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Against the current: an inter-oceanic whale migration event

Cristina Pomilla[1,2,3,*] **and Howard C. Rosenbaum**[2,3]

[1]*Department of Biology, New York University, New York, NY 10003, USA*
[2]*Cetacean Conservation and Research Program, International Conservation-Marine, Wildlife Conservation Society, Bronx, NY 10460, USA*
[3]*Molecular Systematics Laboratory and Center for Biodiversity and Conservation, American Museum of Natural History, New York, NY 10024, USA*
**Author for correspondence (cristina.pomilla@nyu.edu)*

Humpback whales seasonally migrate long distances between tropical and polar regions. However, inter-oceanic exchange is rare and difficult to document. Using skin biopsy samples collected in the Indian Ocean and in the South Atlantic Ocean, and a genetic capture–recapture approach based on microsatellite genotyping, we were able to reveal the first direct genetic evidence of the inter-oceanic migration of a male humpback whale. This exceptional migration to wintering grounds of two different ocean basins questions traditional notions of fidelity to an ocean basin, and demonstrates how the behaviour of highly mobile species may be elucidated from combining genetics with long-term field studies. Our finding has implications for management of humpback whale populations, as well as for hypotheses concerning cultural transmission of behaviour.

Keywords: humpback whales; *Megaptera novaeangliae*; migration; philopatry

1. INTRODUCTION

Humpback whales (*Megaptera novaeangliae*) exhibit distinct patterns of seasonal distribution that range from the tropics, where they breed during winter months, to near-polar waters, where they feed during summer months. Some individuals may travel one-way distances exceeding 8000 km during the intervening migration (Stone *et al.* 1990). Movements of individuals between feeding and wintering migratory destinations within ocean basins have been extensively demonstrated through the hunting of marked whales, photographic capture–recapture, satellite tagging and genetic identification (Mackintosh 1942; Chittleborough 1965; Palsbøll *et al.* 1997; Mate *et al.* 1998).

Genetic studies have shown that maternally inherited fidelity to feeding grounds and presumed migratory routes can sustain long-term population structure in humpback whales (Baker *et al.* 1990), although in different years a small number of individuals may migrate to different wintering sites within the same ocean basin (Mattila & Clapham 1989; Darling & Cerchio 1993; Salden *et al.* 1999; Calambokidis *et al.* 2001). Many wide-ranging mammals do exhibit some degree of male dispersal due to polygyny and associated male competition (Greenwood 1980). However, inter-oceanic migration events appear to be very rare in humpback whales, and prior to our investigation had only been documented for two animals marked off eastern Australia in 1954–1955 and killed off western Australia in 1959 (Chittleborough 1965).

Here we use genetic samples collected from humpback whales in wintering regions of two different ocean basins in the Southern Hemisphere to document direct inter-oceanic movements of individuals by genetic capture–recapture of genotypes constructed from microsatellite markers, an approach applying firm resolution of unique genetic profiles to permit unambiguous identification of individuals (Palsbøll *et al.* 1997).

2. MATERIAL AND METHODS

A total of 1202 skin samples were collected during the austral winter (July–September) from free-ranging humpback whales in the Southwestern Indian Ocean off the northeast coast of Madagascar ($n=722$) from 1996 to 2001, and in the eastern South Atlantic Ocean off the coast of Gabon ($n=480$) in 2001 and 2002 (Lambertsen 1987; table 1 in the electronic supplementary material). Genomic DNA was extracted, and the samples were sexed using ZFX/ZFY markers and genotyped using 11 cetacean microsatellite markers (see electronic supplementary material).

To characterize maternal lineages we used a 486 bp mitochondrial DNA (mtDNA) fragment containing the majority of variable nucleotide positions in the mtDNA control region of humpback whales (Baker *et al.* 1993). Amplification protocols for this fragment and analyses of haplotype diversity are described in Rosenbaum *et al.* (2004).

Duplicate samples within each population were detected either from photographic identification or genotype identity using the Excel add-in Ms_Toolkit package (Park 2001), and were consequently eliminated. The average probability of different random individuals in the populations sharing the same genotype by chance (probability of identity, PI) was estimated using the software Api-Calc v. 1.0 (Ayres & Overall 2004). Since it is more likely that relatives, rather than random pairs, will share genotypes, for a specific case of a genotype match identified between Madagascar and Gabon we also calculated the more conservative PI for siblings (PI_{sib}) and PI for parent–offspring (PI_{pof}) (see electronic supplementary material). Although a relationship of full-siblings is very unlikely in humpback whales (Clapham & Palsbøll 1997), PI_{sib} is more conservative than PI_{pof}, which would be the next closest kin relationship.

