

# ECOLOGY FIELD COURSE

## BIOC52H3



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# SYLLABUS

## Course Description

This course offers the unique opportunity to experience hands-on learning through informal natural history walks, group projects, and individual research projects in a small-class setting (cap at 16 students). The objective of this course is to introduce you to some of the most commonly used field research methods in the study of ecology. Also, you will learn how to design a research project, test a hypothesis and write a scientific paper or poster.

You will study ecological questions related to plants and animals in their natural settings. The major topics will look at the cost associated to male and female reproduction in plants, how succession and the intermediate disturbance regime shape plant diversity, how island biogeography influences plant reproduction, whether and how soil diversity is influenced by plant cover, and how stomatal density can be influenced by ecological conditions a plant individual or the leaf finds itself in.

We will investigate these questions in a wide array of environments from early successional vegetation in old fields, flood plains to closed forests. Most of the field work will happen in the Highland Creek Ravine adjacent to UTSC's campus. To do the field work on one of the projects, we will take a mandatory overnight trip to the Koffler Scientific Reserve KSR, the field station of the University of Toronto in the Oak Ridges Moraine. While field outings will often happen with the whole class, be prepared to venture out to collect data also in groups of two.

## Time and Location

Course lecture time: Mon, 10 am – 3 pm

Location lectures: SW242

Location computer lab: TBA

## Course instructor

Ivana Stehlik

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Email: [ivana.stehlik@utoronto.ca](mailto:ivana.stehlik@utoronto.ca)

Office hours: if my office door is open, please feel free to drop by anytime (open door policy)! My best times to talk to you are Mon 3 - 4, Tue 10 - 12 and Thu 10 - 12, however, I appreciate if you let me know that you want to come. If these times don't work for you, fire off an email to schedule a meeting, I will do my best to accommodate your schedule!

## Course Website

Class information will be provided on the course website on the U of T Portal: [portal.utoronto.ca](http://portal.utoronto.ca). You will need your UTORid and your password to access the site. Please refer to instructions on how to access the course website on blackboard using the information in:

<http://www.portalinfo.utoronto.ca>

### What is needed in the course

- All fieldwork is rain or shine, thus you need sturdy, rain- and cold-proof cloths. In case of rain, rubber boots are highly advantageous, as they will also protect you and your nice shoes from mud (off-trail bushwhacking!). As the time allocated to field work is up to 5 hours, anything not really rainproof will leave you MISERABLE under cold and/or rainy conditions.
- Weather-proof notebook (“rite in the rain”), available from the course instructor for approximately \$8.
- Brown-bag lunches: the course runs 10AM – 3 PM and while time will be allocated to eat, it will mostly not be possible to return to the food court to buy food – often we will eat our lunch outdoors

### Evaluation

Data collection and mailing of data to the TA	5%
Quizzes on required readings (1% each)	5%
4 project reports	80%*
Poster	10%

\*one report (the worst) counts 10%, two count 20% each and one (the best) counts 30%

### Penalty for late submission

There will be a penalty of 10% per day for assignments received late. Unless there are extenuating circumstances (e.g. medical reasons with an official UTSC doctor’s note), a mark of zero will be applied to assignments submitted one week late or more. Workloads, malfunctioning computer equipment, lack of access to data and texts are not legitimate reasons for late submission. Let the course instructor know before the due date if you know you cannot hand it in.

### Required reading (links to the reading can be found on the blackboard course web page)

#### •Project 1 (Jack-in-the-pulpit)

Warner, R. R. 1988. Sex change and the size-advantage model. *Trends in Ecology and Evolution* 3: 133-136

#### •Project 2 (intermediate disturbance hypothesis)

Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. *Science* 199: 1302-1310

#### •Project 3 (milkweed)

Honnay, O., H. Jacquemyn, B. Bossuyt and M. Hermy. 2005. Forest fragmentation effects on patch occupancy and population viability of herbaceous plant species. *New Phytologist* 166: 723–736.

#### •Project 4 (soil biodiversity)

Barrios, E. 2007. Soil biota, ecosystem services and land productivity. *Ecol. Economics* 64: 269-285.

#### •Project 5 (stomata)

Woodward, F. I. 1987. Stomatal numbers are sensitive to increases in CO<sub>2</sub> from pre-industrial levels. *Nature* 327: 617-618.

### **Academic integrity policy**

According to Section B of the University of Toronto's *Code of Behaviour on Academic Matters*, it is an offence for students to:

- use someone else's ideas or words in their own work without acknowledging that those ideas/words are not their own with a citation and quotation marks, i.e. to commit plagiarism.
- include false, misleading or concocted citations in their work.
- obtain unauthorized assistance on any assignment.
- provide unauthorized assistance to another student. This includes showing another student completed work.
- submit their own work for credit in more than one course without the permission of the instructor
- falsify or alter any documentation required by the University. This includes, but is not limited to, doctor's notes.

Violation of the Code of Behaviour on Academic Matters will force the instructor to provide a written report of the matter to the Chair/DeanProvost's and a penalty according to the U of T's guidelines on sanctions will be put into place.

### **Submission of reports to Turnitin**

Students will be asked to submit their reports to **Turnitin.com** for a review of textual similarity and detection of possible plagiarism. In doing so, students will allow their essays to be included as source documents in the Turnitin.com reference database, where they will be used solely for the purpose of detecting plagiarism. The terms that apply to the University's use of the Turnitin.com service are described on the Turnitin.com web site:

<http://www.utoronto.ca/ota/turnitin/ConditionsofUse.html>

Turnitin.com is most effective when it is used by all students; however, if and when students object to its use on principle, the course offers a reasonable offline alternative. The student will then be asked to meet with the course instructor to outline and discuss the report before its final submission to demonstrate the process of creating the report according to the academic integrity policy.

### **Communication policy**

Students are required to regularly and often check their UTOR email to receive announcements relating to the course. To inquire about course-related issues, students are strongly encouraged to solely use their UTOR email, as hotmail or other email providers are spam-filtered on a regular basis. It is the responsibility of the student to make sure his or her email reaches the instructor.

**Overview on course activities and deadlines**

<b>Date</b>	<b>Course activities; important due dates for marks</b>
Mon, Sep. 9	Introduction to the course Natural history hike in Highland Creek Ravine (HLC) Introduction to project 1 (Jack-in-the-pulpit)
Sun, Sep. 15	Online quiz on required reading of project 1 (Jack-in-the-pulpit)
Mon, Sep. 16	Instructions on how to write a good scientific paper Field work on Project 1 (HLC; Jack-in-the-pulpit)
Sun, Sep. 22	Online quiz on required reading of project 2 (disturbance)
Mon, Sep. 23	Field work on project 2 (HLC; disturbance)
Mon, Sep. 30	Lab work on project 2 (disturbance) and stats on Project 1 (Jack)
Fri, Oct. 4	Online quizzes on required readings of projects 3, 4 (milkweed, soil biodiv.)
<b>Oct. 5/6</b>	<b>Natural history hike at the Koffler Scientific Reserve Field work on project 3 (milkweed)</b>
Mon, Oct. 7	Field work on project 4 (HLC; soil biodiversity) and stats on project 2 (disturbance)
Mon, Oct. 14	Report on project 1 (Jack-in-the-pulpit)
<i>Mon, Oct. 14</i>	<i>No class, Thanksgiving</i>
Mon, Oct. 21	Lab work on project 4 (soil biodiversity)
Mon, Oct. 28	Stats on projects 3, 4 (milkweed, soil biodiversity)
Fri, Nov. 1	Report on project 2 (disturbance)
Sun, Nov. 3	Online quiz on selected papers of project 5 (stomata)
Mon, Nov. 4	Field work on project 5 (stomata; TBA)
Mon, Nov. 11	Lab work project 5
Fri, Nov. 15	Report on project 3 (milkweed)
Mon, Nov. 18	Poster making tips; stats on project 5 (milkweed, stomata)
Mon, Nov. 25	Poster making on project 5 (stomata)
Fri, Nov. 29	Poster needs to be printed by today
Mon, Dec. 2	Poster presentation on project 5 (stomata); grading of poster
Fri, Dec. 6	Report on project 4 (soil biodiversity)

# HOW TO WRITE A REPORT

In a tutorial, you will be provided with in-depth information on how to write a good science paper. The information below is complementary to this in-class information, but does not include all tips and tricks on how a good science paper should be written.

## 1. Individual subunits

The length of the text (references, figures and tables excluded) should be 2500 - 3000 words and consist of the parts outlined below. This text length however is an approximation and should not be followed 'religiously'; it can be better to hand in a very clear and concise paper with fewer than 2500 words than a paper within the word limits which 'rambles'. Thus, unless indicated below with an asterisk (\*; where the word limit NEEDS to be observed), please use your best science-writing common sense.

Title

Abstract: maximum of 250 words\*

Key words: 6\*

Introduction: approx. maximum of 1000 words

Methods: approx. maximum of 300 words

Results: approx. maximum of 200 words

Discussion: approx. maximum of 1000 words

References (do not count toward word count of a report)

**Title.** Concise title potentially containing the main finding of your study.

**Abstract.** The abstract should explain to the general reader why the research was done and why the results should be viewed as important. It should be able to stand alone; the reader should not have to get any information from the main paper in order to understand the abstract. The abstract should provide a brief summary of the research, including the purpose, methods, results, and major conclusions. Do not include literature citations in the abstract. Avoid long lists of common methods or lengthy explanations of what you set out to accomplish. The primary purpose of an abstract is to allow readers to determine quickly and easily the content and results of a paper. The following breakdown works well: purpose of the study (1-2 sentences), outline of the methods (1-2 sentences), results (1-2 sentences), conclusion (no introduction to this section, no discussion/guesses, no citations).

**Key words.** List 6 key words. Words from the title of the article may be included in the key words. Each key word should be useful as an entry point for a literature search if your report were to be published. Key words should be listed in alphabetical order and not repeat words used in the title.

**Introduction.** A brief Introduction describing the paper's significance should be intelligible to a general reader. The Introduction should state the reason for doing the research, the nature of the questions or hypotheses under consideration, and essential background. The introduction is the place where you can

show the reader how knowledgeable you are with a given field, without being too lengthy. Close the introduction with your main hypothesis/question(s). A typical introduction should contain four paragraphs (see oral instructions on how to write a good introduction).

**Methods.** The Methods section should provide sufficient information to allow someone to repeat your work. A clear description of your experimental design, sampling procedures, and statistical procedures is especially important.

**Results.** Results generally should be stated concisely and without interpretation. Present your data using figures and tables; guide your reader through them.

