Biochemical Systematics and Ecology 56 (2014) 196–201

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Initial description of short-finned pilot whale (*Globicephala macrorhynchus*) genetic diversity from the Caribbean

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ARTICLE INFO

Article history:
Received 24 February 2014
Accepted 7 June 2014
Available online

Keywords:
Short-finned pilot whale
Globicephala macrorhynchus
mtDNA
Kinship
Strandings
Caribbean

ABSTRACT

Short-finned pilot whales (*Globicephala macrorhynchus)* are known for their highly social cohesive behavior which contributes to the reporting by the public of mass stranding. This species has been hunted in the Lesser Antilles in the Caribbean for generations. Currently, the genetic diversity of short-finned pilot whales has been investigated in the North Atlantic, South Pacific and Japan/Korea, but not in the Caribbean. Here we provide the first description of the genetic diversity of this species in the Caribbean using mitochondrial DNA (mtDNA) control region (CR) sequences and seven microsatellite loci from 25 skin samples collected from four stranding events in Jamaica, Puerto Rico, British Virgin Islands, and Trinidad and Tobago. Overall, three CR haplotypes were identified; of these, one was unique for the Caribbean (Car 1), while a second (haplotype D) was previously reported in the Western North Atlantic, and a third (Haplotype A) was found to be common in the South Pacific. Even though sample size ($n = 25$) was small, microsatellite analysis showed a low level of relatedness between the stranded whales. Our results provide relevant preliminary information on the genetic diversity of short-finned pilot whales in the Caribbean, reporting one previously unknown and unique haplotype for this region. Further research should be carried out in the Caribbean to further our knowledge on this species, particularly since its prone to mass stranding.

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1. Introduction

Pilot whales are amongst the most gregarious of cetaceans, and are often found in groups of up to several hundred individuals. Possibly the most known characteristic of pilot whales natural history is their high propensity to mass strand. There are two recognized species of pilot whales: the long-finned pilot whale (*Globicephala melas*) which is well known for being commonly involved in mass strandings worldwide (Baker, 1981; Evans et al., 2005) and the short-finned pilot whale (*Globicephala macrorhynchus*) which has also been documented in various single and mass stranding events in the north Atlantic and in the Caribbean region (Mignucci Giannoni, 1989).

Mass stranding events are common in species that form long-term social bonds and pilot whales are considered social individuals with long lasting relationships. Their social structure has been described as matrilineal, with groups composed of several generations of maternally related individuals (Amos et al., 1993; Heimlich-Boran, 1993). Most studies on long-finned

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http://dx.doi.org/10.1016/j.bse.2014.06.001
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pilot whales suggest they have a matrilineal social structure, where individuals from both sexes stay with their maternal group (Amos et al., 1991, 1993; Fullard, 2000). Although groups present a matrilineal system, groups at sea can be either composed of individuals descending from a single female ancestor sharing a unique mitochondrial DNA (mtDNA haplotype) (Amos et al., 1993; Fullard, 2000), or be composed by various unrelated matriline units each with different mtDNA haplotypes, suggesting that large groups may be temporal associations of two or more groups (Bloch et al., 1993; Oremus et al., 2013; Ottensmeyer and Whitehead, 2003).

The short-finned pilot whale is distributed worldwide from tropical to warm-temperate waters and considered abundant (Olson, 2009). In the Caribbean, sightings of short-finned pilot whales have been reported from Anegada Passage, Bequia, Cuba, Dominica, Dominican Republic, Haiti, Martinique, Petit Nives, St. Lucia, St. Vincent, and Venezuela (Mignucci-Giannoni, 1989). Short-finned pilot whales have been observed in groups of up to 100 animals with a mean group size of 11.1 ($n = 66$) (Mignucci-Giannoni, 1998) and similar to the long-finned pilot whale, the short-finned pilot whale groups from the oceanic archipelago of Madeira near Portugal, exhibit natal group philopatry and present a matrilineal system, implying relatedness between members of both sexes within the natal group (Alves et al., 2013). The short-finned pilot whale has been hunted in the South Pacific in Japan and Korea (Dalebout et al., 2004; Kage, 1999). Likewise in the Caribbean their gregarious behavior has allowed them to be herded and hunted in the Lesser Antilles for generations (Caldwell and Erdman, 1963; Mignucci Giannoni, 1989)

