APPENDIX – RESOURCES

Who's Who in the Study of the Monarch Butterfly

Professor Dr. Lincoln Brower is an entomologist and research professor at Sweetbriar College in Virginia. He has been "a student and admirer of the monarch butterfly for over 50 years." His current research is on the overwintering, migration and conservation biology of the monarch butterfly. http://texasbutterflyranch.com/2015/02/16/q-a-dr-lincoln-brower-talks-ethics-endangered- species-milkweed-and-monarchs

Chip Taylor, an insect ecologist, is Professor of Ecology and Evolutionary Biology at the University of Kansas and the Founder and Director of Monarch Watch, an outreach program focused on education, research and conservation relative to monarch butterflies. Watch this short documentary, **Saving the Migration** https://www.youtube.com/watch?v=maM2gl30cJc to learn more about his work and the plight of the Monarch.

Dr. Karen Oberhauser is Professor, Department of Fisheries, Wildlife and Conservation Biology at the University of Minnesota. Karen has been studying monarch butterflies since 1984. She works with teachers and pre-college students in Minnesota and throughout the United States using monarchs to teach about biology, conservation and the process of science. http://monarchlab.org/about/staff-and-students/karen-oberhauser

Catalina Aguado Trail was a citizen scientist from the state of Michoacán in México, and part of the original team who discovered the monarch's over-wintering grounds. Under the guidance of Dr. Urquhart, Catalina and her husband Ken Brugger spent two years searching the mountains in Central México for the monarch's winter destination. Their discovery graced the cover of the National Geographic magazine in August 1976. http://texasbutterflyranch.com/2012/07/10/founder-of-the-monarch-butterfly-roosting-sites-in-mexico-lives-a-quiet-life-in-austin-texas

Xerces Society. The Xerces Society is a nonprofit organization that protects wildlife through the conservation of invertebrates and their habitat. For over 50 years, the Society has been at the forefront of invertebrate protection worldwide, harnessing the knowledge of scientists and the enthusiasm of citizens to implement conservation programs. View or download their comprehensive report on the Conservation and Ecology of the Monarch Butterfly in the United States. http://www.xerces.org

U.S. Fish and Wildlife Service works to conserve, protect and enhance fish, wildlife, and plants and their habitats for the continuing benefit of the American people. **USFWS** has

committed to work with its partners, including National Wildlife Federation to restore and enhance more than 200,000 acres of habit for monarch whole supporting over 750 schoolyard habitats and pollinator gardens. https://www.fws.gov/savethemonarch

National Wildlife Federation's Butterfly Heroes campaign is part of NWF's Garden for Wildlife program. Butterfly Heroes seeks to bring awareness to the declining population and connect gardeners, kids and families alike to help the monarch and other pollinators. To take the pledge and create new habitat for monarch butterflies submit your photo pledge and become a butterfly hero. www.nwf.org/Butterfly-Heroes.aspx

Million Pollinators Garden Challenge: A campaign to register a million public and private gardens and landscapes to support pollinators. www.millionpollinatorgardens.org

Monarch Joint Venture. The **Monarch Joint Venture** (MJV) is a partnership of federal and state agencies, non-governmental organizations, and academic programs that are working together to support and coordinate efforts to protect the monarch migration across the lower 48 United States. The MJV is committed to a science-based approach to monarch conservation work, guided by the North American Monarch Conservation Plan (2008). http:/monarchjointventure.org/about-us

Resources for Developing your Monarch Garden

National Wildlife Federation How-To Guide for Schoolyard Habitats

Site selection is key to any successful gardening project. For a great class activity to help you choose the right site while engaging your students in meaningful science and math applications refer to the Site Inventory Activity in Part IV of the National Wildlife Federation How-To Guide for Schoolyard Habitats. http://www.nwf.org/Garden-For-Wildlife/Create/Schoolyards.aspx