**Discussion.** The discussion section should explain the significance of the results. Distinguish factual results from speculation and interpretation. Avoid excessive review. Structure your discussion as follows. 1. First paragraph - restate your major findings concisely and then relate to the literature. 2. Discuss the problems that might have been present to influence your findings. 3. Compare your findings with those of others; examine why differences occurred and why this may have been so.

**References.** Use the correct format (also see the formatting of the literature in the course manual). You should list at least 12 references beyond those provided in the lab instructions.

## 2. Formatting your report, writing tips

Use the formatting style of the journal of "Ecology." It might seem tedious to you to have to follow the many rules the journal prescribes, but adhering to one style makes a paper more organized, increases readability and bad formatting typically is a sign that the contents are also of sub-par quality.

**Formatting of species names.** When mentioning a species in English, also provide the Latin name, at least the first time. Latin names have to be in italics and the first time a Latin name is mentioned, the genus name (first part of the official binary name) has to be spelled out, later on it can be abbreviated, such as in the following example: "Common milkweed, *Asclepias syriaca*, is a hermaphroditic perennial common to Southern Ontario. The leaves of *A. syriaca* are toxic to cattle."

**Formatting of references.** In the body of the text, references to papers by one or two authors in the text should be in full, e.g. Liang and Stehlik (2009) show *blablabla*. Or: *Blablabla* (Liang and Stehlik 2009). If the number of authors exceeds two, they should always be abbreviated; e.g. Campitelli et al. (2008) show *blablabla*. Or: *Blablabla* (Campitelli et al. 2008). If providing more than one reference in brackets, the order should be chronological with the oldest first and the younger ones later. In the case of two studies from the same year, the order should be alphabetical. E.g. *Blablabla* (Zuk 1963; Korpelainen 1998; Stehlik and Barrett 2005, 2006; Stehlik et al. 2008)."

All references cited (and read by you!) in the main text should be included in "Literature cited." References should be in alphabetical order and their formatting should follow the format exemplified below.

Citing articles in scientific journals:

Michaels., D. R., Jr., and V. Smirnov. 1999. Postglacial sea levels on the western Canadian continental shelf: revisiting Cope's rule. *Marine Geology* 125:1654-1669.

Citing whole books:

Carlson, L. D., and M. Schmidt, eds. 1999. *Global climatic change in the new millennium*. 2nd ed. Vol. 1. The coming deluge. Oxford Univ. Press, Oxford, U.K.

Citing individual articles/chapters in books (if the individual chapters have different authors than the book):

White, P.S. and S. T. A. Pickett. 1985. Natural disturbance and patch dynamics: An introduction. Pp. 3-13 *in* S. T. A. Pickett and P. S. White, eds. *The Ecology of Natural Disturbance and Patch Dynamics*. Academic Press, San Diego, California, USA.

Citing a webpage (avoid as much as possible, cite a paper or book instead):

IUCN, Conservation International, and NatureServe. 2004. *Global amphibian assessment*. Available at [www.globalamphibians.org](http://www.globalamphibians.org). Accessed October 15, 2004.

**Formatting of tables.** Tables (if present) should NOT be inserted in your text, but follow, one table per page, after your Literature cited. Give a brief description what the table is about (table caption) and introduce the parameters stated in the table in a text inserted above the table (see examples in all project descriptions). The description should be self-explanatory, thus the reader should not be forced to read the main body of text in order to understand the message of a table. Each column and row in the table should be labeled (with units if necessary). If mentioning a species name, provide the spelled out Latin name (in italics). In the table, round numbers to two meaningful digits.

**Formatting of figures.** The design of a figure should clearly convey a major result, thus scale your data appropriately. Label all axes with sufficiently large font and meaningful labels. Keep it simple; do not use unnecessary elements such as 3D diagrams if not absolutely necessary as based on the data structure. Similarly as tables, figures should NOT be inserted in your text, but follow, one figure per page, after your tables. Give a brief description what the table is about (figure caption) and introduce the parameters stated in the figure in a text inserted below the figure (see examples above). The description of the figure should be self-explanatory, thus the reader should not be forced to read the main body of text in order to understand the message of a figure. Also, each axis in a plot should be labeled (with units) and each bar in a bar chart should be labeled. If mentioning a species name, provide the spelled out Latin name (in italics).

**References to tables and figures in the text.** In your text, refer to figures as follows: 'In the spring, temperatures are higher than in the winter (Fig. 1).' Or: Figure 1 shows that temperatures are higher in the spring than in the winter. In your text, refer to tables as follows: 'In the spring, temperatures are higher than in the winter (Table 1)'. Or: Table 1 shows that temperatures are higher in the spring than in the winter.

**Formatting of statistical references.** In the text, the results of a statistical test should be cited in parentheses, in support of a specific statement. Example: Xylem tension at the top of trees was significantly higher (25 bars) than at the bottom (20 bars) of the tree ( $P < 0.05$ ). When mentioning the result of a statistical test, always provide the P value,  $R^2$  or  $\chi^2$  were applicable, mean values, sample sizes and standard errors or confidence intervals. Format your text according to the following example.

“There was a significant difference in the frequency of flowering between low and high elevation sites, with greater bias among low than high elevation populations (average flowering frequency: low elevation = 0.93, SE = 0.01; high elevation = 0.78, SE = 0.02;  $\chi^2 = 35.04$ ,  $P < 0.0001$ ;  $df = 1$ ).”

**Miscellaneous.** Avoid quotations - paraphrase your sources instead while making sure you are not plagiarizing.

# GRADING SCHEME FOR REPORTS

When writing the report, you should also consider the criteria and grading scheme that will be used to evaluate your report.

The maximum number of points a student can reach is 24.

## 1. Abstract, title, and key words (2 pts. max)

Exemplary (A; 2 pts): Title clearly identifies the main question solved. Abstract includes all sections of the paper and is a coherent whole that can be understood. Key words are informative and complement the title.

Accomplished (B; 1.5 pts): Title identifies the project. Abstract does not include all sections of the report. Key words are OK.

Developing (C; 1 pts): Title does not identify the work. Abstract only a listing of facts. Key words could be more informative

Beginning (D; 0.5 pt): Either abstract, title or key words are missing.

Fail (F; 0 pts): Abstract, title and key words are missing.

## 2. Introduction (4 pts. max)

Exemplary (A; 4 pts): Presents the background information and previous work in a concise manner that directly leads into the question(s) being addressed and the purpose of the research.

Accomplished (B; 3 pts): Gives a listing of the facts and previous work but does not tie them together and show how they lead to the purpose of the present work and the questions being addressed. It does have the question(s) being addressed and some purpose for doing them.

Developing (C; 2 pts): Gives very little background or information. May include the question(s) but does not identify their purpose for addressing them.

Beginning (D; 1 pt): Does not include background or previous work does not identify the purpose, the project, or the question(s) being addressed.

Fail (F; 0 pts): No introduction present.

## 3. Material and Methods (4 pts. max)

Exemplary (A; 4 pts): Presents easy to follow steps which are logical and adequately detailed.

Accomplished (B; 3 pts): Most of the steps are understandable; some lack detail or are confusing.

Developing (C; 2 pts): Some of the steps are understandable; most are confusing and lack detail.

Beginning (D; 1 pt): Not sequential, most steps are missing or are confusing.

Fail (F; 0 pts): No Materials and Methods present.

#### **4. Results (4 pts. max)**

Exemplary (A; 4 pts): The description of the results is concise and complete. The figures and tables contain all the information needed to understand the data. All the figures and tables flow in a clear and understandable fashion and are referred to in the text. Relevant statistical parameters are provided accurately and completely.

Accomplished (B; 3 pts): Most of the descriptions of the results are complete. Some figures and tables information is missing. The student fails to refer to all figures and tables in the text. The sequence of figures and tables could be improved. Some relevant statistical parameters are missing or wrong.

Developing (C; 2 pts): Many of the results are incomplete, missing or in a wrong sequence. Most relevant statistical parameters are confusing and lack detail. Fails to refer to figures and tables in the text. Figures and tables are missing information.

Beginning (D; 1 pt): Figures and tables inaccurate or wrong. Figure or table are missing. Does not provide any relevant statistical evidence.

Fail (F; 0 pts): No Results present.

#### **5. Discussion (4 pts. max)**

Exemplary (A; 4 pts): Presents a logical explanation for findings and addresses the questions. Additionally, suggests what the next experiments would be. Refers to relevant figures and tables.

Accomplished (B; 3 pts): Presents a logical explanation for findings and addresses some of the questions. Fails to refer to all relevant figures or tables.

Developing (C; 2 pts): Presents an illogical explanation for findings and addresses a few questions. Fails to refer to relevant figures and tables.

Beginning (D; 1 pt): Presents illogical explanations for findings and does not address any of the questions suggested in the introduction.

Fail (E; 0 pts): No Discussion present.

#### **6. Clarity (4 pts. max)**

Exemplary (A; 4 pts): The paper is easy to read and flows expertly. Language is sophisticated without being jargonistic. Terms of analysis and argumentation are clearly laid out and well-defined.

Accomplished (B; 3 pts): The paper is well written but suffers from some significant grammatical inconsistencies or spelling errors. Language is clear but lacks scholarly depth. There are some lapses in definition and explication of terms. Segue between points in the analysis are weak.

Developing (C; 2 pts): There are significant but not quite major problems in grammar and spelling. Language is unclear and/or shallow. Terms are not well defined and analysis leaps erratically from point to point.

Beginning (D; 1 pt): Major problems with grammar and spelling. Language is murky, confused and difficult to follow. There is a paucity of definitions or context for analysis.

Fail (F; 0 pts): Language is sub-par for university, riddled with grammatical and spelling errors. The argumentation is difficult to follow and lacks any sense of flow.

**7. Format (2 pts. max)**

Exemplary (A; 2 pts): A cover page provides pertinent information. The bibliography follows a recognized scholarly style. Citations are thorough and well documented throughout the paper.

Accomplished (B; 1.5 pts): Citations and bibliography are solid but not thorough, with some noticeable omissions.

Developing (C; 1 pt): Citations are weak and/or the bibliography is incomplete.

Beginning (D; 0.5 pt): There are next to no citations and/or no bibliography or it does not follow a scholarly style.

Fail (F; 0 pts): The paper does not follow a scholarly format.



