

LAB 1 — INBREEDING DEPRESSION & THE EVOLUTIONARY ADVANTAGE OF OUTBREEDING IN *BRASSICA RAPA*

OBJECTIVES

1. Learn how to estimate fitness in a formal experiment.
 2. Investigate the importance of inbreeding depression in the maintenance of outbreeding in a self-incompatible flowering plant.
 3. Learn some basic statistical analyses.
 4. Learn how to research and prepare a formal scientific manuscript.
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INTRODUCTION

Inbreeding depression can be defined as *the reduction in fitness of offspring derived from mating between relatives (inbreeding) compared to offspring resulting from mating among unrelated individuals (outcrossing)*. The harmful effects of close inbreeding were widely recognized well before any formal scientific investigation into the phenomenon. Indeed, in humans about 42% of offspring from sister-brother marriages die before they reach reproductive age, hence most, though not all, cultures have strong traditions with respect to incest. Plant and animal breeders have also known for centuries of the superior vigor and yield associated with outbreeding compared to inbreeding. The importance of inbreeding depression in evolutionary biology was established in 1876 with the publication of a book entitled *The Effects of Cross and Self Fertilization in the Vegetable Kingdom* by Charles Darwin. His extensive experiments involving 57 species of plants indicated that inbreeding depression is a widespread and significant evolutionary force.

The most likely cause for the reduction of fitness upon inbreeding involves the expression of *deleterious recessive alleles*. Recessive alleles are expressed in homozygotes but remain unexpressed when they occur with a *dominant* allele in heterozygotes. *Deleterious* alleles originate when the underlying DNA sequence of a functional allele is altered by mutation to code for a gene product which is either harmful or simply doesn't work. Since mutation is a universal feature of DNA, all plant or animal populations contain deleterious recessive alleles. At any given locus, however, deleterious alleles are usually so rare that offspring produced through matings among unrelated individuals are almost never homozygous for the harmful gene. With inbreeding the odds of producing an offspring homozygous for a deleterious allele are much higher. Because rare deleterious mutations are transmitted along family lines, brothers and sisters are much more likely to carrying the same deleterious allele than an unrelated individuals.

In plants, very close inbreeding is possible. More than 70% of flowering plant species are hermaphrodite (i.e., they possess both female and male sex organs), so that it is possible for an individual to mate with itself. This is known as *self-fertilization*. Many plant species regularly self-fertilize, and from a genetic viewpoint this is not surprising. Consider a population of outcrossing hermaphrodites in which a self-fertilizing mutant arises. Outcrossing individuals transmit, on average, one copy of their genes by being a mother to their own seeds and another copy by being a father to some other individual's seeds. The selfing mutant, on the other hand, will be both mother *and* father to its own seeds as well as a father to another individual's seeds. Hence the selfing mutant transmits three copies of its genes to the next generation while outcrossers transmit only two (Table 1). This is the *automatic transmission advantage* of self-fertilization.

Table 1. The automatic transmission advantage of self-fertilization.

	Gene copies transmitted through:		Total
	Own seeds	Seeds of others	
<i>No Inbreeding Depression</i>			
Outcrossing individual	1	1	2
Selfing individual	2	1	3
<i>With Inbreeding Depression</i>			
Outcrossing individual	1	1	2
Selfing individual	$2(\omega_s/\omega_o)$	1	< 3

Note: The fitness of outcrossed and selfed offspring are ω_o and ω_s , respectively. With inbreeding depression, $\omega_o > \omega_s$.

Although many plants self-fertilize, the majority possess morphological and physiological mechanisms which reduce the chance of self-fertilization and hence promote outcrossing. For example, male and female sex organs are often separated within a flower (known as *herkogamy*) or function at different times during the life of a flower (*dichogamy*). In species such as your study organism (*Brassica rapa*), self-fertilization is prevented by a physiological mechanism known as *self-incompatibility* through which pollen is incapable of functioning on stigmas of the individual from which it came. The question is: given the automatic transmission advantage of selfing, what selective forces promote the evolution of these outbreeding mechanisms? Or, *what is the evolutionary advantage of outbreeding?* Obviously, inbreeding depression may play a major role here. If the viability of offspring produced through selfing is reduced by inbreeding depression, the transmission advantage of selfing is eroded (see the lower half of Table 1). If selfed offspring are only half as vigorous as their outcrossed counterparts, there is no net advantage of self-fertilization.

MEASURING INBREEDING DEPRESSION

Plants can be ideal study organisms for measuring inbreeding depression. It is relatively easy to regulate who mates with whom, and thereby produce both selfed and outcrossed offspring through experimental hand-pollinations. In addition, the relative fitness of selfed and outcrossed offspring can be compared by growing them in a common environment. Before moving on to the experimental protocol, it is important to become a little more familiar with both the concept of *fitness* in evolutionary biology and your study organism, *Brassica rapa*.

I. Fitness

Fitness is a central concept in evolutionary biology, and is defined as *the relative ability of an individual to leave descendants and hence transmit its genes to future generations*. This has two components. The ultimate component, sometimes called *fecundity*, is the production of viable offspring which, in turn, go on to produce their own offspring. However, before an individual can produce offspring it must survive to sexual maturity. *Survival* is the other component of fitness. Throughout an organism's life, the probability of dying may differ from one *life-history stage* to the next. Biologists trying to measure survival in plants often break down the life span of a plant into four stages: fertilized ovule to seed; seed to seedling (germination); seedling to adult; and reproductive period as an adult (see Figure 1). This approach may, however, prove difficult in long-lived organisms such as trees. Fortunately, your study organism (*Brassica rapa*) is easily grown in the laboratory and completes its entire life-cycle in less than 30 days. You can, therefore, estimate survival for all life-history stages.

