

2017

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Recommended Citation

Warren, Wesley C.; Minx, Patrick; Tomlinson, Chad; Farias, Fabiana H. G.; and et al, "The novel evolution of the sperm whale genome." *Genome Biology and Evolution*. 9, 12. 3260-3264. (2017).
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The Novel Evolution of the Sperm Whale Genome

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Accepted: September 12, 2017

Data deposition: This project has been deposited at NCBI under the Bioproject accession numbers PRJNA89089 and PRJNA237226.

Abstract

The sperm whale, made famous by *Moby Dick*, is one of the most fascinating of all ocean-dwelling species given their unique life history, novel physiological adaptations to hunting squid at extreme ocean depths, and their position as one of the earliest branching toothed whales (Odontoceti). We assembled the sperm whale (*Physeter macrocephalus*) genome and resequenced individuals from multiple ocean basins to identify new candidate genes for adaptation to an aquatic environment and infer demographic history. Genes crucial for skin integrity appeared to be particularly important in both the sperm whale and other cetaceans. We also find sperm whales experienced a steep population decline during the early Pleistocene epoch. These genomic data add new comparative insight into the evolution of whales.

Key words: sperm whale, cetaceans, genome.

Introduction

The sperm whale, made famous by *Moby Dick*, makes some of the deepest and longest dives of any marine mammal: >73 min long and up to 2,035 m deep (Watkins et al. 1993; Watwood et al. 2006) to feed on squid, including the infamous giant and colossal squids (Best 1979; Whitehead 2003). Previous comparative genomic analyses of cetaceans indicated genic adaptation to a marine existence (Foote et al. 2015; Yim et al. 2014), including convergent pathways of metabolism regulation for deep diving (Foote et al. 2015). However, to date, the sperm whale—one of the deepest

diving and earliest branching toothed whales (Odontoceti; Whitehead 2003)—has been excluded from these comparisons. We sequenced and assembled multiple sperm whale genomes to explore genic adaptation. Given the important and broad physiological roles played by proteases, our explorations mostly focused on examining protease loss-of-function (LoF) events important in sperm whale, and cetacean, evolution. We also sought to discover which genes showed signs of positive selection shared with other cetaceans or unique to sperm whale. Finally, as previous analyses suggested that sperm whales

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Table 1

Genes under Positive Selection Enriched by Pathway, Phenotype, or Protein Interactions

Pathway	Source	Genes	Ratio of Enrichment	Adjusted P-value
Focal adhesion	Wiki	<i>CHAD, COL1A2, THBS2, TNC, FLT1</i>	6.9	0.015
Focal adhesion	KEGG	<i>CHAD, COL1A2, PARVG, THBS2, TNC, FLT1</i>	7.5	0.0076
Pemphigus	Disease	<i>PPL, EVPL, DSP, DSG3</i>	38.8	0.0006
Calcium signaling	KEGG	<i>PTK2B, ADCY3, RYR1, NOS2, P2RX3, PHKB</i>	7.6	0.0076
Blood circulation	GO	<i>ALOX5, CHD7, WNK1, EPAS1, CX3CL1, PPP1R13L, COL1A2, AZU1, DSP, GUCY1A3, MYBPC3, TBC1D8</i>	3.4	0.036
Cornified envelope	Reactome	<i>DSP, TGM1, KRT4, PKP1, DSG3, PPL, EVPL</i>	NA	^a FDR 3.65×10^{-11}

^aThe false discovery rate (FDR) calculated within the Reactome software (Croft et al. 2014) is the probability corrected for multiple comparisons. Adjusted P values are not provided.

experienced a global expansion <80,000 years ago (Alexander et al. 2016), we examine the estimated historical effective population size using samples from throughout the sperm whale's range.

