

## **Adaptive shape differences among mammalian carnivore skulls**

In this laboratory, we will measure variation in the skull shapes of a number of fissiped (terrestrial carnivore) species from several families. The purpose of this lab is to investigate the adaptive significance of skull proportions, especially as they relate to the evolution of different hunting styles among the various carnivore families. This laboratory also introduces the use of multivariate statistical techniques to quantify patterns of adaptive variation.

As background, investigate the biology of each family, using any of the references provided. What are the adaptive patterns in each family, especially with respect to use of the jaws in hunting and prey handling? What features of the skull characterize each of these groups?

### **Data Collection**

The data sheet gives the scientific names of a set of carnivore species. Determine the common names and the appropriate taxonomic family for each specimen. Examine each skull as you measure it. What qualitative differences are apparent? Especially, *note differences in the shape of the braincase (cranium) with respect to the snout (rostrum)*. Teeth are the keys to understanding mammals in general and carnivores in particular: note the differences in dental patterns.

Work in pairs. There are six measurements to be taken on each skull; one partner should measure the first three, while the other records the measurements, then switch roles for the last three. Where possible, use digital calipers and record measurements to the nearest 0.01 mm; otherwise, record measurements to four significant figures. **BE ABSOLUTELY CERTAIN THAT THE MEASUREMENTS ARE TAKEN ACCURATELY**; a single mistaken measurement can invalidate the entire analysis. Check that the measurements are reasonable before you leave the lab; re-measure if necessary. Combine the measurements before leaving the lab so that both partners have a complete data set.

Take the following six measurements as instructed (see illustration):

- a) **Greatest Length of Skull (GLS)**: Measure the maximum length of the cranium, from the incisors to the back of the occipital.
- b) **Cranial Breadth (CB)**: Measure the width of the cranium at the paroccipital processes.
- c) **Cranial depth (CD)**: Measure the depth of the cranium at right angles to **GLS** and **CB**.
- d) **Tooth Row (TR)**: On the bottom of the cranium, measure the length of the tooth row from the front of the incisors to the back of the tooth row.
- e) **Rostral width (RW)**: On the bottom of the cranium, measure the width of the snout (rostrum) at the posterior margins of the canine teeth.
- f) **Canine-Nasal Distance (CND)**: Measure the depth of the rostrum from the posterior margins of the canine teeth to the anterior-most symphysis of the nasal bones.

### Calculations with MINITAB

1. The following instructions assume familiarity with MINITAB. The format of a sample command line for each calculation is enclosed in [square brackets]; usually these commands will have to be repeated several times.

2. Enter all data by rows [read c1-c6], one row for each of **n** species. Rename c1-c6 with the appropriate variable names [name c1 'GLS'].

3. **Normalize** the data: divide columns 2-6 by column 1 (GLS), and put the results into columns 7-11 {use the command [let c7 = c2/c1] and so on for c3 through c6}. Rename the normalized columns [name c7 'NCB']. {Note that GLS itself is not normalized: you should end up with five normalized variables}.

4. Determine the **correlation coefficient (r)** for all pairs of normalized variables (there are  $5 \times 4 / 2 = 10$  correlations) [correlate c7 c8]. To test the statistical significance of these coefficients, perform a Pearson Correlation analysis for all pairs of variables [corr c7 c8]. The significance of the correlation is determined by comparing the value to the critical values for **n-2** degrees of freedom. Summarize the correlation coefficients and their statistical significance in a table.

