Michael Schwartz
M3A1: Category II Lab Assignment: Genetic Drift and Bottlenecked Ferrets
SimUText and SimBio Labs

Section 1: Luck of the Draw

The black footed ferret once had large colonies in the American prairie. The primary prey for the ferret were prairie dogs. Black footed ferrets were almost driven to extinction due to habitat loss, hunting and extirpation of prey. Remaining 18 individuals of the last breeding colony in Wyoming were moved into captivity in 1986, and nine individuals are the founders of present day breeding colonies in three states, South Dakota, Arizona and Wyoming.

Qu. 1.5 Answer: I predict that the allele frequencies of the black-footed ferret (*Mustela nigripes*) breeding pen population will not move toward the allele frequency in the larger field population since the breeding pen populations are now independent of the field population.

Explain: Adults in the breeding pen population will pass on a finite amount of gametes, meaning that allele frequencies in their gene pool will likely deviate from allele frequencies in the field population due to sampling error (Andrews, 2010). Allele frequencies within the captive ferret population will change specifically for this population as a result of chance events (i.e. genetic drift) (Andrews, 2010).

(Tips: Use sentences like,
"The simulation examines a fictitious coat color gene in the ferret population."
"The gene has two alleles, ... and ....."
Remember, each individual ferret will have two copies of the gene, one from each parent.)

List all combinations of those alleles in the population and the coat colors associated with the combinations.

- *SS* - Standard (light coats)
- *SC* - Standard (light coats)
- *CC* - Charred (dark coats)

Summarize the set-up of your initial experiments (method). Of a field population of 500 black-footed ferrets, 40 ferret individuals were randomly collected and randomly distributed as eight individuals into five separate breeding pens. Individual sex (male or female), allele combinations (SS, SC, CC), and S allele frequencies (SS - Standard/light coats and SC - Standard/light coats) were recorded per breeding pen. All five breeding pen S frequencies were then measured against the S frequencies of the remaining 460 ferrets in the field population. Finally, and after a period of 100 days, S frequencies from both populations were once more recorded and measured against one another for analysis.

Results:

Field Ferret Population	S frequency
460	0.49

Breeding Ferret Population	40

Breeding Ferret Population Data Upon Capture and Pen Distribution									
Pens	**Sex/ Alleles**	**Sex/ Alleles**	**Sex/ Alleles**	**Sex/ Alleles**	**Sex/ Alleles**	**Sex/ Alleles**	**Sex/ Alleles**	**Sex/ Alleles**	**S freq**
Pen 1	F/SC	F/SC	F/CC	M/SC	F/CC	F/SS	M/SS	M/SS	0.56
Pen 2	M/CC	F/CC	M/CC	M/SS	M/SC	F/SS	F/CC	F/SC	0.38
Pen 3	F/SS	M/CC	M/CC	M/CC	F/CC	M/SC	F/SS	F/CC	0.31
Pen 4	M/SS	M/CC	M/SC	M/SC	F/SS	M/SC	F/SC	M/SS	0.63
Pen 5	M/SC	M/CC	M/SC	M/CC	F/SC	M/SS	M/SC	F/CC	0.38

Population	Initial Frequency of *S*	Freq. of *S* after 100 time steps
Field	0.49	0.52
Breeding Pen 1	0.56	0.0
Breeding Pen 2	0.38	1.0
Breeding Pen 3	0.31	0.0
Breeding Pen 4	0.63	1.0
Breeding Pen 5	0.38	0.0

Discussion: Explain the reason for the result and how the breeding pens differed from the field population: Breeding pen S frequencies were more dramatic than S frequencies in the field population, supporting the prediction that allele frequencies of the captive population would not move toward the allele frequency in the field population since the captive population was independent of the field population. Effects of genetic drift are more prevalent in smaller populations as evidenced in the results. In small populations, genetic drift, caused by phenomena such as the founder effect, can lead to fixation of deleterious mutations, thus

bringing about fitness reduction (Labar & Adami, 2017). Here, genetic drift occurred due to sampling error and nonrandom mating.

