

Hybridization of Southern Hemisphere blue whale subspecies and a sympatric area off Antarctica: impacts of whaling or climate change?

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Abstract

Understanding the degree of genetic exchange between subspecies and populations is vital for the appropriate management of endangered species. Blue whales (*Balaenoptera musculus*) have two recognized Southern Hemisphere subspecies that show differences in geographic distribution, morphology, vocalizations and genetics. During the austral summer feeding season, the Antarctic blue whale (*B. m. intermedia*) is found in polar waters and the pygmy blue whale (*B. m. breviceauda*) in temperate waters. Here, we genetically analyzed samples collected during the feeding season to report on several cases of hybridization between the two recognized blue whale Southern Hemisphere subspecies in a previously unconfirmed sympatric area off Antarctica. This means the pygmy blue whales using waters off Antarctica may migrate and then breed during the austral winter with the Antarctic subspecies. Alternatively, the subspecies may interbreed off Antarctica outside the expected austral winter breeding season. The genetically estimated recent migration rates from the pygmy to Antarctic subspecies were greater than estimates of evolutionary migration rates and previous estimates based on morphology of whaling catches. This discrepancy may be due to differences in the methods or an increase in the proportion of pygmy blue whales off Antarctica within the last four decades. Potential causes for the latter are whaling, anthropogenic climate change or a combination of these and may have led to hybridization between the subspecies. Our findings challenge the current knowledge about the breeding behaviour of the world's largest animal and provide key information that can be incorporated into management and conservation practices for this endangered species.

Keywords: admixture, *Balaenoptera musculus*, biodiversity conservation, endangered species, microsatellite DNA, population genetics

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Introduction

Subspecies are one of the human-defined phases in a continuum towards speciation that have been distin-

guished by morphological, physiological, behavioural and genetic differences (Wilson & Brown 1953). Subspecies tend to be geographically isolated, but are presumed to be capable of admixture when their distribution overlaps (Wilson & Brown 1953), which may lead to the formation of hybrid zones (Barton & Hewitt 1985). The dynamics of hybrid zones between

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species, subspecies or populations has long been a subject of major interest in ecology and evolution (Harrison 1993). The processes underpinning the formation and persistence of hybrid zones need to be reconciled with natural selection and speciation mechanisms. For example, there has been increased recognition of the importance of hybrid zones in the formation of hybrid lineages and novel species in animals, a situation previously well known for plants (Mavárez & Linares 2008). Identification of hybrid zones can become more complicated when the species, subspecies or populations of interest are highly mobile animals such as migratory birds and cetaceans. Assessing the level of genetic connectivity in these animals remains vital for appropriate management practices that take into account ecological and evolutionary timescales.

Baleen whales are known to undergo annual migrations from colder water, higher latitude feeding grounds to warmer water, lower latitude breeding grounds (Mackintosh & Wheeler 1929; Mackintosh 1966). However, there are recorded exceptions including a proportion of the acoustically defined blue whale (*Balaenoptera musculus*) populations off the Western Antarctic Peninsula (Širović *et al.* 2004, 2009), eastern Antarctica (Širović *et al.* 2009), eastern tropical Pacific Ocean (Stafford *et al.* 1999) and the Crozet Islands (Samaran *et al.* 2010). The only clear geographic boundaries to baleen whale movement are large continental land masses separating ocean basins. Additionally, breeding is presumed to be limited between the Northern and Southern Hemispheres because of opposite breeding seasons (e.g. Mikhalev 1997) and warm water at the equator restricting movement between hemispheres (Davies 1963). These barriers are reflected in subspecies and closely related species of baleen whales often occupying separate ocean basins or hemispheres: for example, the Northern and Southern Hemispheres for the two subspecies of fin whales (*Balaenoptera physalus physalus* and *B. p. quoyi*, respectively) (Rice 1998), and the Southern Hemisphere, North Atlantic and North Pacific Oceans for the three species of right whales (*Eubalaena australis*, *E. glacialis* and *E. japonica*, respectively) (Mead & Brownell 2005). An exception occurs for the two recognized subspecies of Southern Hemisphere blue whales, which exist despite no clear geographic barriers.

The two Southern Hemisphere blue whale subspecies were first differentiated based on catches that predominantly occurred during the austral summer feeding season. The pygmy blue whale subspecies (*B. m. brevicauda*) had a smaller size, proportionally shorter tail stock and lower latitude distribution compared with the 'ordinary' or 'true' blue whale (*B. m. intermedia*) (Ichiha-

ra 1966), now referred to as the Antarctic blue whale. They are presently also recognized as having other morphological differences such as nasal bone shape and body weight relative to length (Omura *et al.* 1970). However, the subspecies remain difficult to identify morphologically in free-ranging animals (Donovan 1984) and even stranded animals (Dalla Rosa & Secchi 1997), although photogrammetric techniques on free-ranging animals hold potential (Gilpatrick & Perryman 2008). The subspecies also have different vocalizations, which vary within pygmy blue whales across their distribution (McDonald *et al.* 2006). The subspecies are genetically differentiated, although there are no available diagnostic genetic markers (LeDuc *et al.* 2007). Blue whales currently classified as the pygmy subspecies in the Indian and Pacific Oceans are at least as genetically differentiated as either ocean basin is to the Antarctic subspecies (LeDuc *et al.* 2007).