Returning to fecundity, the number of viable offspring produced is often difficult to measure. Imagine trying to count and determine the viability of all the maple keys produced on even a single maple tree each year throughout its entire reproductive life. Consequently, biologists often resort to measuring variables which are *correlated with* (i.e., show a close association with) the ability to produce offspring. A biologist working on maple trees may, for example, measure the diameter of the tree trunk as an index of fecundity if there was evidence that trunk diameter (as a measure of tree size) was correlated with maple key production.

In theory, the lifetime fecundity of a short-lived annual plant should be relatively easy to measure. You could grow your experimental offspring in a natural setting where they would be visited by pollinators and produce seeds which, in turn, could be counted and tested for viability. Unfortunately, the practical limitations of a teaching lab limit your experiment to the laboratory environment. Consequently, you will base your estimates of fecundity on three *fecundity correlates*.

The expression of inbreeding depression may also be sensitive to the environment in which selfed and outcrossed offspring are compared. Differences between inbred and outbred progeny that occur under harsh conditions (low light or nutrients, competition with other plants) may not be observed in a more benign environment.

LIFE-CYCLE HAZARDS OF A PLANT

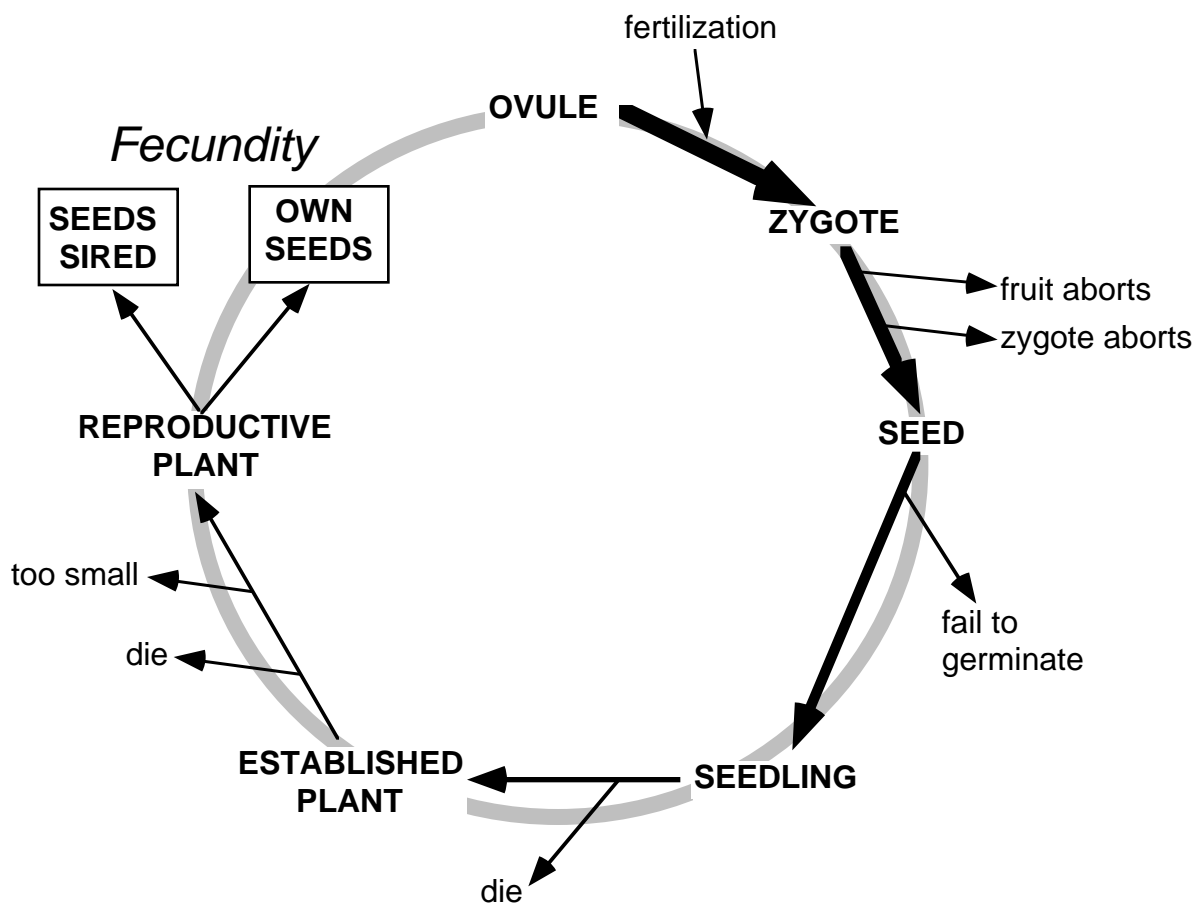


Figure 1. Life-cycle hazards and components of fitness in an annual plant.