Section 2: Random Mating

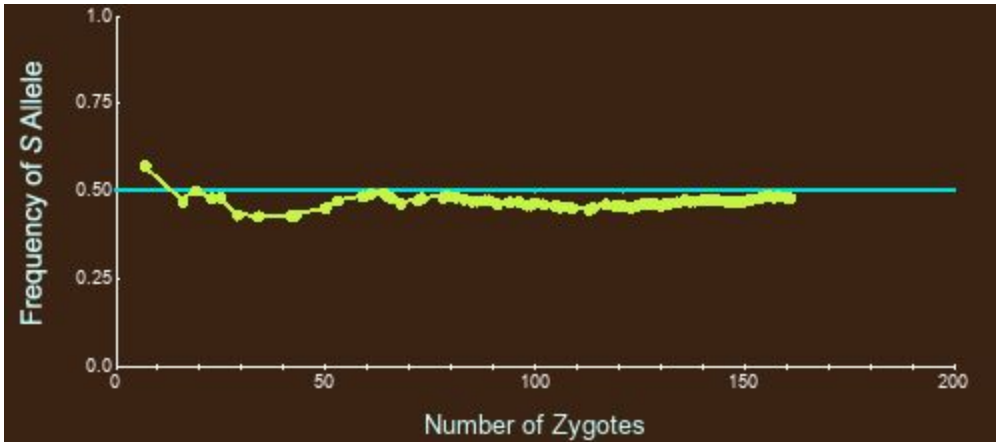
Define sampling error and discuss the problem with sampling error for your experiments.
A sampling error in a sample population—whether natural (e.g. landslide, earthquake) or human caused—is considered an error because it is unrepresentative of the larger population, leading to random changes in gene frequencies (Tave & Food and Agriculture Organization of the United Nations, 1999). As in the prior experiment, genetic drift can occur due to sampling error since the captive population of black-footed ferrets was not representative of the field population.

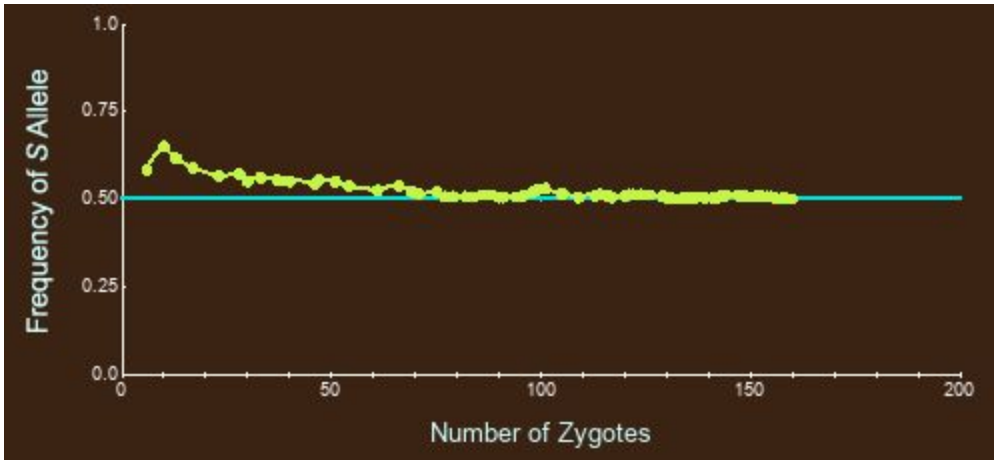
Complete experiments.

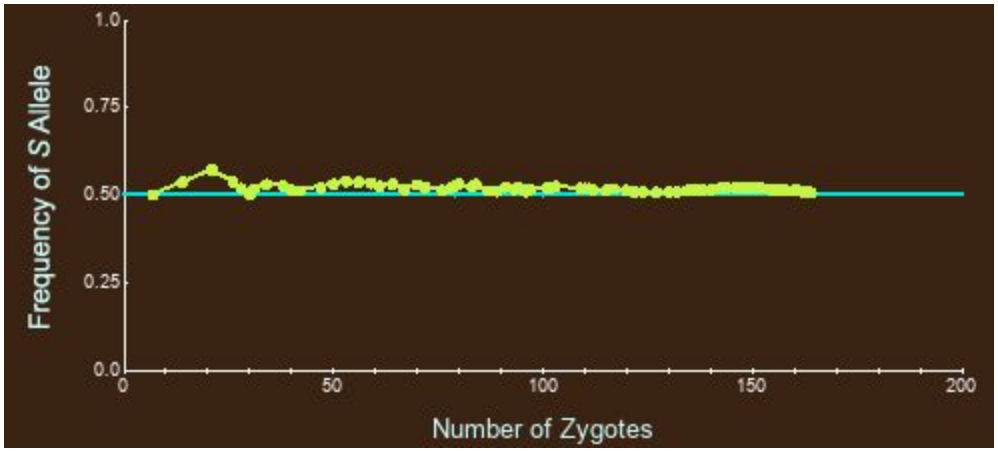
Summarize the set-up of your initial experiments (method). The simulation examined random mating in a randomly collected sample black-footed ferret population to measure the frequency of allele S among the zygotes that were produced. This random collection was then compared with the frequency of allele S in a gene pool with an S frequency measurement of 0.5. This was run at 150 time steps on five separate occasions.

