Biol 475
Vegetation Description and Analysis
Laboratories 10, 11, 12
Ordination using PC-ORD
100 points
Due: Friday, Dec 1

I. Introduction

Labs 10, 11, and 12 will familiarize you with the output from an ordination program called PC-ORD and allow you to use the program to analyze the field data we have collected. This program is quite versatile and will provide most of the types of output that are useful for ordination analysis and numerical classification.

We will use the same species data we used for the sorted table analysis. And we will combine this with the environmental data from the same relevés. Thus, a secondary purpose of the lab is to provide much of the basic environment-vegetation analysis for the oral presentations. Each student will do a series of analyses and these results will be pooled for inclusion in your papers.

If you are unable to complete the exercise during class time, please feel free to come in and use the computers at other times during the week when they are not scheduled for classes. Refer to the PC-ORD manual if you get stuck or ask one of us. The on-line help in the PC-ORD program provides most of the same information that is in the User's Guide.

II. Orientation to PC-ORD

PC-ORD is a DOS program that performs multivariate analysis of ecological data. It is menu driven with windowed output. In addition to utilities for transforming data and managing files, PC-ORD offers many ordination and classification techniques that are not available in major statistical packages. The program is intended for use by those with little or no previous computing experience. However, rank beginners will need to have knowledge of spreadsheet programs, microcomputers, and how to print. We will provide a demonstration of the PC-ORD program to get you through the homework, and go through the various options of the program, including how to print the results.

This handout outlines the demonstration that is used to introduce the lab. Repeat these steps to familiarize yourself with: (a) loading PC-ORD, (b) loading the data matrices, (c) performing Bray and Curtis and DCA ordinations, (d) making dendrograms, and (e) graphing a variety of output from the ordination. Most of this information will be useful for the Homework Exercise below, so read through Part III, Homework Exercise, before beginning the orientation. It is suggested that you also spend an hour or so going through

some of the other options and windows that are not discussed here and browse the PC-ORD manual to get a better idea of some of the functions.

Getting started

Log into Mercury. On the M: drive, you will see a folder labeled "**Working**". Copy the "working" folder to the C: drive on your computer (Highl;ight the folder, **Cntrl C**. Move cursor to the C: drive and **Cntrl V**)..Click **"Novelle Applications" (bottom bar)/PC-Ord/File/Open/Main Matrix/C: drive/Working/Species06Q.wk1**. There should be four data files:

1. ***Species06Q.wk1***. This is a Lotus 1-2-3 version of the species data required for PC-ORD, with all species as **quantitative** variables. Note: that the Braun-Blanquet cover-abundance values have been transformed to mean percent cover scores. Also all the empty cells have a "0" in them.
2. ***Species06C.wk1***. This is a Lotus 1-2-3 version of the species data required for PC-ORD, with all species as **categorical** variables. The categorical variables are necessary for plotting some of the ordinations.
3. ***Environ06QC.wk1***. This is a Lotus 1-2-3 version of the site-factor data required for PC-ORD, with **mixed quantitative and categorical** variables.
4. ***Environ06C.wk1***. This is a Lotus 1-2-3 version of the site-factor data required for PC-ORD, with all **categorical** variables. The categorical variables are necessary for plotting some of the ordinations.

The two original raw data files (species and site factors) have been reformatted into Lotus 1-2-3 files for PC-ORD. The raw species data file has been transposed so that the rows are now plots and the columns are species, this is necessary for PC-ORD. Complete instructions for creating these files are in the PC-ORD manual. Take a look at each file to see what it is. Be aware that if you want to use PC-ORD for other data sets, you will have to first create the Lotus 1-2-3 files exactly as outlined in the PC-ORD manual.

Load PC-ORD

Double click on the **PC-ORD** icon on the desktop.

Load Main and Secondary Matrices

Click on **file/open/main matrix.** Load ***Species06Q.wk1*** as the **Main Matrix**. Click on **file/open/secondary matrix.** Load ***Environ06C.wk1*** as the **secondary Matrix**. The main matrix is the species matrix and the secondary matrix is the matrix of environmental factors. The main matrix will appear on the screen. You want to keep this matrix without displaying it; click on the left hand button in the upper right part of the main matrix window to minimize the window.

