Chapter 8

Evolution By Artificial Selection and Unraveling the Mysteries of Hairy's Inheritance

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Contents

Introduction

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*.

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 **(Figure 8.1)**.

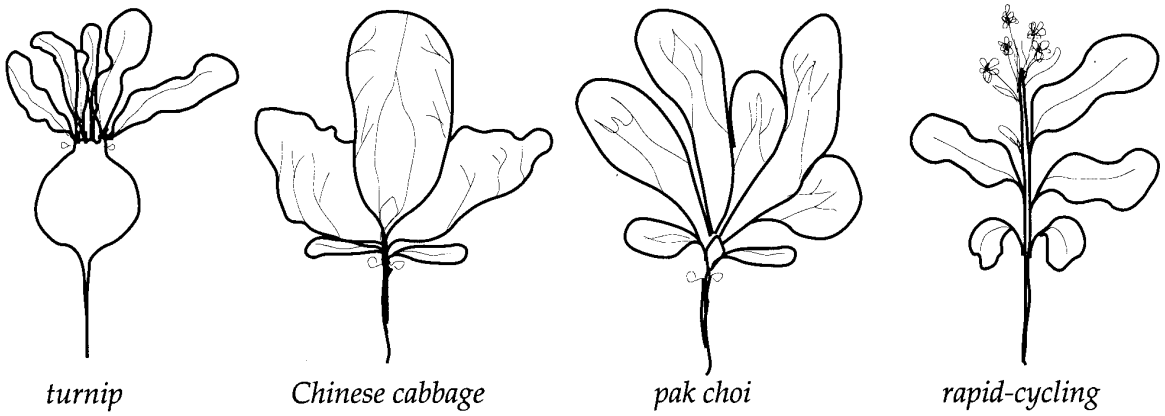


Figure 8.1 *Varieties (cultivar groups) of* Brassica rapa.

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 change the appearance of various lineages of organisms dramatically 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.

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

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 understand this important concept better. 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 other things.

Materials: (This list of material is enough for 40 groups of 4 students, 960 plants.)

1800 Basic Wisconsin Fast Plants Seeds
2 – Light banks (each consisting of 8 fluorescent 40-W cool white bulbs)
Growing System (see Appendix):
- 16 - Standard (11 x 22-inch) plastic greenhouse flats
- 8 – half-flat (11 x 11-inch) plastic greenhouse flats
- 8 - "X"-shaped pieces of plexiglass
- 1,930 35mm film containers
- Cotton string
- 16 – Pieces of pellon fabric to fit into the standard greenhouse flats
- 8 – Pieces of pellon fabric to fit into the standard greenhouse flats

Notes to Instructor

Basic Wisconsin Fast Plants TM seed stock supplies for growing can be purchased from Carolina Biological Supply Company (2700 York Road, Burlington, NC 27215. Telephone: 1-800-334-5551). We designed and constructed our own lighting system and growing apparatus. In each lab we have two permanently mounted light banks end-to-end that occupy a total of about 8.5 X 2 ft of shelf space. Each light bank consists of four standard two-bulb shop lights, with 40-W cool-white fluorescent bulbs (a total of eight bulbs per bank). Under each light bank we grow up to four greenhouse flats of plants (120 plants per flat, 480- total). The lights are on pulleys, permitting us to raise and lower them.

We grow the parental generation plants individually in standard 35-mm film canisters so students can handle them separately. Each canister has a wick inserted into a drilled hole in its bottom, and is filled loosely with potting soil on top of several pellets of commercial fertilizer. Before planting, the canisters are soaked from the bottom in water until thoroughly moist. The planted canisters sit in a standard (11 X 22 inch) plastic greenhouse flat, available at most garden centers; each flat holds 120 canisters. We use two flats, one nested inside the other, the bottom flat is solid and serves as a water reservoir. An "X"-shaped plexiglass spacer keeps the top flat about an inch above the bottom of the reservoir flat. The top flat, which contains the plants, is perforated with holes on the bottom of each end. Strands of cotton rope lie in the bottom of the flat and extend externally through these holes to the bottom of the reservoir. A mat of pellon fabric covers the rope strands in the bottom of the flat. Water travels from the reservoir to the cotton rope strands to the pellon mat to the individual wicks in the canisters and then into the soil. This system is effective and

requires little maintenance. During the first two weeks after planting, the reservoir flat requires refilling only once or twice a week.

Students select about 10% of the plants from the parental generation and place 10 to 12 selected plants in a smaller container, which sits on the top flat. This container is made from a plastic desk organizer. A slit in each end permits a long strip of pellon fabric on the bottom to extend outside the container and underneath it, where it is in contact with the wick system described above. The plants grow to maturity in these canisters; students pollinate them when the plants are 21 days old. One pollination is usually sufficient, but our staff repollinates the plants once, a few days after student pollination. Three weeks after pollination, we remove the plants from the water source. One week after drying, the offspring generation seeds are ready to plant. Usually, hundreds of viable seeds result from these 10-12 parent plants.

Since we don't continue this selection experiment beyond two generations, there is no need to grow individual plants of the offspring in separate containers. Instead students grow these seeds in a single half flat (11 x 11- inch, also available at garden centers) with a cotton-rope / pellon mat wicking system as described above, with the potting soil on top of the mat. A plexi-glass square drilled with a grid of 100 holes (10 x 10) and placed on top of the soil serves as a planting template. Students make a small depression in the soil beneath each template hole, then drop one or two seeds into it. The planted half-flat rests on top of the "X"-shaped spacer, with the cotton rope wicks extending down into the water reservoir. It is possible to grow plants in two half-flats in the same 11 x 22-inch water reservoir – e.g., offspring from the parents selected for both smooth and hairy from the same population. To assess these plants, we cut them with scissors at the soil level and distribute them to students. This (and subsequent) generations could also be grown individually in canisters, but this would add to preparation time. We have conducted the appropriate experimental comparisons, and conclude that there is no difference in the mean trichome density between plants from the same population grown in these two environments.

We soak all growing apparatus thoroughly in a bleach solution after use, and we do not reuse soil. Thus far we have not had any problems because of plant disease.

For our course's lab schedule, students receive the parental generations as 14-day old plants in lab 1; our staff prepares the canisters and plants these seeds two weeks prior to the first lab. However, a different lab schedule would permit students to plant the parental generation seeds themselves and still complete the exercise in 9 weeks. The general schedule of events for this exercise is as follows:

Week - Student Activity

1 - Plant seed in 120 canisters
3 - Assess and analyze parental generation hairiness, select 10-12 parents and predict offspring generation hairiness
4 – Pollination of selected parents
7 – Remove plants from water
8 – Harvest seeds; plant offspring generation (100 seeds)
10 - Assess offspring generation hairiness, analyze data, predict next generation hairiness, discussion

Student Outline

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 is 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 8.2**.