II. Rapid-cycling *Brassica rapa*

Brassica rapa is a herbaceous annual mustard in the plant family Brassicaceae (sometimes also called Cruciferae because of their cross-shaped flowers), a plant family which includes many common agricultural species such as broccoli, cauliflower, radish, cabbage, Brussels sprouts, turnips, and rape seed. In natural populations, mating is mediated by an insect pollinator, usually the honeybee, which is lured to flowers by the bright yellow petals and sugary nectar secreted at the base of the flower (Figure 2). Wild populations of *B. rapa* are annual, that is they complete their life-cycle in one growing season. The plants you will work with have been selectively bred to complete their life-cycle in an even shorter period of time (Figure 3). These 'fast plants' or 'rapid-cycling' *Brassic*as, as they are often called, are ideal for experiments on inbreeding depression.

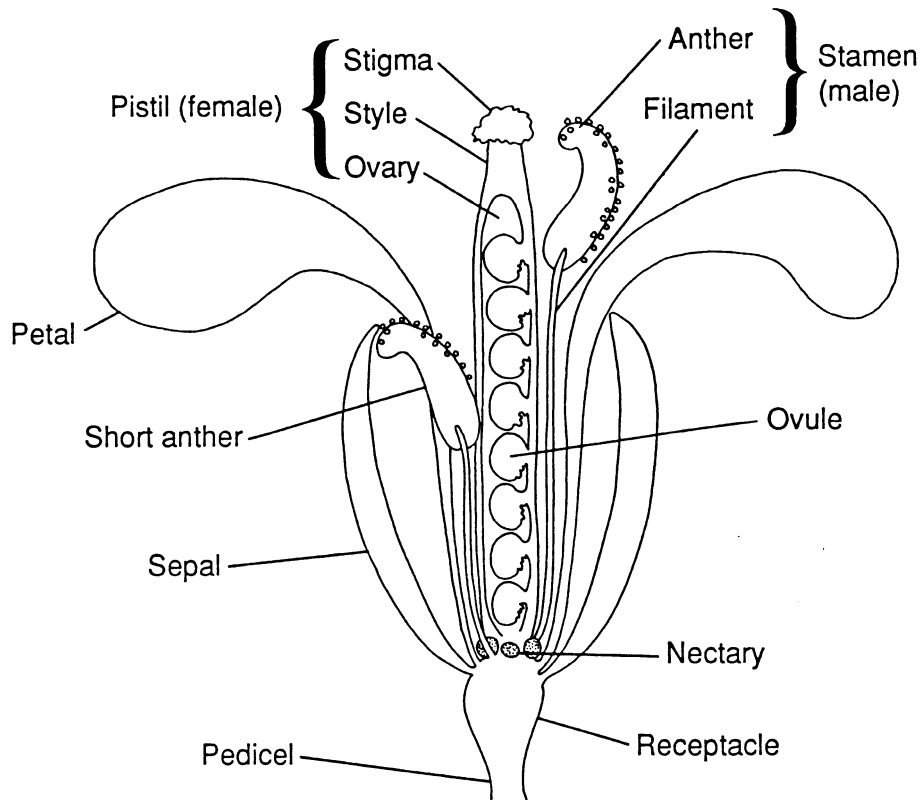


Figure 2. Cross-section of a flower of *Brassica rapa*.

