Understanding the evolution of two species of highly migratory cetacean at multiple scales and the potential value of a mechanistic approach

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ABSTRACT

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An improved understanding of how behavior influences the genetic structure of populations would offer insight into the inextricable link between ecological processes and evolutionary patterns. This dissertation aims to demonstrate the need to consider behavior alongside genetics by examining the population genetic structure of two species of highly migratory cetacean across multiple scales and presenting an exploration of some potential lines of enquiry into the behavioral mechanisms underlying the patterns of genetic population structure observed.

The first empirical chapter presents a population genetic analysis conducted on a data set of new and existing samples of Bryde's whale (*Balaenoptera edeni* spp.) collected from the Western and Central Indo-Pacific and the Northwest Pacific Ocean. Levels of evolutionary divergence between two subspecies (*B. e. brydei* and *B. e. edeni*) and the degree of population structure present within each subspecies were explored. The subsequent three empirical chapters represent a series of population- and individual-level genetic analyses on a data set of more than 4,000 individual humpback whales (*Megaptera novaengliae*) sampled from across the South Atlantic and Western and Northern Indian Oceans over two decades. Patterns of genetic population structure and connectivity between breeding populations are examined across the region, and are complemented by an assessment of genetic structure on shared feeding areas for these populations in the Southern Ocean.

Collectively, these studies demonstrate that a hierarchy of behavioral processes operating at different spatial scales is likely influencing patterns of genetic population structure in highly migratory baleen whales. Notably, for humpback whales, the widely assumed model of maternal fidelity to feeding areas and natal philopatry to breeding areas was found not to be applicable at all spatial scales. From an applied perspective, the complex population patterns observed are not currently accounted for in current management designation and recommendations for applying these findings to the management and protection of these species are presented.

As these empirical studies highlight the importance of behavior as a potential mechanism for shaping the genetic structure of species, the final chapter offers a research prospectus describing how behavioral and genetic data may be integrated using new individual-based modeling techniques to integrate data and information from the fields of behavioral ecology and population genetics.

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DEDICATION

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INTRODUCTION

Behavior is too important to be left to psychologists.

- Donald Redfield Griffin

Genetic population structure is commonly observed in wild populations and arises from variation in the spatial and temporal distribution and movement of individual organisms, which over evolutionary meaningful timescales results in the systematic variation of population allele frequencies across space and time (Jones & Wang 2012). Population structure is therefore, at least in part, driven by complex behaviors operating at the level of the individual organism. An improved understanding of how behavior influences the genetic structure of populations would offer insight into the inextricable link between ecological processes and evolutionary patterns, enabling the interpretation of the mechanisms underlying existing genetic patterns, the forecasting of how these patterns may change in the future (Blair & Melnick 2012) and, in the long-term, may facilitate predictions of the evolutionary trajectories of species (Li *et al*. 2012).

Highly migratory species exhibit a wide range of complex behaviors capable of influencing their genetic population structure at multiple scales. Population-level fidelity to breeding and feeding sites has proven to be an important driver of genetic isolation between populations for a number of migratory species. There is increasing evidence, however, that genetic structure within populations is driven by subtle, and sometimes socially driven, differences in dispersal and migratory behaviors that form barriers to gene flow. This behavioral partitioning within a population may, for example, be linked to differences in the timing of migration on the basis of age, sex, or reproductive status (Sonsthagen *et al*. 2009), habitat and

foraging specializations of certain individuals (Rayner *et al*. 2011; Hoye *et al*. 2012), or different social strategies (Andrews *et al*. 2010).

The properties inherent to different molecular markers enable the testing of explicit hypotheses regarding how behavior may be influencing genetic patterns. When dispersal is biased towards one sex, uniparentally inherited markers would be expected to show incongruent patterns of genetic structure. For example, the general pattern of female philopatry and male-dispersal observed in mammals (Greenwood 1980) is reflected in strong geographic structuring of the maternally inherited mitochondrial DNA (mtDNA), but not the paternally inherited Y-chromosome haplotypes or autosomal markers (Avise 2004). By considering markers with differing mutation rates and coalescence times, one can also discriminate between the timing of dispersal events. For instance, rapidly mutating nuclear microsatellite loci can be informative of recent dispersal and movements of individuals, whereas more slowly evolving mtDNA markers provide insight into population differentiation and connectivity on historic timescales (Avise 2004).

The ensuing four data chapters examine the genetic structure of two species of highly migratory baleen whale at the subspecies, population, and individual scales, and explore some potential lines of enquiry into the mechanisms (i.e. processes) underlying the patterns observed. Akin to all baleen whales, both the Bryde's whale (*Balaenoptera edeni* spp.) and the humpback whale (*Megaptera novaeangliae*) display significant behavioral complexity and plasticity, and recent studies have unveiled corresponding elaborate genetic architectures, the behavioral drivers of which appear to vary at different scales (e.g. Kanda *et al*. 2007; Rosenbaum *et al*. 2009). Thus, baleen whales represent an interesting and relevant test bed for questions concerning how behavior may influence the evolutionary patterns of highly migratory species.

From an applied perspective, the elucidation of population-level management units for these two species is of utmost importance given that baleen whales are in recovery from significant commercial exploitation and illegal Soviet whaling (Rocha *et al*. 2015) and some, including the Bryde's whale, remain a target of scientific whaling by Japan (Kanda *et al*. 2007). These species also are vulnerable to a range of contemporary anthropogenic stressors, such as disturbance to their acoustic environment, increased shipping and pollution (Rosenbaum *et al*. 2014), and the indirect effects of a changing climate (Ramp *et al*. 2015). The interpretation of the genetic analyses conducted in each of the four data chapters therefore also explicitly informs species management. In addition, the geographic regions from which the samples used in these studies were collected, namely the South Atlantic Ocean, Western and Northern Indian Oceans, and the Southern Ocean, are relatively understudied and so this body of work represents an important contribution to the global understanding of these species.

Chapter One (published as Kershaw *et al*. 2013 in the *Journal of Heredity*) presents subspecies- and population-level analyses for two forms of Bryde's whale (*B. e. brydei* and *B. e. edeni*) using mtDNA control region sequences from 56 new samples from Oman, the Maldives, and Bangladesh, and published sequences originating from Java and the Northwest Pacific. This chapter combines nine diagnostic characters identified in the mtDNA control region with a phylogenetic analysis based on maximum parsimony to explore the degree of differentiation between the two forms of Bryde's whale. Genetic diversity and differentiation indices, and a reconstructed haplotype network, are then used to assess population-level genetic structure within each of the two forms. Subsequently, ecological differences between the two forms that may be driving their genetic differentiation are considered.

Chapters Two, Three, and Four focus exclusively on a data set comprising more than 4,000 humpback whales sampled in the South Atlantic, Western and Northern Indian, and Southern Oceans over more than two decades. This data set represents samples from seven breeding populations and sub-populations identified across the region, and which are managed by the International Whaling Commission (IWC) as the following "breeding stocks (BS)" and "substocks": BSA, located off Albrohos Bank, Brazil; BSB1, a breeding population in the Gulf of Guinea; BSB2, a group of feeding and migrating individuals off west South Africa; BSC1, off east South Africa and Mozambique; BSC2, located in the vicinity of the Mayotte and Geyser, and the Comoros Islands; BSC3, that breeds off northeast Madagascar; and the non-migratory Arabian Sea Humpback Whale (ASHW) population sampled from the Gulf of Aden, Oman.

Chapter Two examines the genetic diversity and population structure of seven putative breeding stocks and substocks present in the region using nine microsatellite loci. Genetic differentiation is assessed both with and without *a priori* designation of population units, and gene flow is estimated using maximum likelihood and Bayesian approaches, providing insights into population connectivity on historic and contemporary temporal scales. For all analyses, sex-specific differences are explicitly explored. The results of these analyses are compared and contrasted to those of a parallel study employing a 486 bp consensus sequence of the mitochondrial control region (Rosenbaum *et al*. 2009). Chapter Three assesses the relative contribution and degree of mixing of seven humpback whale breeding stocks and substocks to shared feeding areas in the Southern Ocean. First, feeding areas are defined using a sensitivity analysis based on genetic differentiation indices. A mixed stock analysis is then conducted using ten microsatellite loci and the distribution of haplotypes across feeding areas is examined using a 371 bp consensus sequence of the mitochondrial control region. Genetic diversity is also

assessed for both molecular markers. Chapter Four, the final data-based chapter, presents a genotypic matching analysis using ten microsatellite loci and two quantitative indices to examine sex-specific differences of fidelity to breeding areas, dispersal between breeding areas, and connectivity between breeding areas and feeding grounds, at the individual level.

The preceding four chapters illustrate that the development of a multidisciplinary approach combining the fields of behavioral ecology and population genetics is necessary to developing a mechanistic understanding of genetic population patterns (Habel *et al*. 2015). The fifth and final chapter therefore presents a literature review and research prospectus for advancing multidisciplinary approaches for the integration of data from the fields of population genetics and behavioral ecology using individual-based models (IBMs) as an analytical platform. Chapter Five first reviews recent advances in the field of IBM development that have resulted in these models becoming useful platforms for the integration of data on individual behavior, environmental factors, and genetics. Subsequently, lessons from a variety of applied case studies are synthesized to guide future model implementation, parameterization, and validation, with a particular focus on systems that are generally lacking in rich data sets or where the ability to ground truth model outputs is often not feasible, such as for the majority of highly migratory species.

CHAPTER ONE

Population differentiation of 2 forms of Bryde's whales in the Indian and Pacific Oceans

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ABSTRACT

Accurate identification of units for conservation is particularly challenging for marine species as obvious barriers to gene flow are generally lacking. Bryde's whales (*Balaenoptera* spp.) are subject to multiple human-mediated stressors, including fisheries bycatch, ship strikes, and scientific whaling by Japan. For effective management, a clear understanding of how populations of each Bryde's whale species/subspecies are genetically structured across their range is required. We conducted a population-level analysis of mtDNA control region sequences with 56 new samples from Oman, Maldives, and Bangladesh, plus published sequences from off Java and the Northwest Pacific. Nine diagnostic characters in the mitochondrial control region and a maximum parsimony phylogenetic analysis identified 2 genetically recognized subspecies of Bryde's whale: the larger, offshore form, *B. edeni brydei,* and the smaller, coastal form, *B. e. edeni.* Genetic diversity and differentiation indices, combined with a reconstructed maximum parsimony haplotype network, indicate strong differences in the genetic diversity and population structure within each subspecies. Discrete population units are identified for *B. e. brydei* in the Maldives, Java, and the Northwest Pacific, and for *B. e. edeni* between the Northern Indian Ocean (Oman and Bangladesh) and the coastal waters of Japan.

INTRODUCTION

Barriers to gene flow for cetaceans are rarely evident in marine environments (Mendez *et al*. 2010) meaning that discrimination of lower-level conservation units is challenging, as geographic distribution is not an appropriate proxy for isolation. Genetics can be a powerful tool for discriminating among incipient species and geographical forms, as well as distinct demographically independent populations that are experiencing levels of gene flow too high for local adaptation to occur (Taylor 2005). Notable examples of population-level delineations in baleen whales using genetics include humpback whales (*Megaptera novaeangliae*) in the North Pacific (Baker *et al*. 1998), North Atlantic (Stevick *et al*. 2006), Arabian Sea, and South Atlantic and Indian Oceans (Rosenbaum *et al*. 2009), and blue whales (*Balaenoptera musculus*) in the Southern Hemisphere (LeDuc *et al*. 2007).

Despite these advances, the taxonomy and population structure of many cetaceans remain unresolved. The implications of the existence of undetected conservation units at species and distinct population levels are disquieting, especially for taxonomic groups hunted under scientific permit from the International Whaling Commission, or those recovering from commercial whaling (Clapham *et al*. 2008). There is also the potential for the specialized habitat requirements of distinct lineages to be obscured by being aggregated within larger taxonomic groups. This issue is particularly consequential when lower-level conservation units inhabit areas that can be potentially affected by human activities, such as fisheries interactions and hydrocarbon exploration and development.

Well over a century has passed since the Bryde's whale (*Balaenoptera edeni*) was first described, but the phylogeny of this species complex is still unresolved (Perrin & Brownell 2007). While the nomenclature is unsettled because the species genetics of the holotype

specimen of *B. edeni* has not yet been determined, 2 subspecies are provisionally recognized by their genetics: a larger pelagic form, *B. edeni brydei*, with a circumglobal distribution in tropical and subtropical waters of the Pacific, Atlantic, and Indian Oceans, and a smaller nearshore form, *B. e. edeni*, in the Indo-Pacific region (Committee on Taxonomy 2011). However, others have recognized 2 species rather than subspecies (*B. brydei* and *B. edeni*; Kanda *et al*. 2007; Kato & Perrin 2009; Sasaki *et al*. 2006; Wada *et al*. 2003). For the purposes of maintaining consistency with current nomenclature (Committee on Taxonomy 2011), we refer to the subspecies *B. edeni brydei* and *B. e. edeni,* or "large-form" and "small-form".

A single-species designation of Bryde's whales was broadly accepted until the 1990s. Recently, however, it was discovered that populations in several parts of the range exhibit differences in body size, including a larger offshore form (i.e. *B. e. brydei*) and 1 or more smaller, predominantly coastal forms (i.e. *B. e. edeni*; Best 1997, 2001; Penry *et al*. 2011; Perrin & Brownell 2007; Perrin *et al*. 1996). A new species, *B. omurai*, representing a separate ancient lineage within the Balaenopteridae clade (Sasaki *et al*. 2006), was also recently described in the Indo-Pacific region (Wada *et al*. 2003). As Bryde's whales were previously subjected to commercial exploitation and remain a target of scientific whaling by Japan (Kanda *et al*. 2007), the ability to distinguish Bryde's whale taxa and elucidate their respective genetic population structure is required to avoid overexploitation, develop effective conservation plans, and prevent the loss of irreplaceable evolutionary lineages.

Here, we build upon previous research by combining new genetic samples of Bryde's whales from 3 previously unsampled locations across the Northern Indian Ocean (NIO) with previously published data on samples from the Central Indo-Pacific region and Northwest Pacific Ocean (Kanda *et al*. 2007; Yoshida & Kato 1999). Through the integration of these data sets, we

provide additional insights into the Bryde's whale phylogeny that supports the existing classification of the 2 taxonomic units (here treated as subspecies): *B. e. brydei* (large-form) and *B. e. edeni* (small-form). We then make population-level inferences across the region, which provide an important baseline for understanding the genetic diversity and spatial structure of Bryde's whale populations; information that is vital for effective conservation and management.

MATERIALS AND METHODS

Samples and molecular methods

A total of 409 samples originating from across the Western and Central Indo-Pacific, and the Northwest Pacific Ocean were used for the current study, including those previously published (Kanda *et al*. 2007; Yoshida & Kato 1999). The study region is defined following the Marine Ecoregions of the World (MEOW) schema developed by Spalding *et al*. (2007) and encompasses the Western Indo-Pacific Realm eastwards from the Somali/Arabian Province, the Central Indo-Pacific Realm, and the Warm Temperate Northwest Pacific Province nested within the Temperate North Pacific Realm (Fig. 1). Our dataset includes 56 newly collected samples from Bangladesh, the Maldives, and Oman (see Table S1 for details). 30 samples were from biopsies of whales in Bangladesh (BAN). Of these, 29 were sampled from the rim of the Swatch-of-No-Ground (SoNG) submarine canyon and 1 originated from a stranding at Cox's Bazaar in southeast Bangladesh. Previously unpublished data for the mtDNA control region were obtained for 8 whales sampled off the Maldives (MAL) and 18 individuals stranded or struck by ships along the coast of Oman (OMA). These new genetic data were combined with mitochondrial haplotypes from the south of Java (JAV, n=27), the coastal waters of Japan (COJ, n=16), and

with a large dataset from the Northwest Pacific (NWP, n=310; ACCN: EF068013-048, EF068060-063, Kanda *et al*. 2007; ACCN: AF146378-388, Yoshida & Kato 1999).

Total genomic DNA was extracted following procedures outlined in the QIAamp Tissue Kit (QiaGen). A 407bp consensus fragment of the mtDNA control region was amplified using Polymerase Chain Reaction (PCR), with primers Dlp 1.5 and Dlp 5 (Baker *et al*. 1993). Reactions of 25 µL total volume, containing 21.0 µL H_20, 1.0 µL of each primer at 10µM concentration, 1 Illustra (tm) PuReTaq (tm) Ready-To-Go (tm) PCR Bead (GE Healthcare), and 2.0 µL DNA template were conducted using an Eppendorf Gradient Mastercycler (94˚C for 4 min, followed by 30 cycles of 94˚C for 45 s, 54˚C for 45 s, and 72˚C for 45 s, and a final extension step at 72˚C for 10 min). Amplified PCR products were purified using Agencourt AMPure XP (Agencourt Bioscience) and sequenced with dye-labeled (BigDye ver 3.1 (tm); Applied Biosystems, Inc.) terminators in both directions. Sequence data were collected using a 3730xl DNA Analyzer (Applied Biosystems, Inc.). Geneious ver 5.3.5 (Biomatters Ltd. 2010) was used to edit and create consensus sequences for the forward and reverse reads.

Analytical approach

In fulfillment of data archiving guidelines (Baker 2013), we have deposited the primary data underlying these analyses with Dryad.

Identification of species and subspecies

To identify which Bryde's whale species or subspecies were present in our sample, we selected mtDNA control region reference sequences for *B. e. brydei* (large-form; ACCN: AB201259, AP006469), *B. e. edeni* (small-form; ACCN: AB201258), and *B. omurai* (ACCN: AB201256-7),

based on the phylogenetic analysis by Sasaki *et al*. (2006). Sasaki *et al*. (2006) attempted to phylogenetically verify specimens used in previous studies and obtained new specimens for each taxon that adhered to the classification defined by Wada *et al*. (2003). As all 3 of these taxa were phylogenetically distinct, and while their correspondence to 'small' and 'large' forms requires further work, we consider these sequences to represent the most reliable and consistent reference for defining the species and subspecies in this study. We recognize that this situation could change if the genetic identity of the *B. edeni* holotype is ever determined. *Balaenoptera physalus* (ACCN: NC_001321.1) was selected as the outgroup for the phylogenetic analysis given its basal evolutionary relationship to the Bryde's whale complex (Sasaki *et al*. 2006).

We identified the species and subspecies in our sample using characteristic attribute (CA) diagnosis (Lowenstein *et al*. 2009; Sarkar *et al*. 2002). We accepted the phylogeny of the species/subspecies as described by Sasaki *et al*. (2006) and aligned the reference sequences using ClustalW (Higgins *et al*. 1994) under default settings in MEGA5 (Tamura *et al*. 2011), and trimmed to the consensus 299bp mitochondrial control region (bp position 15545-15843 in the mtDNA genome of *B. e. edeni* [ACCN: AB201258]). To construct a character-based key, we visually inspected the reference sequences for variable sites that could serve as diagnostics for the three taxa (*sensu* Lowenstein *et al*. 2009). We then aligned our unknown sequences to the chosen reference sequences and used the CAs to identify the species and subspecies present in the unknown sample.

Phylogenetic analysis

Sequences were collapsed to haplotypes using DnaSP ver 5 (Librado & Rosaz 2009). Unique haplotypes were combined with the outgroup *B. physalus* and a single *B. omurai* reference

sequence (ACCN: AB201256), and aligned using ClustalW (Higgins *et al*. 1994) under default settings in MEGA5 (Tamura *et al*. 2011). The resulting 304bp alignment (including gaps) was used to estimate lineage relationships using maximum parsimony (Fitch 1971). Maximum parsimony analysis was conducted in PAUP ver 4.0b10 (Swofford 2002) with 1,000 bootstrap replicates using a heuristic search strategy with tree-bisection-reconnection (TBR) branch swapping, random taxon addition with 100 repetitions and one tree held at each step, and a maximum of 1,000 trees saved per replicate in order to decrease the time needed to run large bootstrap replicates (Sessa *et al*. 2012). The resulting bootstrap 50% majority-rule consensus tree was edited using Figtree ver 1.3.1 (Rambaut 2009).

Genetic diversity indices

For the statistical analyses, haplotypes of *B. e. brydei* and *B. e. edeni* were treated separately based on the outcome of the taxon identification and phylogeny, and only sampling regions where n>5 were included to enable statistical inference. Samples were grouped based on their geographic sampling site using DnaSP ver 5 (Librado & Rosaz 2009). Genetic diversity indices (number of haplotypes, haplotype diversity, nucleotide diversity with Jukes-Cantor correction, and average number of pairwise nucleotide differences among sequences) were calculated in DnaSP for the total sample and for each geographic region (when n>5). To further explore the genetic diversity of the newly sequenced samples of *B. e. edeni* from Oman (n=16) and Bangladesh (n=29), diversity indices were separately calculated for the consensus 407bp fragment of the mitochondrial control region (bp position 15500-15906 in the mtDNA genome of *B. e. edeni* [ACCN: AB201258]).

Population-level genetic structure

The Java and Northwest Pacific haplotype frequencies (Kanda *et al*. 2007; Yoshida & Kato 1999) were combined with the new haplotype frequencies from the Northern Indian Ocean. Tests of genetic differentiation between sampling locations (when n>5) were conducted in Arlequin ver 3.5 (Excoffier & Lischer 2010) for *B. e. brydei* and *B. e. edeni*, respectively. A heterogeneity test for haplotype frequencies was calculated using Fisher's exact test of population differentiation (implemented with 10,000 Markov chain steps and 1,000 dememorization steps) at the 0.05 significance level. Pairwise genetic differentiation between sampling sites was calculated using haplotype frequencies (F_{ST}) with 1000 permutations at the 0.05 significance level (Weir & Cockerham 1984) in Arlequin ver 3.5 (Excoffier & Lischer 2010). Pairwise genetic distances were calculated in PAUP ver 4.0b10 (Swofford, 2002) assuming the HKY85 model of nucleotide substitution as selected according to the corrected Akaike information criterion (cAIC) implemented in jModelTest ver 2.1 (Darriba *et al*. 2012). Levels of genetic divergence between samples were then calculated with the fixation index (Φ_{ST}) (Excoffier *et al.* 1992) in Arlequin ver 3.5 using the distance matrix computed in PAUP. Significance of Φ_{ST} for all possible pairwise population comparisons was assessed using 1000 permutations at the 0.05 significance level.

Haplotype network

The dataset for the haplotype network comprised the consensus 299 bp control region sequences for *B. e. brydei* and *B. e. edeni*, representing 48 haplotypes and 348 samples (including sampling regions with n<5). The alignment was converted to Roehl data format (.RDF) using DnaSP. Median-Joining haplotype networks (Bandelt *et al*. 1999), both with and

without maximum parsimony post-processing (Mardulyn 2012), were calculated using NETWORK ver 4.6.0.0 (Fluxus Technology Ltd. 1999-2010) with ε=0 and all variable sites weighted equally. Median-Joining networks have been recommended over maximum parsimony approaches in intra-specific studies as they capture a greater degree of ambiguity, thus enabling more realistic interpretations (Cassens *et al*. 2005).

RESULTS

Presence of Bryde's whale species and subspecies

Phylogenetic reconstruction of available references sequences for *B. e. brydei*, *B. e. edeni*, *B. omurai*, relative to the outgroup *B. physalus*, identified 9 characteristic attributes (CAs) that were diagnostic of the 4 taxa within the 299 bp consensus region. Sequences from our 56 samples matched closely those of *B. e. brydei* or *B. e. edeni*, sharing all CAs with one or the other of these taxa. None of the samples matched the known mtDNA sequence of *B. omurai*, or any other species. These taxon-specific (species or subspecies) clades were supported by the maximum parsimony bootstrap 50% majority-rule consensus tree based on 41 parsimony informative characters (Fig. 2). Bootstrap values for the 2 clades were high (100% for both clades; Fig. 2) and support previous work that has identified the 2 subspecies as sister taxa (Sasaki *et al*. 2006). Samples identified as *B. e. brydei* and *B. e. edeni* were therefore treated separately for subsequent diversity and population-level analyses.

Genetic diversity

The genetic analysis of the mtDNA control region resulted in the identification of 45 unique haplotypes (H1-H45) for *B. e. brydei* that were derived from 348 sequences with 34 polymorphic

sites (2 singletons, 32 parsimony informative) in the 297bp control region (following the removal of gaps and missing data). For sampling locations where n>5, *B. e. brydei* (n=348) was identified at 3 sampling locations: the Maldives (n=8), south of Java (n=27) and offshore in the Northwest Pacific (n=310). In addition, 2 individuals were sampled on the coast of Oman, and 1 individual was sampled from a ship strike offshore of Bangladesh. Genetic diversity (Table 1) was relatively high (*Hd*: 0.845; π(JC): 0.01319; *k*: 3.821) and was generally comparable between samples; the Maldives exhibited a relatively lower *k* value likely related to small sample size, and the south of Java sample exhibited a relatively lower *Hd* value.

In contrast, *B. e. edeni* showed remarkably low genetic diversity with only 3 haplotypes derived in the 299 bp control region from 61 sequences (3 parsimony informative sites) (*Hd*: 0.391; π(JC): 0.00371; *k*: 1.095; Table 1). For sampling locations where n>5, *B. e. edeni* (n=61) was identified at 3 sampling locations: Bangladesh (n=29), Oman (n=16) and coast of Japan (n=16). Notably, no genetic diversity was found among the Bangladesh and Oman samples as all 45 individuals shared a single haplotype for the 299 bp fragment. 3 haplotypes were identified in the coast of Japan sample, 1 of which was identical to the haplotype identified in Bangladesh and Oman. When diversity analyses were conducted on the larger 407bp consensus fragment of the mtDNA control region of the new Oman and Bangladesh samples, we identified 1 additional *B. e. edeni* haplotype in the Oman sample (H49; data not shown).

Overall, 4 new haplotypes were identified for *B. e. brydei* (H01, H06, H07, H44; ACCN: JX090150-52, KC261305) and 1 new haplotype was identified for *B. e. edeni* (H49; ACCN: KC561138). The remaining haplotypes for *B. e. brydei* and *B. e. edeni* have been previously found and presented in other studies (Kanda *et al*. 2007; Yoshida & Kato 1999; see Table S2).

Population structure

Median-Joining networks showed comparable results irrespective of whether or not maximum parsimony (MP) post-processing was included. As expected, the Median-Joining network without MP post-processing captured a larger number of inferred nodes and reticulations (Cassens *et al*. 2005; Mardulyn 2012). However, as the fundamental relationships between haplotypes were not affected, only the more parsimonious network with MP post-processing is shown (Fig. S1).

For the 44 haplotypes identified as *B. e. brydei*, two main clusters are apparent: the Northern Indian Ocean (Oman, Maldives, Bangladesh) and the Northwest Pacific. Haplotypes from off Java are represented across the network (Figs. 2, S1). 2 clusters, NIO and coastal Japan respectively, are also evident for *B. e. edeni*. However, a single individual from the coast of Japan was found to share a NIO haplotype (H46). *B. e. brydei* comprised 11.1% of the total sample in Oman, 100% of the samples in the Maldives, 4.4% of the Bangladesh sample (the single individual sampled from an offshore ship strike), and 100% of the samples from off Java and the Northwest Pacific. In contrast, *B. e. edeni* was only sampled close to the coastline, comprising 88.9% of the Oman sample, 96.6% in Bangladesh, and 100% in the coastal Japan (Figs. 2, S1).

For *B. e. brydei*, pairwise F_{ST} and Φ_{ST} values (Table 2) were highly significant between all sampling sites ($P<0.001$) indicating that populations in the Maldives, off Java, and the Northwest Pacific can be considered genetically distinct populations. In contrast, for *B. e. edeni* pairwise F_{ST} and Φ_{ST} results showed no significant genetic differentiation between Bangladesh and Oman (F_{ST}: 0.000, $p>0.05$; Φ_{ST}:0.000, $p>0.05$). However, highly significant differentiation was found between the coast of Japan and Bangladesh (F_{ST}: 0.866, $p<0.001$; Φ_{ST}:0.923, $p<0.001$), as well as

Oman (F_{ST}: 0.817, p<0.001; Φ_{ST}:0.893, p<0.001). 1 haplotype (H46) was shared between all 3 sampling locations, and is possibly indicative of some unquantifiable degree of gene flow across the region or the retention of ancestral polymorphism (Figs. 2, S1).

DISCUSSION

Our phylogenetic analyses of the mtDNA control region are consistent with previous taxonomic groupings recognized for the subspecies *B. e. brydei* and *B. e. edeni*. Our results provide novel insights into the breadth of the distribution of these subspecies across the Western and Central Indo-Pacific, and the warm temperate Northwest Pacific, and elucidate genetic patterns at the population level. The striking differences between the 2 forms indicated by these analyses, and when considered alongside previously identified morphological and behavioral differences, support the designation of each form as a separate species or subspecies.

Taxon identification and divergence

Using phylogenetic analyses, we confirmed evolutionary divergence in the mitochondrial DNA of Bryde's whale subspecies within our sample: the offshore, large-form, *B. e. brydei*, and the coastal, small-form, *B. e. edeni,* as previously reported by Kanda *et al*. (2007) and Sasaki *et al*. (2006). Due to the limited information available for these taxa, we rely solely on the best available genetic data to define the species and subspecies in our study. Reference sequences should ideally be based on verified voucher specimens that offer corollary information (e.g. morphological data) for taxon designation (Reeves *et al*. 2004), and we recognize this is a limitation of our study; independent classification using morphological data is required for formal taxonomic classification (DeSalle *et al*. 2005; Reeves *et al*. 2004).

Individual genetic loci, like morphological characters, do not necessarily reflect the true phylogenetic history; the gene tree is not always consistent or congruent with the species tree (Page & Charleston 1997). This has been previously demonstrated in Bryde's whales by Sasaki *et al*. (2006) who found inconsistencies in the phylogenetic relationships between *B. e. brydei*, *B. e. edeni,* and *B. borealis* dependent upon the mitochondrial molecular marker employed. Therefore, the phylogeny we identified is likely to be, at least in part, a function of the single mtDNA marker used in the analysis. Future analyses utilizing larger fragments of the mitochondrial genome alongside additional nuclear markers are likely to further resolve the phylogenetic relationships of the Bryde's whale species complex (Morin *et al*. 2010; Sharma *et al*. 2012).

Morphological, behavioral, and geographic information indicate strong differences between the 2 subspecies. This differentiation is not only of ecological and evolutionary interest, but is also of critical importance for informing the conservation and management of these whales**.** Size differences and temporal reproductive phase shifts have been recorded in historical whaling data (Mikhalev 2000). Observations of habitat partitioning (i.e. coastal *vs*. offshore) between the 2 subspecies (Best 2001) indicate the existence of an ecological barrier to gene flow, which may have acted as the mechanism for divergence. These findings are corroborated by field observations of a putative population of coastal small-form whales off South Africa (Best 2001; Penry *et al*. 2011), however the genetic identity of this group still needs to be confirmed. The present study provides further evidence by showing that *B. e. brydei* appears to have a more cosmopolitan distribution in both coastal and offshore areas, likely due to greater mobility and offshore habitat use. In contrast, *B. e. edeni* was only sampled close to the coast of Japan

indicating that the coastal waters of the Northwest Pacific may represent their eastern and northern range extent in the North Pacific.

The original 9 specimens of *B. omurai* were from the Solomon Sea (n=6) in 1976, off Cocos Islands (n=2) in 1978, and Tsunoshima (34˚21'N, 130˚52'E), Sea of Japan, Japan (n=1) in 1998 (Wada *et al*. 2003). More recently additional specimens have been reported from southern Japan, Taiwan, Philippines, and Thailand (the westernmost specimen of *B. omurai* from the Andaman Sea). However, the identification of these new specimens of *B. omurai* is based solely on their morphology and not genetics (Yamada *et al*. 2006, 2008). Omura's whale and *B. edeni*, therefore, appear to be sympatric in parts of their range off southern Japan, Taiwan, and off Thailand in the Andaman Sea. This sympatry may also occur in the waters around Cocos Islands in the eastern central Indian Ocean where two specimens of *B. omurai* were taken under a special research permit in the late 1970s (Wada & Numachi 1991). The exact details of any habitat sympatry are unknown because all the whales from Japan, Taiwan, and Thailand are based on stranded specimens. The lack of *B. omurai* in our sample adds support to the western limit of this species in the Eastern Indian Ocean is the Andaman Sea, off the western coast of the Malay Peninsula (Yamada *et al*. 2008; Yamada 2009).

Population-level diversity and structure

The genetic structure observed for *B. e. brydei* indicates 3 discrete populations experiencing very little gene flow in the Maldives, off Java, and the Northwest Pacific. We note, however, that the small sample size for the Maldives (n=8) limits the statistical inference that can be made regarding this potential 'population' and precludes a definitive conclusion. Given the potential consequences of not recognizing a genetically differentiated group in a species subject to

continued hunting, we chose to include the Maldives as a separate population unit in this study as a precautionary measure with the view to informing management.

The population identity of the whales off Java is not clear, as 3 of the 5 haplotypes were also identified within the Maldives sample (n=1) and the Northwest Pacific sample (n=2), indicating contemporary or historic gene flow. Interestingly, the Java population also exhibits much lower genetic diversity (*Hd*=0.396) than either the Maldives (*Hd*=0.750) or the Northwest Pacific (*Hd*=0.810), suggesting that the population may be small and subject to the effects of genetic drift, perhaps due to the lower ocean productivity found in this region (Longhurst 2007). 2 whales sampled in Oman were identified as *B. e. brydei,* suggesting that another discrete population may exist in the Arabian Sea, or that the population identified in the Maldives may have a broader geographical range than detected by this analysis. Historical Soviet whaling records report large aggregations of both large- and small-form Bryde's whales in the Gulf of Aden (Mikhalev 2000), indicating that this may indeed be an important part of the range for both of these taxa. Increased genetic sampling in this region will be crucial in delineating population boundaries for management purposes.

In marked contrast to *B. e. brydei*, extremely low degrees of genetic diversity (*Hd*=0.391) and population structure were found for *B. e. edeni* across the NIO, at a scale not before seen in baleen whales (e.g. Rosenbaum *et al*. 2000; Patenaude *et al*. 2007). Only a single haplotype (Figs. 2, S1; H46) was shared between the 45 individuals sampled in Bangladesh (*Hd*=0.000) and Oman (*Hd*=0.000) when the 299 bp consensus sequence was examined. As only 1 additional haplotype was identified in Oman (when the larger 407 bp fragment of the control region was considered), these low levels of diversity are likely not fully explained by the limited length of the marker used in our study. Notably, no further diversity was found within the Bangladesh

sample, indicating that levels of genetic diversity can still be considered unusually low for this subspecies.

We observed strong population structure between the Northern Indian Ocean populations of both *B. e. brydei* and *B. e. edeni* compared to those in the Northwest Pacific and in the coastal waters of Japan (Table 2; F_{ST} and Φ_{ST} have significance values of $p<0.001$ for all comparisons). This is consistent with the biogeographic barrier imposed by the peninsulas and islands of Thailand, Malaysia, and Indonesia. However, the shared haplotype between Java and the Northwest Pacific for *B. e. brydei* (Figs. 2, S1; H39), and between the Northern Indian Ocean and coast of Japan for *B. e. edeni* (Figs. 2, S1; H46), provides evidence of inter-oceanic exchange, at least historically, within populations of both taxa. Given our small sample size, it can be assumed that we underestimate the actual rates of genetic exchange between the Northern Indian Ocean and the Northwest Pacific, thus implying a porous barrier to long-range movements.

Conclusion

Evidence from phylogenetic analyses, and corroborating morphological and behavioral studies, supports the presence of 2 taxonomic units of Bryde's whale across the Western and Central Indo-Pacific, and the Northwest Pacific Ocean. The distinctiveness of the 2 subspecies confirms the need to designate each taxon as a separate conservation unit with specific management recommendations for each. Bryde's whales are vulnerable to fisheries bycatch and ship strikes across the study region (Bijukumar *et al*. 2012), and are currently subject to scientific whaling by Japan in the western North Pacific. There is also the potential impact of hydrocarbon exploration and development in coastal waters. Given these stressors, there is a clear need to implement

effective management measures that are fully informed by better defining conservation units at the species and population-level using molecular information.

