

# The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives

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## Chapter 18

# Elasmobranch Genetics and Captive Management

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*Abstract:* The advent of the polymerase chain reaction, or PCR, has rapidly changed the field of genetics. Despite this fact, the field of elasmobranch genetics is in its infancy. Several methods exist for examining questions such as population genetic structure, species identification, paternity exclusion, and evolutionary relationships between species. Captive elasmobranchs can provide insight into the study of wild populations through tissue samples collected during routine exams and by shedding light on genetic mating systems of those species reproducing in captivity. Aquarists should try to minimize the loss of genetic variability in captive elasmobranchs to avoid potential inbreeding.

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In this chapter, we provide a brief overview of genetic techniques. This chapter is by no means an exhaustive review in methodology. There are many genetic techniques now available for molecular ecologists and geneticists and several published volumes (e.g., Ferraris and Palumbi, 1996; Hillis et al., 1996; Hoelzel, 1998) contain detailed protocols for performing these techniques. As elasmobranch genetics is a relatively young field, we briefly review how these methods have been applied to studies on elasmobranchs. We give proper methods for tissue collection and storage and discuss the genetics of captive populations. Although genetic considerations are perhaps not a priority for captive elasmobranchs, they should not be discarded wholeheartedly. Here, we discuss the potential negative effects of the loss of genetic variability in a captive population and suggest ways to minimize such losses. Finally, we suggest ways captive elasmobranchs can contribute to genetic studies.

### PROTEIN-BASED TECHNIQUES

The first molecular technique to gain widespread use in the scoring of polymorphic genetic characters in fishes was allozyme electrophoresis (Utter, 1991). Allozymes are enzymatic proteins that exhibit differential electrophoretic mobility and are generally used to examine genetic structure in populations (Utter et al., 1987), but can also be used for species identification and systematic purposes. As allozymes are proteins, this method is an indirect indicator of genetic variation. Alleles are caused by variation in amino acid sequences reflecting only a fraction of the changes that occur in the nucleotide (DNA) sequence of the protein-coding gene. Allozyme variability is low in sharks when compared to bony fishes (Smith, 1986) and thus allozymes may be of limited use in some elasmobranch species (Lavery and Shaklee, 1989). Because tissue types vary in enzyme expression, a survey

of many loci will require tissue from multiple tissue types (e.g., liver, muscle, nerve, or eye) and hence will typically require lethal sampling. However, for those loci that are expressed in skeletal muscle it is possible to detect sufficient variation from a small biopsy of muscle tissue provided that the tissue is used immediately or frozen at  $-20^{\circ}\text{C}$  or colder (Billington et al., 1996).

Distinct species typically exhibit fixed genetic differences (i.e., no alleles are shared) at one or more allozyme loci. Fixed allelic differences among species are useful for demonstrating the presence of “cryptic” species. For example, Lavery and Shaklee (1991) were able to show that two color morphs of “blacktip” sharks in northern Australia exhibited nearly fixed differences at two allozyme loci and concluded that two species were present. Solé-Cava et al. (1983) demonstrated allozyme differences among two species of angel shark where only a single species had previously been recognized. Solé-Cava and Levy (1987) later demonstrated the presence of a third species. Eitner (1995) suggested the existence of an undescribed species of thresher shark based solely on allozyme data.

### DNA-BASED TECHNIQUES

DNA is present in two organelles in animal cells: the nucleus and the mitochondrion. Whereas nuclear DNA is inherited equally from both parents, mitochondrial DNA (mtDNA) in vertebrates is presumed to be maternally inherited. The nuclear genomes of sharks contain in the order of 6-18 picograms of DNA per cell (see Asahida et al., 1995), corresponding to between  $\sim 3 \times 10^9$  and  $9 \times 10^9$  base pairs of DNA per haploid genome. By comparison, the human genome contains  $2.91 \times 10^9$  base pairs of DNA per haploid genome (Venter et al., 2001); hence sharks tend to have nuclear genomes that range from near equal to several times larger than that of humans. In contrast, the mitochondrial genome of animals (including sharks) is very compact, typically 16,000 to 17,000 base pairs in length (Meyer, 1993). Mitochondrial DNA evolves faster than most nuclear DNA regions (for exceptions see “microsatellites” below) and therefore exhibits considerable variation within and among species. The smaller size and high levels of variation make mtDNA a very useful marker for genetic analyses; however, its usefulness may be limited by the fact that it is maternally inherited. For example, it is impossible to determine paternity using mtDNA, and in species in which males and females exhibit

different migratory tendencies, mtDNA will only reveal information about female migration.

