Sirenian Genetics and Demography

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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 lineages. MtDNA is also one of the molecular markers of choice for studies directed at the application of genetic techniques for conservation of endangered species.

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 genome 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 DNA. This creates easily detectable variability between individuals within populations. The mitochondrial ge-

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 powered 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).

Microsatellites

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

Molecular Sexing

Sex-specific regions of the mammalian genome have been relatively well studied. Thompson 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 mammals.

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.

Homoplasy: 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.

Pannmixia: 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 García-Rodríguez et al. 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 countries. 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). 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 Mexico.

Parfitt 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, 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. 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 manatees. 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 Domning, 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.

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 García-Rodriguez et al. 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. 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. and 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.).

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. 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 examinations and mtDNA analysis. Vianna et al. presented unequivocal evidence of the occurrence of these interspecies hybrids using mtDNA, autosomal microsatellites, and cyrogenetic 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 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 *F₁* (second generation) or further generation backcrosses, meaning some *F₁* (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-
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. are highly structured indicates a need for a population-level approach for future management.

Hybridization can be a very important conservation problem, 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). 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 1964. 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 Reef. However, Palmer 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 Australia.

Molecular Sexing of Dugongs

Thompson utilized exon 11 of the ZFX/ZFY genes to develop a sex-specific molecular assay for the dugong that may be applicable in other sirensians. A fragment in exon 11 of the ZFX/ZFY genes was used in this analysis. Within this gene an Rsal restriction site (a site that can be cut by the Rsal 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. 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

Dunsha 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 analysis with telomere region fragment length. Dunsha 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 confirmed. 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

Tikel 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. McDonald 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 inundation. The lack of subsequent mixing (interbreeding) of these lineages implies some female philopatry, although the geographic scale of this would be regional rather than local. 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 Rsal 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.)
MtDNA Phylogeographic Analysis of the Dugong in Thailand

Palmer\textsuperscript{44} sequenced samples of 40 individual dugongs from Thailand. The genetic diversity observed was lower than that reported by McDonald\textsuperscript{45} 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 smaller\textsuperscript{46}.

Microsatellite Analysis Reveals High Level of Migration and Interbreeding

To date, only one study has published microsatellite loci in sirenians\textsuperscript{47}. This initial screening of three dugongs identified nine of the 14 loci to be polymorphic. However, McDonald\textsuperscript{48} 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 microsatellites developed for the Florida manatee are highly variable in the dugong, 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 a genetic bottleneck. The high allelic diversity of dugongs is also an indication of the large geographic area and large effective population size of the dugong compared with the manatee. The microsatellite loci used indicate a high degree of freedom in tanking systems existing around Australia and between Australia and overseas. A genetic flow and migration occurring. The lack of structure indicates that there is a high level of genetic diversity, which makes long-distance journeys made at their destination site. Isolation-by-distance is not surprising considering the vellacity of the dugong and the geographic scale of sampling. The relative number of alleles in dugongs compared with the manatee is striking. The significant bottleneck observed in the manatee indicates that the lack of structure from microsatellite DNA (which is inherited from both parents) is intriguing. The difference in genetic structure from manatees and dugongs is based on a relatively small sample size, and dugongs were tracked for periods ranging from 15 to 35 days, which is a small proportion of their lifespan. In addition, the genetic diversity is an important issue in conservation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Microsatellite Loci</th>
<th>TmaAO1</th>
<th>TmaAO2</th>
<th>TmaAO4</th>
<th>Tma809</th>
<th>TmaE26</th>
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Source: García-Rodríguez et al. 2000

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

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