Mathematics in Conservation: The Case of the Endangered Florida Panther

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Part I – Endangered Species

As human population sizes increase around the world, we are constantly changing, depleting, and fragmenting habitats of wild species. This habitat change and fragmentation is especially detrimental to large mammals that tend to require large habitable ranges in which to survive, are long-lived, and produce few offspring per year.

As of November 2010, in the United States alone there were 414 animal species listed as endangered (www.fws.gov), meaning that they are "in danger of extinction throughout all or a significant portion of [their] range" (uscode.house.gov/ United States Code, Title 16, Chapter 35). These include species of bears, deer, bats, wolves, seals, whales, and large cats (among many others). The group of large cats includes the interesting case of the Florida panther.

In the mid-1990s, the Florida panther had reached critically low levels, with only 20 to 30 animals remaining in the wild (Johnson et al. 2010; Packer 2010).

It turns out that mathematics is incredibly important in informing our understanding of the biological dynamics of these populations. Mathematical modeling can help us understand the likelihood of extinction in a threatened or endangered population, and is critical in our planning for their continued survival. Here we will investigate some of the important mathematical principles underlying our understanding of the genetics of animal populations, especially as those principles apply to conservation of endangered species, using the Florida panther as an example.

Figure 1. The Florida panther. Photo by Connie Bransilver, USFWS/Southeast, (CC BY 2.0), http://www.flickr.com/photos/usfwssoutheast/with/5164633462/.

Part II – Basic Genetics

All organisms have a set of genetic instructions by which they are built. These instructions are written in the language of DNA. The full set of instructions for any one organism makes up that organism's *genome*. This genome—the entire set of instructions—is found in every single cell of multicellular organisms (with the exception of a few types of cells).

In organisms, including humans and Florida panthers, the genome is made of many long strings of DNA called *chromosomes* (Figure 2). Each of these chromosomes has many *genes*. The genes are the specific sections of the chromosomes that are responsible for making proteins, and so it's the genes that control the vast majority of what happens in the organism developmentally and physiologically. Between these gene regions of the chromosome, there is a lot of DNA that is not part of any gene (Figure 2). Each specific gene is reliably found at a specific location, or *locus*, on a specific chromosome. Because the animal inherits one copy of each chromosome from its father and one copy of each chromosome from its mother, each animal has *two copies of each gene*.

Within a population, each gene may potentially have many variant types of that gene. This assortment of types is the basis of the variability in many characteristics among humans, such as eye color, hair color, and height. These different types or varieties of genes are called *alleles*.

Because each individual has two copies of each gene, each individual can either have two of the same allele, or the individual can have two different alleles. An individual that has two of the same allele at a locus is called a *homozygote*, or is said to be *homozygous* for that gene (Figure 3). An individual that has two different alleles at a locus is called a *heterozygote*, or is said to be *heterozygous* for that gene (Figure 3). This designation of the types of alleles an individual has at a locus is called its *genotype*.

When an individual reproduces, that individual makes new versions of each chromosome for its offspring. This new version of each chromosome is a hybrid of that individual's two copies of the chromosome that the individual inherited from its own parents. Each *gamete* (egg or sperm) receives one of these hybrid chromosomes from the parent. The result of this is that each gamete randomly receives one of the two alleles of each gene from that parent (Figure 4).

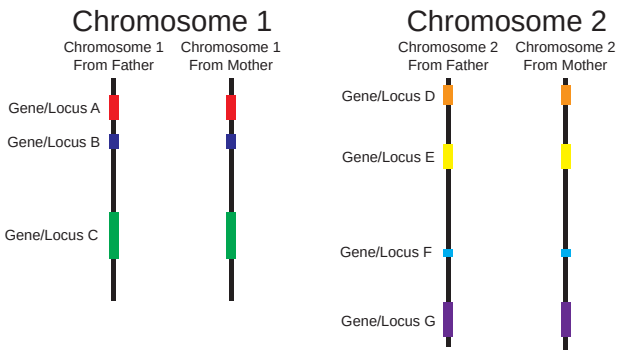

Figure 2. Schematic of a hypothetical animal genome. For each chromosome there is a copy that is inherited from one's mother and a copy that is inherited from one's father. Note: In real animals, there are usually many more chromosomes, each chromosome contains many more genes, and a much larger portion of each chromosome is non-gene DNA than suggested here.

