*
Antarctic Area II	0.00748	–	0.03178*
Brazil	0.05360*	0.04326*	–

*$P < 0.05$ based on 1,000 replications

(see Methods), although the F_{ST} was statistically significant in all comparisons (Table 4). The greater similarity between the two Antarctic areas was also corroborated by the pairwise F_{ST} and Φ_{ST} matrixes that indicated non-significant values between Antarctic Areas I and II but significant differences between Brazil and any of the Antarctic feeding areas (Table 5).

When directly compared to those haplotypes from the Pacific Colombian breeding population studied by Olavarría et al. (2007), the haplotypes from breeding and feeding areas studied here showed that of the 22 haplotypes found in the Antarctic, 16 were also found in Colombia, while only 7 were found in Brazil. This difference is statistically significant using an exact Fischer test ($P = 0.015$). An alternative approach, employing the number of individuals in Colombia and Brazil that carry these haplotypes, further increased the significance of this difference ($P < 0.0001$). This is a conservative estimate, because the sample size in Colombia is lower than in Brazil (148 vs. 171, respectively). Furthermore, when computing the distribution of F_{ST} and Φ_{ST} values between the three main areas based on 100 jackknife replicates with all areas having identical sample size ($n = 77$), it was found that for both statistics, the values estimated between Brazil versus Antarctic and between Colombia versus Antarctic do not overlap. The former comparison was always larger than the latter, which was around zero for Φ_{ST} values (Fig. 3). Similar estimates for these two statistics were found when comparing Brazil and Colombia and when comparing Brazil and Antarctic.

Finally, the two whales sampled near South Georgia Island presented two different mtDNA haplotypes that are identical to two haplotypes found in the Brazilian population, in which they reach frequencies of 4.9 and 1.8%, but that were not found in the Antarctic and the Colombian populations.

Discussion

The Brazilian humpback whale population shows a high level of nucleotide and haplotype diversity, in agreement with other breeding grounds studied in the Southern Hemisphere (Olavarría et al. 2007). The high levels of

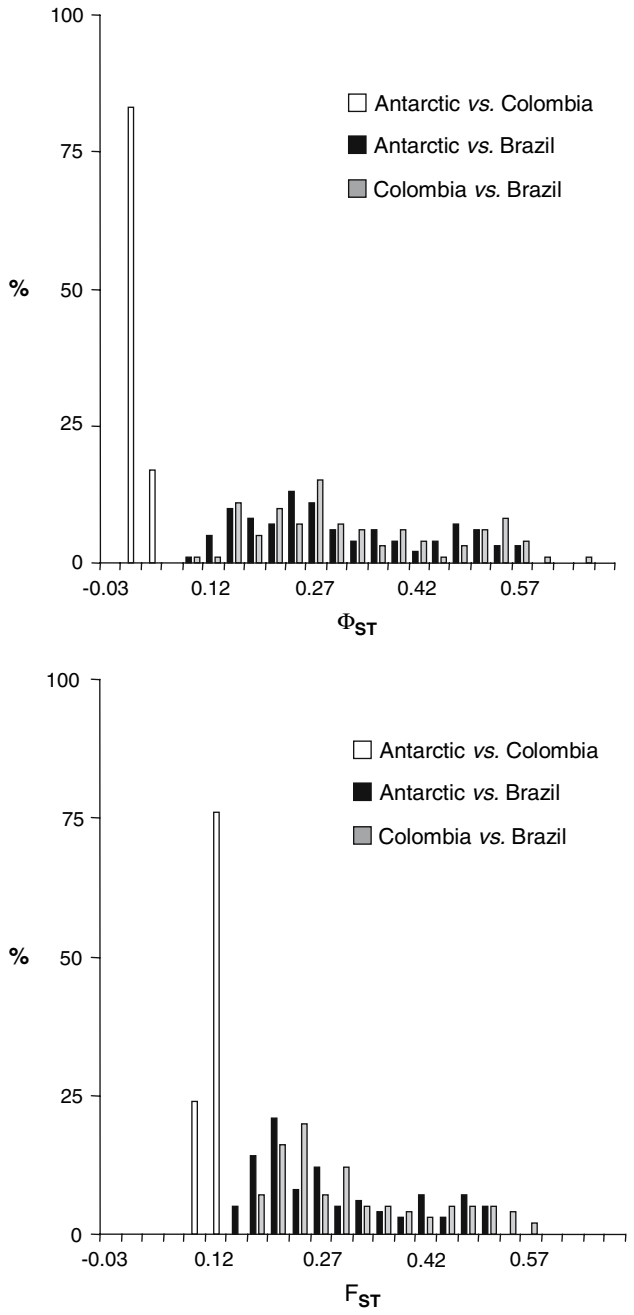

Fig. 3 Distribution of pairwise Φ_{ST} and F_{ST} values based on 100 jackknife resamplings

diversity in these breeding stocks are consistent with the predictions that commercial whaling did not sufficiently reduce the size of the populations nor did it last for enough generations to significantly reduce the genetic variability (Amos 1996). This is in agreement with commercial whaling data that suggest that the most extensive whaling period off the coast of Brazil was relatively recent, and occurring between 1904 and 1967 (Paiva and Grangeiro 1965, 1970). This corresponds to only three to six generations, assuming a humpback whale generation time of 12–24 years (Roman and Palumbi 2003). However, the minimum size reached by the Southwestern Atlantic breeding population during the most intense whaling period is still unknown. Baker and Clapham (2004) have suggested that genetic data, such as the number of mtDNA haplotypes sampled, could be used as an absolute minimum bound on the number of mature females during the whaling bottleneck, and this data could be used as genetic constraints in population size assessments (Jackson et al. 2007). A minimum of 66 different mtDNA haplotypes were found for the Southwestern Atlantic breeding population. In fact, using preliminary data from this study reported to the IWC, results from a Bayesian analyses suggests that the Brazilian breeding population was depleted by commercial whaling to less than 5% of its historical size (Engel et al. unpublished results). However, gene flow between breeding grounds after the whaling bottleneck could also have contributed for the current high mtDNA diversity in the Brazilian and other Southern Hemisphere humpback populations. Unfortunately, this parameter is unknown for the Brazilian population and more data from other Southern Hemisphere breeding grounds, as well as from more informative markers, are needed.