While basic zoogeography and life history information is available for short-finned pilot whales from the northeastern Caribbean, to date there is no information available regarding their genetic diversity or group composition in this region. In this study we present (1) genetic information obtained from analyses of 25 skin samples collected from four single stranding events in Puerto Rico (3) and Jamaica (1) and two mass stranding events at Trinidad & Tobago (11) and British Virgin Islands (10) of short-finned pilot whales in the Caribbean, (2) mitochondrial DNA control region haplotypes identified from the Caribbean samples, and (3) preliminary results from analyses of seven microsatellite loci.

2. Materials and methods

2.1. PCR amplification and sequencing

2.1.1. Mitochondrial genes

Samples ($n = 25$) where collected from four single and two mass stranding events in the Caribbean (Fig. 1; detailed stranding and genetic data collection information is found in Supplementary Material). Genomic DNA was extracted following a phenol-chloroform protocol (Sambrook et al., 1989). A 345-base pair (bp) fragment of the 5′ end of the mtDNA control region (CR) was amplified using primers and PCR protocol previously described by Caballero et al. (2007).

2.1.2. Microsatellites

Samples were genotyped at seven microsatellite loci previously described for long-finned pilot whale studies (Oremus et al., 2013; Table 2). PCR reactions used 1 unit of qARTA Taq HOT DNA Polymerase (QartaBio). The cycling profile consisted of an initial denaturation period of 2 min at 93 °C, followed by 20 cycles of denaturation for 30 s at 92 °C, primer annealing for 45 s at T°_{A}, and extension for 50 s at 72 °C. This was followed by a second cycling profile of 25 cycles of denaturation for 30 s at 89°, primer annealing for 45 s at T°_{A}, and polymerase extension for 50 s at 72 °C. A final extension period of 20 min at 72 °C was included modified from Oremus et al. (2013).

Products were run on an ABI 3100 Genetic Analyzer (Universidad de los Andes) and alleles were scored against a size standard (TAMRA 500) using GeneMapper Software version 4.0 from Applied Biosystems. Two samples were run on all PCR

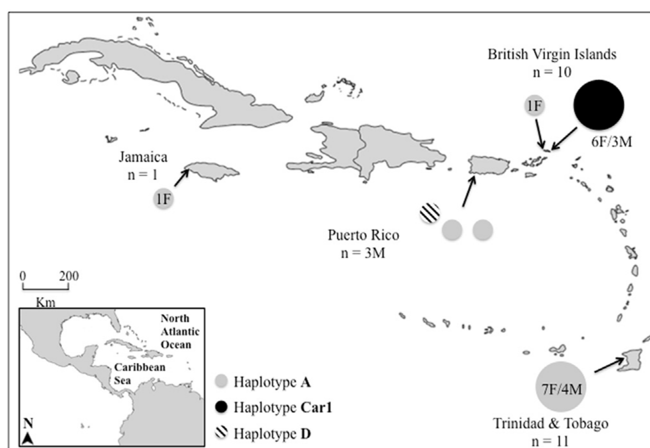

Fig. 1. Distribution and sample size of each stranding (n = sample sizes, F = females, M = males).

Table 1
Two Variable sites ($n = 2$) within a 345 bp consensus fragment of the mtDNA CR of *Globicephala macrorhynchus* used in this study.

Haplotypes	2 0 1	3 4 2
A	C	C
Car1	T	T
D	C	T

plates as internal controls. Additionally, allelic dropout was tested by randomly repeating fragment analysis for five samples per locus.

2.1.3. *Data analysis*

2.1.3.1. Mitochondrial genes. For phylogeographic comparisons, 14 additional haplotypes obtained from short-finned pilot whale mtDNA CR were available from GenBank. These sequences represented 134 individuals previously analyzed (Oremus et al., 2009; FJ513328.1–FJ513340.1), as well as additional sequences from previously published studies (Siemman, 1994; U20921–U20923). All sequences were aligned in Geneious Pro 3.6.1 (Drummond et al., 2011) using the Muscle algorithm (Edgar, 2004) and edited manually. These sequences were trimmed to a consensus length of 345 bp. Unique haplotypes and variable sites were identified using MacClade v. 4.0 (Maddison and Maddison, 2000). Genetic diversity values (nucleotide and haplotype diversity) were estimated with Arlequin V. 3.0 (Excoffier et al., 2005).

