

Genetic Variation and Immune Response in Beluga Whales

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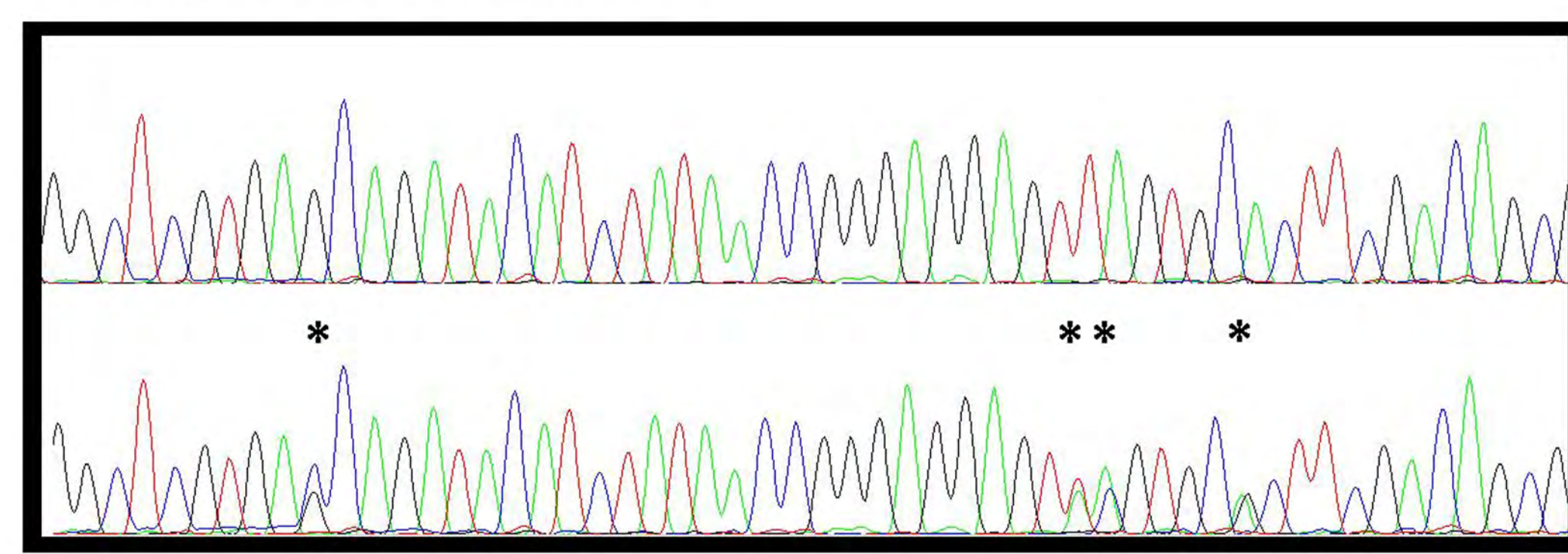
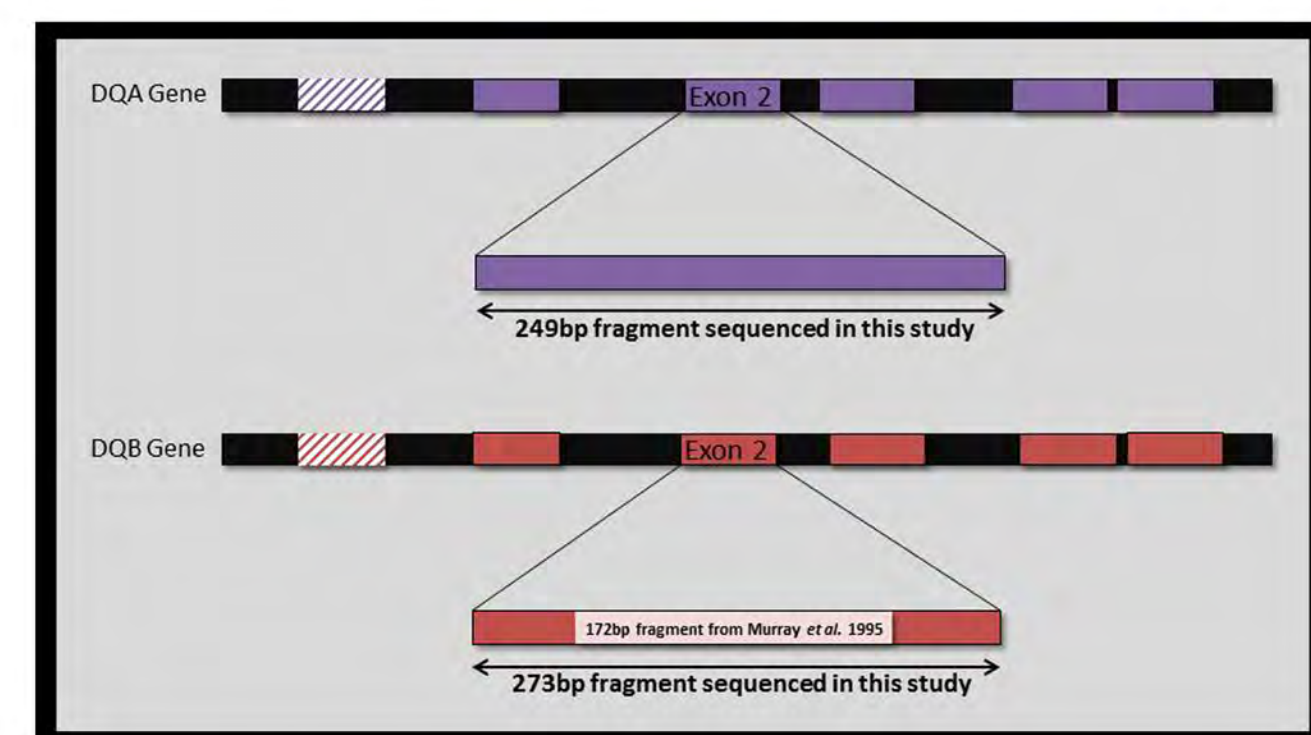
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ABSTRACT