Materials and Methods

We sequenced a Gulf of Mexico female sperm whale (GMX) to high coverage (72×) using short-insert and mate-pair libraries of 100 bp length (detailed in the supplementary material S1, Supplementary Material online) on an Illumina HiSeq2000. We assembled the draft genome of all sequences with ALLPATHS (Gnerre et al. 2011) using default parameter settings, subjecting assembly input reads to quality control as detailed in the ALLPATHS documentation (Gnerre et al. 2011). We obtained RNAseq data from skin biopsies of a different GMX sperm whale to aid gene annotation as described in the supplementary material S1, Supplementary Material online. Gene annotation was performed according to the NCBI gene annotation pipeline as described here: <http://www.ncbi.nlm.nih.gov/books/NBK169439/>. After aligning genes from the sperm whale with other taxa (detailed in supplementary material S1, Supplementary Material online) to establish 1:1 gene orthology, positive selection was detected using PAML4.0 (Yang 2007) and impact on protein structure tested with Provean (Choi and Chan 2015). Canonical pathway enrichment of gene clusters under positive selection was established as detailed in the supplementary material S1, Supplementary Material online. Protease genes were manually annotated and validated for loss/duplication events using BATI (<http://degradome.uniovi.es/downloads.html>). Four additional sperm whale individuals (supplementary table S1, Supplementary Material online) were resequenced to moderate depth (21–28×) and reads were mapped to the draft genome as described in the supplementary material S1, Supplementary Material online. We calculated heterozygosity on a per-individual basis using VCFtools (Danecek et al. 2011). Effective population size was reconstructed with PSMC (Li and Durbin 2011) using the parameters specified in the supplementary material S1, Supplementary Material online.

Results and Discussion

Our sperm whale total assembled sequence was similar in size to other assembled cetacean genomes (supplementary table S2, Supplementary Material online). Using our GMX individual reference assembly (Genbank assembly accession GCA_000472045.1) we inferred 18,686 protein-coding genes—second only to the baiji (*Lipotes vexillifer*) among sequenced cetaceans at 18,906 genes. Using a core eukaryotic mapping method (Simao et al. 2015) we also demonstrated >94.7% of conserved genes were complete in our assembly (supplementary table S3, Supplementary Material online). Of the 18,686 protein-coding genes, 12,717 had single-copy orthologs in both human and other cetartiodactyls (supplementary table S4, Supplementary Material online; additional methods/results can be found in the supplementary material S1, Supplementary Material online). A total of 45 genes found across eight taxa were identified as being under positive selection in the sperm whale lineage; these genes also passed our stringent functional impact tests (default cutoff < −2.5) using Provean (Choi and Chan 2015; supplementary file S1, Supplementary Material online). Several significant pathways emerged from enrichment analyses, which included genes associated with blood-circulation and skin stress responses (table 1). Cetaceans, including the sperm whale, exhibit molting or skin sloughing (Amos et al. 1992), potentially as an adaptive response to fouling by barnacles and other organisms. However, sperm whales face the additional challenge of maintaining skin integrity and blood homeostasis at high water pressures during deep foraging dives.

To complement the analysis of genes under positive selection, we manually annotated the complete set of proteases (i.e., degradome) of the sperm whale. This independent analysis identified several proteases involved in skin function and blood homeostasis that showed LoF events along the lineage leading to sperm whales (fig. 1; additional methods/results in supplementary material S1, Supplementary Material online). We also detected LoF in proteases involved in inflammation, immunity and metabolism within cetaceans, and specifically