2.1.3.2. Microsatellites. The number of alleles (N_A), the observed heterozygosity (Ho) and the expected heterozygosity (H_E) were calculated for every locus. Estimated departure from Hardy–Weinberg equilibrium (Markov chain 100,000, dememorization steps 100) for each locus was obtained and a test of linkage disequilibrium (permutations 10,000) was performed for all pairs of loci using the software Arlequin V. 3.0 (Excoffier et al., 2005). p values were adjusted using Bonferroni corrections for multiple comparisons.

2.1.3.3. Relatedness estimates. The potential frequency of null alleles and the average relatedness among individuals belonging to each mass-stranding event was calculated using the maximum likelihood estimates of relatedness implemented in the software ML-Relate (Kalinowski et al., 2006). This software has an option to account for the effect of null alleles on relatedness (Kalinowski and Taper, 2006). Allele frequencies were estimated using nuclear data from all stranded samples. We used Identix (Belkhir et al., 2002) to determine whether the mean relatedness in each stranding event was significantly different from the mean relatedness expected at random (1000 permutations).

Relatedness (r) is a measure of the fraction of alleles that are identical by shared descent among individuals. Common categories of relationship include parent offspring and full sibling, collectively referred as first-degree relatives (50% of alleles shared identical by descent, on average) $r = 0.5$, and second degree categories as half-siblings $r = 0.25$. Mean relatedness values can range from 0 to 1, where 0 implies no relatedness between sampled individuals (Blouin, 2003).

Estimates of Internal Relatedness (IR) is calculated as the number of alleles shared by locus adjusted for the frequencies of alleles in the sample, allowing the sharing of rare alleles to be weighted more than the sharing of common alleles (Amos et al., 2001). Also, estimates of homozygosity weighted by locus (HL), which weight the contribution of loci depending on their allelic variability, provide more weight to more informative loci (Aparicio et al., 2006). These estimates were calculated with the Excel IRmacroN4 available at: http://www.zoo.cam.ac.uk/zoostaff/meg/amos.htm#ComputerPrograms.

2.1.3.4. Molecular sexing. The sex of four whales was identified or confirmed by amplification of sex-specific molecular markers following the protocol by Gilson et al. (1998). The male-specific SRY gene was amplified and ZFY/ZFX genes of males and females where amplified as positive controls. The sex of all other whales was identified in situ.

3. Results

3.1. *Mitochondrial genetic diversity*

All samples ($n = 25$) were successfully amplified for 370 bp fragment of the mtDNA CR. In order to compare with sequences already published, the length of our sequences was reduced to a consensus fragment of 345 bp. Analyses of this fragment revealed two variable sites defining three haplotypes (Table 1). One haplotype (Car1) was not observed in other regions, being unique to the Caribbean (Genbank accession number KF801471). A second haplotype (Haplotype D) was shared with the Atlantic (found in 11 samples, Siemman, 1994) and a third haplotype (Haplotype A) was shared with the South Pacific (17 samples), the eastern North Pacific ($n = 1$) and the Atlantic Ocean ($n = 1$; Oremus et al., 2009).

Table 2
Statistics for seven microsatellite loci analyzed.# is the number individuals, $T°_A$ is the annealing temperature. N_a is the number of alleles found. H_O is the observed heterozygosity, H_E is the expected heterozygosity. and HWE *p*-value refers to the results of the test for deviation of Hardy–Weinberg equilibrium.

