WFP072402

Investigating Life with the Cabbage White Butterfly and Brassicas in the Classroom

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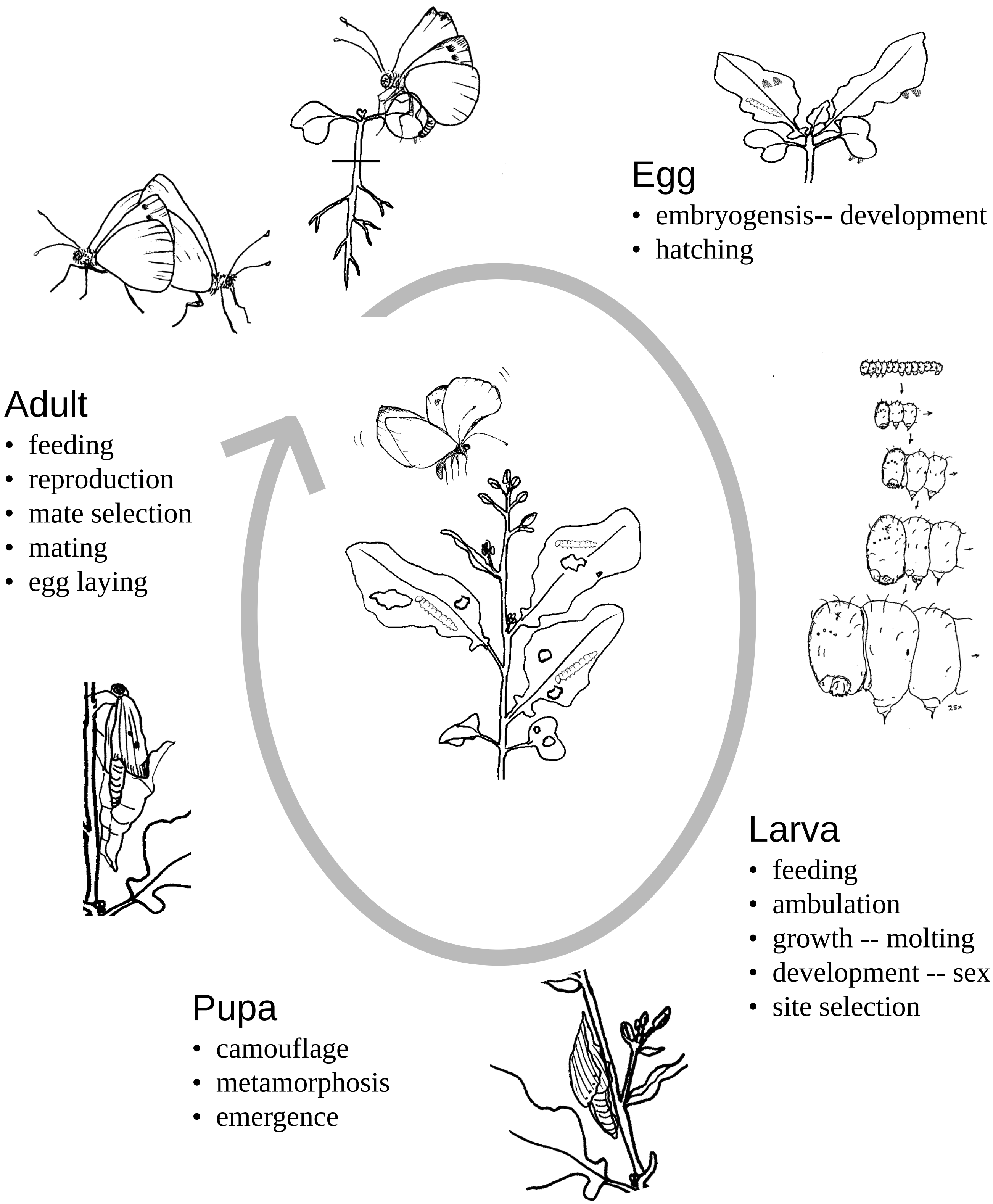
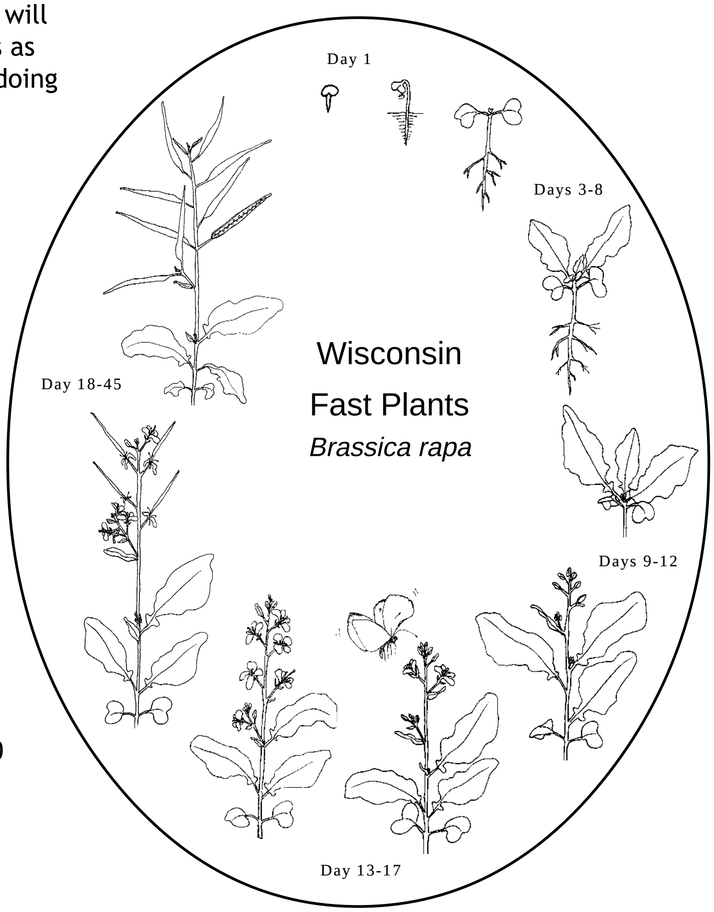


Investigating Life with the Cabbage White Butterfly and Brassicas in the Classroom
- a model of organismal Interdependence

How an insect gets from a tiny egg to an adult butterfly is a fascinating process. In this investigation, students will observe what happens with the Brassica Butterflies as they go through their life cycle on Fast Plants. In doing so, students will learn about the life cycle of an economically important insect and the interdependence between two organisms.

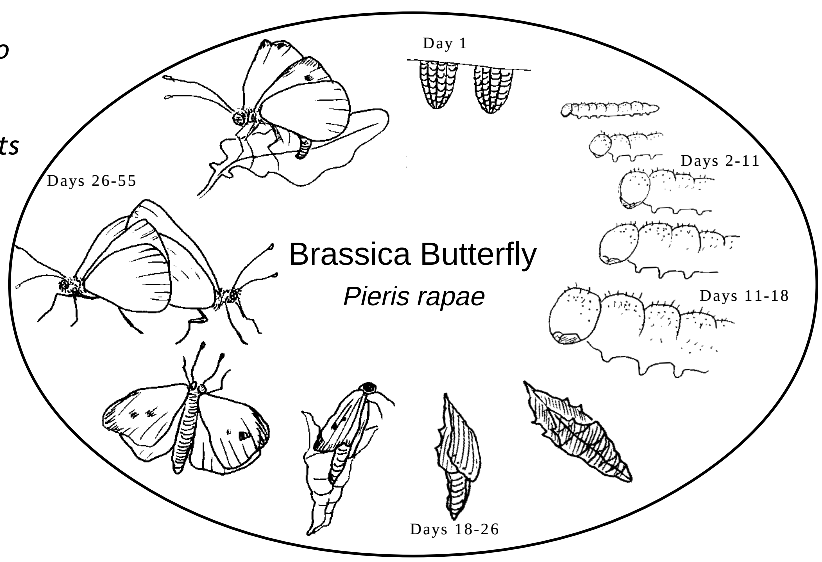


Fast Plants are rapid cycling brassicas, members of the cabbage and mustard family. They have been developed through 25 years of selective breeding to be used by plant researchers and by teachers in the classroom. They have a life cycle of 35-45 days (seed to seed) and can easily be grown in the classroom under continuous fluorescent light.

The Brassica Butterfly is the ubiquitous cabbage white butterfly species found across North America in many natural places and in gardens with cabbage, broccoli, and other crucifers. The butterfly's life cycle begins with an egg, passing through 5 larval stages, and transitioning through a pupae and metamophosisis to an adult butterfly. It progresses from egg to adult in approximately 30 days. Adult butterflies mate, lay eggs, and, with proper nutrition, live for up to 3 weeks.

Teaching Concepts

- *Life cycle of two organisms*
  - *Brassicas*
  - *Cabbage White*
- *Relationships of form and structure to function*
- *Interdependence of organisms*
- *Symbiotic relationships between plants and insects*
- *Environmental conditions influencing the relationships*



The rate of growth and development of Wisconsin Fast Plants and the Brassica Butterfly is affected by temperature and nutrition. (Sketches are not drawn to scale.)

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Plants and Butterflies

They **coexist** in **nature.**

Isn't it time they were brought **together** in the **classroom**?

Investigating Life with the Cabbage White Butterfly and Brassicas in the Classroom - a model of organismal Interdependence

How an insect gets from a tiny egg to an adult butterfly is a fascinating process. The butterfly life cycle investigation begins with eggs. From day one, students are making observations, predictions, collecting data and asking questions about the life cycle. Once the eggs have hatched, the biology of this organism will unfold, challenging students to understand not only the various life cycle stages of the organism but also how this organism interacts with plants (brassicas) and other components of its environment. Each stage of the butterfly's development provides students with opportunities for observations, ideas, analysis, experimentation and creative communication. Through raising butterflies plant material and understanding its life cycle, the foundation is set for students to launch into experimentation and inquiry-based learning.

This manual emphasizes both **science** and **technology** by including the design and construction of the experimental equipment as part of the investigative activities. All of the information and ideas for exploration are presented together so that teachers and students have access to all relevant information as they work together.

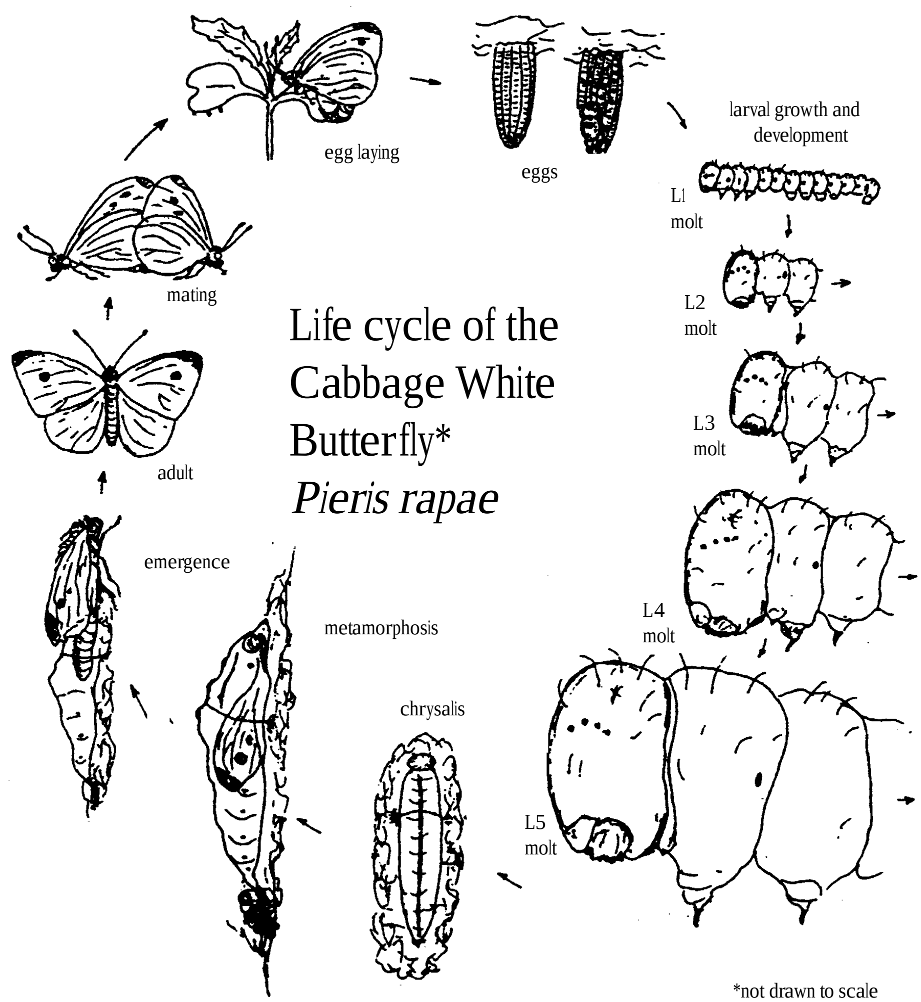
The levels of detail and complexity in this manual are balanced with the middle school classroom as the midpoint audience. At the same time, elementary, high school, and undergraduate college classrooms should find these materials rich and accessible. Many of the activities and concepts are also suited for independent study by students or for use in nonformal education settings.

> *"So many intuitive advances have been made by researchers.... due to an intimate knowledge of their organism. We (can) give the same advantage to our students (with Fast Plants and butterflies.)"*
>
> *Larry Ann Ott*
> *Teacher, Colorado*

Why the Cabbage White Butterfly - (*Pieris rapae)*?

The Wisconsin Fast Plants Program has recently developed the *Pieris rapae* (the Cabbage White or Brassica Butterfly) as a model organism for classroom research and investigations. The Butterflies have successfully guided kindergartners through high school students and college undergraduates into a better understanding of the scientific process of exploration and experimentation. They are also well suited for studying biological principals on the molecular, cellular, organismal, population and ecosystem levels *Pieris rapae* appears to be ideal for teachers, students, and scientists for many reasons.

- The Cabbage White Butterfly can be reared from egg to adult to egg in 35 to 45 days.
- The butterfly can be raised in school or in a lab with low-cost, self-made recycled equipment.
- The insect larvae feed on a wide range of brassica vegetables (Fast Plants, cabbage, broccoli, cauliflower, Brussels sprouts) that are always available in supermarkets, and the adults sip nectar from flowers or artificial nectar feeders containing sugar water and honey.
- The Cabbage White Butterfly can be raised on Fast Plants and synchronized with the Fast Plants life cycle for further investigations.
- By developing the skills for raising one or more cycles of Cabbage White Butterflies in their classrooms teachers will be preparing themselves and their students for virtually unlimited opportunities for high quality, open-ended, investigative learning suitable for all grades and age levels.
- A great virtue of the Cabbage White Butterfly as a model classroom and research organism is that Pieris rapae exists in large numbers in most states and in many other countries around the world. From early spring to late fall students and teachers and parents can observe them in the out-of-doors. The understandings gained through classroom investigations can be enriched when Cabbage White Butterflies are observed in their natural habitats.
- Cabbage White Butterflies may be obtained locally by collecting eggs or larvae from garden plants or they may be purchased commercially.



