Conserv Genet
DOI 10.1007/s10592-010-0121-9

SHORT COMMUNICATION

Genetic diversity and structure of blue whales (*Balaenoptera musculus*) in Australian feeding aggregations

Catherine R. M. Attard · Luciano B. Beheregaray · Curt Jenner · Peter Gill · Micheline Jenner · Margaret Morrice · John Bannister · Rick LeDuc · Luciana Möller

Received: 19 July 2009 / Accepted: 5 August 2010

Abstract The worldwide distribution of blue whales (*Balaenoptera musculus*) has not prevented this species from becoming endangered due to twentieth century whaling. In Australia there are two known feeding aggregations of blue whales, which most likely are the pygmy subspecies (*B. m. brevicauda*). It is unknown whether individuals from these feeding aggregations belong to one breeding stock, or multiple breeding stocks that either share or occupy separate feeding grounds. This was investigated using ten microsatellite loci and mitochondrial DNA control region sequences ($N = 110$). Both sets of markers revealed no significant genetic structure, suggesting that these whales are likely to belong to the same breeding stock.

Keywords Blue whale · *Balaenoptera musculus* · Microsatellite · Mitochondrial DNA · Australia

Introduction

Whaling has reduced the abundance of the largest extant animal, the blue whale (*Balaenoptera musculus*), to a small fraction of its original numbers (Clapham et al. 1999; Branch et al. 2007). In the Southern Hemisphere there are two recognised subspecies of blue whales: the 'pygmy' blue whale (*B. m. brevicauda*) found in lower latitudes and the Antarctic 'true' blue whale (*B. m. intermedia*) found in higher latitudes during warmer months (Rice 1998). In this hemisphere most whaling effort was applied to the Antarctic subspecies, however the Soviets continued illegal hunting of pygmy blue whales after their catches were

Electronic supplementary material The online version of this article (doi:10.1007/s10592-010-0121-9) contains supplementary material, which is available to authorized users.

C. R. M. Attard · L. B. Beheregaray
Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia

L. B. Beheregaray · L. Möller (✉)
School of Biological Sciences, Flinders University, Adelaide, SA 5001, Australia
e-mail: luciana.moller@flinders.edu.au

C. Jenner · M. Jenner
Centre for Whale Research, P.O. Box 1622, Fremantle, WA 6959, Australia

P. Gill · M. Morrice
Blue Whale Study, c/- Post Office, Narrawong, VIC 3285, Australia

P. Gill · M. Morrice
School of Life and Environmental Sciences, Deakin University, PO Box 423, Warrnambool, VIC 3280, Australia

J. Bannister
The Western Australian Museum, Locked Bag 49, Welshpool, DC, WA 6986, Australia

R. LeDuc
Southwest Fisheries Science Center, 8604 La Jolla Shores Drive, La Jolla, CA 92037-1508, USA

L. Möller
Graduate School of the Environment, Macquarie University, Sydney, NSW 2109, Australia

Published online: 22 August 2010

banned by the International Whaling Commission (IWC) in 1966 (Clapham et al. 1999).

Australian feeding aggregations of presumably pygmy blue whales occur at the Perth Canyon off Western Australia (Rennie et al. 2009), and the Bonney Upwelling and adjacent waters off South Australia and Victoria (Gill 2002). These areas are supported by complex seasonal upwelling systems resulting in high prey densities (Gill 2002; Rennie et al. 2009), characteristic of areas where blue whales are known to concentrate (Branch et al. 2007).

