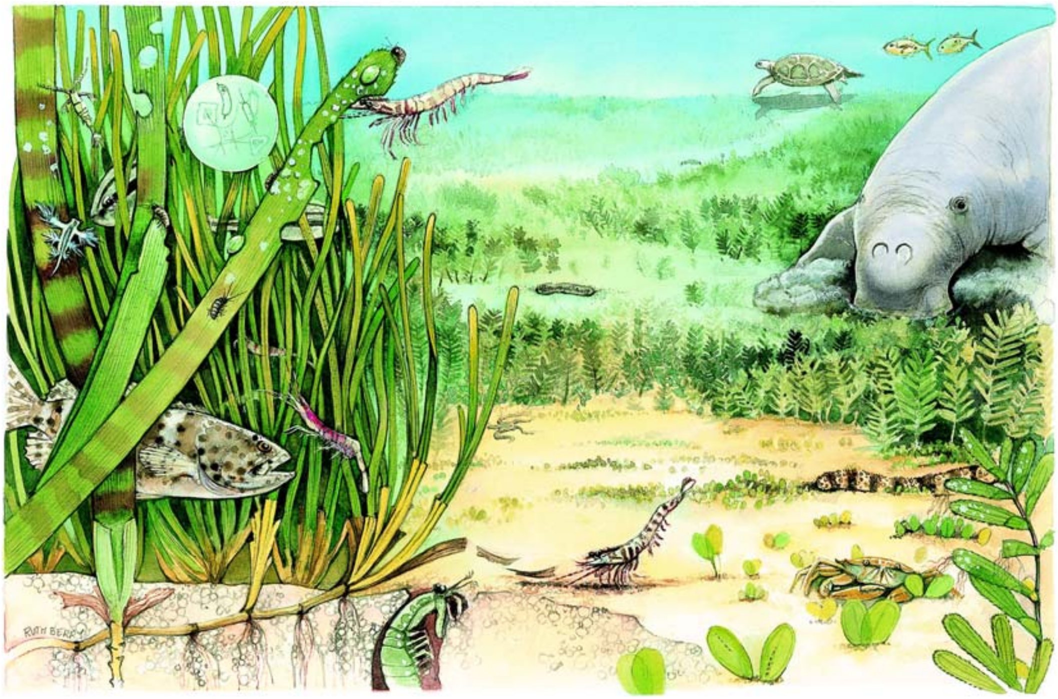
Population genetics of dugongs around Australia:

Implications of gene flow and migration



Thesis submitted by
Brenda McDonald B.Sc. Hons. (JCU)
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in the Schools of Tropical Biology, and Tropical Environment Studies and Geography
James Cook University, Townsville.

STATEMENT OF SOURCES

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given

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Glossary of terms

Definitions are adapted from Weaver (2002), Hartwell *et al.* (2000), or Lincoln *et al.* (1987).

Allele	Alternate forms of a single gene
Allopatric	Populations, species or taxa occupying different and disjunct geographical areas
Amino acid	The building blocks of proteins
Base pair	A pair of bases (A - T or C - G), one in each strand, which occur opposite each other in a double-stranded DNA
Bootstrap	A statistical method used in molecular phylogenetics based on repetitive sampling with replacement from an original sample to provide a collection of pseudo-replicate samples from which sampling variance can be estimated
Bottleneck	A sudden decrease in population density with resulting decrease in genetic variability
Clade	A monophyletic group of taxa sharing a closer common ancestry with one another than with members of any other group of taxa when presented visually as a tree
Cloning	The process of making copies of a specific piece of DNA
Competent cells	The state of bacterial cells able to take up DNA from their medium
DNA	Deoxyribonucleic acid, the molecule of heredity that encodes genetic information
DNA sequencing	The determination of the exact order of the base pairs in a segment of DNA
Evolutionary Significant Unit (ESU)	Populations that have adapted to local conditions and indicates a reservoir of unique genetic variability, demonstrated by divergence at both mtDNA and nDNA (Moritz, 1994a)
Gel electrophoresis	A process by which molecules are separated by electrical current according to size and electrical charge on a gel, usually solid matrix of agarose or acrylamide
Gene	The functional and physical unit of heredity passed from parent to offspring, usually corresponding to a protein or RNA product
Genetic drift	The occurrence of random changes of gene frequencies not due to selection, mutation or immigration
Genetic marker	A segment of DNA with an identifiable physical location on a chromosome, the inheritance of which can be traced
Genome	The entire collection of genetic information in each cell of an organism
Genotype	The actual alleles present in an individual at a locus or across many loci