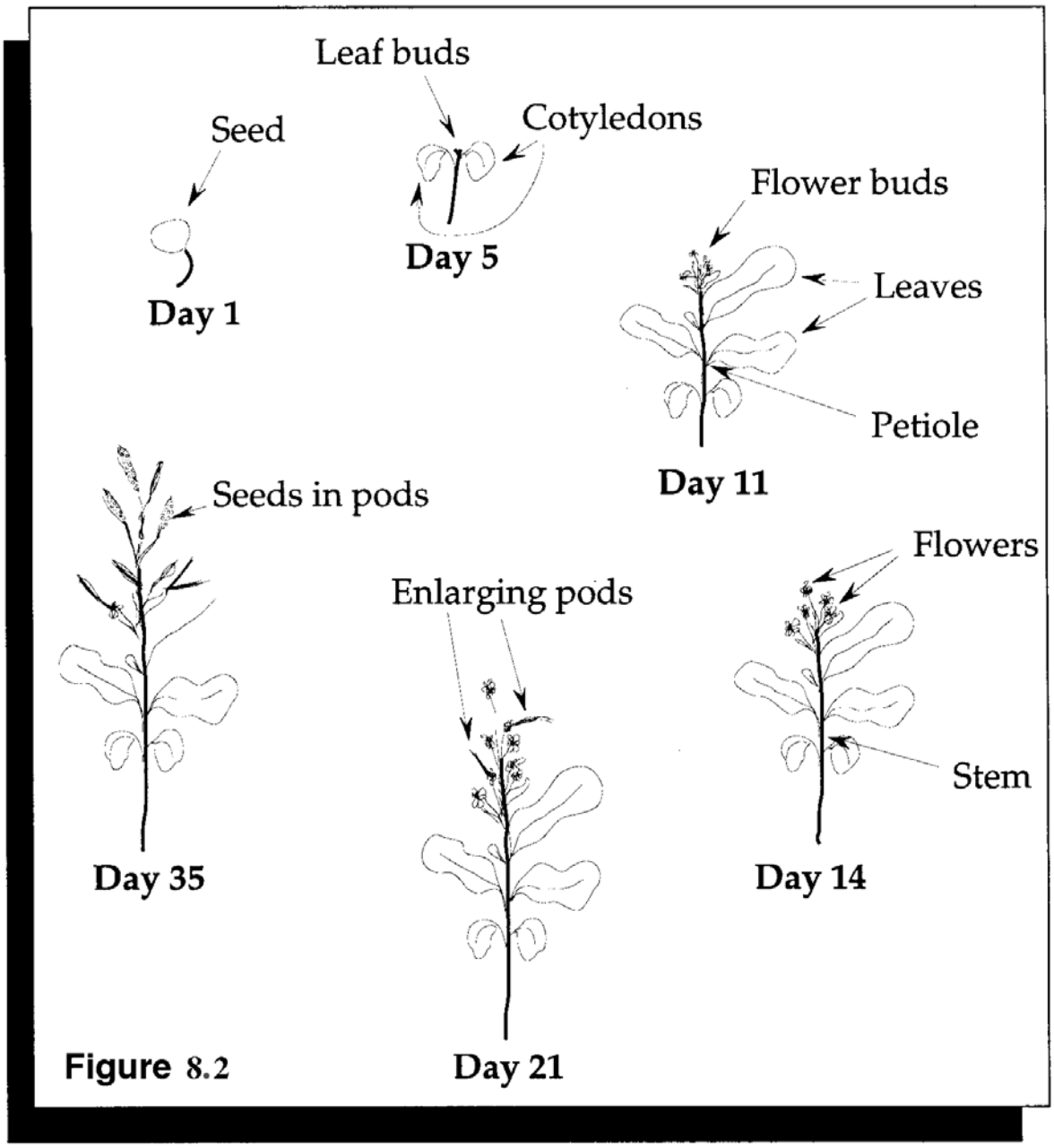


Figure 8.2

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 8.3**). 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.

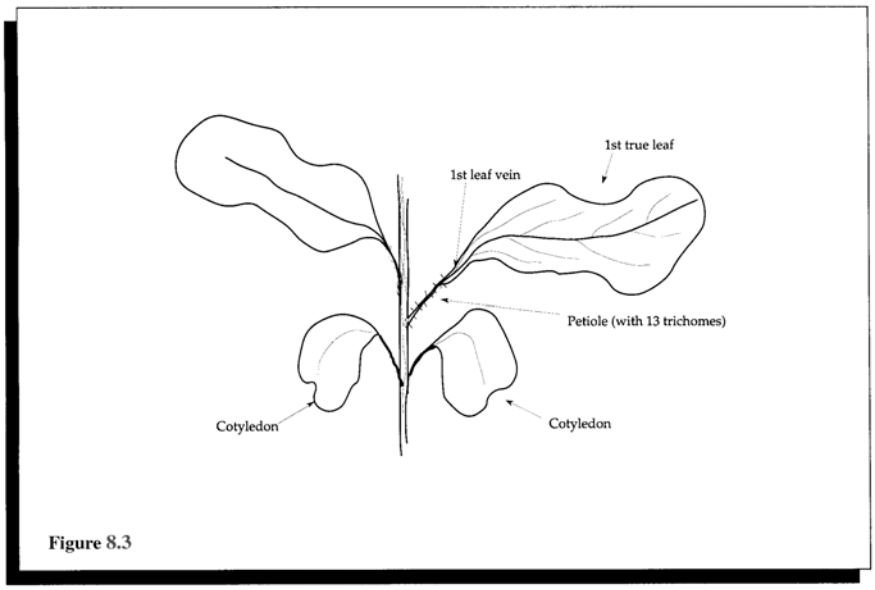


Figure 8.3

Variable traits:

What trait will you attempt to select artificially? 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 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.

What might be the function of "hairs"?

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.

For consistency, we will use the petiole of the <u>first</u> (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 8.4**).

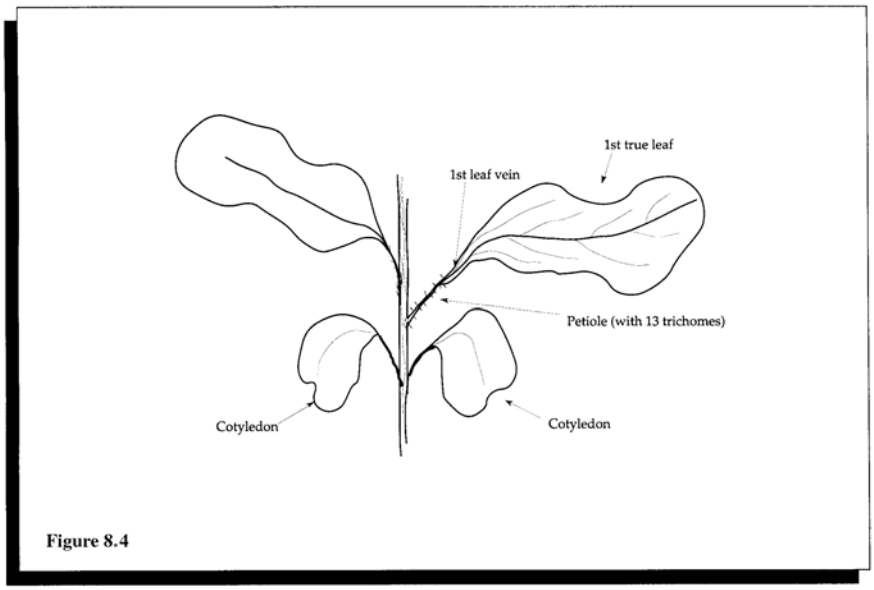


Figure 8.4

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 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):

_____ _____ _____ _____ _____ _____ _____

_____ _____ _____ _____ _____ _____ _____

_____ _____ _____ _____ _____ _____ _____

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 8.5**. Next, plot a frequency histogram (bar graph) of these data in the space provided also in **Figure 8.8**. Mark the population average on the histogram.

Frequency Table

No. of Trichomes Per Petiole

Trichomes	Number of Individuals
0-4	_____
5-9	_____
10-14	_____
15-19	_____
20-24	_____
25-29	_____
30-34	_____
35-39	_____
40-44	_____
45-49	_____
50+	_____

Total _____ individuals in population

Average of population: _____

Frequency Histogram:

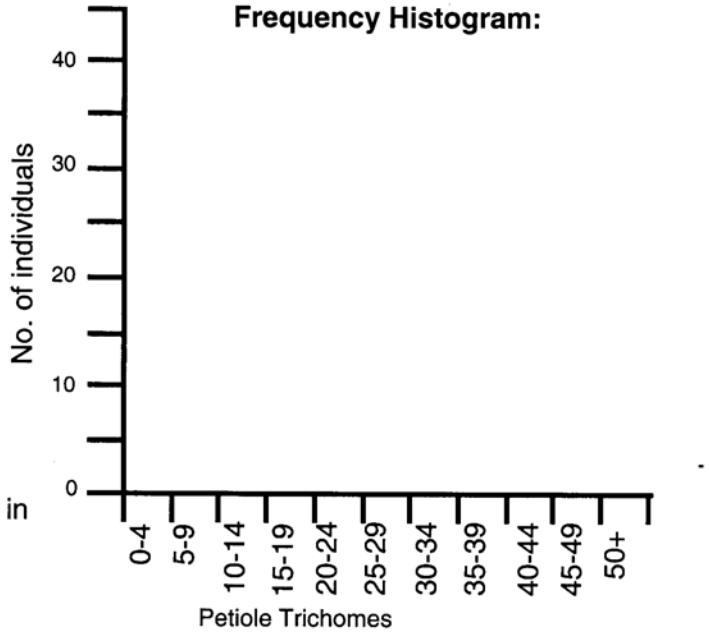


Figure 8.5

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: _____

_____ _____ _____ _____

_____ _____ _____ _____

Data Box 2. Selected parents (number of trichomes on petiole of first true leaf)

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 other possibilities not presented. Discuss these and other possibilities with your lab partner, then draw a frequency histogram in the blank graph at the bottom right of **Figure 8.6** 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.