The Major Histocompatibility Complex (MHC) is the system of antigen recognition and presentation that initiates the immune response cascade in vertebrates. Characterizing MHC diversity is a genetic measure of the adaptive aspects of immune function of vertebrate populations, and is therefore of central importance to species conservation. The polymorphism detected within MHC genes is maintained by positive selection but can also be influenced by drift and gene flow. Class II genes defend against extracellular pathogens, suggesting diversity at these loci may reflect the pathogen environment. Using direct sequencing methods, we isolated the entire exon 2 of the Class II DQB locus in beluga whales from the Chukchi Sea (n=20) and the DQA locus in belugas from the Chukchi Sea (Kasegaluk Lagoon n=17; Kotzebue Sound n=5), Bristol Bay (n=13) and Cook Inlet (n=3). This is the first time the entire exon 2 (273bp) for DQB has been sequenced in beluga whales, and at least 4 alleles were detected. Although there are few cetacean studies who have characterized the entire exon 2 for comparisons, this does appear to be a low amount of polymorphism for this locus. In 1995, Murray *et al.* sequenced 172bp for n=233 beluga whales, and characterized 5 alleles; however, we detected an additional polymorphic site downstream from their fragment, suggesting their allele number is artificially low. To date, DQA has never been sequenced in beluga whales. We analyzed the entire exon 2 (249bp), and, as yet, have found no variation. This is surprising, as we have found substantial polymorphism at this locus in other cetaceans, including bottlenose dolphins, killer whales, and short finned pilot whales. The monomorphism likely reflects strong selective constraints for the observed allele. However, genetic drift and mutational forces may also be at play. These initial findings may provide some genetic insight as to why some beluga populations are not recovering, but they also highlight the need for a more thorough and comprehensive study within and among beluga whale populations and for further comparative work across higher level taxa.



DQB Exon 2 Chromatogram
* indicates heterozygous sites

METHODS

Two sets of cetacean-specific primer pairs were designed to include the entire exon 2, one pair for each locus. Using traditional Sanger sequencing methods, samples were then sequenced on a Genetic Analyzer 3130xl. The sequences were individually inspected and manually aligned using Sequence Analysis v5.22. A three step process was then implemented to identify potential heterozygous sites within individual sequences: (1) manual inspection of chromatograms for the presence of double peaks, the lower peak being at least 25% in height compared to the higher peak, (2) automated detection via reanalysis of sequences in Sequence Analysis v5.2 using a new protocol manager that identified the heterozygote peak using the official IUPAC nucleotide nomenclature, and (3) confirmation of heterozygote peaks in both the forward and reverse strand. Sequences were then aligned using ClustalW Multiple Alignment in BioEdit Sequence Alignment Editor Version 7.1.3.03.

