

Sirenian Conservation

Issues and Strategies in Developing Countries



Edited by Ellen M. Hines, John E. Reynolds III, Lemnuel V. Aragonés,
Antonio A. Mignucci-Giannoni, and Miriam Marmontel

Sirenian Genetics and Demography

LESLEE PARR, FABRÍCIO R. SANTOS, MICHELLE WAYCOTT,
JULIANA A. VIANNA, BRENDA MCDONALD, SUSANA CABALLERO,
AND MARIA JOSÉ DE SOUZA LOPES

As previous chapters in this book make clear, the long-term survival of the four extant species of *Sirenia* is in peril due to many confounding factors, the majority of which are caused, directly or indirectly, by human activity. One of the most critical effects, along with habitat fragmentation and a reduction in manatee and dugong numbers, is a possible loss of genetic diversity. Genetic diversity is the fundamental level of biodiversity. Therefore, a critical first step in developing management programs for endangered or threatened species is the determination of existing genetic variability within and between management units. The accurate delineation of distinct management units and the ability to detect changes in genetic diversity within these units is critical. Box 19.1 gives steps for gathering and preserving samples for genetic analysis. Box 19.2 is a glossary of selected terms in genetics.

Overview of Molecular Techniques

Natural variations at the level of DNA can be used for investigating genetic relationships within and between

populations and for monitoring genetic fluctuations of populations over time and across the range of their natural habitat. The authors of this chapter have successfully employed each of the following classes of DNA for studying the genetic relationships and population dynamics of manatees and dugongs.

Mitochondrial DNA

Direct sequencing of mitochondrial DNA (mtDNA) has been used extensively as a tool for inferring demography, evolutionary past, and geographic distribution of genetic lineages¹. MtDNA is also one of the molecular markers of choice for studies directed at the application of genetic techniques for conservation of endangered species².

Several characteristics of mtDNA make it useful for these studies. MtDNA is small in size—it is about 1/10,000th the size of the smallest animal nuclear genome³ and is therefore easy to distinguish. Mammalian mitochondrial DNA is estimated to evolve at a rate about a five to nine times higher than does nuclear DNA⁴. This creates easily detectable variability between individuals within populations. The mitochondrial ge-

BOX 19.1

Handling Animal Tissue for Genetic Analysis

Ann Marie Clark

- Obtain CITES and/or all relevant regional permits before collection and shipment.
- When possible, it is best to collect samples using sterile instruments (tubes, new scalpel blades for each sample) and wearing latex gloves to prevent contamination of samples and cross-contamination between samples. However, when field conditions make this impossible, careful handling of samples during collection and in the genetics lab can mitigate many of the field contamination issues. Inform the lab of the collection conditions so that necessary precautions can be taken when working with the samples.
- Mark each sample tube or bag with all the available information (date of collection, species, location, gender, sample id#, etc.) using indelible ink on the outside of the container and/or in pencil on waterproof paper, and place the label inside the sample container.
- Keep duplicate log sheets with all sample information.
- Maintain samples at a cool (ambient) temperature and out of direct sun.
- Ship to a cooperating genetics lab as soon as possible (any of the chapter authors would be willing to assist).

Collection vials should be made of high impact, chemically resistant polyethylene (HDPE) and have screw-on caps. These vials are resistant to breakage and impervious to alcohol and buffers such as the SED or blood lysis buffer, and will ensure safe transport of samples. Double bag all samples before shipping.

Collecting Tissue without Refrigeration

SED Buffer for Skin, Muscle, Heart, or Liver Tissue

1. Prepare SED buffer (Saturated NaCl; 250 mM EDTA pH 7.5; 20% DMSO); see preparation instructions following.
2. A tissue sample the size of a large marble is generally sufficient. Remove sample using a sterile or clean scalpel or knife. Biopsy punch samples can also be stored in SED buffer.
3. Cut larger tissue samples a few times to increase penetration of buffer.
4. Add tissue to a pre-labeled tube containing SED buffer.
5. Samples can be stored at room temperature for up to a year or in a refrigerator indefinitely. Avoid extended exposure to heat or sunlight.
6. Prior to shipping, reseal the tubes carefully and double wrap in an airtight plastic bag (Ziploc or equivalent) to prevent leakage.
7. Ship samples by air freight or express mail.

Note: SED buffer is nontoxic, nonflammable, and noncorrosive and can be stored indefinitely at room temperature. Since this buffer is saturated with salt (NaCl), a white precipitate may form in some tubes. This does not affect the ability of the buffer to preserve tissue.

To make SED buffer:

- Dissolve 95 g tetrasodium EDTA in 700 ml distilled water.
- pH to 7.5 with glacial acetic acid.
- Saturate the solution with about 200 g NaCl. Allow salt to dissolve completely.
- Add 200 ml DMSO. Bring buffer to 1 liter with distilled water.

(Protocol modified from Amos and Hoelzel 1991 and Proebstel et al. 1993.)

Desiccant Storage Method for Skin and Muscle

1. Excise a small amount of tissue (~10 g) from each animal. Use a scraping tool or biopsy punch for skin if the animal is living, or cut ~10 g of skin or muscle during necropsy of dead animals.
2. Cut tissue sections to ~0.5 cm cubes or very thin slices.
3. Place each tissue sample in a labeled container (small Ziploc bags or screw-cap tubes work best) and add desiccant. Be sure to cover tissue sample completely. If using 1.5 ml tubes, add ~1.0–1.5 ml desiccant/0.5 cm cube of tissue. Camera and lens desiccant or anhydrous calcium sulfate (Drierite) both work well.