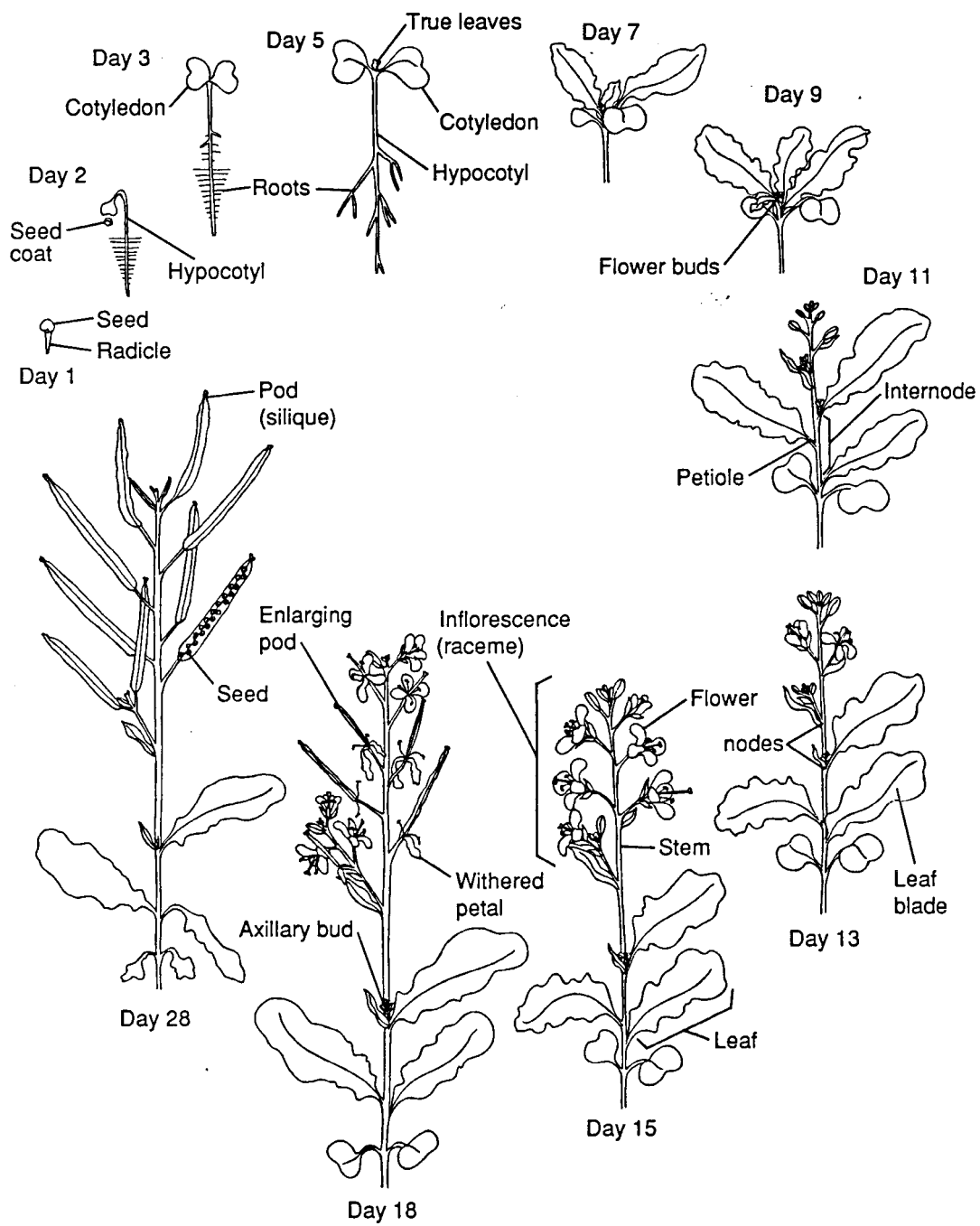


Figure 3. The life-cycle of *Brassica rapa*.

PART A — EXPERIMENTAL POLLINATIONS

In the first part of this study, you will produce seed through both self- and cross-pollination. You will be working with four flowering plants. On two of these, flowers will be self-pollinated; on the other two, flowers will be cross-pollinated. Performing controlled pollinations, whether selfed or outcrossed, simply requires removing pollen from the anthers of the male parent and applying that pollen to the stigma of the female parent. Recall, however, that *B. rapa* possesses a physiological self-incompatibility mechanism which usually prevents successful self-fertilization (see Introduction). To produce selfed offspring you will have to circumvent self-incompatibility.

In *B. rapa*, there are two ways to overcome self-incompatibility. The first method exploits the fact that stigmas are able to receive pollen about three days before the flowers open, yet the self-incompatibility system only develops about two days before the flowers open. This affords one day of bud development during which flowers can be self-fertilized. Finding buds at the right stage is relatively easy since flowers open sequentially every eight hours. However, it is difficult to pollinate young buds since they are very small and delicate. An alternative way of disrupting self-incompatibility is to treat the stigma surface with salt just before pollination. The incompatibility reaction in *B. rapa* occurs on the surface of the stigma, hence altering the stigmatic environment by the application of a weak salt solution abolishes the incompatibility reaction and allows successful self-fertilization.

I. Performing Pollinations

1. With masking tape and a waterproof marker, label each pot with your initials and indicate whether the plant will be selfed (S) or outcrossed (O). You will use at least two plants per pollination treatment.
2. Find the most recently opened flower and locate the first three buds up the stem (the oldest buds). These are the buds you will pollinate.
3. Using forceps and a dissecting needle, carefully open the bud to expose the stigma as illustrated in Figure 4 (A and B). Do not rip off the sepals and petals; simply part them to fully expose the stigma. **Be gentle!!**
4. Inspect the anthers. Note that they are not yet mature and should NOT be releasing pollen at this stage.
5. Apply a drop of salt solution to each stigma using a toothpick. Spread the solution around to fully cover the surface of the stigma. Wait *15 minutes* for the salt to breakdown the incompatibility system, and then dry off the stigma with a tissue to remove any remaining salt solution.
6. Using forceps, gently remove a mature anther from a recently opened flower on one of your *other* plants (for outcrossing) or the *same* plant (for selfing) and rub the pollen-covered surface of the anther lightly across the exposed stigmas of the three buds (Figure 4C), making sure some pollen adheres to the stigma surfaces.
7. Gently mark the base of each pollinated bud with acrylic paint to help identify it in the next lab.
8. Remove all old flowers (i.e. those below the buds you have pollinated).
9. To help support the plant during fruit maturation, attach a stake to the main stem of the plant using the thread and wooden sticks provided.
10. Join your 4 pots together with an elastic band and place them back on the watering container under the lights on the growth bench.
11. **Return 2 days later** and pollinate another 3 buds and mark them with paint.

12. When you have finished all pollinations, remove all *unpollinated* buds plus open flowers that you have not pollinated. Cut off the apical meristem and remove all other inflorescences and axillary buds (see Figure 3). Do *not* remove any leaves. With the flowers, unpollinated buds and other meristems removed, the plant can devote more resources towards maturing the buds you pollinated.
13. Join your 4 pots together with an elastic band and place them back on the watering container under the lights on the growth bench. The selfed and outcrossed seeds will be mature in several weeks.

