Appendix 6

ON THE RELATIVE UTILITY OF BIOPSY, SLOUGHED SKIN AND HISTORICAL SAMPLES IN POPULATION GENETIC ANALYSES

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For over a decade, biopsy sampling has become a widely accepted means of collecting tissue samples for genetic analyses. Biopsy sampling provides small samples of skin and blubber that are useful for a number of genetic and biochemical analyses. The collection of biopsy samples can be integrated into a directed survey design, providing for systematic sampling required for many population-based analyses (e.g., capture-recapture). The quality and quantity of DNA extracted from biopsies is high and creates a permanent archive of the individual whale.

However, due to logistical problems or permitting issues, some researchers have had difficulty with this type of sampling, and have turned to sloughed skin or historical samples as a source of DNA. These latter include bone, baleen and preserved tissues. Some studies have utilized historical materials for addressing specific questions, such as Rosenbaum et al.'s (1997) study comparing haplotypes from museum archived baleen to those from contemporary right whales. This study included a temporal component that necessitated the use of the baleen, the only material available from the whaling era. In Dalebout et al.'s (2002) phylogenetic study of beaked whales, bone material from museums was used for some species, due to the rarity of fresh material from these rarely seen and difficult to sample whales. In other cases (e.g., Clapham et al. 1993), sloughed skin was used in genetic analyses of modern populations of humpback whales. These successes understandably lead some to question the need for obtaining biopsies from living whales for population genetic studies, enquiring instead if sloughed skin and/or museum material would suffice. Here we outline some of the problems encountered with these other materials and the advantage of using biopsy samples.

The primary problem with museum and historical samples is that they usually yield very small amounts of degraded DNA, which can be difficult to extract and work with. The studies mentioned above all generated sequences from the mitochondrial genome, which has a high copy number in cells, which in turn increases the chances for amplification the target sequences. Even this data generation is much more labor-intensive, often requiring multiple extractions and special workspaces in the laboratory (to avoid contamination from other, more robust, DNA sources). In population genetic studies, nuclear markers, such as microsatellites, SNPs and nuclear gene sequences are becoming increasingly important, providing data that are independent of the mitochondrial genome and allowing individual-based analyses through genotyping. The fragmentary nature of the DNA in historical samples usually precludes the examination of nuclear markers, whose lower copy number in the cell significantly decreases one's chances of successful amplification. This problem is not necessarily unbiased, in that larger target segments have a lower chance of amplification than smaller ones. This "allelic dropout" can be a serious source of error in studies that use markers such as microsatellites, in which size polymorphisms are the primary source of variation. These missing alleles would not necessarily be uniformly distributed across the sample set, introducing considerable bias. Some museum materials, especially soft tissues, are preserved in formalin. In addition to the low yield and fragmented DNA, these materials often contain PCR inhibitors, adding yet another layer of difficulty to obtaining usable data.

In some cases, sloughed skin or "scrubbed" skin samples yield high quality DNA, albeit in very small amounts. Their utility appears to vary between species, perhaps even between populations. In some ways, their limitations are similar to those of historical samples, in that studies involving nuclear DNA markers face more difficulties than those targeting mitochondrial DNA. For example, Gendron and Mesnick (2001) had only a 55% success rate in genetically determining the sex of blue whales from sloughed skin. In addition to the technical limitations involving DNA, there are other problems associated with depending on sloughed skin for any particular study. Sloughed skin sampling is opportunistic, in that the researcher has no control over whether a given whale will slough any skin, and when there are multiple whales around, there will be some uncertainty about which whale is the source. The difficulty of genotyping the sample, which involves nuclear DNA, prevents identification of individuals. Determining the molecular identity of individual whales also allows estimation of abundance through capture-recapture, and assessment of relatedness, paternity, male-mediated gene flow and reproductive success. These analyses would be difficult or impossible with sloughed skin samples.

Obviously, none of the problems discussed above apply to biopsy samples. Although some studies have reported some success with sloughed skin and historical samples, and new laboratory techniques are continually being developed for their use, these alternatives should not be considered preferable to biopsy for population genetic studies, especially in light of the increasing emphasis being given to large sample sizes and multiple markers for effective analysis. It should also be mentioned that biopsy samples also enable other important data to be collected from the skin or blubber, such as quantification of contaminants, stable isotope analyses and determination of reproductive status from hormone levels.

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