Preservation of Fecal Material

- Fecal samples can be stored using 70–100% ethanol or they can be frozen. It is best to collect as much as possible. Most feces extractions require at least 1 ml of feces. Be sure to use a large enough container to collect an adequate amount of fecal material or take multiple samples from each individual. Label vials accordingly.

Collection and Storage of Blood Samples

- Blood samples can be collected using a vacuum tube (Vacutainer) treated with EDTA, an untreated tube, or a syringe. Three ml of whole blood are sufficient for DNA extractions. Blood samples in EDTA must be stored in the refrigerator. Alternatively, an EDTA-preserved blood sample can be mixed with a blood lysis solution of 10 mM NaCl, 100 mM EDTA, 100 mM Tris (pH 8), and 1% (w/v) SDS, in a 1:10 ratio (blood:lysis buffer) and kept at room temperature.
1. Draw 1–3 ml of blood using an untreated syringe or Vacutainer tube.
 2. Add blood immediately to a pre-labeled tube, or multiple tubes, containing blood lysis buffer at a 1:10 ratio blood to lysis buffer. Do not add too much blood to the tube!
 3. Immediately but gently invert or rock the tube several times to mix.
 4. Samples can be stored at room temperature for at least 1 year and indefinitely in a refrigerator. Avoid extended exposure to heat or sunlight.
 5. Ship samples by air freight or express mail.

Note: SDS (sodium dodecyl sulfate or sodium lauryl sulfate) may form a precipitate in cool conditions. Warm the vial to get the SDS back into solution before using if possible; however, the precipitant will not affect the effectiveness of the buffer. Blood lysis buffer is nontoxic, nonflammable, and noncorrosive and can be stored for an extended period at room temperature.

(Protocol modified from White and Densmore 1992.)

Collection and Preparation of Bone Samples

- Bone samples can work well for DNA extractions. Sirenian bones do not have a medullary cavity but those bones involved in the production of red and white blood cells (i.e., ribs and sternum) can still be used for DNA isolations. The highest quality and quantity of DNA is obtained from bones that have not been cooked, treated, or lacquered, although all will generally yield some DNA. Bone fragments can be transported in dry plastic bags preferably containing some desiccant. About 20 mg of powdered bone is required for DNA isolations; however, bone DNA extractions are not 100% successful, so it is highly recommended that enough bone is collected for at least three separate DNA extractions. See Tuross (1994) and Holland et al. (2003).

nome is usually maternally inherited (though there is some evidence of “paternal leakage” and contribution during fertilization in some taxa) as a single unit that does not recombine with the paternal genome⁵. This makes mtDNA the preferred molecular marker for descriptions of maternal lineage, population structure, and genetic diversity⁶.

Microsatellites

Microsatellites are short nucleotide sequence repeats (SSRs)⁷ that are distributed throughout the nuclear genome⁸. These repeats, consisting of two, three, or four nucleotides, show high levels of polymorphism⁹. Microsatellites are regarded as some of the most useful molecular markers for the study of population genetic structure and dynamics¹⁰. Assessing microsatellite profiles or “genotypes” allows researchers to assign individuals to particular populations within the species’ range¹¹.

Molecular Sexing

Sex-specific regions of the mammalian genome have been relatively well studied. Thompson¹² utilized a portion of the genes on the X and Y chromosomes to develop a sex-specific molecular assay for the dugong that may be applicable in other Sirenia.

Molecular Aging

Recent advances in our understanding of mammalian chromosome structure have led to the development of potential methods for aging individuals using a DNA assay based on the shortening of telomeres during an organism’s lifespan. Telomeres are located at the end of nearly all animal chromosomes. In mammals, these long repeats of TTAGGG are between 10,000 and 150,000 base pairs (bp) long at birth. A correlation between age and the rate of telomere shortening per year (loss of bp/yr) has been established in both birds and mammals¹³.

BOX 19.2

Useful Terms and Definitions

Allele: A portion of DNA that codes for a functional protein.

Basal: A species, taxon, or clade that is located closest to the root of a phylogenetic tree, indicating it is the ancestral group.

Base pairs: Number of repeating pairs of nucleotides making up the portion of DNA being considered.

Bootstrap values: Technique for estimating the statistical error when the sampling distribution is unknown.

Bottleneck: A sudden, large reduction in population size.

Cluster: In phylogenetic analysis, a group of closely related individuals that appears “clustered” on branches of a phylogenetic tree.

Control region: Highly variable sequence of mitochondrial DNA, also referred sometimes as D-loop.

Diploid (2n): The condition in which a cell or individual has two copies of every chromosome.

Evolutionary Significant Unit (ESU): Genetically distinct populations that are considered to require management as separate units.

Exon: The coding region of a gene.

Founder effect: A loss of genetic variation in a population that was established by a small number of individuals carrying only a fraction of the genetic diversity of the larger population.

Genetic diversity: The extent of genetic variation in a population or species or across a group of species. It can be measured as heterozygosity, haplotype or nucleotide diversity, allelic diversity, or heritability.

Genome: The full complement of genes present in a haploid set of chromosomes in an organism.

Genotype: The genetic constitution of an organism at one, many, or all of the genetic loci.

Haploid (1N): The condition in which a cell or individual has one copy of every chromosome.

Haplotype: Unique mitochondrial DNA sequences/lineages and/or allelic composition for several different loci on a chromosome.

Homoplasmy: Two alleles or characters that are identical in state yet have different evolutionary origins.

Inbreeding: The mating of organisms related by descent.

Introgression: Diffusion of alleles from one population or species into another as a result of interbreeding or hybridization between them.

Mantel test: A statistical test relating geographical distance of samples taken to their relative genetic distance from one another.

Monophyletic: A group of organisms that contains the group's most common ancestor and all of its descendants.