Locus	#	$T°_A$	N_a	H_O	H_E	HWE *p*-value	Null allele Frequency	Source
464/465	20	45	9	0.400	0.769	0.000	0.193	(Amos et al. 1993)
DlrFCB1	19	50	11	0.864	0.842	0.004	0.000	(Buchanan et al. 1996)
DlrFCB6	22	62	4	0.273	0.489	0.009	0.150	(Buchanan et al. 1996)
EV1	25	45	17	0.840	0.900	0.129	0.010	(Valsecchi and Amos. 1996)
EV37	23	50	8	0.609	0.562	0.766	0.000	(Valsecchi and Amos. 1996)
Ppho131	19	60	5	0.368	0.333	1.000	0.000	(Rosel et al. 1999)
GT575	25	50	12	0.920	0.827	0.796	0.000	(Bérubé et al. 2000)

3.2. Microsatellite genetic variation

We were unable to obtain complete genotypes for every single individual at every locus because of unsuccessful amplification possibly due to low DNA quality. A total of 14 individuals were genotyped for all seven loci, five were genotyped for six loci, four for five loci and two for four loci. The mean observed number of alleles per locus was 8.6, mean observed heterozygosity was 0.610, and mean expected heterozygosity was 0.675. Loci 464/465 and DlrFCB1 deviated from Hardy–Weinberg equilibrium (Table 2). There was evidence of linkage disequilibrium for loci DlrFCB6 and Ppho131 and loci DlrFCB1 and GT575. Estimates of null alleles were low for most locus sites, where only loci 464/465 and DlfFCB6 appeared to have a higher frequency on null alleles.

3.3. Relatedness estimates

Mean expected relatedness values can range from 0 to 1, where 0 implies no relatedness between sampled individuals within a group. Mean relatedness values ($r = 0.091$) have been reported for short-finned pilot whale groups in the oceanic archipelago of Madeira. In groups of up to 30 individuals mean relatedness was high (around 0.3) and as group size increase mean relatedness decrease (Alves et al., 2013).

Mean relatedness computed in ML-Relate was slightly higher for the individuals stranded in British Virgin Islands ($r = 0.12$), than the mean relatedness computed for the individuals stranded in Trinidad ($r = 0.04$). The simulations in Identix suggested that mean relatedness values did not differ significantly from those expected at random in Trinidad ($p = 0.13$). However, in the British Virgin Island stranding there was a suggestion of a higher relatedness index than expected at random ($p = 0.04$).

Internal relatedness IR estimates can vary from -1 to 1, whit negative values suggesting relative outbred individuals and high positive values being suggestive of inbreeding. Since the quantity is being measured between parental half genotypes within an individual, it is called internal relatedness (IR). Mean IR estimates for Trinidad (-0.020) suggest that individuals were not so closely related, while mean positive IR estimates for British Virgin Islands (0.0212), are in congruence with higher mean ML-relate estimates, suggesting that, in fact, animals in this stranding were probably closely related among them. The homozigosity weighted by locus (HL) index varies between 0 when all loci are heterozygous and 1 when all loci are homozygous. Estimated values for Trinidad (0.242) and for British Virgin Islands (0.448), suggest individuals stranded in British Virgin Island had an overall higher homozygosis.

4. Discussion

Worldwide mtDNA diversity for the short-finned pilot whale has been considered to be low (Oremus et al., 2009) relative to other whale species with broad distributions and large population sizes (Whitehead, 1998). Atlantic populations have been reported to have low nucleotide and haplotype genetic diversity ($h = 0.25$, $\pi = 0.07\%$, Oremus et al., 2009), while northeast Atlantic populations have reported relatively higher values ($h = 0.692$, $\pi = 0.20\%$, Miralles et al., 2013). For the Caribbean region, mtDNA haplotype and nucleotide diversity ($h = 0.55$, $\pi = 0.33\%$) were slightly higher than those reported by Oremus et al. (2009) but were relatively lower than the values reported for the northeast Atlantic. Considering the low worldwide levels of mtDNA CR diversity reported for short-finned pilot whales (Oremus et al., 2009; Siemman, 1994), the finding of a new haplotype (Car1) for this region is important. Further sample collection and analyses are needed in order to explain the possible local mtDNA CR differentiation that has been observed to date in the Caribbean populations of short-finned pilot whales.

