Update on the evaluation of genetic structure on the feeding grounds and their connectivity to Breeding Regions.

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In SC/60/SH11, we provided estimates of mtDNA differentiation of humpback whales in Antarctic feeding grounds, and evaluated their connectivity with Breeding Stocks A, B and C under multiple scenarios, using different variants of the Naïve and Fringe allocation models. Recently, additional samples became available from 2006/2007 SOWER cruises. Here, we re-evaluate genetic structure of feeding grounds associated with BSB and BSC based on mtDNA data and under Allocation Hypotheses 1 and 2 (Fig 1, Appendix 2, SC 61, Annex H).

A set of 10 microsatellite loci, which have proven to be polymorphic in humpback whales was selected for this study: 199/200, 417/418, 464/465 (Schlotterer *et al*., 1991), EV1Pm, EV37Mn, EV94Mn, EV96Mn (Valsecchi and Amos, 1996), GATA028, GATA053, GATA417 (Palsbøll *et al.*, 1997). Duplicate samples were detected from genotype identity using the Microsoft Excel add-in GENALEX package version 5.1 (Peakall and Smouse, 2001). To evaluate the reliability of the genetic tagging based on the number of loci used, the average probability of two different individuals sharing the same multilocus genotype by chance (probability of identity, PI) was estimated using the Microsoft Excel add-in mentioned above. Based on genetic identity, duplicate samples were excluded from analyses. Genotyping error rates were estimated as described in Cerchio *et al.*, 2009. Standard analyses of genetic variation and differentiation based on mtDNA were performed as described in Loo et al, 2008).

Results and Discussion

Significant differentiation (Fst, phi-st and exact test) was consistently detected between BSB1 and the Nucleus feeding region for BSB (Tables 3 - 5), suggesting that the majority of sampled animals in BSB1 may travel beyond this nuclear area to feed (although, genotypic and Satellite tracking data support BSB1 feeding within the range of nucleus and margin areas). Additionally, significant differentiation (Fst and exact test) was found between all BSC sub-Stocks and the Nucleus feeding region for BSB (Tables 3 and 4). Lack of differentiation between the extent of B and C feeding grounds and BSB2 suggests areas of mixing for these stocks.

Margin B/C and Nucleus feeding regions for C showed a different pattern of differentiation (Fst) under the two Allocation Hypotheses (Table 3). Under Allocation Hypothesis 1, Margin B/C (10E – 30E) did not show significant differentiation from any BS, whereas Nucleus C (30E – 60E) was significantly different from BSB1. Following Allocation Hypothesis 2, Margin B/C (10E – 40E) was significantly different from BSB1 and BSB2, whereas Nucleus C (40E – 70E) did not show significant differentiation from any BS. The variation in differentiation patterns due to the inclusion of wider Margin demonstrates the complexities of these areas and potential ranging patterns of humpback whales on their feeding grounds

In summary, our results shows evidence of the connectivity between BSB2 and the Nucleus feeding region for BSB, and supports mixing of BSB2 and BSC (except for Fst under Allocation Hypothesis 2).

Tables

Table 1. Distribution of humpback whales Breeding Stocks on the feeding grounds

Breeding Stock	Hypothesis 1		Hypothesis 2	
		N		N
B	10W – 10E	110	10W – 10E	110
B/C	10E – 30E	37	10W – 40E	45
C	30E – 60E	30	30E – 70E	23
TOTAL		177		178

Table 2. Sampling sites at the Breeding Regions. N = number of individuals. For detailed information see SC/A06/HW38.

Breeding Regions	N
Region A (Southwestern Atlantic Ocean)	
Abrolhos, Brazil	164
Region B (Southeastern Atlantic Ocean)	
Gabon and Angola (B1)	477
West South Africa (B2)	108
Region C (Southwestern Indian Ocean)	
Mozambique and East South Africa (C1)	151
Mayotte and Geyser, Comoros (C2)	78
Madagascar (C3)	511

Table 3. Mitochondrial differentiation between feeding grounds and Breeding sub-Regions based on different allocation models using fixation indices. Pair-wise F_{ST}. Significant values ($P < 0.05$) are highlighted in bold.

Hypothesis 1	10W - 10E (B)	10E – 30E (B/C)	30E – 60E (C)
B1	**0.00357**	0.00446	**0.00954**
B2	0.00284	0.00616	0.00519
C1	**0.00280**	-0.00388	0.00528
C2	**0.00900**	0.00173	0.00365
C3	**0.00582**	-0.00069	-0.00113
Hypothesis 2	**10W - 10E (B)**	**10E – 40E (B/C)**	**40E – 70E (C)**
B1	**0.00357**	**0.00413**	0.00475
B2	0.00284	**0.00535**	0.0001
C1	**0.0028**	-0.00325	-0.00039
C2	**0.009**	0.00026	0.00019
C3	**0.00582**	-0.00139	-0.00685

Table 4. Mitochondrial differentiation between feeding grounds and Breeding sub-Regions based on different allocation models using fixation indices. Pair-wise ϕ_S. Significant values ($P < 0.05$) are highlighted in bold.

Hypothesis 1	**10W - 10E (B)**	**10E – 30E (B/C)**	**30E – 60E (C)**
B1	**0.00474**	-0.00371	0.01102
B2	-0.00103	-0.00326	-0.00071
C1	0.0049	-0.0079	0.00628
C2	0.00351	-0.00456	-0.00192
C3	**0.00863**	-0.00651	0.00262
Hypothesis 2	**10W - 10E (B)**	**10E – 40E (B/C)**	**40E – 70E (C)**
B1	**0.00474**	-0.0015	0.00787
B2	-0.00103	-0.00279	-0.0039
C1	0.0049	-0.00485	0.00378
C2	0.00351	-0.00332	-0.00583
C3	**0.00863**	-0.00355	-0.00121

Table 5. Mitochondrial differentiation between feeding grounds and Breeding sub-Regions based on different allocation models. Exact Test of Population Differentiation. Significant values ($P < 0.05$) are highlighted in bold.

Hypothesis 1	**10W - 10E (B)**	**10E – 30E (B/C)**	**30E – 60E (C)**
B1	**0.00189+-0.0018**	**0.00518+-0.0022**	**0.00157+-0.0009**
B2	0.12727+-0.0052	0.07026+-0.0089	0.19318+-0.0206
C1	**0.03412+-0.0090**	0.93250+-0.0089	0.10610+-0.0146
C2	**0.00000+-0.0000**	0.21489+-0.0202	0.09222+-0.0100
C3	**0.00000+-0.0000**	0.51166+-0.0362	0.44165+-0.0186
Hypothesis 2	**10W - 10E (B)**	**10E – 40E (B/C)**	**40E – 70E (C)**
B1	**0.00293+-0.0020**	**0.00512+-0.0013**	**0.00259+-0.0011**
B2	0.12313+-0.0095	0.07685+-0.0072	0.33220+-0.0223
C1	**0.02237+-0.0030**	0.86610+-0.0166	0.23800+-0.0241
C2	**0.00006+-0.0001**	0.30383+-0.0196	0.12434+-0.0087
C3	**0.00000+-0.0000**	0.46871+-0.0373	0.67748+-0.0300

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