# PROJECT 1



## **ARE MALES CHEAPER THAN FEMALES?**

### **MALE AND FEMALE COSTS OF REPRODUCTION**

#### **1. Background**

Plant sexual reproduction is costly in terms of the resources required for flowering and fruit set. However, the costs of male versus female reproduction are, in most organisms (including animals), unequal (Lloyd and Webb 1977; Popp and Reinartz 1988). In dioecious plants (male-only or female-only individuals), a female's investment in reproduction is typically much greater than a male's over the course of the growing season (Queenborough et al. 2007). Both sexes encounter the basic cost to produce a flower, but whereas costs for reproduction end after the production of pollen in males, females have to allocate nutrients and energy into seeds, thereby exceeding the biomass and energy requirements to produce pollen by males. This greater resource allocation into reproduction as opposed to vegetative growth (roots, shoots and leaves) in females is reflected in the often observed reduction in vegetative growth of females as compared to males. Thus males can, after the end of flowering, allocate incoming assimilated sugars into growth and defense, whereas females have to continue to allocate resources into their offspring. This tradeoff between growth/defense and reproduction is often reflected in greater height and/or larger annual tree rings in males as compared to females (Vasiliauskas and Aarssen 1992; Cipollini and Whigham 1994; Obeso 1997; Queenborough et al. 2007). Females have also been observed to start flowering later in their life, taking longer to build up the necessary reserves for their more expensive reproduction as compared to males (Bullock and Bawa 1981; Garcia and Antor 1995). In extreme, but not uncommon cases, this higher female allocation into reproduction can lead to higher female mortality (Allen and Antos 1993; Matsui 1995) and hence male-biased population sex ratios (Lloyd and Webb 1977). This is due to the fact that males can afford a costlier defense against herbivores or be better prepared to cope with environmental stress such as drought.

Some species of plants and animals demonstrate the ability to change sex throughout their lives. This sex change is in agreement with the size-advantage model (Warner 1988). The model predicts that an individual should change sex if it can increase its reproduction by doing so. Thus, natural selection will favor sex change in a species if there is differing reproductive success between males and females at different sizes (Charnov 1982; Ghiselin 1969). Policansky (1987) puts size advantage in terms of cost of reproduction, stating that larger individuals are better at bearing the costs of reproduction than smaller individuals.

Jack-in-the-pulpit (*Arisaema triphyllum*) is a long-lived understory herb and a great test case for the size-advantage model for species with labile sex expression. In any given year, a Jack-in-the-pulpit plant is either asexual, male, or female. From year to year, however, an individual has the ability to change its sex expression among all three of those sexual states. As a perennial, excess energy acquired in the past growing season is saved in an underground corm (storage organ) from which the individual regrows in the consecutive spring. This amount of stored energy affects which sexual state a given Jack-in-the-pulpit will express. Males and females face very different expenses for sexual reproduction, as only females grow a large infructescence (i.e. the fruiting stage of an inflorescence) with dozens of fleshy red berries (Fig. 1). The annual amount of stored energy is dictated by the leaf area of the plant, with larger leaves having a greater photosynthetic surface and thus higher capacity for energy production.

Thus, given the fact that (1) reproduction in Jack-in-the-pulpit is generally costly, that (2) females and males face different costs of reproduction and that (3) genders are not fixed, what predictions would you draw for the size of leaves in a given plant, considering that leaf area is a function of how much photosynthesis a plant can do and hence how much energy it can allocate towards reproduction? Or put more simply, which sexual state (non-reproductive, female, male) would have either small, intermediate or large leaves? And how would you go about testing your prediction?

## 2. Data collection

Field work will be done in groups of two. Once the outdoor data is collected, each student will get the whole assembled data set for the analysis and write-up. Use Table 1 as a template for data collection in your field note book.

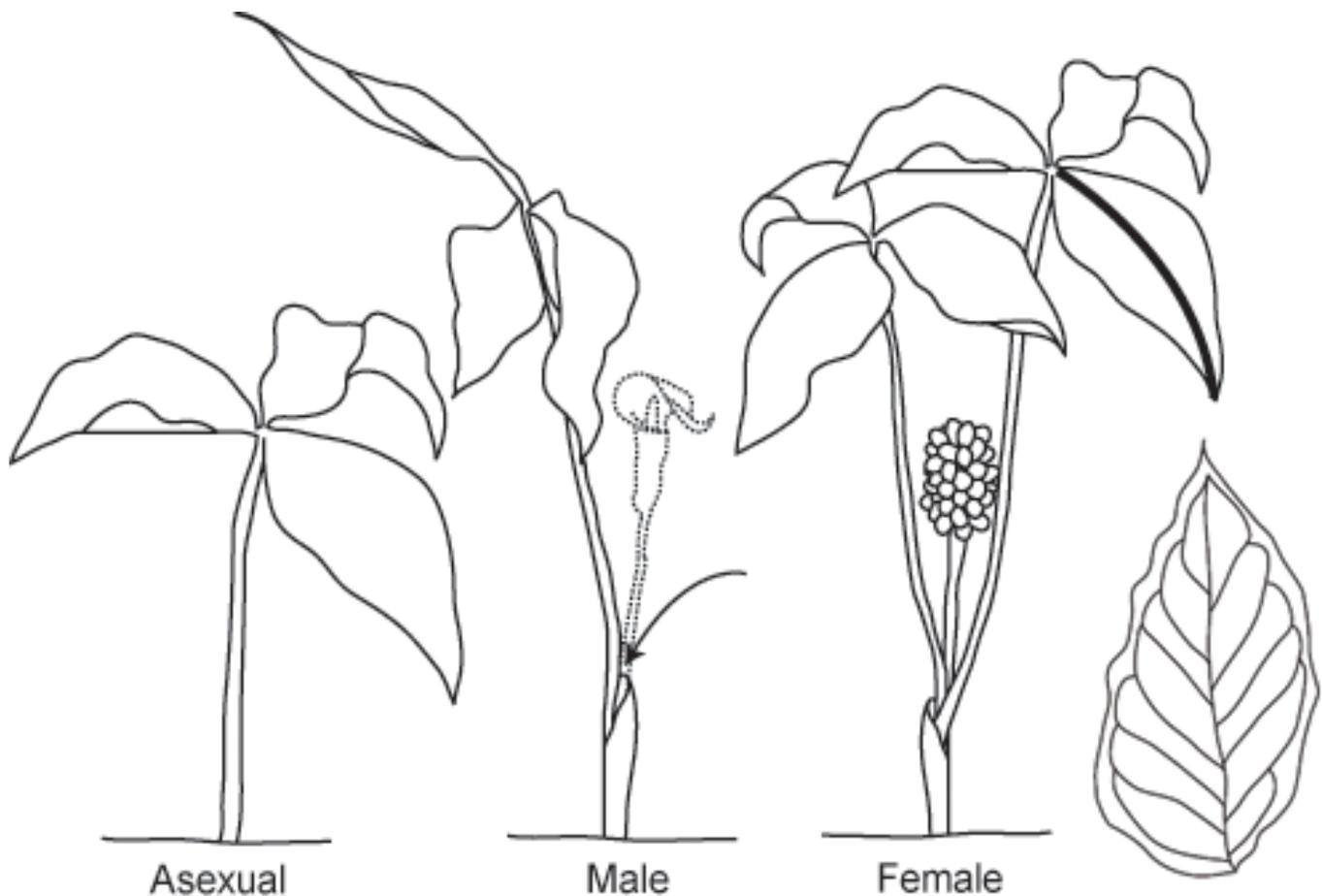
### 2.1. Field work

**Leaf size measurement.** In your “allotted” (by TA or instructor) subpopulation of Jack-in-the-pulpit in the Morningside ravine, measure the length and the widest width of each of the three leaflets and for each of the one to several leaves (Fig. 1).

**Sex expression.** Determine the sex expression of each plant by first looking for an infructescence (stalk with berries; Fig. 1) and where one is found, the individual should be recorded as female. In an absence of an infructescence, search for an evidence of a withered (male) florescence, i.e. a hole near the base of a leaf stem (Fig. 1; arrows for the location of a potential hole). If such a hole can be found, the individual should be recorded as male. Where no fruit nor evidence of inflorescence is found, the individual should be recorded as asexual.

**Table 1.** Layout of the table for entering your field data. Under Sex record the sex expression of each plant. F: female; M: male; A: asexual.

Group	Plant	Sex	Length
Jane&John	1	F	10
Jane&John	2	F	9.5
Jane&John	3	F	10.5
Jane&John	4	F	9.4
Jane&John	5	F	8.5
Jane&John	6	F	8
Jane&John	7	M	7.3
Jane&John	8	M	8.1
Jane&John	9	M	7.2
Jane&John	10	M	7.2
Jane&John	11	M	8
Jane&John	12	M	7.2
Jane&John	13	A	7.5
Jane&John	14	A	8
Jane&John	15	A	7.2



**Fig. 1.** Drawings of Jack-in-the-pulpit (*Arisaema triphyllum*) in the field in late summer to early fall, i.e. when flowering is over. Asexual plants only produce one, typically small leaf (left). Males typically have a sheath with a lateral hole where previously the male inflorescence (now decayed) was inserted (middle; arrow). Females typically have two leaves and a centrally inserted infructescence with many bright, shiny and red berries (right). Note that some males have two leaves and, in such a case, the hole left behind by the withered inflorescence would be central between the two leaves, such as in the case of females (but obviously with no evidence of an infructescence). The thick line on one of the female leaves depicts how you should measure the length of the largest leaflet of the largest leaf. The single leaflet on the far right

### 3. Data analysis

#### 3.1. Class data set assembly

Once you have completed all measurements in the field, enter all this information into an Excel spreadsheet using the same layout as in Table 1.

#### 3.2. Statistical analysis

Using the class data file, specify the predictor LeafSize as continuous and the response parameter Sex (A: asexual; M: male; F: female) as categorical. Run a 1-Way ANOVA for Sex x Leaf Size. From the analysis, retrieve the mean leaf size per sex including standard errors.

#### 3.3. Figures

Plot the relationship between mean leaf size and Sex including standard errors.

### 4. Project report

Please follow the tips and rules outlined in the chapter "How to write a report". Also read and consider the evaluation criteria and grading scheme.

### 5. Literature cited

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- Warner, R. R. 1988. Sex change and the size-advantage model. *Trends in Ecology and Evolution* 3: 133-136. demonstrates the characteristic venation of Jack-in-the-pulpit, i.e. with few very conspicuous large veins on either side of the leaf joining some distance of the leaf margin.

# PROJECT 2



## **HOW MUCH DISTURBANCE IS BENEFICIAL? INTERMEDIATE DISTURBANCE HYPOTHESIS IN THE HIGHLAND CREEK RAVINE**

### **1. Background**

#### **1.1. Intermediate disturbance hypothesis**

Species diversity varies greatly between different habitats and across time. Different factors are been hypothesized to influence species diversity, but disturbance is widely believed to be one of the main factors shaping the variation in species diversity across space and time (Connell 1978). Disturbance has been defined as “any relatively discrete event in time that disrupts ecosystem, community, or population structure and changes resources, substrate availability, or the physical environment” (White and Pickett 1985). The “intermediate-disturbance hypothesis” (Connell 1978) suggests that disturbance prevents competitively dominant species from excluding other species from the community, and that there is a trade-off between species' ability to compete and their ability to tolerate disturbance. At low disturbance levels, diversity is low because the best competitors exclude less strong competitors (competitive exclusion). With high levels of disturbance (very frequent or very strong disturbance), only few species are adapted to withstand such high levels of disturbance and hence species diversity is low. At intermediate intensities or frequencies of disturbance, there is a balance between competitive exclusion and the loss of competitively dominant species by disturbance and conditions hence favor the coexistence of competitive species AND disturbance-tolerant species. Thus, diversity should be highest at intermediate intensities and frequencies of disturbance, as well as at intermediate times since the last disturbance.