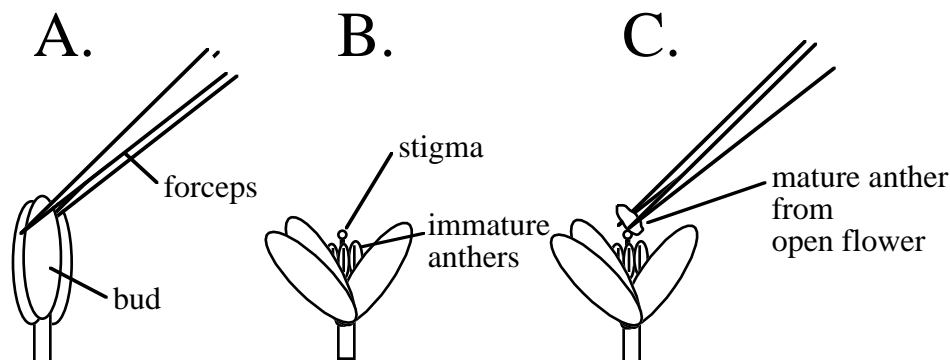


Figure 4. Bud pollination in *Brassica rapa*. Fine forceps are used to carefully expose the stigma (A and B) and lightly brush a mature anther across the stigma surface (C). Note that the anthers in the bud are not mature. Pollen must be obtained from mature anthers on recently opened flowers.

PART B — HARVESTING & PLANTING THE OFFSPRING

In this part of the experiment, you harvest and count the pods and seeds produced by your self- and cross-pollinations. You will then take a sample of seeds and plant them in a growth experiment to compare the relative fitness of selfed and outcrossed progeny.

1. Fruit & Seed Collection

1. Recall that you pollinated 6 buds on each of 4 plants; 2 plants were selfed and 2 were crossed.
2. Remove the mature pods which developed from the buds you pollinated (your T.A. may have already done this if the pods matured early); note those flowers which failed to form pods
3. Carefully split open each pod and count the number of seeds inside. Mature seeds should be brownish and plump. Smaller, shriveled seed-like things are aborted ovules and should *not* be counted as seeds. Record your data in Table 2 below and add them to the class data. **Hand in your selfed and outcrossed seeds to your T.A.; s/he will randomize them and redistribute them to you for planting.**
4. Remove the plants from their pots and discard them. Wash out the pots discard the used watering mat.
5. Copy the class data in Table 3. In the next lab, you'll do statistical tests to assess whether pods/pollination and seeds per pod differ significantly between cross- and self-pollinations.

II. Planting Selfed & Outcrossed Offspring

Your goal is to compare the survival and growth of the selfed and outcrossed offspring under *uniform* conditions. Although growing conditions will always vary to some extent from pot to pot, you should do everything possible to minimize this environmental variation. However, as mentioned in the introduction, the expression of deleterious alleles may depend strongly on the severity of the growth environment. You will examine the effect of intraspecific competition (an extremely important stress in plants) on the expression of inbreeding depression.

1. Set up *eight* pots as follows:
2. Using forceps, pull the thin end of one water wick through the bottom of each pot until half the wick is sticking out of the bottom (see Figure 5).
3. Lightly place some soil in the pot and tap in down by rapping the bottom of the pot on the lab bench. Do not use your finger to jam soil into the pot. *Do not pack soil too tightly.*
4. When the pot is *half* full, place *three* fertilizer pellets in the pot and cover with soil until the pot is almost full. Since the soil you are using is very low in nutrients, fertilizer is critical to plant nutrition. These pellets slowly release nitrogen, phosphorus and potassium over the course of the experiment.
5. You'll plant 4 outcrossed and 4 selfed seeds (one per pot). In two pairs of pots (2 selfed and 2 outcrossed), the plants will grow alone. In the other two pairs, you will plant a seed from a standard outbred line of *B. rapa*. You can refer to this seed as "the competitor". Make masking tape labels to mark each pot with the identity of the seed it will contain (SA = selfed alone, OA = outcrossed alone, SC = selfed with competitor, OC = outcrossed with competitor).
6. Each seed should be planted by grasping it gently with forceps and pushing it into the soil in the center of the pot to a depth of *only 1 or 2 mm*. If you've done all this properly, each of your pots should be set up as illustrated in Figure 5.

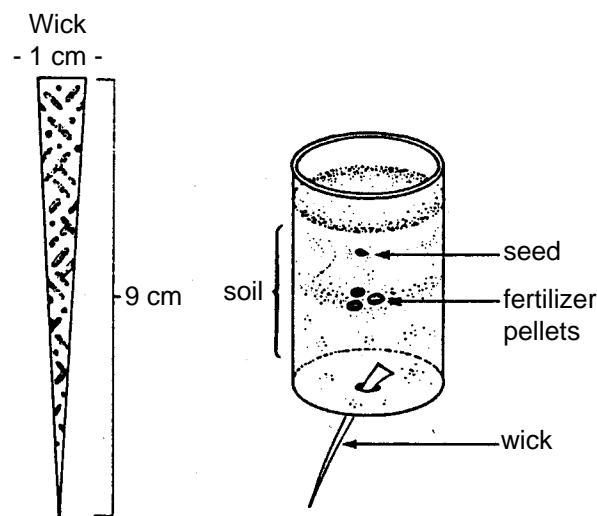


Figure 5. Proper pot set-up.

7. In the pots with competitor, plant the "focal" seed (selfed or outcrossed) slightly to one side and plant the enemy on the other side. Mark the location of the *competitor* with a toothpick.
8. Place your 8 pots on the watering mat, *alternating selfed and outcrossed* so that selfed or outcrossed offspring are not clustered together, and bind the pots together gently with an elastic band. Ensure that the wicks are in good contact with the watering mat.
9. Your T.A. will place the plants under the lights on the growth bench. They will be ready to measure in a few weeks.