What are Cabbage White Butterflies?

Cabbage Whites are imports from Europe. They first appeared in Quebec in 1860 and have since spread as far as south Mexico. They can be seen just about anywhere in the United States from March to November. Several generations are produced each year. Individuals overwinter in the chrysalis stage.

Cabbage Whites are a member of the lepidopteran family Pieridae.

Taxonomy
Family: Pieridae
Subfamily: Pierinae
Genus: Pieris
Species: rapae

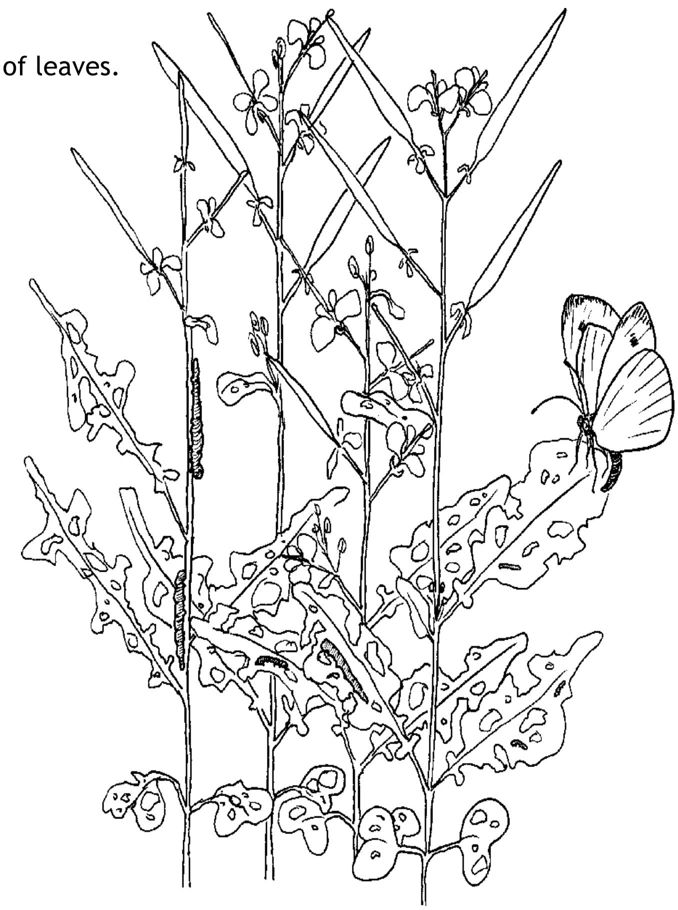
Habitat:
A common species on cultivated lands in almost any type of open space including, cities, gardens, roadsides, suburbs, weedy areas.

Commonly damages field and vegetable crops:
- Brassica spp., Sinapis, Raphanus sativus, etc. (Brassicaceae).

Caterpillar host plants:
leaves of plants in the mustard family (Brassicaceae)
- cabbage, broccoli, and cauliflower are favorites.

Adult Butterflies:
Female butterflies lay eggs singly on the underside of leaves. Males patrol for females.

Adult food:
Brassicas - cabbage, broccoli, and related species
Asters - nectar of asters
Taraxacum - dandelion
Mentha - mints
Nasturium - nasturtiums
Trifolium - red clover



Life Cycle Overview -

Week 1 (0-3 days old)
Within 1-3 days of being laid, the eggs hatch. Once the young larvae chew their way out of a hole on the top side of the egg, they forage for food. If no food is available, the larvae may cannibalize adjacent eggs and unhatched larvae. Brassicas serve as the source of nourishment while the larvae grow and molt.

Week 2 (3-13 days old)
Larvae pass through five stages, called instars. The instars are numbered L1-L5. By the end of each instar, the larvae have outgrown their exoskeletons and need to molt. They seek a dry site and weave a carpet of fine silk to secure themselves. (The silk is spun from a silk gland, located in the head just below the jaws or mandibles.) When the exoskeleton cracks, the larva crawls out and quickly pumps their new exoskeleton with fluid before it hardens. Larvae molt 2-3 times during the first week. As they grow and molt, larvae become voracious eaters, quickly devouring the plant leaves, stems and buds.

Week 3 (13-18 days old)

When the larvae have grown to the final larval stage (L5), they search for a suitable place (protected from the elements) to form a chrysalis. Most of them attach to the stem or the underside of a leaf. Once settled, the L5 larva weave a silken carpet to attach themselves to. First, the larva attach their posterior ends to the carpet with minute hook-and-loop structures then it spins a multi stranded silk belts around its middle.

The pupa (a chrysalises) forms within the exoskeleton of the L5 instar. The pupal stage emerges by splitting the L5 exoskeleton just beneath the head. The old exoskeleton contracts, slipping over the new pupal exoskeleton, similar to pulling off a sock. The old, tightly condensed L5 exoskeletons may be found at the posterior end of the pupa, or they may fall off. The pupa remains belted securely to the carpet in the middle and at the posterior end.

Week 4 (18-26 days old)

Viewed from the outside of the pupa, everything appears to be quiet for the next 8 days. Quite the opposite! Inside the pupa profound changes are occurring as the insect transforms from a caterpillar to an adult butterfly during the process of metamorphosis.

During the first 24 hours, the soft chrysalis hardens and becomes a translucent brown or almost black color. Over the next few days, the outlines of the newly formed wings appear. When two dark wing spots appear on each side of the chrysalises, the butterflies are within 24 hours of emergence.

Weeks 5-9 (26-55 days old)

After crawling out of their chrysalises (sometimes in less than a minute), the butterflies pump up their new wings, rest for their wings to harden, and begin to forage for food. This time, it is nectar from flowers that they crave. Seeking the sweet substance, the butterflies uncoil their proboscises and plunge them into various brassica flowers.

Within 2-3 days of emergence, mating ensues. Butterflies couple, tail to tail, for many minutes to allow the passing of packets of sperm to transfer from the male to the female. Hours later, the female butterflies (two wingspots rather than one) deposit tiny eggs onto the undersides of Brassica leaves, and the butterfly life cycles continue.

What is the difference between a butterfly and a moth?

Butterflies and moths are an evolutionarily related group of insects, called lepidoptera, that share many characteristics, including having wings covered with scales. The word *lepidoptera* means scaly (*lepido*) winged (*ptera*).

Many butterflies are very colorful and1 are active exclusively during the day. In contrast, most moths are fairly drably colored and are active at night. But there are quite a few butterflies that are dull and quite a few moths that are brilliantly colored and fly during the daytime. A better way to distinguish moths and butterflies is to look at their antennas. Butterfly antennas are shaped somewhat like a golf club, with a long shaft that has a "club" at its end. The vast majority of moths have antennas that are either simple filaments, tapering to a point at their ends, or are very complicated structures with many cross filaments, looking like feathers.

Butterflies are vary particular about which plant they use for feeding, egg-laying, and roosting. Simply put, butterflies and native plants depend on each other for survival. As for the plants, the butterflies and other insects aid in their quest for survival by passively helping with the plants pollination process.

In addition to food, the host plants provide shelter for stages of the butterflies life cycle. Many caterpillars are very 'host specific' and would starve rather than eat the wrong plant. The adult female is careful to lay her eggs on the correct host plant so that the larva (caterpillar) may feed and grow.

What are Brassicas?

Brassicas are part of a large family of plants called **crucifers** (Latin = Cruciferae). Crucifers are easily distinguished by the characteristic shape of their flowers—four petals in the shape of a cross or crucifix. A section, or genus, of the crucifer family is the genus ***Brassica***.

Brassicas have great economic and commercial value, and play a major role in feeding the world population. They range from nutritious vegetables, mustards and oil producing crops, to animal fodders and weeds.

Six of the most important *Brassica* species are closely interrelated. The relationship between these species can be represented as a triangle with the three diploid species forming the points of the triangle, and the amphidiploid species (crosses between the first three) on the sides (Figure 1).

Brassica nigra (2n = 16), popularly known as black mustard, is a common weed. It looks very much like the Wisconsin Fast Plants. Seeds of *B. nigra* will stay dormant in the soil for years. Once the ground is disturbed — for instance, by a tractor or bulldozer — black mustard is one of the first plants to appear.

Brassica carinata contains the genome of *B. nigra* and *B. oleracea*. It is a tall, leafy plant found almost exclusively in Ethiopia. The leaves are nutritious and the seed is pressed as a source of edible oil.

Reviewing *Brassica oleracea* is a bit like taking a trip through the produce section of your supermarket. Collards, cauliflower, broccoli, Brussels sprouts, kohlrabi and cabbage all belong in the species *Brassica oleracea*. Members of *B. oleracea* are used extensively in Northern Europe and Central Asia and are an important vegetable in many countries. Cauliflower is the main vegetable in India. One reason is that it can be stored without refrigeration. The outer leaves of the cauliflower wilt and form a protective layer around the head. In other parts of the world where refrigeration is still unavailable or considered a luxury, people also rely on other brassicas that store well (such as heading cabbage or kohlrabi) or those that continue to grow through a mild winter (collard, Brussels sprouts).

The forms of *B. oleracea*, known as kale, are used for a wide variety of purposes, including human food and animal fodder. Kale is used extensively in New Zealand and Scotland for feeding sheep. A particularly tall variety of kale, known as tree kale or Jersey kale, is grown in Portugal as a food crop and dates back to Roman times. On the Isle of Jersey, the tree kale is grown as fodder for the Jersey cattle. Jersey kale is also a source of a cottage industry for island inhabitants who make walking sticks from the eight-foot kale stems.
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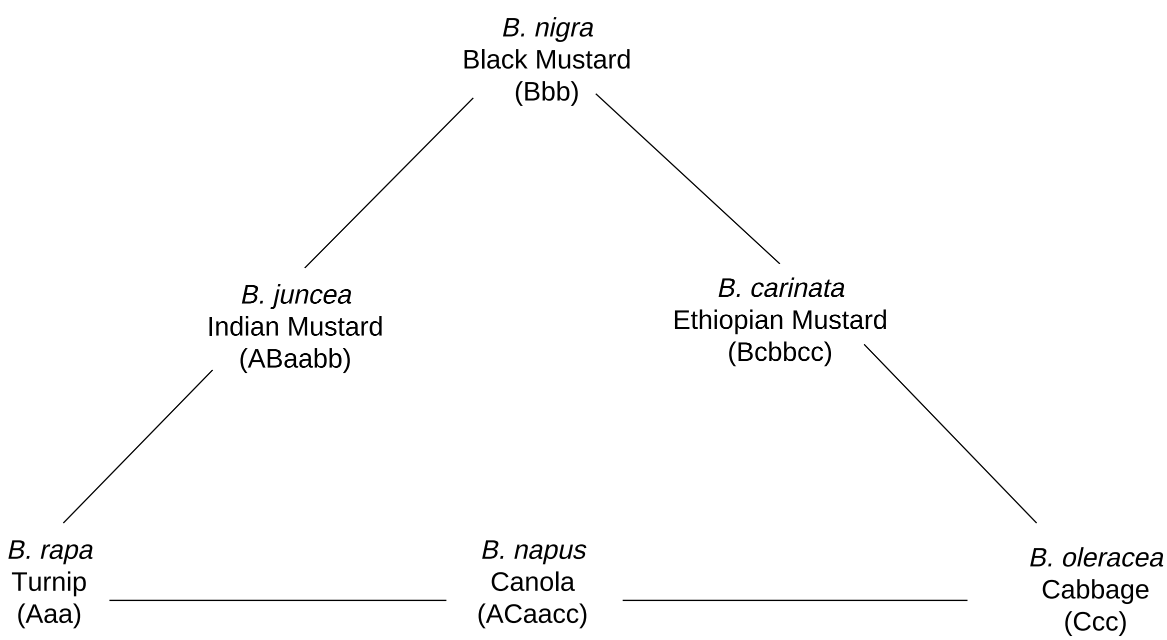


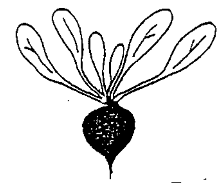
Figure 1: Interrelationships between several *Brassica* species.
The upper and lower case letters are the genomic symbols for each of the species.

Table 1: Names of the species of agriculturally important Brassicas

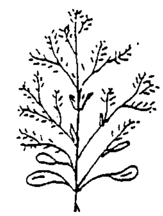
Species (genome)	**Cultivar group or common name**
Brassica nigra (bb = 16)	Black mustard
Brassica oleracea (cc = 18)	Cabbage Broccoli, Calabrese Cauliflower, heading broccoli Brussels sprouts Kohlrabi Collards Savoy cabbage Kales Borecole Chinese kale, Kailan Portuguese cabbage, Tronchuda Marrow stem kale Tree cabbage, Jersey kale Thousand-head kale
Brassica rapa (aa = 20)	Turnip Chinese cabbage, Napa, Bai Tsai Pak choi Broccoletto, Broccoli raab, Rapini Tendergreen, Komatsuna Saichin, Choy sum Tsatsai Mizuna, Mibuna Turnip rape, Toria (oil seeds) Yellow sarson
Brassica carinata (bbcc = 34)	Ethiopian mustard
Brassica juncea (aabb = 36)	Mustard Leaf mustard Cut leaf mustard Head mustard Broccoli mustard Large petiole mustard Multishoot mustard Big stem mustard Root mustard Indian mustard, Raya (condiment)
Brassica napus (aacc = 38)	Swede, Rutabaga Oil rape Fodder rape
Raphanus sativus (rr = 18)	Radish, dikon Oil radish Rat tail radish

The haploid complement of chromosomes is a = 10, b = 8 and c and r = 9.

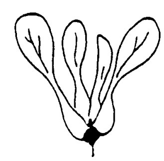
choy sum
Brassica rapa parachinensis



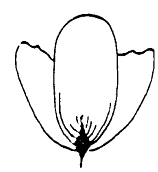
turnip
Brassica rapa rapifera



turnip rape or sarson
Brassica rapa oleifera or B. r. trilocularis



pak choi
Brassica rapa chinensis



Chinese cabbage
Brassica rapa pekinensis

Wisconsin Fast Plants
Rapid-cycling *Brassica rapa*

Morphotypes of the subspecies of *Brassica rapa*

(What are Brassicas? continued)

These walking sticks are very popular in British hospitals, because they are very strong and Kale is used extensively in New Zealand and Scotland for feeding sheep. A particularly tall variety of kale, known as tree kale or Jersey kale, is grown in Portugal as a food lightweight. A different variety of kale, developed in Japan for ornamental purposes, is used for flower beds. This ornamental kale spreads its curly leaves at ground level, and looks like a big red, pink or white flower.

Brassica napus is a cross between *B. oleracea* and *B. rapa*. Rutabagas are a variety of *B. napus* as is oil seed rape. This crop is the third most important source of edible oil behind soybean and peanut oil. In the United States, cooking oils and margarines are produced mainly from corn or soybeans. In other parts of the world, the main source of edible oil is pressed from the seed of oil seed rape. Oil seed rape flourishes in Canada and Europe, where the climate is too cool to produce a corn crop. In the summer, the Canadian prairies are literally 'fields of sunshine,' due to the yellow flowers of oil seed rape, also known as canola.