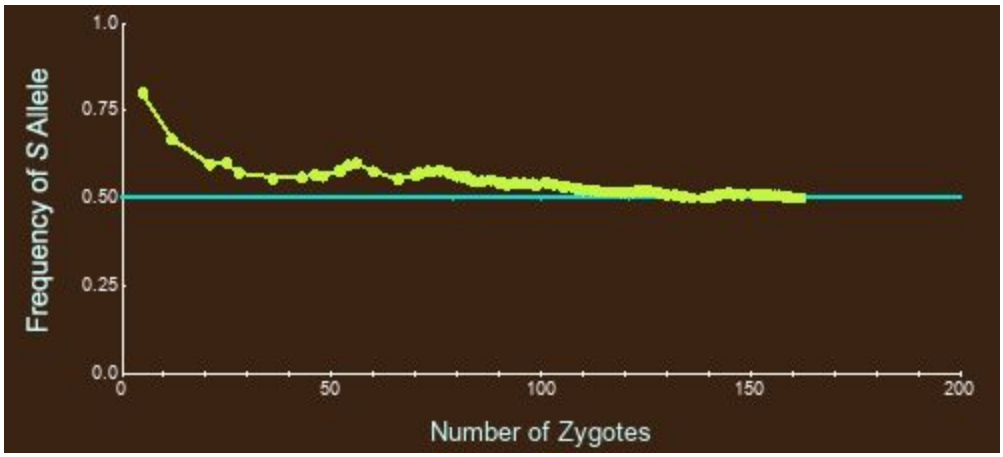
Results:

Experiment 1 Tables 1-5




Frequency of S Allele
1.0
0.75
0.50
0.25
0.0
0
50
100
150
200
Number of Zygotes


Frequency of S Allele
1.0
0.75
0.50
0.25
0.0
0
50
100
150
200
Number of Zygotes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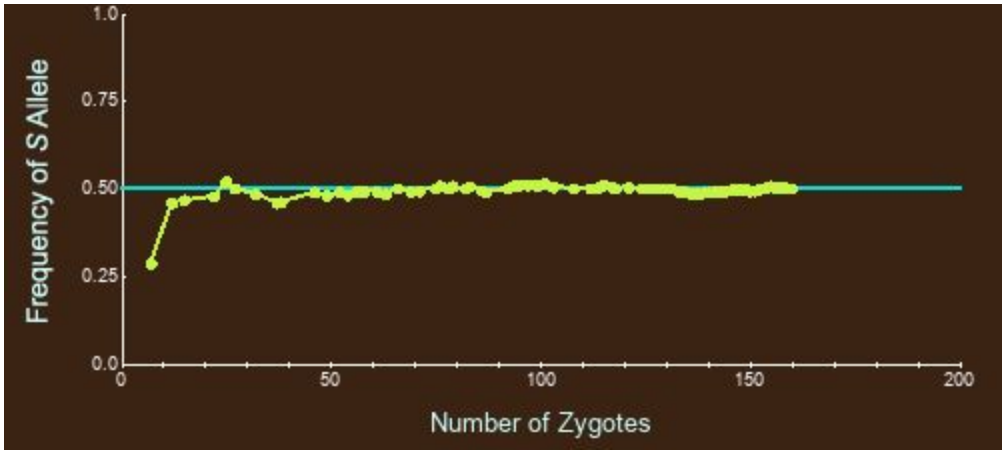
Number of Zygotes

