OBSERVING AND QUANTIFYING BEHAVIOR

Today you will conduct focal sampling and continuous recording using the Jwatcher software package. This tool may be useful for independent projects. You will learn about other sampling and recording rules in future labs but you may want to look at chapter 6 in Martin and Bateson on reserve in the library, or Altmann (1974).

In the past, this lab has used video clips but this year we will observe staged interactions between with live crickets.

While the work today is primarily aimed at familiarizing you with Jwatcher, students should spend the first part of lab considering different hypotheses that could be tested based on similar experimental designs. Each student pair will then determine specific variables for the staged interactions that they will observe today. The crickets will have been housed individually with food and water for 1 week prior to lab. Beyond that there is flexibility for experimental design.

- ☐ There will be both male and female crickets.
- ☐ Crickets can be starved for 24hours prior to lab (requires pre-planning).
- ☐ Crickets could be paired 24 hours prior to observations (requires pre-planning).
- ☐ Morphological data (total body length, cerci length, ovipositor length, antennae length, pronotum width) may be collected.
- ☐ Additional objects could be added to the arena.
- ☐ While pharmacological manipulation is not possible today, it could be incorporated into future independent projects.

PART 0 – DESIGNING THE EXPERIMENT (before lab)

Look at primary literature regarding cricket behavior. You are encouraged to find online resources to determine what questions (beyond those covered in lecture) can be addressed with crickets. Also, are the findings from cricket studies applicable to other organisms? Brain storm ideas on your own or with a lab partner Record your sources in case you wish to consult them again later. Find at least one primary literature research paper that has been published about the staged interaction you have chosen to observe.

Observe the group housed crickets using *ad libitum* recording. (This means that there are no constraints on what is recorded when. You simply note whatever is visible and "seems" relevant.)
Can you tell which are males and which are females?
Can you differentiate aggressive behavior and courtship behavior?
　　　Describe behaviors using objective language.
　　　Categorize behaviors using quantitative measures.
　　　Avoid anthropomorphism and inference.
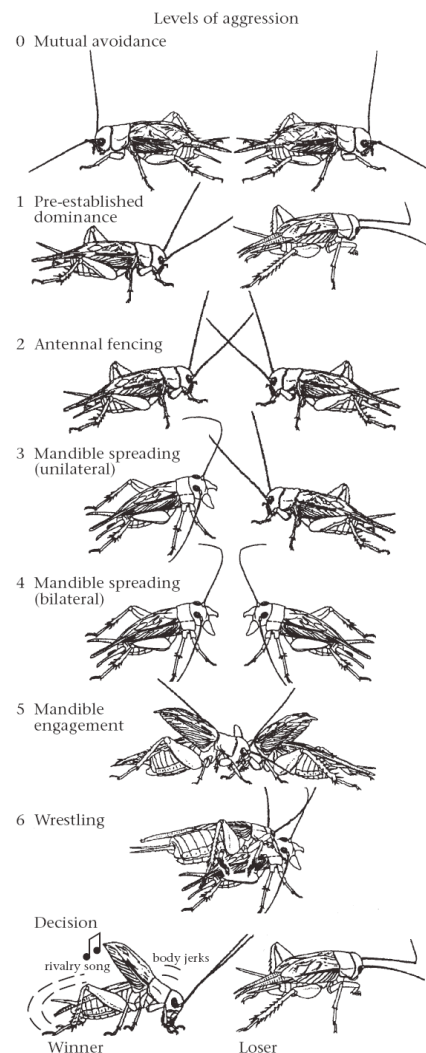Can you identify individual crickets based on morphology?

Establish a protocol and make plans for any pretesting treatment that should be done (i.e. starvation, interaction with another individual, etc.)

PART 1 – DEVELOPING AN ETHOGRAM
Work in pairs. Log onto the computers and the Courses Server as **yourself**.

You should be somewhat familiarized with cricket behavior through *ad libitum sampling* in a group housing and literature review. Based on that knowledge, the available crickets and your interests, student pairs will agree on a relevant hypothesis. Decide on the cricket and experimental characteristics/details that would be required to stage interactions to test your hypothesis. Record this information in your lab notebook with brief explanation about why these characteristics are important. Also consider the optimal details for the experimental design.

Using the literature you read and your own *ad libitum* sampling, develop an **ethogram** to be used for behavioral observations. Remember, an ethogram is a catalog of descriptions of behaviors for a specific species. Your ethogram need not include all behaviors that a cricket may potentially display. Your ethogram need not even include all of the behaviors that crickets display in this staged interaction. Your ethogram should include all behaviors that may be relevant for testing your specific hypothesis. Spend a few minutes discussing with your lab partner, and consulting the primary literature. Record your ethogram in your lab notebook.

