Guide to geometric morphometrics

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Eigenshape analysis

Programs

The key set of programs you'll need is Jim Rohlf's tps suite (http://life.bio.sunysb.edu/morph/).

1. *tpsUtil*. This program will allow you to build tps files with very little hassle.

2. *tpsDig*. This program will allow you to place landmarks on your images and record scale factors, saving this data in the tps file.

You will also need specialized programs to perform the eigenshape analysis.

3. *Eigenshape suite*. You can download N. MacLeod's suite of FORTRAN programs. However, he is actively phasing those programs out in favor of Mathematica-based versions developed by J. Krieger. These can be downloaded from https://www.morpho-tools.net. However, these programs only run on Mathematica, which is expensive. Morpho-tools has an online analysis component, though this depends on the availability of a host server. More information is available on the website. Eigensurface analysis can only be carried out using the Mathematica-based programs.

You may want to consider the investment in Mathematica, especially if you're able to purchase a student version or educational license. Most of the major players in morphometrics use Mathematica: N. MacLeod, F.J. Rohlf, P.D. Polly, and the F. Bookstein lab. It is invaluable for 3D analysis, and it gives you enormous flexibility in making subtle changes to the types of analyses, or the set of analyses performed. You can download the free MathReader software from Wolfram and inspect a few notebooks on the Morpho-tools site. If you're at all comfortable with mathematics, you'll find that the code is very easy to read, as it is written in a higher-level, math-based language.

You will also want a text editor (e.g., *Notepad* or *Word*), a spreadsheet editor (e.g., *Excel*), and a stats package (e.g., *SPSS*, *SAS*, many others, even *Excel*). An image processing program like *Photoshop* is very helpful.

Imaging

1. Leave space around your image during photography. If your image fills the frame, it will likely be distorted near the edges. You can still crop your image afterwards (e.g., in Photoshop).

2. Take photos at max available resolution. Storage is cheap, so don't needlessly shrink your images just to save disk space. You may want that information later. Images should be jpegs or uncompressed tiff files. Other formats are okay, but you risk having problems with the analysis programs. I recommend using lossless compression where possible (e.g., tiffs), then converting to jpegs for the analysis if you want to work with smaller files.

3. Always use a scale bar. Take your photos with a ruler visible in the picture, preferably not on the edge of the image (to avoid distortion).

Building a tps file

Before collecting data, you need a tps file, which is basically a list of your specimens.

1. Place all of the images that you want to use (or copies of them) in the same folder. You don't have to do this, but I find it helpful.

Possible issues: tps programs have had issues with really long file paths (the file name plus all of the names of the folders it's in). To avoid this issue, place your images in My Documents or in the C:\ directory in a folder with a short name (e.g., "analysis"). You can move them out after you're done.

2. Open *tpsUtil* (Start > All Programs > tps > tpsUtil)

3. Click on "Select an operation" and choose "Build tps file" from the drop-down list.

4. Select your input directory:

    a. Click "Input" (which should now be visible).

    b. Find your directory of images

    c. Double-click one image in that directory

    -instead of "Data file = ?", it should show the path to your images

5. Name your output file

    a. Click "Output"

    b. Enter a name that ends in ".tps". I recommend you save this file in the folder with your images.

    -instead of "Output file = ?", it should show the path to your images

6. Build the tps file

    a. Click "Setup" (which should now be visible).

    b. Checked images will be used to build your tps file. Check/uncheck as needed. If the folder contains only the relevant images, your list should be fine.