Mitochondrial DNA (mtDNA): Haploid, double stranded circular DNA molecule located in the mitochondria.

Nucleotides: Molecular subunits of DNA.

Panmixia: Random mating within a breeding population.

Paraphyly: A group of organisms that contains the group's most recent common ancestor but does not contain all of the descendants of that ancestor.

Philopatry: A condition of reproductive behavior where individuals faithfully home to natal sites.

Phylogenetics: The relationship of evolutionary lineages among organisms. Phylogenetic relationships are usually represented in a phylogenetic tree.

Phylogenetic tree: A branching diagram representing the evolutionary relationships of the taxa or individuals analyzed.

Phylogeography: The study of the principles and processes involved in the geographical distribution of genealogical or genetic lineages.

Polymorphism: The percentage of the loci (portion of DNA of interest) that is variable.

Primer: A short nucleotide sequence that pairs with one strand of DNA and provides a free end at which the *Taq* polymerase enzyme begins synthesis of a complimentary segment of DNA.

Single nucleotide polymorphism (SNP): The occurrence of alleles with different nucleotide bases at a specific point of a DNA sequence.

Sympatric: Occupying the same geographic areas.

Telomeres: The terminal regions of chromosomes; in mammals these consist of the repeating nucleotide series, such as TTAGGG.

Vicariance: The separation or division of a group of organisms by a geographic barrier, such as a mountain or a body of water, resulting in differentiation of the original group into new varieties or species.

ZFX/ZFY: Zinc finger chromosomal proteins; genes located in the Y and X chromosome of mammals.

Insights Gained on the Genetics and Demography of Manatees

Subspecies Designation, West Indian and Amazonian Manatees

In 1998 Garcia-Rodriguez et al.¹⁴ presented the first population genetic and phylogeographic study of West Indian and Amazonian manatees. The study was based on mtDNA sequence of 86 manatees from eight coun-

tries. The phylogenetic tree of mtDNA haplotypes identified three distinct, genetically related clusters of West Indian manatees (*Trichechus manatus*) compared to only a single cluster in Amazonian manatees (*T. inunguis*). The division of West Indian manatees into three clusters did not agree with previous morphological analysis (based on cranial characters), which divided this species into two subspecies: the Florida manatee (*T. manatus latirostris*) and the Antillean manatee (*T.*

m. manatus)¹⁵. None of the three genetic clusters detected was made up exclusively of the Florida lineage, as would be expected under the hypothesis of a separate Florida subspecies. Instead, Florida mtDNA haplotypes were grouped into shared clusters with Puerto Rico and the Dominican Republic manatees. In a more recent study, Florida haplotypes were also detected in Mexico¹⁶.

Parr¹⁷ reported a similar level of population structure in West African manatees (*T. senegalensis*). As West African manatee tissue samples have been difficult to obtain, the DNA sequence was analyzed for only 17 individuals. However, the phylogenetic tree of West African manatee mtDNA haplotypes, like that for West Indian manatees, showed three distinct clusters. One cluster consisted solely of animals from Guinea Bissau, a second cluster was derived from Cameroon-Gabon-Ghana in the south, and a third cluster was composed of inland manatees from the lake region of Chad (now landlocked due to recent construction of dams). The Guinea Bissau cluster displayed a marked genetic diversity and was identified as an important region in which to focus conservation efforts.

Cantanhede et al.¹⁸ focused their genetic study on Amazonian manatees (*T. inunguis*), using mtDNA sequence of 68 individuals. They suggested the occurrence of unrestricted gene flow (breeding) and long-distance dispersal throughout the entirety of the Amazon basin. In this case, all Amazon manatees would behave as a single, random mating population. This hypothesis of panmixia was supported by a Mantel test, which did not show any significant correlation between genetics and geographic distances.

Recently, an extensive and international effort to sample and analyze DNA made it possible to do the first comparative study involving phylogeography and phylogeny of all three manatee species based on two separate mitochondrial genes of 189 West Indian manatees, 93 Amazonian manatees, and 6 West African manatees¹⁹. The phylogeny derived in this study suggested the monophyly (shared, common ancestry) of the trichechids, with the Amazonian manatee in position as the basal or ancestral species. This agrees with the morphological analyses of Domning²⁰, who proposed the monophyly of the marine species (the West Indian and West African species, *T. manatus* and *T. senegalensis*) and a long separation time for the Amazonian manatee, *T. inunguis*, which he suggests is the only surviving species of an ancient lineage adapted to the Amazon freshwater environment.

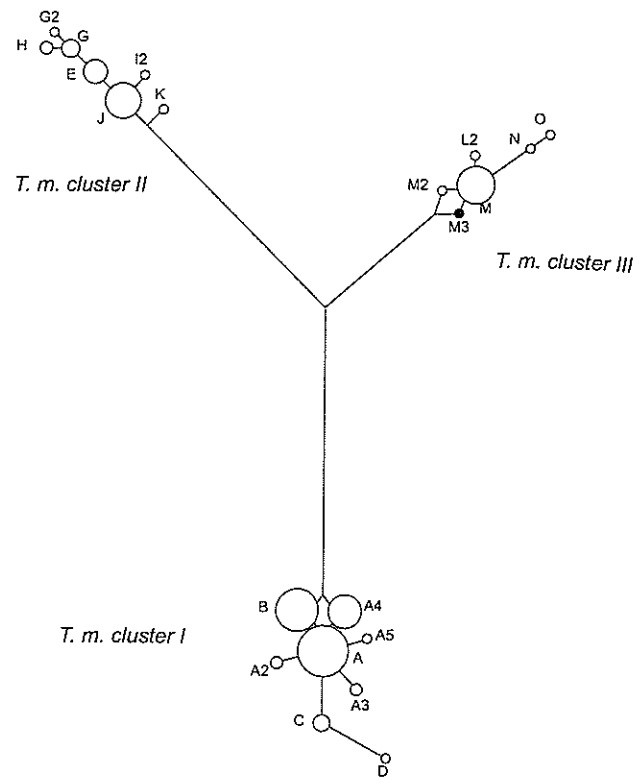


Figure 19.1. Median neighbor joining network showing a three-haplotype cluster pattern identified in mtDNA analysis of 189 West Indian manatees. (Courtesy of Fabricio R. Santos and Juliana A. Vianna.)