5. Do a **principal components analysis (PCA)** on the normalized data in columns 7-11. Use the default correlation matrix. Put the component scores for the **n** species in columns 12-16 [this will make a  $5 \times n$  matrix} and the coefficients of the principal components in columns 17-21 {this will make a  $5 \times 5$  matrix} as follows:

```
PCA c7-c11;  
SUBC> scores c12-c16;  
SUBC> coef c17-c21.
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6. Name the coefficients PC1-PC5 [name c17 'PC1']. Name the component scores I-V [name c12 'I']. Record the eigenvalues (which are the same as the coefficients), proportions, and cumulative percentages.

7. Enter column 22 as identification numbers for each skull [read c22]. Enter another column of family identification codes: assign all members of each family the same number [read c23] {i.e., enter '3' for all canids, enter '6' for all felids, and so on. Re-name column 22 as ID [name c22 'ID'] and column 23 as family [name c23 'Family'].

8. Use the L PLOT option to construct family and individual plots of all pairwise combinations of the scores of the first three principal components: II vs. I, III vs. I, and III vs. II. [L PLOT 'I' 'II' 'id']. Construct family and individual plots of those pairwise combinations of variables *indicated by the instructors*.

9. In the family plots, show the range of variation within each family by connecting the dots for each family as a convex polygon. Include ONLY the plots of families in your report. The individuals plots are for your own use only, to identify any unusual specimens in the family plots. Be sure to include a key to the symbols (only one such is needed).

## Lab Reports

1. In the Materials section, summarize in a table the phylogenetic systematic relationships of these species: determine the suborder (or superfamily), and family of each species.
2. In the Results section, give tables of (a) the normalized data, (b) the correlation coefficients, (c) the eigenvector coefficients, (d) the principal component scores, and (e) the three family plots of PC scores (see the example of this last) as well as (f) any additional plots specified by the instructors. Note any significant correlations between variables.
3. In the Discussion section, *concisely* discuss the biological significance of any trends you observe in the graphs of the scores of the first three principal components.
  - a. **PC1** should contrast skulls shapes of cats and dogs. [Refer to the attached discussion of principal components for help in this interpretation.] Discuss in terms of the contrasting hunting and prey-handling styles of the two families.
  - b. How would you interpret **PC2** and **PC3**? What shapes are contrasted? Which animals fall at the extremes of the plots? Relate this to hunting styles and prey-handling techniques.
  - c. Is there more variation among the skull shapes of some families than others? Do any particular skulls fall closer to another family than to its own? Discuss such patterns with reference to hunting styles, prey-handling techniques, and the evolutionary history of the individual species and families.
  - d. Discuss any patterns of variation specified by the instructors.

### References

Banfield, A. W. F. (1974). *The Mammals of Canada*. Toronto.

Colbert, E. H. and M. Morales (1991). *Evolution of the Vertebrates*, 4th ed. Wiley-Liss. (Chapter 25: Creodonts and Carnivores).

Eisenberg, J. F. (1983). *The Mammalian Radiations*. Univ. Chicago.

Ewer, R. F. (1973). *The Carnivores*. Cornell.

Nowak, R. M. (1992). *Walker's Mammals of the World*, 5th ed. Johns Hopkins.

Vaughan, T. A. (1984). *Mammalogy*, 3rd ed. Saunders. [See Chapter 10 on Carnivores].

### See also

Manly, F. F. J. (1994). *Multivariate Statistical Methods: a Primer*. 2nd ed. Chapman & Hall. [Chapter 5 on PCA Principal Component Analysis. The example with Bumpus' sparrows is especially nice.]

Wiley, E. O. (1981). *Phylogenetics*. Wiley Interscience. [pp. 339-365 on quantitative data analysis, including PCA]

## Data sheet for carnivore skull measurements

ID#	GLS	CB	CD	TR	RB	RD
01) <i>Felis catus</i>	-----	-----	-----	-----	-----	-----
02) <i>Felis rufus</i>	-----	-----	-----	-----	-----	-----
03) <i>Lynx canadensis</i>	-----	-----	-----	-----	-----	-----
04) <i>Viverricula indica</i>	-----	-----	-----	-----	-----	-----
05) <i>Arctogalidia sp.</i>	-----	-----	-----	-----	-----	-----
06) <i>Mustela erminea</i>	-----	-----	-----	-----	-----	-----
07) <i>Mustela vison</i>	-----	-----	-----	-----	-----	-----
08) <i>Melogale personata</i>	-----	-----	-----	-----	-----	-----
09) <i>Enhydra lutris</i>	-----	-----	-----	-----	-----	-----
10) <i>Martes americana</i>	-----	-----	-----	-----	-----	-----
11) <i>Procyon lotor</i>	-----	-----	-----	-----	-----	-----
12) <i>Ailurus fulgens</i>	-----	-----	-----	-----	-----	-----
13) <i>Vulpes vulpes</i>	-----	-----	-----	-----	-----	-----
14) <i>Canis familiaris</i>	-----	-----	-----	-----	-----	-----
