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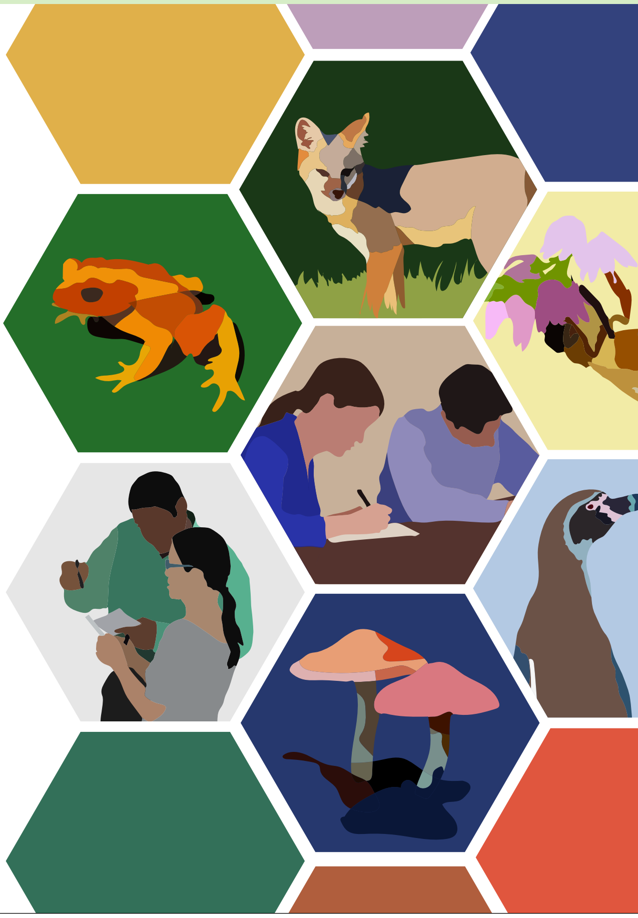

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## ABSTRACT

This exercise is intended to provide students with a real-world example of how museum specimens can provide additional context to challenges and considerations in the broader field of applied conservation genomics. After a brief introduction to the study system—the conservation status of Iberian lynx (*Lynx pardinus*)—students will be asked to review an open-access, peer-reviewed publication that features samples from varied museum, archaeological, and paleontological contexts. Through reading the guide and discussion questions, students will reflect upon study design, concepts, and challenges presented at the intersection between the fields of conservation genomics and museum-based studies. The exercise ends with students breaking down the research into the main components (e.g., research question, independent and dependent variables, hypotheses, predictions) to set a structure for critically reading other scientific studies or designing their own research question.

## LEARNING OBJECTIVES

Through this case-study based exercise, students will critically analyze a conservation genomics/ museomics/paleogenomics project, including understanding study methodologies, interpreting results, and evaluating the significance of findings.

Specifically, students will:
1. Apply conservation genomics principles: students will interpret the case study and apply their understanding of conservation museum genomics to answer comprehension and critical thinking questions.
2. Formulate and interpret scientific hypotheses: students will identify the hypotheses outlined in the paper to reconstruct the research questions and workflow from the case study.
3. Develop experimental design skills: students will identify relevant research questions, define variables and key parameters for measurement, and outline an experimental design encompassing the genetic sampling of both modern and historical specimens.

## PREREQUISITES

Please read this module's synthesis, "Applications of museum collections and genomics to biodiversity conservation" (https://doi. org/10.5531/cbc.linc.14.1.7), prior to completing this exercise.

This exercise assumes that students are familiar with the fundamental principles of conservation genetics and population genetics analyses. For example, students should be comfortable with major principles used in conservation genetics, such as genetic diversity, genetic drift, gene flow, evolutionary systematics, geographic isolation, and inbreeding.

If you would like to review background material on these concepts, we suggest you read the NCEP module "Conservation genetics" (https://doi.org/10.5531/cbc.ncep.0123).

This module also has a companion exercise: "Designing a conservation genetics project incorporating DNA from museum specimens" (https://doi. org/10.5531/cbc.linc.14.1.9), which builds off Part 2 of this exercise and applies the skills to a new hypothetical case study.

## INTRODUCTION: THE CONSERVATION STATUS OF THE IBERIAN LYNX (*LYNX PARDINUS*)

When the number of mature Iberian lynx (*Lynx pardinus*; Figure 1) reached a record-low 62 mature individuals, the species was added to the IUCN Red List (https://www.iucnredlist.org/) as Critically Endangered in 2002 (Nowell, 2002). At this time, *Lynx pardinus* was subject to a combination of multiple threats that included losing over 80 percent of its habitat between 1960 and 1990 (Rodríguez & Delibes, 2002; Palomares et al., 2011). The modification and fragmentation of the mixed grassland and forest habitat likely contributed to the existing scarcity of one of the lynx's main prey species: the European rabbit (*Oryctolagus cuniculus*). This species was already in decline due to an outbreak of the myxomatosis virus (Delibes-Mateos et al., 2009). Additionally, habitat loss also may have led to the contraction of the original nine populations of *Lynx pardinus* in the 1990s to two isolated, remnant populations of the Iberian lynx—one located near Dõnana National Park near the southwestern coast of Spain and the other Andújar-Cardeňa population about 250 miles (~400 km) east in the Sierra Morena mountain (Palomares et al., 2011).