#### **1.2. Natural agents of forest disturbance in southern Ontario**

In deciduous forests such as those of southern Ontario including the GTA, there are three main phenomena causing a disturbance which can influence how species interact: (1) windfall, (2) fire, and, predominantly, (3) human disturbance. (1) As a result of windfall, more light reaches the ground and the soil is disturbed when trees are uprooted. When tree roots are uplifted, the soil around the stump is stirred up; this favors the germination of certain seeds, such as those of the yellow birch (*Betula alleghaniensis*), that would otherwise remain dormant. If the windfall area is little (e.g. one tree), pre-existing adult trees and sapling make use of this injection of light and the local forest dynamics is only little affected. However, a major windfall associated with a violent windstorm can affect many hectares of forest at once, cause the accumulation of debris at the surface of the ground, and a significant disturbance of the ground (creation of holes and mounds) by the uplifting of roots. Such a major event initiates a secondary succession and opens the window of opportunity for competitively inferior species through the temporary release from competition by competitively superior species.

(2) Fire is critical in maintaining the mosaic of forest types across the landscape. Whether the same species predominates after the fire as before depends partly on the fire frequency and partly on the proximity of seed sources. Also, a portion of the nutrients previously held in plant biomass is returned quickly to the soil as biomass burns. In boreal forests such as in northern Ontario, the effect of natural fire is especially well-studied and fire is the disturbance agent that has the greatest impact on forest dynamics (Engelmark et al. 1993). During short fire cycles (50 - 100 yr), the landscape is dominated by postfire species such as aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), or jack pine (*Pinus banksiana*),

whereas at very long fire cycles (> 300 yr), balsam fir (*Abies balsamea*), black spruce (*Picea mariana*), and white cedar (*Thuja occidentalis*) are dominant. Maximum landscape diversity is observed between these two extremes (Gauthier et al. 1996).

(3) An important source of disturbance in Southern Ontario and generally in (Eastern) North America is human deforestation for timber extraction or for the creation of farmland. As a representative example for southern Ontario, the fraction of old growth forest in the York Region has dropped to a mere 4% by the beginning of the 20th century, whereas it has re-grown to about 20% today. Hence, most vegetation in the GTA is recovering somewhere along different successional stages after being clear-cut. Once farmland ceases to be used by humans, first old field vegetation moves in with species of goldenrods (*Solidago*) and asters (*Aster*). Then the first shrubs and trees colonize, such as staghorn sumach (*Rhus typhina*), aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*), typically within 20 years after cessation of human disturbance. Then hardwood trees take over (approx. within 50 years), such as the mid-successional ashes (*Fraxinus*), basswood (*Tilia*) or hornbeam (*Ostrya*) along with the very common sugar maple (*Acer saccharum*), whereas late successional temperate forests are dominated by sugar maple, oaks (*Quercus*), beech (*Fagus grandifolia*) and hemlock (*Tsuga canadensis*). For late successional trees, it can take easily more than 50 years from germination to them reaching the forest canopy, especially in the case of hemlock.

### **1.3. Disturbance and the invasion of non-native species**

Disturbance in plant communities, besides initiating natural succession and an increase of biodiversity, has a profound effect on the invasibility of a habitat by non-native species (Hobbs and Huenneke 1992). Most importantly, open space created by a disturbance (windfall, fire, flooding) is one of the most important general factors responsible for plant invasions (Rejmanek 1989). Disturbance hence can lead to the degradation of the native community by promoting invasion.

## **2. Data collection**

Field work will be done in groups of two. Once the outdoor data is collected, each student will get the whole assembled data set for the analysis and write-up.

### **2.1. Quadrata sampling**

We will assess plant diversity as a function of disturbance in the Morningside ravine, choosing to focus on human disturbance. In the Morningside ravine, we will assess five types of plots representing five approximate sets of durations since human disturbance or intensity of human disturbance. Plot type 1 will be on annually mowed lawn, thus with very intense human disturbance. Plot type 2 will be in old field vegetation. Plot type 3 will be in an early successional forest dominated by staghorn sumach (*Rhus typhina*) whereas plot type 4 will be in deciduous forest. The last plot type 5 will be in forests dominated by hemlock (*Tsuga canadensis*).

**Table 1.** Organization of data collection using fictive plant examples. Everybody should use these exact classes as this will simplify the data analysis of this group project. GroupName refers to people in a group; PlotType refers to the successional stage of the vegetation with 1=lawn, 2=old field, 3=sumach forest, 4=hardwood forest, 5=hemlock forest; PlantID is the name of the plant or reference number in case the ID has to be determined at a later point in time; PlantCover refers to the cover (as a cover class) of a given plant in the quadrat and PlantStatus refers to whether a plant is native (N) or invasive (I). For more explanations on the collected parameters refer to 2.2. and 2.3.

GroupName	PlotType	PlantID	PlantCover	PlantStatus
Jane&Jim	1	Dandelion	1	I
Jane&Jim	1	Herb1	3	N
Jane&Jim	1	Dandelion	1	I
Jane&Jim	1	Beggarticks	1	N
Jane&Jim	1	Grass1	3	I
Jane&Jim	1	Tartarian honeysuckle	3	I
Jane&Jim	1	Canada Goldenrod	3	N
...	...	...	...	...
Jane&Jim	2	Heal-all	1	N
Jane&Jim	2	Common plantain	1	I
Jane&Jim	2	Honewort	3	N
Jane&Jim	2	Alternate-leaved dogwood	4	N
Jane&Jim	2	Bittersweet nightshade	2	I
Jane&Jim	2	Canada Goldenrod	2	N
Jane&Jim	2	Bittersweet nightshade	3	I
Jane&Jim	2	Tartarian honeysuckle	3	I
Jane&Jim	2	Canada Goldenrod	3	N

## 2.2. Species identification

Within each quadrat, plant diversity will be assessed. Identify, with the help of the instructor, TA and plant ID books as many plant species as possible. If a plant cannot be identified in the field, collect a sample as a reference for later species determination (a whole twig in case of a tree or shrub and possibly the whole plant in case of a herb; see example how to deal with an unidentified plant in Table 1). Apply a tag to the unknown plant samples to keep track from what quadrates they originated, collect all tagged reference samples in a large plastic bag (to slow down desiccation) and write down a reference to the collected plants in your sampling sheet. We will make an exhibition of ID-ed reference plants in order for you to try and match your unknown plants with the growing body of ID-ed references. Later determine whether a plant is native or invasive (using plant ID books or online references; Table 1).

### 2.3. Estimation of species cover

For each plant species, estimate its cover in its respective quadrat (Table 1), using the cover classes outlined in Table 2. This assessment is a proxy for how dominant a species is in its quadrat to provide additional information beyond simple presence or absence of a species in the quadrat. Please also include any trees which might be present above your quadrat; if a branch hangs into the vertically extended space above your quadrat, ID it and estimate its cover (estimate as best as you can; a tree or shrub thus does not need to be rooted in your quadrat to be included in the survey). When sampling in the forest, your cumulative cover (sum of all covers of all plants present in your quadrat) will very likely exceed 100%. Do not forget to also estimate the cover of any unidentified plant (in your reference collection).

**Table 2.** Cover classes to estimate dominance of plants in quadrates.

Cover class	Range of cover (midpoint)
1	0% - 1% (0.5%)
2	1% - 5% (3%)
3	5% - 25% (15%)
4	25% - 50% (37.5%)
5	50% - 75% (62.5%)
6	75% - 100% (87.5%)

## 3. Data analysis

### 3.1. Class data set assembly

Once your group has completed all the plant identifications and the determination whether a plant is native vs. invasive, you should enter all this information into an Excel-spread sheet and mail it to the TA (date of mailing TBA).

It is imperative that you use the very exact same format in the Excel-spread sheet (sequence of columns and names), so that the individual files can easily be pasted together for the class data analysis. Thus strictly use the format introduced in Table 1 and if you fail to do so, 5% will be deducted from your mark of this project's assignment. Please note that many statistical programs do not accept column headers and data consisting of more than one word, which is why there should be no gaps between any words in either the collected data points (i.e. Jane&Jim) or in the headers (i.e. GroupName). Please also note that each cell of your final Excel-spread sheet should contain information (it should never be blank), thus you will need to, for example, fill in your group name or the number of the transect into each cell in these columns. Only with a data entry for each and every cell, a data point can be identified unambiguously.

Based on the spread sheet with the raw data (Table 1), construct a new spread sheet which will be used in the statistical analyses. This new spread sheet should have the following columns (Table 3): GroupName, PlotType, #Natives, #Invasives, CoverN and CoverI. GroupName, PlotType, and Distance will contain the same information such as in Table 1, whereas the four new columns are new.

**Table 3.** Organization of data for the statistical analysis, based on fictive plant examples from Table 1. Everybody should use these exact classes as this will simplify the data analysis of this group project. GroupName refers to people in a group; PlotType refers to the successional stage of the vegetation with 1=lawn, 2=old field, 3=sumach forest, 4=hardwood forest, 5=hemlock forest; #Natives refers to the number of native plant species per distance per transect; #Invasives refers to the number of invasive plant species per distance per transect; CoverN refers to the summed cover of native plant species per distance per transect and CoverI refers to the summed cover of invasive plants per distance per transect.

GroupName	PlotType	#Natives	#Invasives	CoverN	CoverI
Jane&Jim	1	1	1	0.5	0.5
Jane&Jim	1	1	2	0.5	15.5
Jane&Jim	1	1	1	15	15
Jane&Jim	2	1	1	0.5	0.5
Jane&Jim	2	3	1	57.5	3
Jane&Jim	2	1	2	15	30

**#Natives.** The number of native plant species per plot type which you can extract from the class data table (which you will have gotten from the TA).

**#Invasives.** The number of invasive plant species per plot type.

**CoverN.** The summed midpoint cover of native plant species per plot type (see Table 2). Add up all the coverages of native plants for each individual quadrat and plot type.

**CoverI.** The summed midpoint cover of invasive plants per distance per plot type.

### 3.2. Statistical analysis

Run four 1-Way ANOVAs, one for each of #Natives, #Invasives, CoverNatives and CoverInvasives against plot type. Use Bartlett's test for homogeneity of variance and post-hoc Tukey test.