The level of admixture between the blue whale Southern Hemisphere subspecies is unknown, and the breeding ground locations are unverified, although evidence suggests they may have the opportunity for admixture. Morphology and other biological data from whaling catches have indicated instances of subspecies migrants during the austral summer (Ichihara 1966; Branch *et al.* 2007a, 2009; Branch & Mikhalev 2008). The distinct calls of the Antarctic and pygmy subspecies have been recorded sympatrically off Antarctica (61°S–62°S, 67°E–70°E) in the austral summer (Gedamke & Robinson 2010), off Cape Leeuwin (35°S, 114°W) in Australia from May to June (Stafford *et al.* 2004, 2011), off Diego Garcia (6°S, 71°E and 8°S, 73°E) in the austral winter (Stafford *et al.* 2004, 2011), and off the Crozet Islands (46°S, 52°E) year-round (Samaran *et al.* 2010). The only published genetic study comparing Southern Hemisphere subspecies found evidence for a potential Pacific Ocean migrant off Antarctica and a potential Antarctic migrant off Chile (LeDuc *et al.* 2007). Their study was limited in the number of genetic markers and samples used, which may have resulted in low power to detect migrants.

It is imperative to investigate the level of connectivity between blue whale subspecies to allow accurate estimates of subspecies abundance and recovery since whaling. Commercial whaling dramatically reduced the abundance of blue whales worldwide (Clapham *et al.* 1999; Branch *et al.* 2007b) with the most intensively exploited subspecies, Antarctic blue whales, dropping to 0.15% of original numbers (Branch *et al.* 2004). Here, we use a large panel of microsatellite DNA markers and 264 sampled individuals representing both Antarctic blue whales and Australian pygmy blue whales to report on migrants and, for the first time, admixed individuals between the two subspecies.

Material and methods

Genetic analyses

Biopsy samples off Antarctica ($n = 186$) were collected from 1990 to 2009 in December to February during the International Decade of Cetacean Research (IDCR) and Southern Ocean Whale and Ecosystem Research (SOWER) cruises of the International Whaling Commission (IWC). Australian blue whale biopsy, stranding and sloughed skin samples were collected from 1995 to 2010 in October to April from the Perth Canyon and Bonney Upwelling feeding aggregations ($n = 113$). Five individuals sampled off Australia had known resamples (Attard *et al.* 2010; only two were inter-seasonal resamples) that were not included within the above sample size. Samples were preserved in either 20% DMSO saturated with NaCl or 70–100% ethanol. DNA was extracted using a modified salting-out protocol (Sunnucks & Hales 1996) or DNeasy[®] Blood and Tissue Kit (Qiagen).

Twenty-one microsatellite markers were genotyped for all samples: GATA028, GATA098 and GATA417 (Palsbøll *et al.* 1997), GT023 (Bérubé *et al.* 2000), GT541 and CA234 (Bérubé *et al.* 2005), Dde09 and Dde70 (Coughlan *et al.* 2006), Bmy1, Bmy8, Bmy11, Bmy14, Bmy33, Bmy41, Bmy42, Bmy53 and Bmy57 (Huebinger *et al.* 2008), Tur4_87 and Tur4_141 (Nater *et al.* 2009), and BM032 and BM261 (Attard *et al.* 2010). Fluorescently labelled PCR products were produced by directly labelling one of the primers, or based on the method of Schuelke (2000). Products were run on an ABI 3100, 3730 or 3130xl Genetic Analyzer (Applied Biosystems) and scored using PEAK SCANNER 1.0, GENEMAPPER 3.7 or 4.0 (Applied Biosystems). The sex of individuals was determined by PCR amplification of a fragment of the genes ZFX and SRY following Fain and LeMay (1995) or Gilson *et al.* (1998).

Tests of assumptions

Resampled individuals were identified by identical multilocus genotypes or those with up to two allele mismatches using EXCEL MICROSATELLITE TOOLKIT 3.1 (Park 2001). When individual multilocus genotypes varied by one or two alleles, their genotypes were rechecked and corrected if appropriate. Genotyping or scoring errors, caused by null alleles, stuttering and short allele dominance, were checked for each subspecies using MICROCHECKER 2.2.3 (van Oosterhout *et al.* 2004) (95% confidence intervals, 10 000 runs). Deviations from Hardy–Weinberg equilibrium were tested using ARLEQUIN 3.5.1.2 (Excoffier & Lischer 2010) (10 000 dememorizations, 100 000 Markov chain steps), linkage disequilibrium between pairs of loci were tested using

FSTAT 2.9.3.2 (Goudet 1995), and significance values were sequential Bonferroni corrected (Holm 1979).

Population structure

Population structure was tested using STRUCTURE 2.3.3 (Pritchard *et al.* 2000) with the admixture model of ancestry, the correlated allele frequency model (Falush *et al.* 2003), and not using sampling locations as priors (Hubisz *et al.* 2009) (burn-in 100 000 iterations then runs of 10^6 , five independent runs of $K = 1–8$). Runs were averaged using CLUMPP 1.1.2b (Jakobsson & Rosenberg 2007) (FullSearch algorithm, G' pairwise matrix similarity statistics). The most likely number of clusters was determined using the ΔK method (Evanno *et al.* 2005) as implemented in STRUCTURE HARVESTER 0.6.7 (Earl & vonHolt 2012). F_{ST} (significance assessed by 1000 permutations) between Antarctica and Australia was calculated using ARLEQUIN.

Detection of migrants and admixture

Migrants and admixed individuals were detected using Bayesian clustering methods implemented in STRUCTURE and NEWHYBRIDS 1.1 BETA 3 (Anderson & Thompson 2002). Genotypic simulations of 'pure' and admixed individuals from the two subspecies were used to determine the power and accuracy of the Bayesian methods in identifying migrants and admixed individuals.