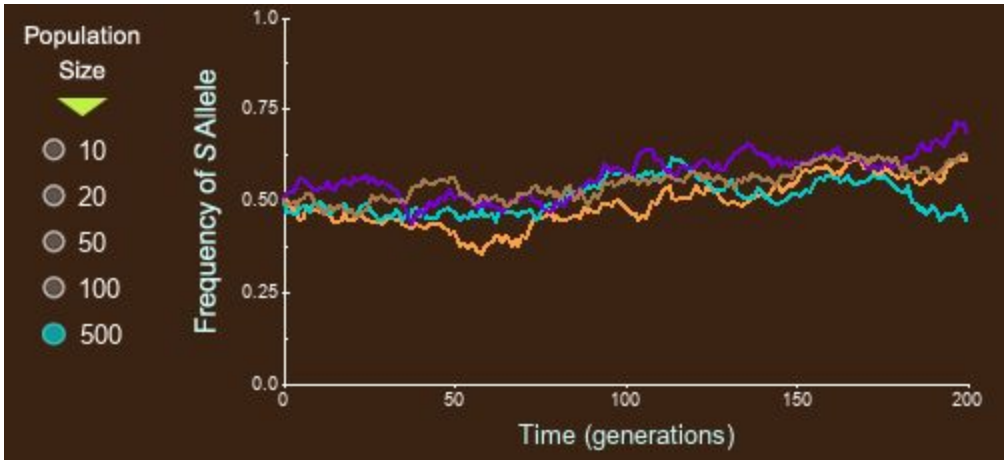


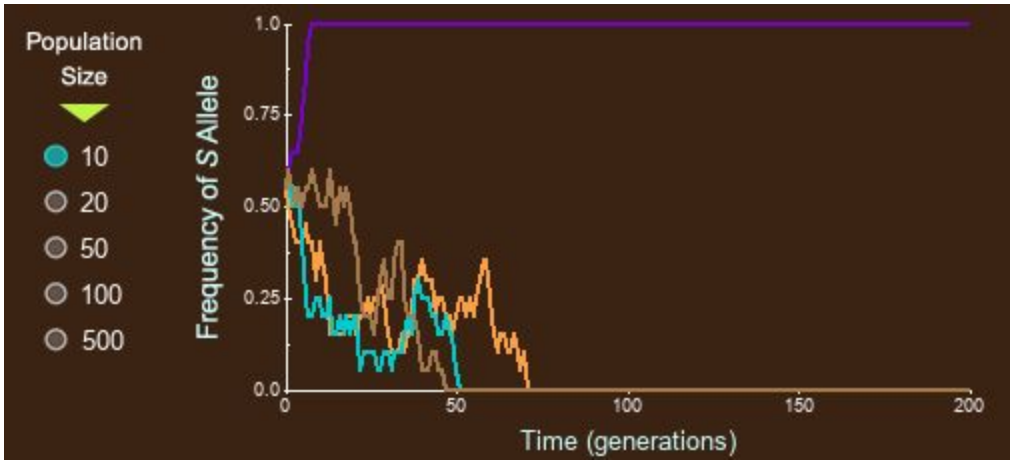
Explain the reason for the result: In the initial experiment, the frequency of the S allele among the zygotes was different from the frequency of the S allele in the gene pool when few zygotes were formed. While small samples were not adequately representative of a larger population (sampling error), over a period of time the S allele frequency moved closer to 0.50 since there was a larger zygote sample that was more representative of the larger population. Ultimately, evolution will occur due to natural selection vis-à-vis individual fitness and genetic drift from random chance resulting from sampling error (Khan Academy, n.d.).

Section 3: Size Matters

Complete experiments. Summarize the set-up of your initial experiments (method). The initial experiment studied the relationship between population size and evolution rate by genetic drift. I predicted that changes in allele frequency would happen faster in small populations due to greater sampling error occurring in small populations rather than large populations. Four fields were stocked with 500 black-footed ferrets each with an initial *S* allele frequency of 0.5. The simulation of random mating was initially run for 200 generations. Population size was then reset to 10, 20, 50, and 100 and run again for 200 generations respectively (see tables below).