### 3.3. Figures

Plot the graphs of each ANOVA analysis, including standard errors in all data points.

**N of plant species per plot type.** The ANOVA analysis will calculate the diversity means and standard errors for each quadrat distance across all plot types. Plot these values (separate for native and invasive diversities) against the time since disturbance (plot types 1 through 5).

#### **Cover of native vs. invasive plant species per plot type.**

The ANOVA analysis will calculate the cover means and standard errors for each quadrat distance across all plot types. Plot these values (separate for native and invasive covers) against the time since disturbance (plot types 1 through 5). Remember that depending on the abundance of shrubs or trees (and herbs), this cover per quadrat can exceed 100%.

## 4. Project report

Please follow the tips and rules outlined in the chapter "How to write a report". Also read and consider the evaluation criteria and grading scheme.

## 5. Literature cited

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# PROJECT 3



## **DANGEROUSLY FEW LIAISONS: MATE FINDING IN A FRAGMENTED WORLD**

### **1. Background**

Plants, in contrast to most animals, are sessile and hence they have to rely on external vectors to pick up and deposit pollen in order to mate. Plants have solved this problem by specializing on abiotic (predominantly wind) and biotic pollinating agents such as bees, flies, beetles or birds. This specialization on a narrow group of pollinators resulted in the evolution of suites of flower traits, including flower shape, size, color, odor, reward type and amount, nectar composition, or timing of flowering. These characters all play together to enhance the effectiveness of biotic and abiotic pollination and to increase the fidelity of biotic pollinators. However, if pollinators fail to deliver an adequate quantity of pollen (pollen limitation), plants will mature fewer seeds than potentially possible. Pollen limitation, a reduction in fruit or seed set, is a common phenomenon among plants (Burd 1994).

Pollen limitation can be due to many factors, but a very important one is the floral density of a population: plants growing at lower densities typically are more pollen limited (Fischer and Matthies 1997). This correlation between population density and the per-individual reproductive output is referred to as the Allee effect (Burd 1994; Gascoigne et al. 2009). The general idea of the Allee effect is that for smaller populations, the reproduction and survival rates of individuals increase with population density. This is due to the fact that when plants occur at low densities, they attract fewer pollinators and/or receive fewer conspecific pollen grains per pollinator visit (Feinsinger et al. 1986; Klinkhamer and de Jong 1990; Wagenius 2006). Also, plants can suffer from insufficient pollen quality if they are surrounded with genetically incompatible individuals, which becomes more likely with decreasing population size or density (Ramsey and Vaughton 2000; Kirchner et al. 2005). However, with very high population densities, plants can again become pollen-limited because pollinators cannot service all available flowers (Fritz and Nilsson 1994; Kunin 1997).

Decreasing population sizes of wild plants (and animals) are currently a large problem with increasing anthropogenic disturbance and human population pressure. A common consequence of human domination of our environment is habitat fragmentation. Habitat fragmentation often leads to a decrease of population density. As a consequence, plant conservation ecologists have become interested in how changes in floral density will affect plant pollinator interactions, and ultimately plant reproductive success and population viability (Hackney and McGraw 2001; Honnay et al. 2005).

We will use common milkweed (*Asclepias syriaca*) to test for a dependence of per-individual reproductive output on population density. The perennial common milkweed is a largely self-incompatible species which upon self-pollination only produces 4% set seed (Kephart 1981). Even though a native species to Southern Ontario, common milkweed behaves somewhat like an invasive species, as it occurs abundantly in disturbed sites but will disappear once old-field species move in. At the Koffler Scientific Reserve KSR, the species occurs patchy in subpopulations of different sizes, densities and degrees of isolation from each other, setting it up as an ideal test case for the Allee effect.

## 2. Data collection

Field work will be done in groups of two, as this eases the assessment of the population density. Once the outdoor data is collected, each student will get the whole assembled data set for the analysis and write-up.

### 2.1. Field work

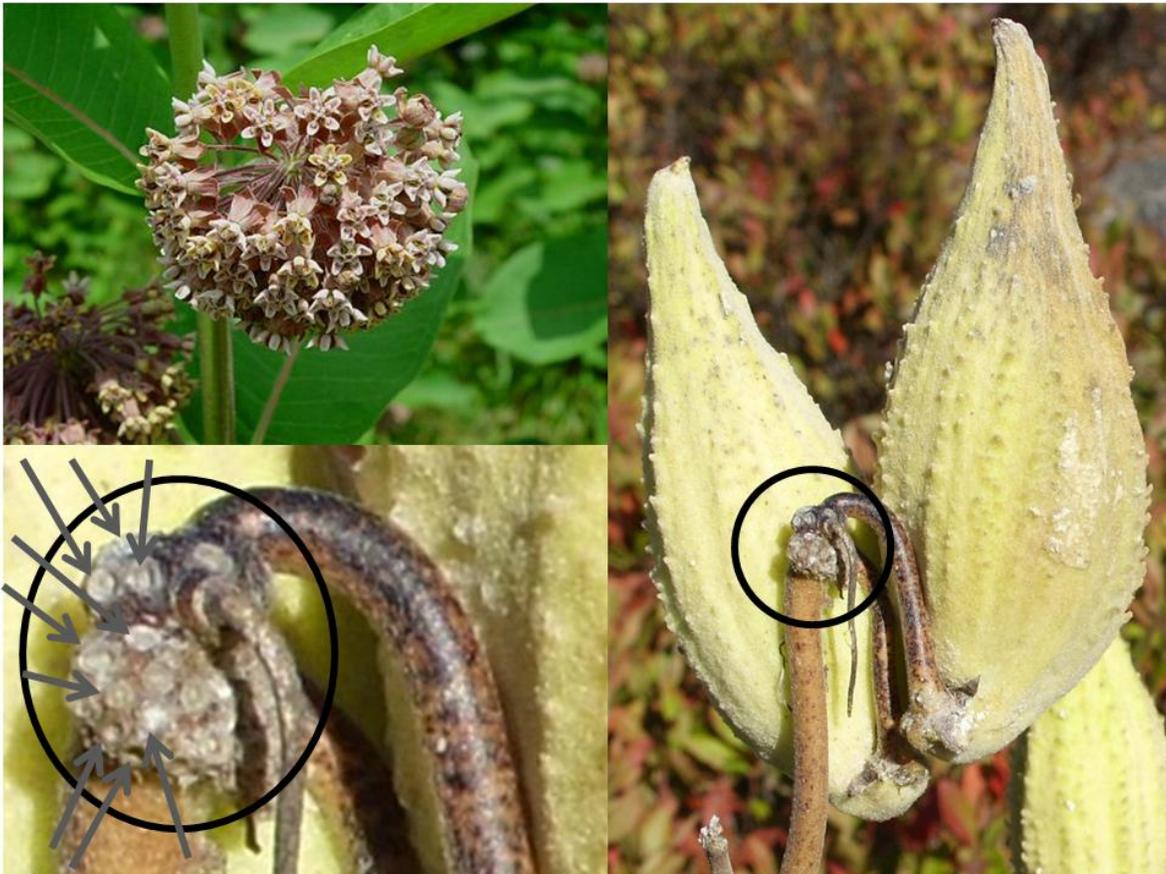
**Fruit set.** In your allotted (by TA or instructor) subpopulation of common milkweed at KSR, first count the number of seed pods per milkweed plant (e.g., the left plant on the cover of this chapter would have 13 seed pods). Then, in a second step, identify all of the bases of inflorescences the plant had produced (including those which have not produced any seed pods). These bases of inflorescences look a little bit like oversized pinheads (Fig. 1). Inspecting each pinhead from up close, you should be able to count the number of scars representing the number of flowers present at flowering time in each umbel (Fig. 1: pinhead-like basis of umbel: circles; flower scars: arrows), thus giving you an estimation of how many seed pods a given plant could have developed potentially. Common milkweed produces large umbels as inflorescences, but mostly only very few of these flowers develop into seed pods (Fig. 1). It might be helpful to use a sharpie to color all the scars as you are counting them to ease the process. Assess as many plants you can in the allotted time. Also, try to cover as many extremes in terms of density/isolation of focal plants as possible, i.e. sample very isolated plants and highly clumped plants.

**Estimation of the subpopulation density and isolation.** For each of the plants you assess fruit set, estimate its mating environment by measuring the distances in m to the *ten closest neighbors* per focal plant (Fig. 2). Thus using the measuring tape, measure and write down the distance from your focal plant to its ten closest neighbors and keep track of these distances in your field book using a layout such as in Table 1. Even if sampling plants within the same cluster, you will have to repeat the measuring of the ten closest distances for each plant individually (Fig. 2).

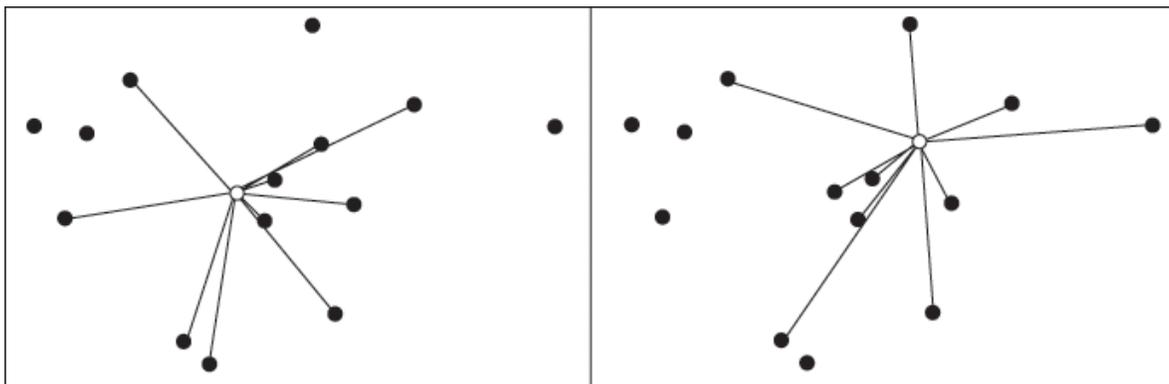
If there are less than ten plants in a subpopulation, estimate (without measuring) the rough distance to the nearest neighboring subpopulation and include this distance how many ever times needed to account for the ten nearest neighbors (Table 1).

**Table 1.** Layout of the data collection in the field. Plant 1 is growing in a larger subpopulation and thus the distances to the closest 10 neighbors (D1 to D10) were measured as indicated in Fig. 2, whereas plant 2 grows in a smaller subpopulation with only three close-by neighbors and hence only roughly estimated distances to the next subpopulation (50 m). NPods refers to the number of seed pods per focal plant, whereas NScars is the total of scar on the pinhead-like basis of all inflorescences per focal plant, including inflorescences which did not set any seed pods.