Spatial Distribution and Gene Flow, West Indian Manatees

The analysis of mtDNA from 189 West Indian manatees originating from ten countries allowed the resolution of a highly structured population in this species. A positive correlation between genetic and coastline geographic distances supported the idea that manatees migrate along the coast. The three-haplotype cluster pattern identified by Garcia-Rodriguez et al.²¹ was also found in this sample set (figure 19.1), and the expansions of each cluster were dated back to the Pleistocene. A discontinuity in the *T. manatus* genetic structure was detected and was regarded as a historical break in gene flow, promoted by a geographic barrier (most likely the continuous chain of islands extending from the Lesser Antilles through Trinidad, near the mouth of the Orinoco River in Venezuela). This historical barrier appears to have isolated the Guyanas-Brazil population from other populations of manatees. The resulting bipartite structure in populations east and west of the Lesser Antilles indicates the existence of at least two independent evolutionary

significant units (ESUs), or two subspecies, in the West Indian manatee.

West African Manatee Population Structure

Although only six *T. senegalensis* samples were analyzed in the Vianna et al.²² study, two different mtDNA clusters were inferred: (1) inland samples from Lake Tchad, which spans the border between Chad and Niger, form a monophyletic group with coastal samples from Ghana (a small, isolated population of manatees that inhabit the lake and the upper reaches of the Benue River, a tributary of the Niger River, likely isolated there after dams were constructed on the Benue); and (2) Guinea-Bissau. Cluster separation was suggested to occur between hydrographic basins, with coastal occupation expanding from each river mouth. However, larger population surveys are needed to properly evaluate the remaining population structure of this species.

Geographical and Genetic Distances Association, Amazonian Manatees

Molecular demographic analysis in the Vianna et al. study revealed a strong bottleneck followed by population expansion in the recent past (Pleistocene). In agreement with Cantanhede et al.²³, all of the 31 different Amazonian haplotypes identified are closely related, forming a single cluster (figure 19.2), despite the fact that the individuals analyzed originated from different Amazon countries and separate regions within Brazil. Moderate population structure was observed (three to four times larger than that detected by Cantanhede et al.²⁴). This could be explained by the larger sample size over a greater geographic distribution and also by the fact that a larger portion of mtDNA sequence was analyzed by Vianna et al.²⁵. However, the genetic correlation with geography is much weaker in Amazonian manatees than in West Indian manatees, which could be associated with a relatively recent expansion in Amazonia.

Hybridization between West Indian and Amazonian Manatees

The existence of possible *T. manatus* × *T. inunguis* hybrids had been previously suggested by morphological examinations²⁶ and mtDNA analysis²⁷. Vianna et al.²⁸ presented unequivocal evidence of the occurrence of these interspecies hybrids using mtDNA, autosomal microsatellites, and cytogenetic analyses. Initially, seven individuals identified as West Indian (from the coasts of Guyana, French Guyana, and north Brazil) possessed mtDNA haplotypes related most closely to Amazonian

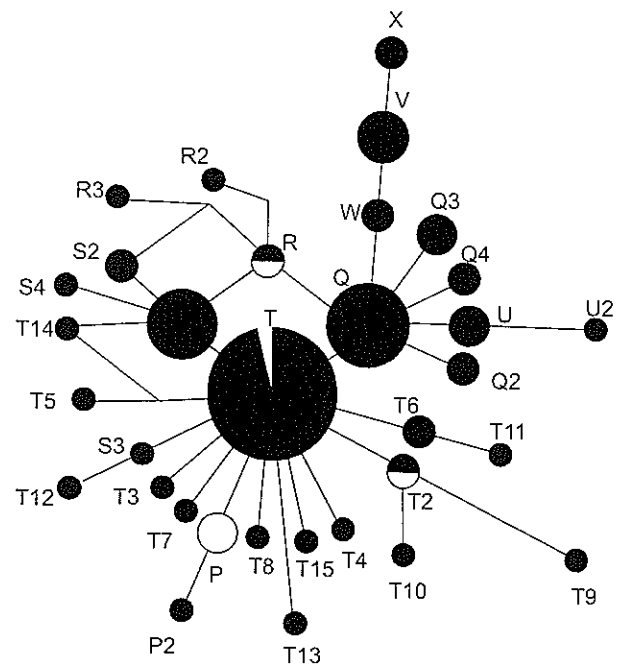


Figure 19.2. Median neighbor joining network showing a single-cluster pattern identified in the mtDNA analysis of 93 Amazonian manatees. The star shape pattern of the cluster indicates a recent expansion following a bottleneck. (Courtesy of Fabrício R. Santos and Juliana A. Vianna.)

sequences. Also, an Amazonian manatee found near the mouth of the Amazon presented a West Indian mtDNA haplotype. Microsatellite loci, used for the first time in this study, allowed the identification of species-specific alleles (making it possible to identify alleles from one or both species) in the eight likely hybrids. The microsatellite data also suggested the occurrence of F_2 (second generation) or further generation backcrosses, meaning some F_1 (first generation) hybrids may be able to interbreed with one of the parental species. One of the likely hybrids identified is a captive animal and has been submitted to a detailed karyotype (chromosome visualization) analysis. This hybrid manatee from the northern Brazilian coast presented an intermediate number of chromosomes (the diploid number of chromosomes, $2n = 50$, figure 19.3) between Amazonian ($2n = 56$) and West Indian ($2n = 48$) as well as microsatellite alleles specific to both parental species. All detected hybrids were from the region around the mouth of the Amazon where both species are sympatric, occupying the same habitat (figure 19.4).