The development of Polymerase Chain Reaction (PCR) techniques has been largely responsible for the explosion in DNA-based technology over the last two decades. Beginning with a minute quantity of genomic DNA, the PCR process uses a thermostable DNA polymerase and 20 to 40 cycles of DNA synthesis resulting in perhaps a million-fold amplification of the target sequence. The amplified fragment can then be sequenced or subjected to restriction fragment length polymorphism (RFLP) analysis. The source of DNA can be any piece of fresh or ancient nucleated tissue including fin clips, blood, or even a museum specimen (e.g., dried jaw). Tips for preserving and taking tissue samples are provided below.

### TYPES OF MOLECULAR MARKERS

Restriction Fragment Length Polymorphisms (RFLPs) use bacterial restriction enzymes that cleave specific (usually 4 to 6 base) sequence motifs in a segment of DNA. Different fragment patterns among individuals are caused by a mutation in the restriction site or a change in the number of nucleotides, through an insertion or deletion, between restriction sites. These different fragment arrays are detected by resolving the fragments on an agarose or polyacrylamide gel that separates the fragments by size. Typically a fragment of DNA is amplified using PCR and then cut with several restriction enzymes. Alternatively, sections of DNA are cut with restriction enzymes, run on a gel and probed with a labeled homologous sequence. The locations of the bands are visualized by the presence of the labeled probe. RFLP analysis of mtDNA has been used to examine the population genetic structure of sharks (Heist et al., 1996a, 1996b).

Numerous “fingerprinting” techniques each produce a large number of bands on a single gel that can be used to infer relatedness among individuals. The multilocus minisatellite technique (Wright, 1993; O’Reilly and Wright, 1995) is a nuclear DNA RFLP approach that uses a probe made from a sequence of DNA known to be present in many copies of the genome of the target organism. When the digested DNA is hybridized to the probe, many fragments are visualized. This technique is commonly used for forensics and paternity-exclusion.

Random Amplified Polymorphic DNA or RAPDs are generated by PCR using short primers (around 10

nucleotides in length) of random sequence. This generates random segments of DNA that are resolved by gel electrophoresis. While this technique is fast and inexpensive (RAPD is an apt acronym), the results are often inconsistent.

A relatively new technique is Amplified Fragment Length Polymorphism or AFLPs (Vos et al., 1995). Like the minisatellite technique, it begins with the use of restriction enzymes to cut genomic DNA. The resultant DNA fragments are then combined with restriction enzyme-specific adapters. Primers complementary to these adapters are then used to amplify fragments using PCR. The use of the adapters removes the inconsistency problem associated with RAPDs and allows the technique to be performed on species for which minisatellite probe sequences have not yet been developed. To date, no elasmobranch study has employed minisatellites, RAPDs, or AFLPs.

What each of these fingerprinting techniques provides is a unique pattern consisting of numerous (typically 20–200) bands on a single gel. Algorithms have been developed to estimate the degree of relatedness among individuals based on the fraction of bands that differ and are shared among individuals. These indices are widely used in captive breeding programs to avoid inbreeding by identifying genetically related individuals. These techniques can be used for paternity-exclusion. Barring a rare mutation, offspring should not possess any alleles not found in either parent. Thus if one parent (typically the mother) can be positively identified, potential sires can be excluded if they lack a band present in the offspring.

Microsatellites are short, tandem repeats of 1-6 base pairs (Ashley and Dow, 1994) that exhibit a high mutation rate (and hence high intraspecific diversity) for the number of repeat units. Primers for these markers must be developed, usually by rather exhaustive and involved protocols (e.g., Dow et al., 1995) for use in PCR. Once primers are developed, PCR is used to amplify microsatellite repeat regions.