Bray and Curtis plot ordination

Pull down the **Ordination** menu in the **PC-ORD** window and click on **Bray and Curtis**. You will get a "Bray-Curtis Setup" window with several options for distance measures, axis projection geometry, residual distances, endpoint selection method, etc. Click on **OK**

for the default parameters. You will then get a window requesting a title that will appear on all results and graph files for this ordination. Type in something like “Bray and Curtis Ordination for Arboretum”. Click on **OK**. You will then get a “Process” window showing progress on the calculations. When the processing is complete, the “RESULTS.FIL” and the “GRAPH.FIL” files will appear briefly on the screen, followed by an ordination graph message. Click on **OK**; and both the Results and Graph files should be on the screen.

Examine the Results and Graph files

Print the **RESULT.FIL** file by pulling down the File menu in the PC-ORD window; click **Print** and then click on **RESULT.FIL**. The results file contains a variety of information describing the details of the Bray and Curtis method. These include the options that were selected, endpoints of the axes, sum of squares of non-redundant distances, regression coefficients, the amount of information extracted by each axis, and the scores for each plot along three ordination axes.

Do the same for the **GRAPH.FIL** file. The graph file lists the coordinates for each plot on the three axes. Hide the RESULT.FIL and GRAPH.FIL windows by clicking on the left-hand box in the upper right corner of the windows.Click the “Graph” menu in the PC-ORD window. **This will show the “Graph” window with many option buttons. We will go through a few options to get a feel for the possibilities. Each button has a descriptive balloon explaining the function. The explanation will appear by touching the cursor arrow to the button**.

Graph the Ordination

Simple Scatterplot. Click on the **Simple Scatterplot** button. An ordination of sample plots should appear on the screen. This may or may not have the points labeled depending on the previous setting in the "Preferences" menu.

Label the plot numbers. If the plot numbers are not labeled, this can be done by clicking on the **Preferences** button (right hand button in the "Graph" window). A "Preferences" window with many font and format options will appear. Click on **Label points/sites** on the "Format" sheet, and then on **OK**.

Group by vegetation type. Click on the **2nd Matrix** button (upper bar, fourth from left)**/Preferences** (right hand button on upper bar)**/groups/color code categories.** The symbols for the plots can be grouped according to the categorical variables in the secondary matrix. We have several categorical variables. One is "VEGTYPE". Click on the **Groups** button, and then on **VEGTYPE**, and then on **OK**.

Change the legend symbols and colors. In the "**Preferences**" window, click on the **Legend symbol shape and color** button. A window will appear with the columns of symbols and colors for each categorical variable. For symbol 1 select a diamond and the color red. For symbol 2, select a circle and the color cyan. Click on **OK**.

Change the legend names. Click on the title of the legend box at the right side of the ordination plot. Change the title to "Broad Vegetation Types". Click on each category title and change the names to a relevant title (1=Upland Spruce Forest; 2=Lowland Spruce Forest; 3=Upland Birch Forest). Click on **OK**. The labels on the axes can also be changed by clicking on them.

Overlay the species matrix. Plots of individual species cover can be shown within the ordination space by clicking on the **Overlay main matrix button**. This will display scaled symbols for each plot according to the relative abundance of the given species in each plot. By examining the attached scatter plots and regression lines, trends of the species cover along the axes and between the categorical variables can be determined. Other species can be selected by using the menu underneath the displayed species name. Explore the patterns by selecting several species (e.g., PICMAR, PICGLA, POPTRE, SPHWAR, ROSACI). When you are done, be sure to remove group coding under **Preferences**.

Overlay the environmental matrix. The distribution of values for environmental variables can be explored in the same manner as the cover values for the species. Click on the **Overlay second matrix** button. To display groups of values for a given variable, go into the **"Preferences"** menu and code all the values of a given class with the same color. Explore the patterns for several site factors (e.g., PH, CHROMA, GMOIS, VMOIS, SAND, etc.). Do the patterns make sense?

Joint plots. Load the ***Environ06QC.wk1*** matrix. Joint-plot diagrams can be generated by clicking the **Joint plot** button.

Change the font size and locations of the biplot arrow labels. You may want to change the location and size of the biplot arrow labels for easier reading. In the "Preferences" window, click on the **Font** sheet and then click on **Joint plot labels**. Change the font size to 8 point. Click on **OK**. Change the location of the labels by clicking on a label and moving it with the cursor.

