Morphometrics

Introduction

In this lab, we hope you will get a sense of the utility of morphometrics, as well as experience working with morphometrrics software. If you are interested, a valuable morphometrics resource is Jim Rohlf's website at Stony Brook Univeristy (http://life.bio.sunysb.edu/morph). The page includes an archive of software (including one program we'll use today), as well as lists of upcoming meetings, a bibliography of morphometrics resources, a glossary of terms, and contact information for people working on morphometrics.

TPSDIG

The first program we will use today is Jim Rohlf's TPSDIG. This is only one of several programs Rohlf has developed, but it is critical for this lab and the next because it allows us to enter the data we will be analyzing.

Because we're conducting geometric morphometric analyses, we're interested in changes in shape as recorded by the changing positions of certain discrete landmarks. Landmarks should be homologous points on our organisms that are consistent, repeatable, and coplanar (i.e., they are all visible in a photograph of a specimen). There is no right number of landmarks, but they should be numerous enough to adequately cover and describe a specimen's shape. The details of what a landmark is and how to choose them is a contentious issue that we will avoid for now.

TPSDIG allows us to define landmarks and save them in a format that the other programs can recognize. To use TPSDIG, we need to have photos of all of our specimens in the same view, and all of the photos must have a scale. To save time today, we'll just do two specimens.

--Open TPSDIG (Start – Programs) and go to the file menu. Go to input source and choose tps file 'test' in c:/Documents and Settings>All Users>Start Menu>Programs>IB200A> TPS>Examples. This file points to the two images, which we will use in our analysis, so that they can be analyzed at the same time (The format of this file is described in the literature). Both images are from the left wing of female *Aedes canadenses*.

--The image file should appear on your screen. Go to the tool bar and select the bulls'-eye tool. You can zoom in on the images using the + button. Each time you click with the bull's-eye a red dot appears representing a landmark. Thus, click on places you want to include as landmarks. In an ideal situation we would have a scale bar in the image, which you could use

to define a standard distance by switching to set scale mode, clicking on the endpoints and entering the length, but we don't. If you make a mistake, click on the pointer to switch to edit mode and move your landmarks around. Select 'Label landmarks' from the options menu to see in what order you made your marks.

--For the next image click the big red arrow. Now click on the homologous landmarks in this image in the same order. If you forget where you put a landmark you can use the big red arrows to toggle back and forth between the two images.

--When you are done, go to the file menu and select save data as. Save your document to the desktop. Type '.tps' at the end of the file name (For some reason the program can't handle this itself). You can open your file in notepad. If you do, you will see it contains the landmark coordinates for your specimens.

The rest of the programs we'll be using today and on Tuesday were developed by David Sheets at Canisius College. They are already on our computers, but they can downloaded at: www2.canisius.edu/~sheets/morphsoft.html. These are probably the most straightforward and easy to use programs available for morphometric analyses.

CoordGen

The first thing we'll need to do before analyzing our data is match all of our specimens up in shape space. In other words, we need to superimpose all of our landmark data for all of our specimens in a common coordinate system, so that differences in the relative position of each landmark are reflected by the differences in their coordinates. The methods that allow us to do this are called superimposition methods because we are essentially superimposing all of our data on top of each other. CoordGen can use several superimposition methods, although we will focus on two, two-point registration ('Bookstein coordinates) and procrustes. Two-point registration requires us two use two landmarks to define a baseline. The rest of our data are superimposed based on their relative positions in regard to this baseline. Procrustes involves using least squares regression to fit the individual points to the mean coordinates for each landmark given all of the data in the data set.

--Open CoordGen6d (the application file!). The path is C:\imp\bin\win32. Keep this window open because we'll return to it for other programs and files. When open CoordGen6f, two windows will appear. The one you want to interact with should be obvious.

--In the tangerine colored box, push the button that's labeled 'load tps file (no ruler/ no scale factor).' Select the file you saved from TPSDIG.