Implications for Conservation and Management

The marked genetic structure and geographic subdivision of *T. manatus* should be considered in its manage-

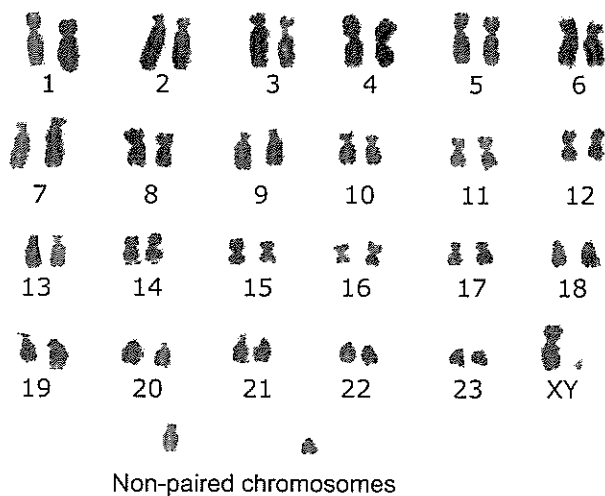


Figure 19.3. Karyotype of the hybrid manatee from the northern Brazilian coast presented an intermediate number of chromosomes. The diploid number of chromosomes, $2n = 50$. (Courtesy of Maria José de Souza Lopes.)

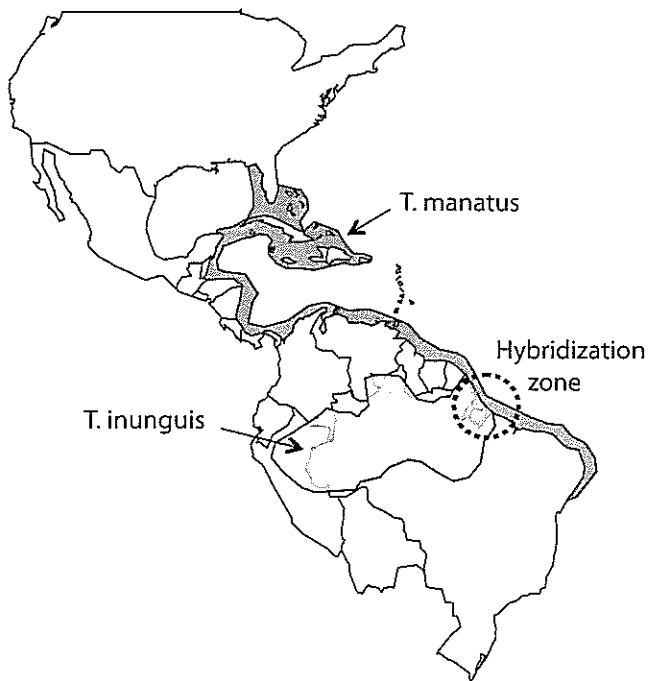


Figure 19.4. All detected hybrids between West Indian and Amazonian manatees were from samples obtained from the region around the mouth of the Amazon (hybridization zone), where both species are sympatric. (Courtesy of Fabrício R. Santos, Juliana A. Vianna, and Maria José de Souza Lopes.)

ment and conservation. The two different ESUs (or perhaps subspecies) identified in *T. manatus* indicate the need for separate management and conservation strategies for each.

There is a special need for careful management of both coastal and Amazonian manatees in Brazil, where the population is under serious threat due to its small size and low genetic diversity. The finding that the ten West Indian populations studied by Vianna et al.²⁹ are highly structured indicates a need for a population-level approach for future management.

Hybridization can be a very important conservation problem³⁰, particularly when it involves populations consisting of a few individuals, like the *T. manatus* from the Brazilian coast. Animals recovered from the mouth of the Amazon in Brazil or nearby regions (Guyanas) should not be translocated to areas where hybrids were not detected, such as the northeastern coast of Brazil and the interior of the Amazon basin.

A genotype/phenotype approach will be important in Puerto Rico also, where the two haplotypes identified were geographically separated (haplotype A occurs on the north coast, haplotype B on the south coast, and both on the east and west coast)³¹. This separation of genotypes could reflect adaptive selection to specific habitats and may indicate that translocation between these areas could fail.

It will be important to expand these phylogeographic studies (especially at the population level, using microsatellite) to include manatees from countries and regions not included in the original analyses, such as Cuba, Jamaica, Honduras, Nicaragua, Costa Rica, and Panama.

The same management attention should be considered in the Panama Canal System, where nine *T. manatus* and one *T. inunguis* from Peru were reintroduced in 1964³². Hybrids could be caused by anthropogenic activities in the area; however, little is known about this population and the survival of the translocated animals. Genetics studies are needed in the area to detect possible hybrids and genetic diversity of this population.

The observed diversity in the small sample set of West African manatees indicates higher geographic differentiation than the observed population structure in Amazonian manatees, which could possibly be equal to or greater than that of the West Indian manatee. Although larger surveys are needed for a more complete understanding, the two or three clusters of West African manatees should also be managed separately, at least between different hydrographic basins. This will require cross-border cooperation, which creates a special challenge because of the evolving state of economic, political, and social considerations faced on the African continent.