In 2003, after several surveys of the lynx by national and international bodies, it was concluded that the Iberian lynx was at risk of extinction. In response, the Spanish Environmental Ministry and the Andalusian Environmental Council in southern Spain signed an agreement to move forward in a captive breeding program for the species (Vargas et al., 2008).

The intended goals of the conservation program were first directed towards *in situ* conservation by

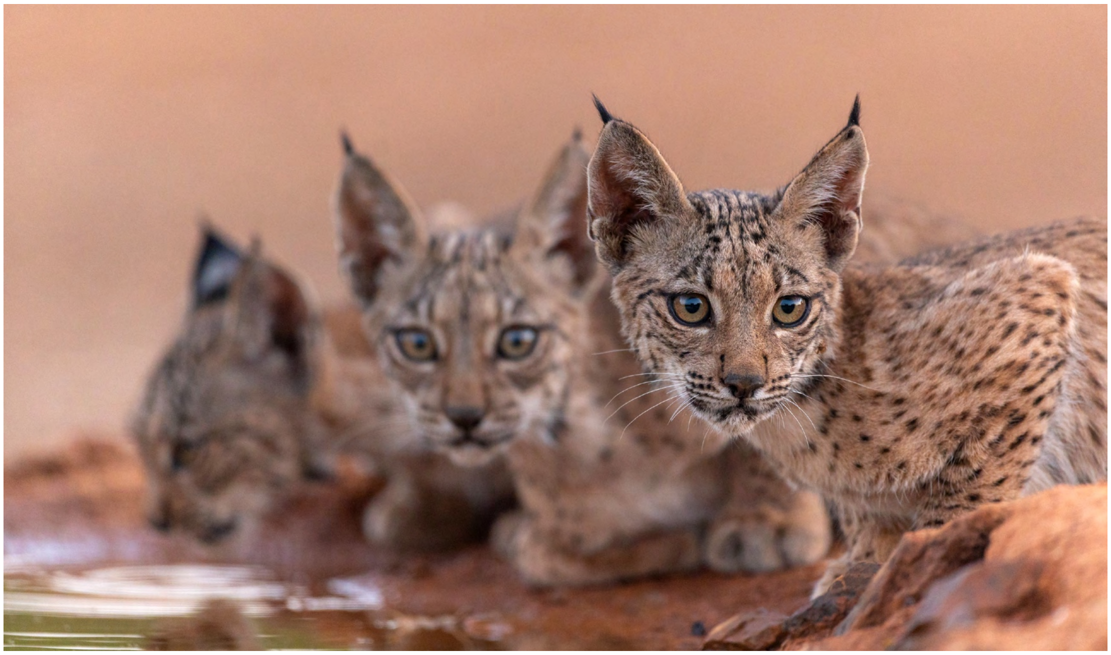

Figure 1: Kittens of *Lynx pardinus*, the Iberian lynx, photographed drinking water in a protected area in Southern Spain. Image credit: Angel Enrique Diaz Martinez.

monitoring and expanding both the Iberian lynx and its prey, the wild rabbit. To accomplish this first goal, the team set out to genotype founders of the lynx captive breeding program to determine which individuals from either Dõnana or Sierra Morena would be most suitable to be the founders of the admixed population. The second goal of the program was to look to the future of *ex situ* conservation that would look to the future by preparing habitat for the free-ranging populations as well as to consider the genetic management of both wild and captive breeding populations as a single metapopulation (Vargas et al., 2008).

In 2005, Saliega and Garfio, two captive parents—one from the Sierra population and the other from the Dõnana population—gave birth to the first three captively-bred, admixed Iberian lynx cubs at the El Acebuche captive breeding center in Dõnana National Park. Once captive-bred individuals reached maturity, it was imperative to establish a wild population of admixed individuals given how vulnerable the two remnant populations were currently (Vargas et al., 2008). Releases of captive-bred individuals began in 2009 and 2010 in two areas of the Sierra Morena mountains in the historic range of Iberian lynx. These individuals had offspring with individuals from the Andújar-Cardeňa population, demonstrating that reintroductions would aid in the goal of forming a meta-population dynamic. Five years later, in 2014 and 2015, reintroductions of hybrids started in four areas outside Andalusia (in southern Spain and Portugal) (Pérez de Ayala, 2019). Around this same time, the IUCN re-classified the Iberian lynx species from Critically Endangered to Endangered (Rodríguez & Calzada, 2014).