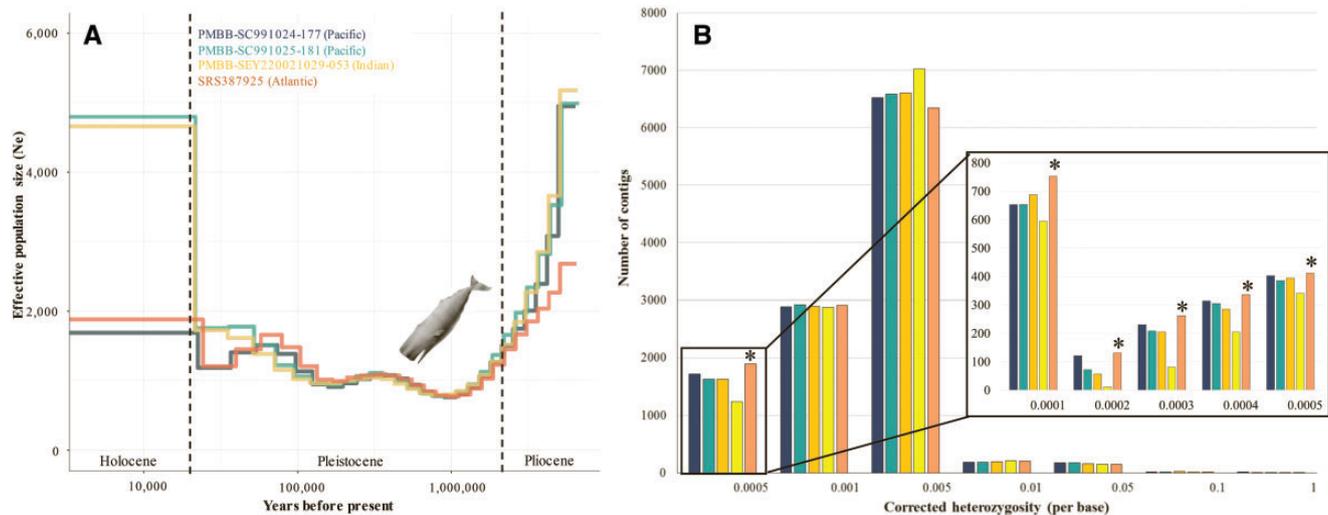


FIG. 2.—Estimated effective population size history and heterozygosity of sperm whales from different ocean basins. Samples are color coded by the key, with blue/green = Pacific, orange/yellow = Indian Ocean, and dark orange = Gulf of Mexico, Atlantic. (A) PSMC reconstruction of effective population size through time by sample (excluding SEY420021031-063, see supplementary material S1, Supplementary Material online), dashed lines represent the estimated start dates for each epoch; (B) Genome wide distribution of heterozygosity for each sample, by contig/scaffold. The Gulf of Mexico sample—characterized by low heterozygosity—is marked by an asterisk where it has the largest number of contigs in a category. The insert emphasizes that this sample has the largest number of contigs with low heterozygosity (<0.0005). Bright yellow in panel (b) is additional Indian ocean sample

Overall, our results suggest positive selection has differentially affected localized portions of the sperm whale genome. In particular, the complex pattern of convergent gene evolution involving skin-related genes suggests they have played an important role in aquatic adaptation, possibly influenced by the somewhat contradictory requirements of heat insulation, buoyancy and deep diving. In comparison to the localized effects of selection on the genome, we infer that the sperm whale experienced a rapid population decline, potentially in response to glaciation, which had a broad effect on genome-wide diversity. Given the apparent influence of past climate change, monitoring the on-going response of sperm whales to anthropogenically mediated climate change will be paramount.

Authors Contributions

S.S.W., J.W.J., J.W.S. isolated genomic DNA and sexed all samples. L.K., T.M.B. performed all population history analyses. J.G.P., C.L.O., V.Q. performed all protease analyses. C.T., P.M. completed genome assembly and curation. J.C., T.J.K., T.G. analyzed population sequences. M.J.M., F.H.F. analyzed gene selection. R.M.W. completed all gene orthology analyses. W.C.W., A.A. wrote the paper and reviewed all analyses. All authors have read and approved the manuscript.

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

Acknowledgments

This work was supported by the National Institute of Health grant 2R24OD011198-04A1 (W.C.W., PI); National Institute of Environmental Health Sciences [ES016893 (J.W. Sr, PI)]; Army Research Office [W911NF-09-1-0296 (J.W. Sr, PI)]; Swiss National Science Foundation grant PP00P3_170664 (R.M.W.); Ministerio of Economia and Competitividad (Spain); and European Union (ERC-Advanced Grant DeAge) (C.L.-O., PI); the Campbell Foundation; the Ocean Foundation; Ocean Alliance; and the many individual and anonymous Wise Laboratory donors. The authors declare no competing financial interests. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Environmental Health Sciences, the National Institutes of Health, the Army Research Office or the Department of Defense. Work was conducted under National Marine Fisheries Service permit #1008-1637-03 (J.W. Sr, PI) and permit #751-1614 (I.K., PI). We thank Kyung Kim for computational support. We would like to thank Catherine Wise, James Wise, Chris Gianios, and all the Wise Laboratory/Odyssey science team volunteers for their help with technical support, whale spotting and sample collection. We thank Iain Kerr, Roger Payne, Bob Wallace, Derek Walker, and all of the Odyssey boat crew for their help with sample collection and logistics. We thank C. Scott Baker for comments on this manuscript. Finally, we thank all the volunteers who supported us in this project.

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Associate editor: Tal Dagan