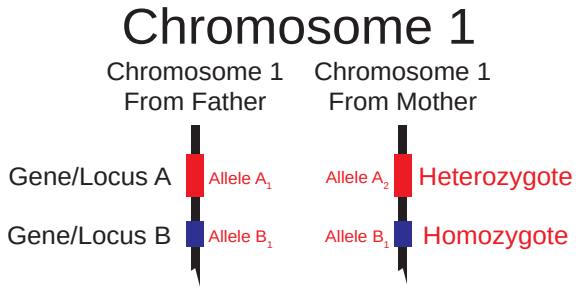

Figure 3. Zoomed view of two copies of chromosome 1 (from top left of Figure 2). Different allele designations at locus A (A_1 and A_2) show that this individual has inherited different versions (alleles) of the gene from its mother and its father at this locus. It is, therefore, called a heterozygote for gene A. At locus B, however, this individual has inherited the same version (allele) of the gene from both its mother and father (B_1 and B_1). It is homozygous for gene B.

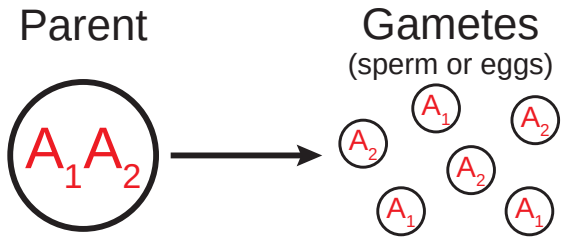

Figure 4. Passing of alleles into gametes. For each gene/locus, a parent passes one of its two alleles into each gamete. Note that every gamete will receive one allele from each locus, but here we are focusing only on locus A.

Question 1

Given that the passing of alleles into gametes is random, if we observe one gamete (egg or sperm) of an individual at a specific gene/locus:

a. What is the probability that the allele in that gamete is the one from the father of the individual making the gametes?

b. What is the probability that the allele in that gamete is the one from the mother of the individual making the gametes?

Wait

When two animals—such as Florida panthers—reproduce to make an offspring, one gamete from the mother and one gamete from the father fuse, so that the offspring has two alleles for each gene/locus. It turns out that if we make a few assumptions about a population (e.g., that mate pairings in the parent generation are random, there is no mutation, and a few others), we can make an abstraction of the population that allows us not to worry about the specific pairings of alleles within specific individuals, and instead only pay attention to frequency (or proportion) of alleles in each population (Figure 5).

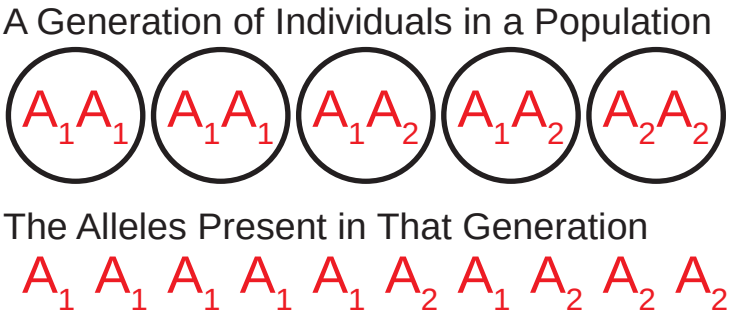

Figure 5. Abstraction of a population represented as only alleles, rather than individuals. The top row shows the allele pairings (genotypes) in individuals. The bottom row shows the alleles present in the population without respect to individuals.

Question 2

a. In Figure 5, what is the frequency of Allele A_1 in the population (i.e., what proportion of the alleles at Locus A are of the type A_1)?

b. In Figure 5, what is the frequency of Allele A_2 in the population (i.e., what proportion of the alleles at Locus A are of the type A_2)?