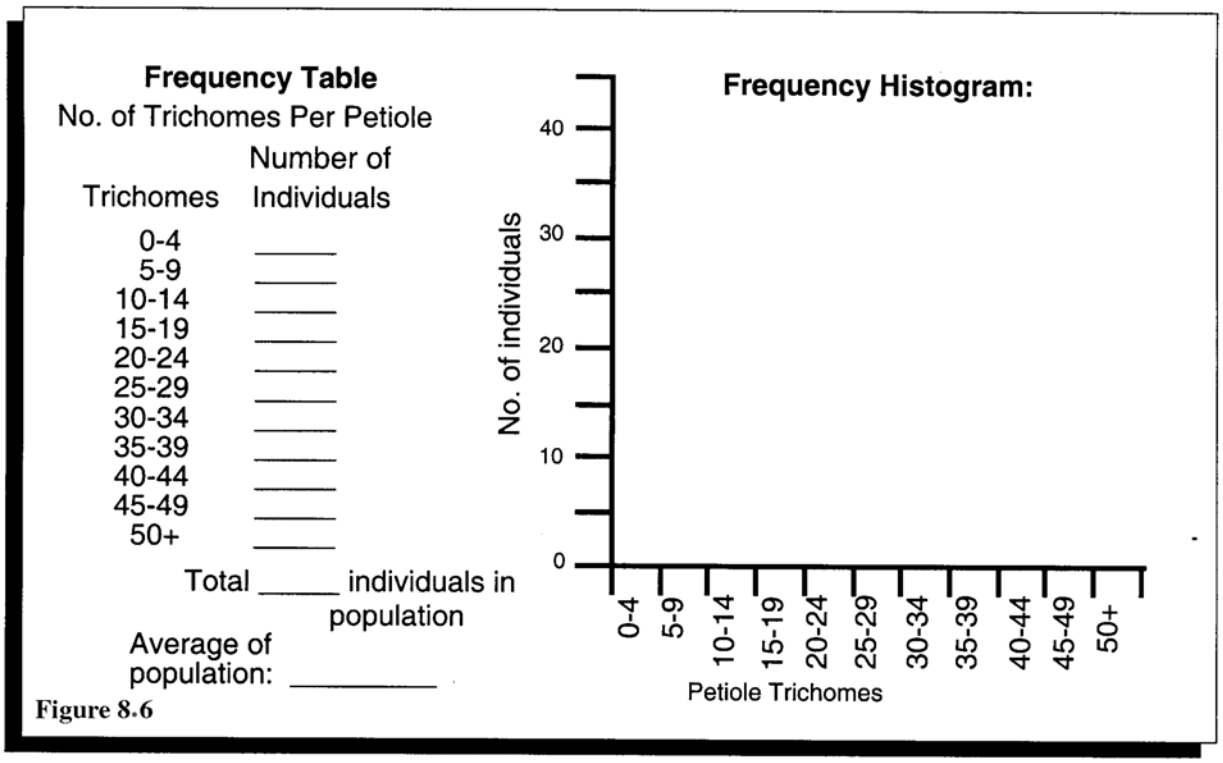


Figure 8.6

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* 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 transfering 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 8.7**.

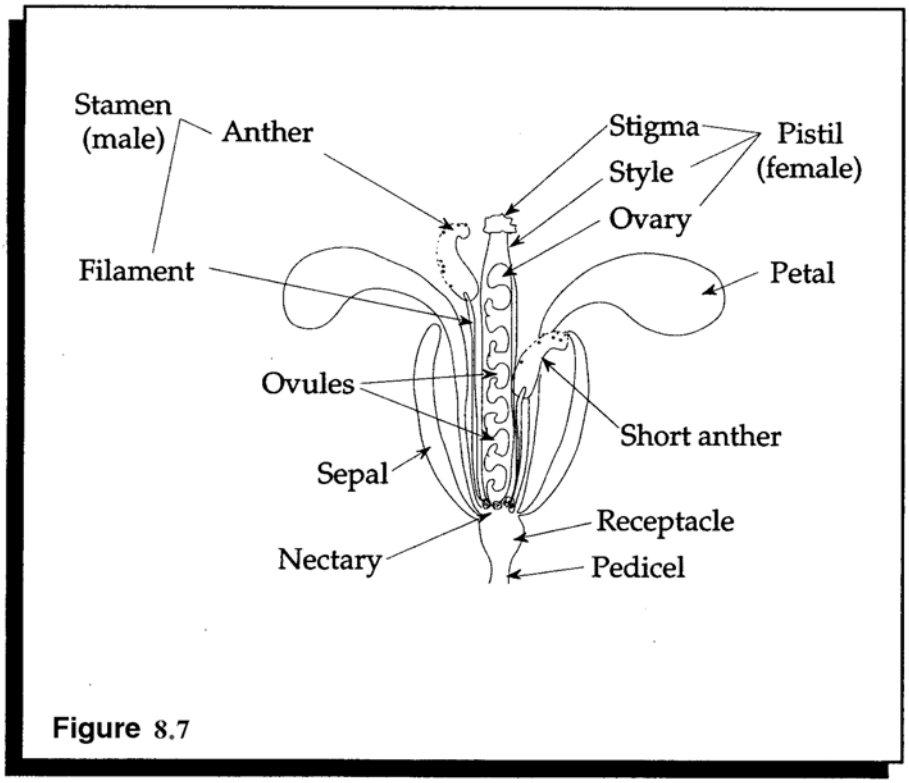


Figure 8.7

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 8.8**). Honeybees are common pollinators of Brassicas in the wild, and we will use honeybees (dead ones, without stingers) to help us pollinate these plants.

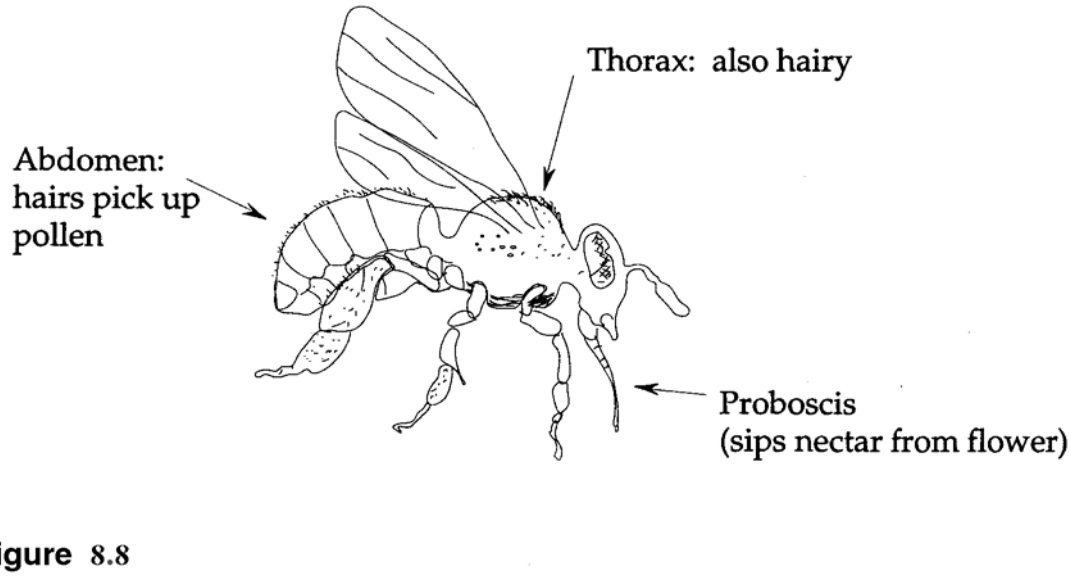


Figure 8.8

"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.

Artificial Selection in *Brassica* 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 to establish the second generation.

Harvesting seeds

The 10 or so Fast Plants 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 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, 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* 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

In other labs, 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 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 8.9**, 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.