Allele frequencies and measures of diversity such as mean number of alleles per locus (A), observed heterozygosity (H_o), and expected heterozygosity (H_e) under Hardy–Weinberg assumptions (Nei 1987) were computed using the Excel add-in MS_Toolkit package (Park 2001). Departure from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between each pair of loci were evaluated using Genepop v. 3.4 (Raymond & Rousset 1995; see electronic supplementary material). Hypotheses of pedigree relationships between pairs of individuals were tested with the software Kinship v. 1.3.1 (Goodnight & Queller 1999; see electronic supplementary material).

To estimate migration rates between populations we used a maximum-likelihood framework based on coalescence theory, implemented in the program Migrate v. 2.0.3 (Beerli & Felsestein 1999; see electronic supplementary material).

3. RESULTS

After removing duplicate samples from each population, detected either from photographic identification or genotype identity, a total of 972 identified individuals were included in the analyses. The mean numbers of alleles per locus (A) were 12.91 for Gabon and 13.09 for Madagascar. The largest

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Table 1. Microsatellite genotypes and mtDNA haplotypes for two matching samples collected in Madagascar (BA-00-S-200) and Gabon (GA-02-S-130), and for the individual associated with BA-00-S-200 (BA-00-S-201).
(*H* indicates the mtDNA lineage (GenBank accession number DQ118384). Microsatellite loci are listed in the top row; numbers refer to basepair sizes of the two alleles at each locus. The genotypic phase is unknown.)

		alleles at each microsatellite locus (bp)								
sample ID	*H*	GATA417	GATA028	GATA053	TAA031	199/200	417/418	464/465	EV1Pm	EV37Mn	EV94Mn	EV96Mn
BA-00-S-200	HBA053	203–226	148–148	249–249	95–116	102–116	196–196	138–142	121–121	194–204	211–215	205–209
GA-02-S-130	HBA053	203–226	148–148	249–249	95–116	102–116	196–196	138–142	121–121	194–204	211–215	205–209
BA-00-S-201	HBA053	203–226	148–148	249–261	95–116	112–116	188–196	142–142	121–125	200–204	211–219	203–209

Figure 1. Humpback whale sampling areas and migration routes around Africa. Stars indicate the locations where the two matching samples were collected on July 17th 2000 in Antongil Bay (16°00′ S, 49°55′ E), Madagascar, and on August 13th 2002 off Loango National Park (1°51′ S, 9°20′ E), Gabon. Shading indicates humpback whale distribution in the wintering destinations (white) and in the feeding grounds (dark grey). Dark grey arrows indicate population seasonal migratory routes; white dotted arrows indicate three plausible alternative deviations from the main routes that the sampled individual might have undertaken during a migration from the Indian to the South Atlantic Ocean passing through the feeding grounds. (Map courtesy of the US National Geophysical Data Center.)

number of alleles (21) was found at locus GATA417 for Madagascar; the smallest (4) was recorded at locus EV1Pm for both sampling sites. No significant differences were found between the observed heterozygosity ($H_o = 0.76 \pm \text{s.d.} = 0.01$ for Gabon, $H_o = 0.75 \pm \text{s.d.} = 0.00$ for Madagascar), and the heterozygosity expected under Hardy-Weinberg assumptions ($H_e = 0.76 \pm \text{s.d.} = 0.05$ for Gabon, $H_e = 0.75 \pm \text{s.d.} = 0.05$ for Madagascar). Deviation from HWE was rejected, and there was no evidence of LD ($p < 0.05$) in the 110 pairwise tests performed.