Group	Plant	N inflor	NPods	Nscars*	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
J&J	1	1	1	35	0.3	0.4	0.8	0.9	1	1.8	2	2.5	3	5
J&J	1	2	3	15	0.3	0.4	0.8	0.9	1	1.8	2	2.5	3	5
J&J	1	3	0	NA	0.3	0.4	0.8	0.9	1	1.8	2	2.5	3	5
J&J	2	1	3	25	0.3	0.4	0.8	0.9	1	1.8	2	2.5	3	5
J&J	2	2	1	16	1.3	1.4	1.4	1.6	2	2.1	2.5	2.8	3.1	3.2
J&J	2	3	0	NA	1.3	1.4	1.4	1.6	2	2.1	2.5	2.8	3.1	3.2
J&J	2	4	0	NA	1.3	1.4	1.4	1.6	2	2.1	2.5	2.8	3.1	3.2



**Fig. 1.** Upper left: two inflorescences of common milkweed (*Asclepias syriaca*). Each individual flower has the potential to develop into a seed pod, but typically only very few flowers do so (right). Right: two seed pods developed from the pinhead-like base of the inflorescence (circle) and scars from the withered flowers are still visible. Lower left: close-up of the base of an inflorescence where some of the scars of withered flowers are highlighted by grey arrows.



**Fig. 2.** The left and right pictures show the same subpopulation of plants with two examples of different focal plants (empty circles) and the mapped distances to the ten closest neighbors.

### 3. Data analysis

#### 3.1. Class data set assembly

Once you have completed all counting in the field, enter all this information into an Excel spread sheet using the same layout of the table as in Table 1. For the analysis of the class data, we are interested in (1) fruit set, thus how many of the potential flowers per plant develop into a pod and (2) the cumulative distance for each assessed plant.

To calculate (1) fruit set, create a new column entitled Fruitset (Fig. 3). Let Excel calculate fruit set for you, with a similar procedure as in project 2. Into the appropriate Excel cell enter the equivalent formula command of  $\text{NPods} / (\text{NPods} + \text{NScars})$  with reference to row/column number of the measured values. Thus, for the example of plant 1 in Table 1 it would be (see Figure 3):

$$=C2/(C2+D2)$$

Then apply the same calculation for all other plants by dragging the little black corner of the Fruitset cell down for all plants (compare instructions in Fig. 2, Project 2).

For calculating (2) the cumulative distance per plant, introduce a new column entitled CumDist (Fig. 3). Into this cell and using the same example of plant 1, enter

$$=sum($$

and then mark with your mouse all the distances for this plant (F2 to O2). Excel will convert this to  $=sum(F2:O2)$  (Fig. 3) and by hitting return, the cumulative distance of 17.6 should show up.

After you are done calculating the fruit set and the cumulative distance using the very same layout as in Figure 3 (5% deduction for non-compliance), mail your table to the TA.

#### 3.2. Statistical analysis

Using the class data, run a simple regression analysis using CumDist as the predictor and Fruitset as the response variable. Before running the regression, logit transform FruitSet by transforming Fruitset to  $\text{Log}(\text{Fruitset} / (1 - \text{Fruitset}))$ . Use the  $R^2$  as the fraction of data explained.

#### 3.3. Figures

Plot the regression in Excel and include the  $R^2$  and the P values in the figure.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Group	Plant	NPods	NScars	Fruitset	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	CumDist
2	J&J	1	10	245	$=C2/(C2+D2)$	0.3	0.4	0.8	0.9	1	1.8	1.9	2.5	3	5	$=sum(F2:O2)$
3	J&J	2	3	185		0.5	0.6	1.5	50	50	50	50	50	50	50	
4																

**Fig. 3.** Calculation of fruit set (Fruitset) and the cumulative distance (CumDist) per plant investigated. The figure represents a screen shot from Excel. See text for detailed explanations.

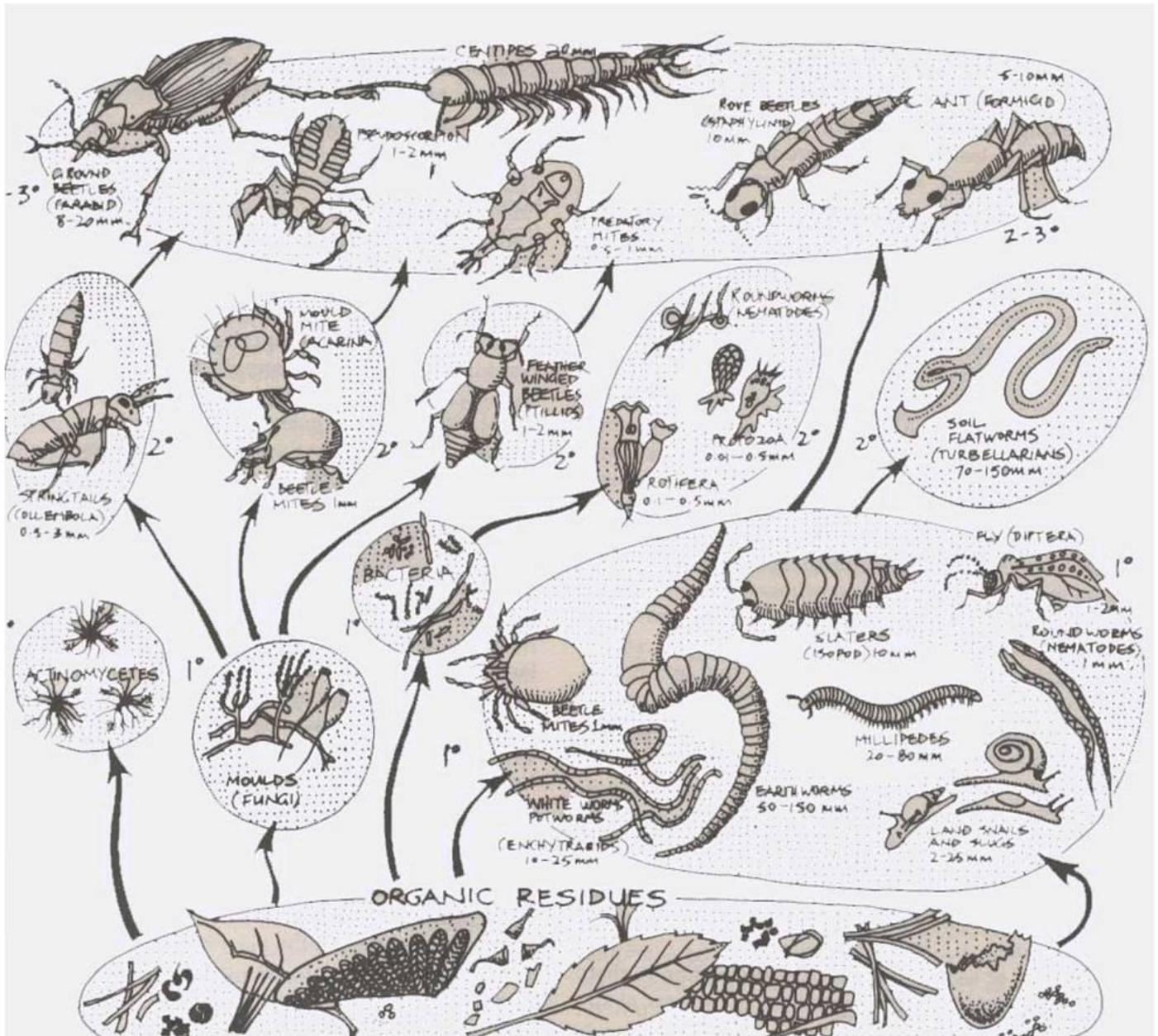
#### 4. Project report

Please follow the tips and rules outlined in the chapter “How to write a report”. Also read and consider the evaluation criteria and grading scheme.

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# PROJECT 4



## SOIL BIODIVERSITY – DO SOIL MICRO-ORGANISMS CARE WHAT HAPPENS ABOVE THEM?

### 1. Background

One of the most important ecosystem services in terrestrial habitats is litter decomposition (Barrios 2007). Litter decomposition ensures that limiting nutrients locked away in dead biomass are returned to soils and can be taken up by living organisms. The decomposition of biomass is carried out through an intricate soil food web. Soil food web analysis shows that soil macrofauna breaks down soil litter into pieces small enough to be accessible to further decomposition by soil bacteria and fungi. These latter organisms are responsible for the mineralization of organic nutrient forms into inorganic nutrients essential for plant growth (Barrios 2007). Detritivorous bacteria and fungi form the food basis for carnivores such as protozoa and nematodes, while higher order carnivores eat these in turn. These food web interactions, critical for energy and nutrient flow through the ecosystem, are graphically represented in Figure 1 (from Hunt et al. 1987).