Strong genetic differences were found at the population-level within *B. e. brydei* and *B. e. edeni*. We found significant differentiation among populations of *B. e. brydei* in the Maldives, Java, and in the offshore Northwest Pacific, and *B. e. edeni* off Oman and Bangladesh in the Northern Indian Ocean, and in the coastal waters of southern Japan. We therefore suggest that each population be considered an independent conservation unit for management purposes. The Arabian Sea may also represent an important priority for management given bycatch and ship strikes of these whales in the region, and the catches of 849 Bryde's whales during the mid-1960s, which based on their total lengths would likely be *B. e. brydei* (Mikhalev 2000). This is a priority for future research as it cannot yet be determined if the whale populations in the Arabian Sea are independent of the Maldives unit identified in this study. Additional genetic sampling is therefore urgently needed in the Arabian Sea and the Maldives, as well as coastal Southeast Asia, particularly along the Malay Peninsula and in the Gulf of Thailand (Perrin & Brownell 2007).

In addition, bi-parentally inherited, neutral microsatellite markers and single-nucleotide polymorphisms identified by high throughput sequencing techniques represent powerful future tools to complement population-level mtDNA analyses. Longer mtDNA sequences are likely to provide greater resolution of haplotypes and more informative estimates of genetic diversity and population differences, as indicated by our identification of an additional *B. e. edeni* haplotype in Oman. It will also be important to collect additional morphological information to validate the findings of phylogenetic and population genetic studies. Photographic documentation of individuals during biopsy sampling and the collection of morphological information from future ship strikes in the Indian Ocean represent two opportunistic methods to gather additional data.

The application of these new data will enable the finer-scale, spatio-temporal analyses essential for ensuring appropriate management and persistence of these whales (Dale & Von Schantz 2007; Gaines *et al*. 2005).

TABLES AND FIGURES

Table 1. Genetic diversity indices for *B. e. brydei and B. e. edeni* haplotypes for the 299 bp consensus region of the total sample and for individual sampling locations where n>5 (OMA, Oman; MAL, Maldives; BAN, Bangladesh; JAV, south of Java; CoJ, coast of Japan; NWP, Northwest Pacific). New samples are indicated by *. N, number of sequences; S, number of segregating sites; *H*, number of haplotypes; *Hd*, haplotype diversity; π(JC), nucleotide diversity with Jukes Cantor correction; *k,* average number of pairwise nucleotide differences among sequences.

Species	Sample	N	S	H	Hd	π(JC)	k
B. e. brydei	All	348	34	44	0.844	0.013	3.752
	MAL*	8	3	4	0.750	0.005	1.536
	JAV	27	12	5	0.396	0.007	2.108
	NWP	310	33	37	0.810	0.012	3.079
B. e. edeni	All	61	3	3	0.391	0.004	1.095
	BAN*	29	0	1	0.000	0.000	0.000
	OMA*	16	0	1	0.000	0.000	0.000
	COJ	16	3	3	0.342	0.002	0.575

Table 2. Pairwise F_{ST} and ϕ_{ST} values for *B. e. brydei* and *B. e. edeni* for each sampling location where n>5 (OMA, Oman; MAL, Maldives; BAN, Bangladesh; JAV, south of Java; CoJ, coast of Japan; NWP, Northwest Pacific). F_{ST} values are shown above the diagonal, ϕ_{ST} results are shown below the diagonal. Significance values are indicated as ***, p<0.001 assessed using 1000 permutations at the 0.05 significance level in Arlequin ver 3.5 (Excoffier & Lischer 2010).

B. e. brydei		**MAL**	**JAV**	**NWP**
	MAL	-	0.479***	0.211***
	JAV	0.561***	-	0.334***
	NWP	0.564***	0.452***	-

B. e. edeni		**BAN**	**OMA**	**COJ**
	BAN	-	0.000	0.866***
	OMA	0.000	-	0.818***
	COJ	0.923***	0.893***	-

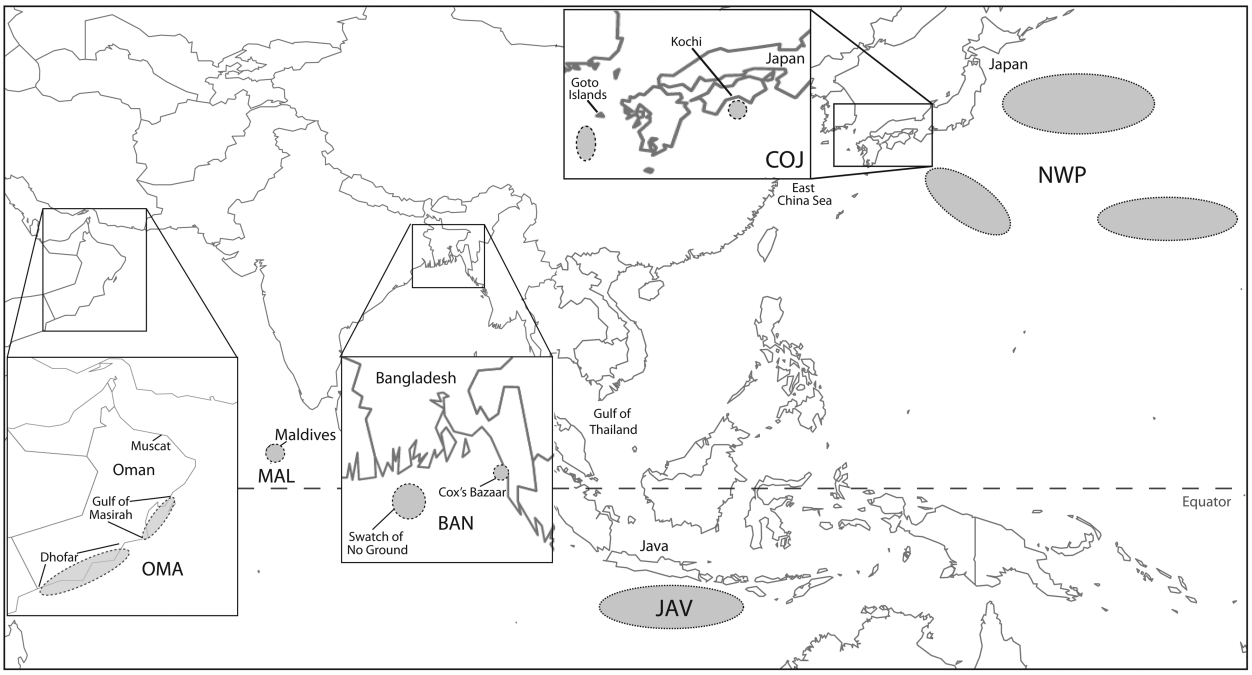
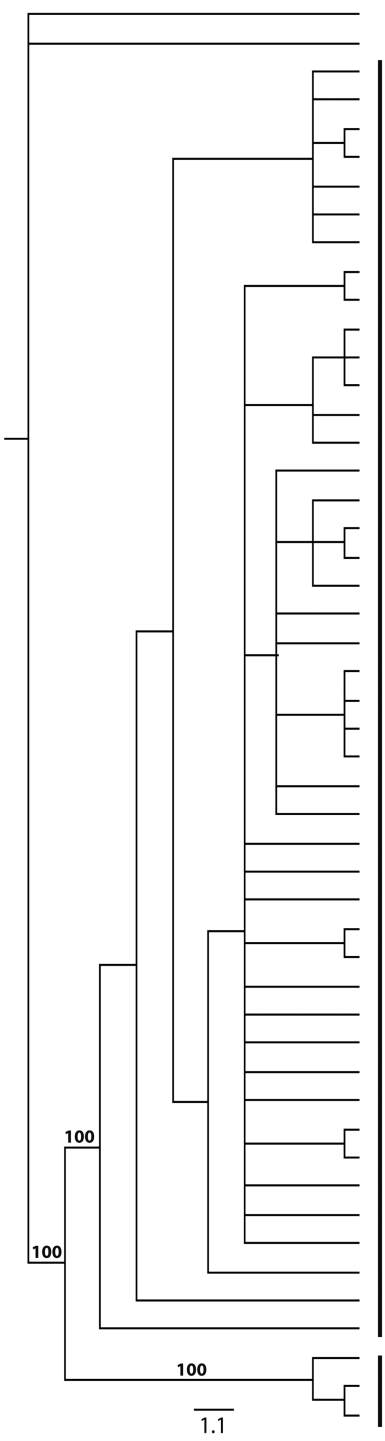


Fig. 1. Schematic illustrating the extent of the study region and approximate sampling locations shaded in gray: OMA, Oman; MAL, Maldives; BAN, Bangladesh; JAV, south of Java CoJ, coast of Japan; NWP, Northwest Pacific. New samples were collected from Oman, the Maldives, and Bangladesh. Existing samples had been previously collected from south of Java, coast of Japan, and Northwest Pacific (Kanda *et al*. 2007; Yoshida & Kato 1999). The eastern portion of the schematic (JAV, COJ, NWP) was adapted from Kanda *et al*. (2007) and Yoshida & Kato (1999).



1556 3	1559 2	1560 9	1561 4	1561 6	1568 1	1572 2	1572 6	1576 9	H	N	OMA	MAL	BAN	JAV	COJ	NWP
C	T	C	G	T	C	T	A	T								
T	*	*	A	*	*	*	*	*								
T	*	T	*	G	*	*	*	A	01	5		4	1			
T	*	T	*	G	*	*	*	A	02	2						2
T	*	T	*	G	*	*	*	A	03	1						1
T	*	T	*	G	*	*	*	A	04	2						2
T	*	T	*	G	*	*	*	A	05	4	1	1		2		
T	*	T	*	G	*	*	*	A	06	1		1				
T	*	T	*	G	*	*	*	A	07	1	1					
T	*	T	*	G	*	*	*	A	08	9						9
T	*	T	*	G	*	*	*	A	09	2						2
T	*	T	*	G	*	*	*	A	10	4						4
T	*	T	*	G	*	*	*	A	11	11						11
T	*	T	*	G	*	*	*	A	12	1				1		
T	*	T	*	G	*	*	*	A	13	5						5
T	*	T	*	G	*	*	*	A	14	3						3
T	*	T	*	G	*	*	*	A	15	28						28
T	*	T	*	G	*	*	*	A	16	1						1
T	*	T	*	G	*	*	*	A	17	6						6
T	*	T	*	G	*	*	*	A	18	3						3
T	*	T	*	G	*	*	*	A	19	3						3
T	*	T	*	G	*	*	*	A	20	1						1
T	*	T	*	G	*	*	*	A	21	7						7
T	*	T	*	G	*	*	*	A	22	3						3
T	*	T	*	G	*	*	*	A	23	4						4
T	*	T	*	G	*	*	*	A	24	5						5
T	*	T	*	G	*	*	*	A	25	1						1
T	*	T	*	G	*	*	*	A	26	6						6
T	*	T	*	G	*	*	*	A	27	1						1
T	*	T	*	G	*	*	*	A	28	34						34
T	*	T	*	G	*	*	*	A	29	1						1
T	*	T	*	G	*	*	*	A	30	126						126
T	*	T	*	G	*	*	*	A	31	5						5
T	*	T	*	G	*	*	*	A	32	1						1
T	*	T	*	G	*	*	*	A	33	11						11
T	*	T	*	G	*	*	*	A	34	7						7
T	*	T	*	G	*	*	*	A	35	5						5
T	*	T	*	G	*	*	*	A	36	1						1
T	*	T	*	G	*	*	*	A	37	4						4
T	*	T	*	G	*	*	*	A	38	2						2
T	*	T	*	G	*	*	*	A	39	3				1		2
T	*	T	*	G	*	*	*	A	40	1						1
T	*	T	*	G	*	*	*	A	41	22				22		
T	*	T	*	G	*	*	*	A	42	1				1		
T	*	T	*	G	*	*	*	A	43	1						1
T	*	T	*	G	*	*	*	A	44	2		2				
T	*	T	*	G	*	*	*	A	45	1						1
*	C	*	A	*	T	C	G	*	46	46	16		29		1	
*	C	*	A	*	T	C	G	*	47	13					13	
*	C	*	A	*	T	C	G	*	48	2					2	
								Total	48	409	18	8	30	27	16	310

Fig. 2. Phylogenetic reconstruction of mtDNA control region haplotypes of Bryde's whales sampled from across the Western and Central Indo-Pacific, and Northwest Pacific Ocean. The bootstrap 50% majority-rule consensus parsimony tree is shown with bootstrap values supporting phylogenetic differentiation of haplotypes identified as *B. e. brydei* and *B. e. edeni*. The 9 characteristic attributes (CAs) used to identify the taxa are shown to the immediate right of the tree. Nucleotide positions correspond to the *B. e. brydei* mitochondrial genome positions 15477-16410 (ACCN: AB201259). Positions 15609, 15616, and 15769 diagnose the *B. e. brydei* subspecies. Positions 15592, 15681, 15722, and 15726 diagnose the *B. e. edeni* subspecies. * represents conserved nucleotides in relation to the outgroup, *B. physalus*. H, haplotype number; N, sample size; and sampling location (i.e. OMA, Oman; MAL, Maldives; BAN, Bangladesh; JAV, south of Java; CoJ, coast of Japan; NWP, Northwest Pacific), are shown adjacent to the termini in the table to the far right. See Table S2 for details of haplotype accession numbers.

SUPPORTING INFORMATION

Table S1. Details of new Bryde's whale samples. Sample ID corresponds to original field sample code managed by the American Museum of Natural History. Taxon was designated as a result of the phylogenetic analysis carried out in the current study. Sampling sites are as follows: BAN, Swatch-of-No-Ground, Bangladesh; BAN (CB), Cox's Bazaar, Bangladesh; MAL, Maldives; OMA, Oman. Latitude and Longitude are in decimal degrees; N, North; E, East. Body length is given in meters or, if this information wasn't available, as life history stage. Sex is coded as: M, male; F, female. ND, no data.

Sample ID	Taxon	Sampling Site	Latitude (N)	Longitude (E)	Body Length	Sex	Sampling Method	Sample Type
163808	B. e. brydei	BAN (CB)	22.28961	91.75449	Adult	M	Necropsy	Muscle
163866	B. e. edeni	BAN	21.31197	89.48166	>12m	M	Biopsy dart	Skin
163867	B. e. edeni	BAN	21.31197	89.48166	>12m	F	Biopsy dart	Skin
163882	B. e. edeni	BAN	21.28040	89.38474	>12m	F	Biopsy dart	Skin
163883	B. e. edeni	BAN	21.28040	89.38474	>12m	F	Biopsy dart	Skin
163884	B. e. edeni	BAN	21.31197	89.48166	>12m	F	Biopsy dart	Skin
163886	B. e. edeni	BAN	21.31197	89.48166	>12m	M	Biopsy dart	Skin
163889	B. e. edeni	BAN	21.31197	89.48166	>12m	F	Biopsy dart	Skin
163890	B. e. edeni	BAN	21.31197	89.48166	>12m	ND	Biopsy dart	Skin
163891	B. e. edeni	BAN	21.31197	89.48166	>12m	M	Biopsy dart	Skin
163892	B. e. edeni	BAN	21.26533	89.50288	>12m	M	Biopsy dart	Skin
163898	B. e. edeni	BAN	21.31197	89.48166	>12m	F	Biopsy dart	Skin
163904	B. e. edeni	BAN	21.31197	89.48166	>12m	M	Biopsy dart	Skin
163906	B. e. edeni	BAN	21.31197	89.48166	>12m	F	Biopsy dart	Skin
163907	B. e. edeni	BAN	21.28040	89.38474	>12m	F	Biopsy dart	Skin
163908	B. e. edeni	BAN	21.30150	89.39712	>12m	F	Biopsy dart	Skin
163910	B. e. edeni	BAN	21.40125	89.55322	>12m	F	Biopsy dart	Skin
163914	B. e. edeni	BAN	21.31197	89.48166	>12m	F	Biopsy dart	Skin
163916	B. e. edeni	BAN	21.31197	89.48166	>12m	M	Biopsy dart	Skin
163917	B. e. edeni	BAN	21.27628	89.56635	>12m	F	Biopsy dart	Skin
163926	B. e. edeni	BAN	21.40125	89.55322	>12m	F	Biopsy dart	Skin
163927	B. e. edeni	BAN	21.32390	89.45966	>12m	M	Biopsy dart	Skin
163932	B. e. edeni	BAN	21.27213	89.41471	>12m	ND	Biopsy dart	Skin
163935	B. e. edeni	BAN	21.26533	89.50288	>12m	ND	Biopsy dart	Skin
163938	B. e. edeni	BAN	21.40860	89.55837	>12m	M	Biopsy dart	Skin
163939	B. e. edeni	BAN	21.33016	89.48374	>12m	ND	Biopsy dart	Skin
163940	B. e. edeni	BAN	21.31197	89.48166	>12m	M	Biopsy dart	Skin
163942	B. e. edeni	BAN	21.31197	89.48166	>12m	F	Biopsy dart	Skin
163954	B. e. edeni	BAN	21.65188	89.23253	>12m	ND	Necropsy	Skin
163957	B. e. edeni	BAN	21.27213	89.41471	>12m	ND	Biopsy dart	Skin
980409-01	B. e. brydei	MAL	7.18333	72.56861	12-13m	ND	Biopsy dart	Skin
980419-01	B. e. brydei	MAL	3.35000	73.70222	12-14m	ND	Biopsy dart	Skin
980419-02	B. e. brydei	MAL	3.25111	73.71916	10-12m	ND	Biopsy dart	Skin

980419-03	B. e. brydei	MAL	3.25000	73.71666	12-14m	ND	Biopsy dart	Skin
980420-01	B. e. brydei	MAL	3.25000	73.58333	12-14m	ND	Biopsy dart	Skin
980420-02	B. e. brydei	MAL	3.25000	73.58333	12-14m	ND	Biopsy dart	Skin
980420-03	B. e. brydei	MAL	3.25000	73.58333	12-14m	ND	Biopsy dart	Skin
980420-04	B. e. brydei	MAL	3.25000	73.58333	12-14m	ND	Biopsy dart	Skin
19-03-01-01	B. e. brydei	OMA	16.94411	54.01592	13m	ND	Necropsy	Skin, Muscle
21-03-02-01	B. e. brydei	OMA	20.40445	58.53280	ND	ND	Necropsy	Muscle
06-03-01-01	B. e. edeni	OMA	21.05246	58.84517	13m	ND	Necropsy	Muscle
11-03-01-01	B. e. edeni	OMA	23.61126	58.31159	12m	ND	Necropsy	ND
12-06-01-05	B. e. edeni	OMA	19.52770	57.69620	ND	ND	Necropsy	Tissue
12-10-00-02	B. e. edeni	OMA	20.52110	58.69620	ND	ND	Necropsy	Tissue
14-03-01-02	B. e. edeni	OMA	20.38003	58.32083	13.5m	ND	Necropsy	Skin
15-03-01-01	B. e. edeni	OMA	23.55497	58.71840	11m	ND	Necropsy	ND
17-10-00-02	B. e. edeni	OMA	ND	ND	ND	ND	Direct	Skin (slough)
28-02-01-01	B. e. edeni	OMA	23.63945	58.49132	ND	ND	Necropsy	Skin
27-10-01-05	B. e. edeni	OMA	ND	ND	ND	ND	Necropsy	Skin, Tissue
30-11-00-06	B. e. edeni	OMA	20.43272	57.99270	ND	ND	Necropsy	Tissue
31-10-02-01	B. e. edeni	OMA	19.43962	57.98106	Juvenile	ND	Necropsy	Skin
Bah001	B. e. edeni	OMA	20.37000	58.26733	Juvenile?	ND	Necropsy	Tissue
Bah003	B. e. edeni	OMA	20.35033	58.43700	Adult	ND	Necropsy	Tissue
Bah006	B. e. edeni	OMA	20.33667	58.41667	12m	ND	Necropsy	Muscle
Mas002	B. e. edeni	OMA	20.43467	58.71217	14.1m	ND	Necropsy	Skin, Tissue
Mas003	B. e. edeni	OMA	20.17367	58.65900	ND	ND	Necropsy	Tissue

Table S2. Table of corresponding accession (ACCN) numbers for haplotypes H01-H49 included in the study. From the 299 bp mitochondrial consensus sequence (bp position 15545-15843 in the mtDNA genome of *B. e. edeni* [ACCN: AB201258]), H01-H45 were identified as *B. e. brydei*, and H46-H48 were identified as *B. e. edeni*. The *B. e. edeni* haplotype H49 was identified from the 407 bp consensus sequence (bp position 15500-15906 in the mtDNA genome of B. e. edeni [ACCN: AB201258]). The total number of individuals (N) for each haplotype is shown and, when two accession numbers are listed, the number of individuals represented by each is indicated in parentheses. * indicates the five new haplotypes described by the current study. The remaining forty-four haplotypes have been previously described and published (Kanda *et al.* 2007; Yoshida & Kato 1999). For details of haplotype frequencies across sampling locations, see Fig. 2 of the main article.

B. e. brydei			
Haplotype	**N**	**ACCN 1**	**ACCN 2**
H01	5	JX090150*	
H02	2	EF068036	
H03	1	EF068044	
H04	2	EF068046	
H05	4	EF068061	
H06	1	JX090151*	
H07	1	KC261305*	
H08	9	EF068013	
H09	2	EF068019	
H10	4	EF068014	
H11	11	EF068030	
H12	1	EF068063	
H13	5	EF068015	
H14	3	EF068032	
H15	28	EF068016 (26)	AF146385 (2)
H16	1	EF068017	
H17	6	EF068020 (5)	AF146384 (1)
H18	3	EF068045	
H19	3	EF068040	
H20	1	EF068025	
H21	7	EF068031	
H22	3	EF068033	
H23	4	EF068038	
H24	5	EF068041	
H25	1	AF146386	
H26	6	EF068037	

H27	1	EF068039	
H28	34	EF068018 (33)	AF146382 (1)
H29	1	EF068021	
H30	126	EF068022 (120)	AF146381 (6)
H31	5	EF068023 (4)	AF146383 (1)
H32	1	EF068043	
H33	11	EF068024	
H34	7	EF068027	
H35	5	EF068028	
H36	1	EF068029	
H37	4	EF068034	
H38	2	EF068035	
H39	3	EF068048 (2)	AF146388 (1)
H40	1	EF068047	
H41	22	EF068060 (19)	AF146387 (3)
H42	1	EF068062	
H43	1	EF068026	
H44	2	JX090152*	
H45	1	EF068042	
B. e. edeni			
Haplotype	**N**	**ACCN 1**	**ACCN 2**
H46	46	AF146379	
H47	13	AF146380	
H48	2	AF146378	
H49 (407 bp consensus sequence)	5	KC561138*	

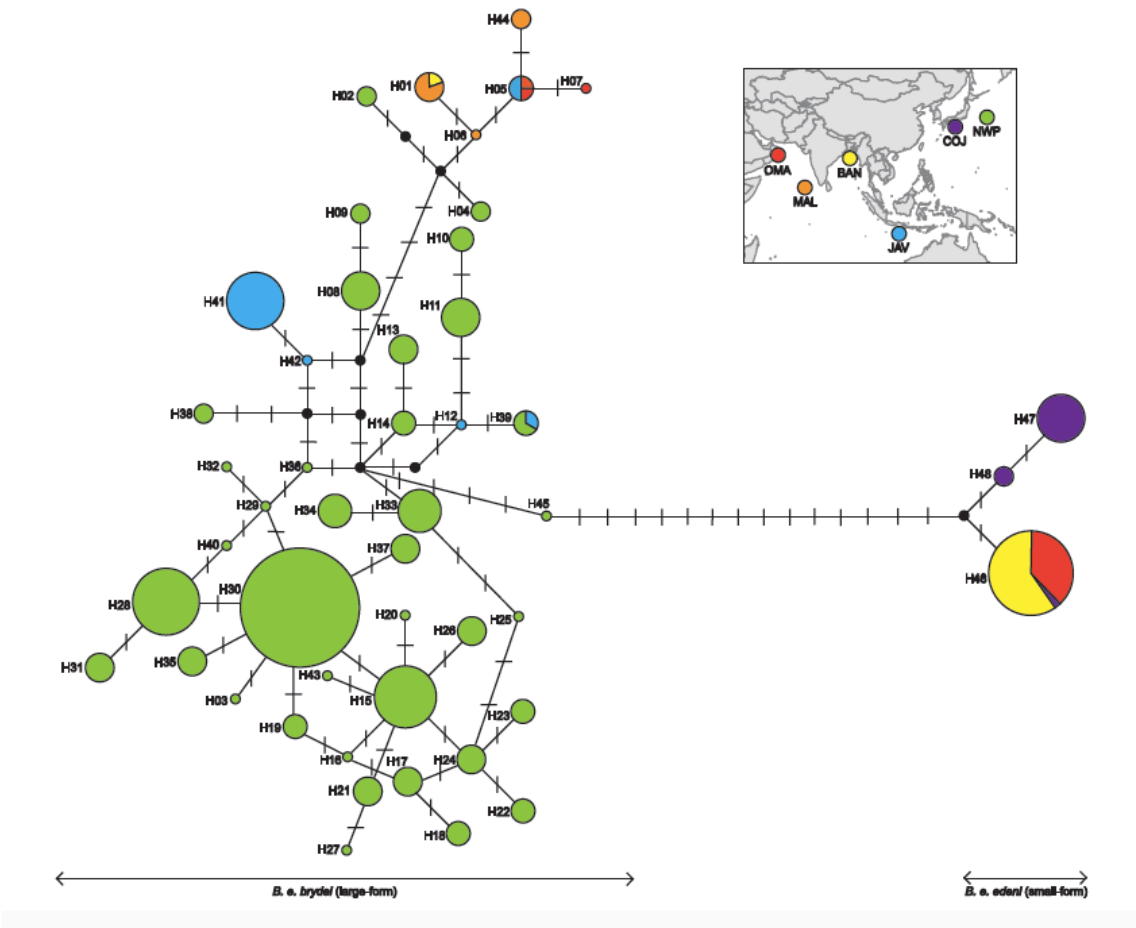


Fig. S1. Haplotype network of *B. e. brydei* (N=348) and *B. e. edeni* (N=61) mtDNA control region sequences created using a median-joining algorithm (Bandelt *et al*. 1999) with maximum parsimony post-processing implemented in NETWORK ver 4.6.0.0 (Fluxus Technology Ltd. 1999-2010) with ε=0 and all variable sites weighted equally. Haplotypes are labelled sequentially H01-H48: H01-H45 represent *B. e. brydei* clustered on the left side of the network; H46-H48 represent *B. e. edeni* on the right side of the network. Nodes are shaded according to sampling location (see inset: OMA, Oman; MAL, Maldives; BAN, Bangladesh; JAV, south of Java; CoJ, coast of Japan; NWP, Northwest Pacific). Size of the node corresponds to the frequency of that haplotype among sampled individuals. Internal nodes represent reconstructed median haplotypes. Notches represent nucleotide differences between haplotypes.

CHAPTER TWO

Multiple processes drive genetic structure of humpback whale (*Megaptera novaeangliae*) populations across spatial scales

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ABSTRACT

Elucidating patterns of population structure for species with complex life histories, as well as disentangling the processes driving such patterns, remains a significant challenge that requires an integrative analytical approach. Humpback whale (*Megaptera novaeangliae*) populations display complex genetic structures that have not been fully resolved at all spatial scales. We generated a data set of nine microsatellite loci representing the most robust sampling of "breeding stocks" across the South Atlantic and western Indian Oceans in order to assess genetic diversity, test for genetic differentiation between putative populations, and simulate the number of genetic clusters without *a priori* population information. We estimated rates of gene flow using maximum likelihood and Bayesian approaches. Our results reveal that patterns of humpback whale population structure vary at different spatial scales. At the ocean basin scale, structure is governed chiefly by geographic distance, female fidelity to breeding areas and male-biased gene flow. At scales within ocean basins, signals of genetic structure exist but are often less evident due to high levels of gene flow for both males and females. Our findings suggest these complex population patterns may not be fully or currently accounted for in management designations, which may have ramifications for assessments of the current status and continued protections for populations still undergoing recovery from commercial whaling.

INTRODUCTION

The field of molecular ecology has contributed significant insights into patterns of population structure for a broad range of terrestrial and marine species (e.g. Wang *et al*. 2009; Mendez *et al*. 2010; Kormann *et al*. 2012). However, understanding patterns of population structure for species with complex life histories, and the processes driving those patterns, remains a significant challenge. Genetic population structure (i.e. the spatial and temporal distribution of allele frequencies) may be influenced by a variety of interacting processes, including behavioral and ecological responses (Andrews *et al*. 2010; Piou & Prévost, 2012), environmental conditions (Kormann *et al*. 2012), and microevolutionary factors such as genetic drift and gene flow (Gaggiotti *et al*. 2009); all of which operate against a background of phylogeographic history (Muscarella *et al*. 2011). Disentangling the processes influencing population patterns therefore requires an integrative analytical approach (Gaggiotti *et al*. 2009).

The genetic architecture of migratory species is often complex due to the evolution of behaviors related to reliance on ephemeral patches of breeding and foraging habitat, such as group cohesion and hysteresis (or "memory") effects (Guttal & Couzin 2010). At regional scales, population-level fidelity to breeding and feeding areas may be a primary driver of genetic structure in these species (Guttal & Couzin 2010); however, at local scales there may be a more nuanced interplay of processes. For instance, genetic divergence between colonies of Cook's petrel (*Pterodroma cookii*) was linked to segregation of different populations during the non-breeding season due to habitat specialization (Rayner *et al*. 2011), and spinner dolphins (*Stenella longirostris*) exhibit two alternative social strategies associated with different levels of gene flow between social groups (Andrews *et al*. 2010). Synthesizing findings from multiple molecular markers is of great utility in shedding light on how patterns of population structure may be

influenced by processes operating across different spatial and temporal scales (Amaral *et al.* 2012a,b).

One of the best-studied migratory marine species is the humpback whale (*Megaptera novaeangliae*), which migrates annually from low-latitude breeding areas to high-latitude feeding areas (Gambell, 1976). Humpback whale genetic structure at the ocean basin scale is driven by a combination of maternal fidelity to feeding areas and natal philopatry to breeding areas (Baker *et al*. 1998, 2013). Patterns of migratory fidelity result from the close dependency of a first-year calf on its mother during the first complete annual migration, and thus vertical cultural transmission of migratory route and destinations (Baker *et al*. 1987; Alter *et al*. 2009; Valenzuela *et al*. 2009; Baker *et al*. 2013; Barendse *et al*. 2013). This mechanism of information transfer from mother to calf contrasts with natal philopatry in the majority of other migratory marine species, such as sea turtles and sharks, which is likely driven by environmental cues or genetic inheritance (Shamblin *et al*. 2012; Baker *et al*. 2013; Feldheim *et al*. 2014). However, as observed for other migratory baleen whale species in both hemispheres (Alter *et al*. 2012; Kershaw *et al*. 2013), genetic studies of humpback whales continue to reveal more complex structure at finer spatial scales than accounted for in current stock designations (e.g. Rosenbaum *et al.* 2009; Schmitt *et al.* 2013; Carvalho *et al.* 2014), indicating that other behavioral mechanisms may be driving humpback whale genetic structure at these scales.

In the South Atlantic and western Indian Ocean, four demographically discrete humpback whale "breeding stocks" (BS) are managed by the International Whaling Commission (IWC) in the southwest Atlantic, southeast Atlantic, southwest Indian Ocean, and northern Indian Ocean (BSA, BSB, BSC, and ASHW, respectively; Fig. 1). BSA shows relatively little diversity or genetic substructure (Cypriano-Souza *et al.* 2010), however, direct movements and song

similarity between BSA and BSB indicate some degree of broad-scale connectivity (Darling & Sousa-Lima 2005; Stevick *et al.* 2011). BSB is partitioned into two substocks; BSB1 breeds in the Gulf of Guinea and BSB2 represents a genetically distinctive group that feeds and migrates off the coast of west South Africa (Rosenbaum *et al.* 2009; Carvalho *et al.* 2014). Differences in levels of migrant exchange and records of individual movements between the four substocks of BSC (BSC1-C4) suggest genetic structure may be more complex than currently considered (Rosenbaum *et al.* 2009; Ersts *et al*. 2011; Fossett *et al*. 2014). The Arabian Sea humpback whale (ASHW) population is the only known non-migratory population globally and is known to be small (approximately 80-200 individuals) and extremely isolated (Minton *et al.* 2011; Pomilla & Amaral *et al*. 2014).

A complete understanding of patterns of humpback whale population structure using multiple molecular markers, and the potential processes underlying those patterns, has therefore not yet been achieved at multiple spatial scales. To help address these ecological and evolutionary questions, we present an analysis of an extensive microsatellite data set to further elucidate population genetic patterns across the south Atlantic and southwestern and northern Indian Ocean. To better understand the potential processes underlying population patterns at different scales, we partition our analyses to undertake a detailed investigation of the influence of sex on dispersal and site fidelity.

MATERIALS AND METHODS

Laboratory protocols

Sample collection, DNA Extraction and Sex Determination

A total of 3,575 humpback whale genetic samples originating from multi-year collections across ten sampling locations were used in this study (Table 1, Fig. 1). Skin tissues were mostly obtained using biopsy darts (Lambertson 1987), but also from sloughed skin and stranded specimens. Samples were preserved in 95% Ethanol or salt saturated 20% Dimethyl Sulfoxide solution (DMSO) and later stored at –20ºC until processed. Total genomic DNA was extracted from the tissue samples using proteinase K digestion, followed by a standard Phenol/Chloroform extraction method (Sambrook *et al.* 1989) or using QIAamp Tissue Kit (QiaGen) following manufacturer's protocol. Sex determination was either carried out by Polymerase Chain Reaction (PCR) amplifications followed by TaqI digestion of the ZFX/ZFY region of the sex chromosomes (Palsbøll *et al.* 1992), or using multiplex PCR amplification of the ZFX/ZFY sex linked gene (Berube & Palsbøll 1996).

Microsatellite molecular analyses

Samples were genotyped at 10 microsatellite loci proven to be polymorphic for this species: GATA028, GATA053, GATA417 (Palsbøll *et al.* 1997), 199/200, 417/418, 464/465 (Schlötterer *et al.* 1991), EV1Pm, EV37Mn, EV94Mn, EV96Mn (Valsecchi & Amos 1996). The 5'-end of the forward primer from each locus was labeled with a fluorescent tag (HEX, 6-FAM, and TET, Qiagen-Operon; NED, Applied Biosystems, Inc). PCRs were carried out in a 20µl volume with the following conditions: 50mM KCl, 10mM Tris-HCl pH8.8, 2.5-3.5mM MgCl2, 200µM of each dNTP, 0.4µM of each primer, and 0.025 U/µl Taq Gold polymerase (Perkin-Elmer). Amplifications were completed in an Eppendorf Gradient Mastercycler, after optimization of published annealing temperatures and profiles. PCR products were loaded with the addition of an internal standard ladder (GS600 LIZ, ABI) on a 3730xl DNA Analyzer (Applied Biosystems,

Inc). Microsatellite alleles were identified by their sizes in base pairs using the software GENEMAPPER v4.0 software (ABI). Specific guidelines were used during laboratory work and scoring procedures to reduce genotyping errors (Supporting Information).

Data analysis

Diversity estimates

Genetic diversity was measured as the mean number of alleles per locus (K), observed heterozygosity (H_o), and expected heterozygosity (H_E) under Hardy-Weinberg assumptions (Nei 1987) using the program Cervus v3.0 (Kalinowski *et al.* 2007). Departure of loci from Hardy-Weinberg (HW) assumptions was tested using Cervus and genotypic disequilibrium (GD) between pairs of loci was assessed using FSTAT v1.2 (Goudet 1995). Significance levels (p=0.05) for departure from HW and GD were corrected for multiple comparisons with Bonferroni correction (Rice 1998).

Analysis of population structure

To test for spatial structure, samples were grouped into seven putative populations, corresponding to the breeding stocks and substocks delineated by the International Whaling Commission (IWC) (Table 1, Fig. 1). To explore the presence of sex-biased dispersal, we partitioned the data set into male and female subsamples and conducted the analyses described below on all three data partitions.

Pairwise genetic differentiation was estimated by counting the number of different alleles between two genotypes, the equivalent of estimating weighted F_{ST} over all loci (Weir & Cockerham 1984), and by counting the sum of the square number of repeat differences between

two haplotypes, the equivalent of estimating R_{ST} (Slatkin 1995). Estimations were made from 1,000 permutations at the 0.05 significance level using Arlequin v3.5 (Excoffier & Lischer 2010). The statistic Jost's *D* (Jost 2008), was estimated using the DEMEtics package (Gerlach *et al.* 2010) in R. Jost's *D* has been shown to produce a more accurate measure of differentiation when using highly polymorphic microsatellite loci (Jost 2008). An analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) was conducted in Arlequin v 3.5 to assess hierarchical population structure. *F*- and *R*-statistics were computed at three levels that considered differences i) among breeding stocks, ii) among substocks within breeding stocks, and iii) within substocks. Estimations were made from 1,000 permutations at the 0.05 significance level.