Note: if you click "include path", you can move the tps file anywhere and it will still "find" the images, but then you can't move the images. If you leave it unselected, you have to keep the tps file in the folder with the images, but you can move that folder anywhere you want.
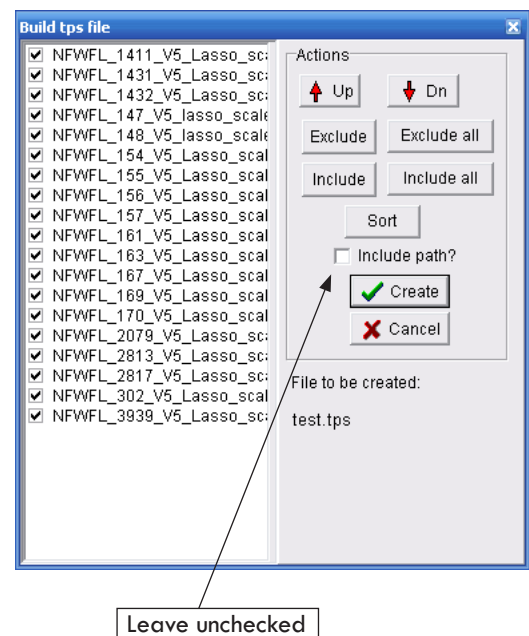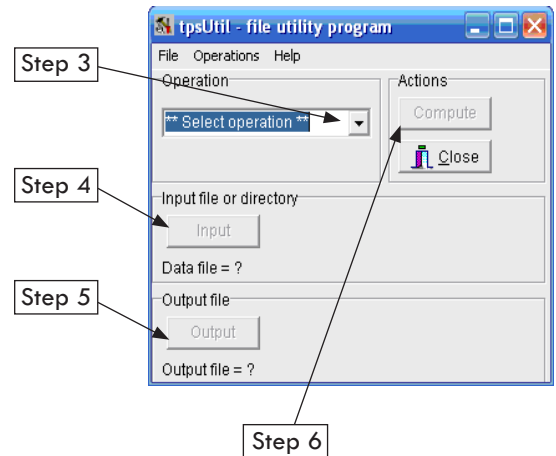
    c. Confirm that you have a file named "[something].tps" under "File to be created". If not, go back to step 5.

    d. Click "Create"

    e. Click "Close" to exit *tpsUtil*.

You should now have a file that you can open in tpsDig (http://life.bio.sunysb.edu/morph/).

What a raw tps file looks like (open in a text editor like Notepad):

```
LM=0
IMAGE=MSB_125_oscoxae_lateral.JPG
LM=0
IMAGE=MSB_54742_oscaxae_lateral.JPG
```

Your specimens (given by the IMAGE= names) currently have zero landmarks (LM=0).

Landmarking your specimens

In this step you place landmarks on your images and save them into your tps file.

1. Open tpsDig (Start > All Programs > tps > tpsDig

2. Open your raw tps file (File > Input Source > File...)

You should now be able to scroll through all of your images using either the right and left arrow keys (on your keyboard) or the red arrow buttons on the top left hand corner of the navigation screen. The file name is listed at the bottom of the screen, and information on the number of landmarks will appear there as you begin collecting data. The help files are worth a look.

3. Place landmarks. Select the crosshairs icon. Left-click to place a landmark at the appropriate location.

Remember: all landmarks must be placed in sequence. If you click a landmark (LM) with the arrow tool, the LM number will be shown at the bottom of the screen. If you've screwed up the sequence, you can move the LMs with the arrow tool. Right-clicking on a landmark with the arrow tool will allow you to delete that LM or insert a new LM before the selected LM.

Make sure you know what your landmarks are before you start and be ready to make adjustments them after you start. This happens sometimes with ambiguous (e.g., non-Type I) landmarks. Consistency in landmark placement is critical, so it is important to spend a good amount of time preparing and thinking about where your landmarks will go.

4. Set scale. Go to Options > Set Scale. In the pop-up window select the length of your scale (e.g., if you have a 10cm ruler, enter 10.0). In your image, place the cursor (now an arrow with a cross) on one end of your ruler. Left-click, then set the arrow at the other end and left-click again. The scale window should now show your scale factor. Click OK.

Do you need to set scales? The ability to set scales in *tpsDig* is extremely useful and I believe it should always be used. It allows you to photograph specimens without worrying about the distance between the lens and the specimen by using the scale bar in your images and inserting a scale adjustment into the tps file. Even if you know that your lens was always at an equal distance, it does not hurt to set a scale. You can set the scale for each specimen as you landmark, or come back to it later.

5. Save your landmark data. File > Save data > Save > Overwrite. Because these programs are made by individuals and not corporations, they are prone to minor glitches (that can make you lose valuable information). Therefore, save often! Or don't, and learn the hard way.