It is thought that Australian blue whales migrate between Australian feeding grounds during warmer months to lower latitude breeding grounds during colder months. The breeding ground location(s) of Australian feeding aggregations is uncertain, but are suspected to include Indonesia (Branch et al. 2007) and/or the Solomon Islands (Ohsumi and Shigemune 1993). There are three possible migration patterns (see Hoelzel 1998): (1) each breeding stock has its own feeding ground (genetically differentiated Australian feeding aggregations), (2) multiple breeding stocks share feeding ground(s) (genetic structure within one or both feeding aggregations), and (3) one breeding stock utilising both feeding grounds (no genetic structure within or between feeding aggregations). Here we use microsatellite and mtDNA control region markers to test these hypotheses. This study is of conservation importance as it will help determine what breeding stock(s) may be impacted by anthropogenic activities occurring at the feeding aggregations (e.g. Hoelzel 1991).

Methods

Sample collection and genetic methods

Biopsy, stranding or sloughed skin samples were collected in the Perth Canyon ($N = 77$) and the Bonney Upwelling ($N = 33$) (Fig. 1). Samples were preserved in 20% DMSO saturated with NaCl or 70–100% ethanol. DNA was extracted using a modified salting-out protocol (Sunnucks and Hales 1996) or DNeasy® Blood and Tissue Kit (Qiagen).

Ten microsatellite loci were analysed (Online Supplementary Material). Microsatellite genotyping either followed the method of LeDuc et al. (2007) or microsatellite fluorescent-labelled PCR products were produced based on Schuelke (2000) (Online Supplementary Material). PCR products were run on an ABI 3130xl Genetic Analyzer (Applied Biosystems) and scored using GENEMAPPER 4.0 (Applied Biosystems).

Sequence data for a 394 bp fragment of mtDNA control region was obtained as described in LeDuc et al. (2007) or Möller and Beheregaray (2001). PCR products were purified using an UltraClean® 15 DNA Purification Kit (Mo Bio Laboratories), sequenced using an ABI 3130xl Genetic

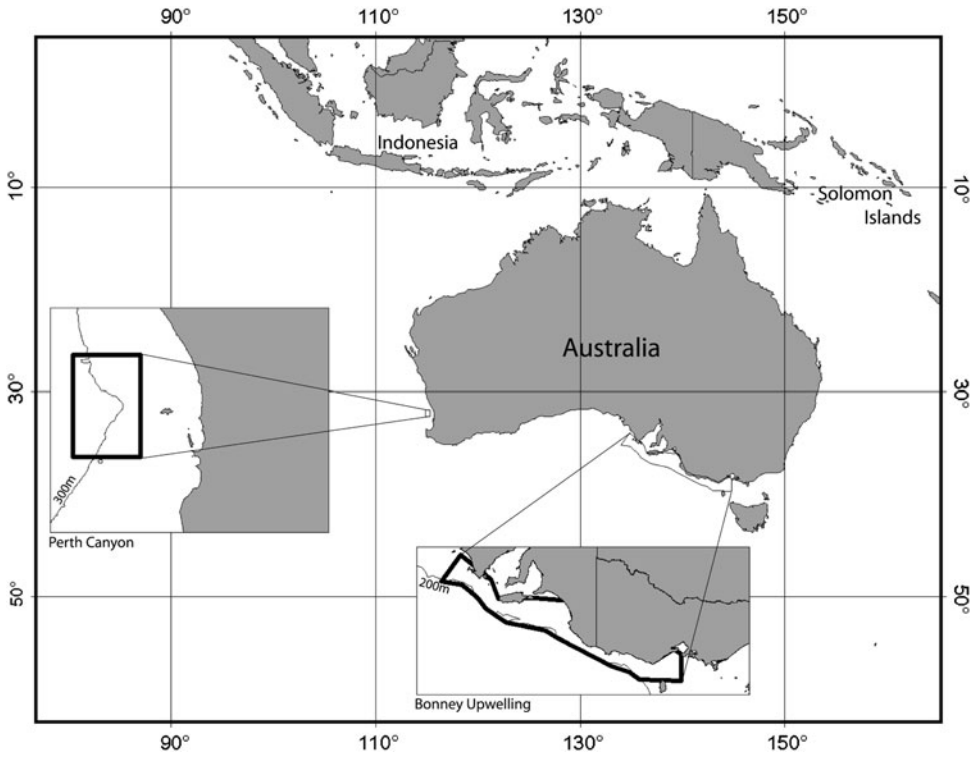

Fig. 1 Map of sampling locations of blue whales, which constitute the known Australian feeding aggregations: the Perth Canyon and the Bonney Upwelling

Analyzer (Applied Biosystems), and edited and aligned using SEQUENCHER 3.0 (Gene Codes Corporation, Ann Arbor, MI).

