Investigating the feasibility of using DNA from sloughed skin for individual identification in humpback whales (*Megaptera novaeangliae*)

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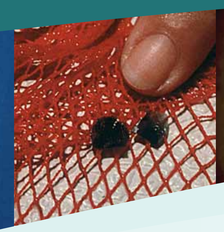

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Introduction

Various methods of DNA collection for cetaceans have been utilized in the past including biopsy darting, fecal sampling and sloughed skin collection. While biopsy sampling promotes an accurate match between observed whale identity and DNA collected, it is an invasive technique. Collection of fecal matter is non-invasive but often does not yield high quality DNA. In this study, we demonstrate that sloughed skin collection provides a non-invasive, low-cost means of acquiring high quality DNA for individual identification of humpback whales (*Megaptera novaeangliae*).

In the past, sloughed skin collection has been used to investigate group composition of humpback whales (e.g. Amos 1992, Valsecchi *et al.* 1998). Valsecchi et al. demonstrated that the same number of genotypes and sex composition in pods was consistent with field observations. In only 1% of cases there were more genotypes than whales observed. In addition, multiple samples from a single collection event were gathered for 15 single whales. Every set of samples for each 'singleton' yielded a single genotype indicating that all skin fragments collected at that one site belonged to the same individual.

In our study, we demonstrate the accuracy of individual identification over time. We have genotyped samples from known whales across years to prove that sloughed skin gives identical, uncontaminated, genotypes over time. Reliance on genotypic data will prove valuable when answering questions about humpback whale ecology. Here, we emphasize the use of sloughed skin for individual identification to investigate reproductive ecology rather than population genetics. Eventually, with the integration of genetic data and photo identification of known whales and associated behavioral observations, we will have the opportunity to better evaluate reproductive success and assess aspects of reproductive biology that no one approach can address in isolation.

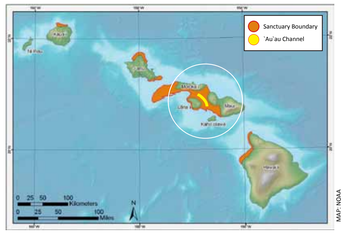

Figure 1. Sloughed skin samples were obtained within the National Marine Sanctuary in the waters of the `Au`au Channel between the four islands region of Maui, Lāna`i, Moloka`i and Kaho`olawe.

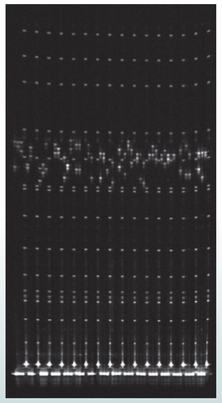

Figure 3. A computer image of a gel ran using a Li-Cor 4200 automated sequencer and M13 labels.

References

Amos W. 1992. Restrictable DNA from sloughed cetacean skin; its potential for use in population analysis. Marine Mammal Science 8(3) 275-283.

Valsecchi E, Glockner-Ferrari D, Ferrari M and Amos W. 1998. Molecular analysis of the efficiency of sloughed skin sampling in whale population genetics. Molecular Ecology 7: 1419-1422.

Abstract

Obtaining genetic samples from cetaceans can be logistically challenging. Various methods of DNA collection for cetaceans have been utilized, including biopsy darting (invasive, strict permit regulations) and fecal sampling (difficult to locate, reduced quality DNA). While sloughed skin has been utilized for genetic analyses of cetaceans in the past, the possibility of misidentification of the animal being sampled has yet to be investigated. In this study, we demonstrate that sloughed skin collection provides a non-invasive, relatively low-cost means of acquiring high-quality DNA for reliable individual identification of humpback whales over time. Using a subset of a large (> 1,500 samples) long-term sloughed skin dataset and a novel combination of 14 microsatellite markers, we compared genotypes from multiple samples of 11 known individuals (confirmed from field observations and photo-ID) that had been seen within and/or across field seasons. For all cases in which we obtained more than one skin sample from a whale identified in the field as the same individual, we confirmed that the genotypes were identical. We also compared genotypes of samples collected from four mothers and their calves (six of these were included in the 11 known individuals with multiple samples) and demonstrated that, in all cases, known mothers shared one-half of their genotype with their observed calves. Careful analysis of genotypic data from sloughed skin enables scientists to address questions about humpback whale social dynamics, reproductive success, ecology and life history. In the future, this protocol can be applied to the comprehensive humpback whale dataset and to other cetacean species where sloughed skin can be collected.

Methods

Data collection:

- Sloughed skin samples were collected as part of a long-term study of humpback whale behavior over the course of fourteen seasons (1995-2009, excluding 1997).
- Sloughed skin was taken from both above and below the water's surface with an aquarium net.
- Samples were collected from February through April in waters off the coast of Maui, Hawaii and were stored in non-iodized table salt (NaCl) since collection.

Sample distribution:

- DNA was extracted (using Qiagen DNeasy kits) from known individuals and any associated calves.
- Genotyping samples collected from the same individual at different sighting events allowed us to test the validity of the sloughed skin collection method.
- We genotyped a total of 47 samples, representing 14 individual whales.