Levels of aggression

0 Mutual avoidance

1 Pre-established dominance

2 Antennal fencing

3 Mandible spreading (unilateral)

4 Mandible spreading (bilateral)

5 Mandible engagement

6 Wrestling

Decision
rivalry song   body jerks

Winner   Loser

PART 2 – RECORDING BEHAVIOR

Today you will use an **event recorder** to quantify the behavior of your chosen organism. **Event recorders** are computer programs (or dedicated pieces of hardware) that record keystrokes as they occur over time. In our case, keystrokes will represent behavioral transitions. For instance, when your animal is foraging, you might type an "f", when your animal is grooming, you might type "g", etc.

We will use an event recorder called **JWatcher** to score your video clips. The JWatcher program includes some analysis algorithms along with the power of the event recorder. With these tools, you will be able to calculate not only the number of events but also the time allocated to each of the behaviors in your ethogram. This can be used to obtain an accurate time budget. The full JWatcher program and manual is freely available on-line at: www.jwatcher.ucla.edu, and it will also be available in class.

This chart describes JWatcher work flow.
By the end of lab today you will:
A) Create a Global Definition File (*.gdf) which will serve as the "ethogram" and assign keys to each behavior.
B) Create a Focal Master File (*.fmf) which includes the behaviors (or a subset thereof) from the global definition file. This file also instructs JWatcher to prompt users for additional data about each observation during data collection.
C) Record behavior. The data file (*.dat) file is created automatically during each observation. It contains the additional notes about the observation as well as the raw data that you have recorded.

D) Create a Focal Analysis File, which specifies how data files (.dat) will be analyzed and specifies the results to be reported.
E) Quantitatively analyze each *.dat file to produce a results file (*.xx.res) that can be opened in Excel. JWatcher is capable of, summarizing several data files (*.rsm), quantifying conditional behaviors, testing observer reliability, and performing complex analysis (behavior combinations, sequence analysis, transitions matrices, Markovian analysis), some of which you will explore today.
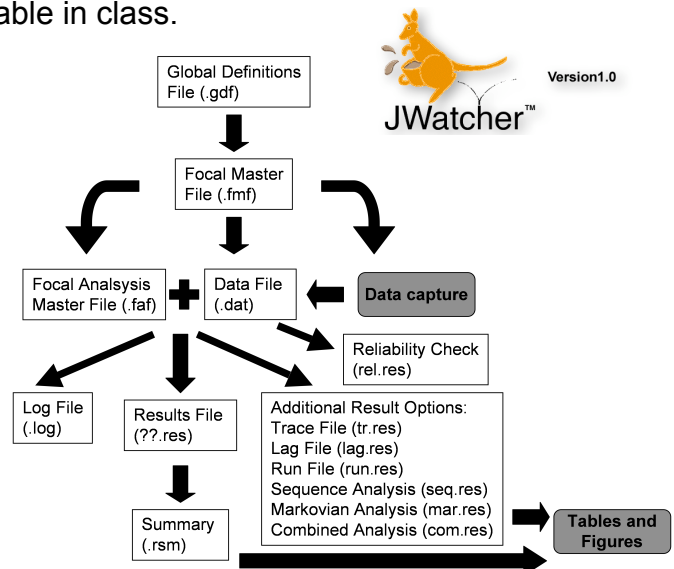
As you work on the computer today, remember to use your lab notebook as a record of the steps you take, the computer files that are generated and used, ideas, hypotheses, or difficulties that you encounter. You should get through part 3 of this exercise during lab, part 4 may take longer than is allotted in one lab section but can be complete outside of lab (recording in your lab notebook still). Part 5 is provided for potential independent projects or students who wish to do more with JWatcher. The **assignment** for today is described on page 17.

2A) Defining your Ethogram in JWatcher

Based upon the written ethogram you have developed you will "define" a JWatcher ethogram. Some people prefer to use **logical key locations**, others prefer to **use logical letters** that match the behavior description. Confer with your partner and decide on a code. You will have a chance to practice and can change the code later.