Microsatellite data analysis

Re-sampled individuals were identified by identical multilocus genotypes (probability of identity = 7.970×10^{-9} calculated using GENALEX 6 (Peakall and Smouse 2006)) using the program EXCEL MICROSATELLITE TOOLKIT 3.1 (Park 2001). Genotyping or scoring errors, caused by null alleles, stuttering and short allele dominance, were checked for each putative population using MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004).

The number of alleles, observed heterozygosity (H_O) and expected heterozygosity (H_E) were calculated for each locus at each feeding aggregation using EXCEL MICROSATELLITE TOOLKIT 3.1 (Park 2001), and allelic richness using FSTAT 2.9.3.2 (Goudet 1995). Deviations from Hardy–Weinberg equilibrium and linkage disequilibrium were tested using GENEPOP 3.4 (Raymond and Rousset 1995) (1,000 dememorizations, 100 batches, 1,000 iterations per batch) and significance values Bonferroni corrected.

Population structure was tested using STRUCTURE 2.3.1 (Hubisz et al. 2009) with the admixture model of ancestry, with and without using sampling locations as priors, and correlated allele frequency model (burn-in 100,000 iterations then runs of 10^6, five independent runs of $K = 1$–5). The number of clusters (K) was inferred from the posterior probability distribution $P(K|X)$ calculated from the posterior probability of the data Log $P(X|K)$. F_{ST} (significance assessed by 1,000 randomisations) was calculated using FSTAT 2.9.3.2 (Goudet 1995) and statistical power estimated using POWSIM 4.0 (Ryman and Palm 2006) (N_e = 2,000, 1,000 dememorizations, 100 batches, 1,000 iterations per batch, 1,000 runs).

A genetic bottleneck was tested using BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) with the two-phase mutation model (95% one-step mutations; 12% variance of multi-step mutations; 1,000 iterations (Piry et al. 1999)), infinite allele model and strict stepwise mutation model, and statistical significance determined with the Wilcoxon signed-rank test. The M ratio was calculated using M_P_VAL (Garza and Williamson 2001) (10% multi-step mutations, average size 3.5, $\Theta = 4N_e\mu = 5$, 10, or 30).

Mitochondrial DNA data analysis

Haplotype diversity (h), nucleotide diversity (π), F_{ST} (significance assessed by 1,000 permutations), Tajima's D and Fu's Fs (1,000 simulated samples) were calculated using ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010). A haplotype network (95% confidence level) was constructed using TCS 1.21 (Clement et al. 2000).

Results

The final data set contained 47 Perth Canyon and 25 Bonney Upwelling samples at microsatellite loci, and 67 Perth Canyon and 32 Bonney Upwelling samples at the mtDNA control region. Samples were not included if they were re-samples ($N = 7$) and, in microsatellite data analyses, if there was greater than three microsatellite loci with missing data. Most low quality sloughed skin samples were unable to amplify at nuclear loci. There was no evidence of null alleles, stutter bands or short allele dominance, and no significant deviations from Hardy–Weinberg equilibrium or evidence of linkage disequilibrium.

Microsatellite and mtDNA variation was comparable between the Perth Canyon and the Bonney Upwelling (Table 1). The microsatellite loci had between two and 12 alleles per locus and the mtDNA 394 bp fragment had 13 variable sites defining 15 unique haplotypes.