The STRUCTURE analysis used to detect population structure also simultaneously estimated for each individual the proportion of their multilocus genotype originating from their sampling population. Individuals with at least 0.95 of their genotype originating from their sampling population were used to simulate the genotypes of 1000 individuals from each parental population (i.e. Antarctic blue whales, Australian pygmy blue whales) using HYBRIDLAB 1.0 (Nielsen *et al.* 2006). This prevented suspect migrants and admixed individuals from contributing to the simulations. From these simulated individuals, the genotypes of 50 individuals from each possible category of admixture after two generations of admixture were simulated: F_1 (Antarctic parental \times Australian pygmy parental), F_2 ($F_1 \times F_1$), backcross to Antarctic blue whales ($F_1 \times$ Antarctic parental) and backcross to Australian pygmy blue whales ($F_1 \times$ Australian pygmy parental). All simulated individuals were run in STRUCTURE (same parameters as above except $K = 2$, runs averaged as above using CLUMPP) to estimate the proportion of their genotype originating from each subspecies. The real and simulated individuals were also run separately in NEWHYBRIDS (minimum burn-in 10 000 then minimum 100 000 sweeps, Jeffreys-like and uniform priors, five independent runs, categories of up to one and up to two

generations of admixture), which estimates the posterior probability of each individual belonging to the parental subspecies and user-defined categories of admixture. Individuals were assigned to the category with the highest posterior probability.

Migration rates over recent (i.e. ecological) and evolutionary timescales were determined. Recent migration rates were estimated using *BAYESASS* 3.0.1 (Wilson & Rannala 2003) (burn-in 10^6 iterations then 10^7 iterations with sampling every 100 iterations, five independent runs), and runs were combined using *TRACER* 1.5 (Rambaut & Drummond 2009). Mixing parameters were altered in trial runs until acceptance rates were between 20% and 40% ($\Delta A = 0.16$, $\Delta F = 0.14$, $\Delta M = 0.07$) according to recommendations in the program's manual. Evolutionary migration rates were estimated using *IMA2* (Hey & Nielsen 2007; Hey 2010) (minimum burn-in 10^6 then minimum 10^6 steps with genealogy saving every 100 steps, geometric heating scheme with 100 chains and heating terms of 0.96 and 0.80, three independent runs combined in load-genealogy mode, 5×10^{-4} mutation rate per generation; six loci were excluded as they did not follow the stepwise mutation model). Migration rates were estimated both with and without samples collected from outside the genetically detected sympatric area (see Results).

Results

Our data set included 155 individual blue whales sampled off Antarctica (71 females and 84 males) and 109 sampled off Australia (59 females, 49 males and 1 unknown) after removal of 35 resamples from 26 individuals. All resamples detected in the current study were intra-seasonal with the exception of one that was inter-seasonal and also found in another study (Sremba *et al.* 2012). None of the microsatellite markers were diagnostic for the subspecies. There was no evidence of linkage disequilibrium in the microsatellite markers after sequential Bonferroni correction (all $P > 0.0001$). BM032 significantly deviated from Hardy–Weinberg equilibrium after sequential Bonferroni correction ($P < 0.0012$) and BM032 and Dde09 showed evidence of homozygote excess based on *MICROCHECKER*, although only within Antarctica. One individual sampled off Antarctica was homozygous for a BM032 allele not found in any other sample, and allele sequencing revealed that all BM032 length mutations were outside the microsatellite region. BM032 was therefore removed because of the likely presence of null alleles. However, homozygote excess in Dde09 might be due to type I error or Wahlund effect (see Sremba *et al.* (2012) for evidence of the latter). Analyses performed with and without Dde09 produced indistinguishable results, and

therefore values from analyses with Dde09 are reported below.

Population structure

There was significant genetic differentiation between Antarctic blue whales and Australian pygmy blue whales. They clustered separately in *STRUCTURE* ($\Delta K_{MAX} = 1886.690$; Fig. 1) and had a significant F_{ST} of 0.112 ($P < 0.001$). This means any admixed individuals detected must be due to admixture between the two subspecies.

Detection of migrants and admixture

Simulated individuals. Parental individuals were simulated from 137 individuals sampled off Antarctica and 107 individuals sampled off Australia that *STRUCTURE* estimated had at least 0.95 of their genotype originating from their sampling population. *STRUCTURE* estimated the proportion of sampling population ancestry as above 0.881 for 99% and 0.948 for 95% of simulated Antarctic parental individuals (minimum 0.678), and above 0.975 for 99% and 0.986 for 95% of simulated Australian parental individuals (minimum 0.903). However, individuals could not be classified reliably into first- and second-generation categories of admixture because of considerable overlap in estimated ancestry for different categories (Fig. 2).

When individuals were classified into categories of up to one generation of admixture, *NEWHYBRIDS* correctly identified 99.8% of simulated Antarctic parental individuals and all Australian parental individuals as

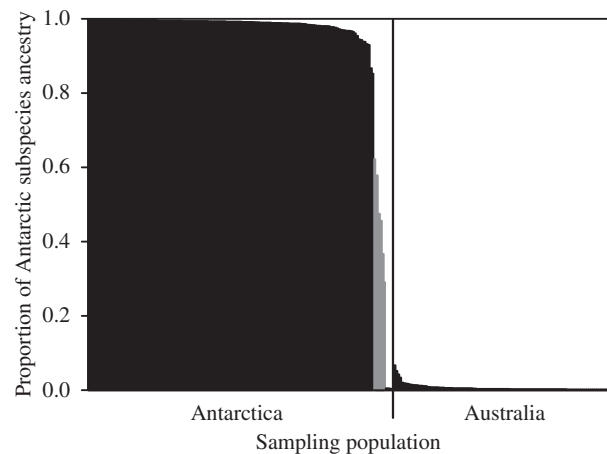


Fig. 1 Clustering results of *STRUCTURE* analysis for blue whales sampled off Antarctica and Australia when $K = 2$. Runs were averaged using *CLUMPP*. Each individual is represented by a column, with individuals within each sampling population in descending order of proportion of Antarctic subspecies ancestry. Detected admixed individuals are indicated in gray.