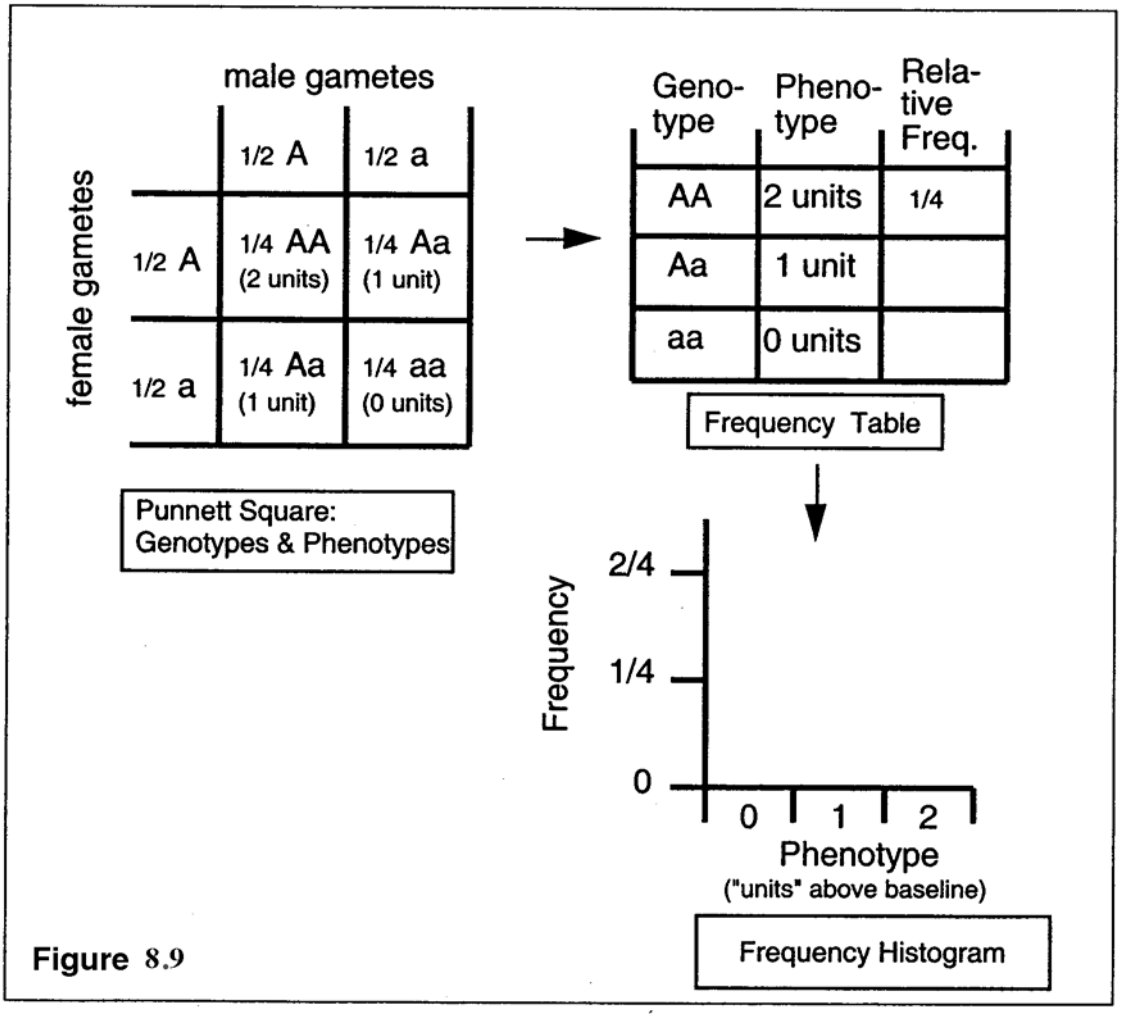


Figure 8.9

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 8.10**, 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.

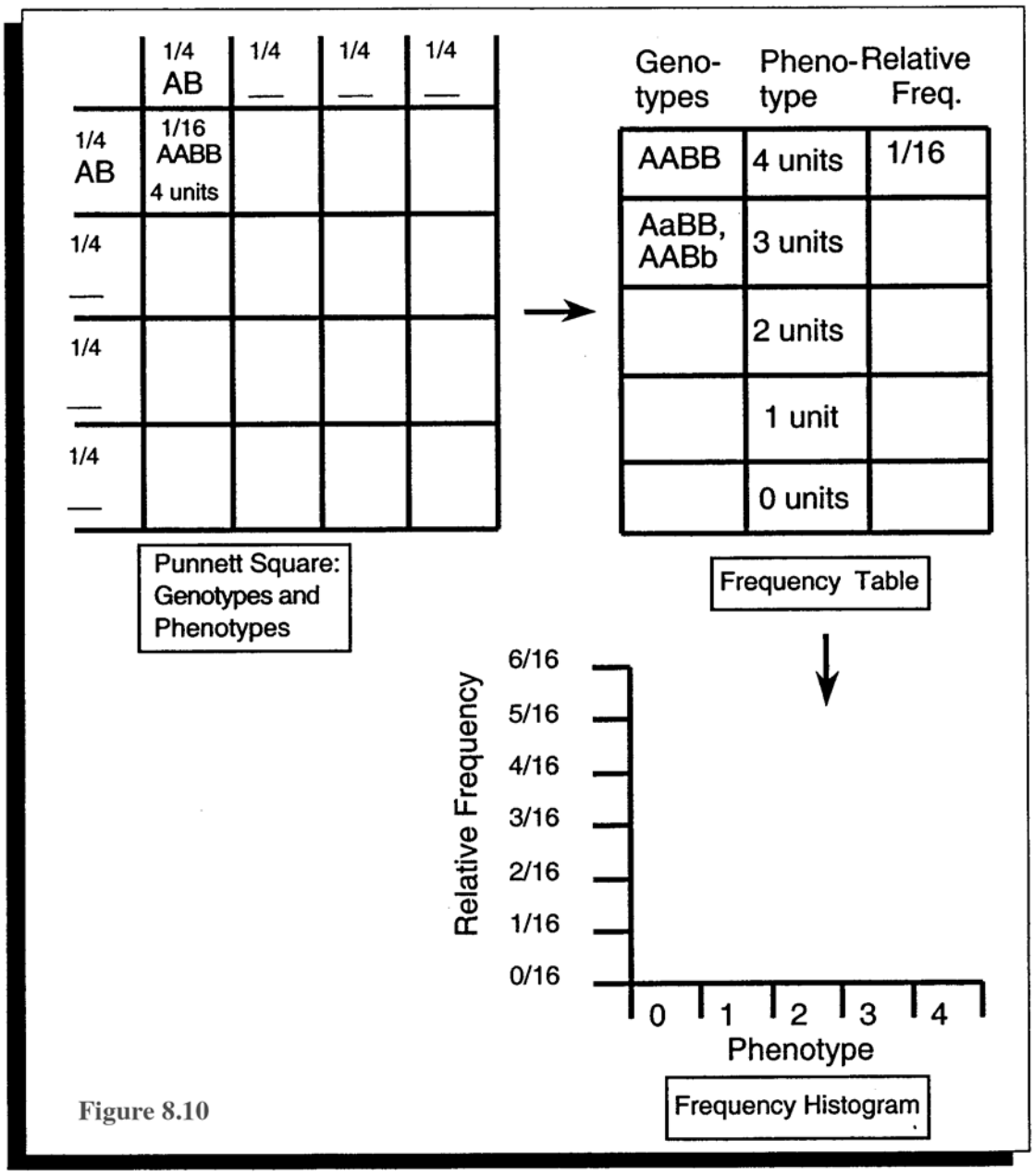


Figure 8.10

Three loci

Now consider <u>three</u> 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 accomodate 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: _____ .

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).