The low estimate obtained for the average PI ($\text{PI} = 1.123 \times 10^{-12}$) allowed us to conclude that any match recovered when searching genotype data for between-site matches would indicate inter-oceanic migration of an individual. One sample collected in 2000 in Antongil Bay, Madagascar, matched a sample collected two years later off Loango National Park, Gabon (figure 1, table 1; $\text{PI}_{\text{sib}} = 3.114 \times 10^{-5}$ and $\text{PI}_{\text{pof}} = 5.382 \times 10^{-9}$ for Madagascar, $\text{PI}_{\text{sib}} = 2.811 \times 10^{-5}$ and $\text{PI}_{\text{pof}} = 5.640 \times 10^{-9}$ for Gabon). Both samples were collected from a male individual and shared a locally rare mtDNA lineage (frequencies: $p = 0.008$ in Madagascar and $p = 0.006$ in Gabon, H. C. Rosenbaum *et al.*, unpublished data). A photographic comparison has not yet been attempted for datasets from these two areas. However, a comparison of dorsal fin characteristics in photographs taken at the time of sampling confirmed the genetic match.

In Madagascar, this individual was accompanied by a female and analyses of genotypes putatively identified her as his mother (relationship log(ratio) = 1.673, $p < 0.001$; table 1; and see electronic supplementary material for kinship analyses). Other behavioural data show that humpback whale calves remain with their mother for their first year, and occasionally for their second year (Glockner-Ferrari & Ferrari 1984; Baker *et al.* 1987; Clapham & Mayo 1987). Therefore, when sighted in Gabon two years later, this individual could have been a three- or four-year-old juvenile, as the mean age at attainment of sexual maturity in male humpback whales is five years (Clapham 1992; Chittleborough 1955).

Gene flow estimates from MIGRATE based on seven sampled humpback whale populations indicated that the eastern South Atlantic and the southwestern Indian Oceans are expected to exchange approximately 35 migrants per generation (N_em) in each direction (95%CI = 27.1–45.1). However, the same analysis estimated that only approximately one individual per generation ($N_em = 0.8$, 95%CI = 0.6–1.3) is expected to migrate from Madagascar to Gabon.

4. DISCUSSION

The worldwide distribution of humpback whales mtDNA haplotypes seems to reflect historical interchange between the hemispheres (Baker *et al.* 1993). Inter-oceanic exchange is also expected to happen to some degree in the absence of geographic barriers, yet its documentation remains an exceptional event, and more so for the circumstances presented here. Since only a modest degree of migration is sufficient to confound structure between populations, dispersal events may be rare and therefore hard to identify. Previous documentation of migrations of this scale have centred on trans-equatorial movements (Stone *et al.* 1990), and the only other inter-oceanic migrants previously identified were the two animals killed off Australia (Chittleborough 1965). Although several statistical approaches permit an indirect estimate of gene flow between populations, it is often very difficult to distinguish between current exchange and retention of common ancestral genetic states, as both scenarios produce a similar pattern of allele sharing. Dispersal events in highly mobile species are therefore valuable when trying to distinguish between the above hypotheses. Given a generation time of 12–24 years (Roman & Palumbi 2003), our direct observation of one migrant over six and two years of sampling for Madagascar and Gabon, respectively, is consistent with the expectation ($N_em = 0.8$, 95%CI = 0.6–1.3), especially considering that N_e (effective population size) is smaller than N (population census size) and in light of the stochastic nature of one resighting.

Acoustic data on the radical replacement of humpback whale song in Australian populations suggested the immigration of adult males from the Indian into the Pacific Ocean (Noad *et al.* 2000). Cultural transmission seems to play an important role in maintaining song homogeneity over entire ocean basins (Guinee *et al.* 1983). Our finding is consistent with episodes of inter-oceanic cultural transmissions possibly occurring through direct movements of animals in addition to hypothesized contact on common feeding grounds or migration routes (Payne & Guinee 1983).

Even though this migrant whale was probably sexually immature, and therefore does not represent a direct vector of gene flow or song interchange, its behaviour is important proof of current inter-oceanic exchange between humpback whale wintering regions. Alternatively, given the young age of the animal, this inter-oceanic movement could be the result of navigational error due to lack of experience.

Despite genetic evidence for rejecting a single panmictic humpback whale population in the Southern Hemisphere, the relationship between populations of different ocean basins and the extent of potential gene flow merit further evaluation (Baker & Medrano-Gonzalez 2002). From a conservation perspective, an improved understanding of large whale migratory behaviour and population connectivity will help improve current definitions of population boundaries, evaluation of protected areas, and assessment of post-whaling recovery of populations. This is essential where revision of international management procedures by the International Whaling Commission may rely on accepted views or traditional notions that lack adequate scientific data.

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