Monarch Joint Venture Schoolyard Butterfly Gardens

For tips on designing and installing your garden refer to the Monarch Joint Venture Schoolyard Butterfly Gardens Fact sheet. You can download this fact sheet and many more wonderful resources from Monarch Joint Venture. http://monarchjointventure.org

Monarch Lab at the University of Minnesota

For information on planting a monarch garden, creating and using a schoolyard garden, and garden grants visit the Gardening for Monarch page.
http://monarchlab.org/education-and-gardening/gardening-for-monarchs

Curriculum Resources

Monarchs and More (Grades K-2, 3-6 and Middle School)
Free lessons from the **Monarch Lab** at the University of Minnesota. The comprehensive curriculum guide includes lessons on monarch behavior, life cycle, a focus on features, adaptations, and migration. Full curriculum is also available for purchase at their Monarch Store. http://monarchlab.org/education-and-gardening/curricula

U.S. Fish and Wildlife Service
The U.S. Fish and Wildlife Service and Protección de la Fauna Mexicana A.C. (Profauna A.C.), a Mexican non-governmental organization, developed ***The Monarch Butterfly Manual, Royal Mail: A Manual for the Environmental Educator*.** This manual was developed for grades Pre-K through 12 and offers activities that promote conservation of the Monarch Butterfly. http://www.fs.fed.us/wildflowers/pollinators/Monarch_Butterfly/documents/royal_mail/monarch_pu b.pdf

Journey North
Categorized by season, and then by topics such as Citizen Science, Background, Conservation, migration, life cycle, video clips and food, **Journey North's** educational resources allows your study of the Monarch Butterfly to be relevant to the season you are in. (http://www.learner.org/jnorth/tm/monarch/indexSpring.html)

- Migration Rate Activities: http://www.learner.org/jnorth/tm/monarch/MigrationRateMathIntro.html

Journey North's Symbolic Migration
A terrific way to engage your students and help create cross-cultural connections is through Journey **North's Symbolic Migration**. Each year over 60,000 students in the United States and Canada create symbolic paper butterflies and send to them to Mexico for the winter. The children who live in Mexico beside the monarch's winter sanctuaries protect the butterflies and send them north in the spring. http://www.learner.org/jnorth/sm/index.html

Rearing Monarchs in the Classroom
Rearing monarchs in the classroom can be a captivating and fun educational experience that can encourage conservation actions, but in order to avoid the spread of disease and harming the monarchs, it needs to be done responsibly. **Monarch Joint Venture** has a great fact sheet on rearing Monarchs responsibly.
http://monarchjointventure.org/images/uploads/documents/Monarch_Rearing_Instructions.pdf

Monarch Watch
Practical tips for rearing Monarchs in the classroom. http://monarchwatch.org/rear

Citizen Science Programs

Citizen science involves everyday people - just like you and your students - who volunteer to help scientists with their research. Using Citizen Science in your study of the monarch butterfly is a meaningful way to involve students in data collection. Students realize that their observations are contributing to real research that is being used to help scientists better understand the behavior, biology and migration of the monarch butterfly.

Journey North: Help scientists learn more about monarchs. Track the monarch migration each fall and spring. Record your observations on real time migration maps.

- Download the Journey North app for Android or iOS from Annenberg Learner.
- Live monarch updates throughout migration: http://www.learner.org/jnorth/monarch/News.html

Monarch Larva Monitoring Project: The Monarch Larva Monitoring Project, MLMP was developed by researchers at the University of Minnesota to collect long-term data on larval monarch populations and milkweed habitat. This citizen science program is run by the monarch Lab at the University of Minnesota.

- Online Training: http://monarchlab.org/mlmp/training/online-training
- Activities: http://monarchlab.org/mlmp/monitoring/activities

Monarch Watch – Migration and Tagging: Monarch Watch engages in research on monarch migration biology and monarch population dynamics to better understand how to conserve the monarch migration.

Funding your Garden

Funding your monarch garden can be a great way to connect to your larger school community and spread the word about the work you are doing on your campus to help the monarch butterfly while providing innovative learning opportunities for your students. Below are just a few organizations that provide funding for school gardens.