STRUCTURE estimated one genetic cluster ($P(K|X) = 1.000$ for $K = 1$; $P(K|X) = 0.000$ for $K = 2$–5) regardless of whether sampling locations were used as priors. Fixation indices based on microsatellite loci ($F_{ST} = 0.002$, $P = 0.319$, 95% CI −0.006 to 0.010) and mitochondrial haplotypes ($F_{ST} = 0.001$, $P = 0.340$) showed no significant genetic differentiation between the two Australian

Table 1 Summary of genetic variability in blue whales of two Australian feeding aggregations based on ten microsatellite loci and mtDNA control region sequences

	Microsatellites					mtDNA control region			
	ns	NA	H_O	H_E	AR	*ns*	NH	*h*	π
Perth Canyon	47	6.70 (2.79)	0.659 (0.022)	0.655 (0.042)	5.927	67	14	0.683 (0.062)	0.003 (0.002)
Bonney Upwelling	25	5.80 (2.25)	0.590 (0.031)	0.625 (0.043)	5.702	32	9	0.758 (0.070)	0.004 (0.003)

Standard deviation shown in parentheses

ns number of samples, *NA* mean number of alleles, *Ho* mean observed heterozygosity, *HE* mean expected heterozygosity, *AR* mean allelic richness, *NH* number of haplotypes, *h* haplotypic diversity, π nucleotide diversity

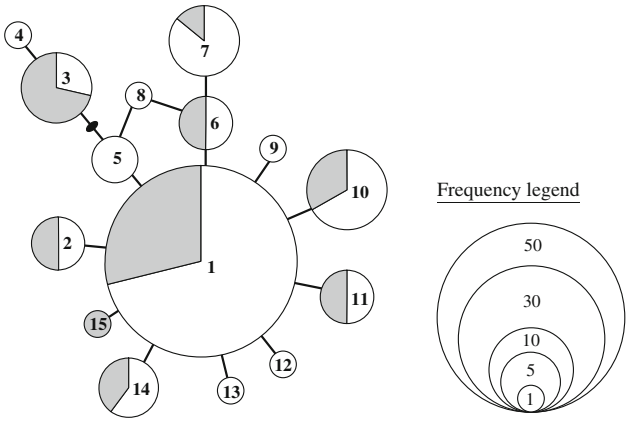

Fig. 2 Haplotype network of 15 unique mtDNA control region haplotypes. The size of each *circle* reflects the frequency that the haplotype was observed, and the *shading* represents the proportion observed in the associated putative population (Perth Canyon, white; Bonney Upwelling, grey). Each *line* represents one mutational difference. Each *black oval* represents haplotypes that were not sampled or are extinct. Haplotype sequences correspond to 21–414 bp positions of blue whale haplotypes submitted to GenBank by LeDuc et al. (2007) (*1* EU093921 or EU093935, *2* EU093934 or EU093953, *5* EU093924, *6* EU093952, *7* EU093956, *10* EU093920, *11* EU09 3919, *12* EU093954, *13* EU093922). New haplotypes are *3* HQ130 726, *4* HQ130727, *8* HQ130728, *9* HQ130729, *13* HQ130730, *15* HQ130731

feeding aggregations. POWSIM indicated that a F_{ST} of $\geq$0.0151 ($t = 60$) could be detected with $\geq$95% confidence (95.5% Fisher's exact test, 96.2% chi-square). The haplotype network revealed that all except one of the Bonney Upwelling haplotypes were also found in the Perth Canyon (Fig. 2).

Both bottleneck analyses, conducted on the combined data set, provided support for a reduction in genetic variability in Australian blue whales. The Wilcoxon signed-rank test indicated a significant excess of heterozygosity using the infinite allele model ($P = 0.001$), and marginally non-significant when using the two-phase and strict stepwise mutation models ($P = 0.065$ and 0.080, respectively). The M ratio was 0.631, with a P value of $\leq$0.001 for all Θ values. Fu's Fs was significant (-8.011, $P = 0.005$) and Tajima's D was non-significant (-1.162, $P = 0.122$) for the combined data set.