PCR products are resolved using polyacrylamide gel electrophoresis. Because of their high degrees of polymorphism and hence great utility, microsatellites are rapidly becoming the marker of choice in many studies of population genetics (O'Connell and Wright, 1997). Two studies on Carcharhiniformes (Heist and Gold, 1999a; Feldheim et al., 2001a), one on an orectolobiform (Heist et al., 2003), and one on a lamniform (Pardini et al., 2000) have developed species-specific primers for microsatellite analysis. The utility of these species-specific primers for an array of taxa (Heist and Gold, 1999a; Pardini et al., 2000) may make microsatellite analysis feasible for population genetic studies in many elasmobranch species.

### TISSUE COLLECTION AND STORAGE

Prior to the development of PCR, the only suitable tissues for genetic analyses were those that were fresh or freshly frozen and maintained at temperatures of -20 °C (or preferably -80 °C). Allozyme analysis still requires tissue samples of high quality, and DNA-extraction yields, in terms of quantity and quality, from fresh and frozen tissue, are superior to tissues that have been preserved via other means. PCR-based techniques require such small initial amounts of target DNA that nearly any preserved tissue (except that stored in unbuffered formalin) is sufficient. Besides freezing, the two most common methods for preserving tissue for genetic analyses are storage in 95% ethanol and 20% DMSO saturated with sodium chloride (Seutin et al., 1991; Table 18.1 and Table 18.2). Tissues stored in these solutions at room temperature (or preferably refrigerated at 4 °C) can provide adequate DNA for amplification for several years, although 20% DMSO may be a superior buffer for subsequent DNA extractions (Seutin et al., 1991; Dawson et al., 1998). Blood can be stored in Queen's lysis buffer (Seutin et al. 1991) in a ratio of one part whole blood to three parts buffer. Unbuffered formalin, perhaps the most commonly

**Table 18.1.** Sample collection and appropriate tissue storage for genetic studies. Asterisk denotes tissue that must be frozen if used for an allozyme study.

Sample collection	When collected	Storage	Use
Fin clip	Exam	Frozen or buffer (Table 18.2)	DNA study
Blood sample	Exam	Frozen or Queens lysis buffer (Table 18.2)	DNA study
Muscle biopsy	Necropsy or Exam	Frozen or buffer*	DNA or allozyme study
Internal organs	Necropsy	Frozen or buffer*	DNA or allozyme study
Oviducal gland	Necropsy	Frozen or buffer	Sperm storage study

**Table 18.2.** Storage buffers for tissue samples used in genetic studies.**TNES urea buffer**

6-8 M Urea

0.125 M (125 mM) NaCl

0.01 M (10 mM) Tris, pH 7.5

0.01 M (10 mM) EDTA

1% SDS

pH=7.5

**For 1 L (6 M Urea solution):**

360.4 grams Urea

7.3 grams NaCl

1.21 grams Trizma Base

3.72 grams EDTA

**Long-term storage buffer**

0.1 M (100 mM) Trizma Base

0.1 M (100 mM) EDTA

2% SDS

pH=8.0

**For 1 L:**

12.2 grams Tris

37.2 grams EDTA

20 ml of 10% SDS

**20% DMSO-salt saturated storage buffer**

20% DMSO saturated with 5 M NaCl

optional: EDTA, pH 8.0 (up to .25 M)

**For 1 L:**

243 grams 5 M NaCl

74.5 grams 0.25 M Na<sub>2</sub>EDTA

Dissolve in 400 ml H<sub>2</sub>O (800 ml for 1 L). Once EDTA and NaCl dissolved, add DMSO to 20%, 100 ml for 500 ml; 200 ml for 1 L.

**Queen's lysis buffer (blood storage)**

0.01 M (10 mM) Tris

0.01 M (10 mM) NaCl

0.01 M (10 mM) EDTA

1% n-laurylsarcosine

pH=7.5

95% ethanol or isopropanol can also be used to store tissue samples. If tissues will be used for an allozyme study, they should be frozen at -20°C or colder. Freezing will also work for subsequent DNA studies.

used tissue fixative in museums and aquariums, causes irreversible chemical damage to DNA, rendering tissues all but worthless for subsequent DNA analyses. While protocols exist for extracting DNA from formalin-fixed tissues, the protocols are long and tedious and typically result in DNA that

amplifies poorly, if at all. While buffered formalin is less harmful to DNA, an appropriate rule of thumb is: if future genetic analyses of a specimen may be desired, either do not fix the animal in formalin or collect a tissue sample for formalin-free preservation prior to fixing the animal.