Monarch Watch – grants for milkweed plugs.
https://docs.google.com/forms/d/1I9lFB__ZQYS9OCgjUkc8PtuncEUPWsXaHpNuVmeCtVI/closedform

Native Plant Societies - http://www.ahs.org/gardening-resources/societies-clubs-organizations/native-plant-societies

Kids Gardening.org - Youth Gardening Grants http://grants.kidsgardening.org

Keep America Beautiful - Check with your local Keep America Beautiful to see what grants they have to offer. https://www.kab.org/resources/beautify-communities

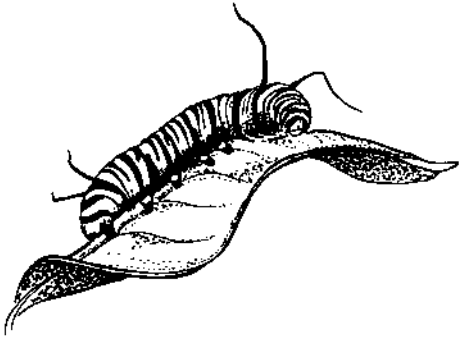
Lady Bird Johnson Wildflower Center - LBJWC has partnered with Native American Seed to provide **seed grants to schools in Texas.** http://www.wildflower.org/wildflowers

Real School Gardens - Tips for proposal writing and comprehensive list of school funders. http://www.realschoolgardens.org

Whole Kids Foundation - School Garden Program - https://www.wholekidsfoundation.org/index.php/schools/school-garden-grant-program)

Annies - Grants for Gardens – http://www.annies.com/giving-back/school-gardens/grants-for-gardens

A Field Guide to Monarch Caterpillars (*Danaus plexippus)*



Karen Oberhauser and Kristen Kuda
Illustrations by Kristen Kuda

Produced with the support of the National Science Foundation. Opinions expressed are those of the authors and not necessarily those of the Foundation.

INTRODUCTION

This guide will aid in recognizing eggs and distinguishing larval (caterpillar) instars of monarch butterflies (*Danaus plexippus*) in the field. We assume that readers have some familiarity with monarch larvae already, and will recognize their bold yellow, white and black stripes on or near their host plants.

Several clues will help you find monarch eggs and larvae. Look for them on plants in the genus *Asclepias* (milkweeds), or on the closely-related *Cynanchum laeve* (Sand Vine) found in the central U.S. Females usually lay eggs on the underside of young milkweed plants, and this is often a productive location to search. A characteristic sign of a new larva is a minute hole in the middle of a leaf, while older larvae tend to eat on the margins of leaves. Learning to recognize "monarch-eaten" leaves will increase your success at finding larvae. They can also be located by the presence of their frass, or fecal matter. If you see adult monarchs (butterflies) in an area with milkweed, there is a good chance you'll find eggs or larvae as well.

Before going into the field to look at monarchs, we recommend reading the anatomy, molting, and distinguishing instars sections of this field guide. After these sections, there are detailed descriptions and drawings of eggs and each of the five instars.

Happy monarch hunting!

ANATOMY

The diagram below shows a generic butterfly larva, with three parts to its body—the *head, thorax* and *abdomen*. The thorax and abdomen each have several segments, which are numbered in the diagram. Many of these segments contain small holes called *spiracles*. The spiracles are connected to a network of airtubes called *tracheae*, which carry oxygen throughout the larva's body. Monarch larvae have two sets of *tentacles* or *filaments* (front and back); these are not antennae, and are not found on all butterfly larvae. They function as sense organs. The thoracic segments each have a pair of jointed *true legs*, and there are five pairs of false legs, or *prolegs*, on the abdomen.