To infer the number of genetic clusters in our data set without *a priori* designation of populations, we analyzed individual multilocus genotypes using the program STRUCTURE v2.3.3 (Pritchard *et al.* 2000), via the University of Oslo Bioportal (Kumar *et al.* 2009). We performed 5 independent iterations of K=2–10 for 5,000,000 Markov Chain Monte Carlo (MCMC) generations with a 500,000 burn-in period, assuming correlated allele frequencies (gamma distribution with mean 0.01 and standard deviation 0.05). Separate runs were performed with and without admixture and a sample location prior (LOCPRIOR). A two-cluster scenario was chosen as the minimum number because when population structure is expected to be low the scenario K=1 may be disproportionately favored, reducing the likelihood of all other scenarios to zero and resulting in a loss of overall resolution (Pomilla 2005). We selected the most probable value of K based on the average maximum estimated log-likelihood of P(X|K) and the ΔK method (Evanno *et al.* 2005), where optimum K has the highest rate of change in log probability in the data between successive K values (i.e. ΔK). All calculations were conducted using

STRUCTURE Harvester (Earl & VonHolt 2012). Clusters were aligned using CLUMPP v 1.1.2 (Jakobsson & Rosenberg 2007) and graphically displayed using the program DISTRUCT v 1.1 (Rosenberg 2004).

To validate the results of the genetic distance and Bayesian clustering analyses, we performed a discriminant analysis of principle components (DAPC) (Jombart *et al.* 2010) on individual allele frequencies using the adegenet package in R (Jombart 2008; Supporting Information). DAPC has been shown to recover complex patterns of population subdivision and has proved robust to deviations from HW equilibrium and GD because it does not rely on an underlying genetic model (Jombart *et al.* 2010). To assess the genetic distinctiveness of each breeding stock, the proportion of correct reassignment of each individual to its putative population was computed (Supporting Information).

Measures of migration rates and gene flow

We estimated relative effective population size (θ) and levels of historical gene flow ($M=m/\mu$), where m represents the immigration rate and μ the mutation rate, using the maximum likelihood algorithm implemented in MIGRATE v3.5.1 (Beerli & Felsenstein 2001). To address the issue of unequal sample sizes between locations, we chose to sub-sample our data set prior to analysis (Beerli 1998; Supporting Information). We used Brownian motion approximation to obtain initial parameter values and implemented a complete pairwise migration matrix model of gene flow between all breeding stocks. The final Markov chain scheme consisted of: 20 short chain searches (50,000 trees sampled, 500 trees recorded) followed by 3 long chain searches (5,000,000 trees sampled, 50,000 trees recorded) after a burn-in period of 10,000 genealogies.

The final long chain searches were averaged over ten independent runs and across subsamples. To aid visualization, results were transformed: $N_e m_T = ((1-(1/N_e m)^2)*100$.

We estimated the magnitude and direction of contemporary gene flow among populations using BayesAss v 3.0 (Wilson & Rannala 2003). To address inconsistencies in the results from initial runs, we again elected to sub-sample our data set (Supporting Information). Apart from the mixing parameters, all other options were left at their default settings (Supporting Information). The final Markov chain scheme comprised 50,000,000 iterations including a 2,500,000 burn-in period, and a sampling rate of 100. Results were averaged over the five independent runs, and across both random subsamples, if convergence was achieved.

RESULTS

Sample description

The 3,575 genetic samples analyzed were determined to represent 3,188 different whales (hereafter, "total sample"; Table 1). Average probability of identity (P_{ID}) for the total sample was small enough to exclude duplicate individuals with high confidence ($P_{ID} = 1.95x10^{-12}$; $P_{ID(sibs)} = 9.2x10^{-5}$; reciprocal of sample size = $2.5x10^{-4}$). Sex was determined for 3,046 individuals, 1,978 males and 1,067 females, resulting in an overall proportion of 1.8:1 males to females (Table 1). Proportionally greater numbers of males were sampled within most breeding stocks, likely due to a sampling bias resulting from breeding behavior differences between the sexes (Smith *et al*. 1999). Conversely, there were almost equal numbers of males and females sampled within BSB2, and a strong female bias in BSC2, with more than three times the number of females sampled than males (Table 1).

Genetic Diversity

All ten microsatellites were highly polymorphic, ranging from 4 alleles per locus (EV1Pm) to 28 (GATA417). No significant differences were found between the observed heterozygosity (H_o) and the expected heterozygosity (H_e) under Hardy-Weinberg assumptions. Two loci, GATA028 and GATA053, were insignificant GD ($p<0.01$). The least polymorphic locus, GATA053, was removed from subsequent analyses (Weir 1990). Values of observed and expected heterozygosity were relatively high across all breeding stocks (H_o=0.702-0.742; H_e=0.678-0.738) and the mean number of alleles per locus ranged from 6.44 (ASHW) to 12.89 (BSB1), although Oman was an outlier with 6.44 while the Southern Hemisphere stocks ranged from 10.11 (BSA) to 12.89 (BSB1; Table 1). Diversity estimates for BSB2 (n=204, k=11.33) and BSC1 (n=203, k=12.00) were disproportionately high relative to sample size.

Population structure

The AMOVA showed that genetic variance was best explained within the substock level for all sample partitions (for total, males, and females, F_{ST}=0.003, $p<0.001$; Table 2). Significant variation was also observed for the total sample among substocks within breeding stocks (F_{ST}=0.001, $p<0.01$). Significant variation among breeding stocks (i.e. the highest level of organization) was found only for females (F_{ST}=0.003, $p<0.003$).

Pairwise F_{ST} estimates ranged from 0 to 0.065, R_{ST} from 0 to 0.088, and Jost's D from 0 to 0.181 (Table S1). ASHW proved the most highly differentiated across all fixation indices and partitions and BSA showed high genetic differentiation from other Southern Hemisphere stocks for F_{ST}; however, results for BSA were more variable for Jost's D (Table 3). BSB1 also showed strong differentiation from all other breeding stocks for F_{ST} in the total sample, a result generally

supported by Jost's *D*; however, this relationship is less pronounced when segregated by sex (Table 3). The relationships between BSB2 and the substocks of BSC are less clear. BSB2 is significantly differentiated from BSC1 for F_{ST}, and Jost's *D* suggests this is driven by the female sample (Table 3). For males, BSB2 was significantly differentiated from BSC2 and BSC3 but only for F_{ST} (Table 3). Within BSC, BSC1 and BSC3 showed significant differentiation for the total sample, which appears to be driven by females. All other comparisons between BSB2 and BSC, and within BSC, were not significant for any indices (Table 3; Table S1).

Genetic structure based on individual allele frequencies without *a priori* designation of populations was only detected by STRUCTURE when a location prior was used with correlated allele frequencies and no admixture. The ln P(K) and ΔK values did not clearly discriminate whether the optimal number of clusters was K=3 or K=4 (Fig. S1). However, the individual assignment plots clearly show K=3 (Fig. 2) as the most likely for all data partitions (see Fig. S2 for K=4 plots). The clusters primarily correspond to the South Atlantic (BSA and BSB1), western Indian Ocean (BSC), and the northern Indian Ocean (ASHW; Fig. 2a-c). BSB2 appears more genetically similar to BSC than to BSB1 for all data partitions. BSA does not appear substantially different in composition from BSB1 for the total sample or for males; however, it does appear to be less admixed for females. The assignment plots also show evidence of population substructure within BSC when sampling location is considered (Fig. 2). The males sampled from southwest Madagascar, for example, appear to be highly differentiated from those sampled in northeast Madagascar (Fig. 2). Attempts to identify the number of genetic clusters using the DAPC failed.

The first two principle components of the DAPC analysis explained 94.18% of the variance in allele frequencies for the total sample (118 PCs retained), 68.61% for the male

sample (115 PCs retained), and only 29.98% for the female sample (43 PCs retained, Fig. 3). For the total sample, the first principle component shows separation between breeding stocks that reflects their longitudinal distribution, at least for BSA, BSB1, and ASHW; the remaining breeding stocks show a significant degree of overlap (Fig. 3a). The second principle component clearly shows the differentiation of ASHW. The longitudinal gradient is not clear for the male sample (Fig. 3b), however BSB2 also shows some separation on PC2 in addition to ASHW. The strongest differentiation of ASHW is observed on PC1 for females (Fig. 3c), whereas PC2 describes the longitudinal separation of BSA and BSB1.

The proportion of individuals correctly assigned to their original putative breeding stock by the DAPC was highest for BSB1 (65-67%), BSC3 (54-68%), driven primarily by the much larger sample from northeast Madagascar, and ASHW (65-74%; Fig. 3d-f). Despite its geographic distance from the other breeding stocks, BSA showed relatively low reassignment success (20-30%). Reassignment to BSB2 (2-9%), BSC1 (3-7%), and BSC2 (5-35%) performed particularly poorly. Individuals from the breeding stocks and substocks with the smaller sample sizes were primarily assigned to the much larger BSB1 and BSC3 samples regardless of sampling locality (Fig. S3).

Gene flow estimation

Historical gene flow (N_em) was estimated to occur to some degree between all pairwise breeding stock comparisons with little bias in directionality of movements (Fig. 4a-c). For the total sample, migration estimates ranged from N_em_T=1.553 (BSB1 to ASHW) to N_em_T=22.345 (BSC2 to BSC3, Fig. 4a). The highest estimates occurred from BSB2 to BSB1 and BSC, and also within BSC. Estimates for ASHW were the lowest of all comparisons; however, some estimates

between ASHW and BSC remained $N_e m_T$>10. BSA showed relatively high exchange for all comparisons apart from ASHW. For males, estimates ranged from $N_e m_T$=0.391 (BSC2 to ASHW) to $N_e m_T$=23.341 (BSC3 to BSC1, Fig. 4b) and approximated the same pattern as the total sample; however, a stronger westward bias was evident for some comparisons. For females, estimates ranged from $N_e m_T$=0.148 (BSA to ASHW) to $N_e m_T$=24.618 (BSC1 to BSC2, Fig. 4c) with high rates of multidirectional exchange ($N_e m_T$≈20) estimated between BSB and BSC (Fig. 4b-c). Notably, westward exchange for females from BSC3 to BSC2 was more than three times that of males (female $N_e m_T$=27.565; male $N_e m_T$=7.669). Overall, females showed less exchange between BSA and the other breeding stocks than males (Fig. 4c).

Levels of contemporary gene flow (proportion of migrants, *M*) estimated using BayesAss were less informative as many of the pairwise comparisons did not achieve convergence: ~31% for the total sample, ~33% for males, and ~40% for females (Fig. S4a-c). For the total sample, *M* ranged from $M=2.7^{10-3}$ (BSB2 to BSC2) to $M=29.7^{10-3}$ (BSB2 to BSB1, Fig. S4a). Comparisons of BSA with all other breeding stocks indicate an eastward bias in migration. Estimates for males were generally higher than females (Fig. S4b-c) with directional gene flow being more evident, notably from BSC2 to BSC3 ($M=135^{10-3}$). Estimates for females support a strong eastward bias from BSA to the other breeding stocks, particularly for BSA to BSB1, with an eastward estimate ~58 times that of the westward (Fig. S4c).

DISCUSSION

Hierarchical assessment of population structure influences

This first examination of the diversity and differentiation of nine microsatellite loci for more than 3,000 individual humpback whales from across the South Atlantic and western Indian Ocean suggests that a hierarchy of processes is likely to be driving patterns of genetic population

structure at different spatial scales. Such a hierarchy reflects the interplay between phylogeographic and ecological processes evident in other behaviorally complex mammals (Wolf *et al.* 2007; Vanderwaal *et al*. 2014). In general, our findings support the current model of maternal fidelity to feeding areas and natal philopatry to breeding areas as the primary driver of population genetic structure (Palsbøll *et al*. 1995; Baker *et al*. 1998, 2013). Previous examinations of the distribution of humpback whale mtDNA haplotypes at the sub-regional scale (i.e. BSA-C, ASHW) indicated strong differentiation between humpback whale breeding stocks for females and less so for males, supporting a model of maternally-directed philopatry to breeding areas, due to culturally transmitted hysteresis (or "memory") of specific locations, combined with male-biased gene flow (Palsbøll *et al*. 1995; Rosenbaum *et al*. 2009; Baker *et al*. 1998, 2013). These patterns are similar to those observed for the southern right whale (*Eubalaena australis*; Carroll *et al*. 2011), where combined genetic and isotope analyses indicate site-fidelity to feeding areas is culturally transmitted along matrilineal lines, providing a mechanism for maintaining genetic population structure (Valenzuela *et al*. 2009; Vighi *et al*. 2014).

Despite the overall congruence of our data, we find it difficult to entirely generalize this model either within or across all spatial scales. At regional scales across ocean basins, processes of isolation by distance (Wright 1943) and phylogeographic history appear to be the primary drivers of genetic structure. Breeding stocks showed some differentiation along the longitudinal axis consistent with a model of isolation by distance and previous studies suggesting the long-term isolation of ASHW (Minton *et al*. 2011; Pomilla & Amaral *et al*. 2014). Both BSA and BSB1 appear distinct from BSB2 and the substocks of BSC, and the ASHW population is clearly isolated (Fig. 3). Pairwise comparisons of genetic differentiation for nine nuclear introns also

revealed regional population structure between Brazil, Gabon, and Madagascar (Ruegg *et al.* 2013). However, our data indicate that even at broad spatial scales there may be additional factors influencing genetic structure: historic and contemporary gene flow estimates for females between BSA and BSB and BSC (Fig. 4c,f), in concert with real-time dispersal records (Stevick *et al.* 2011), suggest that long-distance movements by females should be afforded more consideration.

Transitioning to local scales (i.e. within breeding stocks) we observed additional divergence from this model as complex patterns of isolation and connectivity appeared as the norm, suggesting an array of interacting processes may be responsible for driving population patterns at these scales. The large data set of genotyped individuals included in our analysis enabled the detection of low levels of interchange for both sexes not previously quantifiable from examination of haplotype frequencies alone (due to the inadequate power to detect low levels of gene flow (i.e. <100 migrants per generation); Baker *et al*. 2013) and so provides additional resolution to the population sub-structure previously observed within Breeding Stocks B and C (Rosenbaum *et al*. 2009; Carvalho *et al*. 2014).

Effect of spatio-temporal variation in migratory behavior on population substructure

Genetic evidence supports the existence of two demographically discrete substocks (i.e. BSB1 and BSB2) off West Africa; however, an alternative hypothesis proposes that BSB1 and BSB2 represent two temporal 'ends' of a single population (BSB) widely distributed in space and time (Van Waerebeek *et al*. 2013; Carvalho *et al*. 2014; Rosenbaum *et al.* 2014). In our study, we detected significant genetic differentiation between BSB1 and BSB2 for the total sample for F_{ST} and Jost's D ($p<0.05$), however neither the male or female partitions were found to be significant

(Table 3). Previous analysis of mtDNA haplotype frequencies support the genetic differentiation of BSB1 and BSB2 for females, but not for males (Rosenbaum *et al*. 2009), and combined analysis of mtDNA and microsatellites provide evidence of spatial and/or temporal segregation between the two substocks, and indicate subtle temporal population sub-structuring based on sex (Carvalho *et al*. 2014). This may, at least in part, be related to different migratory groups undertaking coastal *versus* oceanic routes to and from the breeding areas off Gabon (Elwen *et al*. 2014; Rosenbaum *et al*. 2014). The fact that our pairwise comparisons did not detect significant differentiation for males and females between BSB1 and BSB2 may be a result of our sampling scheme not accounting for these fine-scale differences in the distribution of different, and potentially genetically distinct, groups in the region over the course of the breeding season.

The observed genetic differentiation of BSB2 may also be explained by its apparent connectivity with BSC1 off East South Africa, as demonstrated by our findings that BSB2 is more genetically similar to BSC than BSB1, and for males in particular (Fig. 2). Our results demonstrate that BSB2 exhibits high genetic diversity relative to its size (Table 1), a high level of admixture (Fig. 2), and low reassignment probabilities (Fig. 3a-c), all of which are consistent with a migratory population comprising whales from different breeding stocks, including BSC1. While the lack of differentiation observed between BSB2 and BSC1 may be due to retained shared ancestral polymorphism (Rosenbaum *et al.* 2009), increasing evidence for contemporary exchange between populations on the west and east coasts of Africa suggests recent inter-oceanic migration (Pomilla & Rosenbaum 2005; Rosenbaum *et al.* 2009; Carvalho *et al*. 2014), and genotypic matches have revealed direct connectivity between BSB2 and BSC1 and BSC3, and between feeding areas associated with BSB and BSC in the Antarctic (IWC 2009; Amaral & Loo *et al*. in review; Kershaw *et al*. unpublished data).

Migratory overlap and sex-specific differences drive genetic complexity

Substock-scale genetic patterns of structure and connectivity for Breeding Stock C appear to be highly complex and challenging to generalize in terms of maternal and paternal influence or directionality. Significant genetic differentiation was found between BSC1 and BSC3 for the total sample (F_{ST} = 0.001; p<0.05), and this difference appears to be driven by the female data set (Jost's D = 0.001; p<0.05; Table 3). In contrast, no significant genetic differentiation was detected between BSC1 and BSC2, or between BSC2 and BSC3 (Table 3). However, we did detect subtle differences between these patterns for males and females. No differentiation was detected for males between any of the BSC substocks, supporting the general model of male-biased gene-flow between populations that would result in the erosion of signals of genetic differentiation. However, estimates of historical gene flow showed no clear pattern in directionality and were found to be particularly high for females, calling the general model of maternally-driven natal philopatry to breeding areas into dispute (Fig. 4a-c; Palsbøll *et al*. 1995; Baker *et al*. 1998, 2013). Genotypic matching results also suggest high levels of exchange between BSC1-C3 for both females and males (Kershaw *et al*. unpublished data).

It is possible that the BSC substocks have diverged from one another relatively recently or may have remained consistently 'fluid' (Marko & Hart 2011). This latter suggestion is consistent with previous hypotheses regarding the presence of three migratory streams within the southwestern Indian Ocean, one along the east coast of southern Africa (BSC1), one along the Madagascar ridge (BSC3), and possibly a third through the central Mozambique Channel (BSC2; Best *et al*. 1998). It is possible, however, that this third stream comprises wide-ranging animals from coastal Africa and Madagascar (Best *et al*. 1998). Our findings of the lack of genetic

differentiation between BSC1 and BSC2, and BSC2 and BSC3, combined with high gene flow estimates, support the assertion that BSC2 may represent a mixed migratory stream of wide-ranging animals from BSC1 and BSC3. Recent photo-identification studies (Ersts *et al*. 2011), satellite telemetry data (Fossette *et al*. 2014), and genotypic matches (Kershaw *et al*. unpublished data) show that there is indeed considerable movement between BSC2 and BSC3, which are geographically close to one another relative to distances humpback whales are capable of travelling. In addition, long-distance movements between northeastern Madagascar (BSC3) and coasts of Kenya and Somalia in northern BSC1 appear more frequent than previously supposed, and may even represent a second, more northern migratory stream between BSC1 and BSC3. Photographic recaptures also suggest relatively substantial interchange between BSC3 and BSC4 (Dulau-Drouot *et al*. unpublished data), but levels of genetic connectivity have yet to be assessed. These findings call into question the delineation of substock boundaries in the southwestern Indian Ocean (Ersts *et al*. 2011). A similar scenario exists for humpback populations in the Hawaiian Archipelago and within the wintering region off the coast of Mexico (Cerchio *et al*. 1998, Calambokidis *et al*. 2011).

Importantly, we detected additional genetic structure within BSC3 indicating that further discrete demographic units may be present. The small sample of males (n=17) from the southwest of Madagascar (BSC3) shows greater levels of admixture than the large number of whales sampled in Antongil Bay to the north (Fig. 3). A number of factors apart from population structure could be driving these observed differences in allele frequencies between sampling locations, such as non-representative sampling from different years or disparities in sample size (Marko & Hart 2011). So, until larger, more representative samples are available, conclusions drawn from these results should be considered with caution. However, given our increasing

understanding of the behavioral complexity (e.g. alternative migration routes) of humpback whale populations in this region and others (Cerchio *et al*. 1998; Ersts *et al*. 2011; Carvalho *et al*. 2014; Rosenbaum *et al*. 2014), further investigation into the genetic structure of the BSC substocks appears to be warranted.

Implications for management at multiple scales

Our findings indicate an array of ecological drivers are likely responsible for the complex patterns of genetic structure observed within breeding substocks. Further investigation and syntheses of studies of population genetic structure, individual relatedness, environmental conditions, and behavioral ecology will be necessary to disentangle which processes are operating at each hierarchical spatial scale. The regional genetic structure detected by the microsatellite analyses presented here, and previous studies of nuclear introns (Ruegg *et al*. 2013) and the mitochondrial control region (Rosenbaum *et al*. 2009), is generally consistent with current designations of Breeding Stocks A, B, C, and ASHW, by the International Whaling Commission. Furthermore, in light of the extreme isolation and regional distinctiveness of ASHW, and increasing levels of anthropogenic development occurring in the Arabian Sea, our findings support recommendations that this population be attributed international conservation priority (Pomilla & Amaral *et al.* 2014).

Our results for BSB and BSC provide the most definitive evidence to date that the IWC substocks should be treated as hypotheses only, and a precautionary approach should be taken toward the management of whale populations in this region until the number of demographically discrete population units is resolved (Rosenbaum *et al.* submitted). Efforts to accurately elucidate discrete management units and understand their connectivity for this species are particularly

germane as the current listing status for the humpback whale as “endangered” under the Endangered Species Act (ESA) is under review (Bettridge *et al.* 2012). Given the range of contemporary anthropogenic impacts potentially affecting whale populations and important breeding habitat (Rosenbaum *et al.* 2014), the accurate identification of demographically discrete populations is paramount to the effective management of this species, which is still undergoing recovery from commercial whaling.

TABLES AND FIGURES

Table 1. Sample location, size, and diversity indices for nine microsatellite loci across breeding grounds, migratory corridors, and feeding grounds of humpback whales sampled in the South Atlantic and Western Indian Ocean. STP, Sao Tome & Principe; N, sample size; M, number of males; F, number of females; K, mean number of alleles per locus; H_o, observed heterozygosity; H_e, expected heterozygosity. The sum of the number of males and females does not always match the sample size. Duplicate samples have been removed.

Breeding Ground Breeding Stock	N	M	F	M: F	Years	K	H_o	H_e
(A) Southwestern Atlantic Ocean								
Abrolhos, Brazil	50	30	20	1.5:1	1997-98	10.11	0.702	0.715
(B) Southeastern Atlantic Ocean								
(B1) Gabon, STP, Cabinda	1395	826	421	2:1	1999-2006	12.89	0.732	0.735
(B2) West South Africa	204	95	103	1: 1.1	1990, 93, 95 2000-2009	11.33	0.740	0.737
(C) Southwestern Indian Ocean								
(C1) Mozambique & East South Africa	203	112	81	1.4:1	1991 1997-2005	12.00	0.742	0.738
(C2) Mayotte & Geyser, Comoros	75	17	55	1: 3.2	1997-2003	10.44	0.723	0.735
(C3) Madagascar	1227	842	373	2.3:1	1994 1996-2006	12.78	0.731	0.729
(X) Northern Indian Ocean								
Oman	34	20	14	1.4:1	2001-2002	6.44	0.706	0.678

Table 2. Analysis of hierarchical variance (AMOVA) results obtained using *F*- and *R*-statistics at three levels for the total sample (n=3,188), and males (n=1,978) and females (n=1,067) separately. Bold type indicates statistical significance at **p*<0.05, ***p*<0.01, ****p*<0.001.

Sample	**Source of variation**	**% var**	***F*-statistics**	**% var**	***R*-statistics**
Total	Among breeding stocks	0.24	F_{CT} = 0.0024	0.32	R_{CT} = 0.0032
	Among substocks within breeding stocks	0.08	F_{SC} = **0.0008****	- 0.01	R_{SC} = -0.0001
	Within substocks	99.68	F_{ST} = **0.0032*****	99.69	R_{ST} = **0.0031*****
Male	Among breeding stocks	0.27	F_{CT} = 0.0027	0.42	R_{CT} = 0.0043
	Among substocks within breeding stocks	0.01	F_{SC} = 0.0001	- 0.12	R_{SC} = -0.0012
	Within substocks	99.72	F_{ST} = **0.0028*****	99.70	R_{ST} = **0.0030***
Female	Among breeding stocks	0.31	F_{CT} = **0.0031***	0.22	R_{CT} = 0.0022
	Among substocks within breeding stocks	0.02	F_{SC} = 0.0002	0.05	R_{SC} = 0.0005
	Within substocks	99.67	F_{ST} = **0.0033*****	99.73	R_{ST} = 0.0027

Table 3. Significance values for pairwise fixation indices obtained between humpback whale breeding stocks and substocks for F_{ST} and Jost's *D*. F_{ST} values are shown above the diagonal, Jost's *D* below the diagonal. Results are shown for the total sample (n=3,188), and males (n=1,978) and females (n=1,067), separately. * indicates statistical significance at *p<0.05, **p<0.01, ***p<0.001. Estimations of significance were made from 1,000 permutations at the 0.05 significance level. Shaded F_{ST} values indicate a statistically significant result for mitochondrial DNA data, adapted from Rosenbaum et al. (2009). See Table S1 for fixation index values and R_{ST} results.

Total	**A**	**B1**	**B2**	**C1**	**C2**	**C3**	**X**
A	-	**	**	***	***	***	***
B1	ns	-	*	***	*	***	***
B2	**	*	-	*	ns	ns	***
C1	**	**	ns	-	ns	*	***
C2	ns	ns	ns	ns	-	ns	***
C3	**	**	ns	ns	ns	-	***
X	**	**	**	**	**	**	-

Male	**A**	**B1**	**B2**	**C1**	**C2**	**C3**	**X**
A	-	*	*	*	ns	*	***
B1	ns	-	ns	*	ns	*	***
B2	ns	ns	-	ns	*	***	***
C1	ns	*	ns	-	ns	ns	***
C2	ns	ns	ns	ns	-	ns	***
C3	*	***	ns	ns	ns	-	***
X	***	***	***	***	***	***	-

Female	**A**	**B1**	**B2**	**C1**	**C2**	**C3**	**X**
A	-	ns	*	*	*	*	***
B1	ns	-	ns	ns	ns	*	***
B2	*	ns	-	ns	ns	ns	***
C1	*	ns	*	-	ns	ns	***
C2	ns	ns	ns	ns	-	ns	***
C3	**	***	ns	*	ns	-	***
X	***	***	***	***	***	***	-

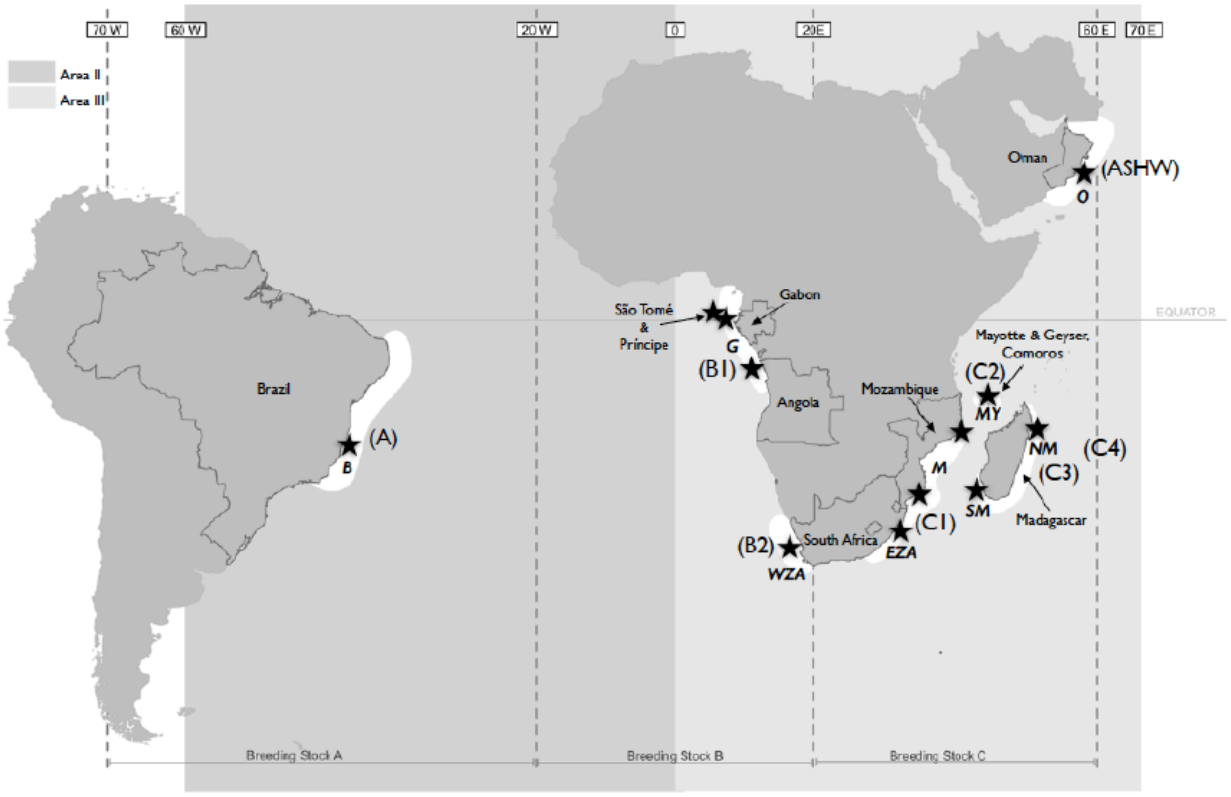


Fig. 1. Map showing sampling locations for the humpback whale breeding stocks and substocks analyzed in this study. The location of breeding stocks and substocks are indicated by white shading and labeled in parentheses. Sampling locations are indicated by stars and labeled as follows: *B*, Abrolhos Bank, Brazil; *G*, comprising samples from Iguela and Gamba, Gabon, Cabinda region, Angola, and São Tomé & Príncipe; *WZA*, Cape Columbine, West South Africa; *EZA*, Richard's Bay, East South Africa; *M*, comprising samples from Cabo Inhaca and Mozambique Island, Mozambique; *MY*, Mayotte and Geyser-Zelee, Comoros Archipelago; *SM*, Tulear/Ft Dauphin, Southwest Madagascar; *NM*, Antongil Bay, Northeast Madagascar; *O*, Gulf of Masirah and Dhofar, Oman.

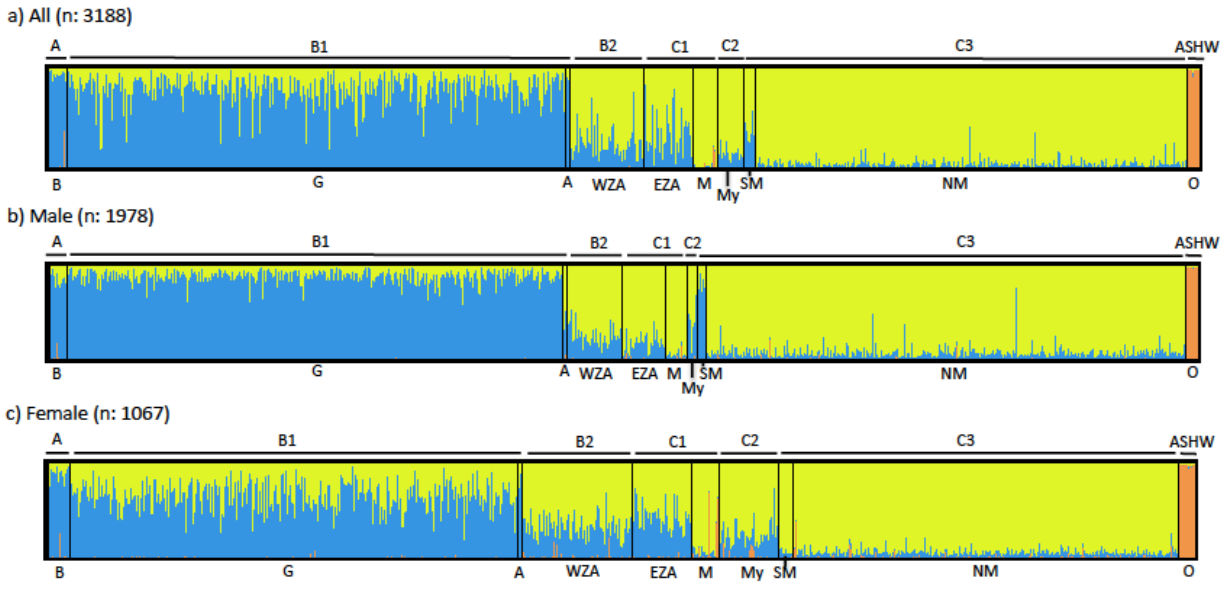


Fig. 2. Distribution of 3 genetic clusters estimated using STRUCTURE for a) the total sample, b) males, and c) females. Vertical lines are partitioned into colored segments showing the proportion of each individual assigned to each K cluster. Breeding stocks are indicated above each figure and sampling locations are below.