The last of the diploid *Brassica* species is *Brassica rapa* (2n = 20). Both turnips and Chinese cabbage fit into this species. Chinese cabbage is consumed by millions of people all over the world. Over 200 different varieties of Chinese cabbage can be found in China. The per capita consumption of brassicas in China is 1/4 pound each day. Multiplied by China's population, this approximates 250 million pounds of brassicas eaten in China every day. In northern China, where winters are long and cold, heading forms of Chinese cabbage are harvested in the fall. Stored anywhere and everywhere there is room (including under the beds), Chinese cabbage provides a major source of vitamins through the winter. One can easily imagine that by spring, no one in China ever wants to see Chinese cabbage again!

In Korea, Chinese cabbage is again a mainstay in the diet, but is processed in a different way. As the Germans ferment cabbage to make sauerkraut, the Koreans ferment cabbage to make kimchee, the national dish. Kimchee is made in large earthenware jars by layering Chinese cabbage, radish, garlic, hot peppers and salt. Every family has its special recipe — some even add fish to the kimchee. Whatever the recipe, kimchee is eaten three times a day, for breakfast, lunch and supper.

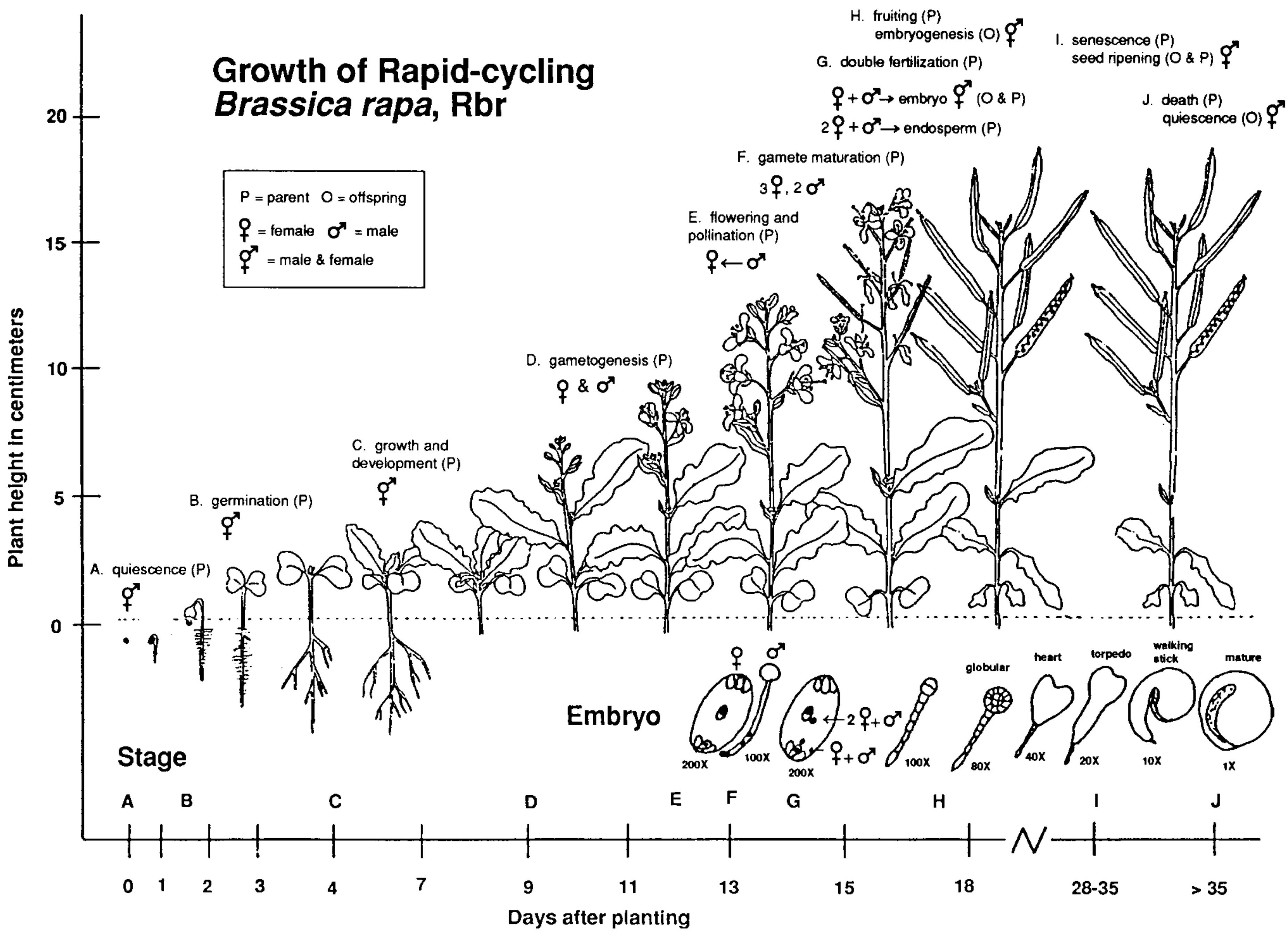
The final of the six *Brassica* species is *Brassica juncea* (2n = 36), the cross between *B. rapa* and *B. nigra*. *Brassica juncea* includes the group known as mustard. In the southern United States, the foliage of *B. juncea* is often eaten as highly nutritious mustard greens. Most people use mustard as a condiment or spice. The distinctive flavor of mustard, present to some extent in all the brassicas, is strongest in the various types of *B. juncea*. All brassicas contain chemicals called glucosinolates, which are activated by saliva and release the strong 'mustard' flavor. These glucosinolates are a help to both plants and humans. Certain pests are repelled and others attracted by glucosinolates. The strong flavor also helps to discourage deer and rabbits from eating brassica crops. In mammals, certain glucosinolates have been found to help detoxify cancer-causing nitrosamine chemicals in the liver.

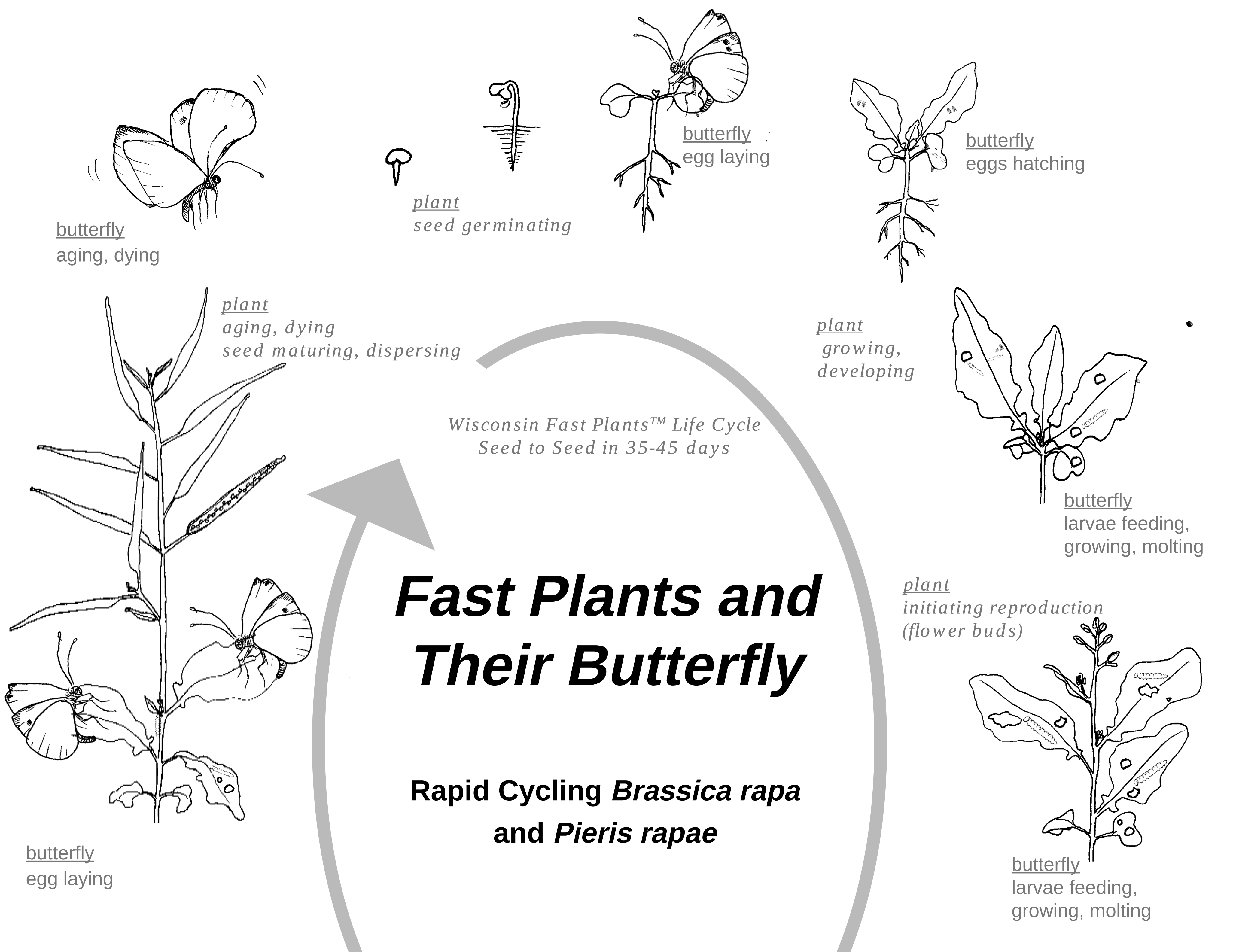
What are Fast Plants?

Selective breeding of a specific Brassica has produced Wisconsin Fast Plants from this tremendous resource of brassicas collected from all over the world (both wild and domesticated forms). Fast Plants (Rapid cycling *Brassica rapa*) were initially bred at the University of Wisconsin-Madison as a model organism to study the genetics of this important group of plants. Bred for their small size, simple growing needs and rapid life cycle (35 days, seed-to-seed), Fast Plants quickly became a popular teaching tool that is now used internationally in pre-K through college classrooms.

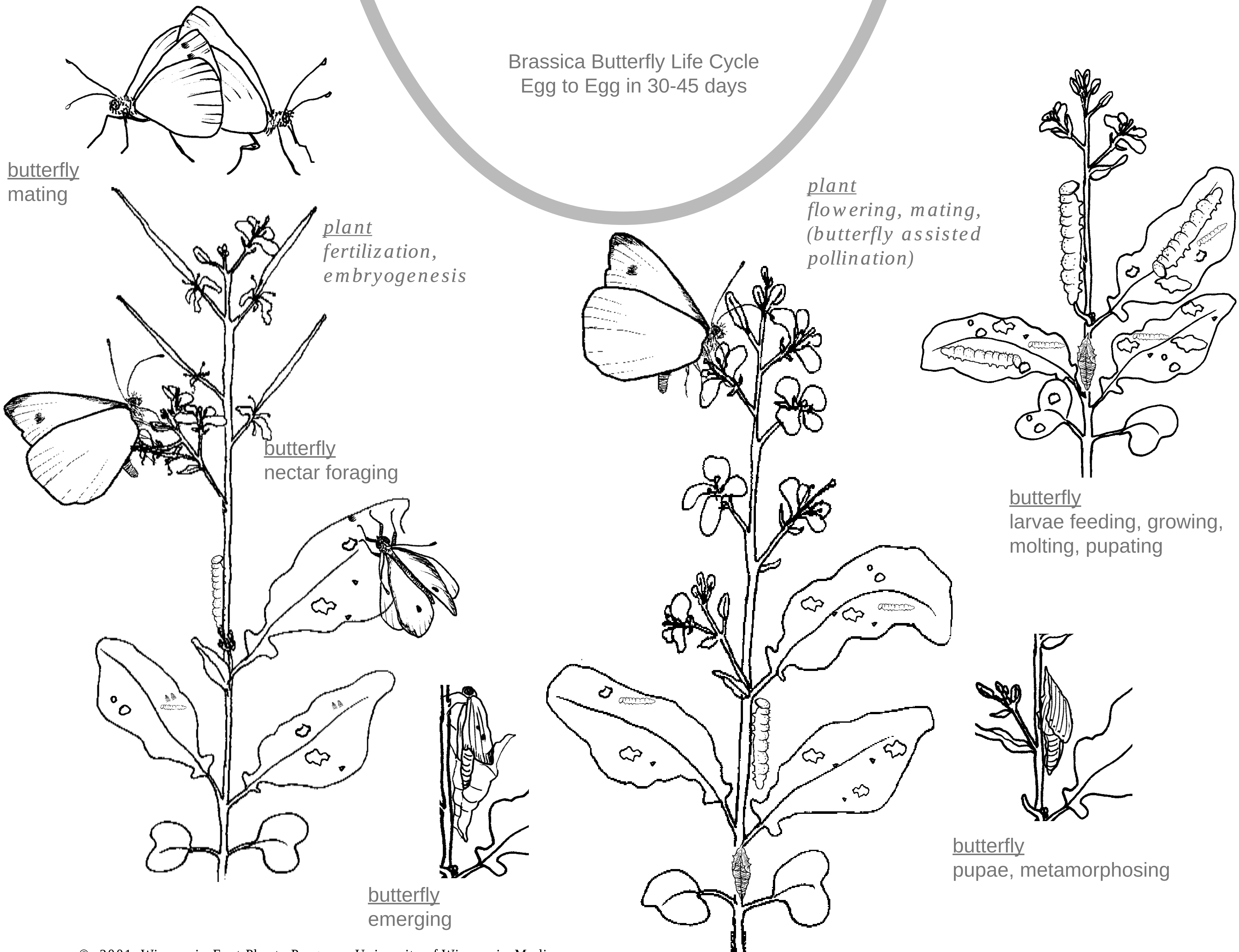
Two Life Cycles Together - Life in Balance?

By growing Fast Plants and the Cabbage White Butterflies teachers and students can investigate the interdependence of two complete life cycles. From day one, students are making observations, predictions, collecting data and asking questions around the two life cycles. Once the eggs have hatched and the plants are growing, the biology of these two organisms will unfold, challenging students to understand not only the various life cycle stages of the two organisms but also how these organisms interact with one another.


