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Digital Coyote: Examining Geographical Variation Using a Virtual Museum Collection

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Measuring geographical variation requires specimens from diverse locations. Hosted by Wikieducator, Digital Coyote is a virtual museum of calibrated coyote (*Canis latrans*) skull photographs. This open educational resource includes 96 specimens from 24 locations from Texas to Alaska, New Brunswick to Washington State. Students use digital or printed images to test the hypothesis that northeastern coyote skulls are larger than northwestern conspecifics. The image collection also includes domestic dog skulls to facilitate comparisons between artificially and naturally selected populations. A rich literature on the topic provides an entry point to species concepts, hybridization, conservation genetics, and wildlife management.

Keywords: evolution; *Canis latrans*; coyote; skulls; open educational resource; zoology

Student Outline

Objectives

1. Test the hypothesis that coyotes in the northeastern United States have larger skulls than those in the northwestern United States.
2. Develop and test a second hypothesis using the Digital Coyote skull database.

Introduction

Vertebrate species can vary dramatically in appearance, size, and behavior across their geographical ranges. Moose from northern populations in Sweden are 15-20% larger than their counterparts in southern Sweden (Sand *et al*. 1995); populations of house finches from across the United States differ markedly in color patterns (Hill, 1993); home range and a number of behavioral and morphological traits vary dramatically among orangutan populations (van Schaik *et al*. 2003).
Coyote distribution was at one time restricted to the western plains but has expanded to most corners of the lower 48 states (Levy, 2012). In New England and the northeastern United States, coyotes can grow twice as large as their western counterparts. Various hypotheses including wolf hybridization have been advanced to explain the size difference. In this exercise, we will test to see if the difference in body size is reflected in differences in skull size.

Studying geographical variation requires work at multiple field sites or access to museum collections of specimens from diverse locations. Digital Coyote (**http://wikieducator.org/Digital_Coyote**) is a collection of calibrated skull images created by undergraduate biology students at Saint Michael's College. Coyote skulls from 24 locations are available and accurate measurements can be made from images printed from the web site or using software packages such as ImageJ. A growing collection of domestic dog skulls is also available (**http://wikieducator.org/Category:DogSkull**).

Methods

Stratification

The skulls presented in the Digital Coyote web site have not been identified by gender. However, male coyotes have larger skulls than females (Elbroch 2006). We might reasonably assume that the largest individuals from each region are male. To reduce variance contributed by gender in this exercise, we can select the largest individuals from a given region and compare them to the largest individuals from a different region.

Student question: Why can we not take a similar approach to identifying female skulls?

Randomization (alternative approach)

Use the random number generator in MS Excel to select six skulls from northeastern states and six from northwestern states.

Student question: What effect would you predict that this approach will have on the outcome of a comparison between skulls from two regions?

Replication

Start with six replicates from each location. With six replicates we should have no difficulty detecting a large difference in size between the regions if it exists. Detecting more subtle differences would require selecting more individuals from each region. An interesting addition to this analysis might be to see how varying the number of replicates used alters your ability to detect differences between regions as opposed to using the stratified approach.

Control and experimental groups

For our purposes the western skulls representing an older coyote population will serve as the control group. The northeastern population will serve as a comparative group representing a younger population that includes a wolf-derived genetic component (Kays *et al.* 2010).

Response variables

The most commonly used measurements of skull size are greatest length (GL), condylobasal length (CB; Fig. 1), and zygomatic width (ZW; Fig. 2).

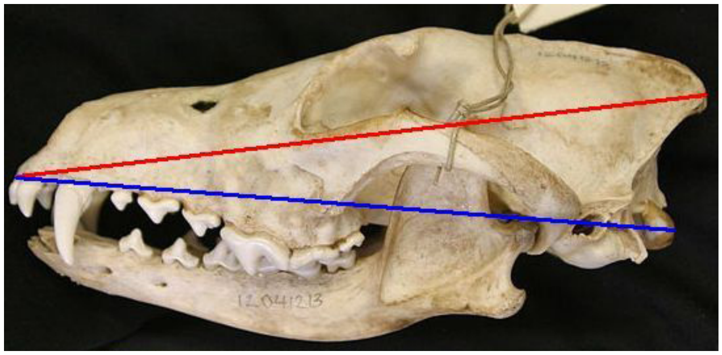

Figure 1. The upper line indicates the greatest length (GL) of the skull; the lower line indicates the condylobasal length (CB).

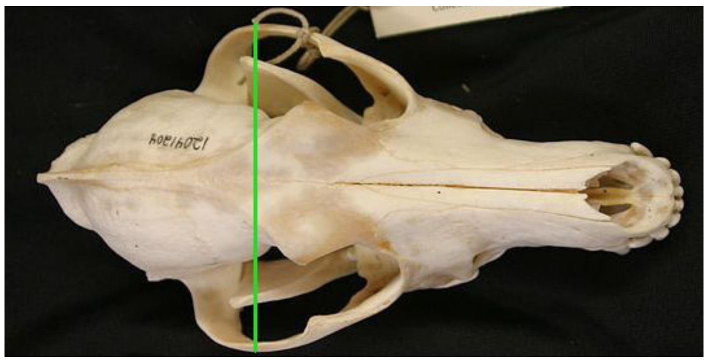

Figure 2. The vertical line indicates the zygomatic width (ZW), or the widest part of a coyote skull.