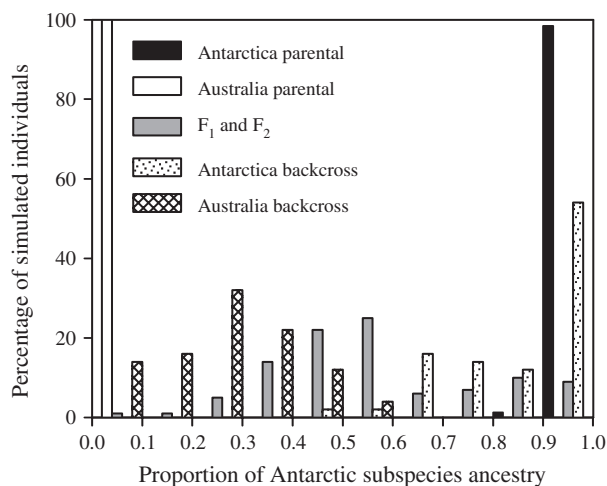


Fig. 2 Percentage distribution histogram of the proportion of Antarctic blue whale subspecies ancestry estimated using STRUCTURE for simulated parental and admixture classes.

belonging to their parental populations for both the Jeffreys-like and uniform priors. This analysis also detected 82% and 84% of simulated F₁ individuals, 66% and 72% of F₂ individuals, 22% and 22% of backcrosses to Antarctic blue whales, and 74% and 66% of backcrosses to Australian pygmy blue whales as admixed for the Jeffreys-like and uniform priors, respectively. The results of the five independent runs of NEWHYBRIDS were identical for both priors. There was insufficient statistical power for reliably distinguishing between categories when individuals were classified into categories of more than one generation of admixture.

Empirical individuals. STRUCTURE (using minimum ancestry estimated for simulated parental individuals as a cut-off) and NEWHYBRIDS (using categories of up to one generation of admixture) produced the same findings for all sampled individuals. There was strong evidence for four migrant pygmy blue whales and six admixed individuals off Antarctica, and no migrants or admixed individuals were detected off Australia. The proportion of Antarctic subspecies ancestry estimated by STRUCTURE and averaged using CLUMPP for the sampled migrants was from 0.002 to 0.006 inclusive, and for the sampled admixed individuals was from 0.291 to 0.623 inclusive. The posterior probability range across independent runs estimated by NEWHYBRIDS for the sampled migrants was from 0.9999 to 1.0000 inclusive for the Australian parental category for both priors, and for the sampled admixed individuals was from 0.926 to 0.999 and 0.935 to 0.996 inclusive for the admixed category for the Jeffreys-like and uniform priors, respectively. This is with the exception of one admixed individual that was categorized in the Australian parental category with low

probability (0.799–0.802 inclusive across runs) when using the less preferable (Anderson & Thompson 2002) uniform prior. The migrants and admixed individuals both had an equal sex ratio.

All detected migrant and admixed individuals, including resamples, were located from 65°31'S to 69°51'S and 0°02'W to 16°14'E inclusive, which is within a dense cluster of 92 samples collected from 64°56'S to 69°52'S and 0°02'W to 19°28'E inclusive (Fig. 3). Only one biopsy resampled individual showed movement into or out of this area; in January 2002 it was sampled at 64°32'S, 137°26'E, and in January 2006 it was sampled at 69°40'S, 5°44'E.

There was a greater recent compared to evolutionary migration rate for pygmy to Antarctic blue whales with no overlap in the 95% credible intervals, but considerable overlap for migration in the opposite direction (Table 1; Fig. 4). Only the 95% credible interval for the pygmy to Antarctic recent migration rate did not include zero. These patterns did not change when animals outside the dense cluster of samples where all migrant and admixed individuals were located (64°56'S–69°52'S, 0°02'W–19°28'E inclusive) were excluded from analyses. The results of the independent runs were very similar, indicating that convergence was reached.

Discussion

Our study substantially impacts the current knowledge of blue whale breeding behaviour. We found evidence for four migrant pygmy blue whales off Antarctica. The presence of migrants off Antarctica does not necessarily indicate that the subspecies are hybridizing, especially because Antarctica is considered a feeding rather than a breeding ground. However, six admixed individuals from the currently recognized blue whale Southern Hemisphere subspecies were identified in the same area off Antarctica (65°31'S–69°51'S, 0°02'W–16°14'E inclusive), which is predominately off the Princess Astrid Coast (5°E–20°E). This is the first record of hybridization between any blue whale subspecies. There may be more admixed individuals than those detected. This is because of the current study's inability to distinguish all simulated admixed individuals from simulated parental individuals and the conservative approach used for identifying admixed individuals. Greater statistical power is needed to detect backcrossed individuals the longer ago the original hybridization event occurred.