Data analysis:

- 29 microsatellite loci were screened and 14 total loci were chosen for analysis.
- A MJ Research DNA engine (Waltham, MA) was used to perform PCRs in 10 µL volumes (10mM Tris-HCl, 50mM KCl, 2.0mM MgCl2, 0.2 mM each dNTP, 0.3 U Taq DNA polymerase, and 100 ng DNA template). PCR profiles involved one cycle at 94°C for 2 min; 30 cycles of 94°C for 30 s, annealing temperature for 30 s, and 72°C for 15 s; and one cycle at 72°C for 30 min.
- Products were visualized using M13 labels and Li-Cor 4200 and 4300 automated sequencers (Li-Cor Biosciences, Lincoln, NE).
- Genotypes were scored using SAGA v 3.3 (Li-Cor Biosciences, Lincoln, NE).
- GENEPOP version 3.4 (Raymond and Rousset 1995) was used to test for Hardy-Weinberg equilibrium. Exclusion probabilities were calculated using CERVUS.

Results

- Seven of 14 loci deviated significantly from Hardy-Weinberg equilibrium after Bonferroni correction. The high proportion of loci out of HWE is currently under re-investigation.
- Exclusionary probabilities demonstrated that the probability of identifying a single parent with no information on the other parent was 0.9999 and the probability of identifying an individual was 0.9999.
- We obtained replicate samples for 11 individuals. In all cases, the individual's genotype was identical.
- In all cases, known mothers shared at least one allele at each locus with their observed calves.

Table 1. Number of samples genotyped for each known individual, years the individual was sampled and number of days they were sampled in years of multiple sampling dates. Note that individuals sampled in multiple years have a separate listing for each year.

Identity	Sex	Sample year	Number of samples genotyped	Number of sampling dates	Group type
Escorts or unknown					
Big Scar	male	1996	4		Mother/calf/singing escort
Calf in group with escort Big Hunk	female	1996	2		Competitive group with calf
Females with calves and associates by year					
Carmen	female	2002	1		Mother/calf/escort
Carmen's calf Rascal	male	2002	6	4	Mother/calf/escort
Carmen	female	2006	5	3	Mother/calf/escort or mother/calf
Carmen's calf	female	2006	1		Mother/calf/escort
Carmen	female	2009	1		Mother/calf then joined by escort
Pineapple	female	2006	2		Mother/calf
Pineapple's calf	male	2006	1		with mother
Sasha	female	1995	7	3	Mother/calf
Sasha's offspring Flood	male	1995	2		with mother
Sasha	female	1996	1		Mother/yearling
Sasha's offspring Flood	male	1996	3		with mother
Spreckles	female	1995	2		Mother/calf/escort
Spreckles' calf	male	1995	1		with mother
Escort with Spreckles	male	1999	2		Mother/calf/escort
Spreckles	female	2008	1		Mother/calf
Tippy	female	1998	2		Subadult female
Yoga's calf	female	2000	3		Mother/calf
Total			**47**		

Table 2. Descriptive statistics of the 14 loci used in analysis. n is the number of genotypes obtained out of 176 total samples. ** indicates significance after Bonferroni correction.

Locus	Allelic range (bp)	Number of alleles	n	Annealing temp.	HW	GenBank accession no.
AC137	138-168	14	168	60	**	DQ151647
GT129	102-114	4	174	55	**	DQ151655
GATA028	164-204	7	172	54	**	U93890
GATA098	95-131	8	152	56	NS	U93892
CA234	201-223	9	163	58	NS	DQ151651
GATA417	207-247	11	171	49	**	U93893
AC045	191-205	5	174	58	NS	DQ151645
GT575	156-182	10	164	61	**	AF309698
CA141	192-204	5	159	62	NS	DQ151659
GT271	114-138	8	150	61	**	AF309694
GT310	122-126	3	173	63	NS	AF309696
GT195	175-187	7	165	54	NS	AF309692
GT023	125-139	8	151	62	**	AF309690
CA232	166-192	10	165	56	NS	DQ151650

Conclusions

- This unique combination of loci, along with the optimized protocol, will provide further insight for future analyses of humpback whale social dynamics, reproductive success, ecology and life history.
- The high-quality DNA demonstrates that storage of sloughed skin in NaCl at ambient temperature is a viable preservation method for skin samples for up to 15 yr.
- Our ability to identify individuals with such high exclusion probabilities highlights the utility of the chosen microsatellite markers.
- Comparing genotypes from multiple samples of known individuals validates sloughed skin collection by proving that field observations of individuals have matched with genetic identification.
- This analysis only includes a subset of samples from a much larger dataset. The protocol that has been optimized in this study, including the validation of sloughed skin collection methods for individual identification, can now be applied to additional samples in order to gain a broader understanding of humpback whale reproductive ecology.
- Integrating long term photo identification and genetic analyses of known individuals with behavioral observations of known individuals will provide the tools necessary to generate paternity analyses and better understand how, and to what extent, different mating strategies influence reproductive success in humpback whales.

Acknowledgments

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