Wait

If we abstract the population to allele frequencies as above, we now have a lot of power to calculate the probabilities of certain genotypes in the next generation. Given our assumptions about random mating, we can say that every allele in individuals in the next generation is selected randomly from the alleles available in the parent generation. Consider the selection of allele A_1 a "success," therefore the selection of A_2 is considered a "failure." Each individual offspring samples twice (once for the allele inherited from each of its two parents) from the alleles that are available in the previous generation. So if A_1 and A_2 are present with equal frequencies in the parent generation, then the probability of "success" is equal to the probability of "failure," which is 0.5. The probability of having a homozygote for allele A_1 would be the probability of getting two "successes." The probability of having a homozygote for allele A_2 would be the probability of getting two "failures." The probability of having a heterozygote would involve getting one "success" and one "failure." Note that there are two ways of getting the heterozygote (A_1A_2 or A_2A_1).

Question 3

Given that the population depicted in Figure 5 reproduces to make a generation of offspring and using the allele frequencies you calculated for that population:

a. What distribution can be used to calculate these probabilities?
b. What is the probability of an individual in the offspring generation being homozygous for A_1?
c. What is the probability of an individual in the offspring generation being homozygous for A_2?
d. What is the probability of an individual in the offspring generation being heterozygous?

Wait

Back to the Florida Panther

Now let's return to our population of Florida panthers. Our goal here, as with most species conservation efforts, is to keep the population from going extinct. From many biological studies, we know that the extinction of a population becomes more likely as a population becomes smaller. This is true for several reasons. One is the obvious fact that, in a population with few individuals, an environmental challenge to the population (e.g., a natural disaster, disease, etc.) is more likely to kill all of the individuals. But this is not the only reason that having a small population is bad for avoiding extinction.

There are two other significant reasons that small populations are bad for avoiding extinction.

1. Small populations are more likely to lose alleles due to genetic drift.
2. Small populations are forced into inbreeding.

Genetic Variation and Genetic Drift

Small populations are more likely to lose alleles due to *genetic drift*. Genetic drift is change in allele frequencies due to chance. Species/populations that reproduce by having sex appear to benefit from having a great variety of alleles in the population (in fact, there's a good chance that sex evolved in order to increase genetic variation in the species/population). Genetic drift exhibits its effect primarily on alleles that are neutral (that is, they are not beneficial or detrimental to the population). The fact that allele frequencies can change due to randomness from one generation to another should be clear from the exercises you did above, where allele frequencies in one generation influence, but do not absolutely determine, allele frequencies in the following generation.

To illustrate genetic drift, let's focus on a specific gene/locus with neutral alleles. For any given allele at that locus, we can determine the probability that it will eventually disappear from the population. Because these alleles are inherited directly from an individual's parents, once an allele disappears from the population, it is gone forever (except in the incredibly unlikely event that it is re-created by another mutation, the probability of which is negligible and can be statistically ignored). We can also determine the probability that a given allele will eventually become the only allele for that gene/locus in the population. If it becomes the only allele, we say that it is *fixed*, or that it has reached *fixation*. It is important to realize that an allele reaching fixation in a population means that all other alleles at that gene/locus have been lost forever from the population!

For any given point in time, the probability that an allele eventually becomes fixed in the population is exactly equal to its proportion in the population. And the probability that it will disappear from the population is exactly one minus its proportion in the population.

Question 4

Given the population we used in the example above, answer the following:

a. What is the probability that Allele A_1 eventually becomes fixed in the population?
b. What is the probability that Allele A_1 eventually disappears from the population?

c. What is the probability that Allele A_2 eventually becomes fixed in the population?

d. What is the probability that Allele A_2 eventually disappears from the population?

Wait

Note that these above probabilities are true regardless of population size. But it turns out that population size has a drastic effect on *how quickly* an allele disappears or goes to fixation—the smaller the population size, the more drastically genetic drift affects the population by eliminating allelic variation from the population. As we discussed briefly above, and will discuss in more detail below, variation is generally good for a population, and so the loss of variation is bad for the population. This is important for our panther population, because the small population of panthers means that alleles across the entire genome will disappear quickly.