Growth of Rapid-cycling
Brassica rapa, Rbr
P = parent O = offspring
♀ = female ♂ = male
⚥ = male & female
A. quiescence (P) ⚥
B. germination (P) ⚥
C. growth and development (P) ⚥
D. gametogenesis (P) ♀ & ♂
E. flowering and pollination (P) ♀ ← ♂
F. gamete maturation (P) 3♀, 2♂
G. double fertilization (P)
♀ + ♂ → embryo ⚥ (O & P)
2♀ + ♂ → endosperm (P)
H. fruiting (P) embryogenesis (O) ⚥
I. senescence (P) seed ripening (O & P) ⚥
J. death (P) quiescence (O) ⚥
Embryo
globular
heart
torpedo
walking stick
mature
200X
100X
200X
100X
80X
40X
20X
10X
1X
Stage
A
B
C
D
E
F
G
H
I
J
0
1
2
3
4
7
9
11
13
15
18
28-35
> 35
Days after planting
Plant height in centimeters
20
15
10
5
0



Fast Plants and Their Butterfly
Rapid Cycling *Brassica rapa* and *Pieris rapae*
Wisconsin Fast Plants™ Life Cycle
Seed to Seed in 35-45 days
butterfly
aging, dying
plant
seed germinating
butterfly
egg laying
butterfly
eggs hatching
plant
growing, developing
butterfly
larvae feeding, growing, molting
plant
initiating reproduction (flower buds)
butterfly
larvae feeding, growing, molting
butterfly
egg laying
plant
aging, dying
seed maturing, dispersing


Brassica Butterfly Life Cycle
Egg to Egg in 30-45 days
butterfly
mating
plant
fertilization,
embryogenesis
butterfly
nectar foraging
butterfly
emerging
plant
flowering, mating,
(butterfly assisted
pollination)
butterfly
larvae feeding, growing,
molting, pupating
butterfly
pupae, metamorphosing

Cabbage White Life Cycle Concepts and Questions

Eggs

- **Embryogenesis -- Development**
  What visual changes are evident?
- **Hatching**
  How long does it take for an egg to hatch?
  Does a new larva know what to eat?

Larvae

- **Feeding Choices**
  Do larvae prefer certain foods?
  How can you tell what a larva has been eating?
  How does a larva process food?
- **Ambulation**
  How does a larva walk around?
- **Growth -- molting**
  How does a larva grow encased in a suit of armor?
  How does a larva molt?
  What is the heart rate of a larva?
  How does a larva breathe?
- **Development -- sex**
  Can you tell the sex of a larva?
- **Site selection for pupation**
  How can you tell when an L_5 larva is getting ready to pupate?
  What is the orientation of a larva beginning to pupate?

Pupa

- **Camouflage**
  How does a pupa adapt to its environment?
  What affects the color of the pupa?
- **Metamorphosis**
  What visual changes are evident?
  How long does metamorphosis take?
  Can you tell the sex of a pupa?
  Why would you want to know the sex of the pupa?
- **Emergence and wing expansion**
  How does the adult emerge?
  How much space does a butterfly need to pump up its wings?

Adult

- **Feeding**
  How does a butterfly feed?
  What attracts the butterfly to the flower?
  What are the feeding preferences of the Butterfly?
- **Reproduction**
  Can you tell the sex of an adult butterfly?
  How and when do butterflies mate?
  How many times do butterflies mate?
- **Mate selection**
  How can a male butterfly determine that a female has mated?
- **Egg laying**
  How does a female butterfly detect a suitable host for egg laying?
  How does she lay her eggs?
  How many eggs can a female lay?
  How long can she lay eggs?
  Are there patterns in the timing of the egg laying?

Environment - The Basics For Success

It is important to remember that to be successful at raising any organism in the classroom through a life cycle, a suitable environment for that organism needs to be provided.

Environmental variations in temperature, humidity, and light (intensity and duration) may affect the success and the length of the butterfly life cycle in the classroom.

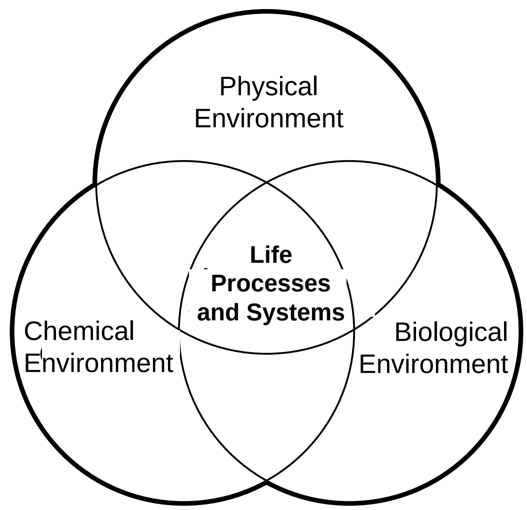
Temperatures between 20-24 degrees C (68-75 degrees Fahrenheit) are optimal.

It appears that the greater the relative humidity the better (up until 90%) No exact percentage has been quantified, nor is it easy to control in a classroom.

The adult butterflies appear to prefer a 16 hour light with an 8 hour dark photoperiod inside the Butterfly Box.

As with all organisms, fresh food and water are essential.

Understanding the Environment



Three broad categories of environmental components interact to influence all life:

1. physical environment - light, temperature, gravity
2. chemical environment - atmospheric relative humidity, mineral nutrients, water (carbon, hydrogen, and oxygen - based chemical energy)
3. biological environment - abiotic and biotic

As with all organisms, there is a potential for disease to occur with the butterflies. Larval and pupal stages of Pieris are susceptible to various viral and bacterial insect pathogens.

Occasionally you may notice evidence of a bacterial or viral infection in a larva or chrysalis. The larvae or chrysalis may look a little soft and then turn brown and "oozy". If this happens, you must maintain rigorous sanitation. The infection can rapidly spread through the population.

If a larva looks soft or dull and develops black spots and then turns black or turns "oozy", remove the larva in such a way that it doesn't touch other larvae, plant leaves or walls of the enclosed container. Use fresh, flat toothpicks and slide them under the caterpillar (don't poke or puncture it.) Then drop the caterpillar and toothpicks into a paper towel and dispose of it. **Wash your hands thoroughly**.

If a chrysalis looks soft or begins to turn uniformly black and looks dry, lift it off with two flat toothpicks and dispose of it. If a chrysalis actually begins to ooze and secrete what one student described as "brown-green goo", more careful sanitation measures should be followed. Touch the pupa only with clean flat toothpicks or small pieces of paper toweling. Wipe any surface that they have touched with paper toweling and then wash your hands. It is recommended that you also move any other chrysalis to a clean container; then wash down the "infected" container with a 5% bleach solution, 1 cup of bleach/1 gallon of water, and finish by washing your hands.

Classroom Activity and Investigation Timeline for the Cabbage White Butterfly

Preparation	**Time Line**	**Brassica Butterflies**	***Optional Investigations***
Make a Plant Light House Make Butterfly Box Prepare growing systems	**Week prior to starting**	Order eggs early to be delivered when the Brassica Nurseries are 5-7 days old. Plant Brassica Nurseries	*Plant one Brassica Nursery with radish to test the difference between radish and other Brassicas*
	Day 1 (Tuesday)	Put egg strip on the plants in one film can to hatch	
	Day 2-10	Larvae hatch and grow Observe Measure Sketch	
Assemble the Brassica Barn or Cabbage Cafe	**Day 9**	Purchase Brussels sprouts, broccoli , or cabbage from the grocery store	

	Day 10-18	Tend larvae Observe Measure Sketch	*Larval feeding studies* *"Brassica Salad Bar"* *"Frass Forensics"* *"Botanical Anatomy"*
	Day 18-20	Larvae will pupate - form a chrysalis	*Sex determination and separation of larvae*
	Approximately-Day 18-26	Observe metamorphosis (spinning of pupa to emergence of butterfly = 8 days) Observe emergence	*View stages of metamorphosis*
Activate Butterfly Box and Film Can Sugar Feeders	**Day 26-27**	Transfer hatched butterflies into Box	
	Day 28-33	Observe feeding, mating, and egg laying (ovipositing)	
Prepare Film Can Ovipositrons	**Day 28-40**	Glue leaves to film can Ovipositrons	*Ovipositing experiments*
	Day 28-40	Remove egg strips from film cans. Transfer to Brassica Nurseries to continue the life cycle	

Rearing Calendar

Day of cycle	Maintenance
Preparation (5 days prior starting)	Order butterfly eggs. Build and assemble light houses and growing systems. Plant nurseries for arriving eggs. Arrange all planting materials. See pages 30-34.
Day 1 (10 min.)	**Start life cycle** Place egg strips onto the five day old nurseries. Check the water level in the reservoir.
Day 4-5* (5 min./day.)	Check the water level in the reservoir.
Day 10* (45 min.)	Set up Brassica Barns or Cabbage Cafes and transfer larvae to barns.
Day 11-18* (5 min./day)	Clean Barns, removing all frass or poop, daily. Conduct larval experiments and investigations.
Day 18-26* (5 min./day)	Observe metamorphosis. Run pupae experiments and investigations.
Day 26*(20 min.)	Prepare Butterfly boxes. Adult butterflies begin to emerge. Prepare sugar feeders.
Day 26-45* (30 min.)	Prepare Ovipositrons. Conduct feeding choice experiments. Run egg laying experiments.

** The developmental timeline for the life cycle will vary depending on environmental conditions in your classroom.*

Preparation

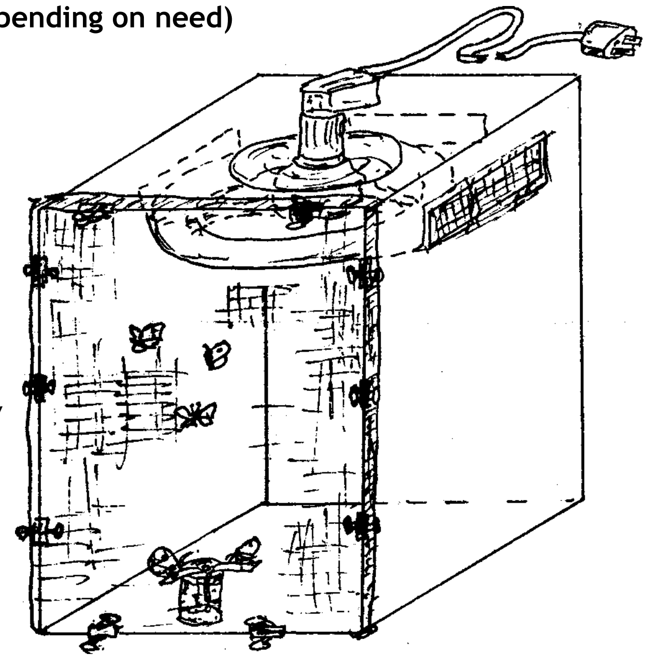
As part of building the concept of science and technology in the classroom and beyond, all materials needed to raise the butterflies successfully in the classroom can be collected and constructed from low-cost, recycleable materials and are designed to be constructed by students.

Materials and Equipment (per group or class depending on need)

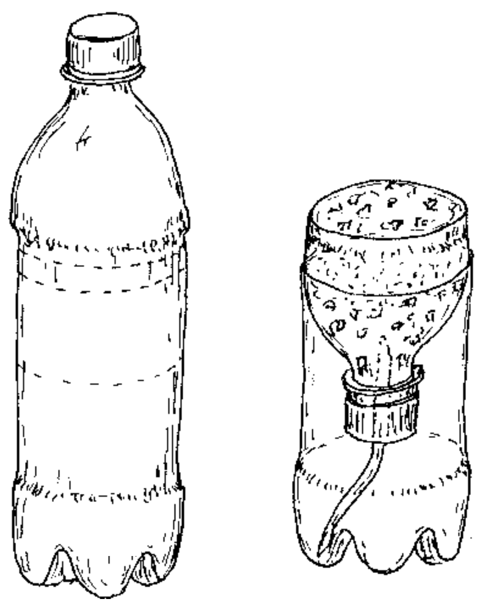
(see page 30 - 34 for construction and materials)

- *1 egg strip (30 - 50 eggs)**
- *1 Butterfly Box / Light House*
- *1 Bottle Growing System*
- *1 Brassica Nursery and Brassica seed mix**
- *1 Brassica Barn or Cabbage Cafe*
- *2 Film Can Sugar Feeders*
- *1 Film Can Ovipositron*

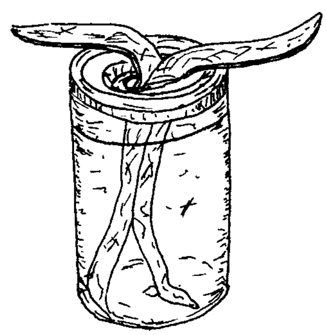
* *Cabbage White Butterfly Eggs, Brassica Nursery Mix and Butterfly Box / Light House are available from Carolina Biological Supply Company - 1-800-334-5551 www.carolina.com*



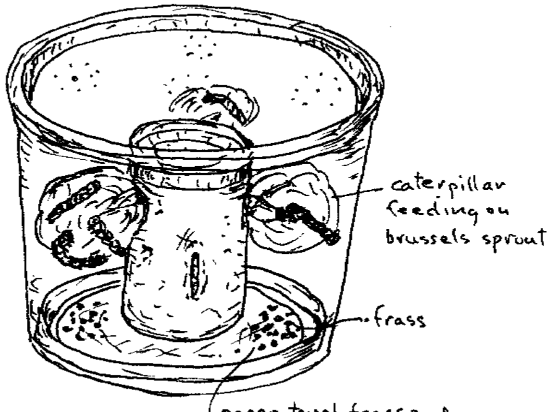
Butterfly Box / Light House



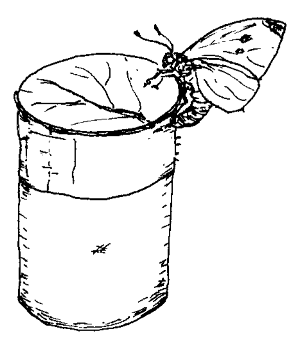
Bottle Growing System



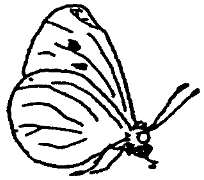
Film Can Sugar Feeder



Brassica Barn



Film Can Ovipositron



ORDERING BUTTERFLY EGGS

Order the Butterfly eggs from the Carolina Biological Supply Company (1-800-334-5551) at least one week prior to starting the activity with your students. The eggs will be shipped overnight .

- The eggs will hatch approximately 2-3 days after they have been shipped.
- It is extremely important that you are notified of the package arrival and that the eggs be placed immediately on the growing Brassica Nurseries.
- Eggs will be on a waxed paper strip and protected in a small plastic container.

Egg Laying

- All insects, including butterflies, begin life as an egg - the female adult butterflies hover near a plant and curl their abdomen under and deposit an egg on the underside of the leaf.
- The egg is fertilized shortly before it is laid. The sex of the offspring is determined at this time.
- Butterflies do not care for their young after the eggs are laid, but females do lay their eggs on or near a food source.
- Most female butterflies secrete a small amount of glue to attach the eggs directly to a suitable host plant.

Instructions for Rearing the Butterflies

Timeline

A Timeline (pages 16 and 17) and Rearing Calendar (page 18) of the activity have been prepared, beginning with planting of the Brassica Nursery and ordering the butterfly eggs. They are intended to help you coordinate your first run through of the activity. As with any timeline concerning a living organism, it is an approximation.

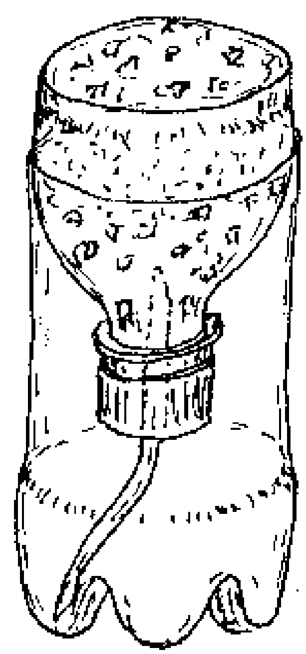
Instructions

The step by step instructions are written with everything that the teacher needs to know in order to be successful in running the butterfly life cycle. Appendices are added for specific instructions on growing plants, and on making the equipment.