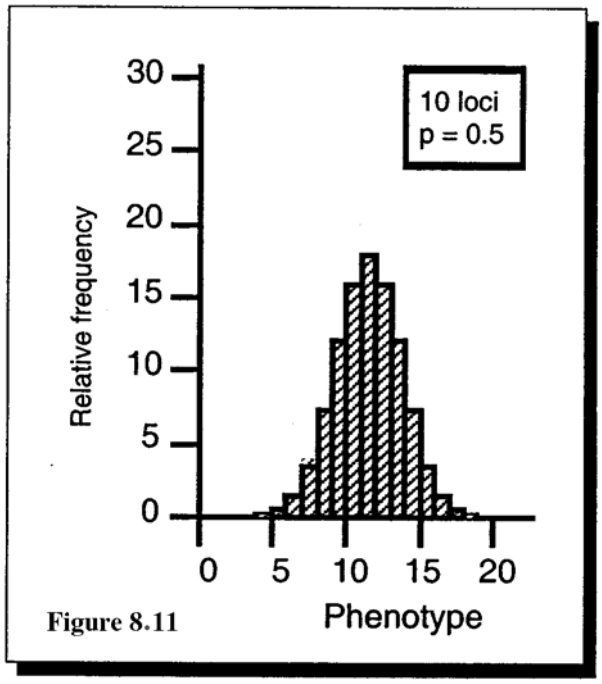


Figure 8.11

Figure 8.11 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 8.12**). 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**.

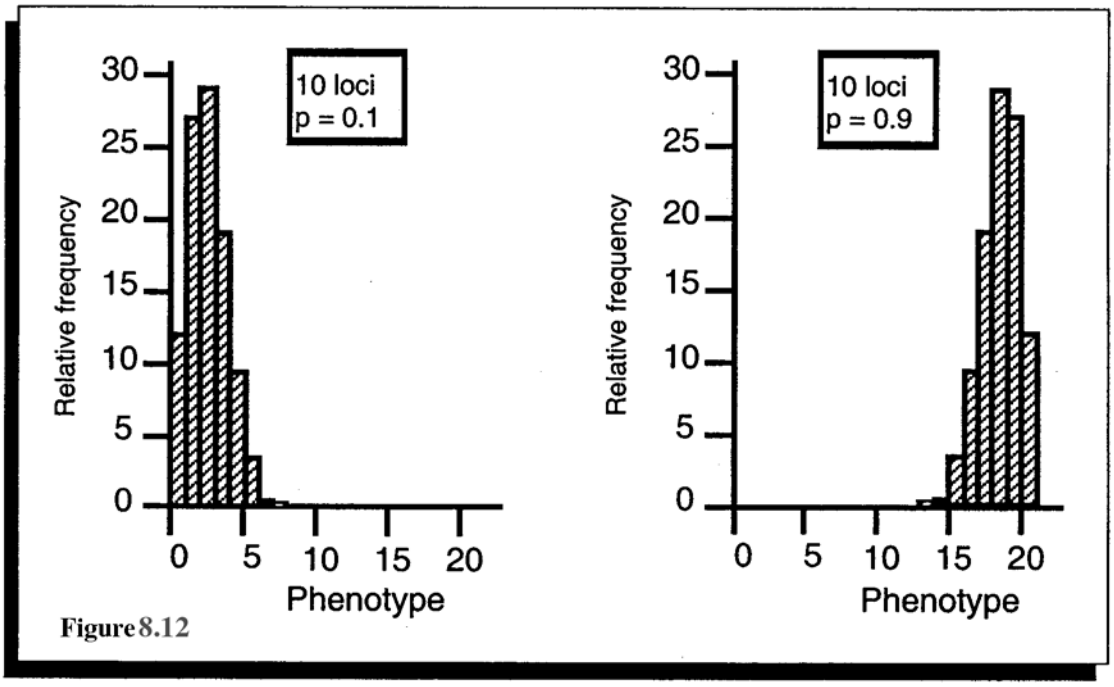


Figure 8.12

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* 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 4**. Then hand in a copy of these data to your instructor.

Data Box 4

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

___	___	___	___	___
___	___	___	___	___
___	___	___	___	___
___	___	___	___	___
___	___	___	___	___

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 8.13** 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.

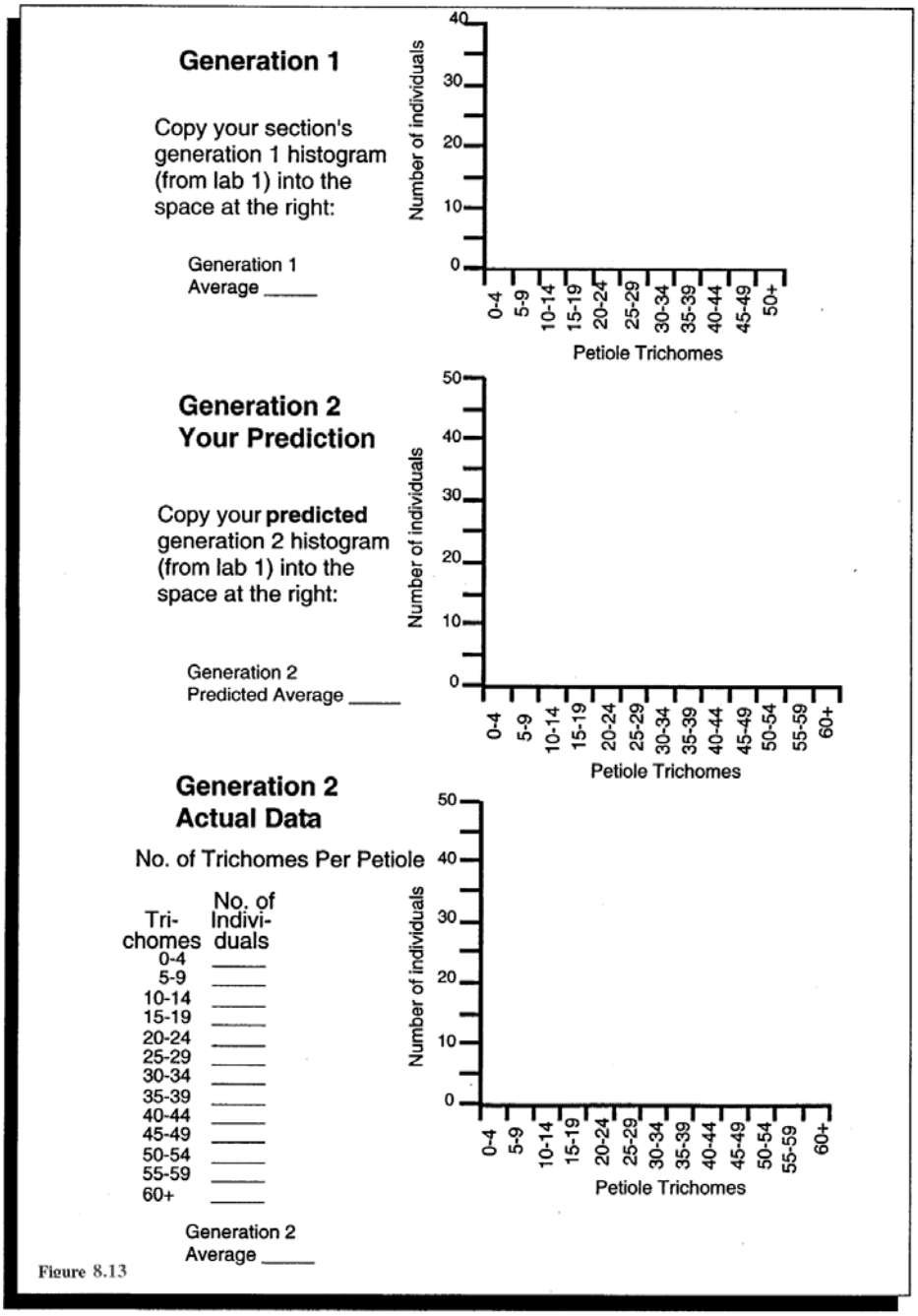


Figure 8.13

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 5**, as well as the mean value.

Data Box 5

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

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

Selected individuals, average: _____

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.

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 8.6**. After you have finished, your instructor will show you an example of real third-generation data from another lineage.