PART C — ESTIMATING FITNESS & INBREEDING DEPRESSION

In this part of the lab, you will compare the relative survival and fecundity of selfed and outcrossed offspring grown in a common environment. First, you will calculate survival from seed to seedling and from seedling to adult. Then, you will measure three characteristics thought to be correlated with fecundity. Finally, you will synthesize the data from the entire lab, perform the appropriate statistical analyses, and draw conclusions on the strength and evolutionary consequences of inbreeding depression in *B. rapa*. **Make sure you review the statistical methods in Appendix 1 before coming to this lab.**

I. Seed & Seedling Survival

1. Retrieve your four pots and closely inspect each to determine whether the focal seed germinated. Also note whether the competitor (in those pots that have one) germinated. Germination is indicated by emergence of the root or shoot from the seed. Note also which of the germinated seeds survived to produce adult plants. Enter the germination and survival data in Tables 4 and 5, respectively.
2. Add your data to the class data and once the totals have been tallied perform a chi-squared (χ^2) test to evaluate whether germination and survival differs significantly between selfed and outcrossed offspring.

II. Measurement of Fecundity Correlates

1. You will measure three characteristics of your plants that may be correlated with fecundity (the ability to produce viable seeds). First measure *plant height* (to 0.1 cm) from the soil surface to the highest apical meristem. Next, count the *number of flowers*; both those that are open and previously opened flowers that are now withered. Finally, cut the stem at soil level and measure the *fresh weight* of the plant (to 0.01 g) using the electronic balance provided. Plants that have prematurely dried out should *not* be weighed but can provide data on both flower number and plant height. Enter your data in Table 6 and add your numbers to the class data.
2. Remove the plants from their pots and discard them. Wash out the pots and the watering container and discard the used watering mat.
3. Copy the class data into Table 7 and perform Student's *t*-tests to evaluate whether the three fecundity correlates differ significantly between selfed and outcrossed offspring.

III. Data Analysis

Differences between selfed and outcrossed offspring are conventionally summarized by the parameter δ (delta) which represents the strength of inbreeding depression, and is calculated as:

$$\delta = 1 - \left(\frac{\omega_s}{\omega_o} \right),$$

where ω_s and ω_o are the fitnesses of selfed and outcrossed offspring, respectively. Note that δ increases with the strength of inbreeding depression. Positive values mean that outcrossed offspring have a higher fitness than selfed offspring; negative values mean just the opposite. When $\delta = 0$ inbreeding has no effect on offspring fitness. You may calculate δ separately for survival during each of the three life-history stages, and for each fecundity correlate by substituting the mean of selfed offspring for ω_s and the mean of outcrossed offspring for ω_o .

An overall estimate of inbreeding depression that makes use of all the data you have collected can be derived by calculating a *multiplicative fitness function* (W) for both selfed (W_s) and outcrossed (W_o) offspring and then calculating:

$$\delta_t = 1 - \left(\frac{W_s}{W_o} \right)$$

The multiplicative fitness function combines the probabilities of survival at each of the life-history stages with your measure of fecundity:

$$W = \left(\frac{\text{pods}}{\text{pollination}} \right) \left(\frac{\text{seeds}}{\text{pod}} \right) (\text{germination})(\text{survival})(\text{fecundity})$$

Since you have not measured fecundity directly, correlates of fecundity will be substituted in its place. Do these calculations separately for plants with and without competitors. Complete Table 8 by calculating δ for all the parameters measured, and δ_t using each of the three fecundity correlates. Calculate the average of the three δ_t 's. This is probably your best overall measure of inbreeding depression in *B. rapa*.

IV. THE WRITE-UP

You will write-up the results of this experiment as a scientific paper. In doing so, you will also review some of the recent inbreeding depression experiments done on other species and compare your results with those obtained by other workers.

Format

When you submit a manuscript to a peer-reviewed journal for publication, you follow a specific format for preparing the paper. Each journal has its own particular format. You will format your paper for submission to the journal *Evolution*. The formatting instructions can be found in the back of each issue. Refer to the 1999 instructions. The instructions will be fairly brief and will ask you to follow the format of recent articles published in the journal. Your manuscript should be no more than 12 pages (not including figures and tables) of double-spaced, 12-point font. ***Be sure to follow this format exactly, as marks will be deducted for improper format.***

Structure

Your paper will start with a title page, followed by a 1-page abstract, an introduction, materials & methods, results, discussion, literature cited, tables, figure legends, and figures. Each of these sections serves a particular purpose. Below, is a brief description of what should be included in each section. You may also find it useful to refer to one of the many books on writing scientific papers.

- 1) *Title page* — This page should bear the title (brief and descriptive, maybe even intriguing), your name (and student number) and your affiliation (department, institution, address).
- 2) *Abstract* — This is a 1-page synopsis of the study. It should be brief and informative, including the rationale for the study as well as the main conclusions. Carefully examine abstracts of papers published in *Evolution* to get an idea of how to construct one.