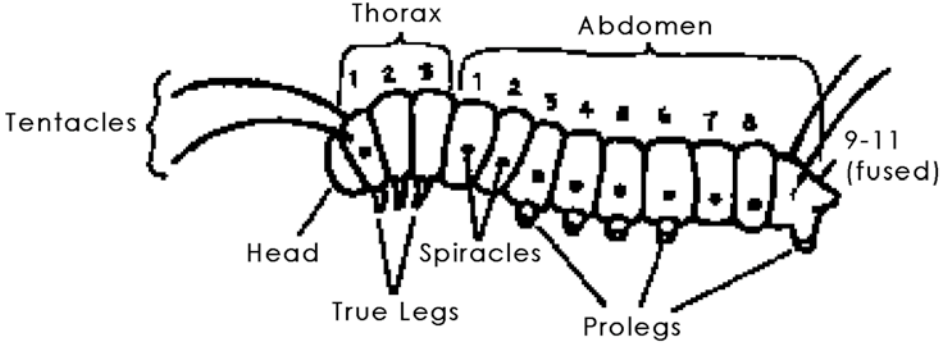


Figure 1. Larva anatomy

The head has a pair of short *antennae*, mouthparts, and six pairs of very simple eyes, called *ocelli*. The *spinneret* produces silk that small larvae use when they drop off a leaf and hang suspended in the air. Larvae in all instars use the silk to anchor themselves during molting, and fifth instar larvae make a "silk button" to which the pupa is attached. The *maxillary palps* are sensory, and also help direct food into the jaws. These features can be seen with the aid of a hand lens, but are difficult to see with the naked eye.

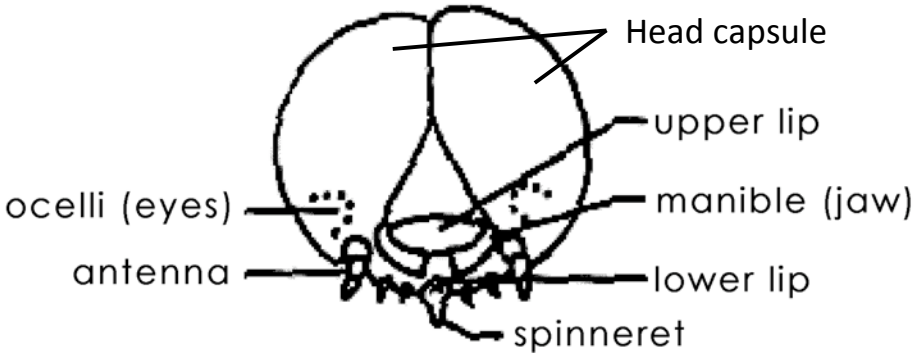


Figure 2. Butterfly Larva head

MOLTING

Monarchs have five larval *instars*, or stages between shedding their *cuticle* (outer layer of skin). The cuticle is made of long protein chains and chitin. It is rigid and hard, and serves to support and protect monarchs and other arthropods. It also restricts water loss. However, the cuticle limits growth and must thus be replaced periodically. The process of replacing the old cuticle is called *molting*. Molting is controlled by a hormone called *ecdysone* produced in glands in the thorax. It actually involves a whole sequence of events, beginning with the separation of the old cuticle from the epidermal (skin) cells that underlie it, a process called *apolysis*, and ending with the shedding of the old cuticle, a process called *ecdysis*. The old cuticle is partially broken down by enzymes, and some of its constituents recycled. When it is first secreted, the new cuticle is protected from these enzymes by a layer of wax. The new cuticle is soft and flexible, thus permitting expansion before it undergoes *sclerotization*, or hardening.

Table 1. Sequence of events in molting

1. apolysis (separation of old cuticle)
2. new cuticle production
3. wax secretion (protects new cuticle)
4. activation of molting enzymes
5. ecdysis (shedding of old cuticle)
6. expansion of the new cuticle
7. sclerotization (hardening of new cuticle)

Monarch larvae remain very still during all the steps of molting, the older instars often move off the milkweed at this time. The first thing that you will notice, besides their motionlessness, is the separation of the part of the cuticle that covers their head from the rest of the cuticle. This *head capsule* is the first part of the old cuticle to be shed, and the larva then crawls out of the rest of the skin. The shed skin is called the *exuvia.* After molting, monarch larvae (and the larvae of many other insects) usually eat the exuvia, thus recycling useful nutrients that it still contains.