Results:


Population Size
10
20
50
100
500
Frequency of S Allele
1.0
0.75
0.50
0.25
0.0
0
50
100
150
200
Time (generations)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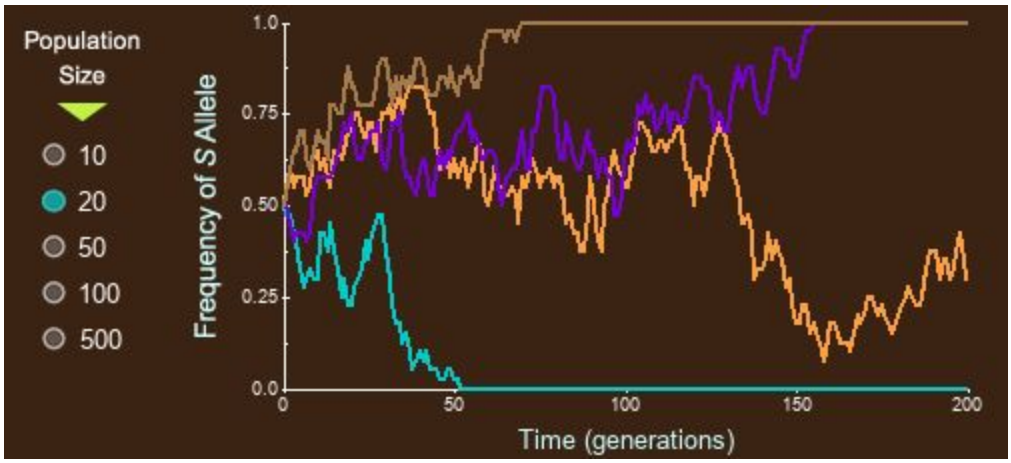
Time (generations)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Frequency of S Allele
