

Artificial Selection in *Brassica*, Part I

Goals of this activity:

1. Gain general familiarity with the process of artificial selection.
2. Become acquainted with some of the diverse products of artificial selection in *Brassica*.
3. Set up an artificial selection experiment involving a variety of *Brassica rapa*.

Introduction

A trip to a supermarket, farm, pet store or garden center will offer nearly endless examples of the products of selective plant and animal breeding by humans. Over hundreds, and in some cases thousands of years, humans have altered various species of plants and animals for our own use by selecting individuals for breeding that possessed certain desirable characteristics, and continuing this selective breeding process generation after generation. In many cases the end results have been dramatic. Domestic dog varieties, from chihuahuas to great danes, trace their separate lineages to a common wild ancestor, the wolf (*Canis lupus*). Domestic fowl varieties are all derived from the wild jungle fowl (*Gallus gallus*), while most modern breeds of domestic cattle originate from the now-extinct giant wild ox (*Bos primigenius*). One example that particularly impressed Charles Darwin was that of domestic pigeon varieties (such as tumblers, fantails, carriers, pouters, and many others), which are derived from wild rock doves (*Columba livia*) over a period of some 5000 years.

There are many similar examples among plants, including those that humans have bred for food

as well as beauty. One plant group especially important to humans for food is *Brassica*, a genus of plants in the mustard family. A wide variety of familiar and highly nutritious vegetables originate from just a few species of wild Brassicas, in particular *B. rapa*, *B. oleracea*, and *B. juncea*. Some varieties have been bred specifically for root production, others for leaves, flower buds, oil production, etc. The group is of great economic importance, and because of this, the genetic relationships among the various forms have been thoroughly studied and are now fairly well understood. It may surprise you to learn that these familiar vegetables so different in appearance have the same species as a common ancestor. Centuries of artificial selection have produced such widely divergent forms. For example, the following varieties originate from these wild species:

Brassica oleracea: kale, cauliflower, broccoli, cabbage, Brussels sprouts, kohlrabi, collards, savoy cabbage.

Brassica juncea: leaf mustard, root mustard, head mustard, and many more varieties of mustard.

Brassica rapa: turnip, Chinese cabbage, pak choi, rapid-cycling (see **Figure 4**).

The next time you're in a grocery with a large produce section, or a large farmers' market, see how many of these different varieties you can find; there are numerous other varieties not listed above.

That plant and animal breeders have been able to dramatically change the appearance of various lineages of organisms in a relatively short period of time is an obvious yet profound fact. It certainly did not escape the attention of Charles Darwin, who devoted the first chapter of *The Origin of Species* to the topic of artificial selection by humans ("Variation Under Domestication"). He used many examples of selection by humans to help support the case for his proposed mechanism resulting in evolution of natural populations—natural selection.

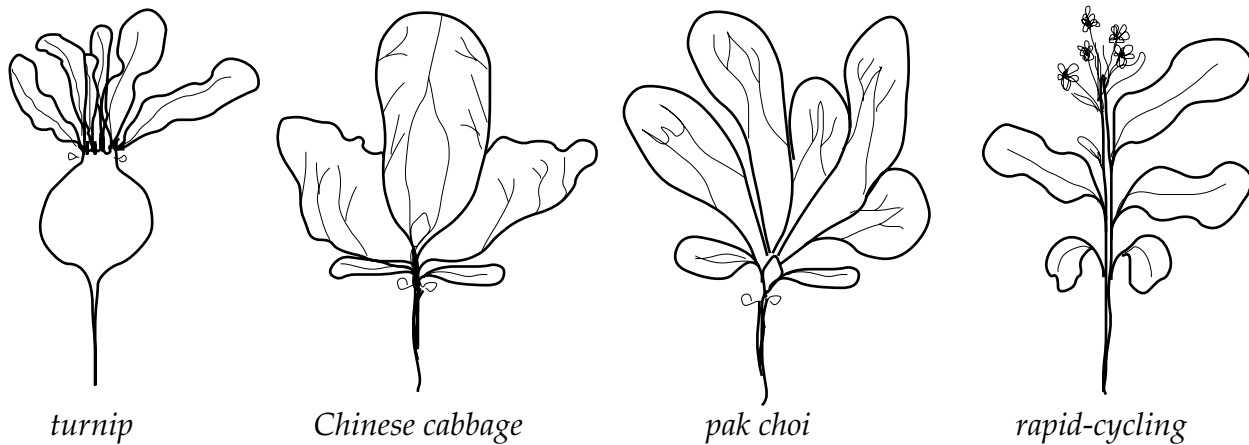


Figure 4 Varieties (cultivar groups) of *Brassica rapa*.

In order to gain better understanding of selection and inheritance, researchers have experimented with artificial selection involving a wide variety of traits in many different species of plants and animals. The results, obtained in a relatively short period of time, are often impressive.

For example, in one experiment (started in 1896), the average oil content of corn kernels was increased from 5% to 19% in 75 generations of selective breeding. In another, average annual egg production in a flock of white leghorn chickens was doubled in only 33 years (from 126 to 250 eggs per hen). Similar examples abound, for characters useful to humans (as above) as well as more esoteric ones (such as the number of abdominal bristles on fruit flies, or the fecundity of female flour beetles).

A general finding from these studies is that most variable traits in organisms respond to artificial selection (i.e., it is usually possible—even easy—to increase or decrease the frequency or average value of a trait in a lineage through careful selective breeding). Starting today in a lab exercise, you will attempt to accomplish the same thing.

How artificial selection is similar to natural selection

You will be learning a great deal about natural selection in lecture and in later labs. Natural selection is a deceptively simple concept, relatively easy to understand at a basic level, but with profound implications that are intellectually challenging. The following exercise in artificial selection will serve as an introduction to natural selection, and we hope will help you to better understand this important concept. Fundamentally, artificial selection and natural selection are quite similar, but there are a few important differences.

Very briefly, natural selection occurs when certain variants within a population of organisms experience consistently greater reproductive success (i.e., leave more offspring) than do other variants. Generally, within a population of organisms (of the same species), there is variation among individuals for a great variety of obvious and not so obvious traits. Some of this variability is a result of genetic differences among individuals, while some is probably a result of different

environmental influences. Here we are concerned only with variability that has a genetic basis (i.e., is inheritable—passed on from parents to offspring). As a general rule, organisms produce more (often far more) offspring than can survive to reproduce themselves (in Darwin’s words, there is a “struggle for existence”). If individuals with certain inheritable characteristics have an advantage in survival and reproduction over individuals with other characteristics, then it follows that the next generation will differ from the previous one—perhaps slightly, perhaps considerably. Why? Because individuals with those certain favored inheritable characteristics are more likely to be parents by virtue of possessing these characteristics; the next generation will contain a disproportionate number of offspring of these “naturally selected” parents, and these offspring will tend to resemble their parents. As a result, over many generations the genetic structure of the population changes (in other words, the population evolves).

Artificial selection is essentially this same process. A population of organisms exhibits considerable variability among individuals for a trait or traits that might be of interest to humans for one reason or another (such as running speed in thoroughbred horses, petal size and shape in tulips, number of kernels in an ear of corn, egg size in chickens, etc.). By virtue of possession of certain traits, some individuals are selected by the plant or animal breeder or scientist to be parents of the next generation, while the remainder of the population not possessing those traits are excluded from

breeding. To the degree that the desired trait is inheritable, the offspring will tend to resemble their parents, and hence on average the next generation will differ from the previous one — i.e., will be faster, or have larger petals, or more kernels, or larger eggs, etc. Over time, substantial differences can be achieved between the original population and the artificially selected subsequent ones.