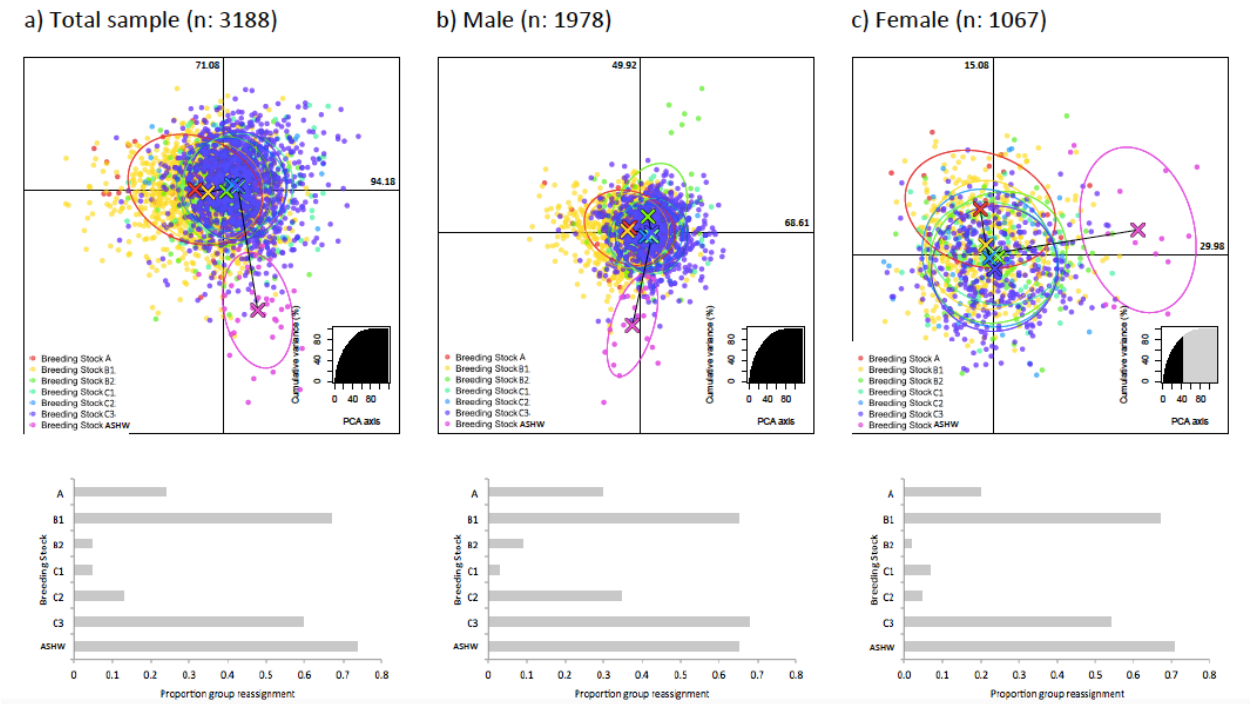
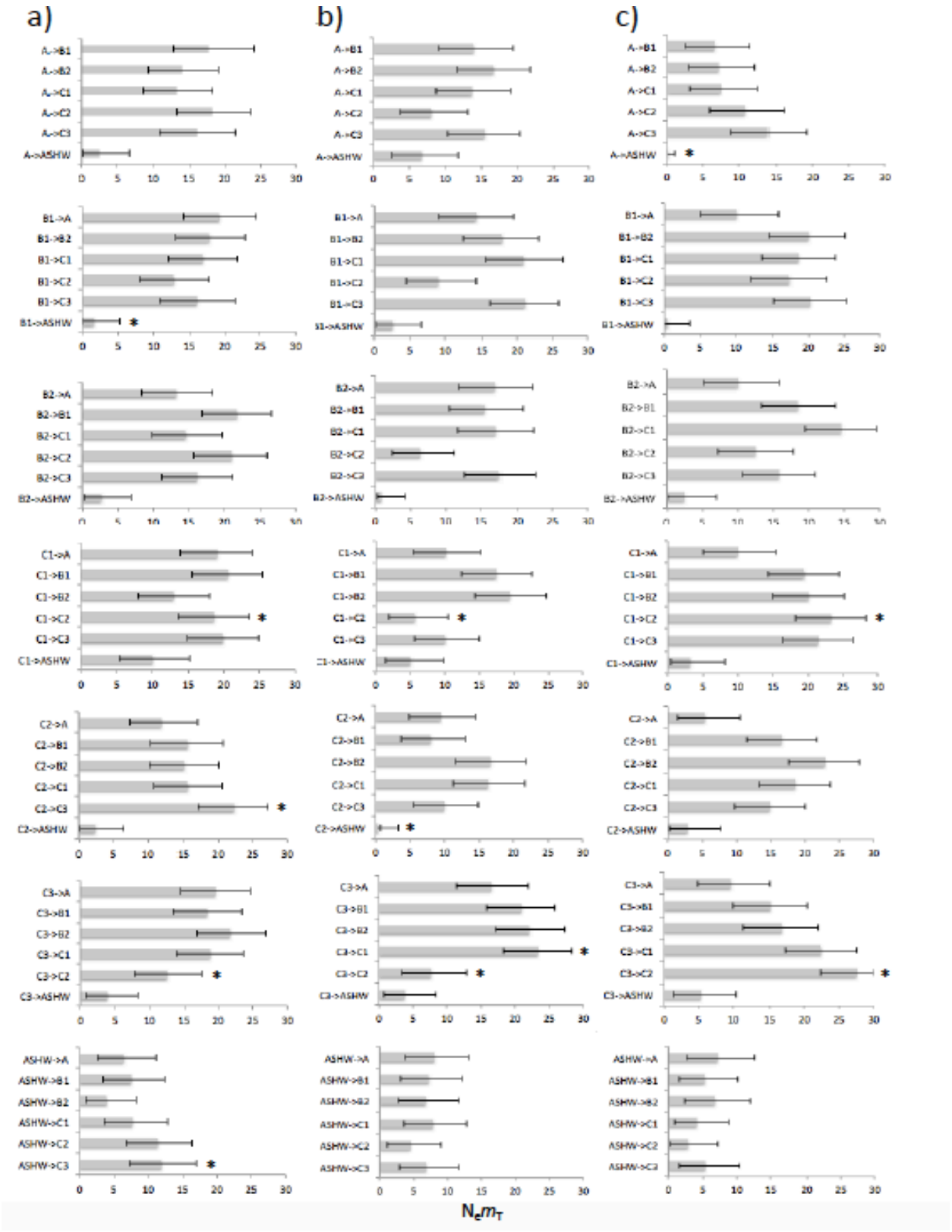


Fig. 3. Discriminant analysis of principle components (DAPC) scatterplots showing the genetic structure between humpback whale breeding stocks for a) the total sample, b) males, and c) females. Key describes the colors attributed to each breeding stock and substock. Eigenvalues for each PC axis are shown (PC1, vertical; PC2, horizontal). The number of PCA axes retained in each DAPC analyses is shown in the bottom-right inset (black bars). Bar charts show the proportion of reassignment of each individual to its original putative breeding stock (group).


a)
b)
c)
A->B1
A->B2
A->C1
A->C2
A->C3
A->ASHW
B1->A
B1->B2
B1->C1
B1->C2
B1->C3
B1->ASHW
B2->A
B2->B1
B2->C1
B2->C2
B2->C3
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C2->C3
C2->ASHW
C3->A
C3->B1
C3->B2
C3->C1
C3->C2
C3->ASHW
ASHW->A
ASHW->B1
ASHW->B2
ASHW->C1
ASHW->C2
ASHW->C3
0 5 10 15 20 25 30
$N_e m_T$

Fig. 4. Magnitude and directionality of historic gene flow between breeding stocks. The historic estimated number of migrants per generation ($N_e m_T$) exchanged between breeding stocks is shown for a) the total sample; b) males; and c) females, as estimated using MIGRATE. Asterisks highlight key results discussed in the main text.

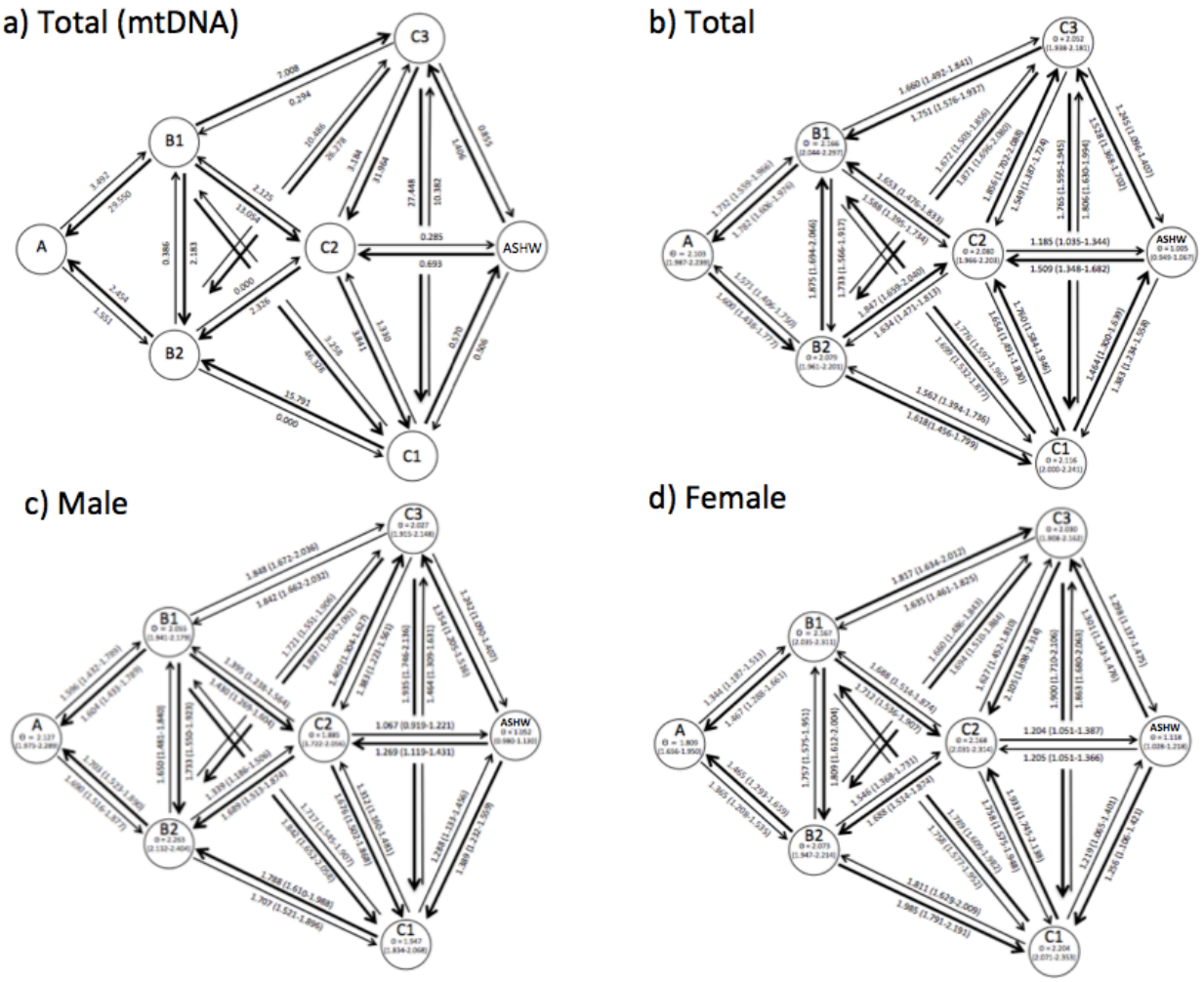

Fig. 5. Estimated number of migrants per generation (N_em) exchanged between neighboring breeding stocks and substocks and using MIGRATE for nuclear microsatellites (this study) and mitochondrial DNA, adapted from Rosenbaum *et al*. (2009). a) Average estimates of N_em for mtDNA for the total sample; b-d) estimates of theta and N_em (standard deviation shown in parentheses) for microsatellites for the total sample, and male and female data partitions. Bold arrows indicate directional bias in migration. Results were not transformed.

SUPPORTING INFORMATION

Procedure for checking genotype errors

First, automation was introduced whenever possible during PCR setup and manipulation of genomic DNA or PCR products. Negative controls were run at the PCR step to control for exogenous contamination. Two reference samples of known allele size were added to each amplification and subsequent analyses to standardize scoring. Scoring was automated in GENEMAPPER, and allele sizing was successively checked by hand. Samples that yielded ambiguous allele peaks were repeated a second time. Genotyping error was checked for the samples by re-amplifying and re-typing 15% of the total, chosen at random. In order to detect errors in our dataset, such as identifying possible non-amplified alleles (null alleles), large allele dropout and scoring errors due to stutter peaks we used the programs DROPOUT v1.3 (McKelvey and Schwartz 2005) and MICRO-CHECKER v.2.2.3 (Van Oosterhout et al. 2004). Overall, 10 cases of allele dropout were detected and solved by duplicate genotyping.

Overview of Discriminant Analysis of Principle Components (DAPC) methodology

The following overview has been adapted from Jombart et al. (2010). Principle Component Analysis (PCA) enables the identification of genetic structures in very large data sets within negligible computational time and the absence of assumptions about the underlying population genetics model. However, PCA does not provide group assessment and would require *a priori* definition of clusters to study population structure. In contrast, Discriminant Analysis (DA) is a multivariate method that defines a model in which genetic variation is partitioned into a between-group and a within-group component, and which maximizes the first while minimizing the second. This method therefore provides the best *discrimination* of individuals into pre-defined

groups. However, DA requires the number of variables (alleles) to be less than the number of observations (individuals) and assumes uncorrelated variables.

Discriminant Analysis of Principle Components (DAPC) is a new method developed by Jombart et al. (2010) that relies on data transformation using PCA as a prior step to DA, which ensures that the variable submitted to the DA are perfectly uncorrelated, and that their number is less than that of analyzed individuals. The method assigns individuals to clusters and provides a visual assessment of between-population genetic structure. When group priors are unknown, the method employs K-means clustering of principle components to identify groups of individuals. The best-supported number of clusters is assessed using the Bayesian Information Criteria (BIC).

Selection of number of PC axes retained in DAPC

The number of PC axes that explain the largest amount of total genetic variability in the data set while achieving the best discrimination between populations was determined using the optima.a.score function. All discriminant analyses (DA) axes were retained to capture the maximum amount of variability within the data set (Warmuth *et al.* 2012).

Sequential K-means clustering in DAPC

The number of genetic clusters in the data set was estimated without *a priori* population information using sequential K-means clustering (Legendre & Legendre 1998; see Supplementary Materials). The Bayesian Information Criterion (BIC) was used to determine the optimal number of clusters by selecting the value of K after which the BIC either increased or decreased by a minimal amount (Warmuth *et al.* 2012). Structure was also tested for each sequential value of K for K = 1 - 20 by examining individual assignment plots.

Sub-sampling protocol for MIGRATE

Given the large number of individuals in our sample, their unequal distribution among populations, and the fact that including more individuals does not necessarily improve estimates but only increases computation time due to the augmented complexity of the genealogies (Beerli 1998), we chose to sub-sample our data set prior to analysis. The data set was randomly sub-sampled without replacement so that a maximum of 50 samples were included for each population (Pomilla 2005). We checked the consistency of results between repeated runs for two different sub-sets of data.

Sub-sampling protocol for BAYESASS

Due to inconsistencies in the results from initial runs, which seemed to be due to the disproportionate sample size of BSB1 and BSC3, we randomly sub-sampled these two populations without replacement resulting in 150 individuals from each population being included in the final data set. The analysis was conducted on two different random sub-sets and the results were compared for consistency.

Selection of mixing parameters for BAYESASS analysis

Short MCMC chains were conducted (0.08% completion) to determine appropriate values for the mixing parameters for allele frequencies (Δ_A), inbreeding coefficients (Δ_F), and migration rates (Δ_M). Mixing parameters were chosen so that acceptance rates remained within the optimal range of 20-60% (Rannala 2007). Final mixing parameter values for each data partition were as follows: total sample, Δ_A=0.3, Δ_F=0.4, Δ_M=0.2; male and female samples, Δ_A=0.6, Δ_F=0.8, Δ_M=0.4.

Table S1. Pairwise fixation index values obtained between humpback whale breeding stocks and substocks for F_{ST}, R_{ST}, and Jost's D. Values are shown for the total sample, and males and females, separately. * indicates statistical significance at *p<0.05, **p<0.01, ***p<0.001.

	Total			**Male**			**Female**		
	F_{ST}	R_{ST}	Jost's D	F_{ST}	R_{ST}	Jost's D	F_{ST}	R_{ST}	Jost's D
A/B1	0.004**	0.001	0.011	0.004*	0.001	0.008	0.004	-0.002	0.018
A/B2	0.007**	0.003	0.026**	0.005*	-0.001	0.013	0.008*	0.006	0.040*
A/C1	0.008***	0.002	0.027**	0.006*	0.006	0.012	0.009*	0.000	0.030*
A/C2	0.010***	0.004	0.024	0.008	0.012	-0.001	0.009*	0.002	0.021
A/C3	0.007***	0.006	0.025**	0.005*	0.006	0.012*	0.007*	0.001	0.038**
A/X	0.055***	0.076***	0.161**	0.044***	0.088***	0.119***	0.065***	0.056*	0.181***
B1/B2	0.001*	0.000	0.005*	0.001	-0.002	0.000	0.000	-0.001	0.001
B1/C1	0.002***	0.000	0.010**	0.002*	-0.001	0.005*	0.001	0.008*	0.006
B1/C2	0.002*	0.004	0.005	0.001	0.006	-0.009	0.000	0.001	-0.004
B1/C3	0.001***	0.001*	0.006**	0.001*	0.001	0.005***	0.001*	0.001	0.006***
B1/X	0.046***	0.056***	0.155**	0.041***	0.062**	0.107***	0.049***	0.033*	0.175***
B2/C1	0.001*	0.000	0.007	0.000	-0.003	-0.004	0.002	0.007	0.009*
B2/C2	0.002	0.003	0.008	-0.002*	0.006	-0.019	0.000	-0.002	0.005
B2/C3	0.000	0.000	0.002	-0.001***	-0.001	-0.004	0.000	-0.001	0.004
B2/X	0.045***	0.056***	0.151**	0.041***	0.063**	0.100***	0.044***	0.030	0.158***
C1/C2	0.002	0.000	0.004	-0.001	0.009	-0.017	-0.001	-0.001	-0.006
C1/C3	0.001*	0.000	0.002	0.000	-0.001	-0.002	0.001	0.004	0.008*
C1/X	0.037***	0.048***	0.125**	0.034***	0.070***	0.080***	0.038***	0.012	0.131***
C2/C3	0.001	0.001	0.002	-0.001	0.003	-0.013	0.000	-0.002	-0.003
C2/X	0.047***	0.025*	0.135**	0.048***	0.013	0.102***	0.046***	0.004	0.145***
C3/X	0.044***	0.047***	0.144**	0.034***	0.054**	0.099***	0.000***	0.025	0.154***

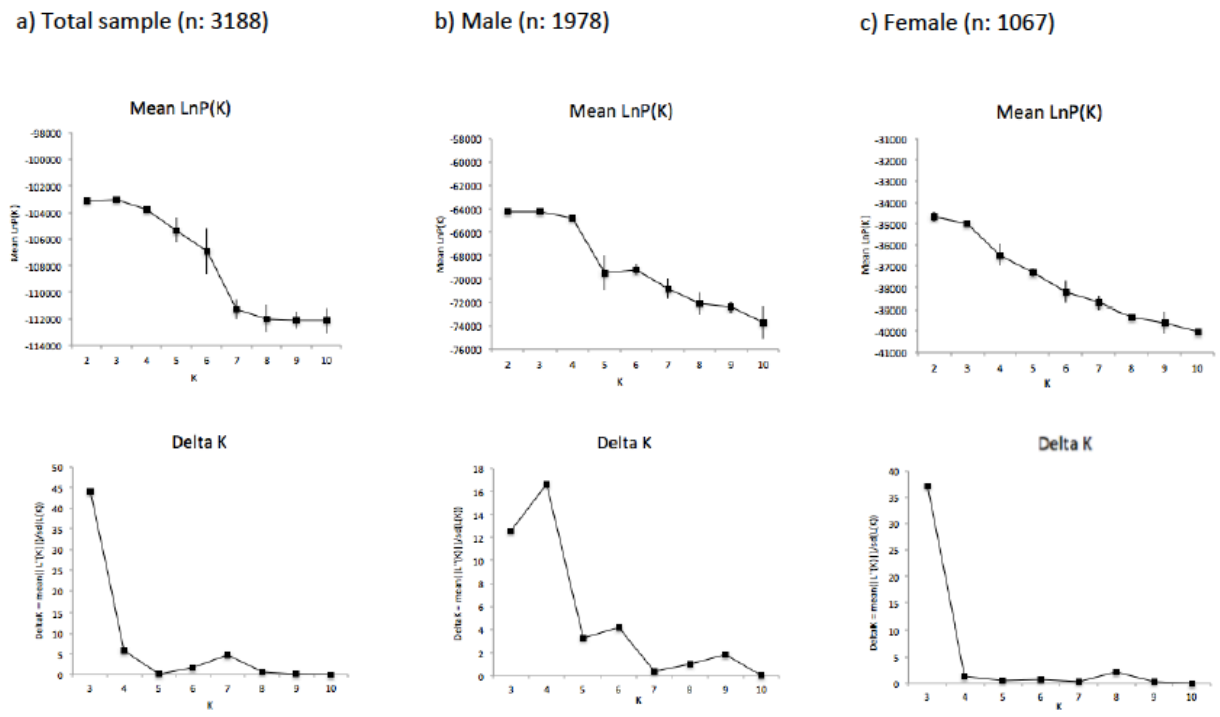

Fig. S1. Mean LnP(K) and Delta K (ΔK) plots for the STRUCTURE outputs for the a) total sample, b) males, and c) females. For mean LnP(K), variance is indicated by error bars.

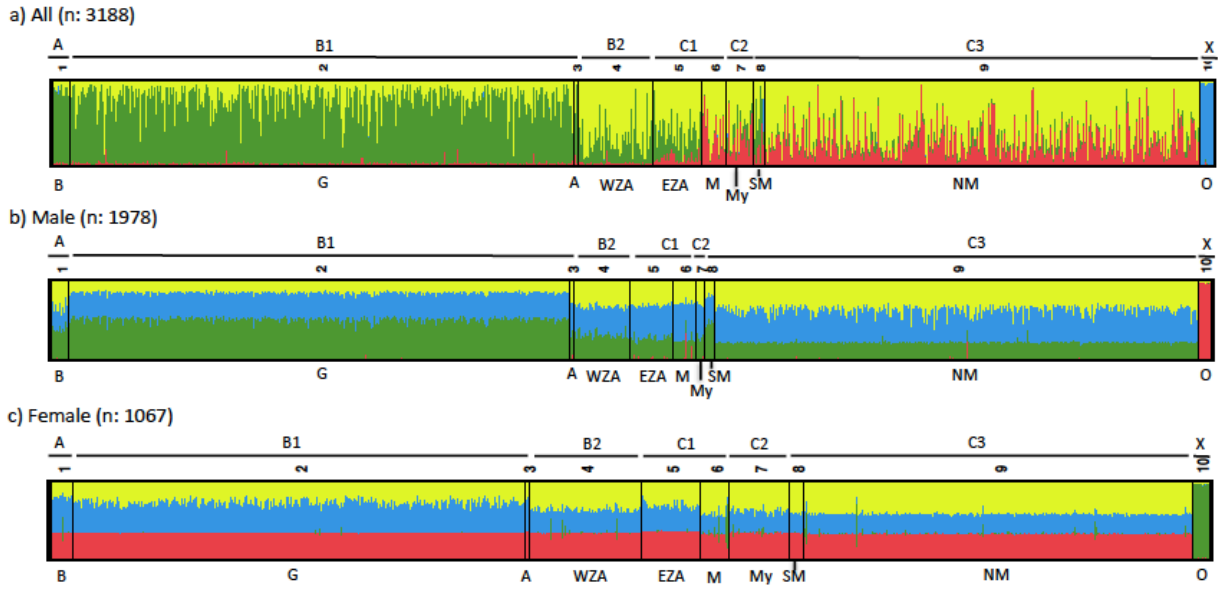

Fig. S2. Distribution of 4 genetic clusters estimated using STRUCTURE for a) the total sample, b) males, and c) females. Vertical lines are partitioned into colored segments showing the proportion of each individual assigned to each K. Breeding stocks are indicated above each figure and sampling locations are below (B, Brazil; G, Gabon; A, Angola; WZA, West South Africa; EZA, East South Africa; M, Mozambique; My, Mayotte & Comoros; SM, South Madagascar; NM, North Madagascar; O, Oman).

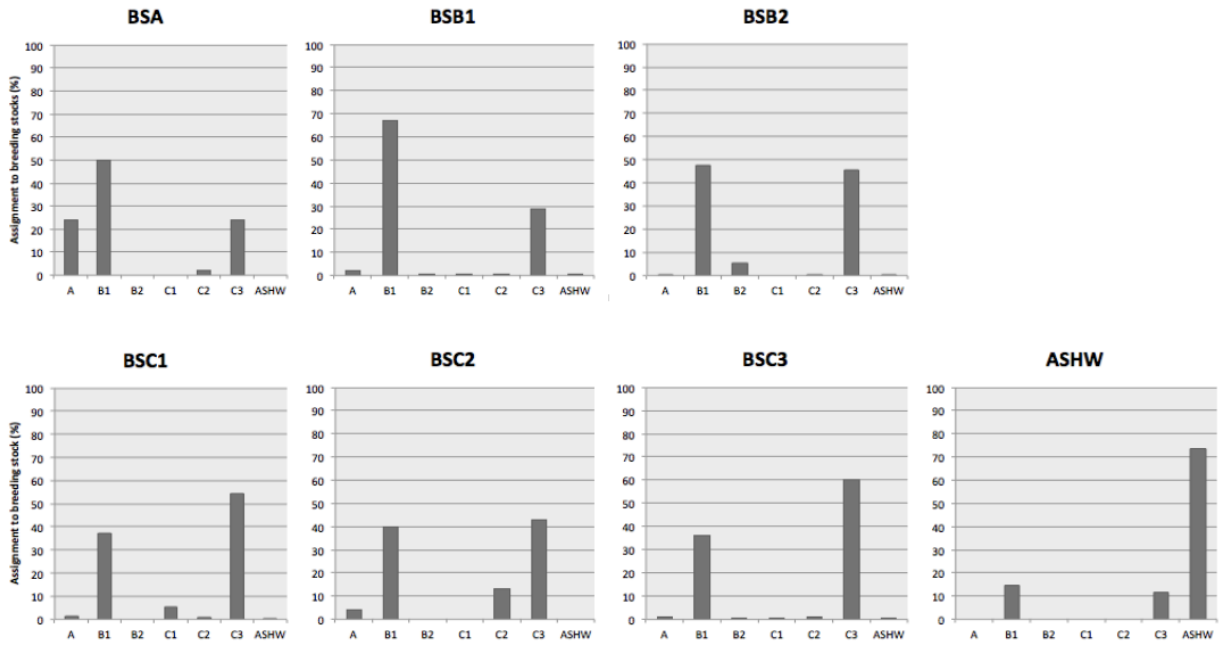

Fig. S3. Distribution of individual reassignment of each breeding stock and substock by the DAPC.

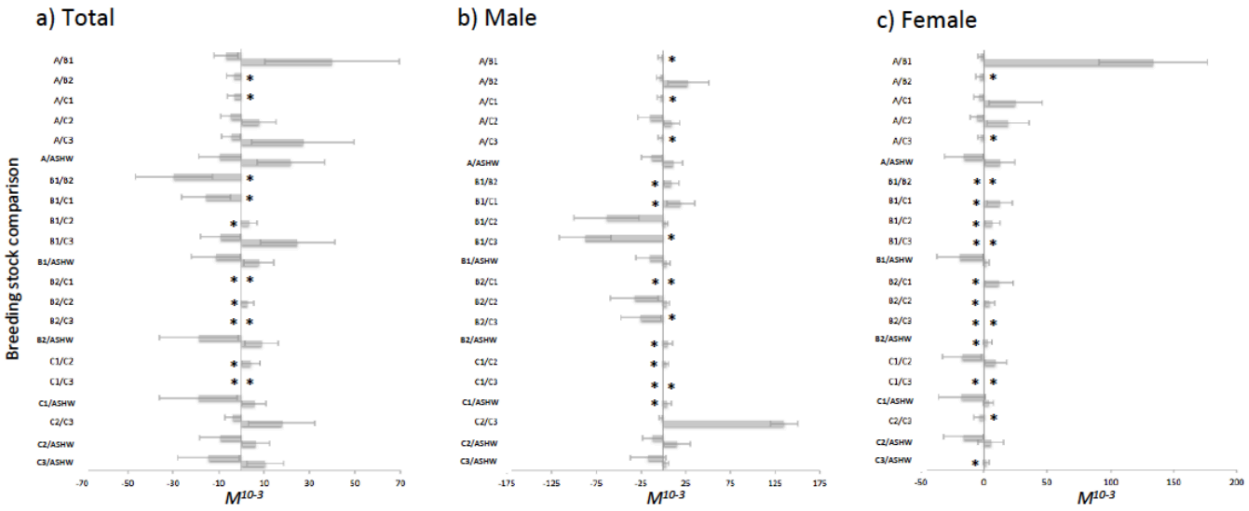

Fig. S4. Magnitude and directionality of contemporary gene flow as estimated using BayesAss. The estimated proportion of migrants from one population to another are shown for a) the total sample; b) males; and c) females. Note the varying magnitudes of *M* for each data partition on the horizontal axes. Left bars indicate a westerly migration direction between the two breeding stocks; right bars indicate an easterly direction. Results were transformed to aid visualization (see Materials and Methods).

CHAPTER THREE

Humpback whale (*Megaptera novaeangliae*) populations show extensive and complex mixing on feeding areas in the South Atlantic and Western Indian Oceans

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ABSTRACT

Elucidating the population structure of migratory species requires an understanding of how life history influences population genetic patterns across space and time. This information is essential for the appropriate delineation of management units for species of conservation concern. To determine the relative contribution of humpback whale breeding stocks in the South Atlantic and Western Indian Ocean to shared feeding areas off Antarctica, we conducted a mixed stock analysis (MSA) using ten nuclear microsatellite loci for 340 individuals sampled across seven feeding areas and a baseline data set of 2,772 individuals sampled on breeding grounds. We used a 371 bp consensus sequence of the mitochondrial (mtDNA) control region to assess the distribution of haplotypes across feeding areas and genetic diversity was assessed for both markers. The MSA demonstrated almost equal representation of the breeding stocks and substocks across all feeding areas with the exception of Breeding Substock B1, which consistently represented the greatest proportion of the allocation, particularly in the feeding area comprising the A/B Margin and B Nucleus. We identified 128 unique haplotypes that were also evenly distributed across feeding areas. Collectively, the relatively high levels of genetic diversity, the allocation of samples to breeding stocks by the MSA, and the distribution of haplotypes, support previous findings of complex patterns of fidelity to feeding areas and extensive mixing of different populations across the Southern Ocean. This study highlights that assessments of population structure for migratory species require an integrative approach encompassing genetic, behavioral, and environmental data from across their geographic range.

INTRODUCTION

Elucidating patterns of genetic structure of populations provides insights into the ecological and evolutionary processes influencing those patterns, and is essential for the appropriate delineation of management units for species of conservation concern (DeSalle & Amato 2004). This task is particularly challenging for migratory species as demographically discrete populations exhibit ontogenetic shifts between breeding and feeding areas over large geographic scales (Bolker *et al*. 2007). Migratory patterns can range from simple, where the entire population exhibits strong connectivity by moving between only two sites (one at either end of the migration route), to complex, where several distinct populations at one end of the migration are weakly connected to, and mix in unknown ways at, the other (Bolker *et al*. 2007). Many migratory cetaceans, such as baleen whales, adhere to this latter scenario of connectivity. For these species, genetically distinct populations (or "stocks") are geographically isolated during the breeding season and migrate to a temporary mixed assemblage during a separate feeding season (Hoezel 1998). Improved understanding of how the migratory cycle influences genetic population structure would be informative from both an evolutionary perspective and for species management (Valenzuela *et al*. 2009; Sremba *et al*. 2012; Constantine *et al*. 2014).

The ability to allocate individuals to their original breeding population is a necessary step when attempting to assess how population structure is influenced by migration, particularly if demographically discrete populations overlap in some geographic regions. The method of Mixed Stock Analysis (MSA) is a useful analytical tool capable of directly assessing the degree of mixing of individuals from genetically distinct populations in a given area (Manel *et al*. 2005; Pella & Masuda 2005). MSA uses population-level genetic allele frequency data and either a maximum likelihood or Bayesian statistical framework to estimate the fraction of the individuals

in a mixed stock that originate from each of a set of source (or "baseline") populations (Pella & Milner 1987; Bolker *et al*. 2007). This method also allows for the estimation of relative exploitation, mortality, and harvest rates (Wennevik *et al*. 2008), and has been applied to management questions pertaining to a range of migratory marine taxa, including Atlantic cod (Wennevik *et al*. 2008), sea turtles (Bowen *et al*. 2007; Clusa *et al*. 2014), and humpback whales (Schmitt *et al*. 2014).

Humpback whales (*Megaptera novaeangliae*) migrate annually from low-latitude breeding areas to high-latitude feeding areas (Gambell 1976) and populations are genetically structured due to a combination of natal philopatry to breeding areas and maternal fidelity to feeding areas (Baker *et al*. 1998, 2013). In the Southern Hemisphere, humpback whales form genetically distinct populations in geographically isolated coastal breeding areas that are managed as separate "breeding stocks" by the International Whaling Commission (IWC), an intergovernmental organization charged with the conservation of whales and the management of whaling (IWC 2007). These distinct breeding populations converge on shared sub-Antarctic and Antarctic feeding areas that were the site of heavy industrial and illegal whaling during the 20th Century (Cotte & Guinet 2011). Based on catch records corrected for heavy illegal Soviet whaling that continued until 1973, almost 216,000 humpback whales are estimated to have been killed by pelagic whaling operations on Antarctic feeding areas after 1900 (Rocha et al. 2015). Assessing the impact of whaling on humpback whale populations and monitoring their subsequent recovery is therefore of key management interest.

In the Southern Ocean, the IWC recognizes six circumpolar Management Areas (I-VI) using longitudinal boundaries that approximate feeding aggregations of the large baleen whale species, including the seven humpback whale breeding stocks (BS) A-G defined for the Southern

Hemisphere (IWC 2011). The Management Areas are used by the IWC to divide catch data of humpback whales for feeding grounds reported by the whaling industry in order to inform estimates of historical abundance and post-whaling recovery of the associated breeding stock. Division of a large number of catches using inaccurate longitudinal boundaries, therefore resulting in the assignment of a feeding aggregation to the incorrect breeding stock, can directly impact the evaluation of current population recovery estimates. Given this caveat, the number and boundaries of the feeding areas have been reviewed and modified several times as new information has emerged. An alternative hypothesis "Allocation Hypothesis 1" (hereafter, "AH1") was recently proposed that better accounts for the biology and behavior of the species (IWC 2010). AH1 comprises "Nuclear" areas, where 100% of catches are allocated to the associated breeding stock, and "Margin" areas, where 50% of catches are allocated to the adjacent stocks to the east and west (IWC 2010; Fig. 1).

The distribution and degree of mixing of the seven different breeding stocks and substocks on feeding areas remains unclear, however. Direct connectivity is supported for BSA and BSG to Management Areas II (60°W-0°) and I (120°W-60°W), respectively (Zerbini *et al*. 2006; Cypriano-Souza *et al*. 2010; Horton *et al*. 2011; IWC 2012, 2013; Fig. 1). In accordance, whales in Area I have been found to be genetically differentiated from all other areas (Amaral & Loo *et al*. in review). However, photo identification (Stevick *et al*. 2013) and acoustic (Darling & Sousa-Lima 2005) studies suggest a low level of mixing between BSA and other distant breeding stocks.

The remaining five breeding stocks show more complex patterns; likely compounded by the fact that population substructure within some of these stocks is still being resolved (Rosenbaum *et al*. submitted). The most recent genetic data suggest that there is some stock

structure in the eastern South Atlantic: whales that overwinter in the greater Gulf of Guinea (termed Breeding Substock B1, "BSB1") differ genetically from the smaller group (~510 individuals; Barendse *et al*. 2011) sampled while feeding and migrating off the west coast of South Africa (termed BSB2; Carvalho *et al*. 2014). While satellite telemetry data indicate some individuals from BSB1 migrate to feeding areas directly south (Rosenbaum *et al*. 2014), BSB1 and BSB2 have been found to show significant genetic differentiation from the Nucleus feeding areas of both BSB (10°W-10°E) and BSC (30°E-60°E) and, for BSB2, the B/C Margin (10°E-30°E; Fig. 1). These findings broadly indicate a high degree of mixing and low fidelity to feeding areas for BSB (IWC 2011).

BSC in the Western Indian Ocean is currently divided into four substocks (BSC1-C4), although considerable historic and contemporary interchange between the substocks is evident (Rosenbaum *et al*. 2009; Ersts *et al*. 2011; Fossette *et al*. 2014; Kershaw *et al*. in prep; Dulau-Drouot *et al*. unpublished data). BSC is broadly associated with Management Area III, which has been found to show significant differentiation from adjacent Areas I, II and IV (Amaral & Loo *et al*. in review). Assessments of population structure show that BSC1-C3 exhibit significant genetic differentiation from the BSB Nucleus feeding area but not the BSB/BSC Margin or BSC Nucleus (IWC 2011). Satellite tracked animals off BSC2 have also been observed to move southeastward towards the French sub-Antarctic Islands and Area III (0°-70°E) (IWC 2013). Collectively, these findings indicate general support for the feeding area designation for BSC under AH1 (IWC 2010).

For BSD located off western Australia, BSE1 off eastern Australia, and BSE2-E3 and BSF1-2 located in the western Pacific islands of Oceania, haplotype distribution analyses and MSA employing mtDNA haplotypes and nuclear microsatellite markers suggest complex

patterns of fidelity and mixing similar to that of BSB and BSC (Robbins *et al*. 2011; Schmitt *et al*. 2014).

Even in light of existing research, few data exist to fully assess the significance of mixing of Southern Hemisphere breeding stocks and substocks on feeding areas and further information, particularly from feeding grounds associated with BSB and BSC, is needed (Amaral & Loo *et al*. in review). Here, we present a mixed stock analysis based on nuclear microsatellite data and an analysis of mitochondrial haplotype distributions to determine the relative contribution of humpback whale Breeding Stocks A, B, and C, and the substocks therein, to the composition of feeding areas designated under AH1, and to assess levels of mixing of different breeding stocks and substocks on these feeding areas. This work has direct relevance for informing estimates of pre-exploitation population sizes and assessments of recovery from whaling.

MATERIALS AND METHODS

Sample information

The feeding area (i.e. "mixture") data set comprises 340 individuals sampled in the Antarctic (south of 60ºS) and sub-Antarctic (between 45ºS and 60ºS; Amaral & Loo *et al*. in review). The samples were collected during two major research efforts: the IWC's International Decade of Cetacean Research (IDCR) and Southern Ocean Whale Ecosystem Research (SOWER) cruises, and opportunistic collections by Southern Ocean - Global Oceans Ecosystems dynamics (GLOBEC) cruises during surveys along the Antarctic Peninsula in 2002. For further details of sample collection see Amaral & Loo *et al*. (in review). GPS data was used to allocate samples to one of 14 feeding areas (with the exception of G/A, where n=0), defined by AH1 to be geographically associated with the seven genetically distinct breeding stocks (BSA-G) in the

Southern Hemisphere (Fig. 1; Table 1). This allocation of samples does not confirm connectivity between breeding and feeding grounds nor does it suggest distinct populations.