Insights Gained on the Genetics and Demography of Dugongs

Approaches and Techniques

Studies to date focus on the Australian region, where the largest known living population of the dugong occurs in Torres Strait and the northern Great Barrier Reef³³. However, Palmer³⁴ analyzed mtDNA sequence of dugongs from the Andaman Sea and the Gulf of Thailand. The analysis of broad-scale population genetic structure and phylogeography utilizing mtDNA and microsatellite markers has provided significant insight into the large spatial scale dugongs inhabit across tropical and subtropical Australia³⁵.

Molecular Sexing of Dugongs

Thompson³⁶ utilized exon 11 of the ZFX/ZFY genes to develop a sex-specific molecular assay for the dugong that may be applicable in other sirenians. A fragment in exon 11 of the ZFX/ZFY genes was used in this analysis. Within this gene an *RsaI* restriction site (a site that can be cut by the *RsaI* enzyme) is present in ZFY copy of the gene region but not in the ZFX. Thus dugong males (being XY), have both the ZFX and the ZFY genotype, but females (being XX) have only the ZFX sequence (figure 19.5). Preliminary analysis of the utility of this DNA segment for sex determination in manatees awaits verification. The advantage of this technique is that it may be applied to a small amount of DNA and is useful for screening of animals for their gender based on skin samples alone.

Molecular Aging of Dugongs

Dunshen³⁷ tested the utility of a telomere assay on dugongs with some success; the test involved comparing the age of dugongs established using age determined by tusk growth layer group analysis³⁸ with telomere region fragment length. Dunshen discovered a significant relationship between age assigned by tusk analysis and

age determined using telomere length in dugongs from Torres Strait. However, the number of samples available where both tusk age and sufficient DNA quantity could be obtained was small, so that a usable assay has not been reliably confirmed³⁹. Future technical developments in this area hold considerable promise for adding to our ability to assess sirenian population age structure without the need for destructive sampling.

Dugong Population Structure in the Australian Region

Tikel⁴⁰ presented the first evidence of phylogeographic divisions in the dugong. Analyzing sequence data of the mtDNA control region from 103 individuals, two distinct lineages were observed within Australia that overlapped geographically in the Great Barrier Reef region. McDonald⁴¹ sequenced a longer segment of the control region from a geographically more representative collection of samples, including many from northern and western populations of Australian dugongs. This more recent study included a larger sample set (115 samples) and supported the existence of two distinct lineages around Australia, one geographically widespread lineage and one more geographically restricted lineage not found in the west coast populations (figure 19.6). In addition, limited samples from outside Australia indicated that additional lineages occur in other countries.

The maternal lineages observed among Australian dugongs are most likely the signature of a series of vicariance events where changes in sea level created isolated populations for significant periods of time. This could be due to the emergence of the Torres Strait land bridge for extended periods of the Pleistocene, and its recent inundation⁴². The lack of subsequent mixing (interbreeding) of these lineages implies some female philopatry, although the geographic scale of this would be regional rather than local⁴³. These findings indicate regional-scale differences in haplotype frequency and demonstrate that historical patterns of habitat connectivity/disconnectivity have had a major impact upon the population structuring of dugongs.

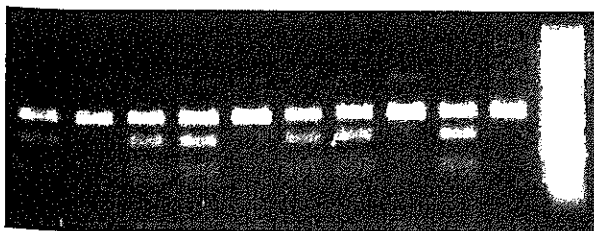


Figure 19.5. PCR products (amplified using paenungulate specific primers) from exon 11 of ZFX/ZFY from known male (lanes 1, 3, 4, 6, 7, 9) and female (lanes 2, 5, 8, 10) dugongs, digested with *RsaI* and fragments separated on a 3% agarose gel. Bands of sizes 236 bp, 171 bp, and 65 bp are seen in samples from males, while only a single 236 bp band is seen in samples from females. Dugong males (being XY) have both the ZFX and the ZFY genotype, but females (being XX) have only the ZFX sequence. (Courtesy of Michelle Waycott and Brenda McDonald.)