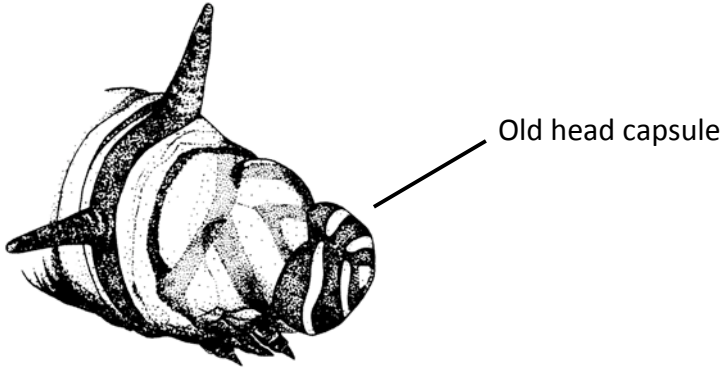


Figure 3. Third instar larva about to shed its head capsule.

DISTINGUISHING INSTARS

While most of the cuticle is quite hard, larvae still grow quite a bit within each instar. This is possible because of the flexibility of the new cuticle, and because parts of the cuticle contain a rubber-like protein which permits it to stretch. Therefore, distinguishing instars by size is not very accurate. Look at the drawings of a first instar larva, all drawn to the same scale, to see how much it changed in size within an instar!

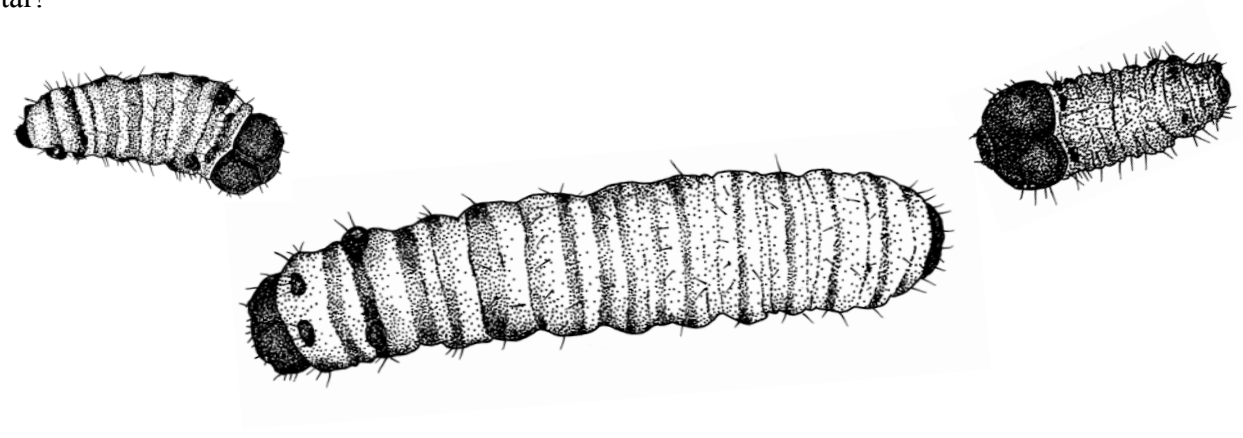


Figure 4. Three drawings of the same first instar larva over a period of 2 days (x25).

The easiest way to distinguish larval instars is by head capsule and tentacle size, since these do not grow during an instar. For example, the front tentacles on a fourth instar larva are about half the length of those on a fifth instar. Also, the size of the tentacles relative to the head capsule and the rest of the body increases with later instars. We have included estimates of the sizes of head capsules and tentacles for each instar in the table on the next page. However, individual monarchs vary in size just like humans do, so the larvae you find may not be exactly the sizes given.