15) <i>Canis latrans</i>	-----	-----	-----	-----	-----	-----
16) <i>Ursus americanus</i>	-----	-----	-----	-----	-----	-----
17) <i>Ailuropoda melanoleuca</i>	-----	-----	-----	-----	-----	-----
18) <i>Ursus maritimus</i>	-----	-----	-----	-----	-----	-----
19) <i>Phoca vitulina</i>	-----	-----	-----	-----	-----	-----
20) <i>Zalophus californianus</i>	-----	-----	-----	-----	-----	-----

## Interpreting a Principal Component Analysis - Theory & Practice

The procedure described in the lab handout generates four matrices:

- [1] Original raw data (6 variables x 20 taxa)
- [2] Normalized data (5 variables x 20 taxa)
- [3] Coefficients of eigenvectors (PC1 - PC5) (5 x 5)
- [4] Principal component scores (I - V) (5 x 20)

The relationship between [1] and [2] is straightforward: the last five variables are divided by the first in order to remove the effect of size differences so as to be able to see the shape differences. In the discussion that follows, references to measurements, variables, or data are to these 'normalized' variables. Each of these normalized variables is an axis that can be graphed, e.g, normalized cranial breadth can be plotted against normalized cranial depth in a bivariate plot. Such a plot would tell you something about head shape.

The Principal Component Analysis converts the normalized data in [2] to so-called 'principal component scores' in [4]. As discussed in the lab, the variables are in essence rotated through multiple dimensions so as to see combinations of variables that describe the major patterns of variation among taxa. The angles of rotation are indicated by the eigenvector coefficient in [3]. Note that the matrix of eigenvector coefficients is identical to the 'eigenanalysis' table produced by MINITAB when the PCA analysis is first run. [Calculation of these angles involves matrix algebra, and the mathematics are beyond the scope of this course: see Manly 1994 for more details]. Once calculated, however, the relationship among the data, the coefficients, and the scores is very straightforward, and is important for understanding and interpreting the results of the PCA analysis.

On each principal component axis, each individual has a single 'score' in [4] to which all five measurements in [2] contribute. The contribution or 'weight' for each measurement is the eigenvector coefficient for that measurement in [3]. That is, the coefficient for each measurement determines how 'important' that measurement is for the particular component. The 'score' of each individual is in essence a new 'measurement' that combines all of the original physical measurements. Each of the principal component axes represents an independent pattern of variation. Like the original data, the scores are axes that can be graphed.

For each individual, the score on any axis is calculated as

$$\begin{aligned} \text{Score} = & \text{measurement } \{1\} \times \text{coefficient } \{1\} + \\ & \text{measurement } \{2\} \times \text{coefficient } \{2\} + \\ & \text{measurement } \{3\} \times \text{coefficient } \{3\} + \\ & \text{measurement } \{4\} \times \text{coefficient } \{4\} + \\ & \text{measurement } \{5\} \times \text{coefficient } \{5\} \end{aligned}$$

where measurement {1} and coefficient {1} are the values associated with the first variable, and so on.

### Where do Principal Component scores come from? - an example

To make this clearer and to explain the interpretation of the eigenvector coefficients, consider the following example, based on an analysis of terrestrial carnivores. The normalized data (from [2]) for one 'cat' species and one 'dog' species are as follows:

Family	ncb	ncd	ntr	nrw	nrd
Felid	0.444150	0.474723	0.349630	0.275515	0.186141
Canid	0.305361	0.340163	0.539170	0.153536	0.116132

When a MINITAB PCA analysis of the covariance matrix of carnivore skulls is performed, the eigenvector coefficients (from [3]) on the first axis are

	PC1
ncb	-0.541367
ncd	-0.371045
ntr	0.670168
nrw	-0.288332
nrd	-0.192320

and the component scores for these two species (from [4]) are

	I
Felid	-0.297520
Canid	0.003203

For the Felid, this score was calculated by MINITAB by the formula above as

$$\begin{aligned} -0.298 = & (0.444) \times (-0.541) + \\ & (0.475) \times (-0.371) + \\ & (0.350) \times (0.670) + \\ & (0.276) \times (-0.288) + \\ & (0.186) \times (-0.192) \end{aligned}$$

Similarly, the score for the Canid was calculated as

$$\begin{aligned} 0.003 = & (0.305) \times (-0.541) + \\ & (0.340) \times (-0.371) + \\ & (0.539) \times (0.670) + \\ & (0.154) \times (-0.288) + \\ & (0.116) \times (-0.192) \end{aligned}$$