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0.75
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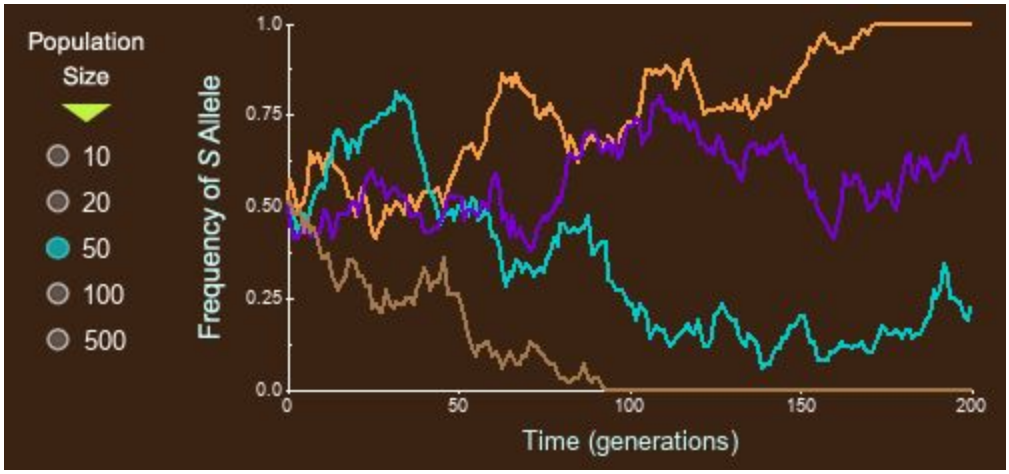
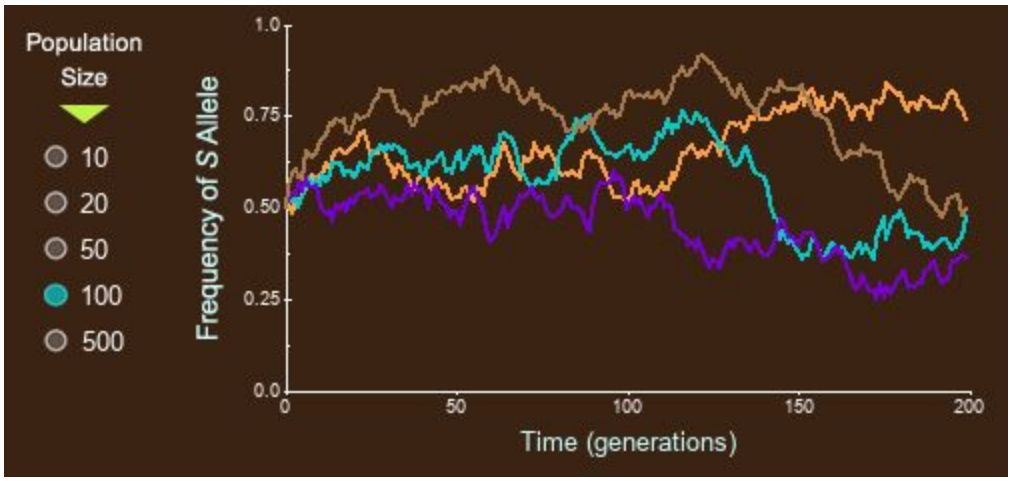
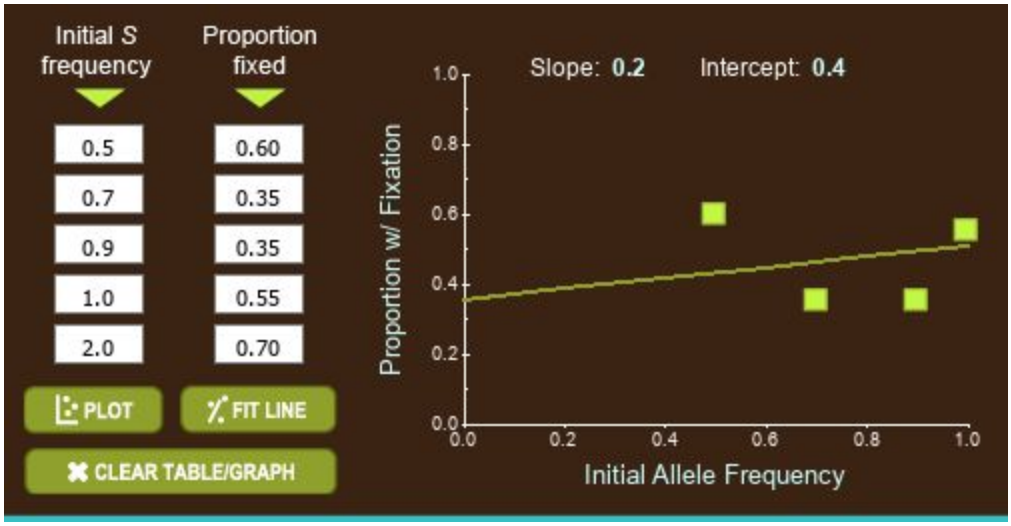
200
Time (generations)