How artificial selection differs from natural selection

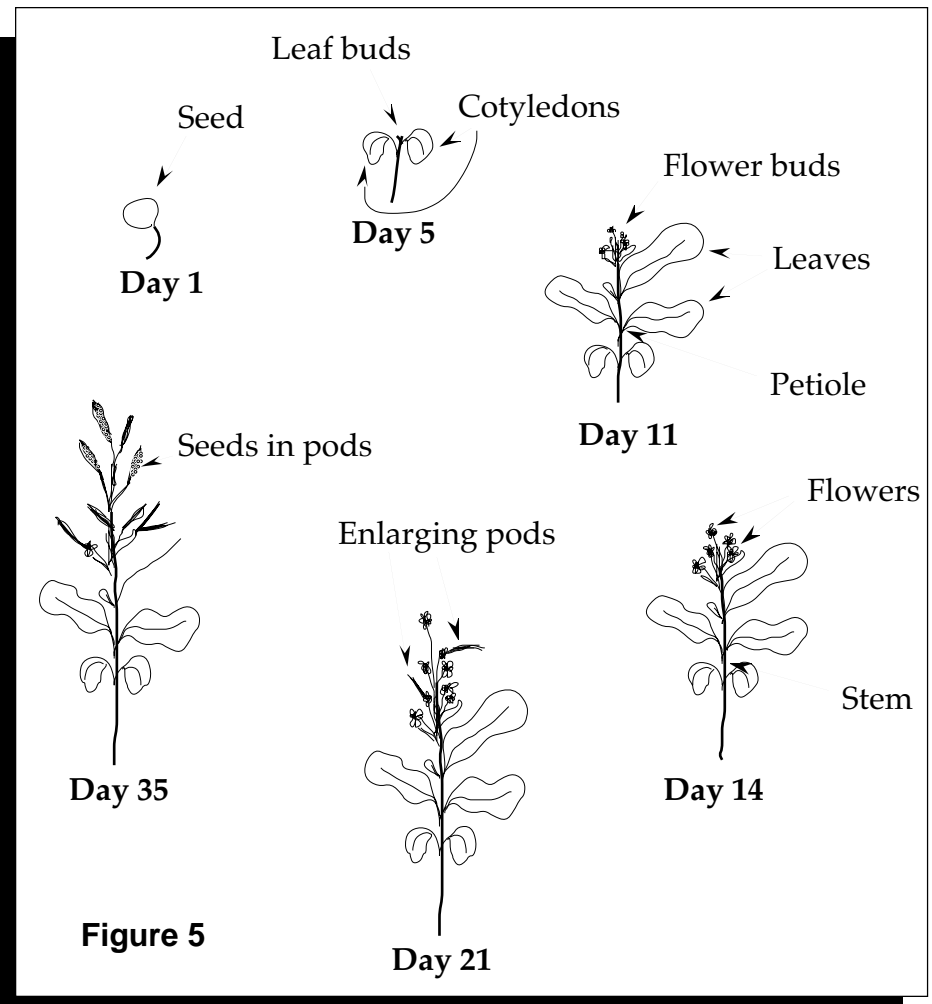
As noted above, there are some important differences between artificial and natural selection. In contrast to natural selection, artificial selection: 1) favors traits that for one reason or another are preferred by humans; 2) has a goal or direction toward which the selection process is directed; 3) generally is much faster than natural selection, because the next generation can be absolutely restricted to offspring of parents that meet the desired criteria (rarely is natural selection such an all-or-none phenomenon). In artificial selection, humans are doing the selecting—purposely restricting breeding to individuals with certain characteristics. In natural selection, the “environment” does the selecting—individuals that survive and reproduce better in a given environment, for whatever reason, are “naturally selected.” The environment can include such things as predators, food supply, climatic conditions, soil nutrients, and many, many others.

An Artificial Selection Experiment Using Wisconsin Fast Plants™

Introduction:

You and the other members of your lab section will participate as plant breeders in an attempt to artificially select for a particular variable trait in a lineage of rapid-cycling *Brassica rapa* (Wisconsin Fast Plants™). This exercise will begin today, but will not be completed until the last lab period this quarter. The organism with which you will experiment belongs to the same species as turnip, pak choi and chinese cabbage, each of which is a different artificially selected variety. The *Brassica rapa* you will be using is itself a product of intense

artificial selection over the past 20 years for the following traits: rapid flowering and maturation; high seed production; short stature; and the ability to thrive under artificial light. The result of these efforts (at the University of Wisconsin) has been a very valuable research and educational tool. The generation time (from seed to mature plant to fertilization to mature seed) is about 6-7 weeks under our lab conditions, short enough to let us study one complete generation in a college quarter. The life cycle of this variety is diagrammed in **Figure 5**. Although remarkably short for a plant, this 6-week generation time is still agonizingly slow for both research and educational purposes, and thus we hope you will appreciate one main reason for our emphasis in Biology 1201 labs on computer simulations, in which many generations can be established and followed in just seconds, rather than months or years.



Prior to the start of the quarter, we established a number of separate populations of about 100 plants each, all from the same basic stock of “wild type” Fast Plant seeds initially obtained from a commercial source. Each population at this point represents the initial generation of a separate lineage or line of descent. We will take considerable care to avoid any cross-breeding between different lineages (different lab sections). Within each lineage, we planted the seeds all at the same time, 14 days prior to this lab, so the plants are still immature but should be close to flowering (see **Figure 6**). This is the initial population (the first generation). Your challenge, as a class, is to: 1) assess or quantify the

variability of one particular trait in this generation; and, 2) attempt to change the genetic makeup of the next generation with respect to this trait, so that the next generation, on average, exhibits the trait to a substantially greater degree than does the present one. If successful, you will have accomplished artificial selection, resulting in evolution within this particular lineage from one generation to the next.

Variable traits:

What trait will you attempt to artificially select? There are a number of fairly obvious variable traits that one can observe in a large population of mature Fast Plants. A brief list could include: total number of flowers, total number of leaves, length of the lower leaf, surface area of the lower leaf, plant height, total number of seeds per plant, total mass of plant, stem length between first and second leaves, etc. In contrast, there are other traits that don't appear to vary at all, such as: number of petals per flower (4); number of sepals (green petal-like structures just below the petals) per flower (4); petal color (bright yellow); and number of cotyledons or "seed-leaves" (2).

Your lab instructor will divide the population of plants into small samples of between 10 and 15 plants, and will give each student group (2 students) one of these samples. **Treat these plants gently; they are young and tender, and easily**

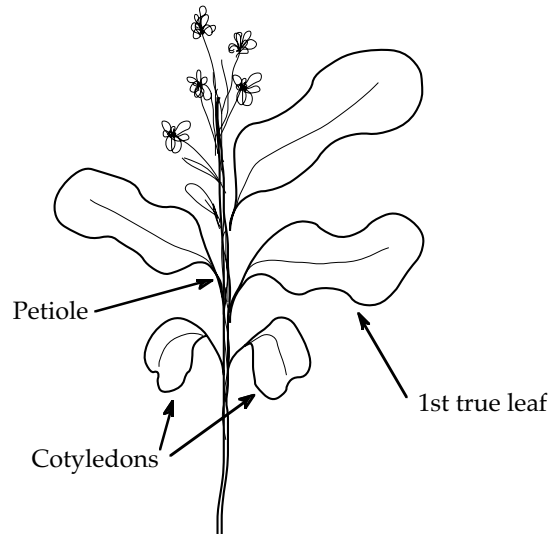


Figure 6 *Fast Plants, about 14 days old.*

damaged! When your group obtains your sample, look carefully at the plants and note the variability you can see. Remember, all your plants are almost exactly the same age, so differences you see (such as height, leaf size, etc.) are not due to differences in age.

Below, list 5 traits that are variable within the small sample of plants you have at your table, and give an indication of the degree of variability:

- 1.
- 2.
- 3.
- 4.
- 5.

One variable trait that you might not have noted in your list is “hairiness” of leaves, petioles (or leaf stalks) and stems, but if you look more closely (especially with a hand lens and desk lamp) you should see these “hairs” on some plants. Technically, plants “hairs” are called trichomes, and they have been shown to have a specific function. In the space below, hypothesize what this function might be.

Counting “hairs”

As you have probably guessed, the variable trait that you are going to attempt to alter in this lineage is “hairiness.” In this “wild type” population, some plants should be noticeably hairy, many are slightly hairy, while others are apparently hairless. But such an observation is too general, and needs further quantification.

To accomplish this, you could count all hairs on all parts of each plant, but this would be a time-consuming task, and an unnecessary one. It turns out that hairiness of one part of a plant (such as the leaf surface) is strongly correlated with hairiness on other parts (such as the leaf margin). In other words, a plant’s hairiness in general can be quantified by assessing hairiness of a specific structure.

The structure we will use for this “hairiness index” is the petiole, or leafstalk, where trichomes are large, conspicuous and rather easily counted, and the structure is relatively small with a defined starting and ending point.

What might be the function of “hairs”?