[NOTE: These formulae apply only to PCA done with the covariance matrix of the normalized data. In the lab exercise, we will use the correlation matrix, and you will NOT be able to repeat the above calculations directly. In the correlation matrix analysis, the data are "standardized": for each measurement of each variable, MINITAB subtracts the mean and divides by the standard deviation of all measurements for that variable. All variables then have identical distributions, with a mean of zero and a variance of one. In a correlation-matrix analysis, the relationship of matrices [2] and [3] to [4] is not as direct as described above, because the standardization of matrix [2] in effect multiplies each variable by a (hidden) constant. In principal, though, the relationships among the matrices in either form of the analysis are identical and the calculated coefficients are usually quite similar. The correlation matrix is used because of its other useful properties, as discussed below.]

### **Interpreting principal components and eigenvector coefficients**

Now that we know where the principal component scores come from, the important questions for this lab are (1) How can the array of eigenvector coefficients be interpreted as a description of a biological shape? and (2) What is the biological meaning of the score for any individual? That is, how do the individual weights contribute to the overall 'score' of the individual? Let us take the first principal component as an example.

Consider first the cat. The most prominent feature is its inflated cranium, indicated by the large cranial breadth and depth. Both of these variables have large, negative coefficients or weights: thus large (positive) measurements are multiplied by large (negative) weights, and the result is a large negative number. Thinking of a number line, these measurements and their respective weights 'push the score to the left', that is, they shift the score to the negative end of the axis. The only weight that is positive (the only one that can push the score to the right) is that for tooth row; the cat however has a relatively short rostrum, so this positive 'push' is relatively small. The last two variables each contribute a small negative 'push'.

Contrast this with the dog. The most prominent feature is the long rostrum, seen as a long tooth row. This contributes a large positive value to the component score: it is 'pushed to the right' and increases the score on the axis. As before, the weights on cranial breadth and depth are negative, however the cranium is fairly compact and the net negative contribution is much smaller than in the cat. As in the cat, the last two variables each contribute a small negative 'push', though less so because the dog's muzzle is not so high as the cat's.

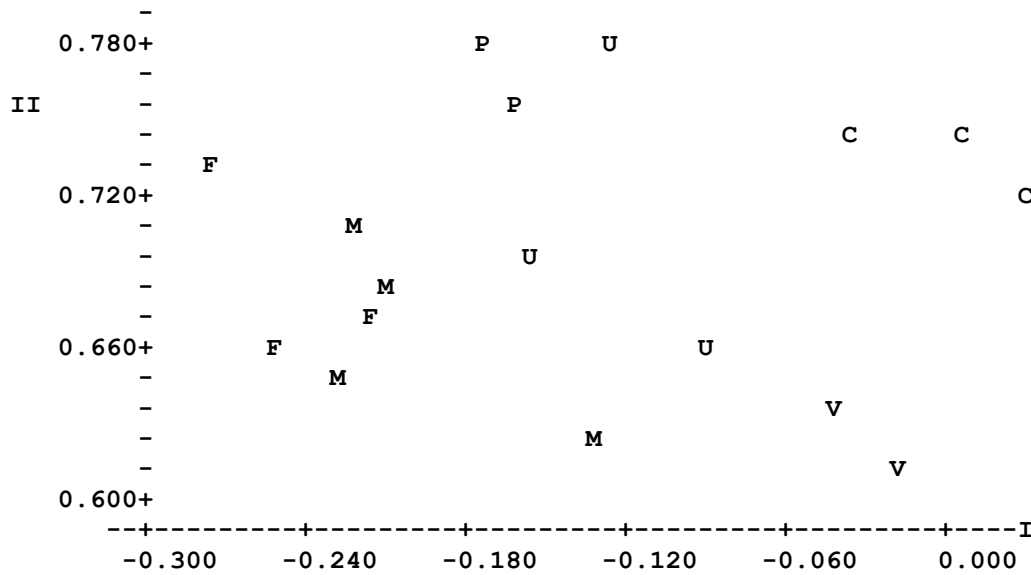
Thus, the three largest weights on the first principal component may be interpreted as contrasting the cross-sectional areas of the cranium (large negative loadings) with the length of the snout (large positive loading). Animals with bulgy craniums and short faces will go toward the negative end of the axis, animals with compact craniums and long faces will go towards the positive end of the axis. That is, animals with "dog-like" proportions will have more positive (in this case, less negative) scores, those with "cat-like" proportions will have very negative scores. The first principal component, which accounts for more than half of the observed variance among skulls, contrasts dog-like carnivores and cat-like carnivores. In Figure 1 (below), Canids (C) are at the right extreme of PCI and Felids (F) at the left extreme. Other species are intermediate, some more cat-like and some more dog-like.

The other principal components can be interpreted in the same manner. Each principal component is completely independent of the others, and represents a completely different pattern of variation. [To prove this to yourself, you could use MINITAB to calculate the correlation between the first and second principal components of your data]. However, each successive component explains a smaller and smaller proportion of the total shape variation [as indicated by the 'cumulative variance' in the PCA table]. Usually the first three components taken together explain 90-95% of the variation, and are the only ones we need worry about interpreting.

An practical consequence of using the correlation matrix is that the component scores calculated from standardized data will be 'positive' or 'negative', depending on whether they are above or below the mean value, respectively, unlike the example in which the extreme cases are less or more negative. A score of zero can then be interpreted as 'neutral': in this case, 'neither cat-like nor dog-like'. The resemblance of other individuals to either extreme is then easier to assess.



### Principal Components plot: an example



**Figure 1: Variation in skull shape among 17 species in six families of fissioned carnivores. First and second PCA axes from five normalized measurements.** For Axis I, note particularly the distribution of the three canid species (C) at the extreme right, and of the three felid species (F) at the extreme left. [Other codes: M = Mustelidae, P = Procyonidae, U = Ursidae, V = Viverridae]