Explain the reason for the result: Allele frequencies change at a slower rate through genetic drift for larger populations, whereas allele frequencies change at a faster rate through genetic drift for small populations due to greater sampling error. As such, smaller populations will show greater allele frequency differences temporally.

Will alleles be lost or fixed more likely in large or small populations? Does this matter for population survival? Considering random chance, alleles are more likely to be lost in small populations (see table below). Large population sizes with even distributions in allele frequencies will usually decrease the probability of allele fixation (American Phytopathological Society, n.d.). This vitally matters for population survival, since smaller species populations are more closely associated with loss in genetic variety. In short, genetic drift could cause variants to disappear, thus reducing genetic variation (Star & Spencer, 2013).



Section 4: Inbreeding and Genetic Drift

Based on your experiments, which factors contributed to large genetic drift of ferret populations? Based on the experiments, the factors that contributed to a large genetic drift in black-footed ferret populations include small population size, less random/nonrandom mating (e.g. inbreeding depression), and a skewed sex ratio. These are strongly associated with a loss in loss heterozygosity in a population over time.

Section 5: Save the Ferrets

How can we maintain genetic diversity in small populations of ferrets?

- Summarize the problem and research needed in order to answer this question.

Considering that all black-footed ferrets (*Mustela nigripes*) are descended from only seven individual ferrets (Wisely, 2002), there has been tremendous loss in genetic diversity due to genetic drift. As the manager of a black-footed ferret reintroduction program, a large responsibility is to set up a breeding program that will ensure genetic diversity prior to reintroduction. This challenge notwithstanding, there is also the added problem of the inability to perform real-time experiments to acquire more information about ferret ethology, genetics, and life history of *M. nigripes* due to it being listed as an endangered species. Given this limitation, simulation modelling of different reserves may prove useful in establishing and running experiments that help predict how a population might behave under different simulated conditions with the ultimate goal of determining how the ferret numbers and connectivity can promote gene flow and concomitant genetic diversity. Moreover, another goal maintaining allele *S* (light coat) and allele *C* (charred coat), while promoting heterozygous ferrets within the wild population, though it should be stressed that these two priorities may conflict with one another. Given this, will genetic diversity be better maintained with one population in a single reserve or a reserve comprised of several populations? (See pros and cons in the graphic provided below.)

- Write down a testable hypothesis and explain the experimental set-up (Method)

I hypothesize that several small, connected reserves (Reserve C) will ensure higher than average heterozygosity for a black-footed ferret population while keeping both alleles. To test this, I utilized a simulated system of three reserve designs (as seen above) with random variability to run three separate tests in order to determine which design would best reinforce genetic diversity within a simulated population of ferrets from a captive breeding colony while maintaining both alleles. The design of the reserves were A) a single large reserve; B) four disconnected small reserves; and C) four connected small reserves, each measuring a total of 100km2. For accuracy, breeding was simulated 20 different times up until 200 generations for each simulated reserve design, while measuring A) runs with heterozygosity; B) average heterozygosity; and C) runs with no alleles lost. Data sets were recorded in the table below (see Results - Reserve Design Data).

Simulation modelling

- Show your results, include raw data, and tables or graphs. Discuss results and limitations of the experiments.

Reserve Design Data			
	Reserve A	Reserve B	Reserve C
Generations	200	200	200
Number of Runs	20	20	20
Runs with $H > 0$	12	9	20
Average H	0.22	0.06	0.30
Runs with No Alleles Lost	12	19	20

The data listed above supports the hypothesis that several small, connected reserves (Reserve C) will ensure higher than average heterozygosity for a black-footed ferret population while keeping both alleles. However, it should be stressed that such simulations are highly idealized, and that environmental stochasticity could limit and/or alter the accuracy of the data as it relates to the proposed hypothesis.

- Make a recommendation for conservation of ferrets.

Taking into account the fact that genetic variety within extant populations of *M. nigripes* is low, one possible *in situ* method of effectively reestablishing genetic diversity is through interspecies somatic cell transfer (iSCNT) via reproductive cloning (Wisely et al., 2015). This would involve transferring the cell of one species to the cytoplasm of another species' enucleated oocyte, also known as an egg cell (Wisely et al., 2015). Wisely et al. (2015) provide a conceptual model, whereby the nucleus is removed from a domestic ferret oocyte, obtaining somatic cells of black-footed ferrets through cryopreservation, and inserting and infusing the black-footed ferret somatic cell with the domestic ferret oocyte, thus allowing embryonic development to occur. This might well provide the genetic diversity so necessary to ensure black-footed ferret conservation.

- Reference

Wisely, S. M., Ryder, O. A., Santymire, R. M., Engelhardt, J. F., & Novak, B. J. (2015). 21st century genetic restoration: Gene pool enrichment of the black-footed ferret. *Journal of Heredity*, 106(5), 581-592. doi:10.1093/jhered/esv041

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Andrews, C. A. (2010). Natural selection, genetic drift, and gene flow do not act in isolation in natural populations. *Nature Education Knowledge*, 3(10), 5.

LaBar, T., & Adami, C. (2017). Evolution of drift robustness in small populations. *Nature Communications*, 8(1). doi:10.1038/s41467-017-01003-7

Khan Academy. (n.d.). Genetic drift. Retrieved from https://www.khanacademy.org/science/biology/her/heredity-and-genetics/a/genetic-drift-founder-bottleneck

Star, B., & Spencer, H. G. (2013). Effects of genetic drift and gene flow on the selective maintenance of genetic variation. *Genetics*, 194(1), 235-244. doi:10.1534/genetics.113.149781
Tave, D., & Food and Agriculture Organization of the United Nations. (1999). *Inbreeding and brood stock management*. Rome, Italy: Food & Agriculture Org.

Wisely, S. M. (2002). Genetic diversity and fitness in black-footed ferrets before and during a bottleneck. *Journal of Heredity*, 93(4), 231-237. doi:10.1093/jhered/93.4.231