1. In the **Applications** folder, find the **JWatcher_V1.0 folder** and double click. Now double click on the **JWatcher icon** (black square, with a gold J and gray W) to launch the JWatcher program.
2. When JWatcher first opens, you will be in the Data Capture tabbed window. Click on the **Global Definition tab** to change windows. The Global Definition window is where you will specify your ethogram by assigning key codes to each behavior.
3. The Global Definition window is divided into two sections: one to define behaviors and another to define modifiers. We will be defining behaviors only.
4. Click on the **Add row** button in the top section to begin assigning key codes to behaviors.
5. It is up to you and your partner to choose the key strokes that correspond to each behavior. You may use **only one single character** per behavior. Try to choose something intuitive and easy to remember, such as **a** for **a**ggression and **f** for **f**orage, etc. **Note that JWatcher is case sensitive**: f will NOT be recorded the same way as **F**. Because it is difficult to switch between upper and lower case, for this exercise, **use lower case letters**. Record the letters that you choose for each behavior in your lab notebook.
6. Type in the single character code in the **Key Code** box. Then either tab or click into the adjacent **Behavior** box to type in the corresponding behavior. You may also include a more lengthy description of each behavior in the **Description** box but remember to avoid anthropomorphisms or "intent" in your descriptions whenever possible.
7. Click on the **Add row** button to continue adding each behavior.
8. When you are done adding all your behaviors, click the **Save** button in the lower right corner of the window.
9. To Save your new Global Definition file to the desktop click the **house icon** in the upper right part of the Save As window. Now double click on the **Desktop** folder icon for local user. Name your file (**gdf_exp_initials**) by typing into the **File Name:** box in the lower part of the window. The extension .gdf will automatically be added when you click **Save (**this stands for **G**lobal **D**efinition **F**ile).

NOTE: When starting a study, it is often difficult to decide how many and which behaviors to record. Your global definition file may include behaviors that you do not end up using in your observations or in your analysis. For now, include all behaviors that you think you might want to quantify.

NOTE: Generally it is good to include a key code for "out of sight" and also one for "other". Think about why these are important categories.

2B) Specifying Focal Details for a JWatcher Observation Project
Based upon your global definition file you will now create the "**focal master file**"
(**.fmf**). This file specifies what data (behaviors, and additional information) will be
collected during each focal observation.

1. Click on the **Focal Master** tab at the upper left to change windows. Be
   careful, as it is right next to Focal Analysis Master. You may find yourself
   in the **Test Details** page (see bottom left tabs). If so, change pages by
   clicking on the **Define Codes** tab.

2. The **Define Codes** page shows the ethogram to be used when scoring
   data for this particular project. Currently it should be blank.

3. To import your pre-existing ethogram from your .gdf file, click on **New**
   (bottom center of the window) and a new selections window will open.
   You should see your .gdf file that you just made (**gdf_exp_initials.gdf**). If
   not, you will have to navigate back to the desktop by clicking the house
   icon and then the desktop icon. **Select** your global definition from the list
   in the top part of the window. Click **Open** to load your ethogram. If you
   decide not to use specific behaviors in your global definition file, use the
   delete_row button to eliminate those. While JWatcher allows you to add
   rows here, it is important to remember that your global definition file is you
   recorded ethogram with the concise behavioral description. It is good
   practice to keep the focal master file consistent with the global definition
   file and make any required changes directly to your .gdf file. For good
   record keeping, the .gdf file should not be saved under the same name if it
   is changed, rather use a new name (ver1.1 or the date).

4. Next, using the lower left tabs, change to the **Test Details** page. You will
   score each staged interaction for **the same amount of time**. In the
   Duration boxes, type 0 in the hours (**HH**) box, and the number of minutes
   (**MM**) box, and 0 in the seconds (**SS**) box. If you expect the interactions go
   be of varying lengths set the time to the maximal amount of time.

5. Finally, in the lower left tabs, change pages again by clicking on
   Questions. This window is where you may define the variables that will be
   associated with each focal sample file, such as individual ID, age, sex,
   time of day, name of observer, location (if you are in the field), etc.. For
   today, you will decide what information you want associated with your raw
   data. You may specify up to 6 questions.

6. Click **Save As** to save your Focal Master File (**fmf_exp_initials**). Make
   sure that this file is being saved to the **desktop** where you saved your .gdf
   file (though you will not currently see that file name). Again, the proper file
   extension for a **F**ocal **M**aster **F**ile (.fmf) will automatically be added for you
   when you click **Save.**

2C) Scoring Behavior with JWatcher

Now you are ready to begin scoring behavior.

Change windows by clicking on the **Data Capture** tab (upper left).