The equal sex ratio of migrants (which was also the case for admixed individuals) suggests that dispersal from Australia to Antarctica was not sex-biased. Despite this, male-biased dispersal is generally the case for mammals (Lawson Handley & Perrin 2007), and different patterns of spatial and temporal segregation by sex have been

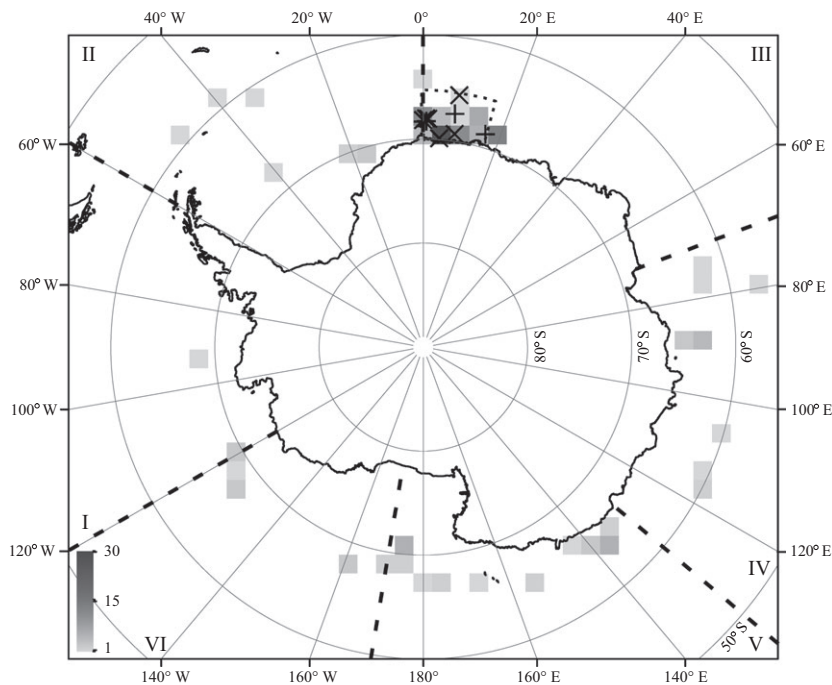


Fig. 3 Polar map showing the location of individual blue whales when biopsy sampled off Antarctica. The first recorded location is used for resamples. Locations of individuals are grouped into squares on a grid. The gradient of shades of gray indicates the number of individuals within each square. Precise locations of detected migrants (black plus symbol) and admixed individuals (black cross symbol) are indicated. The minimum sympatric area, which includes all migrants and admixed individuals (upper bound at 65°31'S inclusive, and 0°02'W to 16°14'E inclusive), is indicated by a black dotted line. Boundaries of the International Whaling Commission's Southern Hemisphere management Areas I to VI are indicated by black dashed lines.

Table 1 Migration rates (proportion of receiving population consisting of migrants) between the Antarctic and pygmy blue whale subspecies from the current study and female whaling catch data by Branch *et al.* (2007a, 2009). Headings in italics indicate migration direction. For these estimates, the Antarctic sympatric area was defined as 0°02'W to 19°28'E inclusive for genetic data and the International Whaling Commission's Southern Hemisphere management Area III of 0° to 70°E for the whaling catch data. The whaling catch studies used data from the 1913/14 to 1972/73 austral summer, defined pygmy subspecies habitat as north of 52°S and from 35°E to 180°E, and Antarctic subspecies habitat as south of 52°S unless otherwise indicated. The migration rate statistic reported varies between recent (mean, median) and evolutionary (mean) genetic estimates and whaling catch data estimates (median) because of the different outputs of analyses.

Data	Time period	Migration rate (%)	95% credible interval (%)	Reference
<i>Pygmy to Antarctic</i>				
Body length	Whaling	0.8	0.7–1.1	Branch <i>et al.</i> (2007a)
Body length*	Whaling	0.7	0.5–1.0	Branch <i>et al.</i> (2007a)
Body length and ovarian corpora	Whaling	0.1	0.0–0.4	Branch <i>et al.</i> (2009)
Genetic	Recent/ecological	2.50, 2.44	1.16–3.94	Current study
Genetic	Evolutionary	0.25	0.00–0.87	Current study
<i>Pygmy to Antarctic sympatric area</i>				
Body length	Whaling up to 1936/37	1.1	0.7–1.8	Branch <i>et al.</i> (2007a)
Body length	Whaling since 1937/38†	1.0	0.6–1.5	Branch <i>et al.</i> (2007a)
Genetic	Recent/ecological	4.23, 4.13	2.04–6.61	Current study
Genetic	Evolutionary	0.37	0.00–0.99	Current study
<i>Antarctic to pygmy</i>				
Body length and ovarian corpora	Whaling	0.5	0.2–1.5	Branch <i>et al.</i> (2009)
Genetic	Recent/ecological	0.30, 0.21	0.00–0.90	Current study
Genetic	Evolutionary	0.12	0.00–0.41	Current study
<i>Antarctic sympatric area to pygmy</i>				
Genetic	Recent/ecological	0.30, 0.21	0.00–0.90	Current study
Genetic	Evolutionary	0.14	0.00–0.41	Current study

*Antarctic subspecies habitat defined as south of 60°S.

†A minimum catch length regulation was introduced in the 1937/38 austral summer.