In 2020, there were an estimated 1,111 Iberian lynx individuals, though in order to achieve Favourable Conservation Status (FCS), a classification from the European Union that ensures protection from extinction, at least eight more subpopulations needed to be formed based on historical population data (Pérez de Ayala, 2019). Due to the significant fragmentation of the habitat of the Iberian lynx over its history and decline in the 20th century, it was necessary to rely on both historic census and paleogenomic data from museum and paleontological samples, for the Iberian lynx to be reclassified once more by the IUCN, as Vulnerable in 2024. This change in distinction finally allowed the *Lynx pardinus* to reach 'green status' according to the IUCN. For more information about the conservation history and current status of Iberian lynx, see the resources provided in Appendix 2.

## OVERVIEW: CONSERVATION MUSEOMICS AND THE IBERIAN LYNX

Museum, as well as archaeological/paleontological (or otherwise 'ancient'), samples can be incredible gateways and "timestamps" of genetic diversity at various time points in the past. In comparing DNA produced from these samples to contemporary specimens, researchers can also get a much clearer picture of fluctuations in past demography (e.g., loss of heterozygosity, increased genetic differentiation between populations, bottlenecks) in the past and how that may contribute to current population structure.

The research case study presented here used both historic (collected between 1920 and 1990) and archaeological (prior to 1920) Iberian lynx samples to understand questions about what was once a critically endangered species with fewer than 100 living individuals (Gil-Sánchez & McCain, 2011).

To complete the exercise below (in Part 1 and Part 2), read the research article by Casas-Marques et al. (2017) published by Molecular Biology and Evolution titled "Spatiotemporal dynamics of genetic variation in the Iberian lynx along its path to extinction reconstructed with ancient DNA" (https://doi.org/10.1093/molbev/msx222). Below are guiding questions as you read through.

Appendix 1 is a worksheet for your answers to Part 1 and Part 2.

## PART 1: CASE STUDY QUESTIONS

### Reading Guide Questions

We recommend you start by reading through all of the questions, actively reading the article with these questions in mind, and then answering the questions on your own.

1. Why do the authors suspect genetic drift played such a large role in the genetic history of Iberian lynx? It may be helpful to refer to Figure 6 in your answer.
2. What is the likely reason for higher ancestral genetic variation observed among the oldest, ancient samples used in this study?
3. How were the authors able to conclude that inbreeding vs outbreeding depression was likely the cause of the current genetic structure? What implications does this have for potential genetic management strategies ("to mix or not to mix")?
4. What conservation strategies are suggested as a result of the paper's findings?
5. Why were museums essential to this study?

### Group Discussion Questions

Discuss the questions below with a partner, a small group, or as a class.

6. What parts of the genome were sequenced and analyzed among the modern, historic, and ancient samples, as compared to only the modern and historic samples used in this study? Why do you think the collection of data differed between the sample groups? Consider the role of DNA degradation and how that may influence different genetic markers (mitochondrial or nuclear DNA) examined in this study.
7. What did the analysis of DNA from historic and ancient samples add to this study? Do you think the authors would have reached the same conclusion if they had only analyzed modern Iberian lynx samples?
8. Extensive metadata (e.g., dates and locations) were available for all of the samples used in this study. In your opinion, if this data were not available, should the researchers have continued with their same research plan? Keep in mind that DNA extraction is an inherently destructive process.
9. The authors mention that low genetic diversity across the species may have made Iberian lynx more resistant to the impacts of genomic erosion and potential extinction. How might this skew public perception of the need to conserve this species? What arguments provided from the paper would you share with the skeptics to protect the Iberian lynx populations mentioned?

## PART 2: RESEARCH FOUNDATION AND PROJECT DESIGN

Now that you've critically read and interpreted the details of a conservation museomics study (Casas-Marce et al., 2017), we are going to go back to basics and think about the main components of this research. Part 2 includes guiding questions to help build the skills needed to identify, develop, and answer a research question. These guiding questions are general and may be applied to your review of any research project or used as a jumping-off point for designing your own research project (see this module's companion exercise "Designing a conservation genomics project incorporating DNA from museum specimens" (https://doi. org/10.5531/cbc.linc.14.1.9).

### Guiding Questions to Interpret the Research Design of Casas-Marce et al. (2017):

1. What were the authors trying to answer in this study? Formulate a research question from the study.

2. In your view, is genetic diversity an independent or dependent variable as it's presented in the paper? Explain. Recall that an independent variable is a parameter that, when altered, affects the dependent variables but is not affected by those variables itself (e.g., time, total area, temperature, flow rate, etc.). The dependent variables are all other components influenced by the dependent variable. What are other independent and dependent variables from the text?
3. Consider the null and alternative hypotheses from the role of genetic diversity you describe in your answer to question 2. Formulate both null and alternative hypotheses with genetic diversity in mind from the publication using "If… then…" statements to connect your research question and experimental variables. For example: "If the hypothesis is correct, then I expect an increase in the independent variable will lead to an increase in a dependent variable." This positive relationship can then be tested through a formal statistical framework. Hypotheses serve as the starting point for any experiment. Therefore, any flaw in their formulation may lead to flaws in the design of the entire experiment.