How drastically does the size of a population affect how quickly alleles are lost or fixed? There are two types of calculations that we can do to understand the effect of small population size.

First, we'll consider how the population size affects the probability distribution of alleles in the next generation. Suppose an allele has frequency p_1. We would like to see how much the allele frequency can change in the next generation. Let p_2 be the allele frequency in the second generation. This probability, p_2, is a random variable, so it has an expected value and standard deviation. One can show that the mean μ and standard deviation σ of this random variable are:

$$\mu = p_1 \text{ and } \sigma = \sqrt{\frac{p_1(1-p_1)}{2N}}\ .$$

Question 5

a. Suppose that the data are bell shaped. How much data lies within two standard deviations of the mean? Explain your answer.

b. Suppose that the allele frequency $p_1 = 0.5$ and the population size $N = 25$ (an accurate estimate of the population size of panthers in the early-mid-1990s). Now consider your percentage answer (from 5a above), y%. For that y% of cases, the allele frequency of p_2 is in what range?

c. Repeat the calculation in 5b above for population sizes 33, 100, and 1000. What does an increased population size do to the range?

d. In words, summarize how population size affects the probability distribution of the trait in the next generation.

Wait

Now let's consider how the population size affects the average rate at which alleles are lost from the population. Using the proportion of an allele in the population, p, we can also determine the number of generations it takes for an allele to become fixed or lost given the population size.

The average time to fixation is:

$$\overline{T}_{fixed} = \frac{-4N(1-p)\ \ln(1-p)}{p}\ .$$

And the average time to loss is:

$$\overline{T}_{lost} = \frac{-4Np\ \ln(p)}{1-p}\ .$$

Question 6

a. Calculate the average time, in generations, to fixation of an allele that starts at proportion 0.4 in populations of sizes 25, 33, 100, and 1000.

b. Calculate the average time, in generations, to loss of an allele that starts at proportion 0.4 in populations of sizes 25, 33, 100, and 1000.

c. Calculate the average time, in generations, to loss of an allele that starts at proportion 0.1 in populations of sizes 25, 33, 100, and 1000.

Wait

So far we've focused on alleles that are neutral with respect to natural selection. That means that none of the alleles we've considered is either beneficial or detrimental to the population in comparison to the other alleles for that gene/locus. *But just because an allele is not beneficial to the organism now, doesn't mean it won't be beneficial in the future of an ever-changing dynamic environment.* This is one of the reasons that maintaining many alleles in the population is generally a good thing, and the high rate of allele loss in a small population is a bad thing.

There is one other significant reason that small populations run a greater risk when it comes to extinction. Understanding this reason requires that we now consider alleles that are currently beneficial or detrimental to the population, and this deals with the fact that *small populations are forced into inbreeding*.

Inbreeding

You probably already have a sense that inbreeding is bad. There is a social stigma against inbreeding in our human species that is shared by essentially every human society in the world. But it turns out there is a clear biological basis for this stigma. Inbreeding is usually detrimental to the health of offspring. To understand why that's true, we need to return to our understanding of genes and alleles. Remember that *genes* make *proteins*. It is these proteins that make the *traits* of an organism. *So if a trait is determined by a single gene, there are two alleles that could potentially contribute to that trait.*

How do these alleles interact to make the trait of an organism?

The way a trait is determined by the alleles for a gene depends on how the alleles, and the proteins they make, interact with each other. Obviously, if an individual is *homozygous* for a gene/locus (i.e., having two of the same allele), that allele determines the trait. If an individual is *heterozygous* for a gene/locus (i.e., having two different allele types), there are two main possibilities: (1) The alleles can both contribute to the trait, or (2) the alleles can interact in such a way that only one allele determines the trait. This latter case is quite common, and is the case on which we will focus here. In this case, we call the one allele that determines the trait the *dominant* allele. The allele that does not contribute to the trait is called *recessive*.