Questions (inquiry strand) and suggestions for additional investigations are included with the step by step instructions along the sides of the appropriate page.

Getting ready

Order the Cabbage White Butterfly eggs and Brassica Mix*
The eggs and seeds for the mix are available from the Carolina Biological Supply Company (1-800-334-5551). Schedule the eggs to arrive one week after the seed. This will allow you to plant the mix at least 5 days prior to the eggs arriving.



Plant two Brassica Nurseries (Brassica Mix seeds) 5 days prior to the arrival of the butterfly eggs.

** The Brassica Seed Mix is a mixture of various seeds, Fast Plants, Turnips, and Radish. You can mix your own turnip and radish seeds from a garden store. (Fast Plants are available through the Carolina Biological Supply*

Day 1 -
Place eggs onto the Brassica Nurseries

The eggs will arrive on waxed paper in a small plastic vial. Gently place the waxed paper strip of butterfly eggs egg side down to cover one or more cotyledons on several brassica seedlings in the Nurseries. Depending on the number of nurseries in the classroom, you may wish to cut the egg strip into several smaller pieces. Be extremely careful not to touch / damage any of the eggs.

The eggs will take between 3-5 days to hatch depending on temperature.

Embryogenesis

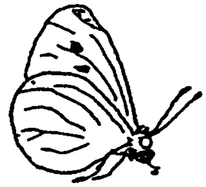
- The new insect is formed on the outside of the yolk mass.
- By 37 hours after fertilization, the body wall begins to broaden and segmentation starts. Rudiments of the mouthpart appendages begin to form and the holes of the new spiracles (entrance to the breathing tubes) become apparent
- By 57 hours, the embryo is taking on the look of a caterpillar.
- During the next 60 hours, the appendages of the insect will take shape. During that time, the insect will undergo a shedding of the cuticle while it is still enclosed within the chorion.

Emergence

- The larva will use its large mandibles to chew a hole in the chorion and climb out to eat the remaining portions of the chorion.
- The emerging larvae are very pale cream color, almost translucent.

Cannibalism

- Once the larvae emerge they are in search of food. If no plant material is available, they will turn to the egg next to them and eat the premature larvae.



Why Does a Female Cabbage White Butterfly lay her eggs on Brassicas?

All brassicas, contain chemicals called glucosinolates. When activated by the saliva in your mouth, glucosinolates give the typical flavors found in all brassicas (such as broccoli, cabbage, Brussels sprouts, and radish).

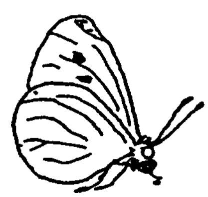
These biologically active chemicals may be beneficial in that they may repel certain insects while attracting others. The Cabbage White Butterfly is one of insects attracted to the Brassicas and has evolved to exclusively live and reproduce on Brassicas.

The strong flavor helps to discourage deer and rabbits from eating brassica crops in the fields. In mammals, some glucosinolates have been found to help detoxify cancer-causing nitrosamine chemicals in the liver.

Chew a Brassica seed and see how it tastes. Is it spicy?

Anatomy of an Egg

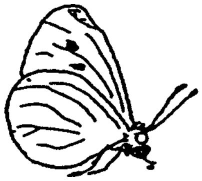
- The Cabbage White Butterfly eggs are conical and creamy white. They stand approximately 1.5 mm tall and are usually found on the undersurfaces of leaves.
- The covering of the egg, the chorion, is impermeable to water, however, there are small holes in the shell to allow sperm to enter to fertilize the egg. These holes are collectively called the micropyle.



Up Close and Personal

Have your students view the egg strips under a microscope or a video flex cam and describe and draw the eggs.

Describe how the eggs are similar or different from one another.



"Where's Pieris?"

A great introductory activity to pique students curiosity and simulate observational skills is to have students seek and find the L_1-L_3 larvae in the Brassica Nursery.

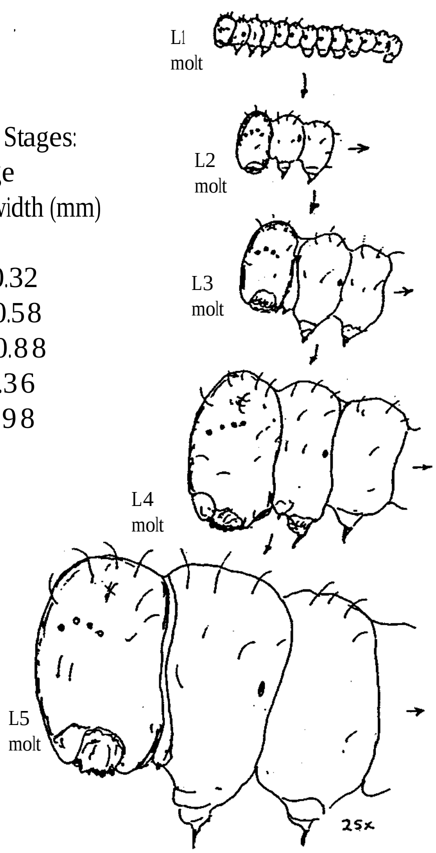
Larval Development

Watch for the larvae to hatch as each larva, L1 stage, chews its way out of the egg through a hole in the mid-top side of the egg.• In the next week the larvae will molt two-three more times. Observations can be made.

• There are 5 larval stages, also called instars. Width of head capsule indicates larval stage.

Larval Stages:
average
head width (mm)

L1 = 0.32
L2 = 0.58
L3 = 0.88
L4 = 1.36
L5 = 1.98



Larva or Caterpillar?

The word larva refers to the growth stage of all insects with complete metamorphosis. Caterpillar refers only to a butterfly or moth in the larval stage. Either word is correct, but most scientists say larva.

Day 2-10 - Larvae hatch, eat, grow, molt, and grow some more

The tiny larvae crawl off the waxed paper and immediately feed on the brassica seedlings. As they forage, they will move onto the plants.

Pieris rapae goes through 5 molts before becoming a pupa or chrysalis. The 5 larval stages or instars are designated L1 - L5. See pages 36 and 37 for a detailed description of each larval stage.

- Larva, L1 stage, chews its way out of the egg through a hole in the mid-top side of the egg.
- L1 may consume egg shell.
- L1 may feed on other adjacent eggs (on the egg strip) consuming part or all of an egg and unhatched larvae.
- L1 head relatively large compared to body length and width.

Molting
- When molting, larvae seek a dry site, weave a fine carpet of silk, attach to it, lie quietly for several hours, then crack and crawl out of their exoskeleton. Once out of their old exoskeleton, they quickly pump up their new exoskeleton before it rigidifies. (All this can be viewed with a hand lens.)

Chewing
- The larvae continue to devour the Brassica Nursery.

Silk production
- Larvae produce a fine strand of silk (protein polymer) from a gland located in the head just below the mandibles (jaws)
- Larvae often produce a silk track on surfaces (e.g., on leaf or plastic) on which they cling with their legs. Note the characteristic movement of the head swaying back and forth to create continuously overlapping figures of 8 using the head silk gland.

Defecation
- Larval excrement is called frass.
- Under the microscope it looks watery. It dries to fine, dark black granules.
- Frass may be dried and used in various kinds of experiments, e.g., as fertilizer for organic amendments for Fast Plants and others or suspended in water drops and viewed under the microscope.

Anatomy of the Larvae

Larvae have three distinct body parts:

Head - The head has a pair of very short antennae, mouthparts (upper lip, mandibles, lower lip, silk gland), and six pairs of very simple eyes, called ocelli. Even with all of these eyes, larval vision is poor. The antennae help to guide the weak-eyed larvae, and the maxillary palps, which are sensory organs, help direct food into the larva's jaws.

Thorax -Each thoracic segment has a pair of jointed, or true legs, while some of the abdominal segments have false legs, or prolegs. There are usually five pairs of prolegs. The prolegs have tiny hooks on them that hold the larva on its silk mat or leaf.

Abdomen - Like other insects, Cabbage White Butterfly larvae obtain oxygen through holes in the sides of their thorax and abdomen called spiracles. The spiracles are connected to a network of long air tubes called tracheae, which carry oxygen throughout the body.

L4 & L5 larvae are voracious feeders and will consume increasing quantities of brassica tissue as they grow.

- Typically, one Brassica Nursery will support 15-25 larvae up to their L4 stage at which point the larvae will be transferred to a Brassica Barn or a Cabbage Cafe.

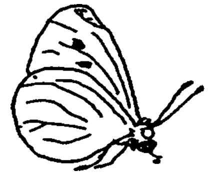
Prepare the Brassica Barn or the Cabbage Cafe

Prepare as many Brassica Barns as you need. Instructions are in Appendix 2. You will need one Brassica Barn per 9-12 larvae. Probably 3-4 Barns is a good number for a class of 25 students. An alternative to the Brassica Barn is the Cabbage Cafe. One Cabbage Cafe will provide enough food for up to 60 larvae.

- The barns and cabbage cafes need to be ready for transferring larvae into the barns at about Day 10.
- If using the barns, cut a good supply of paper towel pads (20-30) as detailed in the instructions.

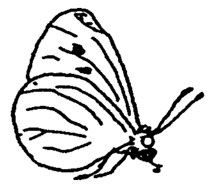
Day 9 - Visit the grocery store

One or two days before transferring the growing larvae, you will need to find food for the larvae. Visit your grocery store and purchase either Brussels sprouts or broccoli stems if using the barn or a head of cabbage for the cafe for the larvae.



What Stage is your larva ?

You can judge the larval stage, e.g., L2 or L3, by estimating or measuring larval length or head capsule width in mm and comparing this measurement to the average figures. (L1 to L3 larvae are very small and delicate and should not be "handled".) See page 22 for average head capsule sizes for the larval stages.



"The Salad Bar "

How does a *Pieris rapae* larva "know" which food to eat?

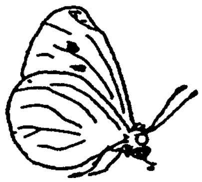
Activity

Observe the feeding preference of larvae among brassicas (cabbage/mustard) family or between brassicas and other plant families.

Materials per group

- 1 petri dish or other covered plastic container
- paper towel
- 1 or 2 living L4 - L5 stage *Pieris rapae* larva
- Mixture of moist leafy greens (For example, celery and carrot tops, lettuces, spinach, collards, mustard or turnip greens, pak choi, Chinese cabbage, chard, or a prepackaged Asian salad mix.) Alternative: select leaves from a garden including leaves from trees, grass, and weeds. (See page 9 for listing of brassicas.)

Keep leaf squares fresh on moist towel



The Poop on Frass --

"Frass Forensics"

What you can learn about plants and their predator from frass?

The size of the frass, larval excrement, is directly related to the size of the larva (e.g., size of the bite and size of the gut). The size of the bite each different instar takes is related to the size of the head (or really, the mandibles.)

Extension ideas:

1. Bite size Frass!
   Take a dried frass pellet and put it onto a glass slide in a drop of water, or of iodine solution, or of safranin stain solution. Let it sit for 5 minutes. Then poke and stir it with a toothpick. The frass pellet will disperse into many "x" sized pieces of tissue, their size being related to the size of the mandible of the larva from which it came.

2. What Color is your Poop?
   Feed one larvae red cabbage tissue and another white cauliflower tissue. Notice the plant tissue color as it is moving through the gut of the caterpillar.

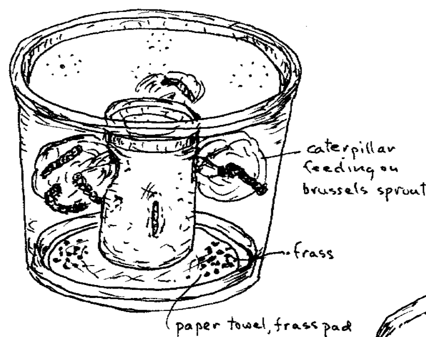
3. Botanical Anatomy via Frass!
   By suspending frass in water drops and viewing under the microscope different plant cell types are observable. Try adding a few drops of IKI to stain the starch granules. IKI is a combination of iodine and potassium iodide (nicknamed IKI). IKI is a reddish-yellow solution that turns dark blue/black when put in contact with starch.

4. Frass Fertilizer
   Consider saving the frass into a spare deli container for a Fast Plants fertilizer experiment. Dry the frass in air.

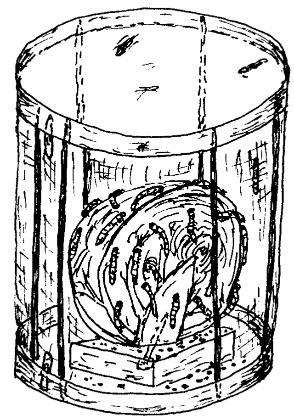
- Brussels sprouts are the suggested food source for the Brassica Barns. They will normally sustain the larvae (without replacing the sprouts) from the time of transferal to the barn until the larvae form a chrysalis.

- Another alternative larval food is broccoli stems, cabbage or cauliflower. Caution: Some of these may have residual insecticide on them and thus may be lethal to larvae. If using cabbage, be sure to remove outer leaves. If using Broccoli stems, remove outer layer.

Day 10-11 -Transfer larvae to the Brassica Barn or the Cabbage Cafe. (See page 32 and 33)



Brassica Barn



Cabbage Cafe

Day 10-18 - Tending larvae

L4 & L5 larvae may be handled with care for experiments. Pick up larvae gently with fingers or with forceps. Do not poke through their soft bodies or squeeze them too hard.

Cleaning Brassica Barn or the Cabbage Cafe

Check your larvae daily to be sure that the their current food source is clean and free of frass.

Day 18-20 - Pupa (Chrysalis) formation

Observe transition of L5 to pupae in Brassica Barn or the Cabbage Cafe. Larvae are usually on the lid or sides of the container at this stage and are easily observed.

- In preparation for pupal formation and metamorphosis, the fifth instar larva, L5, weaves a strong carpet, then a silk belt around its middle. The belt is attached at the ends to the carpet. (This makes wonderful viewing.)

- The pupa forms within the exoskeleton of the L5. The L5 skeleton beneath the head splits. The L5 exoskeleton contracts, slipping over the new pupal exoskeleton, like pulling off a stocking. The old tightly condensed L5 exoskeleton with its head capsule appears at the extreme posterior of the pupa and often falls away from it leaving the pupa belted to the carpet and attached at its posterior (rear) end by minute, Velcro-like hooks called a cremaster.

When all larvae have turned into pupae, clean out all remaining frass and food source from the feeders and allow the pupae to remain in the for metamorphosis to proceed.

Day 18-26 - Stages in Metamorphosis

Transition from larva to adult occurs during the pupal stage within the pupa, or chrysalis, and is known as metamorphosis. Although the pupa appears to be quiescent or sleeping, profound changes in the organism's form are taking place as the pupa remains attached to its silken carpet. See pages 36 and 37 for a detailed description of each pupa stage.