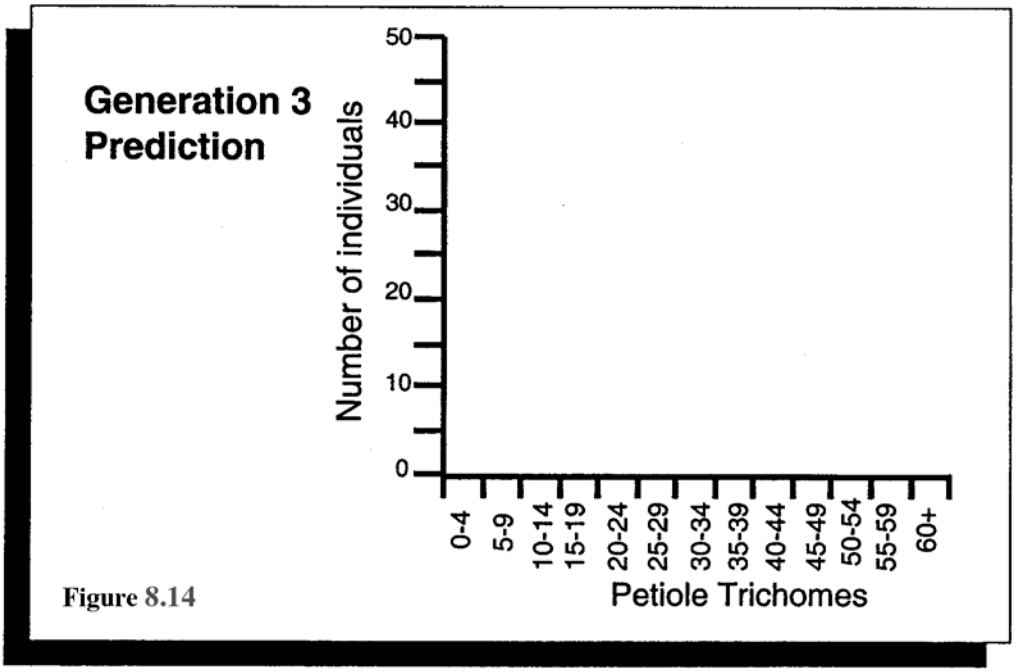


Figure 8.14

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?

Part II: Unraveling the Mysteries of Hairy's Inheritance

Variation is one of the fundamental characteristics of life. All organisms exhibit some variation among individuals. Understanding the ways that variation is manifested in organisms, how it comes to be expressed through the development of the individual, and how it is transmitted from one individual to the next generation of individuals are central themes in biology.

Upon close observation of a population of rapid-cycling *Brassica rapa*, Fast Plants, students will become aware that any particular characteristic observed, (phenotype) on one plant varies more or less on other plants. The Crucifer Genetics Cooperative, CrGC, the genetic stock center of the Wisconsin Fast Plants Program, has developed many stocks of rapid-cycling *Brassica rapa* suitable for genetic investigation of the nature and inheritance of phenotypic variation. Some stocks contain

traits in which only a few easily discernible or discrete phenotypic differences are exhibited and are suitable for Mendelian genetics. Other stocks exhibit phenotypes whose expression may vary continuously and which may be quantified as countable units, estimates of size , or as intensity of color saturation. The inheritance of such quantitative phenotypes maybe conditioned by a few (oligogenic) or many genes, (polygenic) and normally requires numerical descriptors in which statistical notations of population size (n), range (r), the mean (x), and the standard deviation (s) are applied. Understanding the inheritance of quantitatively inherited phenotypes is important to understanding the role of genotype in phenotypic expression, variation, and evolution (**Figure 8.15**).

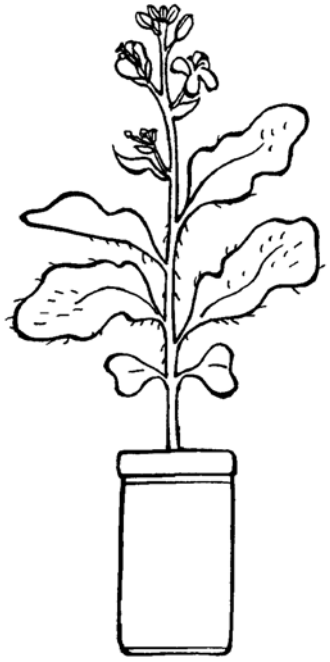


Figure 8.15. Depicts a hairy Fast Plant. The hairs on Fast Plants allow students to investigate both Mendelain and quantitative inheritance.

Hairs found on Fast Plants, are an example of a continuously variable phenotype ideally suited for exploring the modes of inheritance underlying the trait. Hairs are a directly countable (meristic) trait and can be observed on the stem, petioles, leaf blades, margins, and occasionally on the sepals. How hairs are inherited in *Brassica rapa* is still not completely understood. However, the number of hairs on a plant or plant part appears to be conditioned polygenically, and is considered to be qualitatively inherited, (Agren, J. et al., 1992), while the absence of any hair on *Brassica rapa* is conditioned by a single Mendelian recessive allele (Song KM, et al., 1995).

The number of hairs on a specified plant part such as, on the margin of the first true leaf, can be used to represent the hairiness of an individual. Hairiness in a population of plants can be depicted as a statistical summary of individual plant counts. Graphing a frequency histogram of hairs can help students understand how Mendelian and quantitative inheritance contribute to the expression and variation of a trait (**Figure 8.16**).

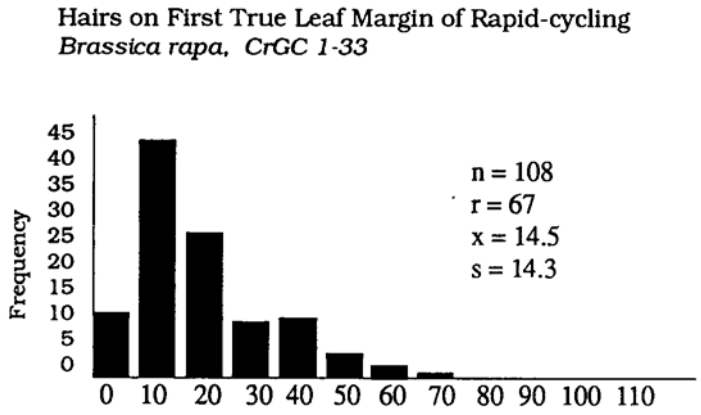


Figure 8.16. Frequency histogram showing the number of hairs in a population of Rapid-cycling *Brassica rapa*, CrGC 1-33

Numerical representation of the population in tables or graphs, (**Figure 8.16**), enables students to understand the variation in the specified phenotype, e.g. hairs, in the population. By selecting and intermating individual plants to have none or a few hairs, or plants having many hairs, the mean number of hairs can be decreased or increased demonstrating that hairiness is a heritable trait. Bruce Fall at the University of Minnesota has demonstrated the heritability of hairiness in an undergraduate biology lab by having his students select for the number of hairs on the petiole of the first true leaf. Starting with a population with a mean number of 8.8 hairs per individual, hairs had been completely eliminated from the smooth-selected lineages after 10 generations of recurrent selection for hairless. By selecting and intermating the 10% hairiest plants in each successive generation, the mean hair number has been increased to over 105 in 10 generations (Fall, B. et al., 1995)

The CrGC has developed and maintains a number of Fast Plants stocks having varying numbers of hairs. Two stocks in particular, CrGC 1-54, hairless, Hir (0-1), and CrGC 1-37, hairy, Hir (3-6), have been developed for students, and researchers to investigate the nature of the inheritance of hairs. Quantitative phenotypes such as hairiness which show a wide range in variation can be described using a scale from (0-9) to represent the relative expression of the phenotype (**Figure 8.17**).

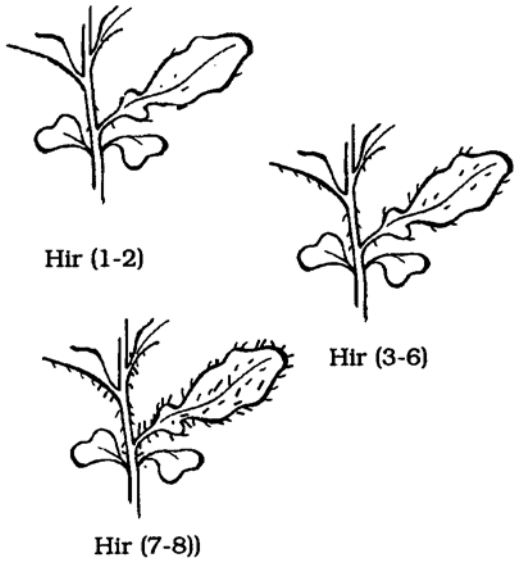


Figure 8.17. A diagram illustrating the (0-9) point scale developed to quantify the number of hairs on a Fast Plant.