Because of the small amount of DNA required for a successful PCR amplification, contamination from other species and other individuals is a serious consideration when collecting samples for genetic analyses. Latex gloves should be worn and either changed or cleaned between samples to prevent the carryover of human DNA or contaminants present on the hands. Instruments should be heat sterilized, not alcohol sterilized, since alcohol will only act to preserve whatever DNA contamination is present on the tools. If heat sterilization is impractical, instruments should be washed vigorously with soap and water to remove foreign tissue, soaked in a 10% bleach solution, and rinsed prior to use on specimens.

### SYSTEMATICS AND TAXONOMIC IDENTIFICATION

Molecular markers are proving to be very useful for determining the phylogenetic relationships among elasmobranch species (Dunn and Morrissey, 1995; Kitamura et al., 1996a; Naylor et al., 1997). Molecular genetics has been used to demonstrate the presence of unrecognized or cryptic species (Gleeson et al., 1999; Martin and Birmingham, 2000). Because elasmobranchs are morphologically conserved and because many species are morphologically similar, it is likely that additional species will be recognized and confirmed using molecular genetics. Molecular genetics can be used for species identification (Heist and Gold, 1999b; Shivji et al., 2003) and perhaps even identify the population of origin of a captive elasmobranch.

A few milliliters of blood or a tissue biopsy provide ample DNA for PCR amplification and DNA sequencing. The sequence obtained from the unknown shark must be compared to one from a positively identified specimen. Perhaps the best technique for identifying a single shark is by sequencing all or part of the mitochondrial cytochrome-b (cyt-b) gene. The cyt-b gene is the single most widely-used gene for systematic analyses in vertebrates (Lydeard and Roe, 1997) and hence there is considerable data available for comparison. While cyt-b exhibits very little intraspecific variation within carcharhinid sharks (Heist, 1999), there is considerable divergence among species.

DNA sequence data for comparison are available from the GenBank internet database (www1). By using this service, it is possible to perform taxonomic searches to download sequences from particular species. "Blast" searches can be performed in which

a sequence submitted by a user is compared to the entire database and the sequences with the greatest similarities are retrieved. Relatively few shark sequences have been deposited into the GenBank database. Many scientific journals now require deposition of the DNA sequence on the database as a condition of publication; therefore, as more studies are completed and published, the database for elasmobranchs will enlarge considerably.

### GENETIC STOCK STRUCTURE

When populations are reproductively isolated, allele frequencies at polymorphic loci diverge due to the stochastic process of random genetic drift within each population. The magnitude of the difference in allele frequencies is represented by various estimates of Wright's  $F_{ST}$ , which can be thought of as the standardized variance in gene frequencies among populations (Wright, 1969).  $F_{ST}$  values typically range from 0 to 1, with values  $<0.05$  generally taken to mean that there is little genetic divergence among stocks (Hartl and Clark, 1997). Estimates of  $F_{ST}$  are routinely used to determine whether or not a species is divided into multiple stocks (Carvalho and Hauser, 1994).

$F_{ST}$  values are expected to be inversely proportional to the amount of migration (gene flow) among populations. Applying the island model of migration:

$$F_{ST} = \frac{1}{(4Nem + 1)}$$

where  $Nem$  is equal to the effective migrants per generation (Wright, 1969). Under this scenario, a single migrant per generation (corresponding to an  $F_{ST}$  of 0.2) is considered sufficient to prevent significant genetic divergence. However, if migrants come from nearby populations that are genetically more similar than members of the species as a whole, if some of the migrants are sexually immature, or if migrants have reduced reproductive success relative to natives,  $F_{ST}$  may underestimate the number of migrants per generation (Wright, 1969; Mills and Allendorf, 1996). Typically this is not a problem since the number of migrants necessary to reduce  $F_{ST}$  to a value very close to zero is so small that a statistically significant value of  $F_{ST}$  may be taken as evidence of multiple stocks. However, with the increased statistical power of multiple highly-polymorphic microsatellite loci, a statistically significant ( $F_{ST} > 0$ ) outcome might not represent biologically significant stock structure