The breeding area (i.e. "baseline") data set comprises a total of 2,772 individuals originating from multi-year collections from the South Atlantic and Western Indian Ocean: BSA located off Abrolhos Bank in Brazil (n=38), BSB located off West Africa, comprising BSB1 (n=1226) and BSB2 (n=176), and BSC located in the southwest Indian Ocean, comprising BSC1 (n=182), located off east South Africa and Mozambique, BSC2 (n=61) in the vicinity of Mayotte & the Comoros, and BSC3 (n=1089), located off Madagascar (Rosenbaum *et al*. 2009; Kershaw *et al*. in prep). No genetic data are available for BSC4. Only those individuals with both mitochondrial and microsatellite loci were included in this study (Table S1).

Laboratory protocols

DNA extraction and sex determination

Total genomic DNA was extracted from the tissue samples using proteinase K digestion, followed by a standard Phenol/Chloroform extraction method (Sambrook *et al*. 1989), or using QIAamp Tissue Kit (QiaGen) following the manufacturer's protocol. Sex determination was either carried out by polymerase chain reaction (PCR) amplifications followed by *Taq*I digestion of the ZFX/ZFY region of the sex chromosomes (Palsbøll *et al*. 1992), or using multiplex PCR amplification of the ZFX/ZFY sex linked gene (Bérubé & Palsbøll 1996).

Mitochondrial sequencing

A 550 bp fragment of the mitochondrial DNA (mtDNA) control region was amplified by polymerase chain reaction (PCR) using the primers light strand Dlp 1.5 and heavy strand Dlp 5

(Baker *et al*. 1993). Reactions of 25 mL total volume containing 50 mM KCl, 10 mM Tris-HCl pH 8.8, 2.5 mM $MgCl_2$, 200 mM of each dNTP, 1.0 mM of each primer, and 0.05 U ml^{-1} Taq polymerase (Perkin-Elmer) were conducted under the following conditions: initial denaturing at 94°C for 2 min, followed by 35 cycles of 94°C denaturing for 45 s, 54°C annealing for 45 s, and 72°C extension for 45 s. Amplified PCR products were cycle sequenced with dye-labeled terminators using conditions recommended by the manufacturer (Applied Biosystems, Inc). Sequence reactions were analyzed using an ABI 3700 or 3730 DNA Analyzer (Applied Biosystems, Inc).

Microsatellite molecular analysis

A set of ten 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 & Amos 1996); and GATA028, GATA053, GATA417 (Palsbøll *et al*. 1997). The 5'-end of the forward primer from each locus was labeled with a fluorescent tag (HEX and 6-FAM, QIAGEN-Operon; NED, Applied Biosystems, Inc). PCRs were carried out in 10mL or 20mL total volume containing: 50 mM KCl, 10 mM Tris-HCl pH 8.8, 2.5 - 3.5 mM $MgCl_2$, 200 mM of each dNTP, 0.4 mM of each primer, and 0.025 U mL^{-1} Taq Gold polymerase (Perkin-Elmer). Amplifications were completed for most samples, after optimization of published annealing temperatures and profiles. PCR products were loaded with the addition of an internal standard ladder (GenScan-500 ROX or GenScan-600 LIZ, Applied Biosystems, Inc) on an ABI 3700 or ABI 3730 DNA analyzer (Applied Biosystems, Inc). Microsatellite alleles were identified by their sizes in base pairs using GENOTYPER software v. 2.1 and GENEMAPPER software v. 4.1 (Applied Biosystems, Inc).

Error checking and duplicate samples

Specific guidelines were used during laboratory work and scoring procedures to reduce genotyping errors. First, automation was introduced whenever possible during PCR setup and manipulation of genomic DNA or PCR products. Negative controls were run at the PCR step to control for exogenous contamination. Two reference samples of known allele size were added to each amplification and subsequent analyses to standardize scoring. Scoring was automated in GENEMAPPER and allele sizing was successively checked by hand. Samples that yielded ambiguous allele peaks were repeated a second time. Genotyping error was checked for the samples by re-amplifying and re-typing 15% of the total, chosen at random. In order to detect errors in our dataset, such as identifying possible non-amplified alleles (null alleles), large allele dropout and scoring errors due to stutter peaks we used the programs DROPOUT v. 1.3 (McKelvey & Schwartz 2005) and MICRO-CHECKER v. 2.2.3 (Van Oosterhout *et al*. 2004).

Duplicate samples were identified from microsatellite genotype identity using Cervus v. 3.0 (Kalinowski *et al*. 2007). The probability of different individuals and siblings sharing the same genotype by chance (Probability of Identity, P_{ID}, and P_{ID} for siblings, $P_{ID(sibs)}$, respectively) were estimated using Cervus (Kalinowski *et al*. 2007). The reciprocal of the sample size was used as the arbitrary cut-off below which the probability values are sufficiently small to conclude that matching genotypes belong to the same individual (Peakall *et al.* 2006). Duplicate samples were removed from subsequent analysis.

Data analysis

Sensitivity analysis to define feeding areas

As samples were unevenly distributed across feeding areas and often low or, in one case, absent within areas, it was necessary to group the samples prior to conducting the mixed stock analysis. A sensitivity analysis was conducted to define the most biologically meaningful longitudinal boundaries for the groupings. Samples were first grouped into the 14 putative core and boundary areas defined by AH1 (Fig. 1), and then were re-grouped into four alternative boundary "sets" of either six or seven areas (Tables S2, S3).

Mitochondrial DNA sequences were aligned using ClustalW (Higgins *et al*. 1994) under default settings in MEGA5 (Tamura *et al*. 2011) and were trimmed to the 472 bp consensus region (bp positions 15484-15955 in the mtDNA genome of *M. novaeangliae* [ACCN: AP006467.1]). Pairwise genetic differentiation between feeding area groupings was calculated using overall differences in haplotype frequencies (F_{ST}) with 10,000 permutations at the 0.05 significance level (Weir & Cockerham 1984) in Arlequin ver 3.5 (Excoffier & Lischer 2010). Levels of genetic divergence between samples were calculated with the fixation index (Φ_{ST}), which takes into account the relationships between haplotypes based on molecular distance (Excoffier *et al*. 1992), using the distance matrix inferred from the data in Arlequin v. 3.5 (Excoffier & Lischer 2010). Significance of Φ_{ST} for all possible pairwise population comparisons was assessed using 10 000 permutations at the 0.05 significance level.

For the microsatellite data set, pairwise genetic differentiation was estimated by counting the number of different alleles between two genotypes, the equivalent of estimating weighted F_{ST} over all loci (Weir & Cockerham 1984). Estimations were made from 10,000 permutations at the 0.05 significance level using Arlequin v. 3.5 (Excoffier & Lischer 2010). The statistic Jost's *D* (Jost 2008) was estimated using the DEMEtics package (Gerlach *et al*. 2010) in R. Jost's *D* has

been shown to produce a more accurate measure of differentiation when using highly polymorphic loci (Jost 2008).

The final boundary set was selected based on congruence in levels of genetic differentiation between the two molecular markers and to account for variation in sample size.

Genetic diversity estimates for feeding areas

The 472 bp mitochondrial control region consensus sequences were collapsed to haplotypes and samples were grouped based on the feeding areas defined by the sensitivity analysis using DnaSP v. 5 (Librado & Rosaz 2009). Genetic diversity indices (number of haplotypes, haplotype diversity, nucleotide diversity with Jukes-Cantor correction, and average number of pairwise nucleotide differences among sequences) were calculated in DnaSP for the total sample and for each feeding area. Genetic diversity for microsatellite data was measured as the mean number of alleles per locus (K), observed heterozygosity (H_O), and expected heterozygosity (H_E) under Hardy-Weinberg assumptions (Nei 1987) using the program Arlequin v. 3.5 (Excoffier & Lischer 2010). Departure of loci from Hardy-Weinberg (HW) assumptions was tested using Cervus and genotypic disequilibrium (GD) between pairs of loci was assessed using FSTAT v. 1.2 (Goudet 1995).

Stock composition on feeding areas

The mixed stock analysis (MSA) was implemented using the Statistical Program for Analyzing Mixtures (SPAM v. 3.7b; Alaska Department of Fish and Game 2003), which is based on maximum likelihood. As the goal of the analysis is to inform management decisions regarding the contemporary use of feeding areas by breeding stocks and substocks, only the microsatellite

data set was used for the MSA due to their rapid mutation rate and bi-parental mode of inheritance. The analysis was carried out separately for each of the seven feeding areas defined by the sensitivity analysis, estimating the proportion allocation of the samples to each of the six breeding stocks and substocks. To account for the number of zeros in any particular region's allelic distribution (i.e. not all of the alleles in the feeding area data sets were represented in the baseline), the Bayesian Pella-Masuda model was implemented in the estimation mode. This method assumes that the absence of an allele from the baseline data set is rare in the baseline rather than nonexistent (Koljonen *et al*. 2005). The analysis was carried out with 100 bootstrap replicates, two random seeds, and a genotypic tolerance of 1.0×10^{-55}.

In addition to the MSA, the distribution of mtDNA control region haplotypes across feeding areas and breeding stocks and substocks was examined. Mitochondrial DNA sequences were aligned using ClustalW (Higgins *et al*. 1994) under default settings in MEGA5 (Tamura *et al*. 2011) and were trimmed to the 371 bp consensus region (bp positions 15559-15930 in the mtDNA genome of *M. novaeangliae* [ACCN: AP006467.1]). Sequences were collapsed to haplotypes and samples were grouped based on breeding and feeding area, using DnaSP v. 5 (Librado & Rosaz 2009). The presence or absence of breeding area haplotypes on each of the seven feeding areas was then quantified.

To infer the number of genetic clusters in the feeding area data set without *a priori* designation of populations, we analyzed individual multilocus genotypes using the program STRUCTURE v. 2.3.3 (Pritchard *et al*. 2000). We performed 5 independent iterations of K=1-7 (to account for BSA-BSG) for 5,000,000 Markov Chain Monte Carlo (MCMC) generations with a 500,000 burn-in period, assuming correlated allele frequencies (gamma distribution with mean 0.01 and standard deviation 0.05). Following a series of initial test runs using alternative

parameter sets, we selected the final parameter set based on the fact that it was the only combination of parameters that achieved convergence for the summary statistics α, *F*, the divergence distances among populations $D_{i,j}$, and the maximum likelihood scores. The final parameter set was therefore performed with no admixture and without a sample location prior (LOCPRIOR). We selected the most probable value of K based on the average maximum estimated log-likelihood of P(X|K) and the ΔK method (Evanno *et al*. 2005), where optimum K has the highest rate of change in log probability in the data between successive K values (i.e. ΔK), and the probability distribution of individual assignments. All calculations were conducted using STRUCTURE Harvester (Earl & VonHolt 2012). Clusters were aligned using CLUMPP v. 1.1.2 (Jakobsson & Rosenberg 2007) and graphically displayed using DISTRUCT v. 1.1 (Rosenberg 2004).

RESULTS

Sensitivity analysis to define feeding areas

Examination of the genetic differentiation indices for the four alternative boundary sets (see Supporting Information) used in the sensitivity analysis led to the designation of seven feeding areas: the BSA core area (A), the margin area for BSA and BSB, and the BSB core area (A/B-B), the BSB and BSC margin area (B/C), the core area for BSC (C), the margin area for BSC and BSD, and the BSD core area (C/D-D), the core and margin areas for BSE and BSF (D/E-E-F-F/G), and the core and margin area for BSG and BSA (G-G/A).

Genetic diversity estimates for feeding areas

The genetic analysis of the mtDNA control region across the seven feeding areas defined by the sensitivity analysis resulted in the identification of 110 unique haplotypes derived from the total sample of 340 sequences with 71 polymorphic sites (*Hd*=0.976; π(JC)=0.020). Levels of genetic diversity were generally high across all feeding areas, the highest diversity being observed for area A/B-B (n=112; *H*=52; *Hd*=0.969; π(JC)=0.020) and the lowest for area A (n=21; *H*=14; *Hd*=0.919; π(JC)=0.20). Levels of diversity generally corresponded to sample size; however, feeding area G-G/A demonstrated relatively low diversity considering its sample size was one of the highest (n=50; *H*=24; *Hd*=0.927; π(JC)=0.018).

All ten microsatellites were highly polymorphic, with a mean number of alleles ranging from 8.3 in feeding area A to 11.4 in feeding area A/B-B (Table 1). No significant differences were found between the observed heterozygosity (H_O) and the expected heterozygosity (H_E) under Hardy-Weinberg assumptions. Values of observed and expected heterozygosity were relatively high across all feeding areas (H_O=0.725-0.750; H_E=0.717-0.756).

Stock composition on feeding areas

The MSA demonstrated representation of all six breeding stocks across each of the seven feeding areas tested (Fig. 2). Individuals sampled from all feeding areas were allocated to BSA, with the highest proportion allocated to feeding area A (10.00%; 90% CI=0.00-17.00) and the lowest proportion to area A/B-B (3.00%; 90% CI=0.00-15.00). Allocation proportions to BSA across the remaining feeding areas were relatively even, ranging between 5.00% and 7.00%.

BSB1 consistently represented the breeding substock with the highest representation in all feeding areas, with the highest proportion allocated in A/B-B (53.00%; 90% CI=7.00-94.00) and the lowest proportion allocated in feeding area G-G/A (9.00%; 90% CI=1.00-26.00).

Feeding area A/B-B also showed particularly low levels of allocation to all other breeding stocks and substocks than any other feeding area, apart from for BSC2. A high proportion of individuals from feeding area A to the west were also allocated to BSB1 (26.00%; 90% CI=6.00-87.00) and from the non-adjacent feeding area C/D-D to the east (24.00%; 90% CI=2.00-76.00).

For feeding areas A and G-G/A, individuals were allocated to BSB2 and the substocks of BSC with relatively equal distribution. Notably for BSB2, feeding area A/B-B showed the lowest proportion allocation (3.00%; 90% CI=0.00-15.00) in contrast with the adjacent feeding area A to the west that showed the highest proportion (11.00%; 90% CI=0.00-17.00). Slightly greater representation of BSB2 was estimated for feeding area B/C to the east (6.00%; 90% CI=0.00-14.00). Notably, BSC2 showed proportionally higher allocation than the other BSC substocks in four of the feeding areas: A/B-B (10.00%; 90% CI=0.00-19.00), C (10.00%; 90% CI=1.00-32.00), C/D-D (11.00%; 90% CI=0.00-38.00), and D/E-E-F-F/G (15.00%; 90% CI=0.00-50.00). In contrast, more similar estimates were observed for BSC1 and BSC3; however, allocation to BSC3 was found to be slightly lower in feeding areas A/B-B, B/C, C/D-D and D/E-E-F-F/G.

The genetic analysis of the 341 bp consensus sequence of the mtDNA control region across the combined data set of six breeding stocks and substocks and seven feeding areas resulted in the identification of 128 unique haplotypes derived from the total sample of 3,112 sequences with 61 polymorphic sites (*Hd*=0.972; π(JC)=0.019; Tables S1, S4). The mtDNA haplotypes identified for each of the six breeding stocks and substocks were relatively evenly distributed across the seven feeding areas (Fig. 1). However, feeding area A showed the lowest proportion of haplotypes from BSA (2.70%) compared to the other six feeding areas (6.09-9.68%; Fig. 1). In contrast to the MSA results, the elevated allocation to BSB1 was not reflected in the distribution of haplotypes for any feeding area. Similarly, greater representation of

haplotypes from BSC2 compared to BSC1 and BSC3 was not observed. Feeding areas A, C/D-D, D/E-E-F-F/G, and G-G/A, included a number of haplotypes (N=5, 3, 7, 11, respectively) that were not identified in the six breeding stocks and substocks, possibly indicating the presence of individuals from unsampled breeding locations.

Genetic structure based on individual allele frequencies without *a priori* designation of populations was suggested by ln(P|K) and ΔK values produced by STRUCTURE as K=3 (Fig. S1). However, no population structure was distinguishable from the individual assignment plots (Fig. S2), leading to the conclusion that K=1.

DISCUSSION

Genetic structure of BSA-C on feeding areas

The lack of geographic structuring of mtDNA haplotypes (Fig. 1) and individual genotypes (Fig. S2) on feeding areas observed here and in previous studies (Amaral & Loo *et al*. in review) indicate shared evolutionary lineages across the Southern Hemisphere and support the recent proposal to designate this group as a separate subspecies (Jackson *et al*. 2014). At the regional scale of the South Atlantic and western Indian Ocean (i.e. BSA-C), the genetic diversity and differentiation analyses, allocation of samples to breeding stocks and substocks by the MSA, and the distribution of haplotypes, indicate that breeding stocks exhibit different patterns of fidelity and mixing on feeding areas in the Southern Ocean. This may indicate differences in the timing of colonization events and establishment of feeding areas, contemporary mixing of different breeding stocks and substocks (Amaral & Loo *et al*. in review), and the genetic impact of industrial whaling (Jackson *et al*. 2008).

Feeding area A showed the lowest levels of genetic diversity (Table 1) and the highest proportion of individuals were allocated to BSA by the MSA (Fig. 2). In addition, the adjacent feeding area to the east comprising the A/B Margin and B Nucleus (A/B-B) showed the least representation of individuals from BSA (Fig. 2), indicating that there may be a 'break' in the distribution of BSA individuals at the boundary between feeding areas A and A/B-B. This is supported by the significant differentiation of feeding area A from other feeding areas based on mtDNA (Table 2), although this on not reflected in the analysis of haplotype distributions that indicates that feeding A has the lowest proportion of haplotypes found for BSA (Fig. 1). These findings therefore show some support for the previous evidence of direct connectivity between BSA and its corresponding Nucleus feeding area encompassing the region from approximately 50°-20°W (Zerbini *et al*. 2006; Cypriano-Souza *et al*. 2010; Horton *et al*. 2011; IWC 2012, 2013).

BSB1 consistently showed the highest representation in all feeding areas for the MSA, with a markedly greater proportion allocated to the A/B-B feeding area (Fig. 2). Given that BSB1 is the only breeding component within BSB (as BSB2 comprises migratory and feeding animals), this finding may indicate that the westward Margin and Nucleus areas for BSB represent the primary feeding aggregation for this breeding stock, as currently hypothesized. Levels of genetic differentiation between the A/B-B and B/C feeding areas were found to be significant for nuclear markers (Table 3) but not for the haplotype data (Table 2), suggesting that male fidelity to the A/B-B feeding area may be driving the genetic structure observed. This contrasts with previous evidence of maternally-driven genetic structure on some feeding areas (Amaral & Loo *et al*. in review).

A previous examination of population structure across all feeding areas in the Southern Ocean based on mitochondrial and microsatellite data detected significant genetic differentiation of IWC Management Area II (60°W-0°), which directly overlaps with the A/B-B feeding area in this study (20°W-10°E; Amaral & Loo *et al*. in review), and Management Area III (0°-70°E), that approximately corresponds with part of feeding area A/B-B, the B/C Margin (10°E-30°E) and the C Nucleus (30°E-60°E; Amaral & Loo *et al*. in review). While our results support significant differentiation between adjacent feeding areas A/B-B and B/C for nuclear markers (Table 3) they did not support differentiation between non-adjacent areas A/B-B and C for nuclear or mitochondrial data (Tables 2, 3). These differences appear to be related to the longitudinal boundaries used to define Management Areas compared to feeding areas, particularly from 20°W to 30°E. As Management Area III overlaps with part of feeding area A/B-B and entirely with feeding areas B/C and C, the high contribution of BSB1 individuals to feeding area A/B-B may therefore be influencing the genetic distinctiveness of Management Area III found by Amaral, Loo and colleagues (in review), as opposed to it being driven by the genetic structure of BSC on feeding areas, of which we found little evidence.

All other feeding areas, and in particular C/D-D, also showed disproportionately high allocation to BSB1 by the MSA (Fig. 2). This pattern may result from the fact that the sample size of BSB1 is very large (n=1,226) compared to other breeding stocks and substocks (n=<200). The allelic composition of BSB1 is therefore overrepresented in the baseline sample used for the MSA, which may potentially result in inflated levels of allocation to this substock. However, the fact that the sample size of BSC3 is comparable (n=1,089) but does not show similar levels of over-representation indicates that differing degrees of population structure and mixing are likely to be at least a contributing driver of the signal for BSB1.

In contrast, BSB2 and the substocks of BSC showed relatively even allocation and haplotype distribution among feeding areas (Figs. 1 and 2). Studies of the genetic structure of these substocks demonstrate high levels of complexity (Rosenbaum *et al*. 2009; Kershaw *et al*. in prep). While the whales observed feeding and migrating off west South Africa (BSB2) likely comprise a component of the BSB1 substock undertaking a coastal migration from breeding areas in the Gulf of Guinea to feeding areas in the Sub-Antarctic and Southern Ocean (Barendse *et al*. 2013; Carvalho *et al*. 2014; Rosenbaum *et al*. 2014), genetic evidence suggests that BSB2 and BSC1 also have high levels of connectivity (Kershaw *et al*. in prep). In addition, genotypic matches have revealed direct connectivity between BSB2 and both BSC1 and BSC3, and between feeding areas associated with BSB and BSC in the Antarctic (IWC 2009; Kershaw *et al*. unpublished data). The low allocation to BSB2 for feeding areas A/B-B and B/C, and the higher allocation to feeding areas further east, support the eastward mixing of BSB2 with the BSC substocks demonstrated by these previous studies.

Within BSC, significant differentiation has been found between BSC1 off the east coast of southern Africa and BSC3 in the vicinity of the Madagascar ridge for mitochondrial (Rosenbaum *et al*. 2009) and microsatellite data (Kershaw *et al*. in prep); however, no significant genetic structure has been detected between BSC2 located in the Mozambique channel, and either BSC1 or BSC3 (Rosenbaum *et al*. 2009; Kershaw *et al*. in prep). Genotypic matches suggest, however, that contemporary exchange does occur between all three substocks (Kershaw *et al*. unpublished data). These genetic findings, in combination with corollary evidence from photo-identification (Ersts *et al*. 2011) and satellite telemetry data (Fossette *et al*. 2014), indicate that BSC2 in fact represents a migratory stream comprising wide-ranging animals from coastal Africa and Madagascar (Best *et al*. 1998). The proportionally higher allocation of individuals to

BSC2 compared to BSC1 and BSC3 in four of the feeding areas (Fig. 2) may also be indicative that BSC2 comprises individuals from multiple breeding substocks. The low proportion of samples allocated to BSC3 despite its larger samples size may be due to the genetic similarity of the BSC2 and BSC3 baseline, meaning individuals on feeding areas that originate from BSC3 may be being incorrectly allocated to BSC2 by the MSA.

Environmental drivers of population structure on feeding areas

Our results suggest that humpback whales from geographically separate and genetically distinct breeding stocks and substocks converge and significantly mix with one another on feeding areas. Our work also supports long distance movements on feeding areas as a plausible mechanism for individuals switching, either temporarily or permanently, between breeding stocks (Rosenbaum *et al*. 2009; Stevick *et al*. 2013). The patterns of structure on feeding areas may be attributed to complex life history and behavior, and oceanographic features that influence prey distribution, such as primary productivity and seasonal sea ice dynamics (Friedlaender *et al*. 2010; Cotte & Guinet 2011; Amaral & Loo *et al*. in review), as is evident in other baleen whale species (Wada & Numachi 1991; Hoezel 1998; Sremba *et al*. 2012).

During the summer, Southern Hemisphere humpback whales feed almost exclusively on Antarctic krill (*Euphausia superba*; Murase *et al*. 2002) and are thought to target certain habitat features that promote prey abundance, such as banks, canyons, and the sea ice margin (Friedlaender *et al*. 2010; Cotte & Guinet 2011). Summer sea ice, rather than prey abundance, has been identified as the dominant parameter predicting catch abundance (Cotte & Guinet 2011), indicating that whales target sea-ice habitat not simply in relation to overall krill abundance or density, but perhaps due to its influence on the 'patchiness' of prey distribution

(Cotte & Guinet 2011). Whales are also often associated with habitats characterized by high levels of primary productivity, such as banks and canyons, and variable frontal systems such as the Antarctic polar front (Stevick *et al*. 2006). Seasonal and inter-annual variability of these oceanographic features, for example changes in the distribution and dynamics of sea ice (Thiele *et al*. 2004), are likely to lead to spatial and temporal changes in prey distribution and abundance (Croll *et al*. 1998). Highly mobile species likely have the capacity to respond behaviorally to these changes by adapting their movements within and between feeding areas (Stevick *et al*. 2003). It would therefore be expected that greater longitudinal movements, and thus higher levels of mixing, would occur in regions where prey distribution was more variable.

The most recent estimate of post-larval krill biomass for the entire Southern Ocean was 379 million tons (Atkinson *et al*. 2008) and more than 50% of this biomass is contained within the Atlantic sector, which has been confirmed as the region with the highest densities of krill in the Southern Ocean (Atkinson 2004). Due the large geographic expanse of the Atlantic sector (3.94 million km^2) however, the maximum density of krill (7.6 million tons/km^2) is markedly lower than the sectors proximate to the Antarctic Peninsula (Peninsula = 131.0 million tons/km^2; South Orkneys = 64.5 million tons/km^2; South Georgia = 151.0 million tons/km^2) (Nicol *et al*. 2000). The differences in maximum krill density between these two regions may provide an explanation for the recorded fidelity of BSA and BSG to reliable feeding areas with high krill densities off the Antarctic Peninsula (Zerbini *et al.* 2006; Cypriano-Souza *et al*. 2010; Horton *et al.* 2011; IWC 2012, 2013), compared to individuals from BSB and BSC (IWC 2011) that are feeding in lower density krill areas and may need to undertake long-distance longitudinal movements to maximize feeding opportunities. Maximum krill density in the Southwest Indian Ocean is estimated to be 2.3 million tons/km^2 (Nicol *et al*. 2000) and may drive individuals from

BSC westwards to exploit the greater feeding opportunities of the Atlantic sector. Prey distribution may therefore be one of the drivers maintaining genetic connectivity between BSB and BSC as individuals mix on feeding areas and potentially switch between breeding areas (Rosenbaum *et al*. 2009; Stevick *et al*. 2013).

The genetic signature of whaling

The hunting of almost a quarter of a million humpback whales on sub-Antarctic and Antarctic feeding areas (Rocha *et al*. 2015) is likely to have reduced levels of genetic diversity in contemporary populations and therefore should be considered in the interpretation of assessments on population structure (Schultz *et al*. 2009). In a bottlenecked population, rare alleles are the first to be lost, lowering the mean number of alleles per locus (Schultz *et al*. 2009). Heterozygosity, on the other hand, is less affected, resulting in a transient excess of heterozygosity relative to that expected given the resulting number of alleles (Cornuet & Luikart 1996). Generally, a bottleneck must persist over several generations to impact heterozygosity significantly (Schultz *et al*. 2009). Given the long generation time of humpback whale, high levels of heterozygosity are expected to be observed in the bottlenecked population and therefore belie the impact of whaling on the erosion of genetic diversity through the loss of rare alleles.

The loss of rare alleles would increase the genetic similarity of humpback whale populations that would have been previously distinguishable by private alleles. This could result in a similar genetic signature to that of historic or contemporary migration of individuals between different populations and potentially inflate perceived levels of connectivity. Therefore, disentangling the effects of each process is necessary to fully determine the extent to which contemporary migration and mixing is influencing connectivity of humpback whale populations.

While such an analysis is beyond the scope of this study, methods based on Bayesian and coalescent-based simulations offer a useful framework for reconstructing historic baselines of genetic diversity and exploring how these may have been influenced by a range of factors, such as whaling and demographic changes (e.g. Alter *et al*. 2007; Jackson *et al*. 2008). Emerging genomic sequencing technologies combined with sequential Markov coalescent-based or Bayesian ABC approaches offer additional flexibility and complexity to these approaches (Palsbøll *et al*. 2013).

Limitations of the mixed stock analysis

The results of the MSA support previous evidence for mixing of different breeding stocks and substocks on feeding areas; however, the levels of mixing occurred at unexpectedly high levels (i.e. almost equal representation of some breeding stocks and substocks across all feeding areas tested). While these results may have been influenced by the presence of shared evolutionary lineages, a number of analytical factors that may have influenced the results need to be evaluated, including the power of the genetic data set, the size and distribution of samples, and the constraints inherent to the MSA statistical framework (Bowen *et al*. 2007).

We elected to use 10 microsatellite markers previously found to be highly polymorphic for humpback whales for the MSA given that their rapid mutation rate and bi-parental mode of inheritance makes them well suited to addressing management questions on a contemporary timescale (Avise 1995). However, it has been questioned whether microsatellite data have adequate power compared to mtDNA sequence data to be useful for MSA in humpback whales (Schmitt *et al*. 2014). Our genetic differentiation analyses for mtDNA and microsatellite data sets (Table 2 and 3, respectively), demonstrate a loss of some discriminatory power between

feeding areas for F_{ST} when ten microsatellites were used compared to the 472 bp consensus sequence of the mtDNA control region. However, a comparison of nucleotide-level differentiation (i.e. Φ_{ST}; Table 2) and the fraction of allelic variation among groupings (i.e. Jost's *D*; Table 3) shows little difference in levels of statistical significance, indicating that the two markers show some consistency. In addition, the analysis of haplotype distribution (Fig. 1) supports the finding of the MSA that high levels of mixing occur across all feeding areas. We therefore consider the data set used for the MSA to have adequate discriminatory power and advocate for the use of nuclear data in similar analyses for species that demonstrate sex-biased dispersal, which may go undetected by mtDNA analyses alone due to its uniparental nature of inheritance (Avise 1995).

Sampling effects can also influence the accuracy and precision of MSA; for example, if sampling of the baseline populations is not exhaustive or if sample sizes are low or unevenly distributed (Bowen *et al*. 2007). The opportunistic nature of sampling baleen whales, particularly on remote feeding areas of the Southern Ocean, leads to generally small sample sizes that are aggregated spatially and temporally. Simulations demonstrate that sample size of the source populations directly correlates with the accuracy of population identification by the MSA (Schmitt *et al*. 2014). The size of the samples used in our study for the source population and mixture data sets in most cases are relatively small, particularly for feeding areas (<200 individuals is recommended by Schmitt *et al*. (2014); Tables 1 and S1). Moreover, SPAM has been found to overestimate the predicted accuracy and precision of the MSA by resampling from the baseline with replacement, particularly for closely related populations (Anderson *et al*. 2008; Schmitt *et al*. 2014). An additional consideration is that sampling location only indicates where an individual is present at the time the sample was collected. For highly mobile species it cannot

be assumed that the sampling location corresponds to a feeding location (i.e. an individual may be in transit) or that an individual would return to that same location over time. These biases may have resulted in overestimates of mixing by the MSA and haplotype analyses, as the true location of the major feeding aggregations is not being captured.

MSA generally assume that the source samples and allele frequency estimates are representative of the populations present in the mixture, and therefore do not take account of unrepresentative baseline samples and alleles or omitted source populations (Schmitt *et al*. 2014). Given the biases inherent in sampling baleen whales, the absence of alleles in the mixed sample or in the baseline data set may be a key factor influencing the results (Wennevik *et al*. 2008). Feeding areas A, C/D-D, D/E-E-F-F/G, and G-G/A included a number of haplotypes that were not identified in the breeding stocks and substock data set (Fig. 1). This finding may be due to incomplete sampling of individuals on breeding areas and the geographical distribution of these unidentified haplotypes likely reflects the absence of samples from BSD-G in the analysis. While we included the Pella-Masuda option in SPAM to adjust for alleles that may exist but were not sampled in the baseline (Alaska Department of Fish & Game 2003), there is no way to systematically account for the possibility of individuals from unsampled stocks and substocks being present in the mixture (Schmitt *et al*. 2014).

We conducted separate analyses for each feeding area (i.e. mixed stock) and this "many-to-one" (*sensu* Bolker *et al*. 2007) approach may have limited the findings of our analysis. As previous research demonstrates, humpback whales may visit a number of breeding areas and feeding grounds, meaning that a "many-to-many" (*sensu* Bolker *et al*. 2007) analysis, capable of simultaneously estimating the origins and destinations of individuals in a metapopulation made up of multiple source populations and multiple mixed stocks (Bolker *et al*. 2007), may be more

appropriate. Hierarchical Bayesian models may offer a useful framework for future "many-to-many" MSA for humpback whales (Bolker et al. 2007).

Recommendations for future research

Our findings demonstrate that patterns of population structure of Southern Hemisphere humpback whales on feeding areas are markedly different than on breeding areas and show that mixing of different breeding stocks and substocks occur at high levels and to differing degrees. Given the constraints related to analytical power, sample size, and model structure, further research is needed to discriminate the mixing of breeding stocks and substocks on feeding areas to ensure the accurate assessment of historical abundance, the impact of whaling on genetic diversity, and population recovery. Including existing samples from the remaining Southern Hemisphere breeding stocks would provide more robust estimates of circumpolar population structure by reducing the likelihood of missing alleles in the baseline. Increased genetic sampling in underrepresented feeding areas would also be beneficial; however, the significant resources required for sampling may be prohibitive. Increasing the power of the genetic data set may represent a more feasible option. Application of genomic data would improve estimates of allocation and mixing on feeding areas, and would increase the resolution of the source populations to which samples are allocated. In addition, implementing a many-to-many model framework using a hierarchical Bayesian modeling approach (Bolker *et al*. 2007) may also lead to more accurate estimates, as it is better able to reflect the behavior of the species. As genetic evidence suggests latitudinal variation in the distribution of BSB whales in the Antarctic (IWC 2011), feeding area fidelity and mixing for areas defined by different latitude should also be explored in further detail. Additionally, models of habitat suitability and physiology (e.g. energy

requirements) may prove a useful complementary tool for predicting whale movements, and therefore the extent of mixing of different breeding stocks and substocks (Friedlaender *et al*. 2011; Braithwaite *et al*. 2015). In sum, assessments of population structure for migratory species require an integrative approach encompassing genetic, behavioral, and environmental data.

TABLES AND FIGURES

Table 1. Genetic diversity based on a 472 bp consensus region of the mitochondrial control region and 10 microsatellite loci for the total sample and the seven feeding areas defined by the sensitivity analysis. N, sample size; S, number of segregating sites; *H*, number of haplotypes; *Hd*, haplotype diversity; π(JC), nucleotide diversity with Jukes-Cantor correction; K, mean number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity.

Area	**N**	**MtDNA**				**Microsatellites**		
		S	***H***	***Hd***	**π(JC)**	**K**	**H_E**	**H_O**
Total	340	71	110	0.976	0.020	12.9	0.741	0.732
A	21	32	14	0.919	0.020	8.3	0.728	0.743
A/B-B	112	52	53	0.969	0.020	11.4	0.753	0.743
B/C	35	44	28	0.978	0.020	8.8	0.718	0.731
C	37	43	25	0.971	0.019	9.3	0.727	0.735
C/D-D	41	44	27	0.977	0.020	9.5	0.756	0.750
D/E-E-F-F/G	44	41	26	0.968	0.020	9.6	0.717	0.745
G-G/A	50	35	25	0.927	0.018	8.8	0.725	0.725

Table 2. Genetic differentiation between the final feeding area boundary sets for the 472 bp consensus sequence of the mtDNA control region. F_{ST} values are shown below the diagonal and Φ_{ST} above the diagonal. * indicates statistical significance at 0.05*, 0.01**, 0.001***. Estimations of significance were made from 10,000 permutations at the 0.05 significance level.

	A	**A/B-B**	**B/C**	**C**	**C/D-D**	**D/E-E-F-F/G**	**G-G/A**
A	-	0.038*	0.026	0.041*	0.028	0.075**	0.011
A/B-B	0.044***	-	0.001	0.006	0.007	0.037**	0.021*
B/C	0.037**	-0.004	-	-0.003	-0.018	0.001	0.013
C	0.050***	0.002	-0.001	-	-0.005	0.040*	0.034*
C/D-D	0.044***	0.006	-0.003	0.004	-	0.004	0.019
D/E-E-F-F/G	0.042***	0.014**	0.003	0.021**	0.004	-	0.050**
G-G/A	-0.004	0.036***	0.029**	0.039***	0.035***	0.032***	-

Table 3. Genetic differentiation between the final feeding area boundary sets for the ten microsatellite loci. F_{ST} values are shown below the diagonal and Jost's *D* above the diagonal. * indicates statistical significance at 0.05*, 0.01**, 0.001***. Estimations of significance were made from 10,000 permutations at the 0.05 significance level.