This pattern of dominance and recessiveness is the basis of a major problem of inbreeding. Mutations in genes cause new alleles to come about, and these mutated alleles are often very bad for the individual organism. We call these bad alleles *deleterious*. If this new deleterious allele is dominant, it is very likely to negatively affect the individual's reproduction, so that an individual is less likely to survive and reproduce, and so the allele will not be passed on to future generations and will quickly disappear from the population. If the new detrimental allele is recessive, it will only affect the trait if there are two copies of the deleterious recessive allele. When an allele affects a trait we say that allele is *expressed*. Therefore, a deleterious recessive allele is only expressed when it is present as a homozygote. The effects of this deleterious recessive allele are hidden when it is present in a heterozygote. These deleterious recessive alleles persist in populations and are rarely expressed unless they become very common in the population, because one of the deleterious recessive alleles must be inherited from each parent in order for it to affect the trait. So what we find in nature is that *many rare deleterious recessive alleles exist at many genes/loci in any population.*

Two close relatives are more likely to have the same genes than are two randomly chosen individuals in the population. So when two close relatives mate, the offspring tend to have more homozygous genes/loci than the offspring of

randomly chosen mates—*inbreeding causes an increase in homozygosity.* This increase in homozygosity also means that *inbreeding causes an increase in traits determined by deleterious recessive alleles.*

Because the size of the Florida panther population is so small, all of the individuals are closely related and so are forced into inbreeding.

Question 7

Let's calculate the effect of inbreeding on the expression of rare deleterious alleles.

a. Consider a rare deleterious recessive allele for a specific gene/locus. In this hypothetical population, the deleterious recessive allele exists at a proportion of 0.01. In an offspring with randomly chosen parents, what is the probability that the offspring will be homozygous for the deleterious recessive allele?

b. Now let's consider that the Florida panther population has 20,000 genes/loci (this is a reasonable estimate, and is about the number of genes that humans have). And let's assume that for every gene/locus there is a deleterious recessive allele that exists at a proportion of 0.01 in the population. If all mating is random, in the average offspring, how many of its genes/loci are homozygous for deleterious recessive alleles?

c. Now consider an offspring with full-sibling (brother and sister) parents. In this offspring, what is the probability that the offspring will be homozygous for the deleterious recessive allele? Note that full siblings share, on average, 50% of their genes. So, in order to calculate this, consider that one allele in an offspring is randomly inherited from the population. Then, given that the randomly inherited allele is the deleterious recessive allele, we can say that there is the normal chance of inheriting the deleterious recessive allele due to randomness, plus an increased chance that the other parent has the allele due to the fact that it is a full sibling of the other parent. This added increased chance must take into account that there is a 50% chance that the other parent has one copy of that allele due to relatedness, and the fact that that parent has two alleles, so there is a 50% chance they pass on that deleterious recessive allele if they have it.

d. Now consider the same mating of full siblings in a Florida panther population with 20,000 genes/loci where each gene/locus has a deleterious recessive allele that exists at a proportion of 0.01 in the population. On average, how many of the offspring's 20,000 genes/loci are homozygous for deleterious recessive alleles?

e. Compare the results of 7b and 7d above and explain what this means about the effects of inbreeding.

Wait

Clearly inbreeding has a drastic effect on how many deleterious recessive alleles are affecting traits! This highlights yet another major problem of small population size, and an incredibly serious problem in conservation biology. Because inbreeding increases the likelihood of homozygosity, this last problem of inbreeding can be approximated by measuring the amount of homozygosity. If inbreeding increases, the average proportion of genes/loci that are homozygous in a species should also increase. Inbreeding can similarly be identified by a corresponding decrease in heterozygosity.

Question 8

Summarize the three major problems with population size.

Wait

We now have a good understanding of the problems that small populations pose for the conservation and preservation of species. This all adds up to the conclusion that, as the remaining numbers of a threatened species decrease, the probability of saving the population does not scale linearly with population size. Rather, as the population depletes, the per-capita effort needed to save a population/species must increase. The fact that it becomes increasingly difficult, per-capita, to save a species as its population's size decreases, is termed the *extinction vortex* (Gilpin and Soulé 1986). This "vortex" tends to exhibit an increasing strength pulling the population towards extinction as the population size decreases.