(Gold and Richardson, 1999). To further complicate matters, Dizon et al. (1995) argued that in cases in which a type II error (failing to reject the null hypothesis of a single genetic stock when multiple stocks exist) is more deleterious to management practices than a type I error (falsely rejecting the single stock hypothesis when only a single stock is present), prudent risk management practices call for a reduction in the  $\alpha$ -level of the test to balance the risks associated with each error type. Thus, a very large value of  $F_{ST}$  unambiguously indicates the presence of multiple isolated stocks while small  $F_{ST}$  values require careful consideration and judgment. Waples (1998) provides further caution when interpreting small values of  $F_{ST}$ , noting that because of the asymptotic shape of the relationship between small  $F_{ST}$  values and  $Nem$ , a small error in the measurement of  $F_{ST}$  will result in a large error in the estimate of  $Nem$ .

Previous studies of population genetics in sharks have detected very small values of  $F_{ST}$  among continuously distributed sharks, but greater divergences among discrete populations of sharks (Heist, 1999). Allozyme studies of the spottail shark (*Carcharhinus sorrah*) and Australian blacktip shark (*Carcharhinus tilstoni*) found no evidence of multiple stocks within Australian waters (Lavery and Shaklee, 1989).

Populations of gummy shark (*Mustelus antarcticus*) from southern and eastern Australia exhibit significant differences in allozyme and mtDNA profiles (Gardner and Ward, 1998). Within the North Atlantic, studies of the sandbar shark, (*Carcharhinus plumbeus*) and Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) detected no significant differences in mtDNA haplotype frequencies (Heist et al., 1995; Heist et al., 1996b). Heist et al. (1996a) reported small but significant differences in mtDNA haplotype frequencies in shortfin mako (*Isurus oxyrinchus*) between the North Atlantic and other ocean basins; however, there was no evidence of evolutionarily distinct stocks. Feldheim et al. (2001a) found small but statistically significant  $F_{ST}$  values in lemon sharks (*Negaprion brevirostris*) from Bimini, Bahamas, and Brazil. Gaida (1997) found significant differences in allozyme allele frequencies among populations of Pacific angel sharks (*Squatina californica*) from different islands in the California Channel Island chain that were isolated by deep channels. Recently Pardini et al. (2001) detected significant differences in mtDNA diversity, but not microsatellite diversity,

among white sharks (*Carcharodon carcharias*) from South Africa and Australia/New Zealand, suggesting male-mediated gene flow accompanied by female philopatry.

## STOCK TRANSFERS AND RELEASE OF ANIMALS

Effects of stock transfers in fishes, which have generally been viewed as deleterious, can be divided into direct or indirect effects (Waples, 1995; Utter, 1998). Indirect effects include competition and disease transfer. For example, a transferred fish that fails to reproduce may compete for scarce resources with native fishes, or it may be a resistant carrier of a disease organism to which the native stock is susceptible. This last scenario is especially dangerous, and there are numerous examples of native stocks of salmonid fishes whose existence has been threatened through the introduction of diseases carried by introduced stocks (Utter, 1998). Direct effects occur when released fish interbreed with native fishes. Traits gained through domestication selection can be passed on to wild animals (Storfer, 1999). Farm-raised trout have developed a shadow following behavior in which animals follow the shadow of the feed truck (Vrijenhoek, 1998). While this trait may be favorable in captivity, this behavior may lead to an increased risk of mortality in the wild (as animals follow the shadow of a raptor for example).

One of the most serious direct effects of stock transfer is outbreeding depression, which can result from two causes: loss of adaptation and breakup of co-adapted gene complexes (Templeton, 1986; Waples, 1995). The offspring of matings between native and introduced fishes, and subsequent generations of progeny, may not be adapted to the local environment. Hence the gene pool may be disrupted through the presence of foreign maladapted genes. In subsequent generations, genes and chromosomes that have co-evolved as a unit will be shuffled via meiotic reductive division resulting in fish that are maladapted to the environment. Mixing of distinct stocks of fishes may be beneficial where a local stock is suffering from inbreeding depression due to a reduction in population size. However, unless signs of inbreeding depression are apparent, (e.g., fluctuating asymmetry, high occurrence of anatomical or physiological abnormalities), stock transfers should be viewed as potentially dangerous (Vrijenhoek, 1998).