	A	**A/B-B**	**B/C**	**C**	**C/D-D**	**D/E-E-F-F/G**	**G-G/A**
A	-	0.036**	-0.000	0.001	0.006	0.023*	-0.028
A/B-B	0.004	-	0.020**	0.005	0.008	0.034***	0.045***
B/C	-0.001	0.004*	-	-0.002	0.004	0.038**	0.027**
C	-0.002	-0.001	0.000	-	-0.002	0.016	0.015
C/D-D	-0.001	0.002	-0.001	-0.001	-	-0.001	0.014
D/E-E-F- F/G	0.001	0.005**	0.008*	0.002	-0.000	-	0.021*
G-G/A	-0.006	0.010***	0.008**	0.002	0.004	0.006*	-

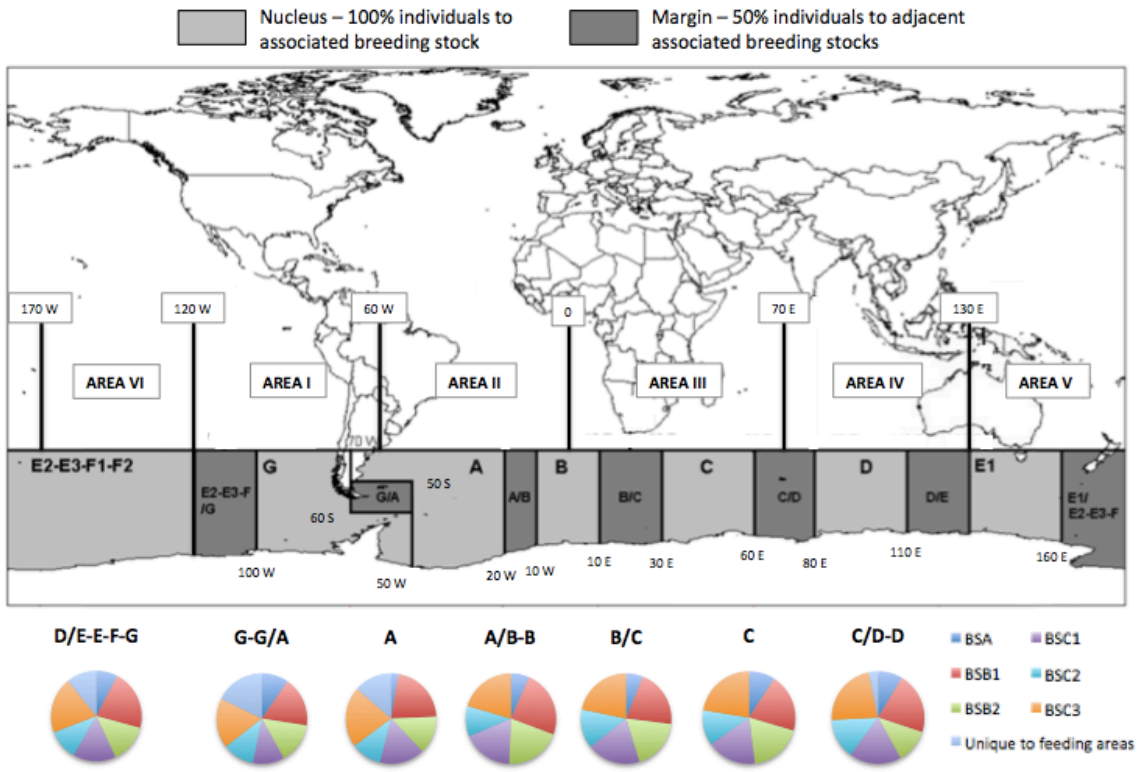

Fig. 1. Map of the longitudinal boundaries of the 14 feeding areas defined by IWC Allocation Hypothesis 1 ("AH1"; IWC 2010; light gray, Nucleus regions; dark gray, Margin regions). Longitudinal Boundaries of the six IWC Management Areas (Areas I-VI; IWC 2011) are shown in the outlined boxes. Note that all feeding areas occur south of 60°S and Areas I-VI are shown north of 60°S for visualization purposes only. Pie charts represent the distribution of haplotypes across feeding areas. Haplotypes present in multiple breeding areas are counted once for each area.

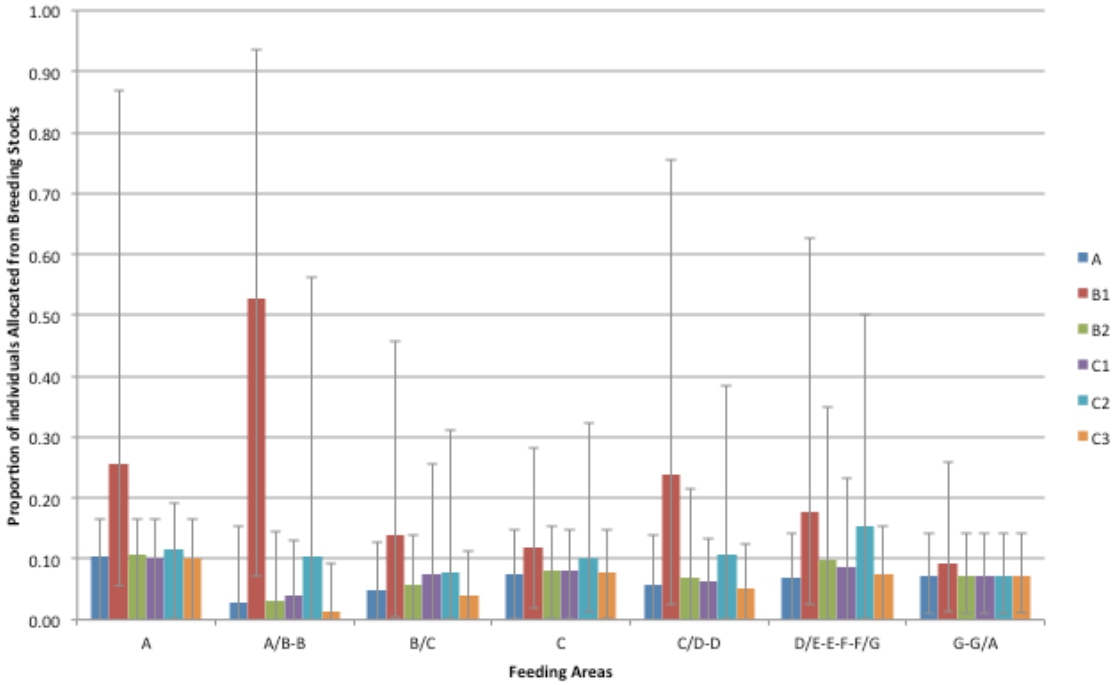

Fig. 2. The proportion of individuals sampled on the seven feeding areas defined in this study estimated by the MSA to be allocated to the six breeding stocks (BSA, BSB1, BSB2, BSC1, BSC2, BSC3) using ten microsatellite loci. Error bars represent 90% bootstrap confidence intervals.

SUPPORTING INFORMATION

Sensitivity analysis to define feeding areas

The BSA core area showed significant differentiation ($p<0.001$) from almost all other groups, including A/B-B, for the mtDNA data set (Table S2a-d) and so was designated as a single grouping. Significant differentiation of A was less evident in the microsatellite data set, however the separation of A from A/B was still supported by the Jost's *D* statistic (Table S3b). Aggregating A/B with the core area B resulted in no loss of resolution for mtDNA or microsatellite data set (Table S2; S3). For the mtDNA data set, B/C showed no significant differentiation from C-D-E-F, however C and C-C/D was found to be significantly differentiated from the areas designated for E and F, indicating that using C or C-C/D would provide additional resolution than B/C-C (Tables S2a; S2d). However, the microsatellite data set demonstrated the opposite, with B/C showing significant differentiation from E-F at both F_{ST} and Jost's D, whereas C and C-C/D did not show differentiation from any other stock (Tables S3a; S3d). As samples sizes were adequate across the boundary and core areas for B and C, the B/C boundary area, C core area, and the C/D-D boundary and core area were designated as separate groupings. Little resolution was found within the core and boundary areas corresponding to the BSE and BSF stocks and substocks (Tables S2; S3), resulting in the designation of a single large area comprising D/E-E-F-F/G. Finally, the core area for G and the G/A boundary (n=0) were aggregated as designated as a separate area due to the significant differentiation from most other areas for both the mtDNA and microsatellite data sets (Tables S2; S3).

Table S1. Genetic diversity based on a 371 bp consensus sequence of the mitochondrial control region and 10 microsatellite loci for the total feeding sample and the six breeding stocks and substocks included in the baseline data set and the haplotype comparison of breeding and feeding areas. N, sample size; S, number of segregating sites; *H*, number of haplotypes; *Hd*, haplotype diversity; π(JC), nucleotide diversity with Jukes-Cantor correction; K, mean number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity.

Stock	**N**	**MtDNA**				**Microsatellites**		
		S	***H***	***Hd***	**π(JC)**	**K**	**H_E**	**H_O**
Total BS	2772	60	107	0.972	0.019	14.0	0.743	
BSA	38	25	18	0.946	0.017	9.4	0.724	0.718
BSB1	1226	55	89	0.965	0.019	13.0	0.745	0.742
BSB2	176	44	54	0.966	0.019	11.1	0.746	0.761
BSC1	182	43	55	0.968	0.018	11.7	0.747	0.750
BSC2	61	34	30	0.969	0.019	10.2	0.736	0.723
BSC3	1089	47	78	0.970	0.019	12.6	0.739	0.742

Table S2a-d. Genetic differentiation values based on the 472 bp consensus sequence of the mitochondrial control region for four alternative feeding area boundary sets (a-d) assessed as part of the sensitivity analysis. F_{ST} values are shown below the diagonal and Φ_{ST} above the diagonal. * indicates statistical significance at 0.05*, 0.01**, 0.001***. Estimations of significance were made from 10,000 permutations at the 0.05 significance level.

a. Boundary Set 1

	A-A/B	**B**	**BC**	**C-C/D**	**D-D/E**	**E-F-F/G**	**G-G/A**
A-A/B	-	0.016	0.007	0.018	0.018	0.047*	0.005
B	0.027***	-	0.001	0.004	0.012	0.040**	0.020*
BC	0.021**	-0.005	-	-0.005	-0.015	-0.001	0.013
C-C/D	0.030***	0.001	-0.002	-	-0.004	0.040*	0.034*
D-D/E	0.023***	0.010*	-0.001	0.006	-	0.002	0.024*
E-F-F/G	0.028***	0.014**	0.001	0.018**	0.003	-	0.047*
G-G/A	-0.002	0.036***	0.029**	0.037***	0.036***	0.032***	-

b. Boundary Set 2

	A	**A/B-B**	**B/C**	**C-C/D**	**D-D/E**	**E-F-F/G**	**G-G/A**
A	-	0.038*	0.026	0.041*	0.038*	0.070**	0.012
A/B-B	0.044***	-	0.001	0.004	0.011	0.039**	0.021*
B/C	0.037**	-0.004	-	-0.005	-0.015	0.001	0.013
C-C/D	0.049***	0.001	-0.002	-	-0.005	0.040*	0.034*
D-D/E	0.042***	0.009*	-0.001	0.006	-	0.002	0.024*
E-F-F/G	0.045***	0.013**	0.001	0.018**	0.003	-	0.047*
G-G/A	-0.004	0.036***	0.029**	0.037***	0.036***	0.032***	-

c. Boundary Set 3

	A-A/B	**B**	**B/C-C**	**C/D-D**	**D/E-E-F-F/G**	**G-G/A**
A-A/B	-	0.016	0.014	0.011	0.050*	0.005
B	0.027***	-	0.004	0.007	0.039**	0.020*
B/C-C	0.026**	-0.001	-	-0.011	-0.022*	0.023*
C/D-D	0.026***	0.006	0.001	-	-0.004	0.019
D/E-E-F-F/G	0.026***	0.014**	0.013**	0.004	-	0.050**
G-G/A	-0.002	0.036***	0.034**	0.035***	0.032***	-

d. Boundary Set 4

	A-A/B	**B**	**B/C**	**C**	**C/D-D-D/E**	**E-F-F/G**	**G-G/A**
A-A/B	-	0.016	0.007	0.018	0.019	0.047*	0.005
B	0.027***	-	0.001	0.006	0.011	0.040**	0.020*
B/C	0.021**	-0.005	-	-0.003	-0.014	-0.001	0.013
C	0.030***	0.003	-0.001	-	-0.002	0.044*	0.034*
C/D-D-D/E	0.023***	0.007*	-0.002	0.006	-	0.004	0.026*
E-F-F/G	0.028***	0.014**	0.001	0.021**	0.003	-	0.047*
G-G/A	-0.002	0.036***	0.029**	0.039***	0.034***	0.032***	-

Table S3a-d: Genetic differentiation values based on ten microsatellite loci for four alternative feeding area boundary sets (a-d) assessed as part of the sensitivity analysis. F_{ST} values are shown below the diagonal and Jost's *D* above the diagonal. * indicates statistical significance at 0.05*, 0.01**, 0.001***. Estimations of significance were made from 10,000 permutations at the 0.05 significance level.

a. Boundary Set 1

	A-A/B	**B**	**B/C**	**D-D/E**	**D-D/E**	**E-F-F/G**	**G-G/A**
A-A/B	-	0.009	-0.009	-0.020	-0.013	0.012	-0.015
B	0.000	-	0.020*	0.001	0.013	0.040**	0.043**
BC	-0.004	0.004*	-	-0.005	0.006	0.034**	0.027**
C-C/D	-0.005	-0.001	-0.001	-	-0.006	0.008	0.011
D-D/E	-0.003	0.003	0.001	-0.002	-	0.007	0.017*
E-F-F/G	0.001	0.053**	0.008*	0.000	0.001	-	0.022*
G-G/A	-0.002	0.009***	0.008**	0.001	0.005*	0.005*	-

b. Boundary Set 2

	A	**A/B-B**	**B/C**	**C-C/D**	**D-D/E**	**E-F-F/G**	**G-G/A**
A	-	0.036**	-0.000	-0.003	0.010	0.024	-0.028
A/B-B	0.004	-	0.020**	0.000	0.011	0.040**	0.045**
B/C	-0.001	0.004*	-	-0.005	0.007	0.034**	0.027**
C-C/D	-0.004	-0.001	-0.001	-	-0.006	0.008	0.011
D-D/E	0.000	0.002	0.001	-0.002	-	0.007	0.017*
E-F-F/G	0.001	0.006**	0.008*	0.000	0.001	-	0.022*
G-G/A	-0.006	0.010***	0.008**	0.001	0.005*	0.005*	-

c. Boundary Set 3

	A-A/B	**B**	**B/C-C**	**C/D-D**	**D/E-E-F-F/G**	**G-G/A**
A-A/B	-	0.009	-0.013	-0.013	0.005	-0.015
B	0.000	-	0.011*	0.009*	0.036**	0.043**
B/C-C	-0.004	0.002	-	-0.008	0.023*	0.020*
C/D-D	-0.003	0.002	-0.001	-	0.001	0.014
D/E-E-F-F/G	0.000	0.005**	0.005*	-0.001	-	0.021*
G-G/A	-0.003	0.009***	0.005*	0.004	0.006*	-

d. Boundary Set 4

	A-A/B	**B**	**B/C**	**C**	**C/D-D-D/E**	**E-F-F/G**	**G-G/A**
A-A/B	-	0.009	-0.009	-0.017	-0.011	0.012	-0.015
B	0.000	-	0.029*	0.007	0.012*	0.040**	0.043**
B/C	-0.004	0.004*	-	-0.002	-0.009	0.034**	0.027*
C	-0.004	-0.000	0.000	-	-0.004	0.015	0.015
C/D-D-D/E	-0.003	0.003*	0.001	0.001	-	0.006	0.018*
E-F-F/G	0.001	0.005**	0.008*	0.002	0.001	-	0.022*
G-G/A	-0.003	0.009***	0.008**	0.002	0.005*	0.005*	-

Table S4: Genetic diversity based on a 371 bp consensus sequence of the mitochondrial control region for the seven feeding areas included in the haplotype comparison of breeding and feeding areas. N, sample size; S, number of segregating sites; *H*, number of haplotypes; *Hd*, haplotype diversity; π(JC), nucleotide diversity with Jukes-Cantor correction.

Feeding Area	**N**	**MtDNA**			
		S	***H***	***Hd***	**π(JC)**
Total	340	53	91	0.971	0.019
A	21	22	14	0.919	0.017
A/B-B	112	40	45	0.959	0.019
B/C	35	32	27	0.976	0.019
C	37	30	23	0.964	0.017
C/D-D	41	31	26	0.974	0.020
D/E-E-F-F/G	44	28	23	0.950	0.018
G-G/A	50	25	23	0.925	0.017

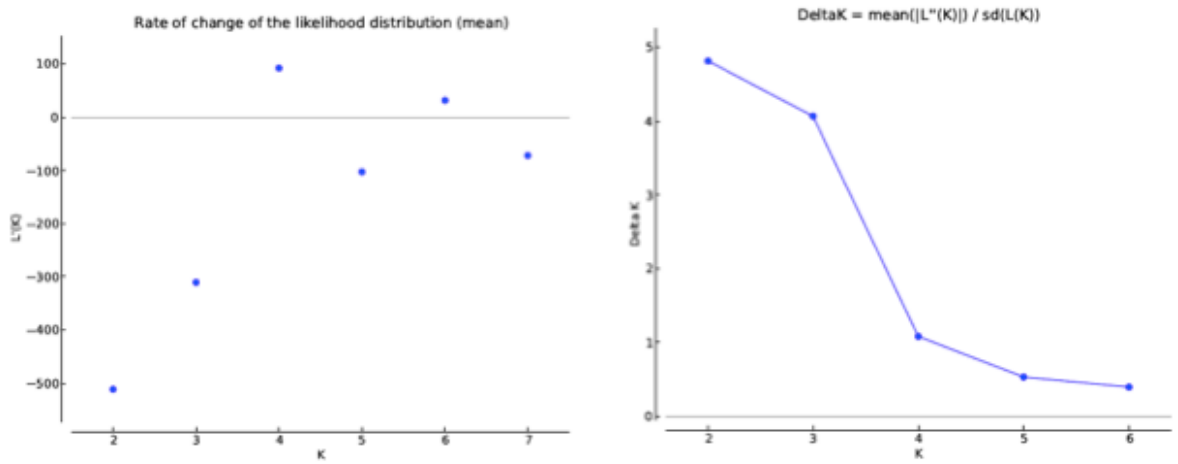

Fig. S1. Mean Ln(P|K) and Delta K (ΔK) plots for the STRUCTURE outputs for K=1-7.

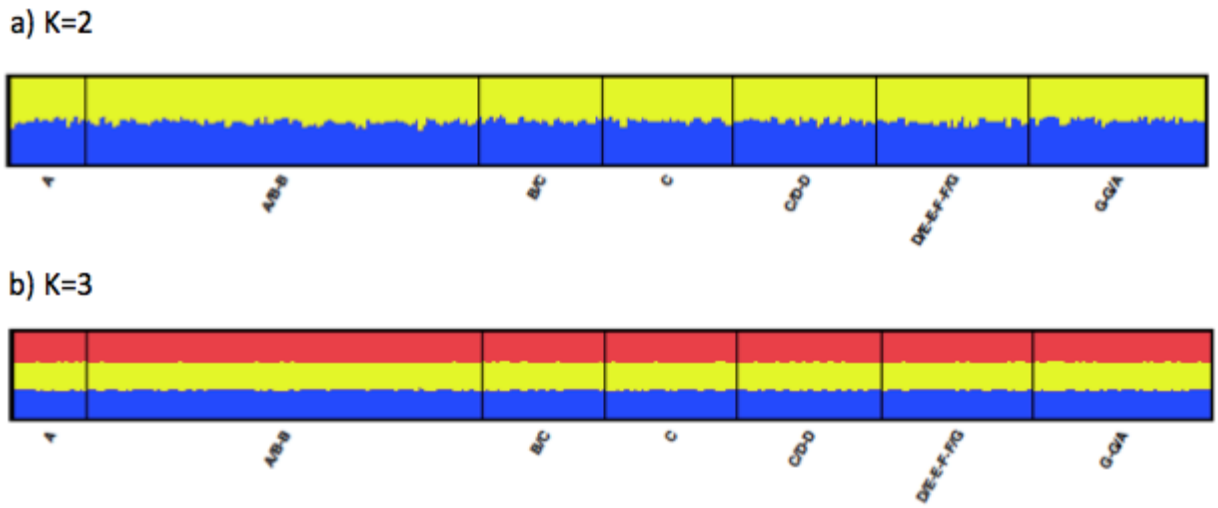

Fig. S2. Distribution of genetic clusters for a) K=2 and b) K=3. Vertical lines are partitioned into colored segments showing the proportion of each individual assigned to each K. The seven feeding areas defined by this study from which individuals were sampled are indicated below each figure.

CHAPTER FOUR

Philopatry and sex-biased dispersal shapes humpback whale population structure at multiple scales

FRANCINE KERSHAW, CHARLOTTE TISCH and HOWARD C. ROSENBAUM.

ABSTRACT

Sex-specific differences in philopatry and dispersal have direct influence on the genetic structure of populations. To better understand the influence of these behaviors on the population structure of humpback whales, we present a genotypic matching (i.e. genetic capture-recapture) analysis for 3,814 individual whales, genotyped at 10 microsatellite loci from breeding stocks and substocks in the South Atlantic (BSA, BSB1-2), the western and northern Indian Ocean (BSC1-3, ASHW), and Antarctic feeding areas. We used a 'return index' (R_i) and 'interchange index' (I) to assess breeding area fidelity and movements between different areas and recorded recaptures between breeding and feeding areas. Site fidelity was observed for 182 individuals recorded at the same breeding area in at least two years, and BSB2 showed the highest R_i values overall. 17 individuals were identified in different breeding areas and interchange was highest between substocks in the same region, however long-distance movements were observed for four males between BSB and BSC. Mixing of individuals from BSB and BSC on feeding grounds was evident. At the regional scale (i.e. between breeding stocks), our results generally support a model of female philopatry and male-biased dispersal as the primary driver of population structure in this species. At the local scale (i.e. within breeding stocks), substocks BSB2 and BSC1-3 experience high levels of contemporary interchange, supporting the high gene flow estimates previously observed at the population-level. Our findings highlight the importance of understanding current demographic exchange and incorporating this information into the delineation of population management units.

INTRODUCTION

The demographic process of dispersal, or any movement of individuals or propagules with potential consequences for gene flow across space (Ronce 2007), is an important factor influencing the structure of populations (Johnson & Gaines 1990). Dispersal is a complex, multi-causal process and the timing and frequency of dispersal events result from a combination of resource dynamics, evolution of life history traits, and inter-individual variation in dispersal tendencies (Cote *et al*. 2010; Baguette *et al*. 2012). In mammals, the majority of species exhibit male-biased dispersal and female philopatry, likely as a mechanism for females to avoid inbreeding with related males and the potentially high cost of dispersal (Bonte *et al*. 2012; Clutton-Brock & Lucas 2012). Instances of female-biased dispersal do occur however, likely due to both competitive avoidance for resources and breeding opportunities, and the need to find unrelated partners (Clutton-Brock & Lucas 2012). In other species, males and females remain in the same population but show mating preferences for individuals from different social groups (Amos *et al*. 1993).

Population-level genetic analyses can be used to tease apart some of these patterns, for example, by examining variation in gene flow for males and females, and by contrasting maternally-inherited (i.e. mitochondrial) and bi-parentally inherited (i.e. nuclear) molecular markers (Avise 2000; Tosi *et al*. 2003). A number of constraints inherent in population genetic analyses, however, pose challenges to understanding how current demographic processes are shaping populations on a timescale relevant for management. Results only reflect historic patterns due to the genetic signature being scaled to the coalescence time of the molecular marker used (Avise 2000). It also is challenging to tease apart signals of true genetic distinctiveness from shared ancestral polymorphism due to recent divergence (Edwards & Beerli

2000; Bulgin *et al*. 2003). Individual-level genetic analyses, for example, assignment tests or genotype matching methods, offer a useful complement to population-level studies as they can provide insights into direct movements of individuals between two populations or between sampling locations (Lukacs & Burnham 2005; Blair & Melnick 2012). These methods are therefore capable of providing information on prevalence of philopatry, the rate of current interchange between two populations, movement capabilities of individuals, and habitat use, among others. Together, population- and individual-level genetic studies can provide a more comprehensive understanding of population structure and the demographic processes driving the patterns observed (Lowe & Allendorf 2010).

The humpback whale (*Megaptera novaeangliae*) is a baleen species that migrates annually from low-latitude breeding areas to high-latitude feeding areas (Gambell 1976). Akin to many wide-ranging mammals (Greenwood 1980), humpback whale population structure is driven primarily by a combination of maternal fidelity to feeding areas and natal philopatry to breeding areas, with low-levels of male-biased gene flow (Palsbøll *et al*. 1995; Baker *et al*. 1998; 2013; Rosenbaum *et al*. 2009). This behavior has led to the species being managed by the International Whaling Commission (IWC) as demographically discrete breeding populations, or "breeding stocks (BS)", and it has recently been suggested that populations in different ocean basins be afforded sub-specific status (Jackson *et al*. 2014). In the South Atlantic and Western and Northern Indian Oceans, there are four stocks termed BSA, BSB, and BSC, and the non-migratory Arabian Sea humpback whale (ASHW). Population sub-structure has been observed within BSB and BSC and has led to the management of two separate breeding "substocks" within BSB (i.e. BSB1, a breeding population in the Gulf of Guinea, and BSB2, a genetically

distinct group observed to migrate and feed off West South Africa) and four substocks within BSC (i.e. BSC1-BSC4) (Fig. 1a).

While natal fidelity plays a predominant role in driving population structure, rare instances of long-distance interchange do occur between breeding stocks and ocean basins. The first inter-oceanic migration was documented for two animals marked off eastern Australia in 1954-1955 and killed off western Australia in 1959 (Chittleborough 1965). Direct connectivity of a single individual has been recorded between BSA off Brazil and the distant BSC in the Western Indian Ocean (Stevick *et al*. 2013), and song similarity between BSA and BSB in the eastern South Atlantic has been observed (Darling & Sousa-Lima 2005). Gene flow between BSB and BSC is also apparent (Best *et al*. 1998; Pomilla & Rosenbaum 2005; Rosenbaum *et al*. 2009), although direct exchange between the west and east coast of South Africa (i.e. BSB2 and BSC1, respectively) appears limited (Carvalho 2011). At the breeding substock scale, however, the model of maternal fidelity and male-biased dispersal is not generally supported (Kershaw *et al*. in prep) as higher magnitudes of gene flow and complex patterns of isolation and connectivity occur for both sexes (Rosenbaum *et al*. 2009; Carvalho *et al*. 2014; Kershaw *et al*. in prep).

Southern Hemisphere breeding stocks and substocks converge on shared sub-Antarctic and Antarctic feeding areas (Gambell 1976). It is generally assumed that temporary or permanent interchange between breeding stocks occurs as a result of long-distance longitudinal movements on feeding areas (Rosenbaum *et al*. 2009). Studies suggest that there are marked differences between the behaviors of different breeding stocks in terms of feeding area fidelity and the frequency and extent of longitudinal movements (e.g. Schmitt *et al*. 2014; Amaral & Loo *et al*. in review; Kershaw *et al*. unpublished data). For example, BSA demonstrates strong fidelity to discrete feeding areas (Zerbini *et al*. 2006; Cypriano-Souza *et al*. 2010; IWC 2012, 2013),

despite evidence of low levels of long-distance interchange (Darling & Sousa-Lima 2005; Stevick et al. 2013). In contrast, BSB and BSC demonstrate lower levels of fidelity and a higher degree of mixing, although significant genetic differentiation does occur between some feeding areas (IWC 2011; Amaral & Loo *et al*. in review; Kershaw *et al*. unpublished data). Given the implications for assessments of stock recovery from industrial and illegal Soviet whaling, there is an ongoing need to define the location of major feeding aggregations and the degree of mixing between different breeding stocks and substocks (IWC 2010).

To complement existing population-level genetic studies, we present a genotypic matching analysis for more than 4,000 genotyped samples, spanning more than two decades of research, from the seven different breeding areas in the South Atlantic and Western and Northern Indian Oceans, and feeding areas in the Southern Ocean. We assess a) the degree of site fidelity of individuals to breeding areas in different years, b) the rate of interchange between different breeding areas, and c) connectivity between breeding areas and feeding areas, as defined by IWC Allocation Hypothesis 1 (IWC 2010; Fig. 1a).

MATERIALS AND METHODS

Sample information

A total of 4,360 humpback whale genetic samples originating from multi-year collections across seven breeding areas and Antarctic feeding areas were used in this study (Fig. 1a). No samples were available for BSC4. Skin tissues were mostly obtained using biopsy darts (Lambertson 1987), but also from sloughed skin and stranded specimens. Total genomic DNA was extracted and samples were sexed using ZFX/ZFY markers following Rosenbaum *et al*. (2009). Samples were genotyped at 10 microsatellite loci proven to be polymorphic for this species: GATA028,

GATA053, GATA417 (Palsbøll *et al.* 1997), 199/200, 417/418, 464/465 (Schlötterer *et al.* 1991), EV1Pm, EV37Mn, EV94Mn, EV96Mn (Valsecchi & Amos 1996) following the protocols described in Carvalho and colleagues (2014).

Identifying genotypic matches

Duplicate samples were identified from microsatellite genotype identity for 9 or 10 loci using Cervus v3.0 (Kalinowski *et al.* 2007). The probability of different individuals and siblings sharing the same genotype by chance (Probability of Identity, P_{ID}, and P_{ID} for siblings, $P_{ID(sibs)}$, respectively) were estimated using Cervus (Kalinowski *et al.* 2007). The reciprocal of the sample size was used as the arbitrary cut-off below which the probability values are sufficiently small to conclude that matching genotypes belong to the same individual (Peakall *et al.* 2006).

Duplicate samples identified in the same breeding area during the same year were assumed to be individuals mistakenly resampled in the field and were therefore removed from subsequent analysis. Genotypic matches of individuals identified in the same breeding area in different years were used as an indicator of site fidelity. The number of and year of each resighting, and the sex of the individual were recorded for each breeding area. To better understand connectivity between breeding and feeding areas, genotypic matches were recorded for individuals found both on breeding areas and the Antarctic feeding areas defined by IWC Allocation Hypothesis 1 (IWC 2010; Fig. 1a).

Site fidelity and interchange indices

To quantify the relative rate of site fidelity for each breeding area, a 'return index' (R_i) was calculated following Carvalho (2011). The return index was calculated for each year that sampling was undertaken in a particular location:

$$R_i = [M_i / (A_i * B_i)] * 1000$$

Where:

A_i = number of genetically identified whales in all the years before year x for breeding area i

B_i = number of genetically identified whales in year x for breeding area i

M_i = number of genetically identified whales in the previous years and resighted in year x

To quantify the relative rate of interchange between breeding areas, an 'interchange index' (I) was calculated following Carvalho (2011):

$$I = [M_{i,j} / (a_i * a_j)] * 1000$$

Where:

A_i = number of genetically identified whales in breeding area i

A_j = number of genetically identified whales in breeding area j

$M_{i,j}$ = number of genetically identified whales in both regions

Both indices are considered to be zero when there are no genotypic matches (i.e. when M = 0) within (for R_i) or between breeding areas (for I).

RESULTS

Sample information

The 4,360 genetic samples analyzed were determined to represent 3,814 different whales (Fig. 1a). Average probability of identity (P_{ID}) for the total sample was small enough to identify duplicate individuals with high confidence ($P_{ID} = 1.92x10^{-12}$; $P_{ID(sibs)} = 9.2x10^{-5}$; reciprocal of sample size = $2.6x10^{-4}$). Sex was determined for 3,590 individuals, 2,267 males and 1,323 females, resulting in an overall proportion of 1.7:1 males to females.

Fidelity to breeding areas

A total of 182 individuals (123 males; 57 females; 2.5:1 sex ratio) were resighted across five breeding areas in at least one year (BSA, n=1; BSB1, n=79; BSB2, n=33; BSC2, n=2; BSC3, n=67; Table S1). The number of times an individual was resighted within a breeding area ranged from 1 to 3 years. Breeding area fidelity, defined by the return index (R_i), ranged from 0.660–44.444 and this range varied notably between breeding areas (BSA, R_i=2.083, number of years (N_y) = 1; BSB1, R_i=0.000-0.116, N_y=8; BSB2, R_i=0.000-44.444, N_y=14; BSC2, R_i=0.870-10.4167, N_y=2; BSC3, R_i=0.000-0.945, N_y=11; Fig. 2). For breeding areas where N_y>1, BSB2 showed consistently higher values of R_i (Fig. 2b), followed by BSC3 and BSB1 (Fig. 2a, c).

Interchange between breeding areas

A total of 17 individuals (10 males; 7 females; 1.4:1 sex ratio) showed genotypic matches with more than one breeding area either in the same year (n=3) or different years (n=14). Interchange occurred primarily between breeding areas in the same region (BSB, n=7; BSC, n=6), and all interchange observed in the same year occurred between BSB1 and BSB2. Long-distance

movements were observed between BSB and BSC (n=4). All four of these individuals were male. No interchange was observed for BSA or ASHW. The interchange index (I) ranged from 0.001–0.046 resulting in a change in magnitude of 1x-45.783x, with the highest I value observed between BSC2 and BSC3, and the lowest between BSB1 and BSC3 (Fig. 1b).

Connectivity between breeding and feeding areas

Genotypic matches for 7 individuals (4 males; 3 females) were recorded between breeding areas and feeding grounds in the Southern Ocean (Table 1). Six individuals were observed within the nucleus feeding area for BSB designated under IWC Allocation Hypothesis 1, however, only three of these individuals were sampled in the BSB breeding area. The remaining matches comprised two females sampled in BSC1 and one male sampled in BSC3. One male sampled in BSC3 was matched to an individual sampled in the nucleus feeding area for BSC.

DISCUSSION

Fidelity to breeding areas

Genotypic matches confirm that humpback whales show fidelity to breeding areas and also, in the case of BSB2, to feeding areas and migratory routes. Counter to expectations that site fidelity would be observed to a greater extent for females due to maternal philopatry, our results demonstrate a skew towards males (sex ratio 2.5:1), above that observed for the total sample (sex ratio 1.7:1). While the skew in sex ratio may be influenced by sampling effects, for example, if there is temporal variation when different sexes arrive at a breeding location (Carvalho *et al*. 2014), our results suggest that males are at least as likely as females to return to the same area to breed in multiple years. Male fidelity has also been observed more frequently than females both

intraseasonally and interannually on breeding grounds in Hawaii (Craig & Herman 1997) and New Caledonia (Garrigue *et al*. 2001), and a male-biased distribution is common to humpback whale wintering grounds worldwide (Herman *et al*. 2011).

The pattern of male-biased fidelity may be due to male seasonal residency on breeding areas exceeding female residency on average, perhaps due to continued searching strategy by for mates, whereas females tend to depart the breeding area once impregnated (Herman *et al*. 2011). In the absence of feeding or predation pressure on breeding grounds, sexual selection is the primary force shaping the breeding behavior of the species (Cerchio *et al*. 2005), which adheres to a mating system of male dominance polygyny (Emlen & Oring 1977) and displays the features of a 'floating lek' (Clapham 1996) involving the establishment of dominance rankings among singing males that are temporarily resident in a specific location (Clapham 1996; Cerchio *et al*. 2005). The existence of a dominance hierarchy and associated lekking behavior among male humpback whales is supported by observations of a reproductive skew suggesting that certain males may have slightly greater reproductive success, although this may be the product of several alternative mating strategies rather than singing alone (Cerchio *et al*. 2005).

The highest return index values were observed for BSB2, supporting previous genetic and photo-identification evidence of long-term fidelity to this area (Barendse *et al*. 2011; Carvalho 2011; Fig. 2b). This finding suggests that migratory and feeding behavior may also be conserved, perhaps as a result of vertical cultural transmission of migration routes and destinations between mother and calf (Alter *et al*. 2009; Valenzuela *et al*. 2009; Barendse *et al*. 2013). In contrast to BSB1 and BSC3, we observed a slight bias towards females (12 males, 21 females) in the genotypic matches for BSB2, possibly due to the samples being collected during

the southern migration of whales from the BSB1 breeding area and therefore comprising a larger proportion of impregnated females and mother-calf pairs (Carvalho 2011; Herman *et al*. 2011).