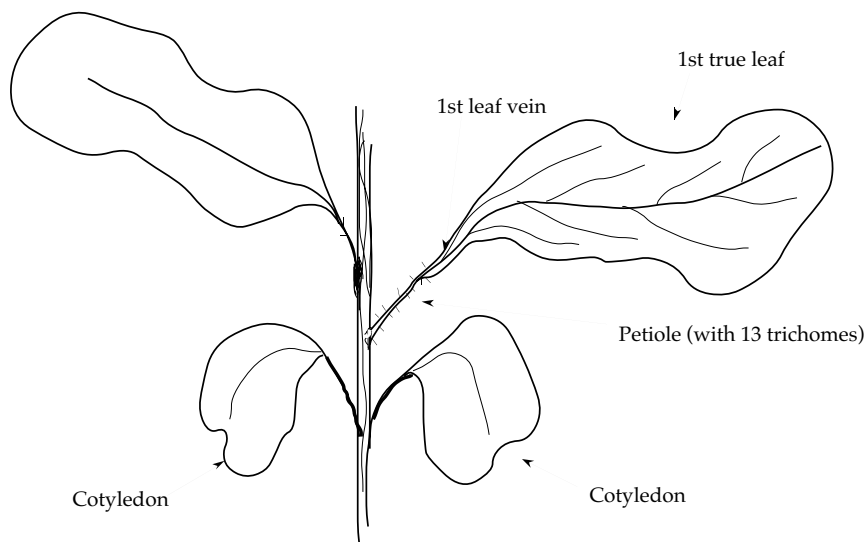


Figure 7

For consistency, we will use the petiole of the first (lowermost) true leaf, and we will define the limits of the petiole as follows: from its junction with the main stem (usually marked by a small bulge or ridge, often differently colored) to its junction with the lowermost leaf vein (see **Figure 7**).

An important note: the two lowermost squarish, two-lobed and rather thick leaves are actually cotyledons (“seed leaves”), not true leaves. The first true leaf is just above these two cotyledons. What you need to do now is to count the total number of trichomes on the petiole of the first true leaf of each plant in your sample. Your data will then be combined with data from the rest of the section.

Use the hand lens and desk lamp; the highest magnification on the lens is the small circle near the handle. The trichomes are most conspicuous if strongly illuminated against a dark background, such as the black table top. If present, the trichomes will generally be concentrated on the

lower side of the petiole, but some could occur on the sides or top as well. Be sure to rotate the plant so that you can see all aspects of its petiole. Count a second time for verification, then record your data in the spaces below.

Also record this number as a temporary mark on the side of each canister; use a marking pen with water soluble ink (green, blue and especially red pens all leave legible marks on the black plastic canisters). These marks are intended to be temporary—they are easily washed or rubbed off (later, you will permanently mark a small number of selected plants). As a reminder: handle these plants gently. They are easily damaged if treated roughly, and bent stems may result in death of the plant.

Collect data on your group’s plants now, and enter those data in **Data Box 1** (the number of plants you assess will probably be fewer than the available spaces).

Data Box 1

Your group’s data (number of trichomes on petiole of first true leaf, per plant):

_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____
_____	_____	_____	_____	_____	_____	_____

Combined data for entire population:

When all groups have finished assessing trichome number of individual plants, your lab instructor will coordinate the effort of combining the separate data into a single data table. After tabulating the section's data, your instructor will make this

summary available to you, and you should copy the data into the following frequency table in **Figure 8**. Next, plot a frequency histogram (bar graph) of these data in the space provided also in **Figure 8**. Mark the population average on the histogram.

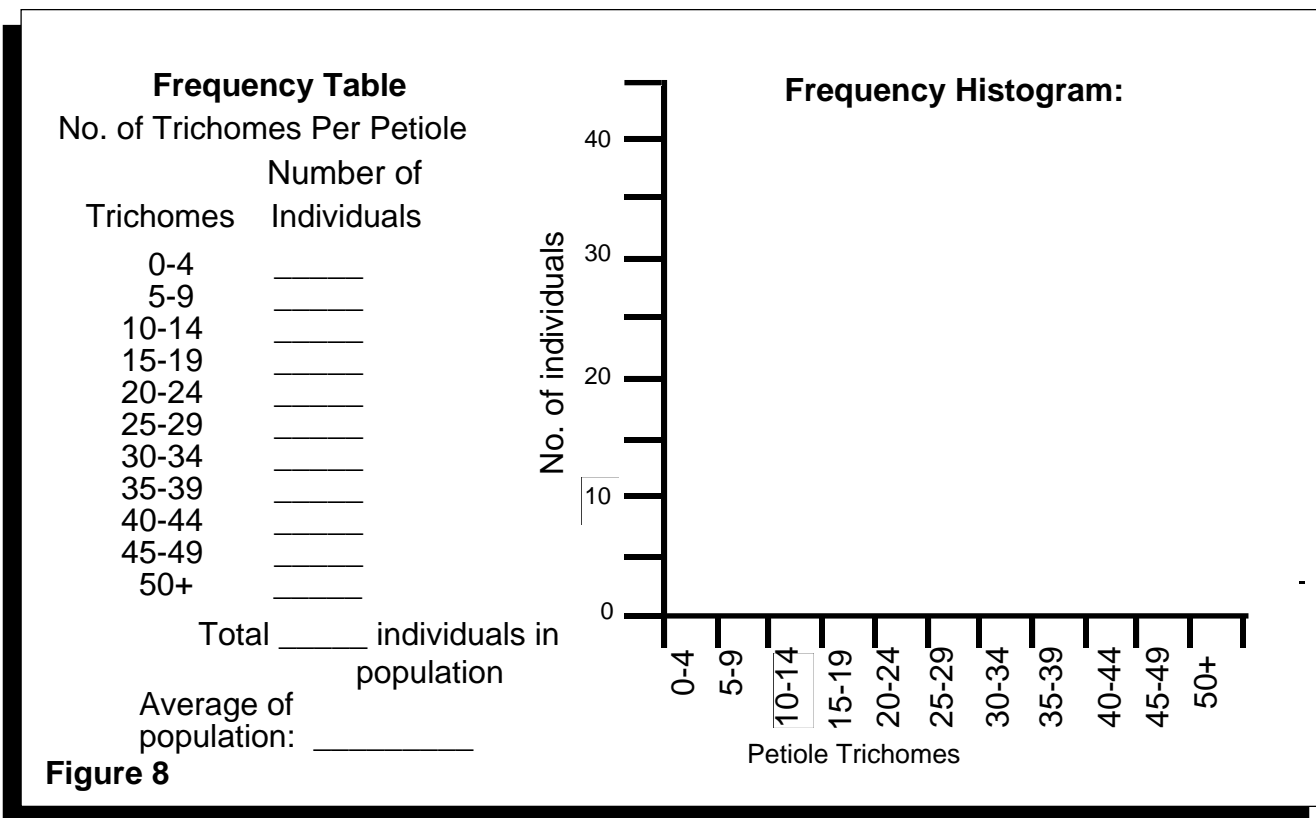


Figure 8

Selecting the parents of the next (second) generation:

Only a small fraction (about 10%) of this population of plants will be selected to be parents of the next generation. These won't be randomly chosen, but instead will be the hairiest plants in the population. Identify the 10-12 plants that had the highest petiole trichome counts, and label each canister with that

plant's trichome count using a small piece of tape and indelible (waterproof) ink. Then place these selected plants on a separate watering tray. Most plants will probably require a stake for support. Record the trichome values of the selected individuals (parents-to-be) in **Data Box 2**, as well as the average value.

Data Box 2

Selected parents (number of trichomes on petiole of first true leaf):

Selected individuals' average: _____

On the histogram on the previous page, indicate (with cross-hatching or stippling) the selected parents-to-be, and mark and label their average value.

Now calculate the difference between the average number of petiole hairs of the selected parents-to-be and that of the population as a whole. Record those numbers in **Data Box 3**.

Data Box 3

selected individuals, average: _____

entire population, average: _____

difference: _____

Finally, discard all of the unselected plants. Dump the soil and plants into a container; place the canister into a separate container. These discarded plants will not be permitted to breed, and thus will not contribute any offspring to the subsequent generation.

Predictions

Now it's time to hypothesize about the outcome of this experiment. How hairy do you think individuals in the second generation will be? Consider the three possible outcomes shown below. These are merely three possibilities — none of them is necessarily "right" and there are many others not presented. Discuss these and others with your lab partner, then draw a frequency histogram in the blank graph at the bottom right of **Figure 9** that you predict will represent the

second generation (which will be about 100 offspring of the selected parents). This histogram should show the range of values (lowest and highest) as well as the frequency (number of individuals that occur within each trichome number interval). Include on this histogram your prediction of the average value. The left ("Y") axis in your "predicted" histogram is purposely unlabeled; you should fill in the interval values (increments of either 5 or 10) so they are appropriate for your predictions.