ACKNOWLEDGEMENTS:

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RESULTS

DQB

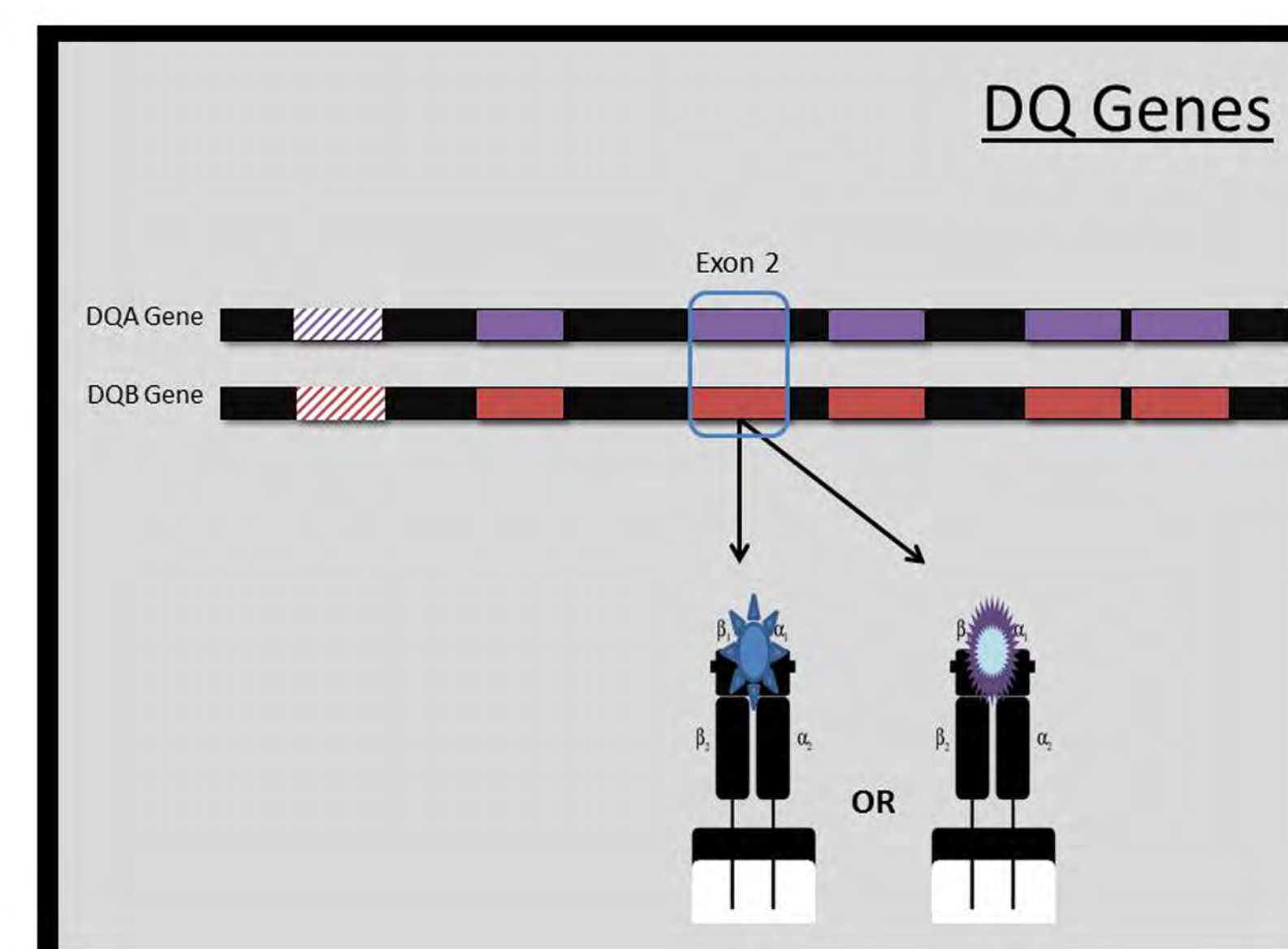
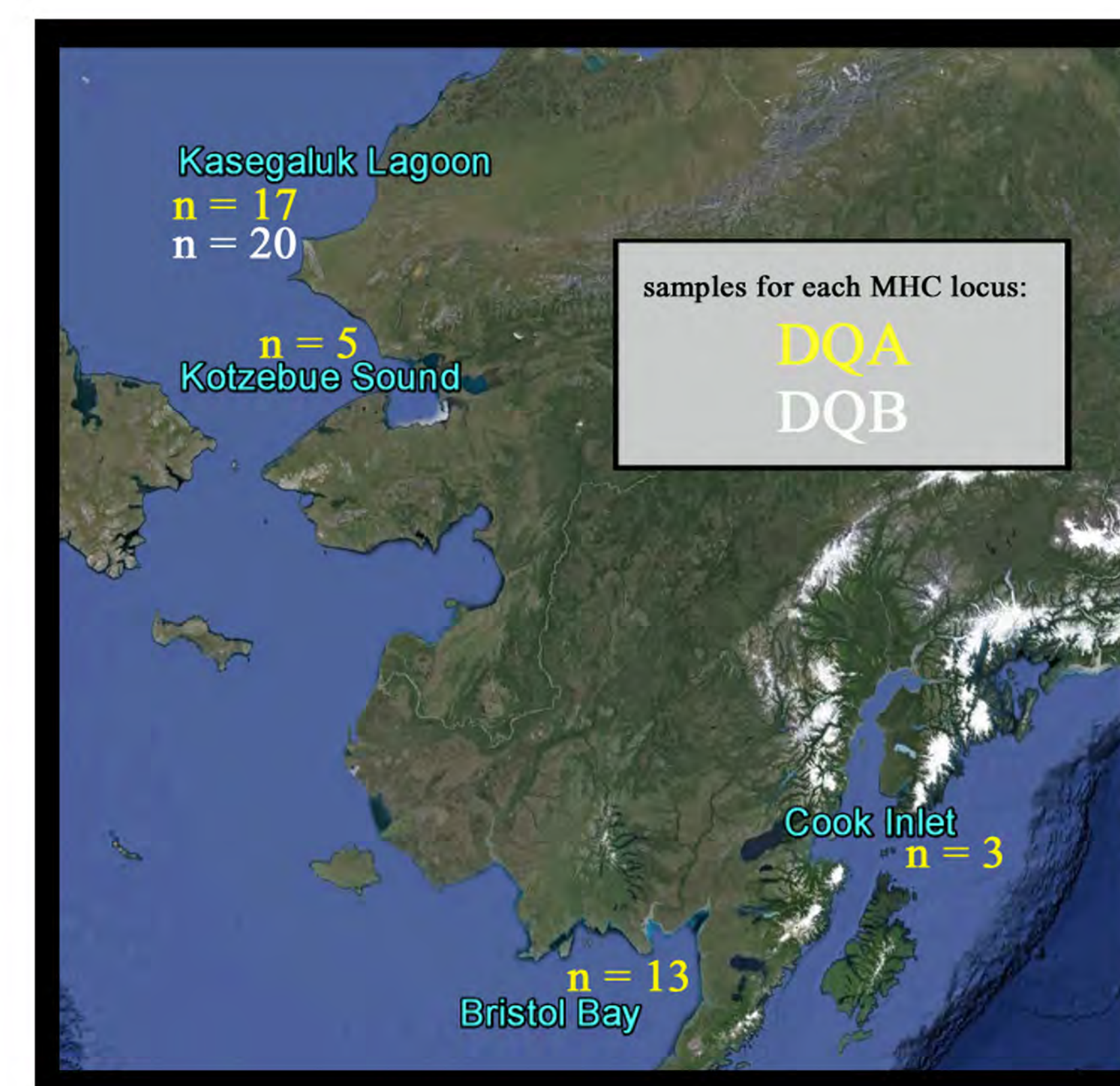
- This is the first study to characterize the entire exon 2 (273bp) for DQB in n=20 beluga whales
- At least 4 alleles were identified
- Detected an additional polymorphic site downstream from the Murray *et al.*, 1995 study, which only characterized 172bp of the exon 2