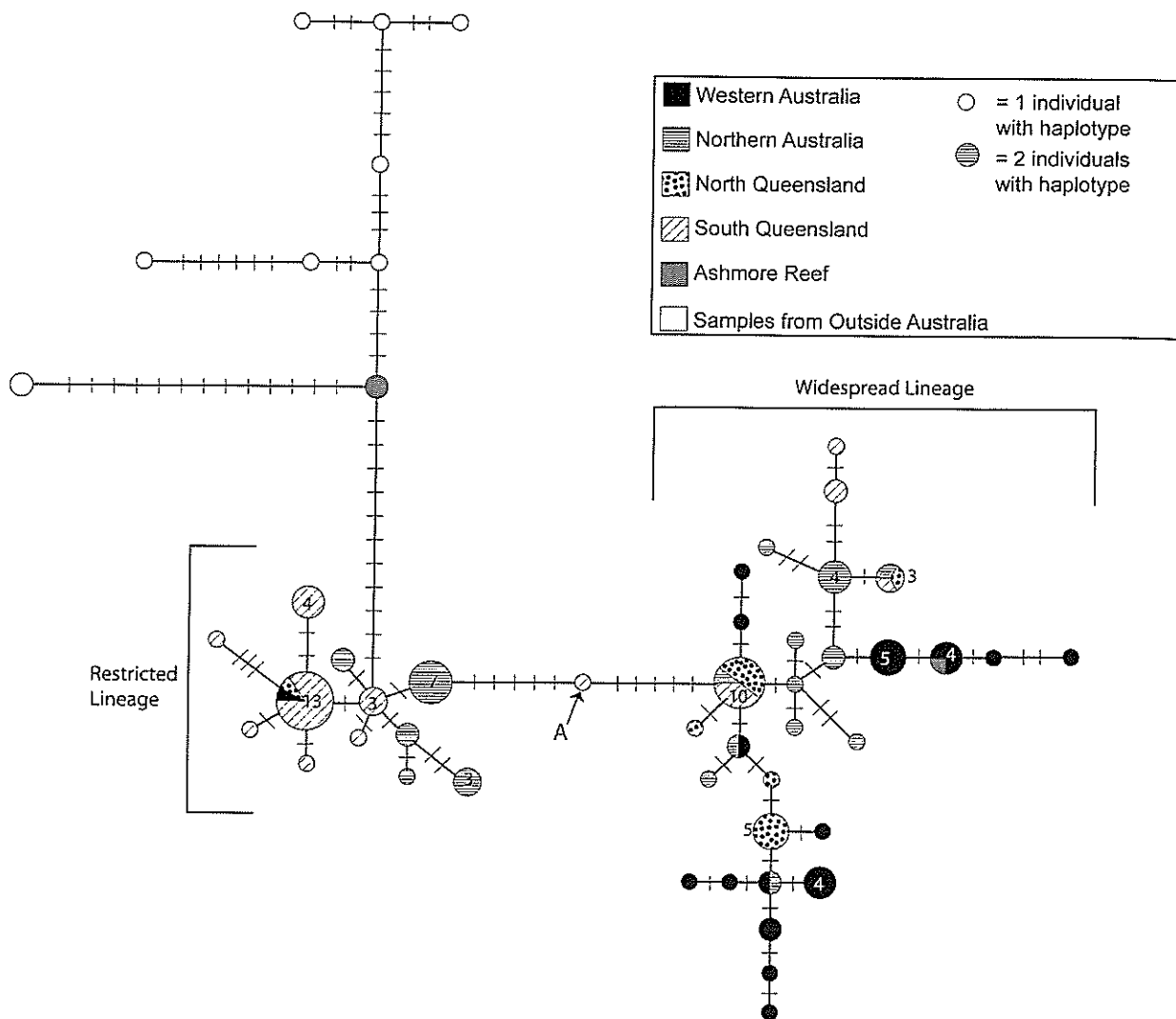


Figure 19.6. Minimum spanning tree showing the relationships between the dugong long haplotypes (492 bp; 115 individuals). Circles indicate the location of samples containing that haplotype. The size of each circle and the number within it indicate the number of individuals with that haplotype. Dashes indicate single base pair differences between haplotypes. The two Australian lineages are identified. (Courtesy of Michelle Waycott and Brenda McDonald.)

MtDNA Phylogeographic Analysis of the Dugong in Thailand

Palmer⁴⁴ sequenced samples of 40 individual dugongs from Thailand. The genetic diversity observed was lower than that reported by McDonald⁴⁵ for Australian dugongs. Mean interpopulation diversity and mean genetic distance between the Gulf of Thailand and the Andaman Sea populations were small, suggesting little differentiation between the dugongs of the east and west coasts of Thailand. Phylogenetic analysis reveals the existence of two maternal lineages within the population of Thailand dugongs.

Though the sample size of this study was small, it is estimated that approximately 200 dugongs remain along

the Andaman Sea, while estimates for Gulf of Thailand are thought to be even smaller⁴⁶.

Microsatellite Analysis Reveals High Level of Migration and Interbreeding

To date, only one study has published microsatellite loci in sirenians⁴⁷. This initial screening of three dugongs identified nine of the 14 loci to be polymorphic. However, McDonald⁴⁸ found that only six of the nine microsatellites were variable, amplified consistently, and were easy to score reliably in the dugong. She screened 452 dugong samples with these six microsatellite loci. These samples included 417 from Australian waters, 31 from Asia (Thailand, Indonesia, Philippines, Sabah, and Japan), and four from the Pacific (New Caledonia and Palau).

The geographic scale of gender-biased dispersal may be very large (a large proportion of the Queensland coast-line, for example), and thus tracking studies will be limited in their applicability.⁵⁷

These two datasets (on an overlapping sample set) indicate that representatives of the different mtDNA lineages in Australia are interbreeding where sympatric.⁵⁸ This result suggests that the two refugia at times of low sea level were not separated for sufficient time to cause a recognizable difference in appearance or behavior of the two matriline, thus facilitating interbreeding between the lineages when reunited. The lineages therefore cannot be termed ESUs but may be broken into management units based on regional geographic boundaries.⁵⁹

Implications for Conservation and Management

Owing to the very large spatial scales dugongs appear to inhabit, breed in, and move in and out of, the scale of habitat management that is required spans political boundaries, posing ongoing challenges in this area.

Historical patterns of habitat connectivity have had a major impact upon the population structuring of dugongs. Currently, the distribution of seagrass beds in the coastal waters of northern Australia is naturally fragmented. It is therefore likely that further fragmentation of seagrass beds around coastal northern Australia as a result of anthropogenic impacts will also have a significant effect on the genetic structuring of the dugong population in the longer term.

Application of Genetic Techniques in Developing Countries

DNA is a powerful tool when used to resolve the taxonomy, biology, and ecology of organisms. Maintaining genetic diversity is an important issue in conservation

The microsatellites developed for the Florida manatee are highly variable in the dugongs⁴⁹ and have a greater allelic diversity in the dugong than in the manatee (table 19.1). The results of these tests suggest that the dugong population has not undergone an extensive bottleneck or founder effect. The high allelic diversity of dugongs is also an indication of the larger geographic area and larger effective population size of the dugong compared with the manatee.⁵⁰ The microsatellite loci used indicate that dugongs most likely engage in nonrandom mating facilitated by a variety of mating systems around Australia and by an asynchronous breeding season.