Haplotype	A unique DNA sequence usually referring to mtDNA
Heteroplasmy	More than one genetic type in an individual
Heterozygous	A genotype in which the two copies of a gene that determine a particular trait are different
Homolog	Genes that have evolved from a common ancestral locus
Homozygous	A genotype in which the two copies of a gene that determine a particular trait are the same
H (heavy)-strand	The outer strand of the mtDNA genome (double stranded)
Intron	The non-coding regions of a gene that is transcribed into RNA but excised during the processing of the primary RNA into mature mRNA
L (light)-strand	The inner circular strand of the (double stranded) mitochondrial DNA genome
Management unit	Populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of alleles (Moritz, 1994a)
Microsatellite	A DNA element composed of 15 - 100 tandem repeats of two to six bp motifs
mRNA	Messenger RNA transcript which comprises the genetic information for making one or more proteins when translated
Mutation	heritable alterations of the genetic material occurring in gamete-producing cells
Negative control	PCR reagent sample to which no DNA has been added, used to indicate contamination of PCR reagents
Nucleotide	A subunit of DNA or RNA consisting of a nitrogenous base, a phosphate and a sugar molecule
Oligonucleotide	Short, synthesised chains of nucleotides used as DNA probes and primers
Panmictic	Pertaining to an infinitely large randomly interbreeding population
Phenotype	The observable traits or characteristics of an organism
Phylogeny	The evolutionary history of lineages or species
Polymerase chain reaction (PCR)	Amplification of a region of DNA using flanking primers by repeated cycles of DNA polymerase actions
Polymorphism	Any variation in the sequence of DNA among individuals
Population	For definitions see section 2.1.1.
Primer	A short oligonucleotide sequence used in the polymerase chain reaction to initiate the synthesis of DNA by a DNA polymerase
Recombination	The exchange of gene segments between non-sister chromatids through the physical exchange of homologous strands of DNA

Restriction enzymes	Enzymes that recognize a specific sequence of double-stranded DNA and cut the DNA at that site
Restriction fragment length polymorphism (RFLP)	A variation between individuals in the number of cutting sites for a given restriction endonuclease in a given genetic locus
Sequencing	Determination of the amino acid sequence of a protein or the base sequence or DNA or RNA
***Taq* Polymerase**	A thermostable DNA polymerase obtained from the thermophilic bacterium *Thermus aquaticus*. Used for amplification of DNA via the polymerase chain reaction
Universal primer	An oligonucleotide designed to be complementary to target sequences that are conserved over a wide range of taxa

Abstract

Dugongs (*Dugong dugon*) live in tropical inshore areas around Northern Australia and throughout the Indo-West Pacific. These marine mammals are obligate seagrass feeders and are thus largely restricted in distribution to areas of seagrass habitat. The turbid environment and the lack of a dorsal fin for individual identification makes it impractical to study these animals using standard observational methods. Consequently very little is known about dugong population structure and migration patterns. From satellite tracking of individuals and aerial surveys it appears that dugongs, like many other marine mammals, can move long distances, but the timing and length of movements vary individually. If dugongs move and mate in their new locality, there will be substantial gene flow across large spatial scales and very little population differentiation around the tropical Australian coast. However, if dugongs maintain a home range and are philopatric (*i.e.* any long distance movements are for food or other reasons not including mating), then there would be very limited gene flow and substantial structuring among populations. In order to ascertain which pattern of population structure is present in the dugong, I used a variety of molecular techniques to assess dugong population genetic structure around the North Australian coast.

A comparison of the two published mitochondrial genomes of dugongs showed that most variation was contained within the control region. I subsequently characterised the entire control region of eight individual dugongs (four available from previous studies and four sequenced in this study) and identified the amount of variation among them. I also made a comparison of the mitochondrial control region of dugongs with available sequences of their closest relatives, the paenungulates (Proboscidea, Hyracoidea, and other members of the Sirenia), in order to assess whether the control region was capable of producing reasonable phylogenies and its usefulness as a marker for phylogeographic and population studies on the dugong.

The 5’ domain of the control region was identified as the most appropriate section of this locus for use in the phylogeographic analysis. This domain demonstrates high diversity in the dugong. Phylogeographic analysis of the 492 bp alignment of 115 dugongs, identified two divergent Australian mitochondrial lineages. I hypothesise that the Australian lineages diverged historically during periods of low sea level that would have reduced habitat availability and produced geological barriers such as the Torres

Strait land bridge between Northern Australia and Papua New Guinea. One lineage is restricted geographically to the coast of Queensland and into the Northern Territory, while the other is more widespread occurring from Shark Bay in Western Australia to Moreton Bay in Queensland (*i.e.* across the entire Australian range). The widespread lineage is poorly represented in Southeastern Queensland. Given the availability of continuous habitat with higher sea level for the past 7000 years and the high mobility of dugongs, I expected that more complete geographic mixing of lineages would have occurred. Mitochondrial DNA sequences of dugongs from Asia are distinct from those of Australian dugongs. These results suggest long-term isolation between dugong lineages and subsequent partial geographic mixing of dugong matrilines.

Nuclear DNA microsatellite loci isolated from the Florida manatee were tested for use as population genetic markers the dugong. These loci displayed considerable allelic diversity in the dugong, significantly greater than observed in the Florida manatee. For example, 27 alleles were identified in the dugong at locus TmaA04, while only one allele was identified in the Florida manatee at the same locus. These microsatellite markers reveal a high level of gene flow among dugongs in Australia and a significant level of isolation-by-distance across Australia.

Comparison of the results from mtDNA and nDNA indicate that members of the two distinct mitochondrial lineages within Australia interbreed in areas where they overlap geographically. The contrasting patterns of structure presented by the mtDNA and nDNA suggest male-biased gene flow in the dugong. This pattern has not yet been identified from ecological studies, but is consistent with common dispersal patterns in mammals.

The major findings of this study are the detection of ‘healthy’ levels of genetic diversity of Australian dugongs, a suggestion of male-biased gene flow, and a demonstration of significant gene flow around Australia. This high level of gene flow makes the allocation of management units difficult. These results indicate the importance of a co-ordinated management strategy at a spatial scale of thousands of kilometres. Dugongs within Australia cannot be managed at a bay level due to the connectivity between bays observed in this study. Co-operation between management agencies at local, state, national and international spatial scales is required in order to conserve this vulnerable species.

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