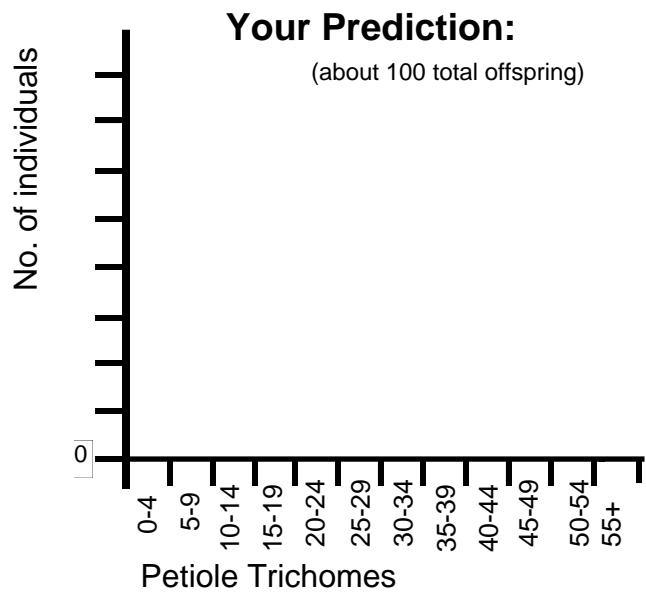
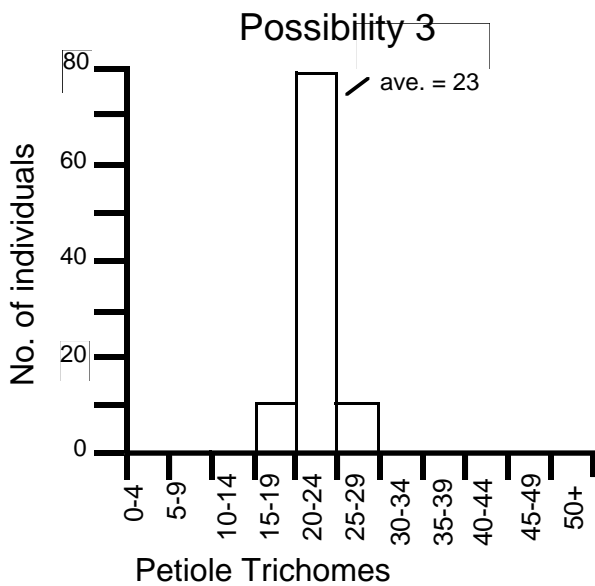
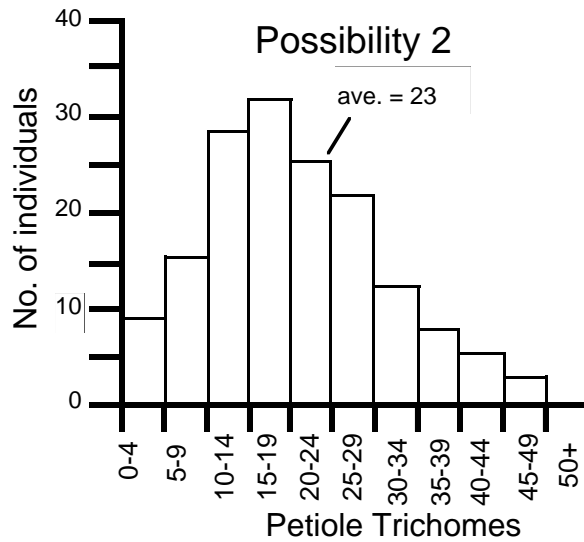
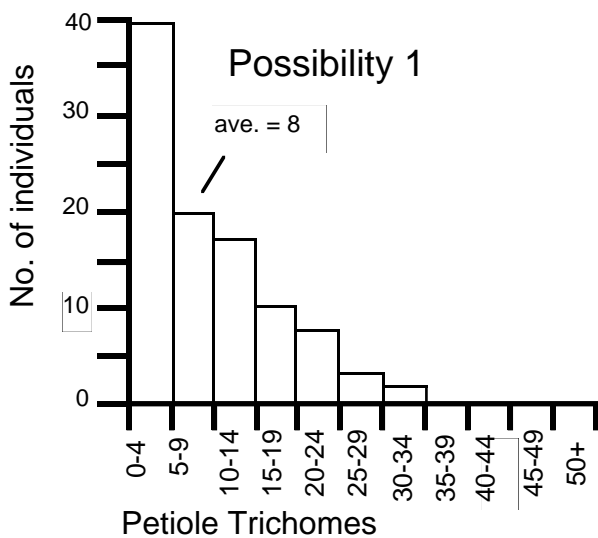


Figure 9

Below, justify your prediction for generation 2, compared with the hairiness of the entire population and the selected parents in generation 1. What reasoning did you use to predict the average hairiness and the range of hairiness in generation 2?

Future schedule for this exercise

The selected plants will be grown in the lab room through the rest of the quarter. The schedule of events will be as follows (today is lab 1):

Lab 2 (next week): The selected plants will be in full flower, and you will cross-pollinate (fertilize) them.

Lab 6: Harvest seeds from the now-mature plants; these seeds are the offspring of the selected parents, and are the next (second) generation. Plant these seeds.

Lab 8: Assess the trichome number of the second generation, now 14 days old. Analyze and interpret the results of the artificial selection experiment.

Artificial Selection in *Brassica*, Part II (continued from lab 1)

Goal of this activity: cross-pollination of experimental plants

Pollination of Selected Plants

The 10 or so plants you selected last week to be parents of the second generation in your section's Fast Plant lineage are 21 days old now, and all plants should be in heavy flower. Today, you will assist these plants in the process of sexual reproduction. *Brassica rapa* plants need assistance because in nature they are totally dependent on certain insects for transferring sperm-bearing pollen from the male part of the flowers of one plant to the female part of the flowers of another plant. In these plants, the most conspicuous floral structures

are the yellow petals. These petals enclose the male sexual structures (stamens, terminating in the pollen-producing anthers) and female structures (pistil, with the pollen-receiving stigma, style and egg-producing ovary), as shown in **Figure 25**. Although each flower has both male and female parts, sperm from one plant are incapable of successfully fertilizing eggs of the same plant. This *self-incompatibility* ensures outcrossing (mating between different individuals). The insect pollinators don't act as pollen couriers just to be nice; the plants lure and reward them with nutrients (nectar and edible pollen), and the insects inadvertently pick up the sticky pollen on various body parts and then carry it with them to the next flower (**Figure 26**). Honeybees are common pollinators of Brassicas in the wild, and we will use honeybees (dead ones, without stingers) to help us pollinate these plants.