If we look at data for the Florida panther population, we find lots of evidence of the existence of an extinction vortex. The increased presence of homozygous deleterious recessive alleles and the overall loss of alleles have serious effects on many characteristics of the panther population. One important trait is whether or not they have descended testes. During mammalian sexual development, testes descend from inside the body into the scrotum, and if testes do not descend, an animal is generally sterile. The failure of one or both testes to descend is known as *cryptorchidism*. Obviously a high proportion of sterile individuals will have an effect on the possibility of increasing population size. Another important trait is *atrial septal defects*. These are defects in chambers of the heart that complicate blood flow through the heart, making it more difficult to survive. An increase in heart defects will also obviously have an effect on the possibility of increasing population size. In this population of Florida panthers, these traits were measured over generations, as summarized in Table 1.

Table 1. Occurrence of deleterious traits in Florida panther population 1970–1995, with standard errors removed for simplicity (Johnson *et al.* 2010).

Heritage Group	*Average Heterozygosity*	*Proportion of Males Cryptorchid*	*Proportion with Atrial Septal Defects*
1970–1984	0.231	0.33	0.33
1985–1989	0.208	0.50	0.16
1990–1995	0.190	0.63	0.21

Question 9

Summarize the observed trends in the traits in Table 1.

Question 10

It turns out that the Florida panther is closely related to a population of panthers (cougars) that still exist in Texas. In fact, the Florida population and the Texas population used to be part of one continuous population of panthers. So it is highly likely that they could and would interbreed. Based on the information available in the mid-1990s, some conservation biologists believed that the only way to save the population of Florida panthers would be to introduce several Texas cougars into Florida to revitalize the population of Florida panthers. Do you think this is a good idea? Also consider factors beyond probability that may influence society's decision on this matter.

Wait

It turns out that conservation managers decided to introduce eight female Texas cougars into the population of Florida panthers in 1995 (hence the reasoning for our doing many of the previous calculations for population size 25 and 33). Aside from simply increasing population size, this increase in genetic variation had notable effects on important traits in the Florida panther, as summarized in Table 2.

Table 2. Occurrence of deleterious traits in Florida panther population 1970–2007, with standard errors removed for simplicity (Johnson *et al.* 2010).

Heritage Group	*Total Number of Individuals Observed Over Time Period*	*Average Heterozygosity*	*Proportion of Male Cryptorchid*	*Proportion with Atrial Septal Defects*
Prior to Texas Cougar Introduction				
1970–1984	33	0.231	0.33	0.33
1985–1989	37	0.208	0.50	0.16
1990–1995	62	0.190	0.63	0.21
After Texas Cougar Introduction				
1996–1998	67	0.220	0.54	0.06
1999–2001	102	0.224	0.42	0.07
2002–2004	139	0.226	0.23	0.06
2005–2007	116	0.240	0.12	0.09

Question 11

a. Summarize the change in the traits in Table 2 after the introduction of the Texas cougars.

b. After seeing these data, do you think the introduction of the Texas cougars was a good idea?

Wait

Some Extra Panther Probability Problems

Question 12

Use the data in Table 2 to answer the following questions. While the "Total Number of Individuals Observed Over Time Period" is not necessarily the same as the population size (because it considers individuals present in the population over a time range rather than a specific point in time), it is highly correlated with the population size, and for our purposes we can use these numbers as the population sizes where needed.

a. Suppose in the early 1990s a doctor volunteers to help one panther with its atrial septal heart condition. She will help the first panther she finds that is in need of help. What is the probability that the first, second, or third panther she finds has this condition?

b. Suppose the son of the early 1990s doctor now volunteers his time to help one panther with this condition during the time period 2005–2007. He will help the first panther he finds that is in need of help. What is the probability that the first, second, third, fourth, or fifth panther he finds has this condition?

c. Interpret the difference in the results of the previous two problems.

d. In your calculations for the doctor and her son, did population size matter? If so, explain how you used the population size. If not, explain why it didn't matter, but mention the significance in terms of how the introduction of Texas cougars helped.

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