- The initial formation of the pupa is referred to as Pg for pupa green.

Pg1 stage - soft transparent exoskeleton and no external coloration. When the pupa, Pg1, is on a dark surface, usually more darkening (melanization) of the exoskeleton occurs.

Pg2 stage - hardening of the exoskeleton with prominent brown & yellow external ridges. Hardening of the exoskeleton (passage from Pg1 to Pg2 stages) takes several hours.

After the Pg2 stage: 1) pupae can either be left attached to the silk mat and observed there until emergence or, 2) they may be detached from their silk mat and secured with double-stick tape onto a piece of paper for viewing with a hand lens or dissecting microscope.

<u>Up Close and Personal</u>

- A portable TV camera with magnifier lens can provide excellent viewing of pupa formation, larval feeding and metamorphosis.)

- Determine how the larva creates and attaches the belt from silk produced from its silk gland.

- Recover the old L5 exoskeleton for observation under the microscope. Soak and stretch it. How did the exoskeleton slip out from under the silk belt to leave the pupa belted to the carpet? This is an excellent place where a hand or arm glove puppet with velcro and elastic would illustrate the phenomenon nicely.

<u>Pupal Coloration</u>

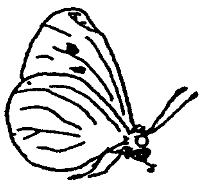
- Not all pupae are green. Pupal external coloration may depend on light, on coloration of surface on which pupation is occurring, or it may depend on the larval diet or on any combination thereof.

- Some pupae that form on a dark surface have a darker gray-brown mottled exterior.

Is this protective coloring? What experiments might be designed to investigate what influences pupal coloration?

Cocoon or Chrysalis?

During the pupal stage the transformation from larva to adult is completed. Many moth caterpillars (but not all) spin a silken cocoon to protect them as pupae. Butterflies do not do this, and their pupal stage is often called a chrysalis. While it's fine to refer to the previous stage as either larva or caterpillar, it is not correct to call a butterfly pupa a cocoon, since it does not have a silken covering.

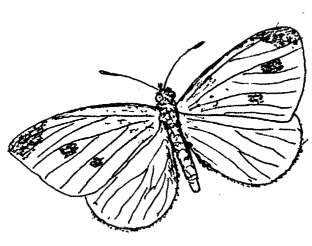


Viewing and sketching under a microscope

- Observe and sketch the external features of the pg2 pupa which is mounted on a Scale Strip. Draw to scale the outline of a side and top view.

- Note the external shape and color of ridges and any surface details such as segmentation lines. Can you identify and draw the location of the spiracles or air pores in the pupal exoskeleton?

- Which is the posterior end? Can you see and draw the tiny "velcro" like hooks of the cremaster that attach the posterior of the pupa to the silk carpet?



- Depending on the temperature, in about 2-3 days, the outlines of the wings will begin to appear as faint yellow-cream areas on each side of the anterior (front) half of the pupa. The cream yellow area intensifies over the next 2 days as the pupa enters the yellow pupal (Py) stage.

Because the exoskeleton of the pupa is relatively transparent, the development of various interesting external and internal features can be easily observed.

- The next distinctive features are the appearance of one (males) or two (females) dark wing spots appearing in the center of the wings followed by a dark wing tip spot. This is not because the pupa becomes transparent; it is because the scale pigmentation only develops at the very end of the pupa stage. Having reached this pupal wing spot (Ps) stage, the butterfly is within 24 hours of emerging.

Note: if pupae are reaching the wing spot stage toward the weekend, their development may be slowed by refrigerating them at 35-40^{1}F for a few days. Upon warming to room temperature, emergence will follow in minutes to a few hours depending on the stage of pupal development at the time they were cooled.

Day 26 - Emergence of adult butterflies

When the pupa reaches the pupal wing spot stage, the strip with pupa can be transferred to an empty barn for viewing of emergence. See page 37 for a detailed description of each larval stage.

- As emergence approaches, the pupa takes in air and accumulates fluids in the posterior region. The pupa appears somewhat swollen. Eyes become prominent and body hair becomes apparent under the anterior ridge between the two eyes.

- Adults emerge quite rapidly, sometimes in less than a minute. The exoskeleton splits above the head and the front legs emerge forward, pulling the antennae, crumpled wings, and body out and away from the pupal case. The newly emerged adult then walks and climbs up wall of the barn, discharges a drop of dark fluid (exuvia) hangs quietly and allows its wings to expand and harden. Wings usually take 15 minutes to expand, then another 1-2 hours to harden.

Day 26 - 40 - Observing Adult Butterfly Behavior

Following the release of adults into the Butterfly Box, many interesting aspects of adult butterfly behavior may be observed and investigated, including:

- feeding
- mating
- egg laying
- interaction
- daily activity relative to time, e.g., circadian rhythms, environmental cues such as light, etc.
- aging
- dying and death

Adult behaviors may be influenced by:

- the number of individuals in the space of the box
- the ages of the individuals
- the sexes of the individuals
- the environment (physical, chemical and biotic) of the box
- possibly the preceding environments of the box

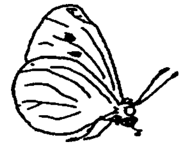
Keep these points in mind when observing butterfly behavior and designing experiments to test your ideas on aspects of butterfly behavior.

Day 26 - 40 - Transfer butterflies to Butterfly Box and observing adult butterfly feeding behavior

Many exciting questions and investigations can come from observing the feeding of butterflies and moths. The sugar, water, minerals and other nutrients found in floral nectar are the primary source of maintaining butterflies during their quest for mates and for suitable plants on which to lay their eggs. Without water and sugar butterflies will die in a few days.

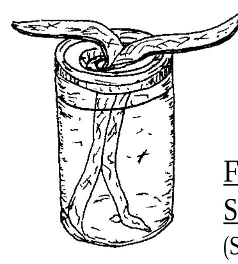
The mouth parts of adult butterflies and moths function solely to take in water and nutrients. The butterfly proboscis is an elongated feeding tube that can be rolled up or extended through the use of special muscles. The length of the proboscis varies greatly depending on the species and on the particular flowers that they have evolved with as their primary food source.

Butterflies can be released into the Butterfly Box after their wings have expanded and hardened (2-24 hours after emergence).



Observing Feeding Behavior

- Put different kinds of flowers in film can vases and compare P. rapae feeding behavior.
- Observe the proboscis probing for nectar.
- Can you quantify feeding behavior? Hint: Number and duration of feeding visitations.
- Does flower color play a role in feeding?
- Does flower age play a role in feeding?
- Are butterflies "attracted" to feeding on one type of flower over another? Can they "learn" to seek nectar from one species of plant in preference to another?



Film Can Sugar Feeder
(See page 34 for instructions).

Investigating with Sugar Feeders:

- Is flower color an important attractant for feeding? Try adding food various colors to sugar solution.
- Does the concentration of sugar in the solution influence feeding?
- Does the addition of honey to sugar solution influence feeding?
- Do other additives to the sugar solution influence feeding, e.g., minerals, alcohols, fruit juices, etc., etc.
- How long will butterflies survive with only water? or with no water?
- Is egg production affected by adult nutrition?

Poop Sheets -
"What Goes in Must Come Out!"

Adult Butterfly Feeding Experiment

Activity -
Observe whether adult butterflies prefer different colored nectar

Materials:
- Population of adult butterflies
- minimum of two Film Can Sugar Feeders
- minimum of two different colors of food coloring

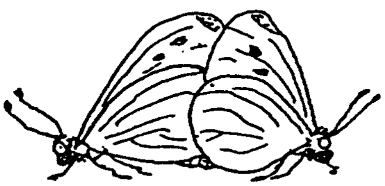
Make the sugar solution for the Film Can Feeder as directed on page 34, except add a different color of the food coloring to each, e.g. yellow, blue, green, or red. Place in the butterfly box and observe .

Questions:
- Do the butterflies prefer one color over the other
- How could you quantify their preference? (Hint: the title of this activity)

Try feeding the butterflies commercial beverages such as red and / or orange Gatorade.

Observe mating behavior

Continue to add newly hatching butterflies into the Butterfly Box whenever they are ready to be released. Try not to leave newly hatched butterflies in a barn more than 24 hours.

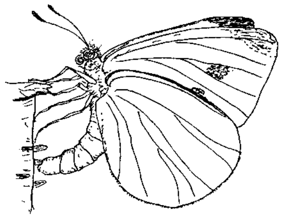


Mating will occur within a day or two if males and females are in the box together.
How likely is it that there wouldn't be any females or any males?

Place a Film Can Sugar Feeder inside the Butterfly Box to provide butterflies with a source of liquid and nutrients. (See Appendix 2 for sugar feeder instructions.) Make two sugar feeders for every Butterfly Box. Keep a sugar feeder in the box and replace it with a fresh one every day or two to keep the solution fresh.

Put a small dish of wet soil in the box for mineral feeding.

Day 28-40- Mating and Egg Laying



Observing adult butterfly mating behavior

As soon as mating behavior has been observed, or after several butterflies have been in the Butterfly Box for a couple of days, an ovipositron can be paced in the box to stimulate egg laying behavior. Female butterflies will continue to lay eggs for up to two weeks. If you want to continue to collect as many eggs as possible, you will want to keep a fresh Film Can Sugar Feeder in the box and introduce the Film Can Ovipositron. (Page 34 for instructions.)

- When you are ready to use the Ovipositron, glue a brassica leaf, e.g., cabbage, Fast Plant, pak choi, turnip, on the bottom of the film can and trim around the rim.
- Place the open end of the film can down, so that the waxed paper strip is at the top, and, using a glue stick, glue the leaf circle to the end of the film can as shown.
- Introduce the ovipositron into the Butterfly Box.
- As the butterflies land on the rim of the Ovipositron, they deposit their eggs on the ring of waxed paper placed around the top of film can.
- After the waxed paper strip is covered with eggs, it can be removed and transferred to a Nursery, or, if you wish to keep the eggs for awhile in the refrigerator before beginning the next life cycle, the waxed paper strips can be stored in a sealed container for two days.

How Does a Female Butterfly Know that she has landed on a Brassica?

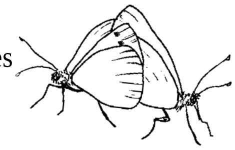
Butterflies get much of their information about the world through chemoreceptors scattered across their bodies. In butterflies, chemoreceptors are nerve cells that open onto the surface of the exoskeleton and react to the presence of different chemicals in the environment. They operate on a system similar to a lock and key. When a particular chemical runs into a chemoreceptor, it fits into a "lock" on the nerve. This fit sends a message to the nerve cell telling the butterfly that it has encountered the chemical. For example, organs on the back of butterfly tarsi sense dissolved sugar; when the dissolved sugar touches these chemoreceptors, the butterfly extends its proboscis to eat the nectar its tarsi have sensed. Humans also have chemoreceptors, which are concentrated on the tongue (tastebuds) and in the nose.

Female butterflies often have important chemoreceptors on their front legs to help them find appropriate host plants for their eggs. These chemoreceptors are at the base of spines on the back of the legs, and they run up along the spine to its tip. Females drum their legs against the plant, which releases plant juices. The chemoreceptors along the spines tell the butterfly whether they are standing on the correct plant.

Termination of the Life Cycle in the Classroom

The Cabbage White Butterflies tend to live in captivity from 1 to 3 weeks. In many agricultural areas, *Pieris rapae* is considered to be a "pest" species. If you have finished investigating with the butterflies prior to their natural death the butterflies can be easily disposed of by placing them into a freezer overnight. The can then be stored for future projects or discarded.

Too Much Lovin'!



In a Butterfly Box, mated females exhibit a very distinctive male avoidance posture which could fool you when you first see it! Can you observe and describe it? (Hint: the positioning of the female abdomen.)

Outdoors in a field or garden you can often see male Pieris rapae chasing after a female. The female is able to avoid the males by their classic "ascending spiral flight". The male follows the female as she spirals up into the air. After a while the male tires and floats back to the ground while the female soars away seeking nectar and plants for oviposition.

"Mother Knows Best" - Egg Laying Choices and Preferences

Activity

Do female adult butterflies prefer to lay their eggs on specific plant types?

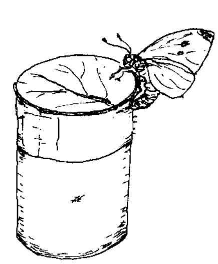
Materials

- minimum two ovipositrons (see page 34 for construction)
- minimum of two different leafy greens. Recommend one Brassica (Fast plant, cabbage, collard) and one other leafy greens (lettuce, spinach, endive).

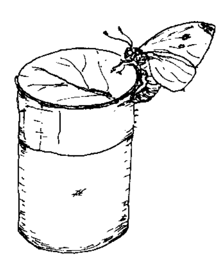
Construct ovipositrons as described, except place different types of leafy greens on each one and place into a Butterfly Box with females that have mated.

Questions:

Which kind or kinds of greens are preferred by female butterflies for egg laying?



Lettuce leaf



Cabbage leaf

The Butterfly Box (a converted Plant Light House)

Materials

- one empty "copy paper" box, e.g., Xerox
- electrical cord and a plug socket
- plastic plate or lid
- glue stick
- clear tape (3/4")
- scissors, cutting blade for card board
- 30 watt fluorescent circle light (Lights of America) or a 39 watt GE circle light
- nylon mesh
- electric 24-hour timer clock switch

Construction

1. Cut a 1 inch hole in the center of a plastic plate or lid and trim off edges to make approximately a 4-5 inch disk with a center hole.

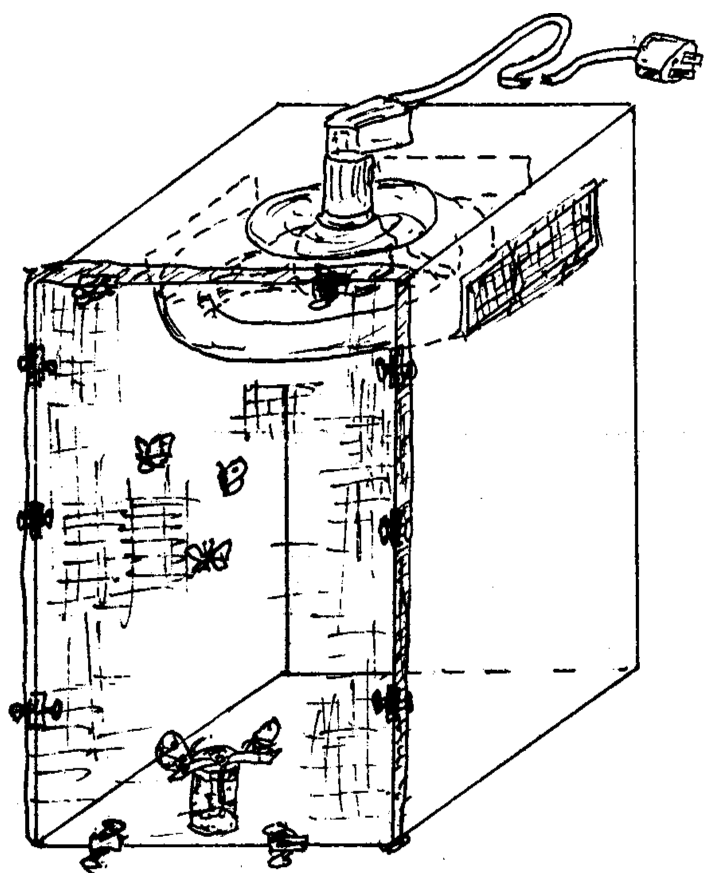
2. Cut several 1 X 4 inch ventilation slots in the upper sides and back of box as shown and cover with nylon mesh.