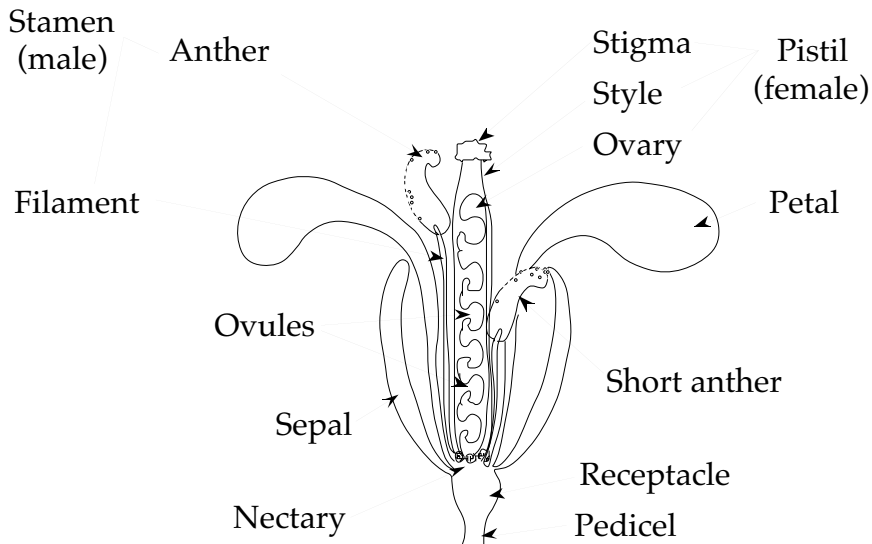


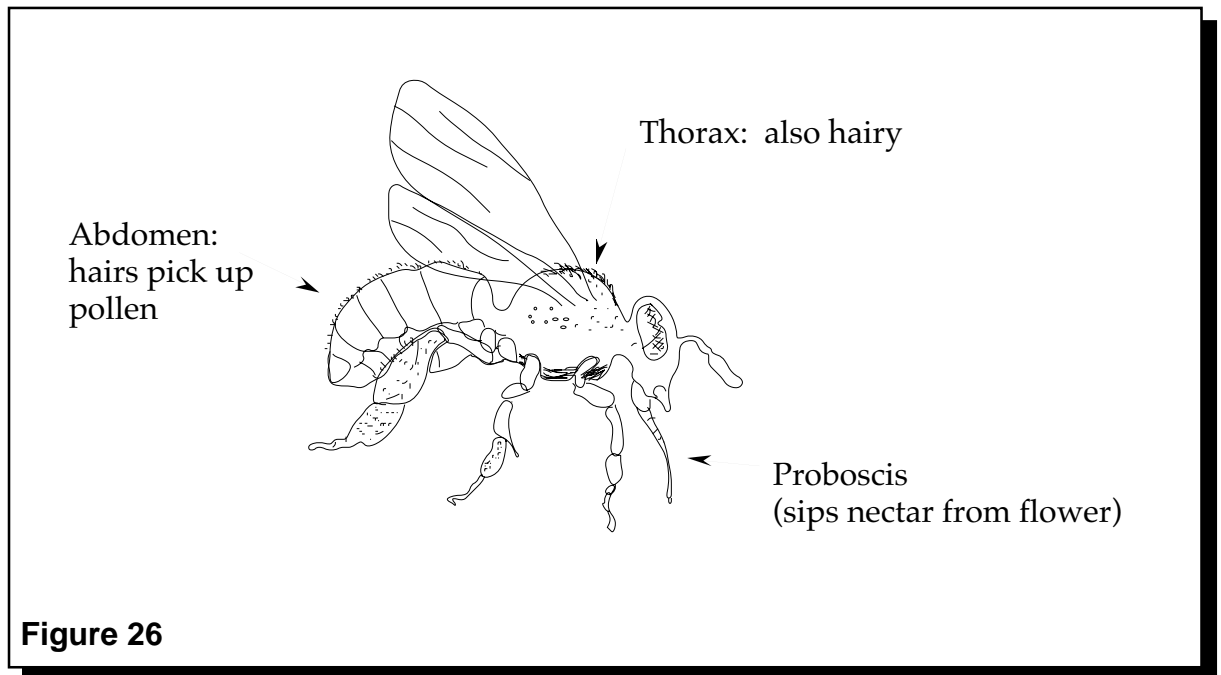
Figure 25

“Bee-sticks” have been prepared for you from the thoraxes of dead honeybees, which were collected from beekeepers after the bees died naturally (individual workers are short-lived). Glued to the end of a toothpick, these bee thoraxes make very efficient pollinating devices; pollen grains cling to the many fine hairs on the bee’s body and are easily transferred to the stigmas of other flowers.

Each student group should obtain one or two of the selected mature plants. The cross-pollination activity need not be done all at once, but can be accomplished over the course of today’s lab while you are doing other exercises. The object is to transfer pollen from each plant to every other plant. This can be done in the following way. Using a single bee-stick, one group will lightly rub and twirl the bee end for several seconds onto the anthers and stigma of each open flower on their plant(s). Then pass the bee-stick, now loaded with yellow pollen, to the next group, who will repeat the process. After the bee stick has made one complete round (all open flowers on all plants have been “visited” by the bee-stick), make a second round in the same manner. When finished,

insert the bee-stick into the soil (pointed end down, bee end up) of one of the pollinated plants—don’t return the used bee-stick to its original container, where other students might confuse it with fresh, unused ones. Finally, return all plants to the watering tray.

Fertilization will result in the development of the ovules (each containing an embryo) into mature seeds, which will be contained in the fruit or seed pod (the elongated ovary). The length of time from fertilization to mature seed is about 3-4 weeks. We don’t need to do anything more with these plants until it is time to harvest the seeds (in lab 6) except to remove them from water in 3 weeks, which hastens the seed-maturation process. Next week, you should easily be able to see the elongating fruits. In two weeks, they will have become even longer (2-5 cm) and the individual seeds inside (perhaps 5-20 per pod) will have become visible. In three weeks, the maturing process is nearly complete. You will plant these seeds (generation 2) in 4 weeks, during lab 6.



Artificial Selection in *Brassica*, Part III

(continued from labs 1 and 2)

Goals of this activity:

1. Harvest seeds from first-generation experimental Fast Plants.
2. Plant a sample of these seeds in order to establish the second generation.

Harvesting seeds

The 10 or so Fast Plants that you selected in lab 1 from a large population and pollinated in lab 2 should have mature seeds ready to be harvested. Each student group should take one or two of the plants to your table. Remove several pods, then break open the pods into a small plastic dish, releasing the seeds. You should be able to retrieve 5-20 or so seeds from each pod. The plant from which you removed the pod is the mother of each of those seeds; the father could have been one or more of any of the other plants in the selected group. Similarly, your plant could have been the father of seeds from any of the other plants in the selected group. Our mass cross-pollination technique ensured that all individuals in the selected group had an equal opportunity to be parents.

Establishing the second generation

The goal is to plant and grow generation 2 seeds in order to assess the average hairiness of this new generation (the offspring of the selected generation 1 parents). We want the second generation to have about the same number of plants (100 or so) as the first. We will not attempt to save any of these generation 2 plants to continue the lineage for a third generation, and so there is no need to grow them in individual containers. Instead, you will sow the seeds in a container about 11 inches square, labeled with your section number. This container has been filled with moist potting soil. A planting template with 100 evenly spaced holes will assist in distribution of the seeds. Each student will be responsible for planting a small number of seeds. Your lab instructor will give you further directions.

After planting, your section's flat will be placed under the lights in the lab room, and maintained by the staff. By next week's lab, most of the seeds will have germinated. In two weeks they will be ready for the final analysis of the experiment.

Artificial Selection in *Brassica*, Part IV (conclusion, from labs 1, 2, and 6)

Pre-Lab Reading

Goals of this activity:

- Discuss a genetic model to explain the inheritance of quantitative traits.
- Assess hairiness of the second-generation plants.
- Analyze the results and compare them with first-generation data (from lab 1).
- Interpret the results in terms of your knowledge of evolution by natural selection.

Overview

The second generation Fast Plants are now 14 days old, the same age as the first generation plants were in lab 1 when you started this experiment. Today, you will assess the hairiness of these second-generation plants, compare the results with those of the first generation, and make some conclusions about the results of your experiment.

As a brief review, you started in lab 1 with a population of “wild type” Fast Plants, from which you selected a small group (about 10 plants) to be parents of the next generation. These parents were characterized by being much hairier than the average for the population as a whole. You then cross-pollinated these selected plants among themselves, harvested their offspring (seeds) and planted these offspring five weeks later. You are now ready for the final phase of this experiment.

Inheritance of hairiness

Earlier in labs 4 and 5 you studied inheritance principles, using computer simulations as well as living organisms (fruit flies). The characters you

studied, such as fur color in rabbits, thorax color in butterflies, and wing shape in fruit flies, all had either two or three different possible phenotypes (e.g., miniature or normal wings in fruit flies; brown, tan or white fur in rabbits). The simplest genetic model that explained the inheritance of these traits involved a single gene locus with two alleles, either with complete dominance (resulting in two possible phenotypes) or incomplete dominance (resulting in three phenotypes). Hairiness in Fast Plants differs from these simple traits in that there are not just two or three phenotypes, but a whole range (from zero to “many”). Explaining the inheritance of such traits involves a more complicated model.

Traits such as body size or skin pigmentation in humans, or milk production in cattle, for which there exists a continuum of phenotypes between two extreme values, are known to geneticists as **quantitative** (or continuous) traits. Traits such as petiole trichome number in Fast Plants, number of bristles on fruit fly abdomens, or number of eggs laid by house fly females, are in a related category called **meristic** traits. These often exhibit wide variability among individuals, but the variability is incremental, not continuous (e.g., an individual might have 5 or 6 hairs, but not 6.3 hairs).