Discussion

Our genetic data support the hypothesis of one breeding stock utilising both feeding grounds as there was no evidence of significant genetic differentiation within or between the two Australian blue whale feeding aggregations. This means potentially harmful anthropogenic activities, such as seismic surveys or ship strikes, at either feeding aggregation will affect the same breeding stock. Genetic methods have also revealed multiple feeding grounds per breeding stock in other baleen whales (e.g. North Atlantic humpback whales, Larsen et al. 1996) as well as mixing of breeding stocks on feeding grounds (e.g. southern right whales, Patenaude et al. 2007). Feeding grounds constituting one breeding stock may still show maternally directed site fidelity at mitochondrial genetic markers (e.g. North Atlantic humpback whales, Palsbøll et al. 1995), though no evidence was found in our study. Management decisions should also take into account limits of our data set (significant F_{ST} below 0.015 could not be detected) and unknown factors such as the degree of site fidelity to feeding aggregations or to sites within feeding aggregations.

The mtDNA variation, significant negative Fu's Fs and the star shape of the haplotype network suggest the possibility of a historical population expansion—a scenario that will be tested in another study with additional genetic markers and samples. For the microsatellites, the levels of genetic variation were comparable to those of non-threatened mammal species (reviewed in Garner et al. 2005), in spite of the detection of a genetic bottleneck. However, the long generation time and overlapping generations of blue whales can result in a slow rate of decline in genetic diversity after a bottleneck, as suggested for other species (e.g. Kuo and Janzen 2004). Therefore, Australian blue whales might be genetically vulnerable as there is the potential for further decrease of genetic diversity due to drift, especially if demographic recovery is slow.

A review of IWC baleen whale Southern Hemisphere stock boundaries by Donovan (1991) emphasised the need to revise stock definitions by considering data collected using modern techniques. The current study builds on the study of LeDuc et al. (2007), which showed marked genetic differentiation of blue whales between ocean basins, but had insufficient sample sizes to resolve regional genetic structure. Further research to elucidate the degree of genetic continuity of Australian feeding aggregations with blue whales in surrounding areas will be extremely important to future management and conservation efforts.

Acknowledgments This study was funded by the Australian Marine Mammal Centre, Australian Antarctic Division. Sampling was conducted during a study program funded by the Australian Defence Department and the Australian Department of Environment, Water, Heritage and the Arts under animal ethics approval from the Department of Conservation and Land Management Animal Ethics Committee (#CAEC/14/2002), Deakin University Animal Welfare Committee (#A19/2006), and/or Macquarie University Animal Research Authority (#2008/017). Permits were supplied by the Department of Environment, Water, Heritage and the Arts (EPBC permits #E2008-0001, #E2003-48230, #E2002-00029) and Department of Sustainability and Environment in Victoria (#10004017).

References

Branch TA, Stafford KM, Palacios DM, Allison C, Bannister JL, Burton CLK, Cabrera E, Carlson CA, Galletti Vernazzani B, Gill PC, Hucke-Gaete R, Jenner KCS, Jenner M-NM, Matsuoka K, Mikhalev YA, Miyashita T, Morrice MG, Nishiwaki S, Sturrock VJ, Tormosov D, Anderson RC, Baker AN, Best PB, Borsa P, Brownell RLJ, Childerhouse S, Findlay KP, Gerrodette T, Ilangakoon AD, Joergensen M, Kahn B, Ljungblad DK, Maughan B, McCauley RD, McKay S, Norris TF, Oman Whale and Dolphin Research Group, Rankin S, Samaran F, Thiele D, van Waerebeek K, Warneke RM (2007) Past and present distribution, densities and movements of blue whales *Balaenoptera musculus* in the Southern Hemisphere and northern Indian Ocean. Mamm Rev 37:116–175

Clapham PJ, Young SB, Brownell Jr RL (1999) Baleen whales: conservation issues and the status of the most endangered populations. Mamm Rev 29:35–60