3. Cut a 1 inch diameter hole in the center of the top of the box (The light socket will be inserted through this hole.).

4. Apply glue stick to the inner surfaces and paste in aluminium foil to cover entire surface. Use clear tape to reinforce corners and edges.

5. Insert light fixture base through hole in top and through plastic plate. Secure fixture by attaching socket. When growing the Brassica Mix leave the light on 24 hours a day. For the Butterflies plug into the 24-hour timer clock switch and set it for 16 hour days.

6. Using small binder clips attach a piece of nylon mesh (large enough to cover the opening on the box with an additional 2 inches on each side) to the front of the box.



Bottle Growing Systems for the Brassica Nurseries

Materials

- 16-, 20-, or 24-oz. soda bottles
- unpolished cotton string (20-cm lengths) or Watermat® for capillary wicks
- planting medium (a soilless mixture of approximately 1/2 peat moss and 1/2 vermiculite, e.g. peatlite, Scotts Redi Earth®)
- Peters 20-20-20 Professional® fertilizer with minor elements or Miracle Grow®

Construction

1. Cut each soda bottle 0.5 cm below the rim of the shoulder to create the growing funnel, which will hold the vermiculite and planting medium. To make the bottom more stable, make a second cut in the bottom portion of the bottle to create a reservoir, 8 to 12 cm tall, for the hydroponic nutrient solution. (A single Plant Light House can accommodate eight soda bottles.)

2. Drill or melt a 5-mm hole in each bottle cap. Screw bottle cap onto bottle tops.

3. Insert string wicks or other capillary wicking, approximately. 0.5 cm X 10 cm, through the holes in the bottle caps. Check your wick before planting to be sure that it draws water well.

4. Invert the growing funnels (bottle tops) and place in the reservoirs (bottle bases). The wick should extend from the funnel to the floor of the reservoir.

Planting

1. Fill the growing funnels loosely with potting mix.

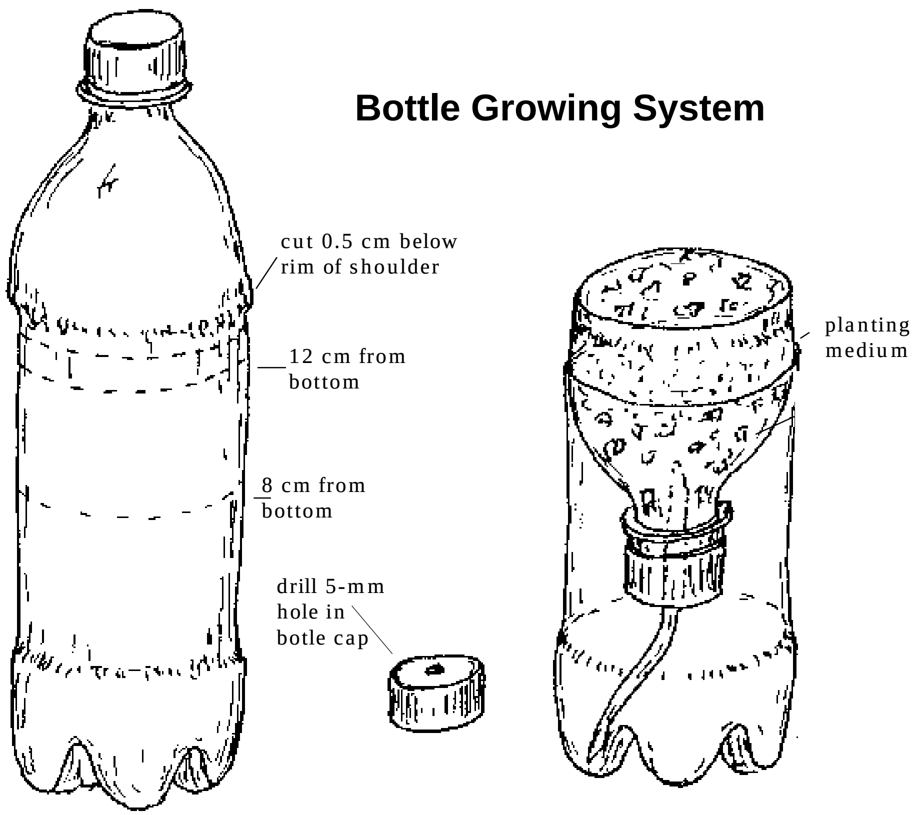
2. Water from the top until water is dripping from the wick below.

3. Distribute 20-30 seeds of the Brassica Seed Mix onto the potting mix.

4. Cover the seeds with a thin layer of potting mix and place in a Plant Light House.

5. Fill the bottle bases (reservoir) with water.

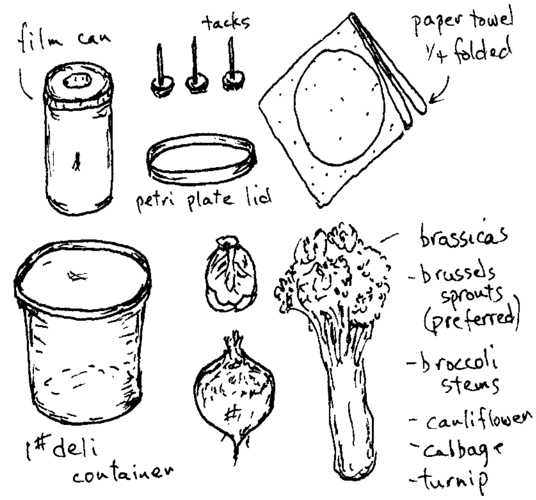
6. Place inside a Plant Light House / Butterfly box with the lights on 245 hours a day.



Brassica Barn

Materials

- clear plastic 1 lb. "deli" food container with lid
- kitchen paper toweling
- one film can
- three upholstery tacks
- flat toothpicks for handling larvae
- sand (enough to fill the film can)
- Brussels sprouts or broccoli stems
- tacks



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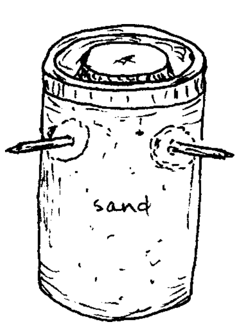
Construction

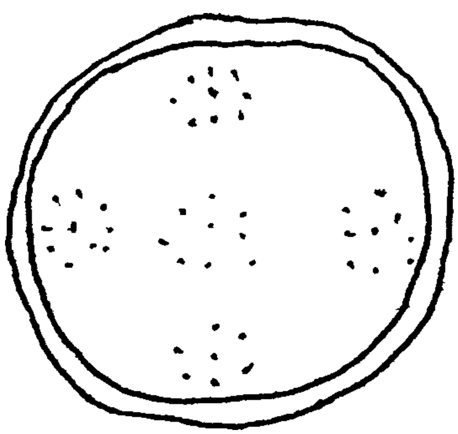


1. Insert 3 upholstery tacks from inside of film can to protrude at approximately 120 degrees apart, and about 1.5 cm from top.

2. Fill film can with sand. Press on lid. This is now the Film Can Feeder.

3. Cut a kitchen paper towel "pad" to fit into the bottom of the deli container.

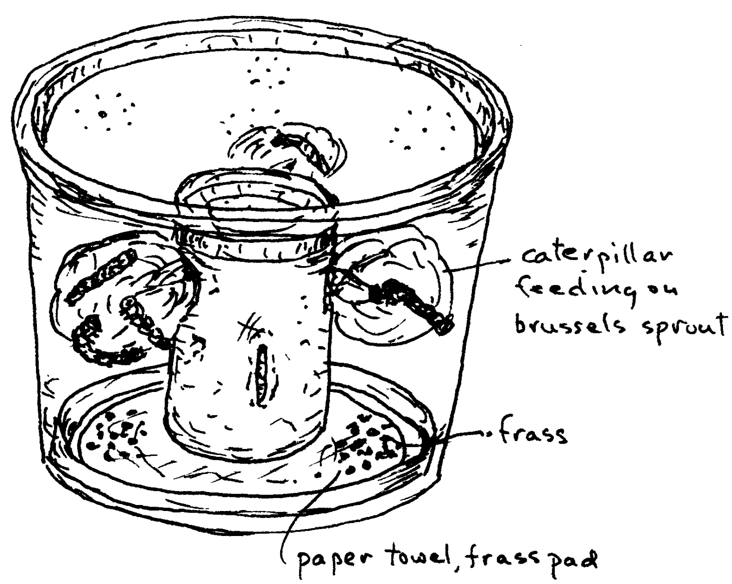
4. With a push tack or pin, make air holes in deli container lid.



5. Before transferring the larvae, you need to fit the Film Can Feeder with food for the larvae.
• Cut 2 small Brussels sprouts in half lengthwise. Remove 3-4 outer leaves.

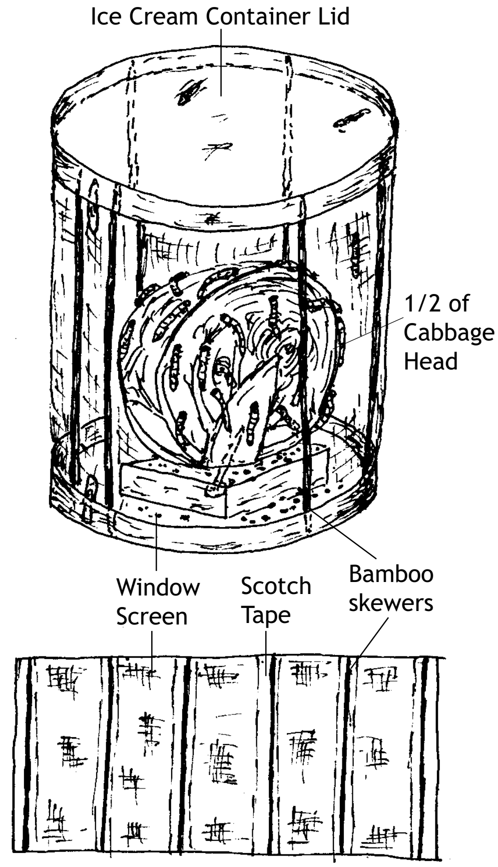
• Press 1/2 of a sprout, stem end, on each tack. Rotate sprouts so flat surface is sloped at 45 degrees from horizontal to allow frass to fall off. Put the feeder with sprouts in center of paper towel pad, in Brassica Barn.

Carefully transfer 3 or 4 *Pieris rapae* larvae, at L4 - L5 stage, onto each 1/2 sprout (9-12 larvae per barn). Secure the pierced deli container lid on top. Keep the barn out of direct sun. Classroom lighting, or a window not in direct sun, will be fine. Check your larvae daily to be sure that their current food source is clean and free of frass and fresh.



Cabbage Cafe

An advantage of the Cabbage Cafe over the Brassicsa Barn is that the frass will dry down and not support mold growth. A Cabbage Cafe works well for 40 - 60 larvae.



Materials

- 2 large ice cream containers lids or the lid and base of a large petri dishes.
- 50 cm x 20 cm piece of window screen
- 6 bamboo skewers, cut to be 20 cm long
- paper kitchen toweling
- 1/2 of one small head of green cabbage, (cut cabbage lengthwise, as shown)
- One roofing nail
- One square of styrofaom approximately 9 cm x 9 cm.

Construction

1. Make several paper towel pads to fit the bottom lid. This will catch the frass and can be changed periodically.

2. The window screen is supported by 6 bamboo skewers attached with scotch tape running down the length of the skewer. The window screen is then rolled to make the side of the cage. Attach two paper clips, one on each end, to secure the rolled window screen together.

3. Insert the roofing nail through the center of the styrofoam square and then place the core of the cabbage onto the sharp end of the nail and press downward to secure the 1/2 head of cabbage in a slightly forward of vertical position. This will allow the frass to fall off of the cabbage. The heavier core base of the 1/2 cabbage head should hold it in the upright position.

4. Place the styrofaom with the cut cabbage attached onto the base "lid".

5. Carefully transfer up to 60 *Pieris rapae* larvae, at L3 - L5 stage, onto the cabbage.

6. Wrap the nylon mesh into a circle and fit into the bottom lid. Secure with paper clips.

7. Place the top lid over all, and the Cabbage Cafe is operational. Keep the barn out of direct sun. Class-room lighting, or a window not in direct sun, will be fine.

8. The larvae will complete their growth to chrysalis inside the Cabbage Cafe and will generally pupate on the sides or top of the Cafe.

Cleaning the Cafe

Most of the frass will fall onto the paper towel pad which needs to be changed regularly (every day or two depending on size of larvae.

- To change the pad, carefully remove the cabbage from the Cafe. If any larvae are on the paper towel pad, carefully remove them with tweezers or fingers and put them back on the cabbage.

- Put in a clean paper towel, replace the cabbage and cover.

Film Can Sugar Feeder

Materials (for one Film Can Sugar Feeder)

- film canister
- 2, 10 cm x 0.7 cm capillary wicks, made from felt or unwaxed cotton string
- small nail, pliers & source of flame
- sugar, honey, and yellow food coloring

Construction

1. Hold nail with the pliers and heat the sharp end in candle (or other) flame and then melt a 1 cm x 0.3 cm slot in lid of film can.

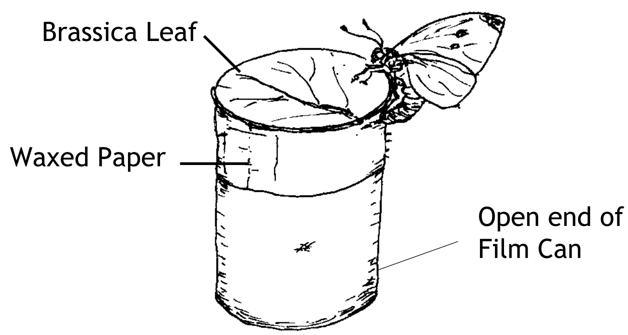
2. Fill film can with warm tap water, almost to the top. Then add 1/4 teaspoon of sugar. Add 3-5 drops of honey and one drop of yellow food coloring. Shake can to dissolve sugar. You can mix in bulk and store in the refrigerator for up to 3 weeks.

3. Soak the two capillary wicks in the yellow sugar-honey solution. Insert them through the slot in the film can lid to protrude about 3 cm. Place lid on can.

4. Place Sugar Feeder in Butterfly Box.

5. Every 2-3 days, discard unused solution in old Sugar Feeder, wash all parts in warm water and soak them for 20 minutes in diluted bleach (mixed 1 cup in 2 gallons of water.) Rinse thoroughly and let dry overnight. (You will need a set of two Sugar Feeders for each Butterfly Box in order to cycle them.)