--If you had a scale you could use the light blue boxes to set it, but we don't.

-- A cloud of points will appear on the graph. This shows the position of each of the landmarks for each specimen as they have been superimposed.

-- Then go to the green box and toggle between the 'show bc' button and "show procrustes" buttons. What difference do you see? Do you know why?

--We could save the results of these superimpositions, in the lavender box you can see the buttons used for saving the results. The saved results files could be input into the other programs we'll be using. For most of the remaining exercises, we'll be using other files that have many more samples. Our example current dataset with only two mosquito wings is not sufficient for the following statistical packages.

PCAGen

The next program we'll be using is called PCAGen. This program starts with superimposition data and then computes partial warp scores based on the loaded data. In other words, the program examines shape changes at a local scale (e.g., enlargement of the head) by comparing the landmark data for each specimen to a mean specimen that it computes based on all of the data using a least-squares procrustes method. The program then conducts a principle components analysis of the covariance matrix derived from the partial warp scores. The program can display landmark positions for all of the specimens, a plot of the data along different principles component axes, and the deformation implied by a particular principle component vector.

--Open PCAGen6n (c:/Documents and Settings>All Users>Start Menu>Programs> IB200A> IMP). Again, two windows will open and the one you want will be obvious.

--Push the button labeled 'load file' and load in the threepir.bd file (in the same path as above). Then push the 'no group list' button. When you do this, a plot of all of our landmark data will appear. These landmarks represent a view of a bunch of fish bodies if you're interested in what you're looking at.

--If you push the 'show pca plot' button, a plot of the specimens on pc axes 1 and 2 will be displayed. This gives you an idea of which specimens are similar or different in regards to these axes. You can also see how much of the shape change each axis explains. You can use the up and down buttons to examine the other PCAs.

--Now go to the blue box labeled 'deformation display format' and select 'vectors on landmarks.' Then push the 'display pc deformation (bc)' button. When you do this, a plot showing you landmarks will appear. The arrows represent the direction and relative amount of

the shape change explained by this pc vector. For pc 1, we can see that most of the shape change it describes is deepening of the mid-section of the body. You have to use your imagination to visualize the orientation of a fish in this cloud of points and vectors. Examine the deformations for some of the other pc vectors and see what shape changes they describe.

--Two other useful features can be found in the 'statistics' menu. The 'screen plot (percentages)' feature shows graphically what percentage of the variation each pc explains. The rule of thumb is to assume meaning for the pc's that occur before the plot flattens out. The 'significant differences in pc components' feature does pair-wise comparisons for all of the pc's and then reports how many are significantly different. In this example, the first pc is significantly different from the rest, but none of the others are significantly different from each other.

CVAGen

The final program we'll use today is called CVAGen. The program performs a canonical variates analysis, which allows us to find a set of axes which allows us to best discriminate 2 or more groups that may be in our data. For example, this program would be useful if you wanted to test whether a series of named species can be discriminated using geometric morphometrics.

--Open CVAGen6j. This time three windows will open.

--Push the red 'load file' box and load the file called 'threepir'. Then go to the 'load group membership list' and load the file 'threepirgrp'. When you are done, a plot showing the landmark data will appear, along with a small dialogue box that will tell you that there were two distinct (i.e., significantly different) canonical variates found.

--Now push the purple 'show cva plot' button. This will show you where your different specimens fall out on the canonical variates axes. As you can see, our data forms three very distinct groups.

--Go to the 'deformation display format' and again select 'vectors on landmarks.' Then push the 'display cva deformation (bc)' button. When you do this, a plot will appear that will show you the shape change that the selected cv describes.

--Another useful feature is the 'show groupings by cva' function found in the statistics menu. Select this function and go to the 'auxiliary results box' (the smaller colorful box that opened when you started the program). In this box you will find a table that shows how many specimens of your original group fell out in each of the groups determined by the cva analysis. In this case, none of our specimens went into any of the 'wrong' groups. However, this is certainly a possibility.