Despite the difference between these two kinds of traits, geneticists feel their mechanism of inheritance is similar. Such traits are very important—most traits in most organisms are quantitative or meristic, and relatively few are simple two-phenotype traits such as exhibited by Mendel’s peas (red/white flowers) or the Macintosh rabbits (straight/floppy ears). Quantitative and meristic traits are also often called **polygenic** traits, because the model that best explains their inheritance involves *multiple* gene loci, with two or more alleles at each locus. The following reading and worksheet will introduce

One locus:

Consider a hypothetical trait coded for by a single gene locus with two *incompletely dominant* alleles, A and a. For this trait, an individual's phenotype is determined by the number of "uppercase" alleles it possesses: each uppercase allele (A) contributes one unit to the phenotype above some baseline value, in additive fashion, whereas each lower-case allele (a) contributes nothing. Thus, individuals with genotype AA have a phenotype of 2 units above the baseline, Aa individuals have a phenotype of 1 unit, and aa individuals have a phenotype of 0 units. A phenotypic "unit" might include an increment of skin pigmentation, or an increment of height, for example. Thus, if a baseline value for plant height was 20 cm, and each uppercase allele added 2 cm above the baseline to the phenotype, then individuals with genotype aa would exhibit a phenotype of 20 cm; Aa individuals = 22 cm; and AA individuals = 24 cm.

In this example, there are just three phenotypes for height: 20, 22 and 24 cm.

What would we predict to be the *frequency* of these phenotypes in a population? As you should know by now, the genotype and phenotype frequencies depend on the frequencies of the alleles in the population. In a large, discrete, randomly mating population, the expected frequency of genotypes and phenotypes can be determined with a Punnett square, as you have done previously. Consider the situation in which the relative frequencies of these two alleles are equal (i.e., $p = q = 0.5$, or $1/2$). In **Figure 1**, the Punnett square on the left shows the expected genotypes and their frequencies; phenotypes (number of phenotypic "units") are in parentheses below each genotype. In the table to the right of the Punnett square, fill in the relative frequencies of each genotype (the first has been done for you). Finally, draw a frequency histogram (bar graph) that corresponds to the data in the table.

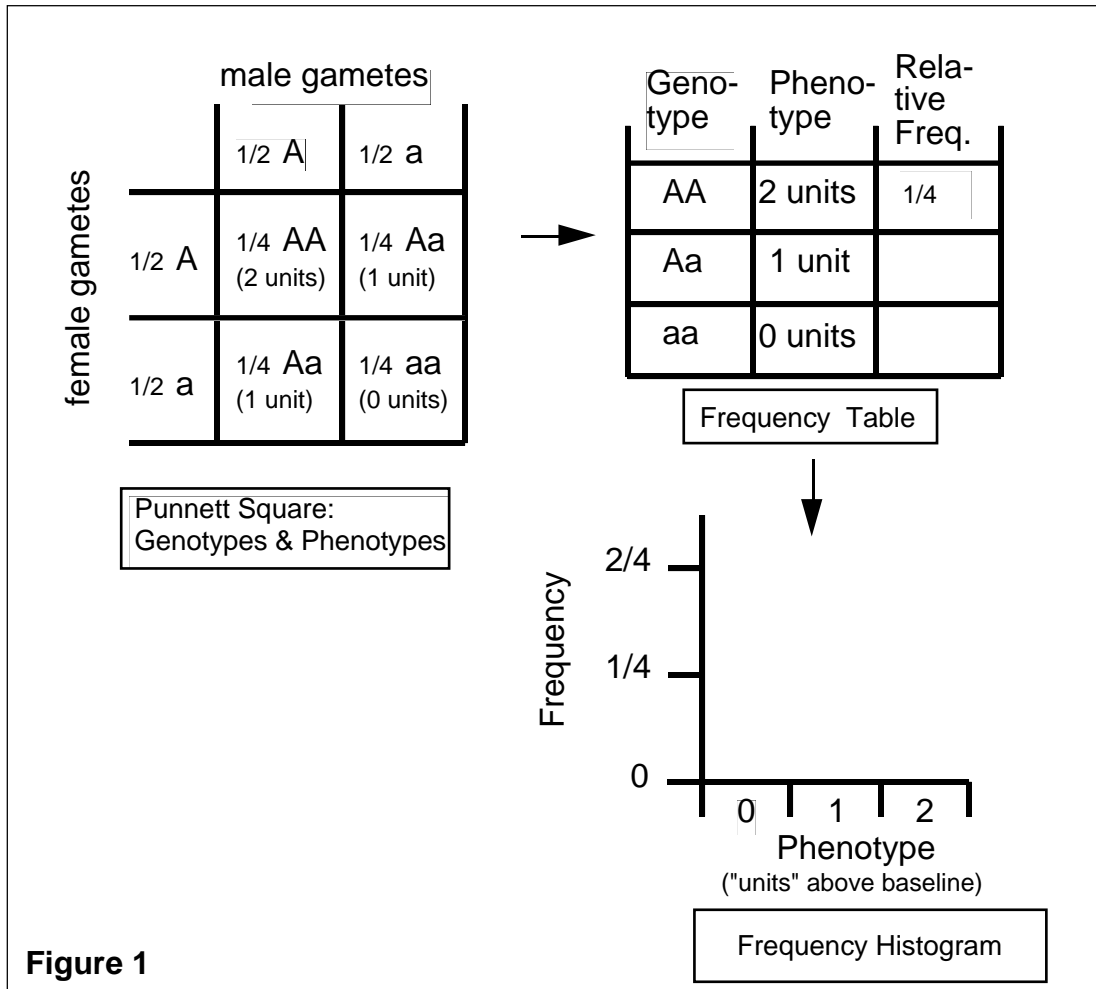


Figure 1

Two loci

Next, consider the situation in which not one, but two loci contribute equally to the phenotype, in additive fashion. As in the previous example, each locus has two alleles (A and a for one locus; B and b for the other), and each uppercase allele contributes one phenotypic unit. Thus, an individual with genotype AABB would exhibit a phenotype of 4 units; individuals with genotype AABb or AaBB would each have a phenotype of 3 units; etc. In **Figure 2**, fill in the Punnett square with the appropriate values. You will have to enter three of the four possible gamete types for males

and females (the first is given), and all but one of the cells (individual genotypes and phenotypes and frequencies). Again, this Punnett square shows the expected frequency of individuals of each genotype in a large randomly mating population in which the frequency of the alleles at each locus is equal (i.e., $A = a = 0.5$, and $B = b = 0.5$). When you have completed the Punnett square, summarize those values in the frequency table to its right (some of the genotype and frequency cells have been filled in for you). Then, in the blank graph below the table, construct a frequency histogram of these values.

