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Genetic Polymorphisms in the Oxytocin Receptor Gene of Beluga Whales and Bottlenose Dolphins

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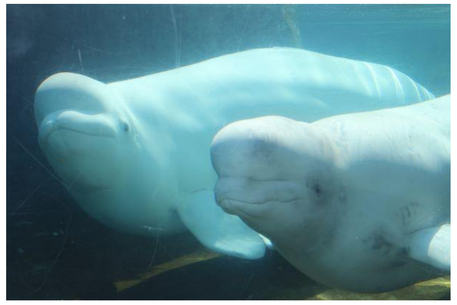
Introduction

Gene polymorphism refers to genes that have more than one occupying allele within a gene's locus. One gene polymorphism that has recently gained the interest of scientists are genes associated with the oxytocin receptor. Recent studies in terrestrial species have found that genetic polymorphisms in the oxytocin receptor are associated with social behaviors in many species.[1]

In dogs, polymorphisms in the oxytocin receptor gene are related to human-directed social behaviors.[2] Specifically, one study shows that the oxytocin receptor gene affects proximity (how willingly a dog approaches and interacts with its owner and stranger) and friendliness (reaction to a threatening stranger and a passive stranger).[2] In humans, polymorphisms in the oxytocin receptor gene are related to the security/insecurity in mother-infant attachments.[2] This can be seen in behaviors such as approach and physical contact towards the caregiver during reunion.[2] Similarly, an oxytocin receptor gene polymorphism in dogs was related to proximity seeking.[2]

However, there is little information available about the oxytocin receptors in various aquatic species and no information on beluga whales (*Delphinapterus leucas)* and bottlenose dolphins (*Tursiops truncatus).* The aim of this research project is to gain a better understanding of the interspecies variation in the oxytocin receptor gene in beluga whales and bottlenose dolphins and to develop tools that could be used to assess intra-species variation in the future. In the future, this work may be integrated with behavioral research to better understand social and maternal behavior, which are important for conservation and management of marine mammal species.



Materials

The DNA used originates from 2 belugas. The published sequences of the oxytocin receptor and non-coding regions near this gene in dogs was used to find the homologous regions on the beluga and bottlenose dolphin genomes, which are published on GenBank. Based on these sequences, PCR Primers were designed so that these regions could be amplified and sequenced. Primers were designed to cover at least 600 base pairs of DNA in the both 5' and 3' direction from the oxytocin receptor. The PCR reaction mixture for each primer pair of consisted of 4 µL of each primer, 3 µL of DNA template, 3 µM dNTPS, 15 µL magnesium chloride, 0.6 µL Taq Polymerase, and 6 µL KB extender. The PCR cycle had 35 cycles of 1.5-minute denaturation at 94 °C, 2-min annealing at 53 °C, and 3 min extension at 72 °C, followed by the final extension step of 5 minutes at 72 °C. The PCR reaction was performed in a total volume of 50 µl. The PCR products were then used for gel electrophoresis. A 2% agarose gel was used. The gel electrophoresis was conducted at 85 volts.

Results

Three different overlapping regions were amplified, which will allow for sequencing to explore for potential polymorphisms in the oxytocin receptor gene's 5' promoter region.

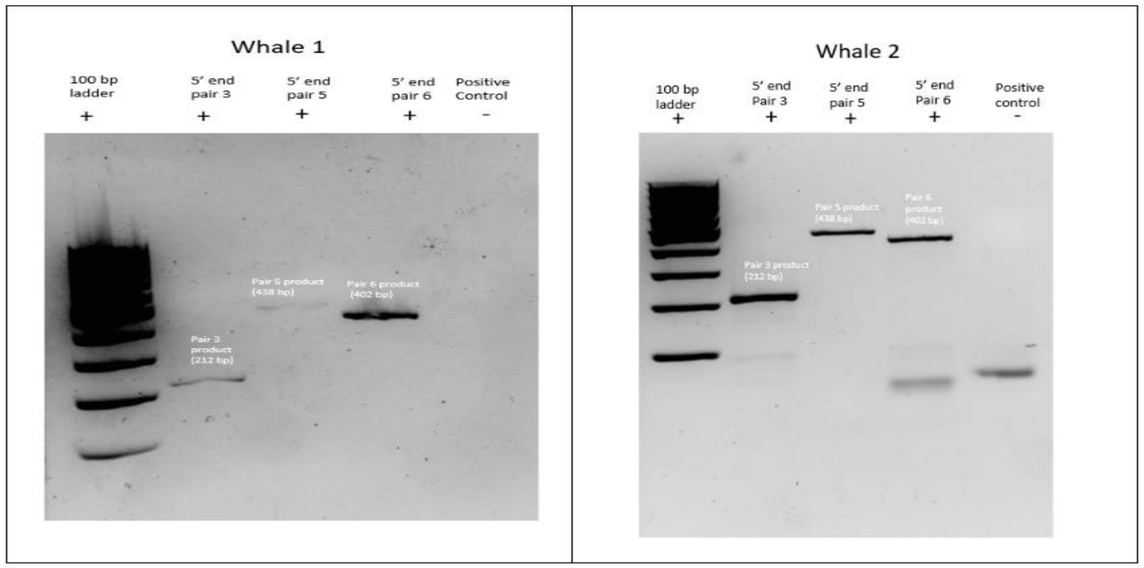


Figure 1: PCR results of working primers from two different DNA samples.