For GL and CB, measure to the end of the bone at the front and do not include the front-most teeth, or incisors; this convention permits comparisons with toothless skulls. Any or all of these response variables can be used to measure skull size. Importantly, all three measurements can be accurately taken from the photographs because the relevant structures and lengths are in the same photographic plane as the rulers in the photographs.

Measuring from scaled photographs

The photographs vary in scale so simply measuring from a printout with a ruler will be inaccurate. The ruler in each photograph can be used to deal with variability in scale.

- Let X = 15 cm. We will use the entire 15 cm of photographed ruler to calibrate our images.

Use a ruler to measure the following from each photograph:

- Y = the measured length of the photographed ruler. Measure the length from the zero mark to the 15 cm mark. Do not include the non-calibrated ends.
- Z = the *raw* measured length of interest (GL, CB, or ZW); use the same units that were used for Y. This is the measurement from the photograph that is corrected below.

Calculate the true skull length using equation 1:

Equation 1 Actual length of interest = $(X * Z) / Y$

Important: repeat this process for each photograph using each photograph's own ruler.

Worked Example:

This example uses the left side view of skull 12091108 from Washington State. $X = 15$; When we measured the 15 cm length of the photographed ruler, we found that $Y = 13.5$ cm. When we measured the greatest length of the skull from the photograph, we found that $Z = 16.3$ cm.

Calculation

Use equation 1 to calculate the actual greatest length of skull 12041209:

$$\text{Actual length} = (X * Z) / Y = (15 \text{ cm} * 16.3 \text{ cm}) / 13.5 \text{ cm} = 18.1 \text{ cm.}$$

Using calipers we found that the actual skull length is 18.1 cm so this measurement from the photo is about as good as it gets. Some slight inaccuracies are inherent in this process but we are unaware of any systematic biases that would alter a result in a meaningful way. What inaccuracies exist will slightly increase variance.

Student question: How might increased variance alter your ability to detect patterns or differences?

This true measurement of the greatest length of skull 12041209 is quite a bit longer than the 16.3 cm raw measurement taken from the photograph and illustrates the importance of correcting the measurement.

First Assigned Data Set

Measure the greatest length (GL) of each selected skull using the procedure above and enter the data below (Table 1).

Table 1. Greatest lengths (cm) of 6 eastern and 6 western coyotes

Eastern coyotes	Western coyotes
Average =	Average =
Standard Error =	Standard Error =

Graphing and Statistics

Length is a continuous variable and we measured animals from two categories (East and West). A bar graph with standard error for error bars would be one appropriate way to present the data. A *t*-test assuming equal variances can be used to interpret the data and answer the question: Are western coyote skulls smaller than eastern coyote skulls?

Alternative Approaches to Measuring Skulls

- Instructions are provided on the Digital Coyote website for using ImageJ to measure without printouts (**http://wikieducator.org/Coyote/Project_tools#Measuring_from_photographs**)
- Many other software packages including Photoshop can be used to measure from photographs.
- The ruler in each printout can be cut out and used to measure each skull directly. Be sure to number the ruler to match the skull. The paper rulers will only work on the images from which they came.

Your Second Hypothesis

Use your imagination; there are coyote skulls online from 24 locations ranging from Texas to Alaska; Washington to New England and more will be added as time permits. There are 28 domestic dog skulls and 6 wolf skulls.

Types of Questions

- Geographical variation; differences between species.

Quantitative Response Variables

- GL; CB; ZW plus any other measured trait you may choose. Even if a trait is not in the same plane as the calibration ruler, whatever small error is introduced will be similarly introduced in all individuals facilitating a good comparative data set.

Qualitative Response Variables

- Address shape by measuring ratios between variables. For example, if you were interested in snout length, measure the length of the snout forward of whatever marker you select and divide that by total skull length to give a ratio that expresses relative snout length. Using ratios has the advantage of normalizing for overall skull size such that the snout of a relatively small boxer dog breed (Specimen number: Dog12103123, **http://wikieducator.org/Dog12103123**) could reasonably be compared to a wolf snout or to that of a Chihuahua.

Materials

- Computer lab with internet access and ImageJ installed
- Web link: **http://wikieducator.org/Digital_Coyote**
  OR
- Printed sets of selected skulls for each lab group; example: **http://wikieducator.org/NE_NW_skull_comparison**
- Rulers
- Pencils
- Student outline handouts

Notes for the Instructor

Use of Images

All of the images can be freely used for educational purposes and are shared on Wikieducator and Wikimedia Commons under a creative commons attribution share-alike license.

Future Data Sets

- We welcome contributions of calibrated skull photographs directly to the wiki site.
- We have a collection of skulls from 13 canid species to be added to the web site.
- Our intent is to also add a modest collection of diverse species (20+ mammalian species) as time permits.
- We cannot return skulls that are sent to us to photograph.

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