- 3) *Introduction* — There are millions of scientific papers published each year. Why would anyone want to waste their valuable time reading your particular paper? The introduction is where you convince a potential reader that it's worth reading on. In your introduction, you want to identify the general question(s) you are addressing and tell the reader why that question is interesting, and to what extent your paper is a novel approach to that question. You should also give a brief description of your study system (species) and point out the reasons why this particular species is appropriate for answering the question at hand. In writing *your* intro, you can assume that no one has measured inbreeding depression in *Brassica rapa* before, and certainly no one has compared the level of inbreeding depression with and without intraspecific competition. End your introduction by clearly stating the specific questions you are investigating.
- 4) *Materials & Methods* — This should be a clear, concise description of the methods you used. Another researcher should pretty well be able to replicate your experiment based on the description of the methods included in this section. Examine the Materials & Methods sections of some of the other inbreeding depression papers you have read for this write-up (see below). **This section should not include verbatim quotes from the lab manual.** Describe what you did in your own words.
- 5) *Results* — The Results are just what they are: results, presented clearly and without editorializing or interpretation. Appropriate tables and figures should be cited as they become relevant. Writing an engaging and honest results section is a fine art. You needn't describe the contents of all the figures and tables in excessive detail. Rather, you want to gently lead your reader through the mass of data you're trying to present. You also want to clearly present the statistical results (i.e. results of chi-squared and *t*-tests) that provide formal tests of the hypotheses being investigated.
- 6) *Discussion* — The discussion is the really interesting part of the paper. In this section you have a chance to interpret the data with respect to the questions you started off asking and elaborate on your interpretation. This is where new ideas are presented and old ideas are put through the wringer. You should also compare your results to those of other workers, talk about methodological problems that might have biased your results, and point out future research directions. When preparing to write your Discussion ask yourself "O.K. what do I want to talk about here? What is interesting about these data? How well did I answer my original questions? What work could I do in the future to build on this paper?". Here are some questions to ponder when planning your discussion:
- i) Based on your results, would a self-fertilizing mutant spread? Express this in terms of the automatic transmission advantage of selfing and the number of gene copies transmitted to future generations.
 - ii) How many other studies have estimated inbreeding depression in a self-incompatible plant species or compared the strength of inbreeding depression in different environments?
 - iii) Is inbreeding depression stronger with intraspecific competition? Why might one expect a difference?. Do your results, therefore, suggest that the potential spread of a mutation for self-fertilization is dependent on the severity of the environment?
 - iv) How well have you estimated *fitness* in your experiment?
 - v) You measured inbreeding depression under controlled growth-bench conditions. Would your results have been different if you conducted the same experiment under natural conditions? Back up your discussion of this point with results from other studies.
 - vi) Do your results have any applied significance with respect to agriculture or conservation biology?

- 7) *Literature cited* — This is simply a list of the papers you have cited in your manuscript. Examine the literature citations in recent issues of *Evolution* to get an idea of how various types of articles (e.g. journal papers, books, book chapters, theses) are cited. Make sure you get the format right. **Do not cite the lab manual.** All the ideas found in the lab manual are from the primary literature and you will find the appropriate references to these ideas in the other inbreeding depression articles you have read (see below).
- 8) *Tables* — Tables are used for particularly detailed presentations of data. It's usually always better to use figures than tables, since figures are often more readily comprehended. Most of your results can be presented in figures. But feel free to use tables when you think they are more appropriate. Follow the format of the tables presented in recent issues of *Evolution*. In your manuscript, each table will be presented on a separate page.
- 9) *Figure legends* — Figures are extremely powerful tools, and making good figures is always an enjoyable challenge. Peruse recent articles in *Evolution* and notice the diversity of figures. Sometimes figures are simple. Sometimes they are multi-paneled and complex. Examine other papers on inbreeding depression to get ideas on how to best present your data. Note that each figure has a concise legend or title associated with it. In manuscript form, all these legends are grouped together on one or two pages, and each figure is presented on a separate page.

Literature

In preparing your write-up you are required to **find, read and integrate at least 6 papers on inbreeding depression in plants published within the last 10 years** (i.e. 1989–1999). The best way to find these papers is to browse the indices of recent volumes of journals such as: *Evolution*, *Heredity*, *American Journal of Botany*, and *Ecology*. Once you find a recent paper, other will be listed in the literature cited. You can also use CD-ROM searches and searches in *Current Contents* and *Biological Abstracts*. However, this route is not always as successful as chasing down citations. There has been much theoretical and experimental work published on inbreeding depression in the last decade. In choosing your 6 papers, you should try to include studies on a diversity of species (i.e. predominantly selfing and predominantly outcrossing species) conducted under a diversity of experimental conditions (greenhouse experiments vs. experiments conducted in natural habitats). **You should start your literature search at least a month before your experiment is finished since it will take a while to read and understand your 6 papers.**

General tips

- Start researching and preparing your report early so that your TA can help you with any problems you encounter.
- Don't get bogged down in the complex statistics that may appear in your 6 papers on inbreeding depression. Try to cut through the statistical haze and focus on the main results of the papers.
- When writing your paper, be clear and concise. Don't wallow in jargon and "scientific sounding" gibberish. Write simply and directly.
- You can organize information within the main sections of the paper using subheadings. Note how these are used in other papers.
- The best way to learn how to write a scientific paper is to critically read other scientific papers. Ask yourself what you like and don't like about particular papers. Make note of particularly good turns of phrase or ways of organizing ideas.
- Make sure you weed out all typos and grammatical problems before handing in your report. Up to 10% will be deducted for bad writing, spelling and formatting.
- Do not leave this report to the last minute. It's worth 25% of your final grade (half as much as both exams combined) and will require considerable effort.