1. In this window, click on the file navigator icon (**it looks like a sheet of paper**) to the right of the **Focal Data** box in order to name the data file you will be creating. Navigate to the desktop by clicking the **house icon** then double click the **desktop** icon.
2. Type in the name of your new file **dat_exp_initials_datetime** in the **File Name** box and click **Open**. Keep the names simple. **(Jwatcher sometimes has problems with long names and spaces in names so try to keep file names short and use underscores for spaces)**
3. Specify your Focal Master File (fmf_exp_initials.fmf) by clicking on the file navigator icon (**it looks like a sheet of paper**). You may need to navigate to the desktop.
4. Select your **.fmf** file. Click **Open.**
   (Remember that this file contains the ethogram along with additional specifications such as the duration for recording the focal observation.)
5. Click the **Next** button at the bottom right to tab into the next page.
6. Answer the questions by typing into the box below the question and click the next button to advance.
7. Click the **Next** button.

If you wish to see a list of the behavioral codes on your computer screen, click the **Behaviors** box in the upper right corner of the JWatcher screen.

8. You should be looking at the **JWatcher – Data Capture – data_yourfile.dat** window.
9. You are now ready to start your first staged interaction. Gently introduce your two crickets to the arena.
10. Click the **Start** button in the center of the Data Capture page.
11. Immediately type the key code representing the behavior the focal subject is currently engaged in.
12. **Whenever** the behavior changes, type the key code for the new behavior.
13. JWatcher will time out at the time specified in your focal master file or you can end the recording.
14. JWatcher will automatically save your data in the **.dat** file that you named above)

If you made a data entry mistake, look at the timer and make a mental note of it. You can fix it later.

15. Stage a second interaction but have your partner record the behavior.
16. give the new data file a new name that ties it to the second interaction.

Remember that even though you are working on the computer, you should be recording critical steps, difficulties encountered, as well as the name and location of data files and analysis results. At the end of the day, your lab notebook should serve as a record of what you have done to reach specific conclusions about your organism's behavior.

2D) Editing your data

If you made mistakes while recording behavior you should have noted those errors in your lab notebook when they occurred. It is always good practice to edit your data immediately after scoring while the details are fresh in your mind. Use your lab notebook to indicate which errors have been corrected.

1. Click on the **Edit Data** tab.
2. Use the **Open** button to select the *.dat file that contains errors.
3. On the **Key Codes** tab you can **Insert Rows**, **Delete Rows**, edit the time (with elapsed (ms) or Time) and change key codes.
4. Save your data file using the original name.

2E) Checking Your Reliability

Eliminate this step but consider why it is important for a research project.

If you have several assistants collecting data for you, it would be necessary to demonstrate that they are all coding behaviors in the same way and that there is no confusion between the behaviors defined in the ethogram. If your assistants are not reliable you would want to offer further training before sending them out in the field. If you are collecting all of your own data, it would be helpful to demonstrate to yourself that you are accurately able to code the behaviors. If you are not reliable you would want to redefine your behaviors, choose different keys, or record fewer behaviors.

1. Click on the **Analysis** tab and select the **Reliability** page.
2. Select the two data files you wish to compare (simultaneous observations of the same individual).
3. Name the reliability output file and indicate where this file should be created.
4. Click **Calculate Reliability** to perform the calculations. The file extension. rel.res will automatically be added to your file name and this comma delimited text file can be opened in excel.

There are three main parts to this file:

1. The **confusion matrix** tabulates the number of agreements between two data files, broken down by key code. Relatively large values found in an off-diagonal cell would indicate a systematic bias that needs to be addressed.
2. The **statistics section** tabulates results from the confusion matrix. The table includes the percent agreement and kappa coefficient. Cohen's Kappa adjusts the percent agreement by incorporating a correction for the probability of agreement due to chance. It is based on the percent agreement for keycodes without respect to the time at which those key codes occurred. For more discussion of reliability measures see Martin and Bateson pg 120 - 124.
3. The **list by line** is a simple juxtaposition of the two data files.

If you and your partner did not score the behaviors reliably, discuss the cause and make adjustments in your ethogram if necessary.

Read ahead through the analysis procedure before coding anymore behaviors. You may decide to add or subtract key codes from your ethogram depending upon how you intend to analyze your data. If this were research you intended to publish you would expect to go through several iterations of observation, recording, analysis before settling on the most appropriate protocol.

Part3 ANALYZING BEHAVIORAL DATA WITH JWATCHER
Once you have scored several observation periods, you are now ready to use JWatcher's analysis algorithms. Analyzing data files can be a complex process and it is necessary to carefully think through the relationships between the different behaviors you have recorded (in fact it is best to do this before collecting a lot of data).

For example:
Are all behaviors mutually exclusive, or can your organism be doing two things at once?
Are all behaviors discrete events, or are they states (such as "resting")?
Are some behaviors modifiers of other behaviors? (such as aggression which might be "modified" by an intensity score)
Are there behaviors that change their meaning in a conditional fashion? (such as an aggressive display when an animal is in its own territory on in another territory)
Are you interested in behavioral sequences or transitions?
Are you interested in the interval between behavioral events?
Are you interested in the lag time between two specific behaviors?