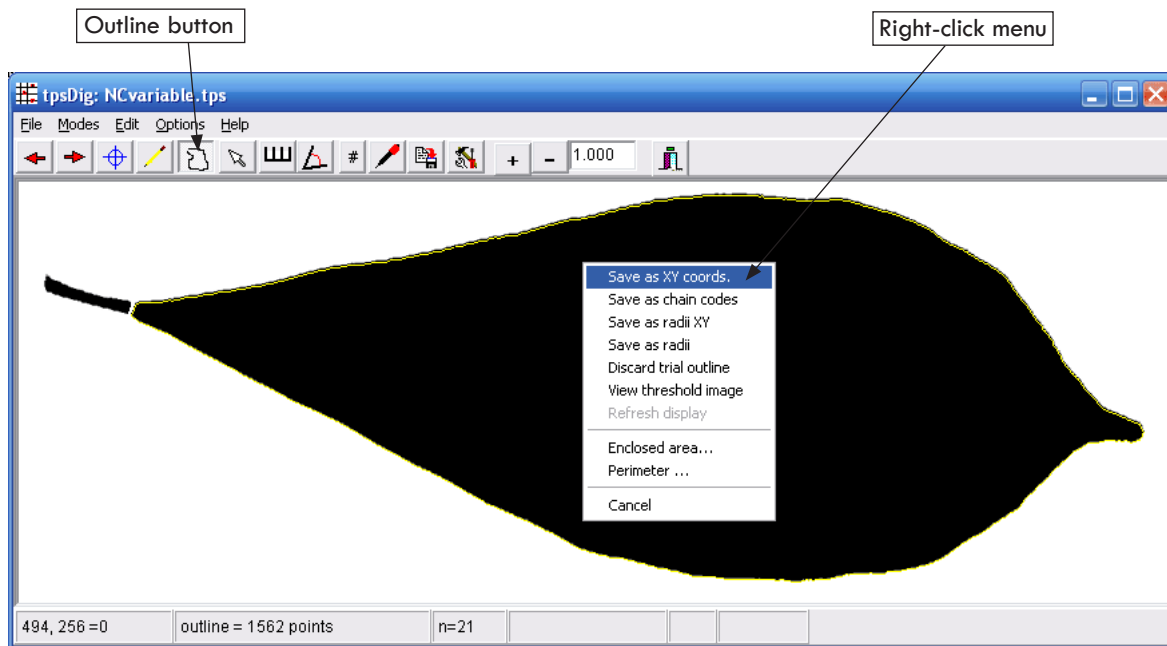Repeat this process for each specimen. Your tps file should now look like this:

```
LM=4
563.00000 732.00000
432.00000 749.00000
435.00000 678.00000
572.00000 685.00000
IMAGE=MSB_125_cranium_lateral.JPG
ID=1
SCALE=0.006664
```

Note that each specimen starts with "LM=" and ends with the scale factor. Occasionally, you may find yourself manually editing files in Notepad. It can be confusing having the specimen name at the end of the specimen data. Be careful about this. The ID number doesn't matter; you can concatenate files so that there are multiple specimens with the same ID. I haven't yet seen a problem with doing so.

Outlining your specimens: closed outlines

In this step you generate outlines for your images and save them into your tps file.

1. Open tpsDig (Start > All Programs > tps > tpsDig

2. Open your raw tps file (File > Input Source > File...)

You should now be able to scroll through all of your images using either the right and left arrow keys (on your keyboard) or the red arrow buttons on the top left hand corner of the navigation screen. The file name is listed at the bottom of the screen, and information on the number of landmarks will appear there as you begin collecting data. The help files are worth a look.

3. Identify an outline. Select the outline icon. Left-click within your shape to find an outline. The program starts where you've click, searching to the left until it finds a boundary. It then follows that boundary around your specimen until it completes a loop of the shape.

Note: all of your outlines will need to start from the same point. You can do this with careful outline capture. An alternate approach is to place a landmark at the start of the outline. If you do this, make sure your outline start point is not the same as the landmark point, which may cause problems with the programs that interpolate your outline.

4. You should see a yellow line around your specimen. To create the outline, right-click. On the resulting menu, select *Save as XY coords*. A window will pop up. Save the outline with the recommended number of points. If it is a round number, your shape may require more points than the maximum allowed by tps-Dig. Click OK and examine the outline to see if it surrounds your shape. If not, you will need to resize the image and try again.

5. Save your data file (File > Save data > Save > Overwrite) and continue with other specimens.

Once you have your outlines, you may want to place landmarks on them, either to record the position of the outline start, or to split the outline for Extended Eigenshape analysis. Follow the section on collection landmark data (Landmarking Your Specimens).

Eigenshape analysis

This is actually very straightforward in Mathematica. Using MacLeod's code is a bit more laborious.

Further analysis

You will want to run analyses using statistical programs like SPSS, or even EXCEL.

Suggestions???

Other tips

1. Subscribe to the morphmet listserve, and don't be shy about posting questions there if you're stuck. Also post any problems you have with the programs. Chances are very good someone's experienced and solved your problem. If not, Jim Rohlf will incorporate a fix into the next version of his programs, which everyone will appreciate.

2. Read N. MacLeod's papers. Don't hesitate to email him, or J. Krieger. Both spend a great deal of time assisting people with eigenshape analysis.