McDonald⁵¹ found a lack of population differentiation over a large geographic scale for dugongs around Australia and between Australia and overseas. This lack of structure indicates that there is a high level of gene flow and migration occurring.⁵² More important, it indicates that a percentage of dugongs that make long-distance journeys are mating at their destination site.⁵³ Isolation-by-distance is observed over the Australian scale, which is not surprising considering the vagility of the dugong and the geographic scale of sampling.⁵⁴

Limited Concordance between mtDNA and Microsatellite Data

The significant haplotypic divergence observed with mtDNA (which is inherited maternally) contrasts with the lack of structure from microsatellite DNA (which is inherited from both parents). It is tempting to invoke the explanation that there is sex-biased dispersal and/or gene flow.⁵⁵ This interpretation is in conflict with satellite tracking evidence, which shows that both sexes can move long distances.⁵⁶ However, Sheppard's study is based on a relatively small sample size, and dugongs were tracked for periods ranging from 15 to 551 days, which is a small proportion of their lifespan. In addition,

Table 19.1 Allelic diversity, as indicated by the number of alleles in dugongs compared with the manatee.

Species	n	TmaA01	TmaA02	TmaA04	TmaE08	TmaE26	TmaM79
Dugong	372	6	25	27	8	17	18
Florida manatee	223	1	3	1	3	2	3
Antillean manatee	21	2	3	3	4	8	3
Amazonian manatee	7	2	5	1	2	1	3

Sources: Garcia-Rodriguez et al. 2000.
 Note: Dugong (*Dugong dugon*); Florida manatee (*Trichechus manatus latirostris*); Antillean manatee (*T. m. manatus*); Amazonian manatee (*T. inunguis*); n = number of samples.

ial
 ype
 ype
 rals
 ype

ais).
 the
 tra-

Thailand

llite loci
 dugongs
 lower,
 rosatel-
 ere easy
 dugongs
 samples
 a (Thai-
 and four

management. At its best, molecular analysis can provide vital information relevant to the management of species of concern, and it can do so quickly and without intrusive or lethal means. However, molecular reagents and especially the equipment required to process such information are often prohibitively expensive tools for scientists in developing countries.

Hope resides in the fact that reagent and equipment prices are decreasing with the development of new technologies. Also, the formation of new national genome research programs (for example, in Brazil) has allowed many laboratories to acquire expensive sequencing equipment to be shared cooperatively by many research

groups, allowing molecular techniques to be applied in conservation studies.

The most important solution may be international collaboration. Collaborative efforts have been paramount to the success of the genetic studies reported in this chapter. The authors have enjoyed the personal and professional benefits of joining forces (sharing samples, sequence data, funding procurement, and reporting efforts) in an attempt to understand manatee and dugong biology better. We look forward to our continual collaboration and offer our collective assistance to readers seeking to undertake genetic research applied to conservation efforts in their own countries.

Of
of
a p
abi
of:
wh
sur
dug
]
the
area
whe
and
ous
pres
with
addi
such
cano
strati
tion
of ma
Be
advan
rectly
and l
land-i
logica
telem
count
is imp
gies th
multai
distrib
count
photo-
Res
spatial

"A practical 'state of the science' review of the conservation of manatees and dugongs in developing countries. The editors have done an excellent job of selecting an international team of more than eighty authors, including researchers and managers who have the responsibility of working with stakeholders to ensure conservation of both the cultural and biological diversity of sirenians. An excellent primer that provides both strategies and tools for continued research progress."

—ANNALISA BERTA, San Diego State University

Manatee and dugong populations in developing countries are dwindling. In these areas, people occupying rivers or coastlines still capture sirenians for food and other uses (oil, bones for carving, leather). In addition, disruption, erosion, or complete loss of sirenian habitat occur because of dredge and fill, coastal run-off, chemical pollution, and damage from boat propellers.

Sirenian Conservation features contributions from an international group of scientists working to address the many challenges to manatee and dugong food supply, environment, reproduction, and survival. They share stories of programs that rescue, rehabilitate, release, and monitor these animals; offer reports on practical, replicable, and cost-effective management techniques; and summarize current research strategies.

This important scientific volume comprehensively explores the biology and ecological status of manatees and dugongs in all of the geographic regions where they can be found today, from the Caribbean to Eastern Africa, from Arabia to the Amazon, and from Japan through the South Pacific to Australia.

ELLEN M. HINES is associate professor of geography and human environmental studies at San Francisco State University. JOHN E. REYNOLDS III, senior scientist at the Mote Marine Laboratory in Sarasota, Florida, and chairman of the United States government's Marine Mammal Commission, is coauthor of *The Florida Manatee* and *The Bottlenose Dolphin*. LEMNUEL V. ARAGONES is associate professor at the Institute of Environmental Science and Meteorology at the University of the Philippines. Biological oceanographer ANTONIO A. MIGNUCCI-GIANNONI is a research professor at the Inter American University of Puerto Rico. An expert on the ecology of Amazonian manatees, MIRIAM MARMONTEL is a senior researcher with the Mamirauá Institute for Sustainable Development in western Brazil, where she leads the Aquatic Mammal Research Group.

Front cover: Young West Indian manatee with tracking device off the coast of Puerto Rico. Back cover: West Indian manatee with sea star. Photographs by Antonio A. Mignucci-Giannoni, 2010.



University Press of Florida

www.upf.com

ISBN 978-0-8130-3761-5



9 780813 037615