DQA

- To date, this is the first study to characterize DQA exon 2 in beluga whales
- Sequenced the entire exon 2 (249bp) n=38
- Only one allele has been identified
- Low diversity compared to *Tursiops truncatus* (n=93), *Orcinus orca* (n=3), and *Globicephala macrorhynchus* (n=12)

CONCLUSIONS

1. There appears to be a low amount of polymorphism in MHC for beluga whales compared to other cetaceans, including bottlenose dolphins, killer whales, and short finned pilot whales
2. The additional polymorphic site downstream from the Murray *et al.* DQB fragment indicates that their reported alleles are not fully resolved and the number could be artificially low
3. The monomorphism seen in DQA exon 2 may indicate:
 - a). a dramatic loss in diversity in recent evolutionary history that may reflect increased species vulnerability and possibly provides insight as to why some populations are not recovering
 - b). the observed allele provides some critical benefit to the species and is under strong selective constraints
4. It is important to take into account neutral variation as well, as genetic drift and mutational forces may also be impacting the species
5. This pilot study demonstrates why it is important to simultaneously look at multiple genes and their regulatory regions. For more information on promoter regions in cetaceans, see Pagán *et al.* complimentary study poster in **Row 18 #208**



FUTURE WORK

- Applying the next generation sequencing method developed by Pagán *et al.* to type DQB alleles
- Increase sample number and populations for both loci
- Relate patterns of MHC class II diversity in beluga whales to patterns of pathogen exposure and whale health
- Participate in the **Cetomics Initiative** to identify antigen epitopes for specific DQ alleles