4.1. mtDNA haplotype distribution

Two mass stranding events in the Caribbean region took place in the British Virgin Islands and Trinidad (Fig. 1). In Trinidad, previous sightings at sea estimated group size to be 25 individuals, but only 11 individuals from this group where stranded on October 13, 1999 at Cocos Bay along the southeast coast. All individuals at this stranding event shared haplotype A. The

stranding event in the British Virgin Islands was progressive, occurring between 28th and 30th of August 1999. Additionally, nine of ten individuals shared the Car1 haplotype with the exception of one female, which had haplotype A. This female was stranded during the same time period but was found at a different location (Pear Point, Anegada), relatively far away from the other individuals (found in East End, Anegada). It is possible that this female may have belonged to another sub-group differing in its haplotype sequence by only a single transition. However there is no information available regarding the complete group size at sea. The mtDNA information from both stranding events is supportive of some level of matrilineal social structure, although information from these events must be interpreted cautiously; these mass strandings represent an unusual event and pre-stranding social disruption may have further separated related individuals before the actual stranding events (Oremus et al., 2013).

4.2. Stranding mean relatedness

Short-finned pilot whales have long lasting relationships, and small groups are usually composed of related individuals suggesting some degree of social philopatry, while large groups may be temporary associations of smaller groups (Alves et al., 2013). Therefore one would expect higher relatedness estimates among whales in the same group or among stranded individuals from the same group.

Mean relatedness estimates for the stranded whales at Trinidad ($r = 0.04$) and for whales stranded at British Virgins Islands ($r = 0.12$) have similar values to relatedness estimates (mean = 0.097) for 31 groups of pilot whales from the oceanic archipielago of Madeira. This lower mean relatedness estimated for Trinidad is in agreement with Alves et al. (2013), indicating mean intragroup relatedness was negatively correlated with group size, since only 11 from 25 whales stranded. Of course, the small sample size available for this study requires that these initial relatedness results be confirmed with increased sampling in the area.

Mean group size at sea for short finned pilot whale has been estimated in 11 individuals in the Caribbean region (Mignucci-Giannoni, 1998) and 15 for the short-finned pilot whales encountered in Madeira (Alves et al., 2013). Stranded groups at Trinidad ($n = 11$) and British Virgin Islands ($n = 10$) are of similar size, however pre-stranding social disruption may have occurred, thus affecting the stranded groups as was seen in Trinidad where only 11 from 25 whales stranded.

In this study we provide preliminary results to understand the genetic diversity and population structure of short-finned pilot whales in the Caribbean. Additional studies, with increased sample sizes and a higher number of molecular markers are warranted in the near future for this geographic area. Establishment of genetic indices for pilot whale populations throughout their range will provide better clues to potential changes in population distributions and vulnerability to human activities.

Acknowledgments

Carcass salvage was conducted under the authority of the British Virgin Islands' Department of Environment and Fisheries (DEF), Jamaica's Natural Resources Conservation Authority, Puerto Rico's Department of Natural and Environmental Resources (DNER), and Trinidad and Tobago's Environmental Management Authority. Collection, import and export of samples was carried out in the US under Marine Mammal Protection Act permits and CITES permits issued to the National Marine Fisheries Service (NMFS). We would like to thank S. Swartz, L. P. Garrison, K. D. Mullin, R. Brownell and K. Robertson (NMFS), B. Lettsome (DEF), and M. A. García (DNER) for their assistance with these permits. Salvage efforts and sample collection was funded in part by the National Science Foundation and a grant from the Commonwealth of Puerto Rico Legislature. We are grateful for the assistance of personnel, participants, students and volunteers of the DEF, DNER, Trinidad's Manatee Conservation Trust, University of The West Indies' School of Veterinary Medicine, and of the Caribbean Stranding Network in documenting these cases, rescuing efforts and the collection of samples, particularly J. Khan, G. Lutchmedial, M. Alsina, M. Cardona, B. Carrasquillo, N. Jiménez, L. Guzmán, J. Pérez, E. Rivera, M. Rodríguez, V. Rosado, R. Rosario, M. Tacher, G. Toyos, and M. Vargas. We would also like to thank D. Heimeier and The Ecology and Evolution Research Group at the University of Auckland (New Zealand), for allowing us access to microsatellite primers. Our gratitude also goes to Carlos Mora for sample sequencing. Financial support for this project was provided by “Proyecto Semilla” and “Proyecto de Profesor Asistente” by Universidad de los Andes, Bogotá, Colombia.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bse.2014.06.001.

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