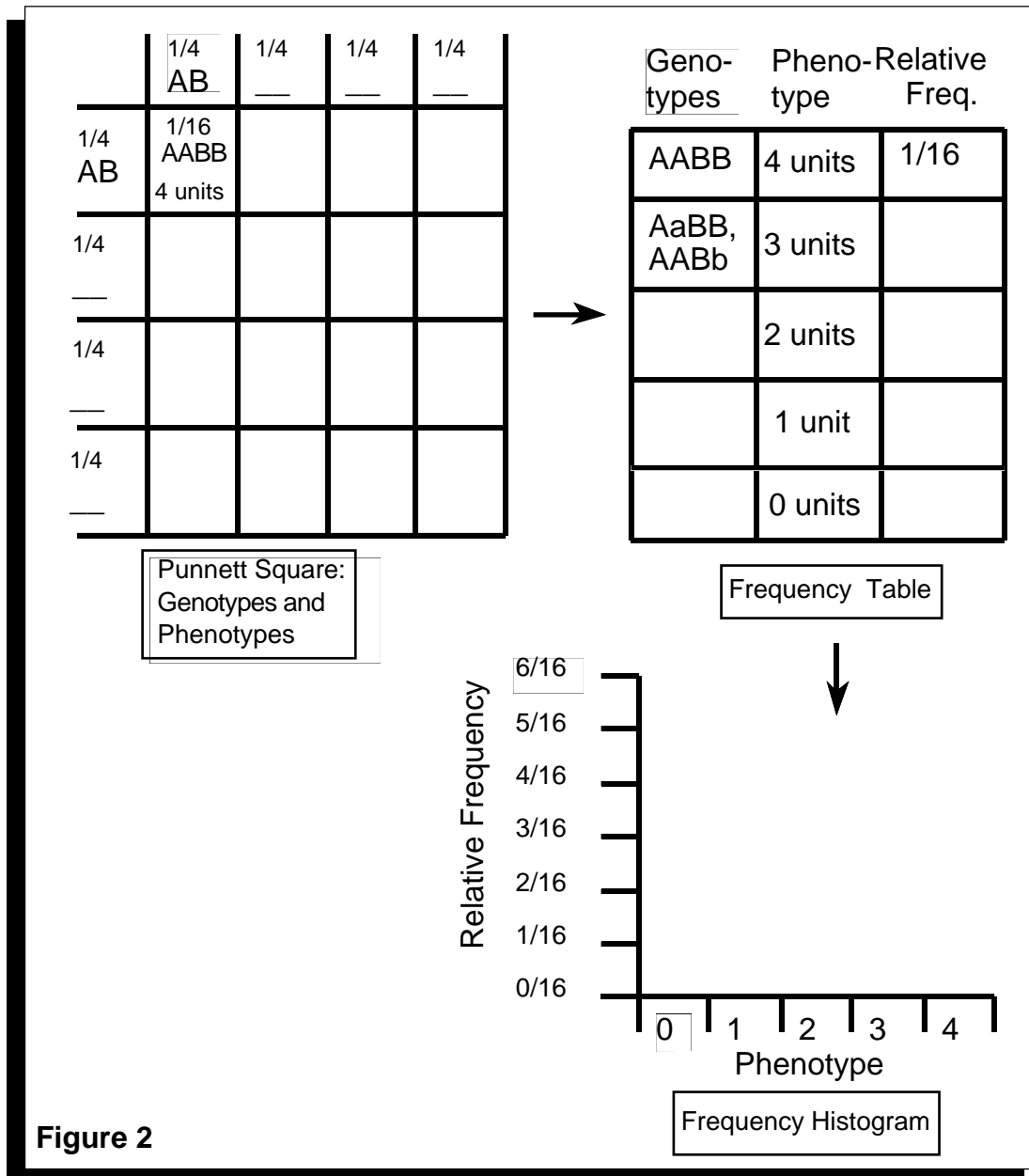


Figure 2

Three loci

Now consider three unlinked loci with additive effects, each locus with two alleles (e.g., A/a, B/b and C/c).

List all the possible gamete types (the first is given):

ABC, _____

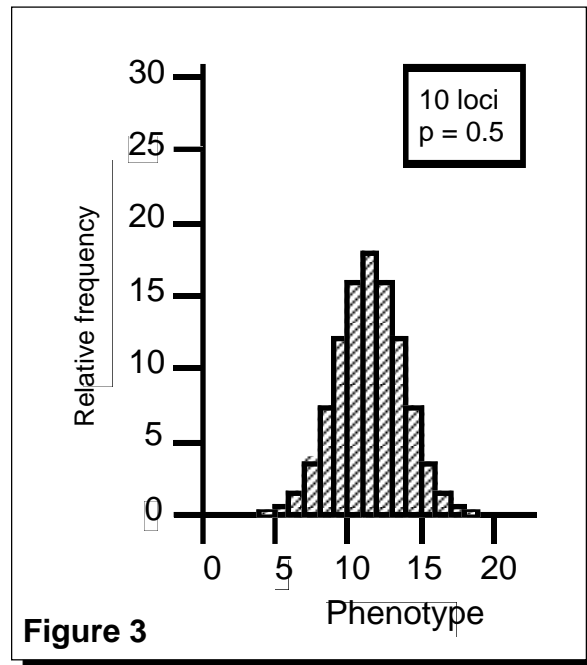
How large (i.e., how many rows and columns) would the Punnett square be to accommodate all the different gamete types? _____ rows and columns. How many cells would this Punnett square contain? _____ cells

How many *phenotypes* would be possible in this situation? [Hint: for one locus, three phenotypes are possible — from 0 to 2 uppercase alleles per genotype; for two loci, five phenotypes — from 0 to 4 uppercase alleles per genotype]. Number of phenotypes with three loci: _____ .

An illustration and explanation of this 3-locus situation (using a partly hypothetical example of skin pigmentation) is given on p. 271 of your textbook (Campbell).

Ten loci

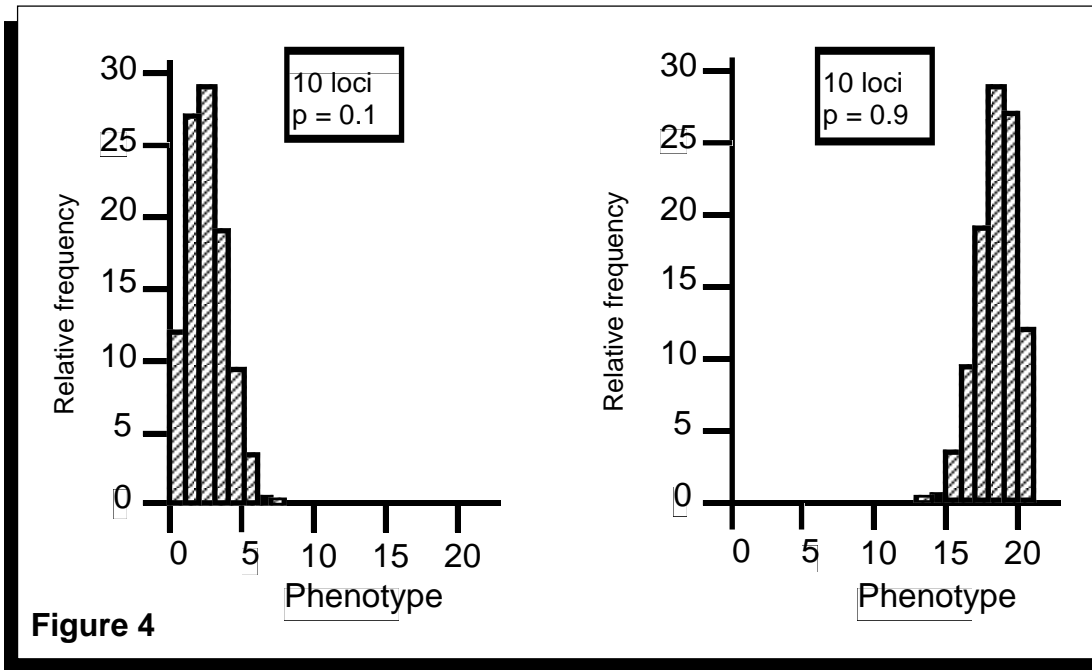
Above 3 loci, the Punnett square becomes increasingly large and unmanageable, but the same principles apply. For ten loci, the square is 1,024 rows X 1,024 columns and contains over a million cells; there are 21 phenotypes possible (from 0 to 20 uppercase alleles per genotype). Although obviously we can't draw the Punnett square, we can calculate (using statistical tables) and illustrate the resulting frequency histogram (again assuming that for each of the 10 loci, the relative frequency of each of its two alleles equals



0.5).

Figure 3 illustrates the situation when the frequencies of alleles at each of the loci involved are equal (i.e., for each locus, $p = 0.5$). But what if the uppercase allele at each locus was rarer, say $p = 0.1$, or more frequent, such as $p = 0.9$?

The expected frequencies of phenotypes in these situations are shown below (Figure 4). If natural selection favored the uppercase alleles in this 10-locus model, such that over time the frequency of such alleles at each locus increased from $p = 0.1$ to 0.5 to 0.9, we would expect a shift in the distribution of phenotypes, from the initial condition in the figure below, left ($p = 0.1$), to the intermediate condition above ($p = 0.5$) to the later condition in the figure below, right ($p = 0.9$). This is an example of **directional selection**.



The **polygenic model** introduced above is an explanation for the genetic basis of traits that show a continuous or near-continuous distribution of phenotypes. It is a widely accepted model, because it best explains a lot of different observations about the inheritance of quantitative traits. The precise number of loci involved in such traits is generally unknown (and probably

unknowable also), but estimates for some traits are as high as 30-100 (or perhaps more) loci. In the case of Fast Plants, we do not know the genetic basis of the phenotypic expression of trichome number. However, our class selection experiments, as well as other experiments we have conducted, indicate that this basic polygenic model does explain, in a general way, the inheritance of hairiness.

Artificial Selection in *Brassica*, Part IV

Laboratory Worksheet Assessment of hairiness of second generation plants

Your lab instructor will distribute to each student group a sample of plants (about 10-15) from the large second generation population. As before, you will assess the hairiness of each of these plants by counting the hairs (trichomes) on the petiole of the first true leaf (refer back to the figures and explanation in lab 1 if you are unclear about any aspect of this data collection process). Use the hand lens (high magnification) and desk lamp; enter the data (number of petiole trichomes on each plant) in the spaces in **Data Box 1**. Then hand in a copy of these data to your instructor.

Before continuing with the analysis, go back to the first generation data that you collected and summarized in lab 1 and copy the histogram into the blank **generation 1** histogram on the following page. Also copy your **generation 2 prediction** from lab 1 into the blank generation 2 prediction histogram.