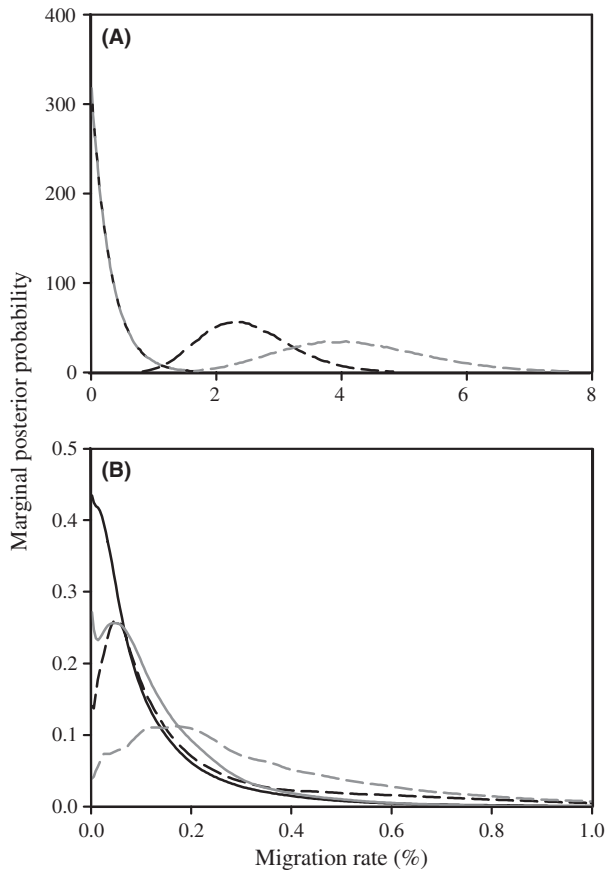


Fig. 4 Marginal posterior distribution of (A) recent or ecological migration rates and (B) evolutionary migration rates per generation between Antarctic and pygmy blue whales. Migration rates from Antarctic to pygmy blue whales (solid line) and the opposite direction (dashed line) were estimated using all individuals sampled off Antarctica (black line) and excluding individuals sampled off Antarctica outside the detected sympatric area (gray line). Antarctic to pygmy blue whale recent migration rates did not change whether all sampled individuals off Antarctica or only individuals within the sympatric area were used (solid line with alternating black and gray shading).

found in cetaceans including baleen whales (Wearmouth & Sims 2008). Lack of sex-biased dispersal in the current study requires confirmation through more samples.

Possible breeding locations

Australian pygmy blue whale breeding grounds are thought to include Indonesia based on timing of sightings off Australia and Indonesia, and acoustic recordings of possibly migrating whales off Western Australia (Branch *et al.* 2007b). Antarctic blue whale breeding grounds are thought to include low latitudes of the Indian and eastern Pacific Oceans based on seasonality of acoustic recordings (Stafford *et al.* 2004), and temperate latitudes of the eastern Atlantic Ocean during whal-

ing based on catch seasonality (Branch *et al.* 2007a,b). Our results suggest that the Australian pygmy blue whales using waters off Antarctica may migrate and then breed during the austral winter with the Antarctic subspecies, or the subspecies may interbreed off Antarctica outside the expected austral winter breeding season. These possibilities take into consideration the unidirectional gene flow from Australian pygmy blue whales to Antarctic blue whales and the presence of both migrants and admixed individuals in the Antarctic sympatric area. The latter suggests site fidelity to the sympatric area, as has been suggested previously for different areas off Antarctica (Branch *et al.* 2007b). Possible migratory pathways and breeding ground locations have only recently been inferred using modern techniques, as previous knowledge of blue whale distribution was predominately based on whaling catches that occurred on feeding grounds. This means migratory pathways and breeding grounds are currently little understood.

Australian pygmy blue whales and Antarctic blue whales may migrate together from off Antarctica to shared northern breeding grounds through waters off Cape Leeuwin in Australia. Their calls have been recorded sympatrically off Cape Leeuwin (35°S, 114°W) from May to June (Stafford *et al.* 2004, 2011). Though, the pygmy blue whale calls detected may be blue whales that were feeding off Australia. Antarctic blue whale calls were also recorded off Cape Leeuwin from July to November (Stafford *et al.* 2004) and Australian calls from December to April (Stafford *et al.* 2011) in the absence of calls from the other subspecies.

Concurrent calls from the subspecies have also been detected off tropical Diego Garcia (6°S, 71°E and 8°S, 73°E) (Stafford *et al.* 2004, 2011) and the sub-Antarctic Crozet Islands (46°S, 52°E) (Samaran *et al.* 2010) during the austral winter breeding season. Therefore, these locations may be shared breeding grounds. The pygmy blue whale calls were of the unique Madagascan and Sri Lankan types during the austral winter, although the Australian type was also detected off the Crozet Islands but from January to April. This means a sympatric breeding ground in either of these locations would only explain the admixed individuals if there is high genetic connectivity between Australian blue whales, and those thought to feed off southern Madagascar (Best *et al.* 2003) and/or those currently thought to feed and breed in the northern Indian Ocean (Anderson 2005). Small genetic sample sizes from off southern Madagascar ($n = 6$) and the northern Indian Ocean ($n = 2$) (LeDuc *et al.* 2007) have prevented determining whether the different call types reflect genetically differentiated populations (McDonald *et al.* 2006). Connectivity between areas during the feeding season is suggested by the almost continuous distribution of

whaling catches from off Australia to off southern Madagascar and the nearby sub-Antarctic Crozet Islands (Branch *et al.* 2007b), and the concurrent detection of Australian, Madagascan and Sri Lankan calls off the Crozet Islands from January to April (Samaran *et al.* 2010).

An alternative explanation to shared breeding grounds is the subspecies interbreed during the feeding season in the sympatric area off Antarctica, and potentially other areas of subspecies sympatry during the feeding season such as off the Crozet Islands (Samaran *et al.* 2010). This would be an exception to traditional thinking that there is specific breeding and feeding grounds for blue whales and other baleen whales with seasonal migration between these locations (Mackintosh & Wheeler 1929; Mackintosh 1966). Foetal lengths of pygmy blue whales from catches have indicated that a small proportion breed in the austral summer (Ichihara 1966), and a proportion of blue whales seem to not migrate including Antarctic blue whales off Antarctica (Širović *et al.* 2004, 2009) and both subspecies off the Crozet Islands (Samaran *et al.* 2010). According to what is known of the baleen whale reproductive cycle and gestation period (Lockyer 1984), the hybrid offspring may be born in the following feeding season rather than during the breeding season, and therefore possibly in the sympatric area if there is site fidelity to Antarctic feeding areas. Breeding may also occur during migration when the distribution of the subspecies could have increased overlap, such as may occur off Cape Leeuwin.