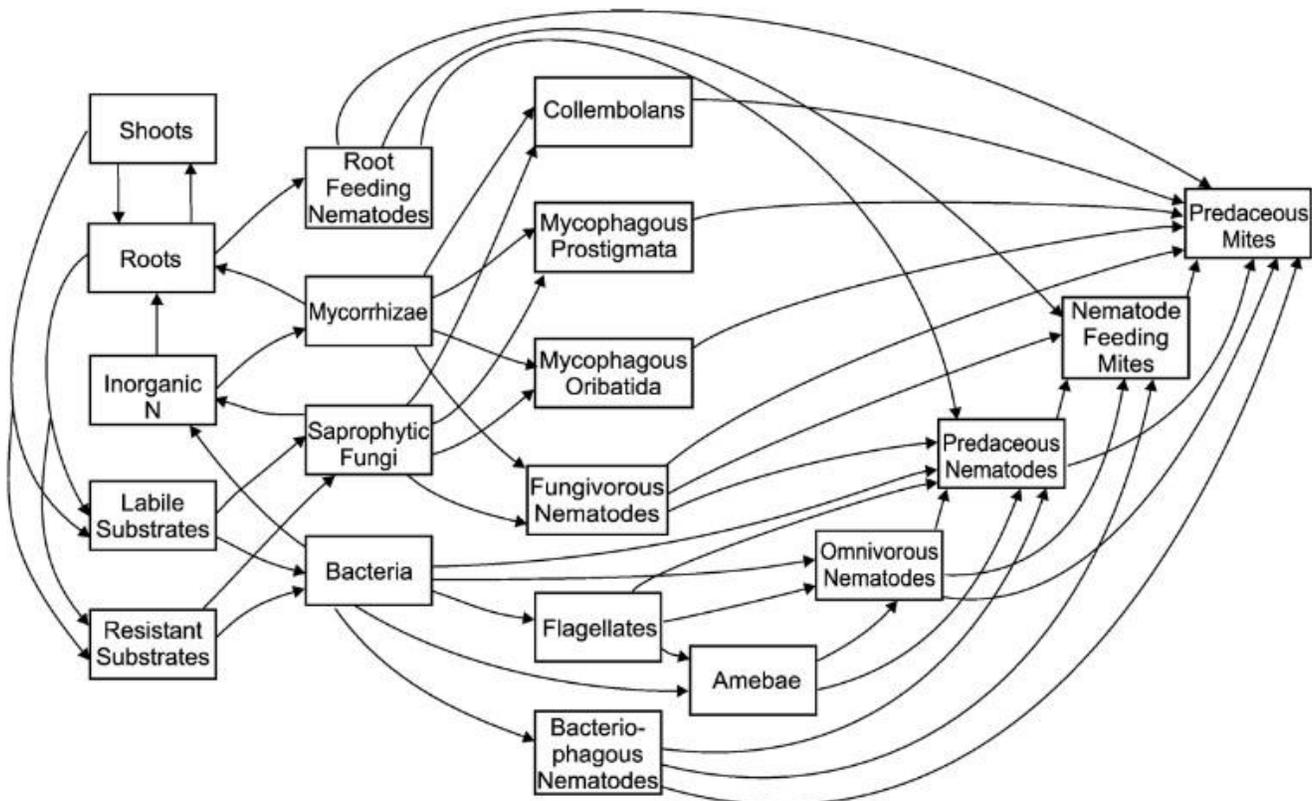


Fig. 1. Graphic representation of a detrital food web in a shortgrass prairie (from Hunt et al., 1987).

Various biotic and abiotic factors influence the speed and efficiency of litter decomposition (Swift et al. 1979; Fig. 2). The three main factors which influence biomass decomposition rates are (1) the composition and activity of the living soil organisms (biota), thus the bacteria, invertebrates, and

vertebrates; (2) abiotic factors such as climate, habitat, soil moisture or bedrock composition (physico-chemical environment); and (3) the composition of the litter, primarily plant species diversity and tissue chemistry (resource attributes).

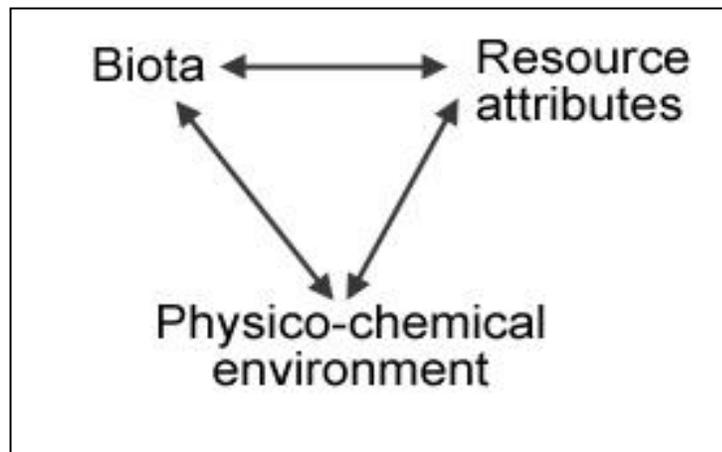


Figure 2. Interactions among factors that control litter decomposition (Swift et al. 1979).

Many studies have shown how resource attributes (Fig. 2) and abiotic parameters affect soil community structure and diversity (Swift et al. 1979, Ingham et al. 1982, Freckman & Virginia 1997). For example, decomposition of plant litter high in lignin and/or low in nutrients is mainly undertaken by fungi, as such biomass is harder to decompose for other organisms. This leads to dominance by soil organisms which feed on fungi (e.g. nematodes, mites and springtails). In contrast, more easily broken-down biomass is decomposed primarily by bacteria, again with effects on the composition of soil organisms higher up the food chain (Coleman & Crossley 1996). On the other hand, the soil organisms can directly affect the vegetation, as it has been shown that the composition of the soil bacteria and fauna can influence successional turnover and plant diversity in a grassland community (de Deyn et al. 2003).

This project provides the opportunity to study the influence of the abiotic and plant-cover attributes on the diversity and composition of soil invertebrates. Soil invertebrates, the organismal group targeted in this project, are key decomposers influencing primary production and other ecosystem services related to soil ecosystem function through their effect on the rate of litter decomposition, soil porosity and hence aeration, and nutrient mineralization (e.g., Six et al. 2002). These organisms are also key players to provide and maintain productive agricultural soils. Soil invertebrates are also important in shaping below-ground or root-root competition among plants. Also, because they form the food basis for vertebrates such as birds and mammals, they play an important role for above-ground food webs as well.

## 2. Invertebrate collection

In groups of 4 students, decide what type of environmental gradient you would like to investigate. You will then go to the field (Highland Creek ravine) to the appropriate site to sample according to your objectives. You will then collect samples of soil and/or litter, using bulb planters/shovels/trowels and plastic zip-lock

bags. The samples are then brought back to the lab and, with the help of the TA and/or the teaching assistant placed in Berlese funnels. A Berlese funnel (Fig. 3) contains a piece of screen, with a light heating system mounted above and a collecting jar below provided with alcohol to catch small soil invertebrates that escape and fall from material that is placed on the screen. In these funnels, the light source is allowed to drive invertebrates into the bottom of the funnel, where they fall through the screen and into alcohol or other preservative. Sample collections run for a week (interval between class meetings), but the actual migration of invertebrates from the soil sample through the screen probably take no longer than 3-5 days.

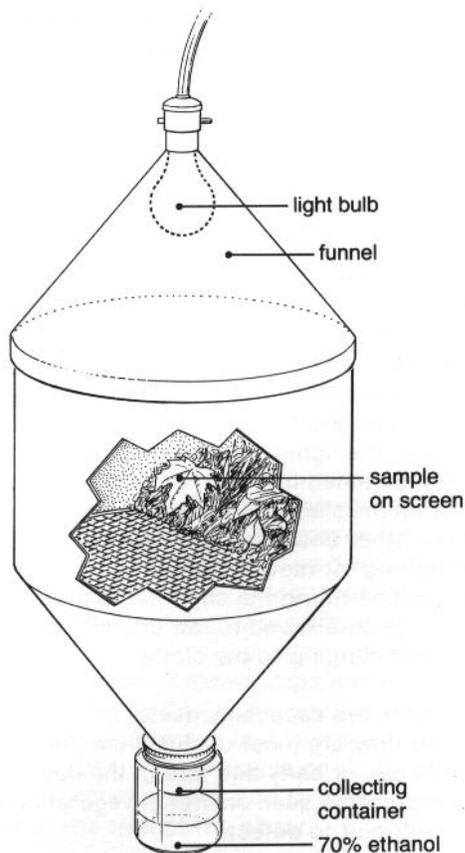


Fig. 3. Berlese or Tullgren Funnel

### 3. Invertebrate identification

**BRING YOUR LAPTOP!** In the second week of this project, you will identify the invertebrates in your samples, using dissecting microscopes. Identification down to species is quite difficult, but identification to higher taxa and then to morphotype is relatively straightforward. We will use a dichotomous key which can be found under

<http://www.cals.ncsu.edu/course/ent591k/kwikey1.html>

Record abundances in each sample by counting total number of each taxon or morphotype. Enter your data into an excel spreadsheet using the layout of Table 1.

Table 1. Setup of the excel table for the data collection.

Species	Number of Observations	
	Site 1	Site 2
a	2	14
b	4	2
c	6	0
d	1	0
e	8	0
f	0	0
g	9	12
h	3	7
i	4	6
j	6	1
k	3	1

#### 4. Statistical analysis

Soil invertebrate biodiversity and evenness will be calculated using the Shannon index ( $H'$ ), one of the most popular diversity index. Shannon's index measures both richness (the number of species) and evenness, or how evenly individuals are distributed among species. High values of  $H'$  denote high biodiversity. Shannon's index is advantageous over simply counting the total number of different species, because the latter is greatly affected by sampling effort (plot size and total number of individuals sampled). The greater the sample, the more rare species you find.  $H'$  is superior because it is calculated from proportions and rare species contribute very little. Therefore, this index is relatively insensitive to the random inclusion or omission of rare species that happens with any sampling effort. The equation for Shannon's index is:

$$H = -\sum_{i=1}^S p_i \ln p_i$$

or

$$H' = -[p_1 \ln(p_1) + p_2 \ln(p_2) + p_3 \ln(p_3) + \dots + p_s \ln(p_s)]$$

where the  $p_i$ 's are the proportion of all observations in the  $i$ th species category, and  $S$  is the total number of species.

Consider two sample soil communities below

Soil sample 1

Species	A	B	C	D	E	Total
# obs.	70	10	10	5	5	100
$p_i$	0.7	0.1	0.1	0.05	0.05	

$$H' = -[(0.7) \times \ln(0.7) + 2 \times (0.1) \times \ln(0.1) + 2 \times (0.05) \times \ln(0.05)]$$

$$H' = 1.01$$

Soil sample 2

Species	A	B	C	D	E	Total
# obs.	20	20	20	20	20	100
$p_i$	0.2	0.2	0.2	0.2	0.2	

$$H' = -[5 \times (0.2) \times \ln(0.2)]$$

$$H' = 1.61$$

Even though both soil samples contain the same amount of species (5), soil sample 1 is dominated by species A, whereas in soil sample 2 all five species are distributed more evenly and thus  $H'$  of sample 2 is higher.

It is possible to carry out a t-test to compare the Shannon indices from two different communities provided that you have numerous replicates (and although it's hard to say how many is a minimum number, a dozen samples per site would be a good target) and the data is normally distributed. Since diversity indices are rarely normally distributed, a non-parametric test, such as the Mann-Whitney U test would be a safer bet. However, an easier, more straightforward, and more elegant way involves the non-parametric Kolmogorov-Smirnov test.

## 5. Report format

Please follow the tips and rules outlined in the chapter "instructions to the report". Also read and consider the evaluation criteria and grading scheme.