Then, after your lab instructor has summarized the data from all groups in the section, fill in the **generation 2** data in the appropriate table in **Figure 5** and construct a histogram from these data.

On the generation 1 and generation 2 histograms, mark and label the average phenotype of the population as a whole. Also identify (by cross-hatching or stippling) on the generation 1 histogram those individuals selected to be parents of generation 2, and mark and label the average value of the selected parents.

Data Box 1

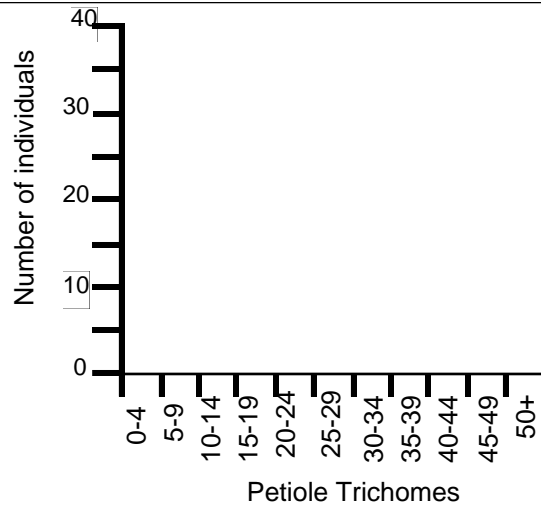
Your group's data from the second generation
(number of trichomes on petiole of first true leaf, per plant):

_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____

Generation 1

Copy your section's generation 1 histogram (from lab 1) into the space at the right:

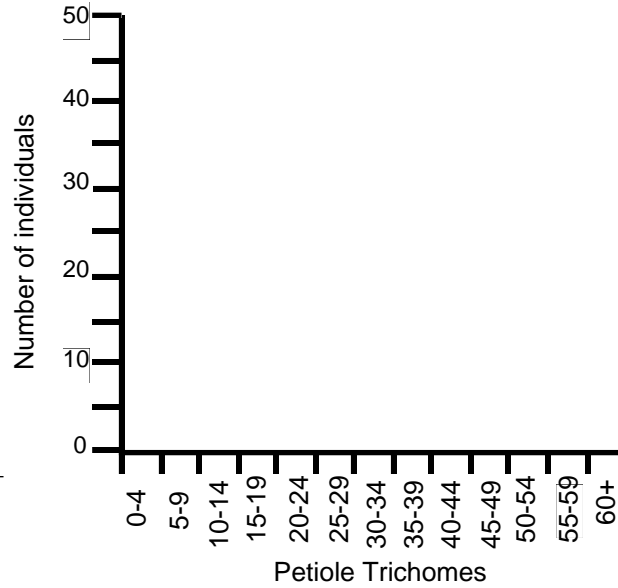
Generation 1
Average _____



Generation 2 Your Prediction

Copy your **predicted** generation 2 histogram (from lab 1) into the space at the right:

Generation 2
Predicted Average _____



Generation 2 Actual Data

No. of Trichomes Per Petiole

Tri- chomes	No. of Indivi- duals
0-4	_____
5-9	_____
10-14	_____
15-19	_____
20-24	_____
25-29	_____
30-34	_____
35-39	_____
40-44	_____
45-49	_____
50-54	_____
55-59	_____
60+	_____

Generation 2
Average _____

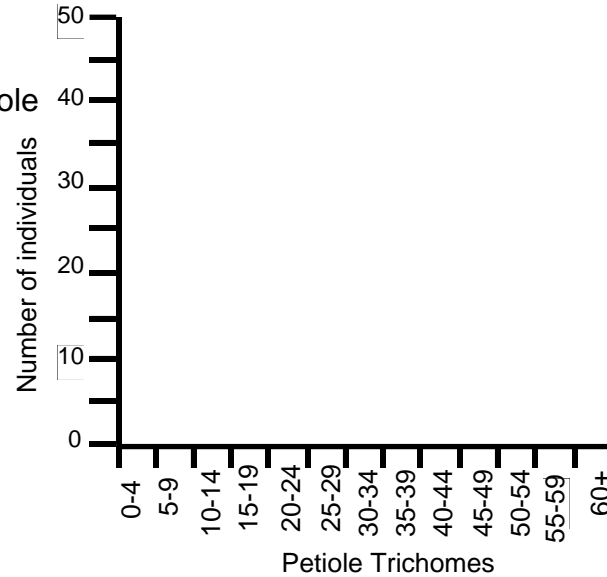


Figure 5

Selecting the parents of the next (third) generation

Although we don't have time in this course to continue the experiment for another (third) generation, you should select the plants that would be parents of that generation if we were to continue this lineage. Use the same criteria as you did for the first generation plants to select the hairiest 10% of these generation 2 plants. Record the trichome values of the selected individuals (parents-to-be of generation 3) into the spaces in **Data Box 2**, as well as the mean value.

Then, as you did for generation 1, identify these selected plants on the **generation 2** histogram on the previous page (by cross-hatching or stippling) and mark and label the average value.

Data Box 2

Selected Individuals of Generation 2 (no. of trichomes on petiole of first true leaf):

_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

Selected individuals, average: _____

Answer the following questions about this experiment:

1. Compare the **generation 1 and 2** histograms.

a. Was there a difference in average phenotype between the two generations, and if so, what was the magnitude and direction?

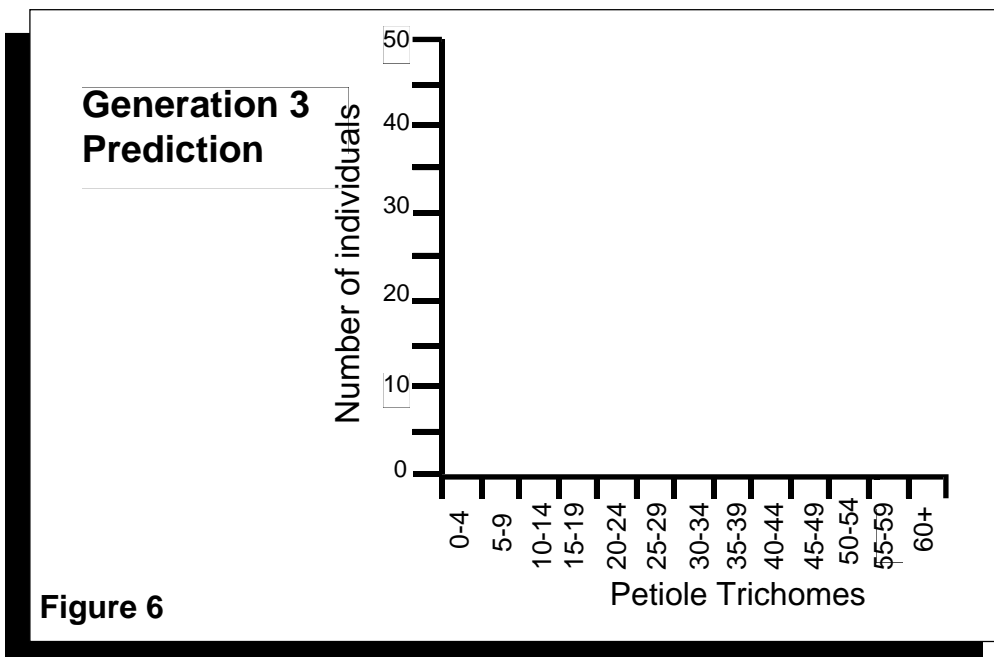
b. Was there a difference in the *distribution* of phenotypes? Explain.

c. Compare the average phenotype of the *selected* individuals in generation 1 with the average phenotype of their *offspring* in generation 2. Were these values equal, or was the average offspring phenotype greater than or less than that of their parents?

2. Did evolution occur in your lineage of Fast Plants? Justify your answer.

3. Compare the **generation 2** results with your **predicted** results from lab 1. How did the actual results differ from those you predicted? Why did they differ (i.e., what assumptions did you initially make that proved to be incorrect)?

4. Use the information from generations 1 and 2 above to make a *prediction* about the average phenotype and distribution of phenotypes in generation 3, if the parents of that generation are those you selected above from generation 2. Draw your predicted histogram in **Figure 6**. After you have finished, your instructor will show you an example of real third-generation data from another lineage.



5. In what ways was this experiment similar to the process of natural selection? In what ways was it different?
6. If you were to continue this selection process for a large number of generations, would you expect to see the same results continued? What factors might contribute to a diminishing or end of the success of artificial selection?
7. Do you think hairiness in “wild type” *Brassica rapa* is an adaptation? For what? How could you test your hypothesis?