The hairy phenotype is described as Hir (3-6). This particular symbol, Hir, is for *hirsute* (after the Latin for hair). On the scale from (0-9), 0 = no expression (no hair), (1-2) = low expression (few hairs), (3-6) = intermediate expression (hairs), (7-8) = high expression (many hairs) and (9) = very high expression (very hairy), (see Figure 3). Hairless, Hir (0-1), and Hairy, Hir (3-6), are ideal stocks for researchers and students to conceptualize, hypothesize, and experiment with the notions of how the hairy phenotype may be inherited.

Crossing hairless and hairy parents to produce an F1 and then F2 progeny, should provide information indicating how hair number is inherited in *Brassica rapa*.The number of hairs on the margin of the first true leaf is used to characterize CrGC 1-54, Hir (0-1), and CrGC 1-37, Hir (3-6), for hairiness. Figure 8.4 shows the frequency histogram of both parents. Notice the difference in the standard deviation of the hairy stock compared to the hairless. What questions about inheritance do these frequency histograms raise? (**Figure 8.18**).

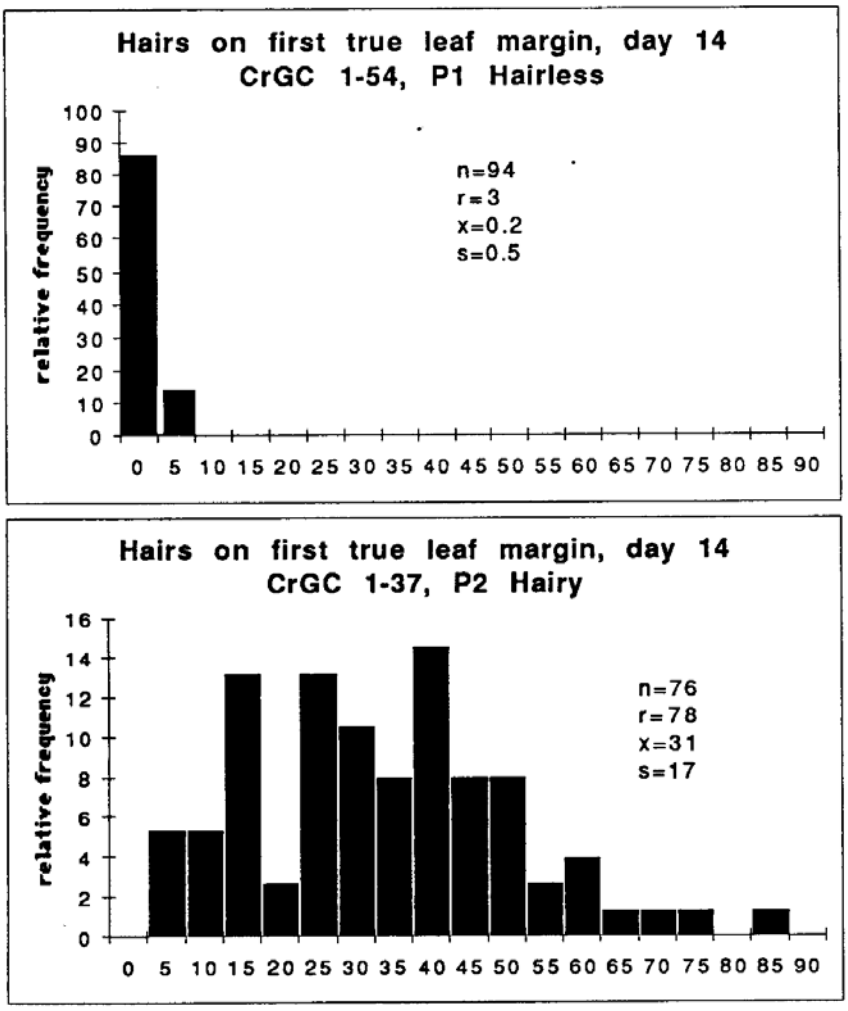


Figure 8.18. Frequency histograms depicting the number of hairs on:

Parent 1: CrGC 1-54: *anl/anl*, Hir (0-1), mst2/(1:1)
(anthocyaninless, hairless, Hir (0-1), and
50% of the plants male sterile)
Parent 2: CrGC 1-37: *anl/anl*, Hir (3-6), mst2/(1:1)
(anthocyaninless, hairy, Hir (3-6), and
50% of the plants male sterile)

The CrGC has also produced and characterized the stocks of the F1, CrGC 1-71, and the F2, CrGC 1-73, generations from the CrGC 1-37 and CrGC 1-54 parents. The production of the F1 seed was facilitated by incorporating in the parental stocks a single recessive allele, mst_2 , which prevents any pollen formation thus, conferring male sterility on the plant. The mst_2 allele is maintained in the hairless, CrGC 1-54, and hairy, CrGC 1-37, parental stocks in order to ensure that one half of the plants will be male sterile and the other half male fertile. Removing the male fertile

plants from one of the parents before pollen is shed enables strict pollen control in the production of the F1 generation.

Analysis of the F1 and F2 generation data, (**Figure 8.19**), provides interesting insight into the inheritance of the hairy phenotype. When F2 progeny are scored for the absence or presence of any hairs, the results support the model of a single recessive allele designated as *hir* for hairlessness. Chi-squared analysis of the F2 data in Figure 8-5 has a P value of approximately 0.05 for a 3:1 of hairy vs hairless. Observed values were 23 hairless of the total population of 95.

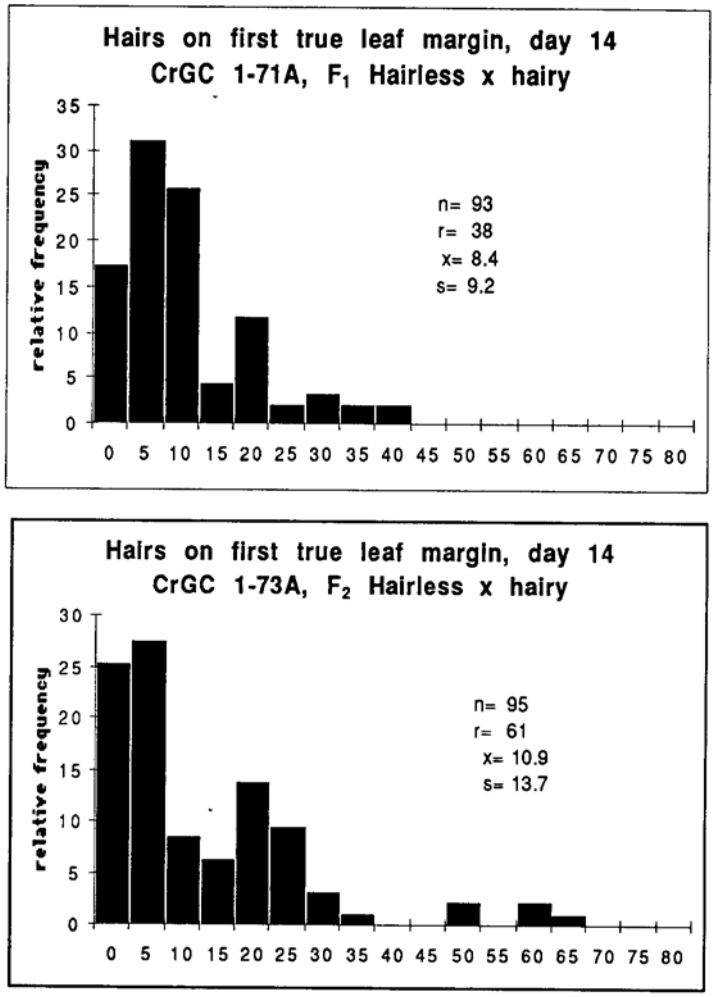


Figure 8.19. Frequency histograms depicting the number of hairs in the F1 and F2 populations from a cross between a hairless (CrGC 1-54) and a hairy (CrGC 1-37) Fast Plant.