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014

Donovan GP (1991) A review of IWC stock boundaries. Rep Int Whal Comm (Special Issue) 13:39–68

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetic analyses under Linux and Windows. Mol Ecol Resour 10:564–567

Garner A, Rachlow JL, Hicks JF (2005) Patterns of genetic diversity and its loss in mammalian populations. Conserv Biol 19: 1215–1221

Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. Mol Ecol 10:305–318

Gill PC (2002) A blue whale (*Balaenoptera musculus*) feeding ground in a southern Australian coastal upwelling zone. J Cetacean Res Manage 4:179–184

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. J Hered 86:485–486

Hoelzel AR (1991) Whaling in the dark. Nature 352:481

Hoelzel AR (1998) Genetic structure of cetacean populations in sympatry, parapatry, and mixed assemblages: implications for conservation policy. J Hered 89:451–458

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Mol Ecol Resour 9:1322–1332

Kuo C-H, Janzen FJ (2004) Genetic effects of a persistent bottleneck on a natural population of ornate box turtles (*Terrapene ornata*). Conserv Genet 5:425–437

Larsen AH, Sigurjónsson J, Øien N, Vikingsson G, Palsbøll PJ (1996) Populations genetic analysis of nuclear and mitochondrial loci in skin biopsies collected from central and northeastern North Atlantic humpback whales (*Megaptera novaeangliae*): population identity and migratory destinations. Proc R Soc Lond B Biol Sci 263:1611–1618

LeDuc RG, Dizon AE, Goto M, Pastene LA, Kato H, Nishiwaki S, Leduc CA, Brownell RL (2007) Patterns of genetic variation in the Southern Hemisphere blue whales and the use of assignment test to detect mixing on the feeding grounds. J Cetacean Res Manage 9:73–80

Möller LM, Beheregaray LB (2001) Coastal bottlenose dolphins from southeastern Australia are *Tursiops aduncus* according to sequences of the mitochondrial DNA control region. Mar Mamm Sci 17:249–263

Ohsumi S, Shigemune H (1993) A sightings survey of larger whales in lower latitudinal waters of the Pacific in austral winter, with special reference to the blue whale. Paper Blue/WP6 presented to the *ad hoc* Steering Group Intersessional Meeting on Research related to Conservation of Large Baleen Whales - Blue Whales - in the Southern Ocean, Tokyo, October (unpublished)

Palsbøll PJ, Clapham PJ, Mattila DK, Larsen F, Sears R, Siegismund HR, Sigurjónsson J, Vásquez O, Arctander P (1995) Distribution of mtDNA haplotypes in North Atlantic humpback whales: the influence of behaviour on population structure. Mar Ecol Prog Ser 116:1–10

Park SDE (2001) Trypanotolerance in west African cattle and the population genetic effects of selection. Dissertation, University of Dublin

Patenaude NJ, Portway VA, Schaeff CM, Bannister JL, Best PB, Payne RS, Rowntree VJ, Rivarola M, Baker CS (2007) Mitochondrial DNA diversity and population structure among southern right whales (*Eubalaena australis*). J Hered 98: 147–157

Peakall R, Smouse P (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Piry S, Luikart G, Cornuet J-M (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. J Hered 90:502–503

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86: 248–249

Rennie S, Hanson CE, McCauley RD, Pattiaratchi C, Burton C, Bannister J, Jenner C, Jenner M-N (2009) Physical properties and processes in the Perth Canyon, Western Australia: links to water column production and seasonal pygmy blue whale abundance. J Mar Syst 77:21–44

Rice DW (1998) Marine mammals of the world: systematics and distribution. Special publication no. 4. The Society for Marine Mammalogy, Lawrence, Kansas

Ryman N, Palm S (2006) POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. Mol Ecol 6:600–602

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233–234

Sunnucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). Mol Biol Evol 13:510–524

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4: 535–538