As compared to the Brazilian breeding population, both feeding areas showed lower genetic diversity (at both the haplotype and nucleotide level), with values similar to those observed in the Southeast Pacific breeding ground (Colombia, Table 1). This is likely to be the result of the migratory connection between the Colombian population and the Antarctic Peninsula (Area I and west of Area II) (see below). Our study also suggests the existence of a new mitochondrial clade (SH), represented by five haplotypes found in the Brazilian coast plus the Eastern Australia haplotype EA11. This clade has also been found in low frequencies in other South Pacific breeding grounds with the exception of Colombia (Olavarría et al. 2007), suggesting it is geographically widespread in the Southern Hemisphere. Our results show for the first time the presence of the clade AE in a breeding ground other than Colombia (Olavarría et al. 2007), and corroborates its occurrence in the Antarctic Peninsula (Olavarría et al. 2000). The CD clade was the most common (>50%) in the three studied areas followed by the IJ clade, similar to other reported Southern Hemisphere breeding grounds (Olavarría et al. 2007).

While the relatively low bootstrap values supporting the main mtDNA clades is not unexpected given the low sequence divergence found in populational studies, it also suggests that conclusions based on the existence and distribution of these clades should be regarded with caution. Considering these clades have been used in several studies, it would be important to test their authenticity, which will require much more sequence data, such as whole mtDNA genomes. However, it should be noted that clade information

was not used in most of our analyses (e.g. shared haplotype approach, AMOVA, and pairwise F_{ST} and Φ_{ST}).

Analyses supported the differentiation of the Brazilian breeding stock from the Antarctic feeding ground studied here (the Antarctic Peninsula on the border of Areas I and II). This high differentiation between these two regions becomes clearer when contrasted with the high similarity between the Colombian breeding ground and the Antarctic Peninsula feeding area. So far, these two populations are the only in the Southern Hemisphere where the clade SH is absent. These results are suggestive that the Antarctic Peninsula is unlikely to be the main feeding ground for the Breeding Stock A. One alternative hypothesis for the difference found between Brazil and Antarctic Peninsula samples is the possibility that assemblages of different stocks may occur in the Antarctic waters (Omura 1953), therefore increasing the genetic dissimilarity from its corresponding breeding grounds. However, the existence of such a mixed population in the Antarctic Peninsula area would similarly affect the comparison with the Colombian breeding population, but the results showed that the Antarctic Peninsula and the Colombian populations are virtually indistinguishable. Therefore, with regards to the Brazilian and Colombian breeding populations, the hypothesis of a "mixed" humpback whale population in the Antarctic Peninsula region is not supported by the present mtDNA results (see also below).

Antarctic feeding Areas I and II, near the Antarctic Peninsula, were genetically indistinguishable. Olavarría et al. (2000), who also studied Antarctic Peninsula individuals near the Area I–Area II border, obtained a similar result. Both these studies therefore suggest that the humpback whales that feed near the Antarctic Peninsula are likely to form a single population that presents a migratory link with the Southeastern Pacific breeding population (stock G). Olavarría et al. (2000) also suggested moving the boundary between these two Areas from 60°W to at least 58°W. This suggestion is consistent with the lack of genetic differentiation between these two areas and the apparent absence of natural barriers at 60°W near the Antarctic Peninsula. However, it should be noted that this lack of differentiation only applies to the region near the Antarctic Peninsula, where these samples were mostly taken, and should not be interpreted as meaning that the entire feeding Areas I and II are not differentiated. Any decisive suggestion concerning the change of stock boundaries requires substantial temporal sampling along the proposed boundaries for Areas I and II, as there may have temporal shift in the feeding areas as suggested for other areas (Omura 1953), as well as the study of additional genetic markers from the nuclear genome.

In summary, these results strongly suggest the genetic distinctiveness of the Brazilian humpback whale breeding population, when compared to the Antarctic Peninsula population in the border of Areas I and II, indicating that this region does not constitute the main feeding ground for breeding stock A. This is further supported by the lack of photo-identification matches between the Brazil and Antarctic Peninsula humpback whales (Stevick et al. 2004), and is fully consistent with genetic and photo-identification evidence linking breeding grounds in Colombia and Ecuador to a feeding area in the Antarctic Peninsula (Olavarría et al. 2000; Caballero et al. 2001; Garrigue et al. 2002; Stevick et al. 2004, this study).

The feeding ground for the Brazilian population is likely in the South Georgia/South Sandwich area in the Scotia Sea, as indicated by recent photo-identification (Stevick et al. 2006) and satellite tagging data (Zerbini et al. 2006). In support of this hypothesis it is noteworthy that the two whales sampled near South Georgia Island showed mtDNA haplotypes identical to haplotypes exclusively found in the Brazilian breeding ground. To further assess this hypothesis, a large sampling effort off South Georgia/South Sandwich Islands and examination of both uniparental and biparental molecular markers will be necessary.

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