Outbreeding depression will only occur when stock transfers occur among genetically distinct stocks. Because many sharks are highly migratory and many species are pelagic, a single shark stock may range over thousands of kilometers, or there may be a single worldwide stock. If two stocks are reproductively isolated, and therefore have the potential for outbreeding depression, there will be significant frequency differences in polymorphic genetic characters (e.g., allozyme or microsatellite alleles, mtDNA haplotypes). In each of the studies cited in the section on stock structure above, the similarity of gene frequencies between populations indicates that there is sufficient genetic exchange among the surveyed locations so that outbreeding depression would not likely accompany a stock transfer. However, Utter (1998) argued that in pelagic species that have large effective population sizes (e.g., shortfin mako), adaptive differences among stocks can develop even in the presence of considerable gene flow. Many sharks, including species commonly exhibited in aquariums (e.g., sandbar sharks and sand tiger sharks, *Carcharias taurus*), exhibit highly discontinuous distributions. In these cases, there may be significant genetic differences among populations. Heist (1994) observed that sandbar sharks from western Australia and the eastern United States have diagnostically different mtDNA profiles, indicating a long period of isolation and perhaps local adaptation. Populations that are genetically and geographically isolated may exhibit selective differences in terms of physiology, behavior, or disease-resistance, that would make transfers of stocks harmful.

Given the great migratory potential and connectivity of shark populations, coupled with the small numbers of sharks that are likely to be released via aquariums, the likelihood of deleterious results from captive releases of elasmobranchs is small. However, based on the information from captive releases in other fish species, the threats to native stocks outweigh the benefit that would be gained to the population by the addition of captive releases. Thus, captive sharks should not be released into the wild environment, particularly in those situations in which a release will result in a transfer of a shark from one discrete population to another.

#### **GENETIC CONSIDERATIONS OF CAPTIVE BREEDING**

For most species, there are genetic detrimental effects associated with captive breeding, including inbreeding and loss of genetic variability through

genetic drift (Storfer, 1999). Inbreeding results in an overall loss of heterozygosity as well as an increase in the expression of recessive deleterious traits (Lande, 1988). Ralls et al. (1988) examined captive populations of mammals and found that juveniles from inbred pedigrees suffered higher mortality than non-inbred lines. To decrease inbreeding, careful pedigree analysis should be used to avoid matings by related individuals. If pedigree analysis is not an option, relatedness of individuals either by band sharing or sharing of alleles (see fingerprinting methods and microsatellites above) can be used to identify potentially related animals that should not be bred to one another. On average, full siblings share 50% of their genes, while half siblings share 25% of their genes, above the background sharing of alleles by unrelated individuals.

Captive populations suffer a loss of genetic diversity due to genetic drift, chance fluctuations in allele frequencies over time (Hartl, 1988). Small populations are especially prone to this phenomenon due to few individuals and resulting low overall genetic variability. This can lead to the rapid loss or fixation of alleles. The effects of genetic drift may be reduced in a captive species if several populations are kept in different aquariums. One large captive metapopulation can maintain genetic variability, even though single captive populations may be losing alleles due to drift. For example, if captive populations drift to fixation for different alleles, allelic diversity can still be maintained over the whole captive metapopulation. In cichlids (*Prognathochromis perrieri*), genetic diversity is preserved over several captive populations worldwide (Fiumera et al., 2000).

Other factors may further exacerbate the loss of genetic variation, including unequal family sizes and disproportionate mating of males and females. It is widely accepted that equalizing family sizes will help keep genetic change, over time, to a minimum (Tave, 1993; Falconer and Mackay, 1996), thereby reducing genetic drift. This way, the genes of no single male or female are over-represented in the following generation. Unequal numbers of breeding males and females can reduce genetic variation. If the sex ratio of breeding adults is unequal then the effective population number ( $N_e$ ), or number of adults contributing genes to the next generation, is actually less than the total number of adults. This is represented by the following equation (from Hartl, 1988):

$$N_e = \frac{4NmNf}{(Nm + Nf)}$$

where Nm is the number of breeding males and Nf is the number of breeding females. Equalization of family sizes and breeding adults may not work for captive animals that do not accept forced breeding or are not amenable to change in breeding structure (Snyder et al., 1996).

### GENETIC STUDIES AND CAPTIVE POPULATIONS

Table 18.3 summarizes the types of genetic studies that may be conducted using tissue taken from captive elasmobranchs.