Extent of Antarctic sympatric area

Pygmy blue whales in the sub-Antarctic were found during austral summer whaling between 0° and 80°E (Ichihara 1966; Kato *et al.* 1995). A sympatric area for the pygmy and Antarctic subspecies off Antarctica may most parsimoniously occur in areas adjacent to the sub-Antarctic pygmy blue whale range. In the context of the current study, this assertion may be possible as all migrants and admixed individuals were between 0°02'W and 16°14'E inclusive, and it is unknown whether pygmy blue whales in the sub-Antarctic are the same genetic population as those using waters off Australia. However, the current study has limited capability to determine the extent of the sympatric area as the genetic samples are not uniformly distributed throughout the Antarctic.

Acoustic evidence indicates that the sympatric area may include the easternmost extent of the sub-Antarctic pygmy blue whale longitudinal range with two occurrences of the unique Madagascan pygmy blue whale call detected in January 2006 (61°S–62°S, 67°E–70°E) (Gedamke & Robinson 2010). This is the only published record of non-Antarctic blue whale calls off Antarctica.

It suggests that the sympatric area encompasses the International Whaling Commission's Southern Hemisphere management Area III (0°–70°E) (Donovan 1991). However, using acoustics to aid in determining the extent of the sympatric area should be undertaken with caution. The detection of Madagascan calls off Antarctica may not be associated with the sympatric area genetically detected in the current study if blue whales off Australia and Madagascar are genetically differentiated. Additionally, it is unknown whether calls can be learnt between populations (McDonald *et al.* 2006) as has been shown for humpback whales (Noad *et al.* 2000). The worldwide decrease in tonal frequency of blue whale calls since the 1960s (McDonald *et al.* 2009; Gavrilov *et al.* 2011) indicates that their calls have a degree of phenotypic plasticity or microevolutionary potential.

The patterns of individual movement off Antarctica have the potential to determine the extent of the Antarctic sympatric area, although they are largely unknown. Mark-recapture data have shown that blue whales off Antarctica have moved between the sympatric area (whether classified according to only genetic data, or both genetic and acoustic data) and other areas off Antarctica both during (Branch *et al.* 2007b) and after (current study; Sremba *et al.* 2012) whaling. Nevertheless, only the unique Antarctic call has been recorded in areas off Antarctica outside Area III (Ljungblad *et al.* 1998; Širović *et al.* 2004; Rankin *et al.* 2005; McDonald *et al.* 2006), resamples of migrant and admixed individuals in the current study were all found within 0°02'W to 16°14'E inclusive, and there is mark-recapture evidence of a degree of site fidelity between seasons to Area III during whaling (Branch *et al.* 2007b).

Genetic population differentiation based on F_{ST} estimates within Antarctica has been found when putative populations were defined by the International Whaling Commission's management Areas (Sremba *et al.* 2012). This may also be a reflection of differences in the extent of gene flow between Australia and genetically differentiated areas off Antarctica. However, the biological importance of these management Area designations to blue whales is questionable (Sremba *et al.* 2012) given that the Areas are not specific to blue whales and were designated based on little information (Donovan 1991).

This together indicates that the sympatric area and possibly associated hybridization extends from at least 65°31'S to 69°51'S and 0°02'W to 16°14'E inclusive (encompasses all genetically detected migrants and admixed individuals), probably up to 64°56'S to 69°52'S and 0°02'W to 19°28'E inclusive (additionally encompasses the dense cluster of 92 genetic samples collected), and possibly up to the International Whaling Commission's Southern Hemisphere management Area III of 0° to 70°E (additionally encompasses Madagascan calls and limited evidence of site fidelity within Area III).

Recent proportion of pygmy blue whales off Antarctica

The mean recent migration rate estimate from the pygmy to Antarctic subspecies was 2.5%, or 4.2% when animals outside the sympatric area were excluded. Previous estimates have been based on female body length (Branch *et al.* 2007a) or female body length and ovarian corpora data (Branch *et al.* 2009) collected from whaling catches (Table 1). Estimates from whaling catch data are consistently less than the estimates from genetic analyses. Additionally, the 95% credible intervals from whaling catch and genetic analyses do not overlap whether they both include all animals off Antarctica or both exclude animals from outside the sympatric area.

The discrepancy in pygmy migrant estimates between the whaling catch data and genetic data could be due to differences between the two methods used. Migration rates can be based on the dispersal of individuals, such as may be measured by mark-recapture. Such dispersal does not necessarily lead to gene flow. Gene flow-based measures of migration rates may be termed effective migration rates (Broquet & Petit 2009). The whaling data estimates are noneffective migration rates. Genetic assignment methods are also capable of estimating noneffective migration rates. BAYESASS is an assignment method, although it attempts to incorporate migration events from recent, previous generations that were not sampled by determining the immigrant ancestries of the sampled individuals. Therefore, a component of the analysis involves assessing migration rates through gene flow. An alternative is to calculate a point estimate for the noneffective migration rate by dividing the number of migrants (four) by the sample size (155 for all samples off Antarctica or 92 for the dense cluster of samples between 0°02'W and 19°28'E inclusive that comprises the sympatric area) (Broquet & Petit 2009). Such a calculation produces estimates that are slightly greater than those of BAYESASS: 2.6% (instead of 2.5%) for the pygmy to Antarctic subspecies, or 4.3% (instead of 4.2%) when animals outside the cluster of 92 individuals are excluded. This means that both genetic effective and noneffective migration estimates are greater than the whaling data noneffective migration estimates. However, the genetic noneffective migration estimates are provisional as point estimates with no confidence interval would benefit from a greater sample size.

