Lab 3: Paternity Analysis Using Molecular Genetics Methods

Many aspects of animal behavior are difficult to address with observational data alone, especially under field conditions. Animals may not behave naturally in presence of observers, or may be altogether difficult to find or observe in nature. Today we will focus on the use of methods from molecular genetics to infer basic aspects of animal reproductive behavior. We will begin with a brief review of the genetic principles and associated molecular techniques used to infer patterns of relatedness among individuals in a given sample. You will then apply these methods to explore the mating systems of red-winged black birds and house mice.

BACKGROUND – PATERNITY ANALYSIS USING MOLECULAR METHODS

Paternity analysis relies on reconstructing the inheritance of genetic markers from parents to offspring. In a sexual reproducing diploid animal most genetic material exists in two copies with one copy inherited from each parent. In this context, a genetic marker is any region of DNA that is potentially informative with respect to inheritance. We refer to variants of markers as alleles and the particular combination of alleles that an individual has at one or more markers as their genotype. Consider the following example involving the inheritance of alleles from a single genetic marker, the “B” locus.

Reproduction between a female with a Bb genotype and a male with a bb genotype can produce offspring with two possible genotypes, Bb or bb. Many questions regarding animal mating behavior use paternity analysis to test the hypothesis that one of several potential sires is the true father of an offspring. These tests rely on exclusion analysis; spurious candidate males are excluded as sires based on mismatches between their genotype and the genotype of the offspring. Consider the example above but with three possible sires with genotypes BB, Bb, and bb. If the female with the Bb genotype has an offspring with the bb genotype then we know that at least one of the offspring’s b alleles came from the father. Based on this information we can immediately exclude the BB male as a potential sire, leaving the bb and Bb males. In the same fashion, we can examine additional markers to exclude either the bb or Bb male.

Scientists usually generate multilocus genotypes using particular kinds of DNA markers called microsatellites. Microsatellites are sequences of DNA that have a simple repeat structure (e.g., CACACA). When simple repetitive DNA is copied within the cell it tends to change or mutate frequently by reducing or increasing the number of repeats present (CACACA mutates to CACACACA). These changes in length can be surveyed using molecular assays based on the Polymerase Chain Reaction or PCR. The microsatellite alleles that we discuss today will labeled as numbers to reflect their length in a PCR assay.

PROCEDURE

The purpose of this exercise is to highlight the power and limitations of molecular markers for resolving basic aspects of reproductive behavior. Each case study is motivated by recent studies in animal reproductive behavior. At the start of lab you will receive microsatellite datasets for two different ecological case studies. You will use these data to gain insights into the mating
behavior of two different species. You are free to work on each exercise in groups of two or three; the simple calculations involved will go faster if at least one member of your group has a calculator.

**CASE STUDY 1: ESTIMATING REPRODUCTIVE SUCCESS IN RED-WINGED BLACKBIRDS**

**Background:** In many bird species, males and females form monogamous, long-term breeding pairs; however, the red-winged blackbird (*Agelaius phoeniceus*) represents a well known exception. These birds are socially **polygynous**, meaning that males commonly have multiple mates. Males expend considerable energy in vocal and visual displays when competing for territories and then mate with all of the females who choose to create a nest in their territory. For decades scientists generally assumed that mating in birds only occurred among social partners, so the male red-winged blackbird who could attract the most females to its territory would have the most mates. Since the male provides little parental care in red-winged blackbirds, the number of mates is an important determinant of a male’s fitness. Historically, the number of females in a male’s territory has been used as a proxy for the number of mates; however, it is now known that male and female birds sometimes mate with individuals other than their social partner. These matings are called **extrapair copulations** (or, **EPCs**). This alternative strategy, if present in red-winged blackbirds, could have a profound effect on the fitness of male blackbirds and at least partially undermine the profitability of investment in defending a territory.

**The Dataset:** You have been provided with the genotypes of four microsatellite markers collected from four families of red-winged blackbirds during a single breeding season. The nesting localities were all sampled within close proximity of each other. Use exclusion analysis of these data to answer the following questions:

1. Does the social father sire all of the offspring of a given family?
2. Is there more variation in overall reproductive success among sampled males or females? Discuss the potential evolutionary implications of your answer.
3. On average, what proportion of male reproductive success comes from extra-pair copulations?
4. What are the limitations of your data and how might these addressed? Design an experiment/study to address these major issues and to further resolve the mating system of red-winged blackbirds.

**CASE STUDY 2: INFERRING THE MATING SYSTEM OF HOUSE MICE**

**Background:** Competition among sperm from different males to fertilize the eggs of a given female can be a strong evolutionary force. In most mammals, females do not store sperm prior to fertilization and **sperm competition** can only occur if females mate with multiple males in a relatively short period of
time. Because copulations are often difficult to observe directly, the frequency of mating, and thus the intensity of sperm competition, is not known for many species. House mice (*Mus musculus*) are nocturnal rodents that live in close association with humans. In nature, males are thought to defend small territories that include one or more resident females. The eggs of inseminated females are fertilized within 20 hours after copulation. Litter sizes typically include between six to eight pups. Sperm are hooked (photo left), a morphology thought to reflect adaptation to intense sperm competition.

**The Dataset:** You have been provided with the genotypes of five microsatellite markers sampled from ten females and their offspring. Females were pregnant at collection and the potential sires have been sampled. Use exclusion analysis of these data to answer the following questions:

1. How many litters were sired by multiple males?
2. Mice often breed with close relatives. How might this influence your analysis?
3. Choose two litters and try to reconstruct the genotypes of sires. What are the limitations of your inference?
4. Is the frequency of multiple paternity in mice likely to be an accurate estimate of the intensity of sperm competition in mice (e.g., of multiple mating by females)? Why or why not?

**Lab Report**

For your lab report, choose one of the two case studies. Focus your introduction and discussion on the listed questions. Your methods section should include a brief statement on the nature of the data that were collected and your method of analysis. Include one or more tables summarizing the results of your study. In your report include some discussion of the assumptions and limitations of your study as well as potential future data you would collect to address these issues.

**Further Reading**