One strength of JWatcher is that you can re-analyze behavioral data in many different ways. You are encouraged to explore the following options and consult your professor, the TA, or the manual for assistance. Consider what additional questions could be addressed with the data you have collected.

3A) Basic Analysis (For today, read, but do not execute, steps in grey.)
For a basic analysis you must first create a file called a **focal analysis master file (*.faf)**
1. To create a **focal analysis master** file, click on the **Focal Analysis Master** tab in the upper left menu to open the window.
2. Click on the **New** box, at the bottom center.
3. You must now load your project's ethogram by choosing the same focal master file that you used to capture data (**fmf_exp_initials.fmf**) (If you do not see your *.fmf file currently displayed, then navigate to the desktop by clicking the house icon and then the desktop icon.) Select your file and click **Open**.
4. Once your ethogram has been loaded, immediately change pages by clicking on the **Modifiers** tab on the bottom series of tabs. Today you probably didn't include modifiers since we haven't discussed it until now,

but this is where you would indicate which key codes would be followed by a modifier.

5. Next change pages by clicking on the **States** tab at the bottom. States are behaviors that occur over time. Some states are mutually exclusive (such as eating and sleeping). This will greatly affect the time budget calculations. Only behavioral states that are mutually exclusive will stop the timer for the previous behavior. Use the check boxes to specify which behaviors will turn each other off.

6. Open to the **Time** tab. This is where you determine the time during which behavior is analyzed. If all of your clips are exactly the same duration, and you always start recording at the beginning of the clip you can use the default "**Score from the beginning of the session**". However, if it takes ~ 10 seconds to start recording once you start the clip you may want to use a **Fixed offset**. Or you may want to start from the first behavior recorded, or you may want to start the analysis with a specific behavioral event (such as awake). The "**Time bin duration**" specifies how the observation period will be subdivided. (0 means the entire observation period is one bin). Time bins might be useful if you were studying a response over time after a specific treatment. They may also be useful if your observation periods are not a standard duration.

7. Click on the **State Analysis** tab (Note: there is also an Analysis tab above and a States tab below, you want **State Analysis**). If you are interested in creating the most accurate time budget for the interaction, it is appropriate to use "all durations", however if you are interested in precise timing of behaviors it is most appropriate to select "**natural duration**" (this will be explained in lab, or in the manual). If you are interested in the interval between specific behavioral states or events you will also select one of the interval options. While it is possible to select all options and calculate all measures you may choose to run analyses separately in order to keep output files to a manageable size. If your cricket was out-of-sight for some part of the observations, then JWatcher can correct for this by calculating the proportion of time in sight.

8. Look at the **Exclusions** tab. This is where you could decide to ignore some of the behavioral events that you recorded, but be careful, this can produce erroneous results if you do not adjust the mutually exclusive behaviors accordingly. Here you can also subtract certain behaviors from the time budget calculation (for example you may choose to subtract all of the time that an animal was asleep and create a time budget only for awake behaviors). Likely you will ignore this for today.

9. The **Conditional States** tab allows you to specify combinations of behaviors as simultaneous streams. These behaviors cannot be mutually exclusive behaviors (as defined in the States tab). Conditional states are indicated as "a|b" which provides the statistics for a the state "a" given that "b" is already on. You can specify " a|b|c " and Jwatcher will calculate both the proportion of time that "a" is on given that both "b" and "c" are both on and also the proportion of time that "a" and "b" are on given that

"c" is on. (a more clear explanation will be given in lab if you are interested in this type of analysis for your observations).
To define conditional states you can either:
Add row and **type** your condition
               or
click **Conditional states** to see non-mutually exclusive states.

10. The **Events** tab allows you to specify which behaviors should be counted as an instantaneous event, these will be counted but will not contribute to the proportion of time, thus they will not be a part of a time budget. JWatcher permits a behavior to be simultaneously analyzed as an event and also a state.

11. The **Conditional Events** tab is similar to Conditional states except that the first behavior must be an event rather than a state, and therefore a "rate" can be calculated (see Events tab). Again you can only use non-mutually exclusive behaviors. (Likely these conditional analyses are beyond the scope of your behavioral observations today but the goal is to appreciate the complexity of behavioral analysis that is possible).

12. When you are done exploring your options and have settled on a basic analysis method, click on the **Save As** button. Specify the location to save your new **f**ocal **a**nalysis **f**ile, and give it a name (**faf_exp_initials_version1**) by typing into the **File Name**: box. Navigate to the desktop if necessary and click **Save.** Again, the proper file extension **.faf** will automatically be added.