### MATING SYSTEMS OF CAPTIVE ELASMOBRANCHS

Little is known about the genetic mating system of most sharks. Nurse sharks (*Ginglymostoma cirratum*), lemon sharks, and blue sharks (*Prionace glauca*) are known to produce litters sired by multiple males (Ohta et al., 2000; Feldheim et al., 2001b, Feldheim et al., 2002a), while most bonnethead (*Sphyrna tiburo*) litters are

produced by a single male (Chapman et al., 2000). With the exception of the bonnethead study, these reports are based on one or two litters from each species. Testing the genetic mating system of sharks in captivity may shed some light on what occurs in wild populations. Parental testing will help with the reconstruction of pedigrees. Parental testing is usually best achieved with a co-dominant marker such as microsatellites (Ashley and Dow, 1994), although dominant markers, such as AFLPs, have been used successfully in paternity assignment (Mueller and Wolfenbarger, 1999).

Female elasmobranchs are able to store sperm in their oviducal gland (Pratt, 1979) and stored sperm may be viable for over a year (Castro et al., 1988). This ability to store sperm in some species may lead to multiple males siring a litter of a female. Captive elasmobranchs may shed some light on both the duration sperm remains viable in the oviducal gland (Castro et al., 1988) and how many male ejaculates are stored in the oviducal gland at one time. Microsatellite genotyping of stored sperm would indicate the minimum number of males represented in the sperm sample. For example, if five alleles amplify at a particular microsatellite locus, this would indicate that at least three males had inseminated the female, as each male can have a maximum of two alleles per locus. Oviducal glands should be carefully dissected and stored during any necropsy of a female elasmobranch.

**Table 18.3.** Types of genetic studies that may be conducted using tissue taken from captive elasmobranchs.

Genetic marker	Study type	Examples
Allozymes	Systematics	Eitner, 1995
	Species designation	Lavery and Shaklee, 1991
	Population genetics	Lavery and Shaklee, 1989; Gaida, 1997
DNA sequencing	Phylogenetics	Naylor, 1992; Martin, 1993; Naylor et al., 1997
	Population genetics	Kitamura et al., 1996b
	Species identification	Heist and Gold, 1999b; Shivji et al., 2003
Fingerprinting	Paternity exclusion	
	Relatedness	
mtDNA RFLPs	Population genetics	Heist et al., 1996a, 1996b
Microsatellites	Genetic tagging	Feldheim et al., 2002b
	Marker development	Pardini et al., 2000; Heist et al., 2003
	Parentage tests	Feldheim et al., 2001a, 2002a
	Population genetics	Heist and Gold, 1999a; Feldheim et al., 2001b
	Relatedness	

## CAPTIVE ELASMOBRANCH CONTRIBUTION TO GENETICS

Elasmobranch tissue is relatively difficult to obtain, and the field work involved is often cost-prohibitive. Most genetic studies undertaken would not have been possible if not for collaboration with field researchers, fishermen, etc. Therefore, during routine veterinary examination of captive animals, or during necropsy, extra tissue or blood samples should be taken for genetic studies. Ideally, blood or tissue samples should be kept frozen. Should freezer space be limited and storage at room temperature become necessary, several storage buffers for both tissue and blood samples are available (Seutin et al., 1991; Asahida et al., 1996).

Captive elasmobranchs can contribute a significant aspect to an ongoing or newly developed genetic project. Screening a genomic library for microsatellites only requires genomic DNA from one individual. A captive individual can obviously provide the DNA for this method. In addition, it is often desirable to test both amplification and variability of microsatellite PCR primers across many taxa. Often, sequences flanking a microsatellite repeat are conserved across genera, families, and even orders (Heist and Gold, 1999b; Pardini et al., 2001), and microsatellite primers developed for a particular species may be useful across a suite of species. Testing these primers for amplification and variability only requires a handful of specimens from each species.

For genetic projects not yet underway, having several samples already in storage would be advantageous to researchers worldwide. In addition, stored specimens would increase the sample size of projects already underway. Regional testing of genetic variability based on sequence data is often only comprised of a handful of samples from each population. Stored specimens may provide the researcher an extra area of the species's range to examine and compare to other populations.

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