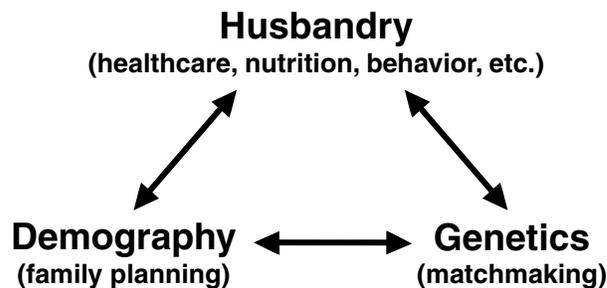


## 1.1 Different Methods and Procedures Used, Gender Determination

There is much to consider when managing animal populations in zoos, especially today, when zoos engage in many cooperative breeding programs. Zookeepers and managers must abide by carefully researched husbandry guidelines, which typically include dietary requirements, training and enrichment strategies, and behavioral observations. Demography also plays a major role insofar as keeping records of breeding and offspring helps eliminate inbreeding and maintains a healthy gene pool for each managed population. Genetics, the final cornerstone of effective population management, is absolutely imperative when maintaining healthy genetic diversity among populations.



### Why does genetics play such an important role in conservation?

In today's genetics lab, much more can be learned about how animals live than ever before. DNA, the genetic molecule that serves as a blueprint to build all living things, can reveal many of the mysteries that animals keep. Among the commonly used purposes and methods are:

#### Purposes:

- *Paternity testing* – This is a method by which a father can be determined by comparing his genetic material with that of his possible offspring.
- *Relatedness testing* – This process compares genetic material from two or more individuals to assess if they are related to one another.
- *Gender determination* – Many animals are monomorphic, where genders appear alike, and genetics can now be used to ascertain who is male or female.
- *Species identification* – Some species appear very much like other species, and separating them usually can be difficult, so specific DNA sequences can tell us who is who.

#### Methods:

- *Karyotyping* – This technique allows researchers to observe the chromosomes, the packages of DNA in each cell, in order to view patterns unique to each species.
- *DNA fingerprinting* – This procedure allows researchers to view the DNA fragments that form a pattern unique to every individual.
- *Genotyping* – This practice involves finding unique patterns in DNA and comparing an individual's pattern with another individual's pattern.

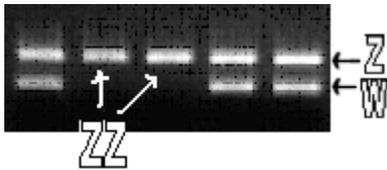
The role of genetics is a monumental one, and the information obtained from the methods above are very important to maintaining a healthy animal population. Zoos that work together contribute updated information to the central person who coordinates the management of a given species, and proper records are kept to maximize the genetic diversity within each group.

## Gender Determination Using Polymerase Chain Reaction (PCR)

DNA sexing has become the preferred method of determining the sex of many monomorphic species. With accuracy rates greater than 99%, it is no surprise that this method of sexing has become the most commonly used protocol in zoos and conservation organizations worldwide. Over the years, the genetics laboratory at the Chicago Zoological Society (which manages Brookfield Zoo) has conducted thousands of DNA-based tests for an array of institutions all over the world, ranging from private individuals and breeders to entire zoo populations.

Why is DNA gender determination so accurate? The answer to this question can be found in the technology used. Advances in molecular diagnostics have led to the development of a technique known as Polymerase Chain Reaction, or PCR. This technique takes advantage of the self-replicating nature of DNA, which allows one to amplify (replicate) DNA molecules. In this technique, double-stranded DNA is heated to a high temperature so that the strands separate. Then short segments of DNA, known as primers—with sequences complimentary to the ends of the region that one wishes to amplify—are allowed to locate and attach to the longer template molecules. A special version of the polymerase enzyme begins the next step. The DNA molecules replicate exponentially, resulting in quick and efficient amplification of the selected DNA segment. Using PCR in the laboratory, the DNA of male and female birds can be amplified and differences can be detected.

Here's one way DNA sexing works. DNA sexing of birds takes advantage of the fact that males and females have different sex chromosomes, much like mammals. The main difference between female and male birds is that females carry a ZW chromosome pair, so they determine the sex of the offspring. Male birds carry a ZZ chromosome pair and can contribute



only a Z chromosome. This is just the opposite of mammals, including humans. Male humans possess an XY pair and females carry the XX pair. For many species, a coding region of DNA that differs in size between the Z and W chromosomes is copied many times. A single type of PCR product is observed for males and two PCR products are present for females, one for the Z and one for the W chromosome, as shown in the picture above.

There are different very safe and noninvasive sources for getting DNA from an animal. These sources include shed feathers, hair with bulbs, and feces. It is a common misconception that blood samples are more accurate than feather samples for determining gender. In reality, using blood versus feathers or hair yields a 100% identical result with regards to accuracy. Since both methods ultimately result in isolating and identifying specific fragments of DNA, the source of the DNA should have no impact on the final results of this test.

Why PCR? First and foremost, as mentioned, this method is noninvasive and does not result in any harm to animals. Even a very small sample can yield a sufficient quantity of DNA. Conservation geneticists also use these genetic techniques to answer questions about the interbreeding of populations or to determine if these populations are isolated from each other by some type of barrier. These answers are extremely important to conservation efforts. Small, isolated populations are more likely to become extinct faster, or certain managed populations should be maintained separately to minimize hybridization between species and thereby maintain maximum diversity within each species.

## 1.2 Conservation in Today's Zoos, Pedigrees

### Conservation Genetics in Today's Zoos

Many years ago, zoos became concerned with husbandry and providing the right environment so that species would be comfortable enough to breed and produce young. Today, zoos have programs that monitor which individuals are mating so that they can maintain a healthy gene pool and sustainable populations. They keep careful records to limit inbreeding and to maintain the valuable genetic make-up of the population's founders: the original individuals who have genes unrelated to the genes of others in the population.

Below is a pedigree, or family tree, of a group of golden lion tamarins (*Leontopithecus rosalia*). Each letter represents an identified allele, or version of a gene, which helps to determine parentage. This pedigree was determined before genetic monitoring was in place. The mean kinship value (MK), is a number calculated to estimate how related an individual is to the rest of the population. Mean kinship is the average of all pairwise kinship values between a given individual and all other living individuals in the population. The higher the number, the more relations and the less desirable it is to breed that individual. Using the pedigree below, answer the questions below to determine how genetics is used to manage this species.