13. The Summary button at the bottom right will open a text window containing all the specification in the current focal analysis master file (*.faf). JWatcher will provide "warnings" for any parameters that are likely to cause problems. The summary text can be copied (using shift/control/command/4 to copy and create a new file from clipboard in preview) to be saved or printed out.

3B) Analyzing Raw Data Files
You are now ready to Analyze your results. JWatcher allows you to analyze files one at a time or in a batch processing mode.

1. To generate results files, click on the **Analysis** tab in the center upper menu.

2. Use the file navigator icon (looks like a piece of paper) to select your **faf_exp_initials_version1.faf** file. Remember that the focal analysis file specifies the types of analyses to be calculated. Click **Open**.

3. Use the file navigator icon (it looks like a piece of paper) to select the data file (**dat_exp_initials_timedate.dat**) to analyze.
   OR
   Use the folder navigator icon to specify an entire folder with multiple *.dat files to be analyzed in a parallel fashion..

4. Specify the **Desktop** as the appropriate location as the destination for the results file by clicking on the folder icon and then click **Open** (note: this file

is automatically created and named, you are just verifying that you know where it will be placed. However you can give the folder a name that is more informative than simply "results").

5. Select the **Print results for all behaviors** button. This will ensure that all of your output files have uniform layout even is not all behaviors occurred in all observations.

6. Click the **Analyze** button at the bottom of the file window to analyze your data.

7. If you get a warning message telling you that there were unrecognized key strokes in your file. These will be ignored.

8. Notice that JWatcher creates a log file. This is akin to a lab notebook. It will be saved in the same folder as your results files.

3C) Viewing the Results Files
You can use Excel to view the results files.
There are two results files, *.cd.res, and *.tr.res.
>   The ***.cd.res** file has the quantitative results.
>   The ***.tr.res** has a file that you can open and graph the behavioral traces.

View the ***.cd.res** file by opening it through **Excel**. Excel will not automatically recognize JWatcher files; you will need to tell Excel do it. The ***.cd.res** file is a comma delimited text file.
>   Launch Excel
>   Use **File /Open** and navigate to the desk top.
>   At the top center of the Open dialog box **Enable: All Documents** needs to be set.
>   Select: your analyzed results file
>   Click **Open**
>   Select the **Delimited** radio box
>   Click **Next**
>   Check the **Comma** box
>   Click **Next**
>   Click **Finish**

Once opened, you should see a list of the behavioral codes and several summary statistics for each behavioral code. Depending on the focus of your research you may be interested in different measures of the behavior.

Each of your result files will relate to just one of your video observations.

If your cricket never went out-of-sight, then proportion of time and proportion of time in sight should be the same. The proportion of time in sight for your behaviors is the time budget.

It is a good idea to check your results files and familiarize yourself with their contents before going on to more sophisticated analyses. JWatcher includes

several algorithms for summarizing and quantifying behavioral data, however, in your own research you may want to perform statistical tests that are not a part of the JWatcher package and you would use these results to do that. For analysis outside of JWatcher it may be necessary to reformat results either by cutting-and-pasting in Excel or using your own computer script (possibly written in PERL). This is beyond the scope of today's lab.

3D) Summarizing Analysis Results
JWatcher includes algorithms to provide summary statistics for the analyzed result files. You may also summarize subsets of your observations, for example you might want to summarize the results for females separate from males.
1. Click the **Summarize Results** tab.
2. Select the folder containing your results files to be summarized. All files to be summarized MUST have been analyzed using the same focal analysis master file (.faf)
3. Choose a file name and folder for the summary.
4. Generally, **Analyze Results in Rows** provides a format that is easiest to work with in excel.
5. Choose the appropriate summary statistics
6. Click **Summarize Results Files**.
7. The resulting file is a comma delimited file that can be opened in Excel.
8. Use this file to make graphs, or figures that are informative regarding your hypothesis. A pie chart works well for a time budget, though a bar graph allows you to include error bars. It is up to you to present you data in the most informative and effective format.

Part 4 SEQUENCE ANALYSIS WITH JWATCHER

For many behavioral questions it is sufficient to calculate time budgets or to study the frequency of events. However, these measures do not tell us about the structure of behavior. Another important way to study behavior is to look for temporal patterns in a sequence of behaviors. For example, animals often engage in a series of behaviors that are quite stereotyped (remember Niko Tinbergen's studies and fixed action patterns). Also behavior can be analyzed for structure that communicates meaning, such as bird songs, or complex ritual displays of aggression.