Ovipositron



Materials

- Fresh Brassica leaf
- glue stick
- film can
- waxed paper strip, cut 1 X 4 inches

Construction

1. Place the open end of the film can down, apply a spot of glue with the glue stick on the side near the top (bottom) of the film can. Attach one end of the waxed paper strip in the spot of glue and wrap the rest of the piece around the film can and affix to the glue.

2. Glue a brassica leaf, e.g., cabbage, Fast Plant, pak choi, turnip, on the bottom of the film can and trim around the rim. As the butterflies land on the rim of the Ovipositron, they deposit their eggs on the ring of waxed paper.

3. After the waxed paper strip is covered with eggs, it can be removed and transferred to a Brassica Nursery, or, you may keep the eggs for 3-6 days in the refrigerator in a sealed container with a moist paper towel.

Tips and Suggestions from Classroom Experience

In the Cold -

Tips for Classroom Management of the Life Cycle

As with all cold-blooded insects and animals, the life cycle rate of *Pieris rapae* can be slowed down by reducing the temperature or speeded up at warmer temperatures. All stages of the life cycle can be manipulated by temperature. For example, if, on Friday, you find that all of the pupae are at the later stages of development and are going to emerge over a weekend, place them in a refrigerator (~39 degrees Fahrenheit) and their developmental rate will slow enough so that they will not have emerged prior to Monday. Monday, pull them out, and they should resume a normal developmental rate.

If you do not have a Nursery ready when the eggs arrive -

For best results, the tiny larvae emerging from eggs require tender, succulent brassica or radish leaf tissue. If you do not have a Nursery ready, Lori Gillam, a teacher in Anchorage Alaska, recommends purchasing radish sprouts form the grocery tore and placing the egg strips on them. (Be sure to keep the sprouts moist!)

Detailed Life Cycle Stages and Timeline

Stages and Approximate Timeline (Temperature ~23^0 C)

Egg - E

		Day
E1	creamy white, within 24 hrs.	<1
E2	white, sometimes with orange tint	>1
E3	pink-purple, mandibles and eye spots visible	3
E4	larval movement within egg	3
E5	larva chewing shell and emergence	3

Larva - L

L1-1	emergent larva, beginning to feed and ambulate, head diameter 0.32 mm, greater than body diameter	4
L1-2	feeding larva, body elongates and swells	5
L1-3	larva quiescent, preparing to molt	5
L1-4	larva molting	6

L2-1	larva ingests cuticle, not L1 head capsule, head diameter 0.58 +/-mm, large compared to body	6
L2-2	feeding larva, body elongates and swells	7
L2-3	larva quiescent on silk rug, preparing to molt	7
L2-4	larva molting	8

L3-1	larva ingests cuticle, not L2 head capsule, head diameter 0.88+/-mm, large compared to body diameter	8
L3-2	larva feeds, body elongates and swells, testes visible on males	9
L3-3	larva quiescent on silk mat, preparing to molt	9
L3-4	larva molting	10

L4-1	larva ingests cuticle, not L3 head capsule, head capsule diameter 1.36+/-mm, head, large compared to body diameter	10
L4-2	larva feeds, body elongates and swells, larva move actively to fresh food sources, testes visible on male	11-12
L4-3	larva quiescent on silk rug, preparing to molt, body diameter large compared to head diameter	13
L4-4	larva molting	13

L5-1	larva ingests cuticle, not L4 head capsule, head capsule diameter 1.98+/-mm, head, large compared to body diameter	13
L5-2	larva feeds voraciously, body swells and elongates, larva move actively to fresh food sources, copious frass is produced, testes visible on males	14-16
L5-3	ceases feeding, actively seeks a suitable site for final molt and pupation, final frass pellet in hind gut is visible and salmon pink	17
L5-4	at pupation site larva lays down a substantial silk mat to surface, attaches proleg crochettes and forelegs to the mat and releases final pink frass pellet, and weaves a multistranded silk belt over the midbody attached to the silk mat on either side of the body; larva appears quiescent prior to molting	18

Larvae - L *(continued)*

		Day
L5-5	molting, the L_5 cuticle splits from the head first. With active undulation and extreme contraction of the cuticle, the head capsule and cuticle retract posteriorly under the silk belt drawing trachea from the spiracles (air pores) in the sides, which appear as white bands. With active undulations the L_5 cuticle with head capsule slips from the new pupal cuticle beneath the silk belt and contracts to approximately 2x2x3mm and is cast off. A relatively active series of twisting undulations of the flexible pupa results in the attachment of a new set of posterior crochettes (the cremaster) to a tuft of silk loops in the posterior portion of the silk mat. The pupa is firmly attached to the silk mat by the cremaster and the silk band.	18

Pupa - P

Pg-1	pupal cuticle is soft and pale green to dark with characteristic ridges on dorsal side; cuticle begins to harden	18
Pg-2	pupa cuticle becomes hardened and assumes characteristic shape and reveals on clear cuticle the outline of where wings, legs, antennae and eyes will develop; color may be clear pale green to darkly speckled with melanized pigment; dorsal ridges are light tan to dark.	19-23

Py-1	first appearances of faint cream to light pigmentation of wing outline	24
Py-2	wing coloring more distinctly cream to light yellow	25
Py-3	wings cream to yellow with distinct darkened veins	26

Ps-1	appearance of one or two faint dark spots in center of wing outline	27
Ps-2	intensification of darkening of wing spots, dark wing tip appears	28
Ps-3	wing spots and tip black, darkening of head and thorax visible, emergence in <1 day	29
Ps-4	wing spots dark, hairy scales on head thorax and abdomen visible, emergence imminent. (< 6 hours)	19-30
Ps-5	emergence, cuticle splits, forelegs emerge and rapidly pull adult out of pupal case. Adult crawls upward, wings expand in a few minutes, brown exuvial fluid is released, and wings harden in an hour or two	29-30

Adult - A

A1	adult remains relatively quiet for a day, neither feeding nor flying	31
A2	adult begins to fly, forage for nectar and seeks mate	32
A3	mating; females 2 forewing spots, males 1 forewing spot, copulation is tail to tail and may persist for several hours	32-33
A4	egg laying, females lay up to 300 eggs over a 7-14 day period, peak activity appears to be around midday.	33-->
A5	adults die between 1-20 days after emergence	30-50

Science and Technology in the Classroom and beyond

Science is All About Questions

Science begins when a person of any age is curious about something and begins to question and explore the relationships of a phenomenon in relation to his or her understanding of the world. The scientific process begins with an observation and questions and proceeds through a process of inquiry involving exploration, investigation, experimentation and analysis, and communication and persuasion. That process engages the creative energy of the individual and leads to deeper understanding, a sense of pleasure and increased self-worth. Even young children quite naturally say: "Look what I found!"

As you and your students proceed with the brassica butterfly and Fast Plants activities, you will be progressing through the stages illustrated in the Exploration Flowchart. The following questions are designed to assist you. Remember the power of writing as an assistance to learning. Have your students pose questions and answers, document ideas and diagram relationships.

1. **What do you observe?**

2. **What is your question about your observations? What is the question you are exploring?**

3. **How would you convert the question into an assertion, which is the idea you are experimentally testing (your hypothesis)?**

   - Can you also write this as a *null hypothesis* in which you may state the hypothesis having the opposite, or null, outcome?

4. **What variable will you change in your tests? What is your treatment? What potential variables will remain constant?**

5. **What are your control treatments? How will each serve as a control?**

6. **How many observations for each result are enough? Is n = 1 enough to be representative? If not, what is enough? Why?**
7. **Is there any special experimental design of the treatments and/or replicates needed in the experiment?**

8. **What equipment, tools, etc., will you need for your experiment?**

   - Draw your experimental setup.

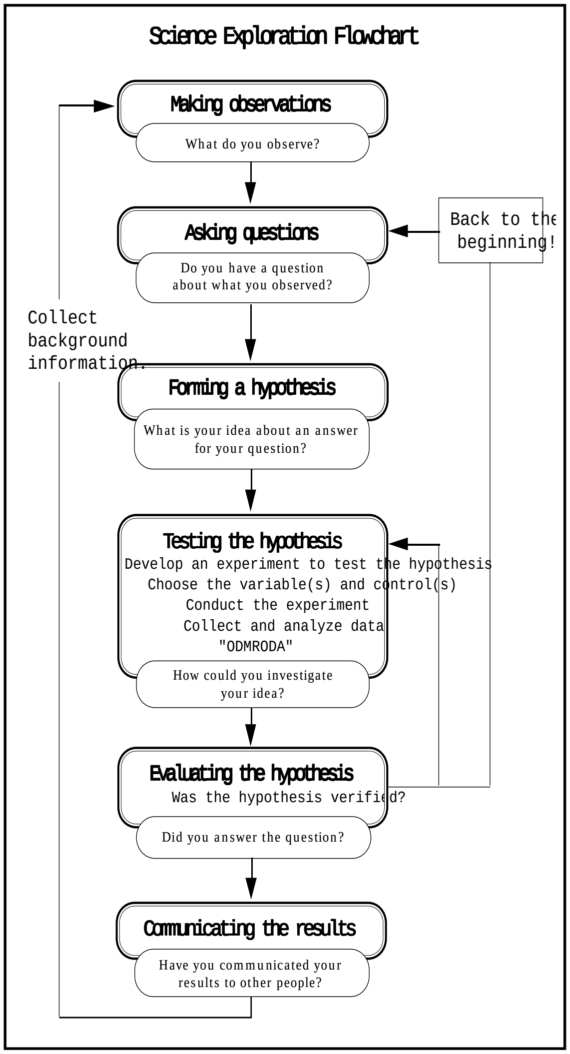
9. **What form will your observations take? How will you describe or measure your observations?**

   - Use descriptors, comparators, scales and quantitative estimates.

10. **How will you record or tabulate your data?**

11. **How will you organize your data? How will you display your data?**

    - Use statistical summarization.



12. What is your conclusion relative to your hypothesis? What further conclusions can you draw from your analysis of your experiment?

13. What other questions come to your mind as the result of this experiment?

14. What is the next experiment that you plan to run? Why?

The Science-Technology Partnership

As students design and execute experiments the need for technological assistance from tools and equipment is ever-present, from the moment of the first observation to the time when new insight is shared with someone across the ocean or across the classroom. Technological innovation, like science, follows a logical progression, resulting in a successful invention and its application to a need or problem.

Design of the Experiment – Testing the Hypothesis

The heart of science activities lies in the design and execution of the experiment developed to test a hypothesis. It is in this phase of the process of science that technology plays an essential role. To conduct any experiments, technological requirements will arise and will need to be addressed. If the question and hypothesis have been carefully thought out and refined to be experimentally testable, then the design and execution of the experimental phases should yield satisfactory results. As you plan your experimental design, consider the following:

¥Keep focused on the question and hypothesis.

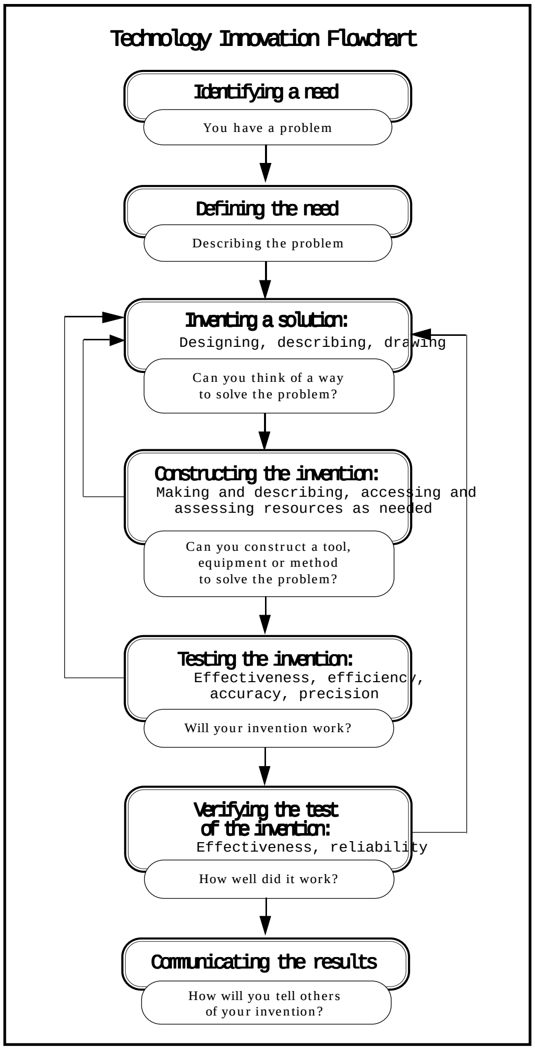
¥Think of the simplest way, both in the design and in the equipment needed, to run the experiment.

¥Alter one variable (treatment) with each experiment and analyze the results.

¥Always run control treatments for each experimental treatment such that for each variable in the experimental treatment there is an adequate basis for interpreting the information from the treatment.

¥The careful choice and execution of the control treatments is as important in the experiment as that of the experimental treatments.

¥Information from the control treatments serves as the basis for determining whether information from the experimental variables is valid and, thus, guides the researcher in conclusions as to the validity of the hypothesis.



Who is "OD MR ODA"?

Progressing through the experimental investigation of an hypothesis.

Observe and Describe:
Using your eyes and other tools to assist in observation (lenses, microscopes, etc.) together with insight from your brain, observe various phenomena or characteristics associated with the experiment and determine the way that you will describe them.

Measure and Record :
Using tools and devices (eyes, brain, rulers, scales, comparators and experience), measure (quantify) and record numeric and descriptive characteristics as *data*. Estimate, count or compare what you observe while adhering to an understanding of the precepts of *accuracy* and *precision*.

Organize and Display:
Organize and display recorded data in various ways (tables, charts, graphs, diagrams, drawing, photographs, videos, audios, multimedia, etc.) that will provide insight into phenomena associated with the experiment.

Analyze:
Observe the data displays (tables, graphs, etc.) for comparisons among treatments, including controls. Apply statistical analysis to the data that provides information from which to derive and develop inferential insight that will be useful in the evaluation of the hypothesis.

Brassica and Butterfly Life Cycles align with the Science Standards

Science as Inquiry

K-12

Life Science Content

K-4
- Characteristics of organisms
- Life cycles of organisms
- Organisms and environments

5-8
- Structure and function in living systems
- Reproduction and heredity
- Populations and ecosystems

9-12
- Biological evolution
- Interdependence of organisms
- Data organization and analysis

Addressing the Standards

(From Stephen Nakano)

Several Science Standards/Benchmarks were met:

1. Analyze, evaluate and propose possible solutions in sustaining life on Earth, considering the limited resources and fragile environmental conditions.
2. Analyze the factors that affect the carrying capacity of an ecosystem.
3. Analyze the interdependence within and between terrestrial, aquatic, and atmospheric systems.
4. Develop and clarify questions and hypothesis that guide scientific investigations.
5. Formulate scientific explanations and conclusions using logic and evidence.
6. Communicate and defend scientific explanations and conclusions
7. Revise scientific explanations and conclusions based on additional information / data gathered.