   Let's define the null hypothesis ($H_0$) and the alternative hypothesis ($H_1$). In the scientific method, we can only falsify hypotheses. Usually, $H_0$ assumes there is no change or relationship between the observations, which researchers try to disprove. $H_1$ is accepted if there is sufficient evidence to reject the null.
4. Now, let's create a graphical representation of our expectations for genetic diversity over time, based on the results described in the paper, connecting the independent and dependent variables you identified in the first part of question #2 above. Contrast the expectations for each hypothesis using a pair of illustrative graphs depicting the projected outcomes for $H_0$ and $H_1$. These could be a line graph, scatter plot, boxplot, etc. Don't forget to label your axes!
5. Write a concise paragraph explaining how these contrasting results, as indicated in the graph drawn above, can be interpreted. In your explanation, be sure to mention a research question, your null and alternative hypotheses, and conclusions the researcher might make depending on the observed relationship between genetic diversity and time in your graph.

## ACKNOWLEDGMENTS

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## APPENDIX 1

### Applications of Conservation Museomics to the Protection of the Iberian Lynx (*Lynx pardinus*)

Please read this module's synthesis, "Applications of museum collections and genomics to biodiversity conservation" (https://doi. org/10.5531/cbc.linc.14.1.7) prior to completing this exercise.

This module also has a companion exercise: "Designing a conservation genetics project incorporating DNA from museum specimens," (https://doi. org/10.5531/cbc.linc.14.1.9) which may be a helpful resource.

Date: ____________ Name/Group: ______________________________________

*Part 1: Case Study Questions*

Reading Guide Questions: we recommend you start by reading through all of the questions, actively reading the article with these questions in mind, and then answering the questions on your own.

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9. The authors mention that low genetic diversity across the species may have made Iberian lynx more resistant to the impacts of genomic erosion and potential extinction. How might this skew public perception of the need to conserve this species? What arguments provided from the paper would you share with the skeptics to protect the Iberian lynx populations mentioned?

*Part 2: Research Foundation and Project Design*

1. What were the authors trying to answer in this study? Formulate a research question from the study.

2. In your view, is genetic diversity an independent or dependent variable as it's presented in the paper? Explain. Recall that an independent variable is a parameter that, when altered, affects the dependent variables but is not affected by those variables itself (e.g., time, total area, temperature, flow rate, etc.). The dependent variables are all other components influenced by the dependent variable. What are other independent and dependent variables from the text?

| **Independent variables** | **Dependent variables** |
|---|---|
| • | • |
| • | • |

3. Consider the null and alternative hypotheses from the role of genetic diversity you describe in your answer to question 2. Formulate both null and alternative hypotheses with genetic diversity in mind from the publication using “If… then…” statements to connect your research question and experimental variables. For example: “If the hypothesis is correct, then I expect an increase in the independent variable will lead to an increase in a dependent variable.” This positive relationship can then be tested through some formal statistical framework. Hypotheses serve as the starting point for any experiment. Therefore, any flaw in their formulation may cause a flaw in the design of an entire experiment.

   So, let’s define the null (H0) and alternative hypotheses (H1). In the scientific method, we can only falsify hypotheses. Usually, H0 assumes there is no change or relationship between the observations, which researchers try to disprove. H1 is accepted if there is sufficient evidence to reject the null.

H0:

H1:

4. Now let’s make a graphical representation of our expectations of genetic diversity over time, based on the results described in the paper, connecting the independent and dependent variables you identified in the first part of question #2 above. Contrast the expectations for each hypothesis using a pair of illustrative graphs depicting the projected outcomes for H0 and H1. These could be a line graph, scatter plot, boxplot, etc. Don’t forget to label your axes!

5. Write a concise paragraph explaining how these contrasting results, as indicated in the graph drawn above, can be interpreted. In your explanation, be sure to mention a research question, your null and alternative hypotheses, and conclusions the researcher might make depending on the observed relationship between genetic diversity and time in your graph.

## APPENDIX 2

### Additional Resources on Iberian Lynx:

- Lynxconnect project: https://lifelynxconnect.eu/en/

### Popular Media Articles:

- Mongabay: Dasgupta, S. (2024). Incredibly rare Iberian lynx making a "dramatic recovery." Mongabay. https://news.mongabay.com/short-article/incredibly-rare-iberian-lynx-making-a-dramatic-recovery/
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