On the east coast of Africa, BSC1 provides an interesting contrast to BSB2. Similar to BSB2, the southern portion of BSC1, from Mozambique Island (15°S) to Cape Agulhas (~34.5°S) is considered primarily a migratory route towards breeding areas north of Mozambique Island to at least the southern border of Tanzania (4°S) (Findlay *et al*. 1994; Banks 2013). Unlike BSB2, however, no genotypic matches were found within BSC1 in multiple years in this study or in previous work (Carvalho 2011). One explanation for the lack of matches for BSC1 is that animals show lower levels of fidelity to the southern portion of BSC1 than to BSB2 indicating that whales in this region exhibit different behavior to those off the west coast. Alternatively, animals may be more rapidly moving through the region towards breeding areas north of Mozambique Island, thereby reducing the likelihood of being resampled (Carvalho 2011; Banks 2013). Observations of the timing of migrating whales (Best *et al*. 1998), greater swim speeds, and very few observations of opportunistic feeding (Banks 2013) off east South Africa suggest that, if behavioral differences are a contributing to the lower the number of individual recaptures for BSC1 than BSB2, this latter scenario may be the most likely.

However, a number of factors related to sampling bias cannot be overlooked as a possible explanation for the differences observed. The estimated population size of BSB2 is approximately 510 individuals (95% CI: 230-790; Barendse *et al*. 2011) and therefore comprises many fewer animals than BSC1 currently estimated at 7,035 (90% CI: 5,742-8,824; IWC 2010). Therefore, even if site fidelity between the two locations is comparable, the likelihood of resampling the same individual is considerably lower in BSC1 than BSB2. Moreover, the geographic expanse of the range of BSC1 is significantly greater than BSB2, that likely only

comprises whales undertaking a coastal migration route south of Namibia (i.e. south of 23°S) (Elwen *et al*. 2014) and for which samples were collected in one location off west South Africa (~33°S). Use of larger geographic areas by individuals would be expected to result in shorter occupancy times in a given location and therefore fewer opportunities for sampling the same individual would be expected (Banks 2013).

Humpback whale migratory behavior appears to be relatively plastic and individuals have been observed to delay or change their migration in order to exploit productive feeding areas (Gales *et al*. unpublished data). The difference in migratory behavior observed for BSB2 compared to BSC1, if real, may therefore be explained by oceanographic conditions (Carvalho 2011). The waters of west South Africa are rich in nutrients as a result of the Benguela Current system and an extensive coastal upwelling system (Ansorge & Lutjeharms 2007). This region therefore represents a persistent feeding area for many species, including southern right whales (*Eubalaena australis*; Best & Schell 1996) and Bryde's whales (*Balaenoptera edeni*; Penry *et al*. 2011). No similar upwelling system is found off the east South African coast. It is therefore likely that BSB2 whales show increased residence time and long-term fidelity to the waters off West Africa given the presence of a stable food source, whereas whales from BSC1 travel directly to breeding areas in order to conserve energy during the migration (Carvalho 2011).

Interchange between different breeding areas

The rate of interchange between BSB1 and BSB2 was relatively high (n=7; I=0.018; magnitude=17.8) and included all individuals matched in the same year, indicating that BSB2 comprises at least a portion of the animals migrating south from BSB1. This rate of interchange is also supported by the photo-identification of three different individuals at both locations

(Barendse *et al*. 2011). Satellite telemetry data suggests that whales breeding in the Gulf of Guinea may take at least two separate migratory routes to Southern Ocean feeding areas, one close to the coast and another offshore (Rosenbaum *et al*. 2014). The high levels of interchange we observed for BSB1 and BSB2 may be representative of this division, with BSB2 whales representing the inshore migrants, and provide additional support to the assumption that migratory routes are also conserved across generations. Notwithstanding the evidence of interchange demonstrated here, significant genetic differentiation has been found between BSB1 and BSB2 at the population level (Rosenbaum *et al*. 2009; Carvalho *et al*. 2014; Kershaw *et al*. in prep). This indicates that the individuals recorded in both BSB1 and BSB2 may not be contributing genetically to both populations but rather are only traveling through or utilizing habitat in both regions, as would be expected given that BSB2 represents a migratory corridor and feeding area (Best *et al*. 1998).

High levels of gene flow have been observed between the three substocks of BSC (Rosenbaum *et al*. 2009; Kershaw *et al*. in prep) and our results suggest that current interchange may play a significant role in driving this connectivity, although the effect of retained ancestral polymorphism cannot be discounted. Interchange was highest between substocks BSC2 and BSC3 (Fig. 1b), and one female was found to switch from BSC2 to BSC3 and back to BSC2 over a period of 6 years. Photo-identification studies (Ersts *et al*. 2011) and satellite tracking data (Fossette *et al*. 2014; Cerchio *et al*. unpublished data) also show considerable movement between BSC2 and BSC3, which are in relatively close geographic proximity. We also observed interchange, although to a lesser degree, between BSC1 and BSC3 (Fig. 1b), supporting suggestions that a northern migratory stream may be present between the east coast of Africa (BSC1) and northeastern Madagascar (BSC3) (Cerchio *et al*. unpublished data). Despite

relatively high gene flow estimates between BSC1 and BSC2 (Rosenbaum *et al*. 2009; Kershaw *et al*. in prep), no direct interchange was detected.

Long-distance movements were observed between BSB1 and BSC3 and, for the first time, between BSB2 and BSC1 (Fig. 1b). All individuals were male, supporting the general model of long-distance male dispersal (Baker *et al*. 1998, 2013). That these are the first records of connectivity between BSB2 and BSC1 (Banks *et al*. 2011) is surprising given their geographic proximity and indicates that more complex behavioral drivers, such as the influence of different oceanographic conditions on fidelity and migratory behavior, may be maintaining the isolation of these two substocks (Carvalho 2011). Previously recorded genotypic matches between BSB1 and BSC3 (Pomilla & Rosenbaum 2005) and estimates of gene flow based on mtDNA (Rosenbaum *et al*. 2009) have suggested a westward bias in movements from BSC to BSB. This westward bias was not supported by gene flow estimates based on nuclear microsatellite data (Kershaw *et al*. in prep) however, and our results show both eastward and westward movement of males between BSB2 and BSC1.

Despite previous evidence of rare interchange between the western South Atlantic and the eastern South Atlantic and Western Indian Ocean (Darling & Sousa-Lima 2005; Stevick *et al*. 2013), we found no genotypic matches between BSA and any other breeding stock. This was not unexpected as BSA shows significant genetic differentiation from other stocks at the population-level (Rosenbaum *et al*. 2009; Kershaw *et al*. in prep), likely due to the population being isolated by geographic distance from stocks in the eastern South Atlantic; although may also have been influenced by the relatively small sample size for BSA. The lack of interchange we observed for ASHW in consistent with this breeding stock being non-migratory and extremely isolated from

other stocks and substocks in the Western Indian Ocean (Minton *et al*. 2011; Pomilla & Amaral *et al*. 2014).

Connectivity between breeding and feeding areas

The seven genotypic matches we recorded between breeding and feeding areas support existing evidence for a high degree of mixing of individuals from BSB and BSC on feeding areas (IWC 2011; Amaral & Loo *et al*. in review; Kershaw *et al*. unpublished data). Of the six genotypic matches observed within the B "Nucleus" feeding area (where 100% animals are allocated to BSB; IWC 2010), three were matched to individuals sampled within BSB and three to BSC (Table 1). While the single match between the C Nucleus region and one individual sampled in BSC3 cannot provide insights into mixing within the C Nucleus, it does confirm direct connectivity between these two locations (Table 1).

The B Nucleus region therefore appears to be a shared feeding area for animals from BSB1 and BSC. This finding is supported by genetic data showing that Management Area II (60°W-0°), which in part overlaps with the B Nucleus (10°W-10°E), has little significant differentiation from other Management Areas across the Southern Ocean (Amaral & Loo *et al*. in review), apart from the eastward adjacent Management Area III (0°-70°E) that encompasses the C Nucleus feeding area (30°E-60°E) (Amaral & Loo *et al*. in review). However, a direct comparison of the B and C Nucleus regions indicates that they are not significantly differentiated (Kershaw *et al*. unpublished data). These contrasting findings indicate that genetic differentiation is sensitive to the longitudinal boundaries used to divide the feeding areas associated with BSB and BSC, particularly between 20°W and 30°E. In additional to longitudinal boundaries, genetic

evidence suggests latitudinal variation in the distribution of BSB whales in the Antarctic (IWC 2011) indicating that this should also be explored in future studies.

Given the relatively small sample sizes for feeding areas used in this study, our results suggest that individuals from BSB and BSC may commonly mix in the B Nucleus feeding area. This is supported by satellite telemetry data for individuals tagged off west South Africa that demonstrate wide westward (~15°W) and eastward (~40°E) movements on sub-Antarctic and Antarctic feeding areas (Seakamale *et al*. 2015). Evidence suggests that baleen whales are capable of significant behavioral plasticity with regards to environmental variability, including opportunistic feeding during migration (Gales *et al*. unpublished data) and adjusting the timing of their seasonal migration to feeding areas in response to climate-change driven shifts in prey availability (Ramp *et al*. 2015). On feeding areas, these species are often associated with habitats characterized by high krill abundance and density, such as banks and canyons, the sea-ice edge, and variable frontal systems such as the Antarctic polar front (Wada & Numachi 1991; Hoezel 1998; Stevick *et al*. 2006; Sremba *et al*. 2012). Seasonal and inter-annual variability of these oceanographic features, for example changes in the distribution and dynamics of sea ice (Thiele *et al*. 2004), are likely to lead to spatial and temporal changes in prey distribution and abundance (Croll *et al*. 1998). For example, inter-breeding area movements by humpback whales between eastern and western Australia has been associated with the atypical distribution of prey during the intervening feeding season (Chittleborough 1959), and the first documented movement of an individual humpback whale between the eastern South Pacific breeding stock off Ecuador (BSG) and BSA in the western South Atlantic coincided with a particularly strong El Nino Southern Oscillation (ENSO) event (Stevick *et al*. 2013). ENSO events affect the entire food web in the eastern South Pacific and may also extend into polar regions, potentially leading whales to

forage in atypical locations or move more widely in search of prey (Stevick *et al*. 2013). It would therefore be expected that greater longitudinal movements and higher levels of mixing would occur in regions where prey distribution was less dense and more variable, as is the case in the Atlantic and Southwest Indian Ocean sectors of the Southern Ocean (Nicol *et al*. 2000).

Management implications

This individual-level analysis has provided important insights into the contemporary drivers of the genetic structure and connectivity of the humpback whale breeding stocks and substocks managed by the IWC in the South Atlantic and Western and Northern Indian Oceans. At the regional scale (i.e. between breeding stocks), the observed fidelity to breeding areas and the low levels of long-distance interchange between breeding stocks echo the findings of population-level genetic studies (Rosenbaum *et al*. 2009; Kershaw *et al*. in prep) that show general support for the designation of the four breeding stocks (BSA-C, ASHW) by the IWC. At the local scale (i.e. within breeding stocks), the high levels of interchange observed between BSB2 and BSC, and between each of the BSC substocks , corroborates estimates of high gene flow for both sexes between these substocks at the population level (Kershaw *et al*. in prep). Our findings indicate that the high estimates of gene flow at the population-level are, at least in part, the result of ongoing demographic exchange rather than as a product of historic connectivity or shared ancestral polymorphism. This work highlights the importance of current research into understanding current demographic exchange using multiple methods and incorporating this information into the delineation of population management units.

TABLES AND FIGURES

Table 1. Sex and date of genotypic matches found between breeding areas and Antarctic feeding areas as defined by IWC Allocation Hypothesis I. *indicates matches identified in IWC (2009).

Individual	Sex	Breeding area	Date	Feeding area	Date
1*	M	BSC3	2000	B Nucleus	2006
2*	M	BSB1	2001	B Nucleus	2006
3	M	BSB1	2002	B Nucleus	1998
4	F	BSC1	2003	B Nucleus	2006
5	F	BSB1	2003, 2005	B Nucleus	2007
6*	F	BSC1	2004	B Nucleus	1997
7	M	BSC3	2004	C Nucleus	1999

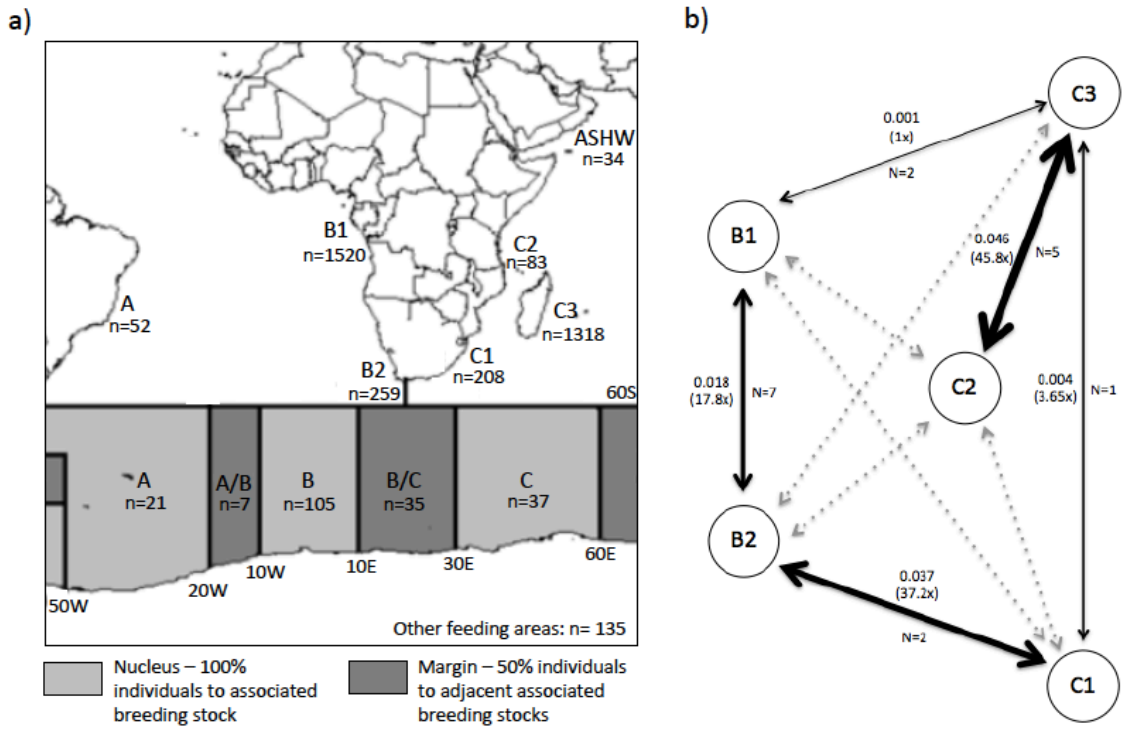

Fig. 1. a) Location and sample sizes of the seven breeding stocks and substocks, and their associated "Nucleus" and "Margin" feeding areas; b) Interchange index (I) values between Breeding Stocks B and C. Magnitudes are shown in parentheses and reflected in the width of the arrow. Gray arrows indicate potential connections but where no interchange (I=0) was observed in the sample.

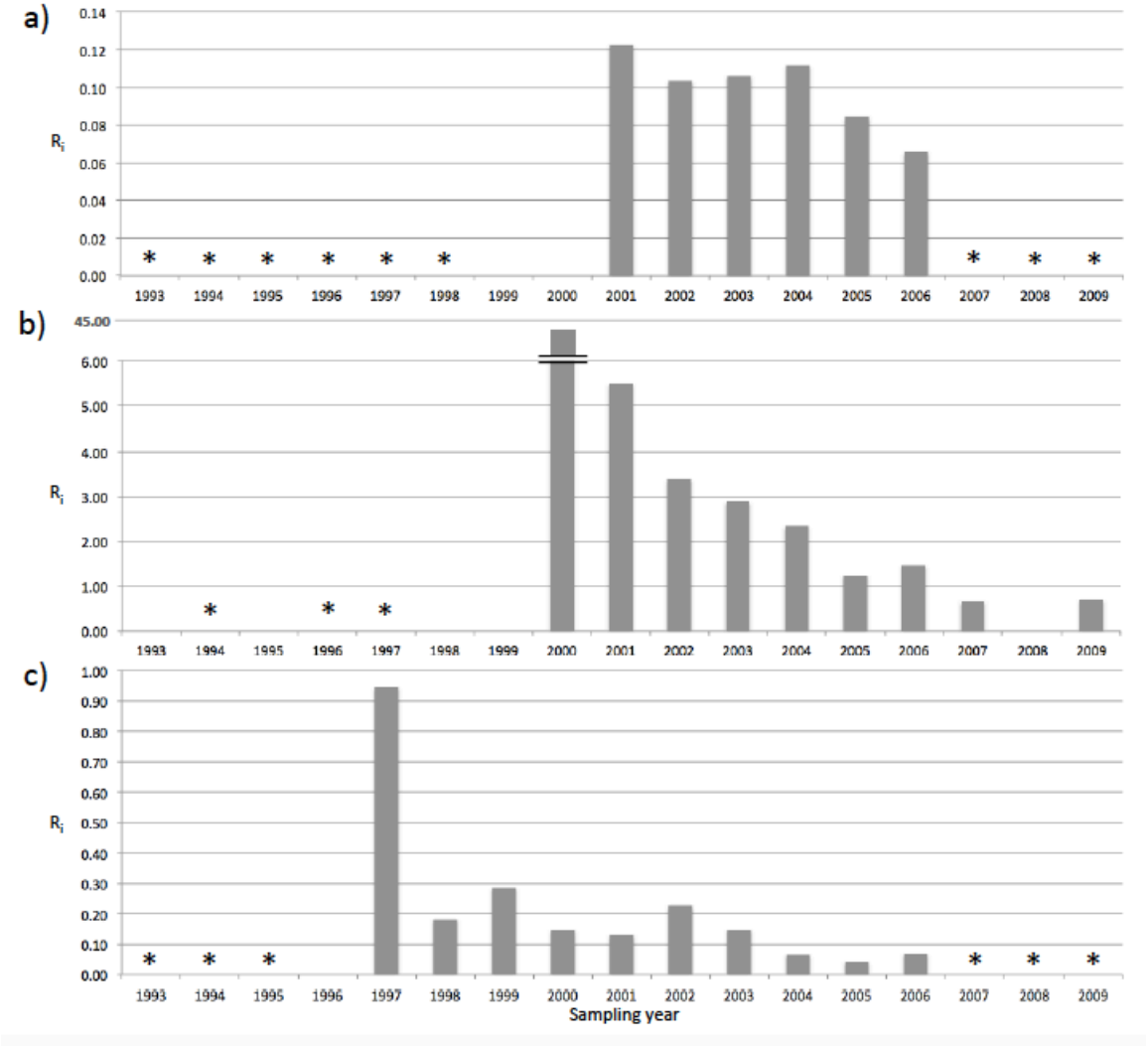

Fig. 2. Return index (R_i) values for a) BSB1, b) BSB2, and c) BSC3, for the entire duration of sampling for this study. Note the different scales for R_i for each breeding area. R_i values can only be calculated from the second year of sampling onward. Asterisks indicate years where no sampling was carried out.

CHAPTER FIVE

Achieving a mechanistic understanding of genetic population structure

FRANCINE KERSHAW and HOWARD C. ROSENBAUM

ABSTRACT

The ability to identify and quantify the multiple factors influencing genetic population structure would offer mechanistic insights into how ecological processes are linked to evolutionary patterns. New methods in individual-based modeling enable such investigations by providing a flexible platform upon which behavioral, environmental, and genetic data can be integrated. Here, we present a research prospectus for developing individual-based models (IBMs) capable of advancing our understanding of the interactions between ecology and genetic architecture. We focus on highly migratory species (HMS) due to their diverse range of genetic population structures resulting from complex interactions between life history and environmental conditions. We begin by reviewing recent advances in the field of IBM development, which have resulted in this approach now offering a framework for the integration of individual movement behavior with environmental and genetic data. To guide model implementation, we transfer lessons from a number of applied case studies of recently developed IBMs from a variety of fields**.** We first provide examples of how genetic parameters, such as genetic diversity, within-population variation, and genetic connectivity, may be included in an IBM framework. We then consider the integration of parameters for physiological condition and life history stage, which may directly influence an individual's dispersal or breeding behavior. We discuss how environmental conditions, which may play an important role in determining when and where an individual chooses to move, can be incorporated. The use of multiple gridded environmental data sets enable models to account for the temporal dynamism of natural systems, and also allows for the testing of the relative influence of different environmental parameters at different spatial scales. We subsequently describe methods for simulating the movement behavior of individuals to enhance biological realism. The challenge of developing models for HMS is their potential

complexity, and so we present a number of recent advances in model parameterization and validation, which will enable the development of IBMs in the absence of rich data sets or where the systematic ground truthing of model outputs is not feasible; both of which represent key constraints when developing models for HMS. Finally, we emphasize the utility of developing IBMs for data poor species. As IBMs are not constrained by existing knowledge of the system they therefore represent a useful tool to explore and generate hypotheses regarding the mechanistic processes underlying observed patterns. They also offer an alternative to impractical field studies and are therefore particularly useful for studies of HMS, which are logistically challenging to study throughout the entirety of their range. We conclude that, while achieving a mechanistic understanding of patterns of genetic population structure still represents a significant challenge, new modeling techniques are now capable of facilitating a cautious yet concerted effort towards establishing a solid foundation for this field of research.

INTRODUCTION

The ability to identify and quantify the behavioral drivers of genetic population structure would offer unique insights into questions concerning how ecological processes are mechanistically linked to evolutionary patterns. Genetic population structure is commonly observed in wild populations and arises from variation in the spatial and temporal distribution and movement of individual organisms, which over evolutionary meaningful timescales results in systematic variation in population allele frequencies through space and time (Jones & Wang 2012). Population structure is driven by complex interactions between processes operating at the level of the individual organism, including behavioral responses to internal state (Piou & Prévost 2012), ecological factors (Andrews *et al*., 2010), environmental conditions (Schunter *et al.* 2011; Kormann *et al.* 2012), and micro-evolutionary factors, such as genetic drift and gene flow (Gaggiotti *et al.* 2009); all of which operate within the broader context of historical phylogeography (Scoble & Lowe 2010; Muscarella *et al*. 2011).

Identifying and quantifying the relative influence of each type of process is fundamental to gaining an understanding of the mechanisms underlying patterns of genetic structure and connectivity observed in natural populations. Such an understanding would not only enable our interpretation of the mechanisms underlying existing genetic patterns, but also enable us to forecast how these patterns may change in the future (Blair & Melnick 2012). In the long term, these insights may also facilitate our ability to predict evolutionary trajectories at the species-level (Li *et al.* 2012). Achieving such a task will require a research prospectus that fully integrates behavioral, environmental, and genetic data.

As the burgeoning field of landscape genetics (Manel & Holderegger 2013) and the growing field of seascape genetics (e.g. Galindo *et al.* 2010; Mendez *et al.* 2010, 2011; Selkoe *et*

al. 2010; Amaral *et al.* 2012a,b; Treml *et al.* 2012) demonstrate, our understanding of how the environment shapes genetic population structure continues to broaden. It is becoming increasingly apparent, however, that focusing on environmental influence alone is inadequate to fully explain the genetic patterns observed for certain species that may select for particular habitat types but are able to tolerate a wide range of environmental conditions. In these cases, population boundaries do not always align with environmental boundaries.

Population-level fidelity to breeding and feeding sites, for example, is an important driver of genetic structure for a number of migratory species. For instance, humpback whale populations in the Southern Hemisphere are genetically structured as a result of strong site fidelity to winter breeding areas in the tropics and summer feeding areas in the nutrient rich Southern Ocean (Rosenbaum *et al.* 2009; Ruegg *et al*. 2013). Within regions, however, there is increasing evidence that population structure is driven by subtle, socially driven dispersal and migratory behaviors. Fine-scale differences in measures of population substructure across time provide evidence for temporal segregation on the basis of age, sex, and reproductive status (Rosenbaum *et al.* 2013; Carvahlo *et al*. 2014). A similar scenario exists within the migratory corridor of the Pacific common eider, which breeds along twelve barrier islands in the Beaufort Sea. Here, nesting location is likely determined by female philopatry that, in turn, is correlated with time of arrival following migration (Sonsthagen *et al*. 2009). Due to the strong hysteresis (or 'memory') effects (Guttal & Couzin 2010) associated with philopatry, species that exhibit strong fidelity to core habitats may be particularly sensitive to anthropogenic impact and environmental change. Populations may fail to recolonize suitable habitat following local extirpation (Clapham *et al.* 2008) or may be unable to respond to shifts in prey distribution by

finding and switching to new feeding areas, resulting in an increased risk of malnutrition and demographic decline (Leaper *et al.* 2006).

Behavioral partitioning within populations has also been noted as an important driver of genetic structure (Taylor & Friesen 2012). For example, genetic divergence between colonies of Cook's petrel has been linked to segregation of different populations during the non-breeding season due to habitat specialization (Rayner *et al*. 2011). Similar partitioning has been observed at the individual level in the highly migratory Bewick swan, where current and future reproductive success was found to correlate with individual foraging specializations along a terrestrial-aquatic gradient (Hoye *et al.* 2012). Social dynamics may also play an important role. In the Hawaiian archipelago, spinner dolphins exhibit two different social strategies, stable versus dynamic group membership, which are associated with low and high levels of gene flow, respectively (Andrews *et al*. 2010).

Integration of behavioral factors into studies of the mechanisms driving the genetic population structures of these species therefore proves essential. The central goal of this review is to provide an overview of the emerging field of individual-based modeling (IBM), which is capable of supporting the necessary integration of behavioral, environmental, and genetic data (Grimm & Railsback 2011). We focus on describing these approaches in the context of understanding the mechanisms underlying patterns of genetic population structure in highly migratory species (HMS) due to their often complex genetic structures, driven not only by environmental influences, but also innate and learned behaviors, life history stages, and conspecific interactions. As many of the case studies herein demonstrate (Table 1), however, these approaches are widely applicable to investigate the behavioral mechanisms underlying patterns of genetic population structure in any marine or terrestrial species.

THE INTEGRATION OF BEHAVIOR

The multitude of examples described in the Introduction demonstrate that it is essential for individual-based behavioral ecology to be considered in attempts to quantify the different mechanisms underlying patterns of genetic population structure in HMS. Challenges of data limitation and computational power have impeded our ability to meet this need in the past; however, recent advances in individual-based (or "agent-based") modeling now offer a framework for the integration of individual movement behavior with environmental and genetic data (Epperson *et al.* 2010; Grimm & Railsback 2011).

Individual- (or "agent-") based models (IBMs) follow the fitness-maximizing behavior of individuals and enable the prediction of population-level consequences (e.g. rates of gene flow) (Grimm & Railsback 2011). Computer simulations can be used to model discrete individuals within a population, including components of the individual's life cycle, variation among individuals, interactions between individuals, and the dynamics of the resources they use (Fig. 1; Stillman & Goss-Custard 2010). In contrast to other types of deterministic models, IBM's are based on the concept of emergence, where behavior is not imposed by programmed empirical rules, but rather emerges from the model based on the simulated individual's behavioral decisions determined by the set of fitness-maximizing decision rules (Stillman & Goss-Custard 2010; Grimm & Railsback 2011). IBMs are therefore not constrained by existing knowledge of the system and represent a useful tool to explore and generate hypotheses regarding the mechanistic processes underlying observed patterns (Grimm & Railsback 2011). They also offer an alternative to impractical field studies by representing "virtual experiments" (Tamburino &

Bravo 2013), which are particularly useful for studies of HMS that are logistically challenging to study throughout the entirety of their range.

Complexity is an important feature of IBMs, as it is their ability to synthesize a broad range of knowledge that can lead to previously unforeseen emergent properties (Piou & Prévost 2012). Early IBMs had little standardization of model code or structure, leading to criticisms of opacity and over-complexity (Topping *et al*. 2010). The recent publication of an Overview, Design concepts, and Details (ODD) protocol offers a framework for standardizing published descriptions of individual-based models (Grimm *et al.* 2010). The application of ODD has been shown to improve the rigor of model formulation and helps make the theoretical foundations of large models more visible, therefore improving their reproducibility (Grimm *et al*. 2010). In addition, the 'pattern-oriented modeling' (POM) strategy of Grimm and Railsback (2012) offers model development guidelines that explicitly account for the multi-criteria design, selection, and calibration of models of complex systems. This approach makes the selection and use of modeled patterns more explicit and rigorous, thus facilitating the development of models that have appropriate levels of complexity and predictive ability (Grimm & Railsback 2012).

DEVELOPING IBMS FOR HIGHLY MIGRATORY SPECIES

Capturing the complex behaviors of HMS within an IBM is not a simple task, and it will likely be infeasible to parameterize all relevant behaviors. These challenges are exacerbated by general scarcity of data and information for HMS throughout all stages of their life history. There is good reason for optimism, however, at least for developing IBMs for better-known species. In our view, the goal of developing IBMs for HMS should not necessarily be to fully replicate the system, but rather to develop a useful hypothesis-generating tool for exploring the possible

mechanisms underlying the genetic structure of populations. Below, we outline some ways to approach the task of developing IBMs for explaining the genetic population structures of HMS. We also present a number of case studies that transfer lessons from recently developed IBMs in the wider field of ecology and evolution to address questions concerning HMS specifically (see Table 1).

(1) Integrating genetics and evolutionary history

IBMs are now being used to explicitly explore interactions between ecological (co-occurrence of individuals in space and time) and evolutionary (reproductive interactions between individuals and micro-evolutionary processes) paradigms (Frank & Baret 2013). Advances in the field of individual-based genetics (Planes & Lemur 2011) enables modeled individuals to be parameterized with genotypic information, providing a useful baseline for explorations into the effects of mutation, genetic drift, migration, and natural selection on the genetic composition of a modeled population (Piou & Prévost 2012; Frank & Baret 2013). Recently, the terms "demo-genetic" and "eco-genetic" have been adopted to describe IBMs specifically aimed at understanding the relative importance of population genetics and quantitative genetics, respectively, on life history traits and population dynamics (Piou & Prévost 2012; Frank & Baret 2013).

(a) Understanding the genetic diversity of populations

The genetic structure of populations reflects the distribution of genetic diversity across different groups of individuals (Jones & Wang 2012). IBMs that account for both the demography and genealogy of a population can be used to elucidate the mechanisms underlying observed levels

genetic diversity, such as isolation and/or a reduction in population size (Kekkonen *et al*. 2012). Further, such models can be used to simulate a population's historic diversity, or project how it may change in the future. For example, Kekkonen and colleagues (2012) developed an individual-based population genetic model to explore whether the relatively high level of genetic diversity in a population of white-tailed deer could be understood based on historical information that the founder population comprised only four individuals (Kekkonen *et al.* 2012). This information appeared to be conflicting as the level of genetic diversity for a population with such a small number of founders would usually be expected to be extremely low. However, observed levels of heterozygosity were found to be concordant with the model's predictions based on the recorded founding population, suggesting that a small number of founders does not necessarily cause a significant reduction in heterozygosity in iteroparous species (Kekkonen *et al.* 2012).

The ability to simulate the potential processes leading to observed levels of genetic diversity is particularly useful for migratory species that have been subjected to commercial harvest. Alter and colleagues (2012) employed a similar combined demographic and genealogical modelling approach within an Approximate Bayesian Computation (ABC) framework to estimate the pre-whaling population size of the eastern Pacific gray whale, a species heavily impacted by commercial hunting in the 19th and 20th centuries. The model estimated the pre-whaling population to be three to five times its current size and supported a single bottleneck coincident with the height of the whaling activity for this species, countering claims of an earlier bottleneck driven by climatic factors (Alter *et al*. 2012). IBMs may help refine this type of population-level model by incorporating individual-level information into the demographic parameters, leading to more realistic simulations of population dynamics. Where information is more limited, IBMs represent a useful tool to explore potential processes driving

observed levels of genetic diversity. In a recent study on Bryde's whales (*Balaenoptera edeni*), the level of genetic diversity for the subspecies *B. e. edeni* was relatively low compared to that generally observed in Balaenopterids (Kershaw *et al*. 2013). IBMs could be used to simulate a range of different scenarios to elucidate whether this low level of diversity is the result of a small founding population, historic whaling activity, or a selective sweep. The application of IBMs in this way would be particularly useful for understanding the long-term trajectory of the genetic diversity of HMS that are recovering from commercial hunting (Ruegg *et al.* 2013) or to gain insights into possible isolating mechanisms leading to sympatric speciation (Amaral *et al*. 2012b).

(b) Accounting for within-population variation: time-selection

Studies into the genetic structure of populations continue to reveal increasing levels of complexity, often resulting from inter-individual behavioral differences within the same population. In some highly migratory species, there is evidence of plasticity in the timing of different life history stages among individuals (Conklin *et al*. 2013; Rosenbaum *et al*. 2013). These inter-individual differences in the timing of migration, or "time-selection" (Conklin *et al.* 2013), represent one of the mechanisms capable of driving fine-scale temporal population genetic structure and also reflect the capacity of a species to respond evolutionarily to environmental change (Reed *et al.* 2010).

Examples of variation in time-selection occur across a diverse range of taxa. New Zealand bar-tailed godwits undertake one of the longest recorded annual migrations, departing from New Zealand in the austral summer to breed in Alaska between May and July (Conklin *et al.* 2013). In a two-year study, Conklin and colleagues (2013) observed that the timing of pre-

breeding movements was conserved across the population, indicating increased fitness benefits of a time-constrained northern migration. In contrast, post-breeding movements exhibited much greater inter-individual variation, with departure from breeding grounds representing the most variable annual movement; however, at the individual level, godwits maintained relatively consistent timing of these post-breeding movements across years (Conklin *et al.* 2013). High heterogeneity in the timing of migratory movements has also been observed in the population of humpback whales that breed off the west coast of Africa. Satellite telemetry data demonstrate that, within the same time period, groups of whales are either still migrating north, residing in a breeding area, or have commenced their southbound migration to feeding grounds (Rosenbaum *et al*. 2013). This variation is reflected in the genetic structure of the population (Rosenbaum *et al.* 2009).

IBMs offer great utility for incorporating complex life history parameters into simulations of genetic population structure and quantitative genetic responses to environmental change. To understand how the population structure of salmonids may be affected by variation in life history stages, Piou & Prévost (2012) developed an integrative demo-genetic IBM. The model realistically captures the complex life cycle of the Atlantic salmon, including the timing of maturation and resulting commencement of the oceanic migration, and also accounts for environmental factors during each migratory stage, such as river and ocean climate. The model's structure incorporates both individual variability and potential microevolution of life histories, enabling the parsing of microevolutionary processes and plastic responses, and therefore allowing explorations into how the population structure of salmonids could be modified as a result of to environmental change (Piou & Prévost 2012).

(c) The nuances of genetic connectivity

Quantifying how different habitats may facilitate and constrain dispersal, and therefore gene flow, has been a key focus of landscape genetics (Wang et al. 2009; Landguth *et al.* 2010a; Zhu *et al.* 2010). The application of circuit theory to this question has enabled exploration into how different habitats may pose resistance to gene flow and provided a means to spatially map likely dispersal pathways (i.e. those of 'least resistance') across the landscape (Blair & Melnick 2012). However, dispersal models that only consider environmental resistance remain relatively simplistic. For example, long distance dispersal events across habitat generally perceived as 'resistant' may play a significant role in maintaining genetic connectivity (Berkman *et al*. 2013), even if such events occur only rarely (Landguth *et al.* 2010b). Individuals may also choose to disperse as a result of physiological (Domeier *et al*. 2012; Burns *et al.* 2013) or socially-mediated factors, resulting in groups dispersing based on the movement decisions of other individuals within their social network, rather than the condition of the surrounding environment (Premo & Hublin 2009; Guttal & Couzin 2010).

Integrating IBMs into existing habitat resistance frameworks offers an opportunity to explore these complexities in more detail. Hargrove and Westervelt (2012) developed a modified Pathway Analysis Through Habitat (PATH) computer simulation model to identify the essential mechanisms that determine animal migration corridors. The model converts expert knowledge about habitat patch locations, and individual-based information on the energetic cost of traversal and probability of mortality associated with non-habitat, into information about the relative connectivity of all pairs of habitat patches and the most favored pathways (Hargrove & Westervelt 2012).

Simulated individuals can also be attributed with parameters relating to conspecific interactions that determine socially influenced movement behaviors. Using IBM simulations, Guttal and Couzin (2010) demonstrated that generally only a small proportion of the population (i.e. the "leaders") actively acquire the information that determines dispersal movements from their environment, or retain the memories of previous dispersal routes. Rather, the majority of individuals exhibit socially facilitated movement behavior through their attraction to "leaders" (Guttal & Couzin, 2010). This type of model would be particularly useful for investigating the structure of migratory species, where only a few individuals may lead their characteristic collective, long-distance migrations.