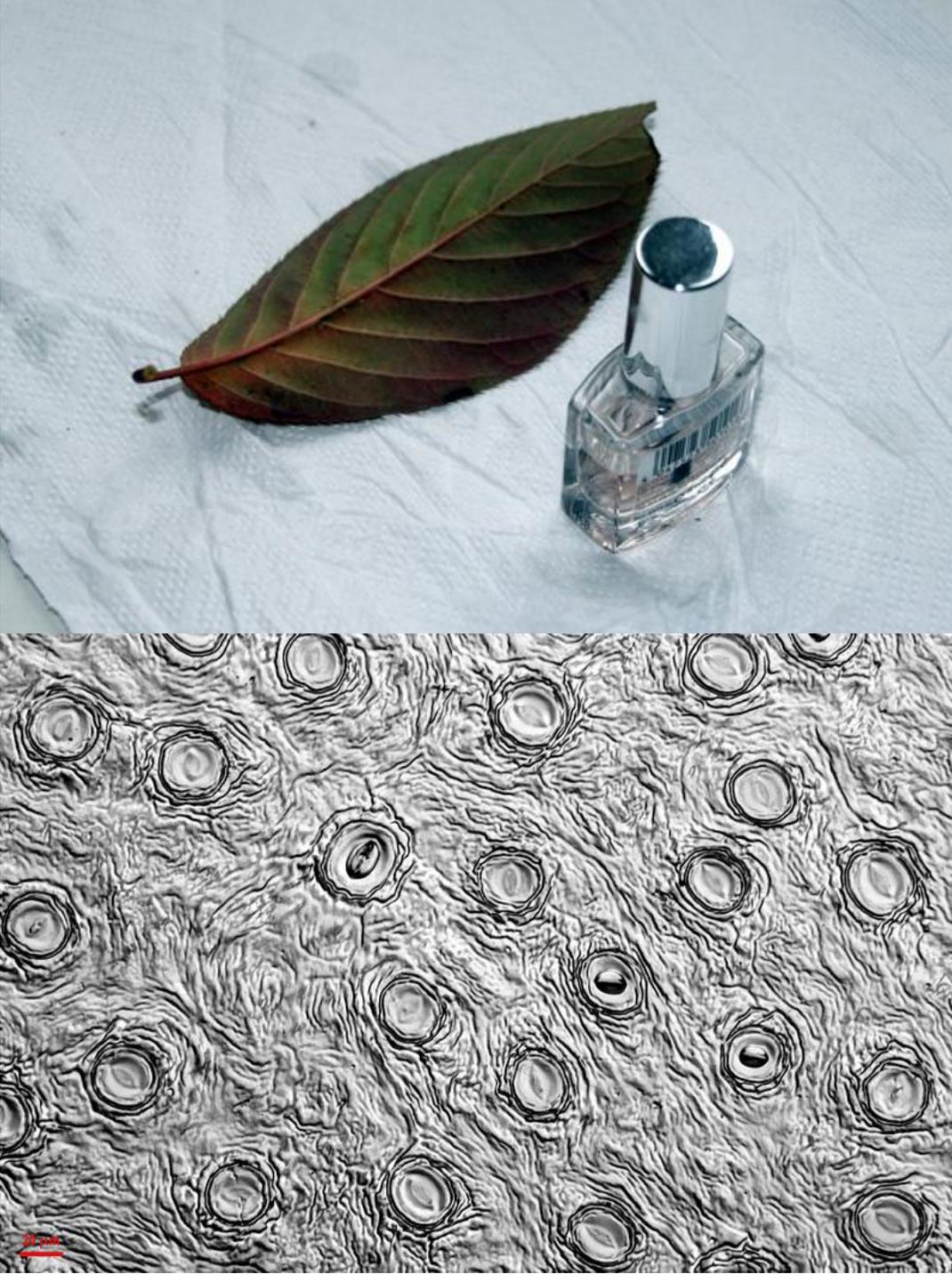
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# PROJECT 5



# ENVIRONMENTAL CORRELATES OF LEAF STOMATA DENSITY

## 1. Background

Vascular plants engage in gas exchange using leaf stomata which are typically located on the lower side of leaves. In order to photosynthesize, plants take up  $\text{CO}_2$  for the production of glucose, while water and oxygen escapes from the leaves. The two adjacent guard cells (Fig. 1) control gas exchange through actively opening and closing the stomates. In addition to this behavioral control, plants can exert control over their gas exchange by varying stomata density during leaf development. The higher the stomatal density, the more  $\text{CO}_2$  can be taken up, and also the more water is transpired.

Generally, a species-specific plant photosynthetic apparatus functions optimally over a rather narrow temperature range. When too hot, cytochromes, pigments, and membranes critical to phosphorylation and carbon fixation rapidly denature. To avoid this, an individual plant may open its stomata and evaporate water to cool its leaves. Thus, all else being equal, leaves in the sun could be hypothesized to have higher stomata density than do leaves in the shade. However, under water limitation and dry environmental conditions, excessive evaporation can lead to desiccation and an equally severe disruption of photosynthesis. Thus, leaves exposed to drought conditions should have fewer stomata in sunlit environments.

In this project and in groups, you will test how leaf stomatal density can vary under different environmental conditions. You will come up with your own hypothesis for a specific abiotic contrast and plant species, design your own sampling design, and collect data using the technique of making clear nail polish impressions of leaves, analyze your data and present it to the class in a poster presentation session.

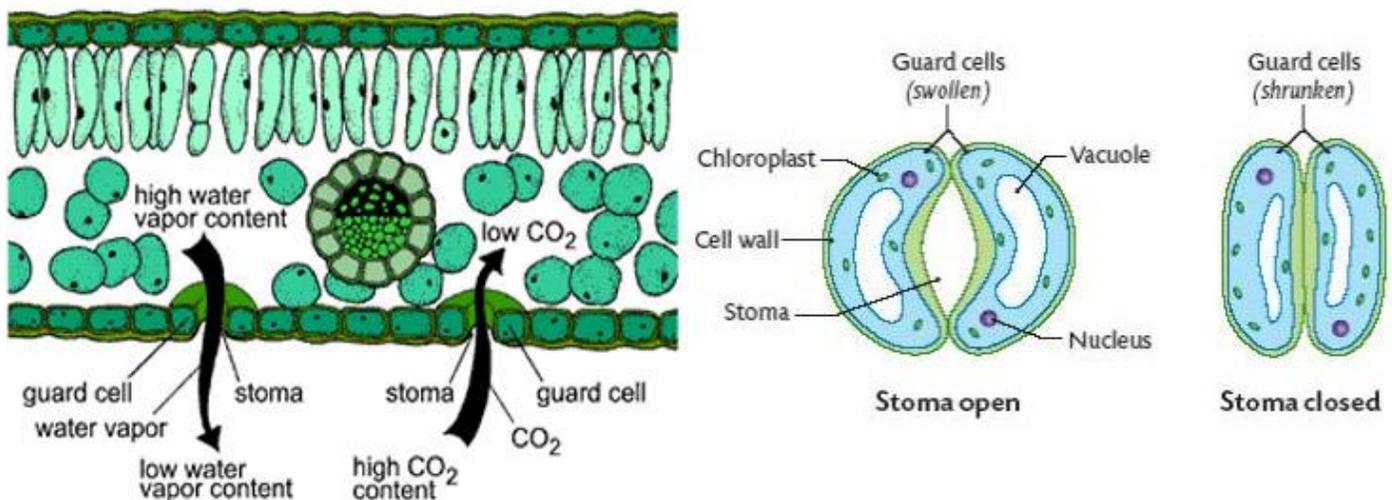


Fig. 1. Location of stomata on the lower leaf side (left) and guard cells controlling gas exchange in a stoma (right).

## 2. Materials and Methods

### 2.1. Study sites

You can collect your data from plants located either on the UTSC campus, in the Highland Creek ravine or in nearby residential gardens. Your choice of plants includes ornamental evergreen ground cover plants, shrubs, and trees (such as grasses and weedy perennial forbs, hollies, yews, and conifers). Data collection

does not work well on dead (and thus dry) plant material, because it is a bit tricky, but not impossible, to obtain the stomata samples (see below).

## **2.2. Hypothesis creation and data collection (Nov. 4)**

In conversation within your group, come up with an environmental contrast that might affect stomata density and formulate a hypothesis about which way you would expect stomata density to vary and WHY. Discuss these in detail with Ivana Stehlik PRIOR to going out and collecting any data. Next, decide on a place where you intend to collect leaf samples and go and get them. Bring your leaf samples back to the lab and start counting stomata densities, using the full length of the lab session (see 2.3. Counting of stomatal densities). Sample 30 individuals (with one leaf each) per environmental category, for a total of 60 samples.

## **2.3. Counting of stomatal densities (Nov. 4 and 11)**

- i. In the field, obtain the leaf upon which you wish to census stomata. Keep track under what environmental conditions and in which location you collected all samples and apply an ID code to each leaf sample, keeping track of these data in your notebook.
- ii. Back in the lab, apply a rather thick swath of clear nail polish on the side you wish to census stomata (typically the leaf underside).
- iii. After the nail polish has dried (several minutes), obtain a square of the provided tape (package sealing tape, NOT scotch tape). Stick your tape piece to the area that contains the dried nail polish swath.
- iv. GENTLY, peel your nail polish swath from the leaf completely. You will see a cloudy impression of the leaf surface now attached to your tape piece (hereafter referred to as your "leaf impression").
- v. Tape your leaf impression to a clean slide and use scissors to cut off the excess tape.
- vi. Use a pen and write the ID code of each leaf directly on the slide.
- vii. Focus your leaf impression under at least 400x power and observe the stomata (see Fig. 1 and title page of this project).
- viii. Search around on your impression to find an area that subjectively appears to have a high density of stomata. That is, move the slide around until the field of view is away from the edge of the impression and so that there are no dirt blobs, no thumbprints, no damaged areas, and no big leaf vein impressions in view.
- ix. Count all stomata you see and record it using the layout shown in Table 1.
- x. Repeat the previous two steps three times, and the highest number of the three will be your one data point from this impression.
- xi. Repeat all steps above for leaf impressions in each treatment group.

## **2.4. Statistical analysis (on your own and outside lab time)**

To test whether leaves collected under your two environmental extremes indeed differ in their stomatal densities, you can carry out the Student's t-test. You can do this either in any stats program of your choice or use free online T-test calculators (e.g. <http://www.graphpad.com/quickcalcs/ttest1.cfm>; using the setting of up to 50 rows and unpaired t-test). Contact your TA if you have any problems or questions.

Table 1. Setup of the excel table for the data collection.

# stomata envir 1	# stomata envir 2
7	4
9	6
11	2
8	4
6	6
8	5
... (30 samples)	... (30 samples)

### 2.5. Poster making tips, the actual poster making and the poster presentation (mini symposium)

On Nov 11, you will be given instructions on how to prepare an effective scientific poster. The lab session of Nov 18 is dedicated to the preparation of your poster VISUALS according to these guidelines. Thus make sure you are done with your stomatal counting and data analysis. Please also note that you will need TEXT UNITS for an Abstract, an Introduction, Materials and Methods, and Conclusions (including the results of your statistical analysis), along with at least three references to feature on your poster. Thus, you need to write these text units OUTSIDE CLASS and bring them along into the poster making session on Nov 18. Also, please bring along at least one laptop per group, so that you and your group partner can work on your poster.