Display axes 1, 3 and 2, 3. Click on the **1 vs 3** button to display axes 1 and 3. Click on the **2 vs 3** button to display axes 2 and 3. Examine the trends in environmental variables along each axis. Print the joint plot diagram with axes 1 and 2 displayed.

Calculate the Kendall's tau correlations between the environmental variables and the plot coordinates. Click on the **Correlations with second matrix (∑2)** button. Click **OK** for the descriptive title. The results of the correlation analyses will appear with the a list of r, r-sq, and Kendall's tau for axes 1, 2, and 3. Kendall's tau is a rank correlation coefficient similar to Spearman's rank correlation coefficient. The values closer to 1 or -1 are the strongest correlations. Values above about ± 0.4 are probably significant. Unfortunately, the significance of the correlations are not given in the output.

Delete the "Graph" window. Click on the **x** in the upper right corner of the window.

Dendrogram of Bray and Curtis results

Click on the **PC-ORD** window and pull down the **Groups** menu, and click on **Cluster analysis**. This will give a "Cluster setup" window. Click on **group average**, and then **OK**. A "Cluster" window requesting a descriptive title will appear. Write in "Group Average Dendrogram: Arboretum" and click on **OK**. A "Results modified" window will appear. The results from the cluster analysis will overwrite the previous results from the ordination. If you do not want to save the previous results (chances are you do not want to save them), then discard the previous results. Print the new RESULTS.FIL and examine the dendrogram.

Bray and Curtix species ordination

To do a species ordination in Bray and Curtis, it is first necessary to transpose the Main Matrix so that the columns are plots and the rows are species. This can be done by pulling down the **Modify Data** menu in the PC-ORD window, and then clicking on **Transpose Main Matrix**. Save the transposed main matrix in your folder as **Spec06_Q_Transpos.wk1**. Click on **File/Open/Main Matrix/ Spec06_Q_Transpos.wk1.** Use the transposed matrix as the main matrix. Follow the same procedures for graphing the oridination as for the plot ordination. The ordination space should contain the locations of the central tendencies for all the species.

DCA Ordination

Repeat the above analysis using the **DCA** option under the **Ordination** menu in the "PC-ORD" window. Print and examine the Results and Graph files. Note how many s.d. units occur along axes 1, 2, and 3. Also note the eigenvalue scores for each axis. Graph the DCA ordination. Produce a scatter plot of the plots only (go into the preferences menu to delete plotting of the species points). Print the graph of the DCA joint plot output with the plots only.

III. Homework Exercise (100 points)

Sample (plot) ordinations:

(1) Each person make ordinations of the samples using the following ordination routines:
Emily: Detrended Correspondence Analysis (DCA)
Ken: Nonparametric Multidimensional Scaling (NMS)
Garrett: Polar ordination (Bray and Curtis)
Mark: Canonical Correspondence Analysis (CCA) (Note: for CCA you will first have to modify the environmental matrix ***Environ06QC.wk1***. to contain fewer than 11 quantitative variables. I suggest you use only the environmental variables such as soil moisture and not plant variables as plant height or moss cover.)

(2) Share the ordinations between the groups and visually compare the four ordinations. Using your knowledge of the Arboretum vegetation, which of the ordinations makes the most sense? Do they provide the same basic patterns? If not, what do you think is the reason for the differences?

(3) Plot Axes 1 and 3 using the same ordination routines. Does this provide additional insight to the distribution of plots?

(4) Label all three DCA axes with number of sd units. These values can be found in one of the results files of the DCA output. What does this information provide you?

Species ordinations:

From here on, everyone should use the DCA ordination method. If time permits, explore the species and environemtal plots using NMS, Polar Ordination, and CCA.
(1) *Species ordination plot:* Make a species ordination. This will require first inverting the releve matrix. This will be crowded so try plotting only a few key species. This can be done in the "Modify data" menu by deleting unnecessary variables.