To understand how these behaviors directly affect levels of genetic connectivity, genotypic information can be assigned to each simulated individual and used to generate a simulated genetic data set that could be analyzed and directly compared with observed measures of gene flow (Frank & Baret 2013). Moreover, genetic sub-models that account for other evolutionary forces that influence population differentiation (e.g. drift, mutation, divergence time) can be embedded, providing a more complete understanding of the population genetic relationships observed (Marko & Hart 2011).

The spatiality of individual-based models means that resulting maps of genetic connectivity are useful for defining population boundaries to inform species-based management, particularly in terms of protected area network planning and the designation of habitat corridors (Klein *et al*. 2009; Decout *et al.* 2012; Hargrove & Westervelt 2012). Further, by integrating models of genetic population structure and connectivity with a quantitative genetic framework, insights can be gained into both genetic and plastic responses to environmental change, serving

to inform the adaptive management of species influenced by climatic change (Piou & Prévost 2012).

(2) Accounting for physiological condition and life history

Important physiological influences on HMS population genetic structure may include, although are certainly not limited to, decisions related to internal state (e.g. physiological condition, life-history stage, evolutionary history and cultural memory) and inter-individual interactions (e.g. cohesiveness of kin-groups, competition, mate-selection). For example, an individual may make the decision to migrate to feeding grounds before its energetic resources fall below the threshold of the energetic requirements of the journey (Moriguchi *et al.* 2010). The age and sex of the individual may influence dispersal behavior, perhaps due to competitive or social interactions with conspecifics (Domeier *et al*. 2012; Burns *et al.* 2013). An individual may make decisions based on those of other members of its kingroup or social network (Guttal & Couzin 2010). If these groups were conserved through time, such cohesiveness may be reflected in the genetic substructure of the population (Rosenbaum *et al*. 2009; Carvahlo *et al.* 2014). Alternatively, an individual may simply memorize and consistently make the same decision (Burns *et al.* 2013).

Simulated individuals can be programmed with submodels (Grimm & Railsback 2011) that have the description of the physiological condition and life history stage embedded within them; essentially, the individual-based model enables mathematical submodels to take on spatial form and behavior (Vincenot *et al*. 2011). Submodels can comprise deterministic (i.e. modeled using mathematical functions) or stochastic elements (i.e. drawn from a pre-set probability distribution) (Hedger *et al.* 2013). For example, Hedger and colleagues (2013) used a combination of approaches to simulate salmon population abundance within a river in western-

central Norway (Hedger *et al.* 2013). Parameters such as recruitment and weekly growth were derived deterministically, whereas individual characteristics (e.g. sex, body mass, growth variation) and return from at-sea migration were derived probabilistically (Hedger *et al.*, 2013). A hybrid of individual-based and System Dynamics (SD) modeling (i.e. models based on ordinary differential equations) can be also be used. It has been suggested that SD submodels may be better suited to handle a given task (e.g. modeling energetic balance, demographic processes, etc.) or simply may offer a way to streamline the model (Vincenot *et al*. 2011).

(3) Integrating the environment

Environmental cues (e.g. seasonal prey distribution, presence of breeding habitat, barriers to dispersal resulting from environmental gradients) may also play an important role in determining when and where an individual chooses to move (Friedlaender *et al.* 2011; Visser *et al.* 2011; Bailleul *et al*. 2012). A number of individual-based modeling platforms (e.g. NetLogo) allow the direct importation of grids of spatial environmental data, with which individuals can also be programmed to interact. Railsback and Johnson (2011) simulated the foraging habitat selection of populations of migratory birds to investigate how land use and habitat diversity affect the ability of migratory bird populations to suppress an insect pest on Jamaican coffee farms (Railsback & Johnson 2011). Within the modeled study region, birds selected which neighboring grid cell they would move to (i.e. forage from) based on its environmental 'quality', as defined by the supply of the pest insect and other arthropod food (Railsback & Johnson 2011). In a more complex application, Guichard and colleagues (2012) modeled spatio-temporal patterns of invasive moth dispersal behavior by combining appetitive and pheromone anemotaxis (i.e. oriented movement) in response to wind, temperature, and pheromone conditions (Guichard *et*

al. 2012). Simulated individuals of HMS might be expected to consistently move towards grid cells with higher values of primary productivity in order to maximize fitness by exploiting more abundant food resources (Doniol-Valcroze *et al.* 2012). Alternatively, individuals could be programmed to avoid steep gradients in environmental conditions that correlate with genetic discontinuities and thus may represent physical barriers to dispersal (e.g. for coastal cetacean species; Mendez *et al*. 2010).

(4) Simulating movement behavior

If insights into the spatial and temporal patterns of gene flow are sought, then once the decision to move (e.g. to migrate to feeding grounds) has been made by a simulated individual, additional submodels would be required to parameterize *how* the individual moves to the chosen location. Accurately representing movement behavior, particularly those relating to dispersal or that are consistent among kingroups or subpopulations, is important for understanding patterns of gene flow and, as a result, population structure.

Movement submodels range from simple stochastic or random walk simulators (Palmer *et al*. 2011; Yackulic *et al.* 2011), to more realistic state-space models that partition movements into multimodal behavioral states (e.g. searching vs. transiting) based on the distribution of turning angles (Breed *et al.* 2012), to models that also account for the sensory perception of the individual to environmental conditions (Guichard *et al.* 2012). Simple versions of these latter models founded upon random walk and Brownian motion, including least-cost path and habitat resistance analyses, are commonplace in the field of landscape genetics (Landguth *et al.* 2010a; Palmer *et al.* 2011; Koen *et al*. 2012); however, their application in marine systems is currently limited. Akin to submodels for physiological condition and life history, movement submodels

can be programmed and embedded within simulated individuals. Movement submodels can also interact with other types of submodel within the same individual (e.g. different habitat selection by adult and juvenile life stages), or other individuals in the population (e.g. to correlate movements of parents and offspring).

When selecting a movement submodel the shape of the environmental gradient and the specific biological mechanism underpinning the behavior for the species of interest needs to be considered (Watkins & Rose 2013). Realistically modeling animal movement is very challenging due to a lack of knowledge of the underlying mechanisms and insufficient calibration data at the scale of interest (Watkins & Rose 2013); both of these issues are pertinent for HMS. The distance at which the individual can perceive environmental conditions (i.e. how many neighboring grid cells should a simulated individual base its decision upon) is a key point of consideration (Fletcher & Sieving 2010). The transfer of social information may also be an important factor, particularly for highly migratory populations in which the majority of individuals may be spatially naïve and make movement decisions solely in relation to the movements of a few 'leader' individuals (Guttal & Couzin 2010; Simpson & Sword 2010). In addition, a single movement submodel might not suffice at all scales of analysis (e.g. local movements on a breeding or feeding ground compared to long-distance migrations to and from these critical habitats); in this case, multi-scalar analyses represent a useful approach for identifying submodel inconsistencies (Yackulic *et al.* 2011).

TECHNIQUES FOR MODEL DEVELOPMENT

(1) Parameterizing IBMs for HMS

The challenge of developing models for HMS is their potential complexity; however, the process of parameterization promotes simplicity as the model that reproduces the observed pattern with a minimal set of parameters is considered to be optimal (Grimm & Railsback 2011). The influence of each model parameter on the modeled pattern can then be explored through sensitivity analysis (Grimm & Railsback 2011). Parameters are generally derived from a combination of information from published literature and empirical observations; however, acquiring enough information to parameterize the model is a significant challenge for many species.

Recent advances in model calibration tools that build on the fitness-maximizing principles of individual-based models offer a possible solution for a lack of calibration data. For example, Watkins and Rose (2013) proved that genetic algorithms (GAs) could be successfully used to calibrate a variety of movement models. A GA adjusts the values of a set of model parameters through selection, mutation, and recombination of a population of parameter vectors over the course of many simulated 'generations', with the goal of maximizing high fitness movement in a particular training environment (Watkins & Rose 2013). The GAs were found to effectively explore parameter space and consistently identify parameter values that produced high fitness (Watkins & Rose 2013). For a model of invasive moth dispersal, Guichard and colleagues (2012) successfully used GAs to explore the ranges of different parameters and to fit the final parameters on four model replicates (Guichard *et al.* 2012). Alternatively, a Bayesian approach to sensitivity analysis can be implemented. Parry and colleagues (2012) introduced a new methodology (Bayesian Analysis of Computer Code Outputs, BACCO) to rigorously analyze the sensitivity of an IBM's parameters. By making use of the general property that model outputs of interest tend to be smooth functions of their inputs, BACCO offers significant efficiency gains over typical Monte Carlo (MC) methods, as each model run can be evenly

dispersed through the input parameter space and information from each run is used more efficiently (Parry *et al.* 2012).

Due to the data limitations, it is likely that individual-based models will initially be developed for the species with the richest body of qualitative and quantitative data. Yet, it is important to note that for species depauperate in information, individual-based models can be very useful as an explorative tool to understand how different parameters and parameter values may be contributing towards any observed ecological or genetic pattern of interest. Indeed, one of the first stages in developing a model is to generate as many hypotheses as possible on what mechanisms might be important in driving the patterns in the study system (Grimm & Railsback 2011); as such, the process of developing individual-based models offers, in itself, a useful framework for thinking about the mechanisms driving complex systems.

(2) Validating mechanistic models of population structure

Following the successful parameterization of the IBM, the model can then be implemented and used to explicitly test hypotheses of the mechanistic underpinnings of observed genetic population patterns. Validating the results of such models, however, can pose a significant challenge. How can the modeler be sure that the processes quantified by the model truly reflect the processes occurring in the natural study population? This problem is particularly pertinent for non-model and wide-ranging organisms such as HMS, where detailed physiological and ecological is rarely available, and would require manipulative laboratory experiments and long-term field observations. Rather, for these species, the process of model validation will, by necessity, require the interdisciplinary examination of multiple lines of evidence (e.g. expert

assessments, field observations, genetic data, and satellite telemetry). Even then, it is likely that gaps will exist and highlight areas for future research.

There are, however, at least two increasingly available data sources that may act useful starting points for model validation: genetic and satellite telemetry data. Models developed for the purpose of understanding an observed genetic pattern are built on the assumption that population level genetic data (e.g. genetic distances such as F_{ST}, migration rates, etc.) already exist for the population of interest. The facility of IBMs to assign genetic information to each simulated individual essentially provides the modeler with the ability to construct a parallel simulated genetic data set that can be directly compared with the data set from the natural population (Fig. 2). This means that the same genetic analysis that was carried out for study population can be conducted on the simulated population. If the simulated data set reproduces the same genetic patterns as observed in the natural population then this would provide at least partial model validation (i.e. the model is successfully reproducing the genetic patterns observed in the natural population).

Satellite telemetry studies are increasingly being carried out for highly migratory species and offer detailed insights into movement behaviors at both the inter-and intra-individual level (Klassen *et al.* 2010; Block *et al.* 2011; Costa *et al*. 2012). Specific locations of animal movements are useful for model validation across multiple scales. At local scales, information such as turning angles (as facilitated by state-space models) and collective behaviors can be used to fine-tune the movement submodels embedded within simulated individuals (Breed *et al*. 2012). At larger scales, satellite telemetry may elucidate behavioral differences between groups of individuals within the same population, providing evidence of spatial and temporal variation that may be directly influencing genetic patterns (e.g. Rosenbaum *et al*. 2014; Carvahlo *et al*.

2014). It should be noted, however, that as satellite telemetry data can only ever provide a snapshot of the behaviors of the few sampled individuals within the population, generalizations of the observed behaviors to the population-level should be carried out with due caution. It is here that genetic data, which provides insights into broader population patterns, represents a useful complement to satellite telemetry studies; together, this information provides at least a useful starting point for validating IBMs for HMS.

CONCLUSIONS

(1) Achieving a mechanistic understanding of patterns of genetic population structure represents a significant challenge; however, new individual-based modeling (IBM) techniques are now capable of facilitating a cautious yet concerted effort towards establishing a solid foundation for this field of research.

(2) Combined use of behavioral information, genetic markers, spatial data on marked animals, and population simulations, within an IBM framework, offers an improved understanding of the ecological and behavioral mechanisms that drive population complexity; knowledge that is essential for effective species- and ecosystem-based management.

(3) Initially, analyses will be restricted to data-rich species, however it will be important to test how informative the resulting models are for other highly migratory species and biodiversity more generally. In order to overcome current data limitations, a concerted effort to routinely gather and analyze genetic data, as well as information on animal movements and social behavior, at multiple spatial and temporal scales both within and outside critical habitats, is required. Notwithstanding this need, IBMs offer a useful

hypothesis-generating tool for investigating and testing possible parameters that may be influencing observed evolutionary patterns in lesser-known species.

(4) In a conservation and management context, this work is particularly pertinent as consideration of evolutionary information is notably absent from international policy mechanisms driving protection initiatives, particularly in the marine realm (Klein *et al*. 2009; Sagarin *et al.* 2009; Laikre *et al*. 2010). Individual-based models also represent an important education tool (Rebaudo *et al*. 2011) for students, evolutionary biologists, and other stakeholders involved in natural resource management.

TABLES AND FIGURES

Table 1. Overview of the lessons that can be transferred from existing case studies for the development of individual-based models (IBMs) for highly migratory species (HMS). The table lists a brief summary of the IBM approach undertaken by the case study and the ways in which this approach can by applied to the development of IBMs for HMS.

Case study	Summary of IBM approach	Application to developing IBMs for HMS
Frank & Baret (2013)	Demo-genetic model to study the medium term impacts of human activities (i.e. migration barriers, stocking) on a population of brown trout.	Demo-genetic IBMs enable an exploration into the importance of population genetics on life history traits and population dynamics.
	Genotypic data were assigned to each simulated individual to examine the following demo-genetic output indicators: annual evolution of trout abundances, inbreeding coefficients (F_{IS}), and fixation indices (F_{ST}).	Assignment of genotypic data to simulated individuals can result in the generation of a simulated genetic data set that can be analyzed and directly compared with a data set derived from the 'real' study population.
		Enables an exploration into the effects of mutation, genetic drift, migration, and natural selection, on the genetic composition of a modeled population, and how these effects may be altered by a range of different scenarios (e.g. climate, disturbance, etc.).
Guichard *et al*. (2012)	Modeled the spatio-temporal patterns of invasive model dispersal by combining appetitive and pheromone anemotaxis (oriented movement) in response to wind, temperature, and pheromone conditions.	Simulated individuals may be programmed to behave/move in response to environmental gradients (e.g. net primary productivity, sea surface temperature, oceanographic current systems). Gradients may also be used to model social behaviors (e.g. maximum distance from a "leader" individual during migration (see Guttal & Couzin, 2010).
Guttal & Couzin (2010)	Simulated social interactions and showed that generally only a small proportion of the population (i.e. "leaders") actively acquire the information that determines dispersal movements. Rather, the majority of individuals exhibit socially-facilitated movement behavior through their attraction to "leaders".	Modelling alternative social interaction scenarios (e.g. minimum number of "leaders" required to maintain a migratory connection, strength of attraction to "leaders"). The conservation of social- or kin-groups through time would be expected to be reflected in the genetic substructure of the population.

Hargrove & Westervelt (2012)	Developed a modified Pathway Analysis Through Habitat (PATH) simulation model to identify essential mechanisms that determine the location of migration corridors. PATH integrates expert knowledge of habitat patch locations with individual-based information on the energetic cost of habitat traversal and probability of mortality, to quantify the relative connectivity between habitat patches.	Maps of connectivity between habitat patches are useful for understanding the likely spatial and temporal distribution of individuals as a result of short-term movements decisions, as well as longer-term patterns of genetic connectivity. Such maps are useful for species-based management (e.g. protected area network planning, designation of habitat corridors, etc.). Understanding the mechanisms underlying these patterns also enables the prediction of how the distribution of populations and species, and their genetic architecture, may be altered by forecasted environmental change.
Hedger *et al*. (2013)	Modeled the complete life cycle of the Atlantic salmon (*Salmo salar*) to estimate population abundance using a spatially explicit IBM that combined deterministic (i.e. based on mathematical functions) and stochastic (i.e. based on a pre-set probability distribution) elements.	Using a combination of deterministic and stochastic elements can produce a more realistic IBM capable of including both deterministic parameters when empirical data is most appropriate/available (e.g. recruitment, growth rate), and stochastic parameters when there is greater uncertainty or the process being modeled is stochastic by nature (e.g. return to breeding grounds following long-distance migration).
Kekkonen *et al.* (2012)	Developed an individual-based population genetics model to explore whether the current levels of heterozygosity in a population of white-tailed deer could be understood based on recorded size of the founder population.	Identifying the processes most likely underlying observed levels of genetic diversity would be useful in discriminating between mechanisms such as the size of the founder population, a selective sweep, or commercial harvesting (e.g. historic whaling activity). For data rich species, IBMs can be used to refine population-level demographic models (e.g. Alter *et al.* 2012), leading to more realistic simulations of population dynamics and more accurate estimations of pre-harvest population size.
Piou & Prévost (2012)	Developed an integrative eco-demo-genetic IBM to understand how the population structure of salmonids may be affected by variation in life-history stages. The model's structure incorporates both individual variability and potential microeveolution of life histories, enabling the parsing of microevolutionary processes and plastic responses. The model therefore allows exploration into how the	Integrating models of genetic population structure and connectivity within a quantitative genetic IBM framework (i.e. eco-genetic IBM), enables insights into both genetic and plastic responses to environmental change, and serves to inform the adaptive management of HMS influenced by climate change.

	population structure of salmonids could be modified as a result of environmental change.	
Premo & Hublin (2009)	Simulated cultural and environmental influences on the genetic diversity and population structure of Pleistocene hominins.	Modelling the relative influence of social interactions and environmental conditions may provide insights into the extent to which a HMS will be affected by a changing climate.
	Identified that dipersal was due to socially-mediated factors, as groups dispersed based on the movement decisions of other individuals within their social network, rather than the conditions of the surrounding environment.	The composition and conservation of social- or kin-groups through time would be expected to be reflected in, and so explain, the genetic substructure of the population.
Railsback & Johnson (2011)	Simulated the foraging habitat selection of populations of migratory birds to investigate how land use and habitat diversity affect the ability of migratory bird populations to suppress an insect pest on a Jamaican coffee farm.	Simulated individuals may be programmed to behave/move in response to environmental or social gradients (e.g. food availability, presence of a "leader" individual).
	Within the modeled study region, birds selected which neighboring grid cell they would move to (i.e. forage from) based on its environmental 'quality', defined by the supply of pest insect and other arthropod food.	For lesser known HMS, IBMs can be used to understand what parameters may be useful in defining "environmental quality", and important component in planning place-based protection and management efforts (e.g. marine protected areas).
Rebaudo *et al.* (2011)	Modeled the human-induced spread of an invasive insect pest in the agricultural landscape of the tropical Andes.	IBMs represent an important education tool for students, evolutionary biologists, and other stakeholders involved in research and management of HMS. In particular, the spatiality of map-based IBMs may be particularly useful in the process of marine spatial planning and marine protected area network design, and for simulating and assessing the impact of alternative management scenarios on HMS.
	The model was then used as an effective educational tool to train farmer communities facing pest risks.	
Stillman & Goss-Custard (2010)	Review a number of IBMs developed for coastal sea birds aimed at simulating components of an individual's life cycle, variation among individuals interactions between individuals, and the dynamics of the resources they use.	HMS exhibit complex life histories and significant inter-individual variation in behavior within the same population. IBMs enable explorations into how this individual-level complexity may influence the simulated individual's behavioral decisions by varying a defined set of fitness-maximizing decision rules.
Tamburino & Bravo (2013)	Developed the IBM "Wonderforest" to understand patterns of mast seeding by mice at the forest-scale. IBMs offer alternatives to impractical field studies by representing "virtual experiments".	"Virtual experiments" are particularly useful for studies of HMS, that are often logistically challenging to study directly, at least through the entirety of their range.

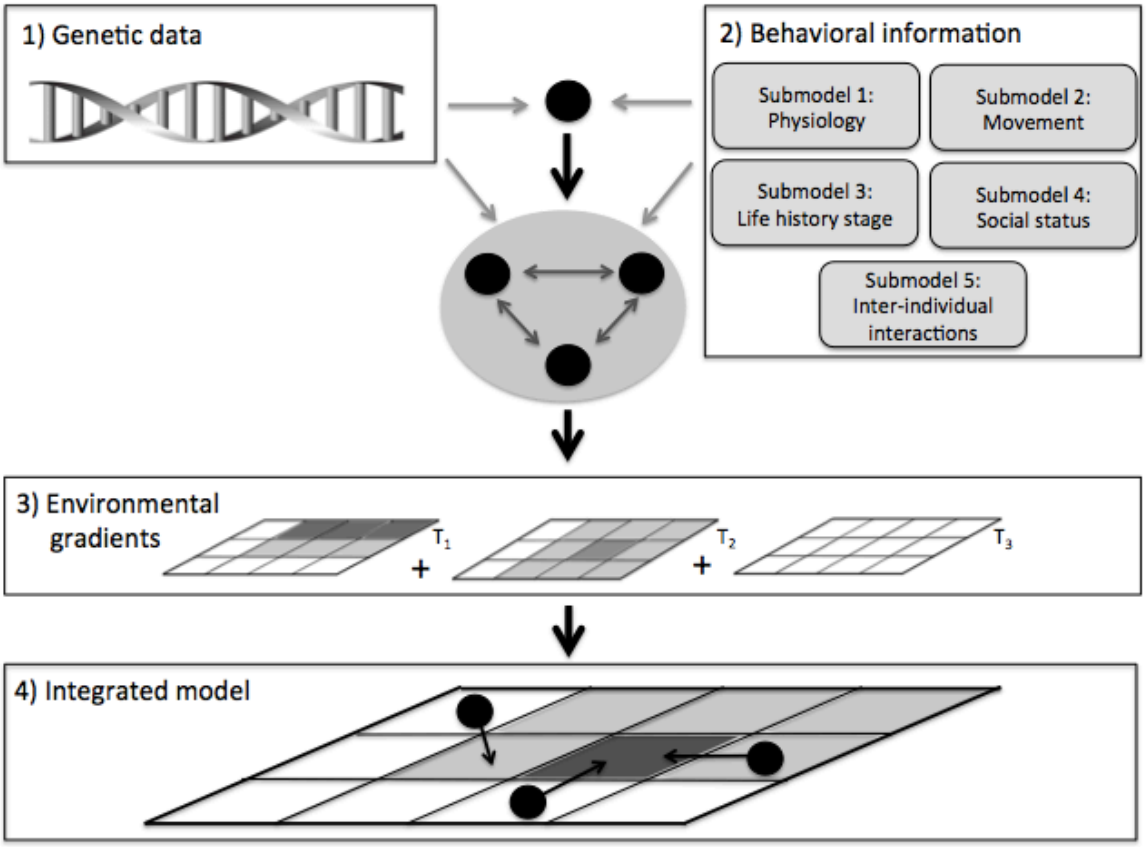

Fig. 1. Conceptual diagram showing potential layers of an integrated individual based model. An individual (black circle) is parameterized first with genetic data and then with a range of submodels that provide the fitness-maximizing 'rules' that influence the individuals emergent behavior during the model run (e.g. physiological condition, movement behavior, life history stage, and social status (e.g. "leader" or "follower")). Individuals are also parameterized to interact with other individual within the model. Spatial IBMs can incorporated 'gridded' data defining information duch as environmental gradients (e.g. habitat suitability). These grids can be static or temporally dynamic (T_1, T_2, $T_3 \ldots T_n$) to capture variation in the system through time (e.g. seasonal shifts in food distribution).

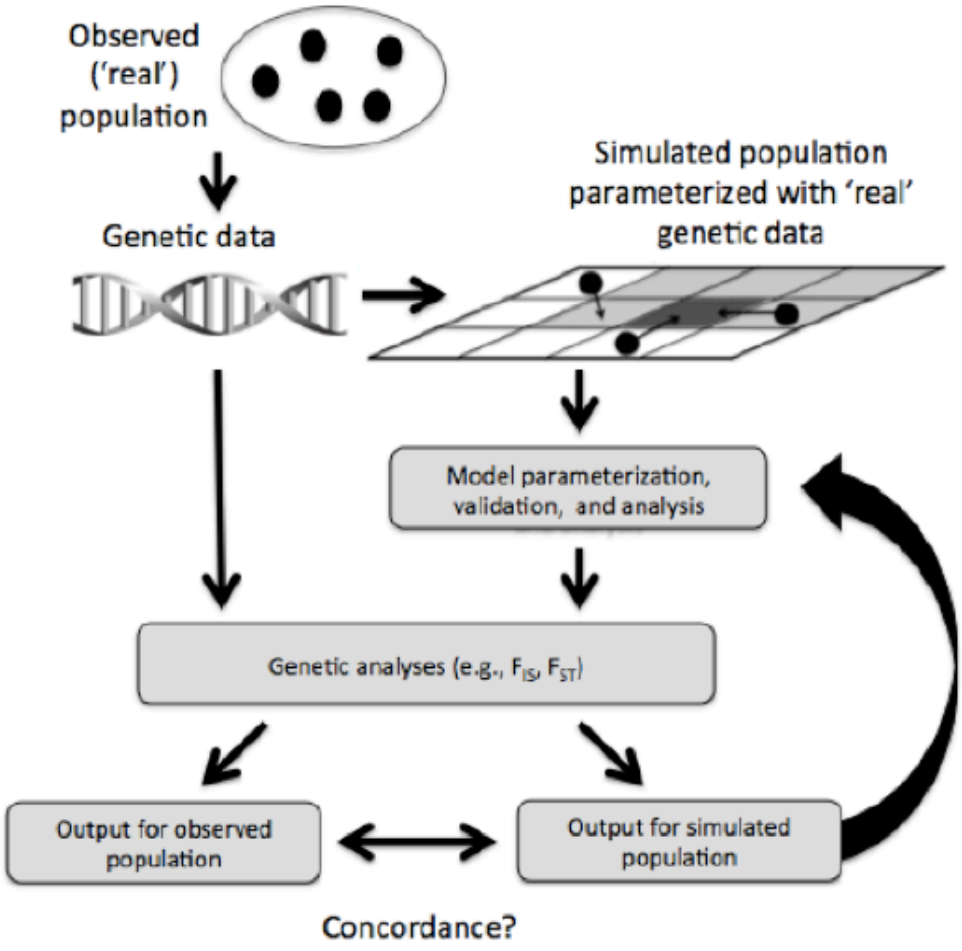

Fig. 2. Diagram showing the work flow for how an individual-based model can be used to understand the mechanisms influencing the genetic patterns in an observed ('real') population. Genetic data is collected from the observed population and is used, in addition to behavioral data, to parameterize individuals in a simulated population within an IBM. Following model parameterization and validation, the model runs will produce a parallel, simulated population genetic data set that can be analyzed in the same way at the data set for the observed population. The results for the observed population and the simulated population can then be directly compared. If the data sets are not concordant then the model parameters can be adjusted and the process repeated. When concordance is reached (i.e. when the model is successfully reproducing

the genetic patterns observed in the ‘real’ population), the modeller can conclude that the parameter values in the model may potentially reflect those mechanisms influencing the genetic structure of the observed population.

SYNTHESIS

The preceding four empirical chapters present new information on the genetic population structure of two species of highly migratory baleen whale across multiple scales. These chapters reveal complex genetic architecture for both species and explore some of the potential influences of behavior in shaping their evolution. The findings presented in this dissertation also directly inform the management and protection of these species. This work therefore highlights the need to integrate behavioral and genetic information, and offers guidance on how this might be achieved using new individual-based modeling techniques. This final synthesis serves to highlight the main findings of the presented studies and evaluate their influence on future research.

The phylogenetic analysis presented in Chapter One (Kershaw *et al*. 2013) confirms the evolutionary divergence in the mitochondrial DNA of two subspecies of Bryde's whale: *B. e. edeni* and *B. e. brydei*. Observations of morphological differences and habitat partitioning (i.e. the coastal distribution of the smaller *B. e. edeni* vs. the cosmopolitan offshore distribution of the larger *B. e. brydei*) between the two subspecies (Perrin *et al*. 1996; Best 1997, 2001; Perrin & Brownell 2007; Penry *et al*. 2011) raise the possibility that these behavioral differences may have resulted in an ecological barrier to gene flow, acting as the mechanism for evolutionary divergence. Striking differences were also found between the two subspecies in relation to their respective genetic population structures, with *B. e. edeni* showing remarkably low levels of genetic diversity and differentiation in comparison to the high diversity and significant structure observed for *B. e. brydei*. The distinctiveness of the two taxa confirms the need to designate each as a separate conservation unit and develop taxon-specific management recommendations.

The population genetic analyses conducted in Chapters Two and Three, and the individual-level analysis carried out in Chapter Four, represent an examination of the genetic population structure of the humpback whale (*M. novaeangliae*) across four major breeding areas (BSA-C, ASHW) in the Southern Hemisphere and afford consideration to the behaviors that may be driving patterns observed.

Chapter Two presents the first examination of the diversity and differentiation of nuclear microsatellite loci for the more than 3,000 individual humpback whales across the region. This work provides a direct comparison with the findings of a parallel study that employed a 486 bp sequence of the mitochondrial control region (Rosenbaum *et al*. 2009). The results of this chapter suggest that a hierarchy of ecological processes operating across spatial scales is likely driving patterns of genetic structure observed. The widely accepted model of maternal fidelity to feeding areas and natal philopatry to breeding areas as the primary driver of population genetic structure (Baker *et al*. 2013) was generally supported by this study; however, this model was found not to be generalizable at all spatial scales. Notably, at the substock scale, the tendency towards male-biased dispersal diminishes and high levels of gene flow with no clear pattern in directionality are observed for both sexes.

Chapter Three extends the study presented in Chapter Two by examining the population structure on feeding areas shared by BSA-C in the Southern Ocean, and the degree of connectivity of each breeding stock to their hypothesized corresponding feeding area (IWC 2010). Collectively, high levels of genetic diversity, the allocation of samples to breeding stocks by the MSA, and the distribution of haplotypes, indicate complex levels of fidelity to feeding areas and extensive mixing of different populations across the Southern Ocean. These findings agree with corollary evidence based on a range of data types (Pomilla & Rosenbaum 2005;

Pomilla *et al*. 2006; Razafindrakoto *et al*. 2001; Amaral & Loo *et al*. in review). The results also provide additional support for the hypothesis that long distance movements on feeding areas are a plausible mechanism for individuals switching between breeding stocks, either temporarily or permanently, to an extent that would result in the low levels of gene flow observed between geographically distant populations in Chapter Two and by Rosenbaum and colleagues (2009).

A number of constraints inherent in the population-level genetic analyses presented in the previous two chapters pose challenges to understanding how current demographic processes are shaping populations on a timescale relevant for management. The individual-level genotypic matching analysis described in Chapter Four therefore offers a useful complement Chapters Two and Three by providing insights into the degree of fidelity to sampling locations across time, and direct movements of individuals between two populations, on a contemporary timescale.

Chapter Four confirms that humpback whales show fidelity to breeding areas and also, at least in the case of BSB2, to feeding areas and migratory routes. Counter to expectations that site fidelity would be observed to a greater extent for females (Baker *et al*. 2013), the results echo previous findings (Craig & Herman 1997; Garrigue *et al*. 2001; Herman *et al*. 2011) that demonstrate a skew towards males, suggesting males are at least as likely, if not more so, to return to the same breeding area in multiple years. Long-distance movements were observed between breeding stocks for four males (Rosenbaum *et al*. 2009); however, interchange between the substocks of the same breeding stock was more common and was found to occur for both sexes. Connectivity to feeding areas was also observed and supports mixing of individuals from BSB and BSC on the Nucleus region for BSB (where 100% of whales are assumed to originate from BSB), therefore support the patterns of population structure on feeding areas observed in Chapter Three.

A synthesis of the findings of Chapters Two, Three, and Four support the primary conclusion of Chapter Two that there is a hierarchy of processes operating at different spatial scales that influence patterns of genetic population structure in humpback whales. At regional spatial scales (i.e. between breeding stocks), maternal fidelity to feeding areas and natal philopatry to breeding areas represents the primary driver of structure, reinforced by the mechanism of isolation by distance between the most geographically distant stocks (e.g. BSA compared to BSB and BSC). Chapters Two, Three, and Four indicate that the low levels of gene flow observed between breeding stocks appear to be primarily driven by long-distance male dispersal events, as predicted under the model of male-biased dispersal; however, it should be noted that that previous studies have also recorded movements of females between breeding stocks (e.g. Stevick *et al*. 2013).

The high degree of mixing on feeding areas observed in Chapter Three, at least for some breeding stocks, provides support for the hypothesis that gene flow between adjacent and non-adjacent breeding stocks may result from long-distance longitudinal movements across feeding areas (Rosenbaum *et al*. 2009). It has been suggested that the notable behavioral plasticity in feeding area fidelity and extent of mixing of different breeding stocks, as observed in this chapter and other studies (Schmitt 2014; Amaral & Loo *et al*. in review), may have evolved in relation to differences in the variability of prey distribution in some regions of the Southern Ocean (Friedlaender *et al*. 2010; Cotte & Guinet 2011; Stevick *et al*. 2013).

At local spatial scales (i.e. between breeding substocks), the influence of maternal fidelity and male-biased dispersal appears to diminish as both males and females show high levels of fidelity, gene flow, and interchange. The different processes operating at this scale may be attributed to social complexity within populations of this species, such as temporal segregation of

dispersal based on life history parameters (Carvalho *et al*. 2014), habitat preferences of breeding females (Barendse *et al*. 2013), and social organization (Barendse *et al*. 2010), among others. Further research employing more powerful genetic data sets is needed to better elucidate these subtle levels of population sub-structure. In addition, the integration of genetic data with other types of information, such as acoustics, habitat suitability, photographic capture-recaptures, and satellite telemetry, among others, will be essential to disentangling the multiple behavioral and other ecological processes underlying these patterns.

From an applied perspective, the complex population patterns observed for humpback whales in this dissertation are not currently accounted for in management designations by the IWC. At the regional scale, this research shows general support for the designation of the four breeding stocks included in these studies (BSA-C, ASHW) by the IWC. However, the more complex relationships observed within BSB and BSC suggest that the IWC substocks do not truly reflect the number and boundaries of demographically discrete population units and support previous suggestions that the IWC substocks should be treated as hypotheses only (Rosenbaum *et al*., submitted). Moreover, the distribution and mixing of humpback whale breeding stocks on feeding areas do not fully support the boundaries designated by the IWC as corresponding to BSB and BSC (IWC 2010). The incongruence between the current IWC management units and genetic population units need to be addressed to ensure accurate assessments of the current status of these populations, which are still undergoing recovery from commercial whaling.

Through four empirical chapters, this dissertation suggests that behavior is an important mechanism shaping the genetic architecture of populations of highly migratory species, thereby presenting a strong rationale for advancing interdisciplinary approaches aimed at uniting the fields of behavioral ecology and population genetics. Similar efforts aimed at integrating genetic

and environmental data in a theoretical and applied context have already been made, as embodied by the field of seascape genetics (Selkoe *et al*. 2008; Kershaw & Rosenbaum 2014; Mendez *et al*. 2014). The literature review presented in Chapter Five offers a methodological contribution on how such an interdisciplinary approach could be achieved through the use of individual-based models as an analytical platform upon which to integrate behavioral, environmental, and genetic data, and to explicitly test hypotheses regarding the mechanisms underlying patterns of genetic population structure. Notwithstanding the need for rigorous parameterization and validation procedures, Chapter Five highlights how IBMs can represent a useful tool for studies of highly migratory species, counter to assumptions that they are of limited use for data poor species. Notably, IBMs are not constrained by existing knowledge of the system and therefore provide a flexible framework for exploring and generating hypotheses regarding the mechanistic processes underlying observed patterns. Moreover, they offer an alternative to impractical field studies and so are particularly useful for species that are logistically challenging to study throughout the entirety of their range.

In conclusion, the empirical studies in this dissertation address a gap in knowledge regarding the genetic population structure of two highly migratory baleen whales across multiple scales. This body of work secondarily presents an exploration into the role that behavior may play in influencing genetic population patterns and, in doing so, highlights the importance of considering behavioral information alongside genetic data in efforts to understand the evolution of species with complex social structures (i.e. when ecological relationships, included related communication and cognition, varies considerably within a species; Whitehead 1997), and to better manage and protect them. The final chapter and literature review proposes that new individual-based modeling techniques may be used to facilitate a cautious yet concerted effort

towards this objective. This dissertation is presented in the hope that it will inform current efforts to unite behavioral and genetic research and contribute to the methodological advancements necessary to realize this goal.

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