Name: _____ Student # _____

Lab TA: _____ Time: _____ Room: _____ Day: _____ Bench # _____

DATA TABLES, ANALYSES & QUESTIONS

(show sample calculations for statistical tests on the back of these sheets)

*****These data tables (2–8) must be handed in along with your report.**

Table 2. Pod and seed production (your data – this table MUST be filled in)

	No. buds pollinated	No. pods matured	Total seeds	Seeds per pod
<i>Outcrossed</i>				
Plant 1
Plant 2
<i>Selfed</i>				
Plant 3
Plant 4

Table 3. Pod and seed production (pooled class data).

	Outcrossed	Selfed
<i>Pods/pollination</i>		
buds forming pods
buds not forming pods
Pods/pollination
<i>Seeds/pod</i>		
Mean
Standard deviation
Sample size

****Compare pods/pollination for selfed and outcrossed progeny (for each growth condition separately) using chi-squared tests. Compare the mean seeds/pod using *t*-tests. See Appendix 1 of this lab manual for more information.**

Name: _____ Student # _____

Lab TA: _____ Time: _____ Room: _____ Day: _____ Bench # _____

Table 4. Germination.

	Number of seeds:	Proportion
	Germinated	germinated
	Not germinated	

Your data

Growing alone

Outcrossed.....

Selfed.....

With competitor

Outcrossed.....

Selfed.....

Class data

Growing alone

Outcrossed.....

Selfed.....

With competitor

Outcrossed.....

Selfed.....

**Compare the proportions for selfed and outcrossed progeny (for each growth condition separately) using chi-squared tests. See Appendix 1 of this lab manual for more information.

Name: _____ Student # _____

Lab TA: _____ Time: _____ Room: _____ Day: _____ Bench # _____

Table 5. Survival.

	Number of seedlings:	Proportion
	Alive Dead	survived

Your data

Growing alone

Outcrossed.....

Selfed.....

With competitor

Outcrossed.....

Selfed.....

Class data

Growing alone

Outcrossed.....

Selfed.....

With competitor

Outcrossed.....

Selfed.....

**Compare the proportions for selfed and outcrossed progeny (for each growth condition separately) using chi-squared tests. See Appendix 1 of this lab manual for more information.

Name: _____ Student # _____

Lab TA: _____ Time: _____ Room: _____ Day: _____ Bench # _____

Table 6. Fecundity correlates.

	Plant height (cm)	Number of flowers**	Fresh weight (g)
<i>Growing alone</i>			
<i>Outcrossed</i>			
Plant 1
Plant 2
<i>Selfed</i>			
Plant 3
Plant 4
<i>With competitor</i>			
<i>Outcrossed</i>			
Plant 1
Plant 2
<i>Selfed</i>			
Plant 3
Plant 4

**Number of flowers = buds + open flowers + spent and/or withered flowers

Name: _____ Student # _____

Lab TA: _____ Time: _____ Room: _____ Day: _____ Bench # _____

Table 7. Statistical analysis of fecundity correlates.

	Growing alone		With competitor	
	Outcrossed	Selfed	Outcrossed	Selfed
<i>Plant height (cm)</i>				
Mean
Standard deviation
Sample size
<i>Number of flowers</i>				
Mean
Standard deviation
Sample size
<i>Fresh weight (g)</i>				
Mean
Standard deviation
Sample size

**Compare the means for selfed and outcrossed progeny (for each growth condition separately) using *t*-tests. See Appendix 1 of this lab manual for more information.

Name: _____ Student # _____

Lab TA: _____ Time: _____ Room: _____ Day: _____ Bench # _____

Table 8. Estimates of inbreeding depression in *Brassica rapa*.

	P/P	S/P	G	S	PH	NF	FW	for δ_i 's		
								W_{PH}	W_{NF}	W_{FW}
<i>Without competition</i>										
Outcrossed
Selfed
ω_s/ω_o
δ (or δ_i)
Average of the three δ_i 's:	_____									
<i>With competition</i>										
Outcrossed
Selfed
ω_s/ω_o
δ (or δ_i)
Average of the three δ_i 's:	_____									

Note: P/P = proportion of buds forming pods; S/P = mean seeds/pod; G = proportion of the seeds germinated; S = proportion of seedlings that survived; PH, NF, and FW = mean plant height, number of flowers, and fresh weight, respectively. Each of the W 's is the multiplicative fitness based on the subscripted fecundity correlate.

☆☆☆ **THIS REPORT SHOULD BE HANDED IN DIRECTLY TO YOUR T.A. AT THE BEGINNING OF YOUR REGULAR LAB PERIOD DURING THE WEEK OF MARCH 15–19. THERE IS A PENALTY OF 5 MARKS (OUT OF 25) FOR EACH 24 HOURS THE REPORT IS LATE. NOTE THAT T.A.'S ARE NOT RESPONSIBLE FOR REPORTS THAT ARE NOT SUBMITTED IN PERSON.**

☆☆☆ **YOU WILL HAND IN YOUR ORIGINAL DATA TABLES (2–8) ALONG WITH (BUT NOT INCLUDED IN) YOUR REPORT, EVEN THOUGH THE CLASS DATA FROM THESE TABLES WILL BE PRESENTED AGAIN IN YOUR REPORT. REPORTS WITHOUT ORIGINAL DATA TABLES WILL NOT BE ACCEPTED.**