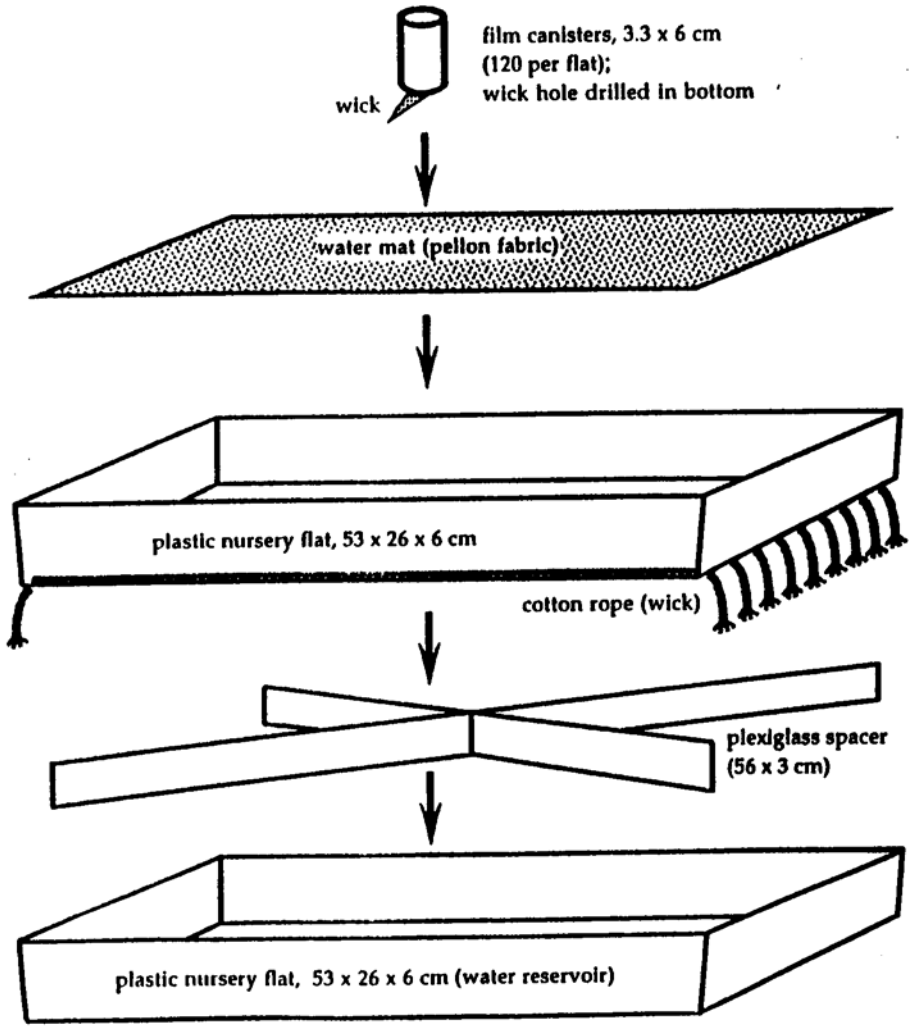
The F1 data are of interest in that they do not support the model of a single recessive allele for hairlessness with a completely expressed dominant, wild type allele conditioning presence of hair. The presence of 17 hairless in the F1 population of 93 suggests that interaction between the alleles for hairless and alleles for hairy may be occurring when the *hir* allele is in the heterozygous condition.

The data in Figure 8-19 and the above preliminary analysis are designed to raise a number of questions relating to the *Unraveling the Mysteries of Hairy's Inheritance*. It appears that the absence of hairs in Fast Plants is controlled by at least one allele having a major effect. The presence of one or more alleles conditioning hairlessness cannot be ruled out since the crosses between the parental stocks were always made with massed pollen from a minimum of 32 plants. Thus, the contribution of multiple alleles at the hairless, *hir*, locus cannot be ruled out. If individual hairless and hairy plants were selected from the parent populations and crossed would their progeny provide more explicit information on the inheritance of hairy? For example, would you expect the same results if you crossed a hairless plant with a plant with a few hairs as compared to a cross between a hairless plant and with a very hairy plant? These types of questions and experiments will provide insight into the Mysteries of Hairy's Inheritance.

Acknowledgement

Part I of this chapter was developed by Bruce Fall, General Biology Program, University of Minnesota, 225 Pleasant Street, SE, Minneapolis, IN 55455. This section is used here by permission and is copyrighted by Burgess International Group, Inc.

Appendix: Growing System Setup



Parental Generation Growth Apparatus

Figure 8.20

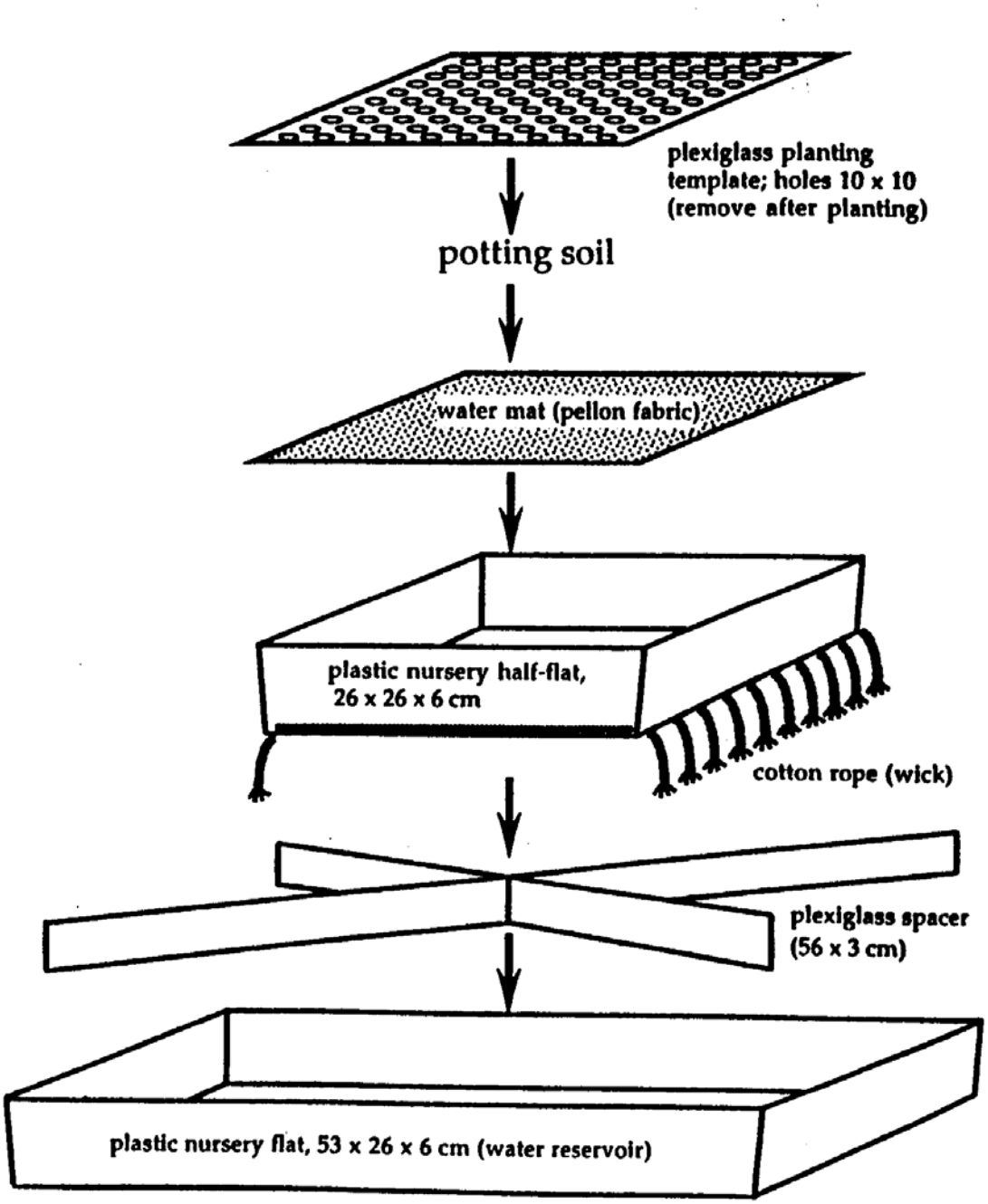


Figure 8.21 F1 Generation Growth Apparatus

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Wisconsin Fast Plants Program, WFPID "Hairy's inheritance," University of Wisconsin - Madison, 1992

Wisconsin Fast Plants Program, WFPID "Variation and Fast Plants," University of Wisconsin - Madison, 1998

Additional information available at these web sites:

Wisconsin Fast Plants Program Website: http://www.fastplants.org

General Biology Program, University of Minnesota Website: http://genbiol.cbs.umn.edu/staff/BAF.html