There are other possible reasons for the discrepancy between whaling catch and genetic data estimates. The current study showed a strong ability to detect individual pygmy migrants based on simulations. However, backcrosses to Australian pygmy blue whales incorrectly detected as migrants off Antarctica would inflate noneffective (but not effective) migration estimates to Antarctica. The whaling catch data studies instead used

a Bayesian mixture model that does not require subspecies identification of each individual. The extent admixed individuals would influence whaling data estimates is uncertain because their morphological and ovarian corpora characteristics are unknown. Additionally, differences in the distribution of samples between the studies may cause artefacts in the results. This was rectified as much as possible in the current study by including a comparison of the genetic estimates from the sympatric area with those estimated by Branch *et al.* (2007a) from body length measurements of catches in Area III. Whether the discrepancy in estimates is attributed to differences in the methods could be tested by taking both photogrammetric and genetic data from free-ranging animals in the sympatric area and comparing the results obtained from the two measures.

Impacts of whaling or climate change?

The discrepancy between the whaling catch data and post-whaling genetic data recent migration estimates may reflect real, temporal differences in the proportion of pygmy blue whale migrants off Antarctica. This is not definitive given other described possible reasons for the discrepancy. However, it is corroborated by the 95% credible interval of the evolutionary (unavoidably effective) migration rates from the pygmy to Antarctic subspecies in the current study overlapping with those from whaling catch data but not recent genetic estimates. The evolutionary migration rate is problematic as the mutation rate needed to convert evolutionary migration rates into demographically relevant values is unknown for blue whales. The current study used a high standard mammalian microsatellite mutation rate of 5×10^{-4} per generation that would overestimate the migration rate compared with other standard rates such as 10^{-4} . This together would suggest that pygmy blue whales have very recently expanded south or increased in proportion in areas historically frequented by Antarctic blue whales during the austral summer. This may also have resulted in hybridization between subspecies, especially if blue whales are able to breed outside their expected breeding season. No biological studies based on catch data have reported the possibility of hybrids (Ichihara 1966; Branch *et al.* 2007a, 2009).

Anthropogenic causes are a possible reason for the formation of a sympatric area within the last four decades. Blue whales have been severely impacted by whaling practices in the 20th century. Antarctic blue whales were the most heavily exploited subspecies, reduced from a pre-whaling abundance of 239 000 (95% credible interval 202 000–311 000) to a low of 360 (150–840) in 1973 (Branch *et al.* 2004). As Antarctic blue whales declined, the proportion of pygmy blue whales caught dramatically

increased from the late 1950s (Ichihara 1966) and continued to be caught illegally by the Soviets after International Whaling Commission protection in the mid-1960s (Zemsky & Sazhinov 1982). Pygmy blue whales in the sub-Antarctic were estimated to have reduced in abundance from 7598 to 3996 (Zemsky & Sazhinov 1982), although this is unsubstantiated. Whaling could have caused the findings of the current study in a variety of ways. Severely altering the ratio of subspecies abundance may have increased the proportion of pygmy blue whales off Antarctica without changing the emigration rate from the pygmy subspecies. Alternatively, the reduction in blue whale numbers off Antarctica to well below carrying capacity may have increased opportunities for migration of pygmy blue whales to the Antarctic.

Another possibility is recent climate change causing a poleward shift in pygmy blue whale distribution. This has been predicted for cetaceans limited to warmer waters (MacLeod 2009) and is thought to have occurred for Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) (Salvadeo *et al.* 2010). Since the mid-1900s there has been a net warming of the world's oceans (Levitus *et al.* 2000) with the Southern Ocean greatly affected (Gille 2002, 2008). The Southern Ocean climate change is largely caused by anthropogenic activities (Fyfe 2006; Liu & Curry 2010). Climate caused ecological changes have been shown for other Southern Ocean predators (Barbraud & Weimerskirch 2006) with the sub-Antarctic area south of the Indian Ocean particularly affected (Weimerskirch *et al.* 2003). Other potential consequences of climate change include change in migratory timing and length, prey distribution and availability, breeding schedules, reproductive success and survival (Learmonth *et al.* 2006; Simmonds & Isaac 2007; Robinson *et al.* 2009). The prey of Antarctic blue whales, Antarctic krill (*Euphausia superba*), have already shown a decline in abundance (Atkinson *et al.* 2004) and may undergo a range contraction (Whitehouse *et al.* 2008).

Conclusion

We have provided the first recorded cases of hybridization between the two recognized blue whale Southern Hemisphere subspecies and have confirmed the existence of a sympatric area of Antarctic and pygmy blue whales off Antarctica. The occurrence of both migrants and admixed individuals in the sympatric area indicate the pygmy blue whales using waters off Antarctica may migrate and then breed during the austral winter with the Antarctic subspecies, or the subspecies may interbreed off Antarctica outside the expected austral winter breeding season. There is discordance between estimates of the recent proportion of pygmy blue whales off Antarctica based on morphological differences from

whaling catches and genetics of post-whaling samples. This may be due to differences in methods, or together with evolutionary genetic migration estimates may indicate a change in proportion of pygmy blue whales off Antarctica within the last four decades. Possible causes for the latter include whaling, anthropogenic climate change or a combination of these. This information is crucial for management and conservation of the world's largest animal. Estimates of contemporary Antarctic population numbers now need to take into account genetic evidence of migrants and admixture, and a potential range shift needs to be considered when designating management areas.

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Data accessibility

Microsatellite and sex data: DRYAD entry doi:10.5061/dryad.8m0t6.