Open the Sequence Analysis window
You will see 6 tabs, one for **General**, in which the focal master file is specified and 5 additional tabs for different types of sequential analysis. Today you will focus on **Sequential Analysis** for simple transitions. Sequential analysis requires larger datasets so you may need to combine several of your observation files if your animals were not very active. This will provide sufficient input to conduct Sequence Analysis. In your lab notebook, record which files have been combined and indicate whether this is inappropriate and why (if your clips all come from the same individual, at roughly the same time of day, doing the same behavior it may not be inappropriate).

Combining data files
Using Excel or Text pad, open the *.dat files that you wish to combine
Copy Paste your data one below the other changing the End Of File to a "break" (use the character that codes for "out of site") save this file with a new name and the ending .dat (**exp_initials_seq.dat**)

#Begin Data	
479	a
21512	b
23865	a
34545	c
60000	EOF

#Begin Data	
238	b
1555	c
23880	d
60000	EOF

#Begin Data	
129	a
2500	d
36544	a
60000	EOF

#Begin Data	
479	a
21512	b
23865	a
34545	c
60000	**X**
238	b
1555	c
23880	d
60000	**X**
129	a
2500	d
36544	a
60000	EOF

The **Sequential Analysis** tab can be used to count the number of transitions between each pair of events found within each sequence. Furthermore JWatcher will calculate the probability that an event pair occurs in the sequence as well as the probability with which a specific transition occurs. JWatcher will also calculate a z-score and p-value that tells us which transitions occur more or less often than expected by random chance. All of this information can be used to draw a kinematic diagram.
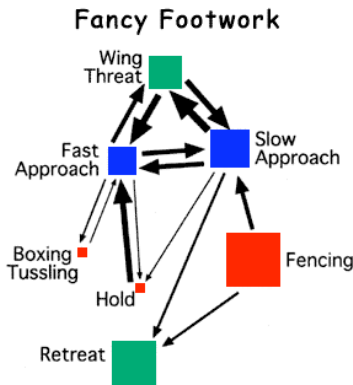
Fancy Footwork

Figure 2: Example of a Kinematic Diagram (also called a behavioral state transition diagram) from *Drosophila* aggression. Aggressive (red) neutral (blue) and submissive (green) behavioral time is indicated by box size. Frequency of transitions between behaviors is indicated by arrow width.
Kravitz lab

4A Transition Matrix

Set the general parameters for sequence analysis.
- Click on the **General** tab
- open your focal master file
- Decide which behaviors you are interested in analyzing for transitions. To keep it simple, select only events and not states. Think about how this will affect your transition matrix.

Define the sequence analysis to be performed.
1. Open the **Sequence Analysis** tab.
2. For **Focal data file(s) or folder** select your combined data (**exp_initials_seq.dat).**
3. Indicate the Desktop location to create a folder with the results.
4. Specify **Lag** for analysis (for a sequence ABCD, Lag1 = AB, BC, CD and Lag2= AC, BD). It is logical to look at lag1 for most simple transition sequence analysis.
5. For the desired output select:
   - **Observed Matrix**,
   - **Transitional Probability Matrix** and
   - **Expected Z-score and p-value**.
6. The observed Matrix is a count of how many times each transition occurred.
7. The Transitional Probability Matrix reports the probability of each transition normalized to the number of occurrences of the first event of the transition pair ("row total").
8. The Z-score is a measure of the degree to which the observed transition occurs more or less frequently than would be expected by chance and is used to determine the p-value.
9. The statistical analysis necessary to compare two different kinematic diagrams is complicated and beyond the scope of today's lab.

4B Create a Kinematic diagram
1. open the seq.res file in Excel
2. adjust the columns widths so you can view your data easily.
3. Print this out to put into your lab notebook.

In PowerPoint (or another graphics program)
1. Create a node for each behavior. (color code these if appropriate)
2. Create arrows between these behaviors

- Depending upon how many behaviors you have scored, it may not be possible (or at least not pretty) to include an arrow for every transition.
  - you may choose to omit transitions that rarely occurred.
3. Use line width to indicate the relative frequency for each transition.
4. Place an asterisk next to those transitions that occurred more often than would be expected by chance (look at the p-value in your sequence analysis file and decide what your threshold for significance is).
5. Save this file as a Powerpoint presentation but also as a jpeg file (so that you can easily insert it into a word document later)

Below I have included a brief description of other available sequence analyses in JWatcher. These descriptions are not intended to provide sufficient instruction for full analysis. You should read the handout and look at each tab to appreciate the sophistication of JWatcher's capability. Use the JWatcher manual if you are interested to try further analysis of your data.