(2) *Species diagrams:* It is also possible to show the distribution of any single species within the ordination space. Click on "**Overlay from the Main Matrix**". A plot of a various sized symbols will be displayed for a given species. The relationship between the

size of the symbols and cover-abundance scores can be interpreted by reference to the regression plots. Make diagrams for the following species:

Emily: ***Alnuvir, Arctlat, Arctuva, Arctrub, Aulapal, Aulatur, Betupap, Carebig, Chamcal, Chamang, Cladarb, Cladran, Cladgra, Cladsqu.***

Ken: ***Dasifru, Dicsco, Empnig, Equarv, Equisci,Eriovag, Galbor, Geocliv, Geraeri, Goodrep, Hylospl, Impanol, Larilar, Ledugro, Linnbor.***

Garrett: Mertpan*, Moehlat, Orthsec, Peltaph, Peltcan, Petafri, Picegla, Picemar, Pleusch, Polistr, Popubal, Poputre, Pticri, Pryospe.*

Mark: ***Rhytrug, Rosaaci, Rubucha, Salibeb, Salipul, Saniunc, Shepcan, Sphaang, Sphacap, Sphasqu, Tomenit, Vacculi, Vaccvit.***

When you are done, make sure you know what each of these species is. Share your plots with the other students so everyone has a complete set. Refamiliarize yourself with these species from Hultén and other floras, and see if the patterns make sense. Examine the diagrams with respect to the species in your sorted table (Labs 8 and 9). You might want to select some key differential species for each type and also some of the ubiquitous species for comparison. For your presentations you may want to import the results from some of the more interesting plots into a drawing program for a more polished product. We can show you how to do this.

Environmental correlations:

(1) *Correlation between environmental variables and 1st and 2nd axis scores:*
Use the Kendall's Tau (rank correlation coefficient) to calculate the correlation of each environmental variable with 1st and 2nd DCA axis. Label the DCA axes 1 and 2 with arrows showing the direction and strength of some of the highest correlations.

(2) *Environmental variable diagrams.* Click on **the Overlay second matrix** button. Keep the plots grouped according to vegetation type so you can better see the distribution of environmental variables for each type. Look at the side plots and see how the environmental variable varies across both axes of the ordination. Also note the size of the symbols on the ordination increase with the magnitude of the variable. The scaling of the size of the symbols varies according to the range of the variable within the data set. So be careful in interpreting the meaning of these differences. Variables with small possibly meaningless ranges for a given variable will have the same range of symbol sizes as variables with large ranges for a variable.

As a minimum, examine the following environmental variables:
Emily: Site moisture, soil moisture, stability, topographic position, disturbance, water cover, soil pH, sand, silt, clay
Ken: Soil color (value, hue, chroma), Organic horizon thickness, soil bulk density.

Garrett: Tree height, cover of deciduous shrubs, cover of evergreen shrubs, cover of mosses.
Mark: Cover of lichens, tree canopy cover, basal area of trees, density of trees. Examine any other variable you think might show an interesting pattern.

(3) *Biplot (joint plot) diagram.* Make a biplot diagram for the DCA ordination for axes 1 and 2. Play with the biplot labeling so that the vectors are clearly labeled. You may want to reduce the number of vectors displayed by increasing r^2. Select the **Joint Plot Cutoof** from the **Graph** submenu or click on the toolbar button the displays the current cutoff value. Which environmental variable accounts for most of the variance in the ordination data? What is the strength of these correlations and are they positive or negative? What other environmental variables have high correlations with the ordination space.

Dendrogram:

Make two dendrograms using a pair of the following methods:

(a) nearest neighbor, (b) farthest neighbor
(c) median and (d) group average
(e) centroid, (f) ward's method
Compare the all the dendrograms from other members of the class. Do they all show the same clustering? Are the results logical? Which best represents the vegetation types as we observed them in the field? What other insights can be gathered from the dendrograms?

IV. Written Report (5 pages plus literature cited, tables, and figures)

Each student should have a xerox copy of the following on which to base the report:
1. **Sample** ordinations produced by (a) Bray and Curtis, (b) DCA, (c) NMS, and (d) CCA with biplot diagrams for axes 1, 2, results file, and graph file for each ordination.
2. DCA **sample** ordination with joint plot for axes 1, 2 and 1, 3, results file, and graph file.
3. DCA **species** ordination with joint plot diagram for axes 1,2.
4. Species cover plots (using the main matrix) for all the species in the DCA sample ordination space (axes 1,2).
5. Environmental variable plots (using the 2nd matrix) for all the environmental variables in the DCA sample ordination space.
6. The six dendrograms.

Each person should take the results from the entire group and write a 5 page report that contains the following:

Introduction: Keep this brief and simply state the overall project goals and objectives of the analyses.