Primer	Sequence	Length	TM	Expected Product	GC Content
5'-1F	AGGAAGAAACCAAGCGGATTA	21	54.1		42.857
5'-1R	CAGTGTGATAGGATGCTGAGG	21	54.9	363	52.381
5'-2F	GCTATCCAAGCCTCCCATTT	20	55		50
5'-2R	CGGTACCCAGTGTGATAGGA	20	55.8	421	55
5'-3F	CCCTGAAGTTGACCTGATGATTG	23	55.7		47.826
5'-3R	CGCGGTAACAAACAGGCTAAT	21	55.8	212	47.619
5'-4F	CTTTAGGTTCGCCTAACGACT	21	54.4		47.619
5'-4R	GGCTATACTCAGGTGCTTCAT	21	54.2	428	47.619
5'-5F	CTTCCCTCTTGCTGCTTGAA	20	55.3		50
5'-5R	TCGTTAGGGGAACCTAAAGTTG	22	55	438	45.455
5'-6F	TGCAATTAGCCTGTTTGTTACC	22	53.8		40.909
5'-6R	GAACCTAAAGTTGGCTCCCT	20	40.9	402	50
3'-1F	GAACCCAGGATGCCAGATATT	21	54.6		47.619
3'-1R	TGGACCTTGATGTCCCAAAG	20	54.8	342	50
3'-2F	GGTGCTGGTCTTCGTTTCT	19	55.1		52.632
3'-2R	GGACTTGGAACTTCCTGGTATC	22	54.9	324	50
3'-3F	ATGGAGACCAGATTCAGTGATG	22	54.3		45.455
3'-3R	CTCCGAAGGTGGGAAGAATATC	22	54.8	225	50
3-'4F	GCCAGATATTCTTCCCACCTTC	22	55.3		50
3'-4R	CAACGATGAGTGAGGGACTTG	21	55.5	389	52.381
3'-5F	TGCTGGACGCCATTCTTT	18	54.9		50
3'-5R	TGGATGCAATTTCCAACATCAC	22	54.1	215	40.909
3'-6F	CAGCTGTGACTGCCTTCTT	19	55.2		52.632
3-6R'	CCTGGATTCACTGAAGGTTTCT	22	54.7	295	45.455

Figure 2: Primers pairs designed to span 5' and 3' regions surrounding the oxytocin receptors.

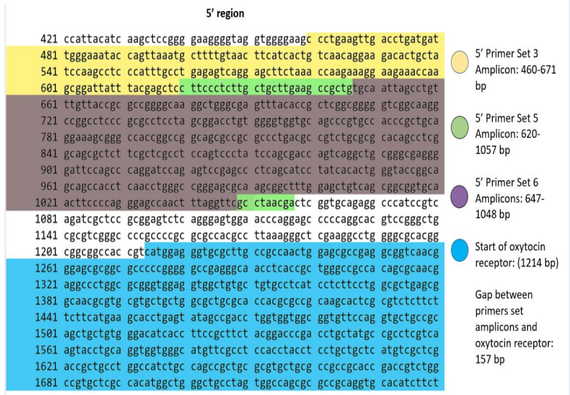


Figure 3: Area of coverage of functioning primers on the 5' region surrounding the oxytocin receptor.

The primer pairs used in this project varied widely in their ability to amplify the correct region of DNA. Three of the primers developed for the 5' promoter region in beluga whales showed successful amplification of the expected size product. Together, these primer pairs covered most of the promoter region; However, a small gap of 157 bp remained uncovered by the existing primers. New primers pairs were designed to cover this gap in beluga whales, and separate primers were designed to cover this gap for bottlenose dolphins. Future experiments will test the utility of these primers. If the functioning 5' beluga whale primers do not adequately amplify the homologous regions in the bottlenose dolphin genome, new dolphin specific 5' end primers were designed to be tested. In contrast to the 5' region primers, none of the primer pairs designed for the 3' region were able to properly amplify the targeted region. New primers for the 3' region in Beluga Whales were designed to attempt to amplify this region properly and will be tested in future experiments.

Although several of the designed primers showed some amplification, they were largely nonspecific. Multiple factors may cause the formation of nonspecific and smeared banding patterns. Too many PCR cycles may have been used, which may have increased the chances of nonspecific amplification. Also, an excessive Mg^{2+} concentration may have caused nonspecific PCR products. Furthermore, insufficient denaturation of the targeted sequences may have occurred due to too short of a denaturation time and/or too low denaturation temperature. In future experiments, it would be useful to continue to vary thermocycler conditions and quantities of reagents to obtain clearer bands.

Conclusions

The three products produced by the 5' primer pairs will be useful in future experiments.

Additional primer pairs were also designed to further explore genetic variations in the oxytocin receptor gene in beluga whales and bottlenose dolphins.

By developing tools to detect genetic differences between individuals within aquatic species, it will be possible in the future to integrate this work with behavioral research to better understand social and maternal behavior, which are important for the conservation and management of marine mammal species.

Future Research

The next step for this project would be to test the newly designed primers to get complete coverage of the oxytocin receptor gene in both beluga whales and bottlenose dolphins. After testing the primers on several DNA samples, single nucleotide polymorphisms can be identified by aligning and comparing the sequence data via Sanger sequencing in the Genomics and Sequencing Center at URI. This sequenced data from the beluga and dolphin data should be aligned to look for differences between the two species and other mammals that are published in the literature.

Lastly, if these regions can successfully be amplified, the DNA from the different belugas will be analyzed to determine individual differences using a real time PCR approach described in Kis et al. (2014).

References

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Acknowledgments

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