Part 5 ADVANCED ANALYSIS IN JWATCHER
The following analyses are beyond the required scope of today's lab. However, if you are interested to try them please consult the manual for more detailed instructions.

5A Markovian Analysis in JWatcher.
Markovian analysis examines the overall structure of a series of behaviors, as opposed to specific pairs that are analyzed with transition matrices. Markovian analysis determines whether serial dependencies exist within the sequence. For example in the English language the letter "q" predicts that the following letter will likely be "u". This is an example of a first order Markovian model: one event determines the event that immediately follows. A second order Markovian model is one in which an event is predicted by two immediately preceding events. A second order process is the limit of analysis available in JWatcher. (note: many protein structure and gene finding algorithms rely on more complex Markovian models).

5B Substituting or Combining.
After viewing your data you may decide that your ethogram was too detailed and you would like to combine 2 or more behaviors (for example you might decide to count both bite and chase as "aggression"). Note that combinations may generate a series of repeated behaviors that could affect state statistics.
1. Click the **Combinations Master** window tab.
2. Click **New**
3. Select the **focal master file** that you want to modify
4. Use **add row** in order to create a new key code and behavior name for the combination and the key codes that are to be combined (Note: this key code is not part of your global definition file. It is important to track such changes).

5. Save this new focal master file. (**newname.fmf**)
6. Click **Next** to open the **Combine Key Codes** window
7. Select the focal data files or folder for which you want to combine behaviors. This will create new data files and leave the originals in place.
8. To analyze the new combined data files you will need to create a new focal analysis master file (*.faf) using the codes defined in the newly created focal master file (*.fmf).

5C Alternate Sampling and Recording with JWatcher.
Time Recording
During Bio102, in addition to **Continual Recording**, we used a **Time Recording** in which one behavior was recorded when the timer beeped every 10 seconds. We recorded only the behavior that was in progress at the beep and omitted behaviors that occurred before or after the beep. We used a pencil and paper rather than an event recorder for Time Recording. Time Recording is a way of condensing information. It does not give true frequencies nor an absolute time budget. Therefore, Time Recording is most appropriate when the duration of a behavior is substantially longer than the time interval. Also, because time recording is less labor intensive, it is often possible to record a greater number of behaviors. Alternately, one could record a greater number of individuals recording a behavior for one of 12 animals every 15 seconds, thus sampling the entire group in 3 minutes. One could use JWatcher for Time Recording. Although Time Recording with JWatcher would not take advantage of the time budget power of the event recorder, it would have the advantage of directly recording behavior to a computer file. The analysis for this data would need to be modified.

Behavior Sampling
With either Continuous or Time Recording one could employ a **Behavior Sampling** technique. In Behavior Sampling the observer records each occurrence of one or a few types of behavior either for an individual or for a group. This is not an appropriate measure with which to generate a time budget, but it can be very useful for recording rare events, or focusing on a few behaviors that happen very often. Ideally, Behavior Sampling for a group would include information about which individuals are engaged in the behavior of interest, however in field studies or with unmarked individuals it may not be possible to identify individuals. In JWatcher one could use "modifiers" to designate which individual, or simply the sex of the individual engaged in the behavior of interest. Keep this option in mind for independent projects.

CONCLUSIONS:
Summarize today's work in 1 page that includes the following items. Attach this to your lab notebook and hand in a copy at the beginning of lab next week.

1 paragraph introduction including
- the area of behavior you are observing (mating, fighting etc.)
- the hypothesis to be tested

1 paragraph methods including
- the characteristics of the two crickets
- the number and length of interactions
- the behaviors that were analyzed (may be a subset of the ethogram)

1 (or more) summary graph(s) of your data that relate to your hypothesis.
- include a figure legend stating what the dataset is, how it is summarized, and what the graph tells us. (Do you present state duration or number of events? do you present averages with standard error bars, or do you present a scatter plot of individual values? Do your data demonstrate that behavior A is more common than B?)

1 kinematic diagram of the observed behavior.
- briefly describe the "take home message" from this diagram
- include a figure legend indicating the significance threshold and any other information necessary to interpret the figure.

Concluding remarks interpreting your data with respect to your hypothesis.
1-2 sentences suggesting future research in this area using similar behavioral observations.

also

Every student team should create a folder on the courses server in which they will save the video clips, a global definition file (.gdf), a focal master file (.fmf), and a focal analysis file (.faf), all raw data files (.dat), the analyzed result files (cd.res), the summary (.rsm), and the sequence analysis file (seq.res), the kinematic diagram, and the summary document described above.