Methods: Briefly describe the methods used in each portion of the ordination and dendrogram analysis. Do not describe the methods used for collection of the field data. The methods should refer to the PC-ORD manual and specify the options that were used for the various ordinations. Also cite the original sources for the various ordination methods (e.g. Bray and Curtis (1956) for the polar ordination method, Hill and Gauch (1980) for DCA. You can find these references in the PC-Ord manual.) There should be separate subheadings within methods for the following:
1. Comparison of the ordination methods (select two from Bray and Curtis, DCA, NMS, and CCA)
2. DCA species ordination
3. Relationship between species and the DCA ordination space. Select 3-4 species that show typical patterns (widely distributed species, species occurring mainly in the Upland Spruce Forests, Species occurring mainly in the Lowland spruce forest, etc.) You might use your sorted table to help you selecting which species to present),
4. Relationship between environmental factors and the DCA ordination space. Show the environmental plots for several of the most important variables. The joint plot diagram and you Kendall's Tau values can help you in selecting these variables.
5. Dendrograms.

Results: The results section should present the results under the same five subheadings listed under "Methods". The text should present the various graphs and point out key features from each set of ordinations. Be sure to point out key elements of each ordination, such as the number of sd units on the DCA ordination. **Notes:** (1) Do not include <u>all</u> the species diagrams in your report, but present examples of species showing some clear patterns as described above. (2) For the environmental variables, also present the most relevant diagrams. Your report should <u>not</u> contain all the ordination diagrams, just ones that represent typical patterns. Please make figures that are neat and readable. You can save the ordinations and tables as jpg files and inport them into Power point or Word and include in the text of your report. Reduce 4-6 diagrams onto a single page to save paper and make easier comparison between the diagrams. Do the same for comparing the four ordination methods. For the environmental correlations, include a table of the Kendall correlations, and describe the meaning of these correleations. Point out differences seen in the results of the two dendrogram methods that you used.

Discussion: Discuss the results from each portion of the ordination exercise, again use subheadings parallel to those in the methods and results section. For the sample ordination, compare the ordination diagrams for at least two of the methods (e.g. B&C and DCA). For the species ordinations, discuss how the species are related to the ordination space. Which have broad distributions and which have restricted distributions? Does the ordination make sense in light of our preliminary vegetation classes?

With respect to the environmental correlations, how is the ordination space related to the environmental factors? Is the first axis strongly correlated with any environmental variable? Are there several environmental variables that are strongly correlated with the axes and which form complex environmental gradients? Does the biplot diagram make

sense? What portion of the ordination space has the highest species richness, and how is this related to the environmental variables. How are our preliminary vegetation types related to the ordination space? How well do the dendrograms depict the preliminary vegetation classes.

All reports should have at least the following tables and figures:

Tables:

(1) A table explaining the meaning of the environmental variable abbreviations and the values used. (This will be given to you, but should be in all the reports.)
(2) A table of the Kendall correlation coefficients for the environmental correlations with the axes of the DCA ordination.
Number the tables, and give them captions that explain the contents. Table captions should go above the tables.

Figures:

(1) Bray and Curtis sample ordination for axes 1 2 with the biplot diagram overlaid.
(2) DCA sample ordination with the biplot overlaid for axes 1, 2 and 1,3, showing the sd units along the axes.
(3) NMS ordination for axes 1 and 2.
(4) CCA ordination for axes 1 and 2. (Note: The first four figures could be combined on one page to make the comparison between the ordinations easier to see. Select two of the four ordination diagrams and compare them.)
(6) Several species isoline plots demonstrating various types of species distributions within the ordination space. Use only a few diagrams that show meaningful patterns. (Again, combine several of the species ordinations into single figures that show distinctive patterns. You might want to go to your sorted table and examine the ordination diagrams for the differential species in each vegetation type.)
(5) DCA species ordination for axes 1, 2. This will be crowded so try plotting only a few key species. This can be done in the “Modify data” menu by deleting unnecessary variables.
(7) Environmental variable isoline diagrams for variables that show clear relationships to the ordination space.
(8) The best dendrogram.
Include clean figures with neat lettering. Each figure should have a figure caption, that clearly explains what analysis was performed and any other information that is necessary to understand the figure. All abbreviations in the figures should be explained in the figure caption. Place the figure captions below the figures. You might want to reduce several of the environmental and species isoline diagrams to a single page for easy comparison. You may also want to show the results from the ordination methods on a single page.

Note: The results from this lab, the sorted table analysis lab, the and the forest structure lab should form the primary data sources for your final presentations.