The drawings below compare head capsule sizes in the five instars. Of course, real larvae have much smaller heads! The lines above each drawing give the actual measurement of the real heads. We measured several larvae with a calipers accurate to 0.1 mm, then took the average size, to get these measurements. Note that the head capsules increase in size by a factor of from 1.3 to 1.6 between each instar.

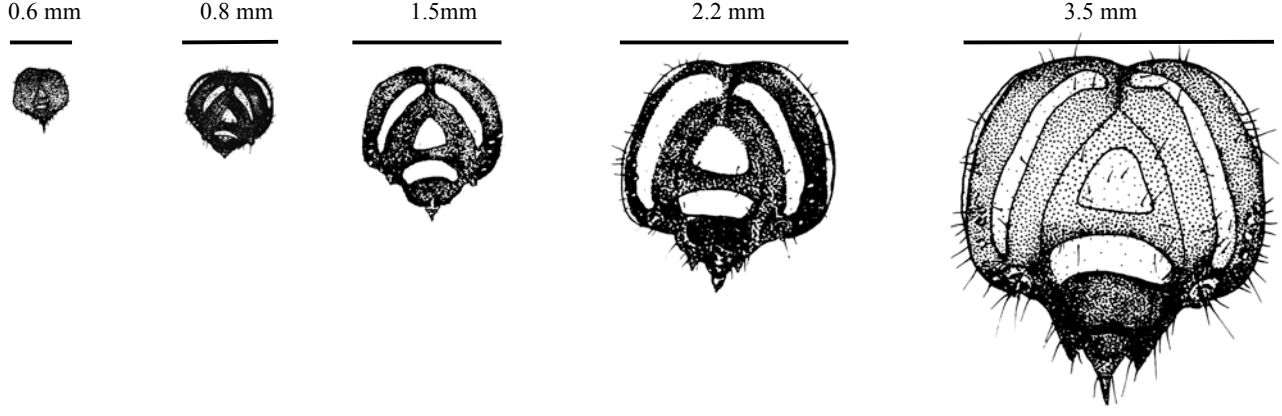


Figure 5. Head capsules, of the five larval instars (all drawn to the same scale, x12.5).

A note on measurement. We report the sizes of monarch eggs and larvae in millimeters (mm). There are 10 mm in a centimeter, so when something is 13 mm long, it is also 1.3 cm long. Sizes of body parts are most useful in distinguishing third and higher instars, since it is difficult to distinguish 0.6 from 0.8 mm (the sizes of head capsules on first and second instars) with the naked eye. It is best to use other characteristics described in the guide for the younger instars. The lines on the table below show the actual head widths and tentacle lengths for each instar. Whenever we show a drawing of a larva, we tell you how many times it has been magnified. For example, the heads shown on the previous page are 12.5 times larger than actual heads; we noted this by putting x12.5 in the figure caption.

Table 2. Comparison of head and tentacle sizes from the five instars. Lines show the actual length of these body parts, and numbers show how long the lines are (in mm). Starred spaces for the tentacles mean that these are too short to measure accurately.

	Instar				
	1	2	3	4	5
Head	(0.6)	(0.8)	(1.5)	(2.2)	(3.5)
Front tentacle	*	(0.3)	(1.7)	(5.0)	(11.0)
Back tentacle	*	*	(0.9)	(2.0)	(4.0)

EGG

Height: 1.2 mm

Width: 0.9 mm

Appearance: Monarch eggs are usually attached to the underside of young milkweed leaves. They are laid singly, and it is uncommon (though not unheard of) to find more than one on a single plant. The eggs look off-white or yellow, and are marked with a series of longitudinal ridges. The hard outer shell, or *chorion*, protects the developing larva.

Figure 6. Scanning electron microscope (SEM) image of a monarch egg

FIRST INSTAR

Body Length: 2 to 6 mm

Body Width: 0.5 to 1.5 mm

Front Tentacles: Small bumps

Back Tentacles: Barely visible

Head Capsule: 0.6 mm in diameter

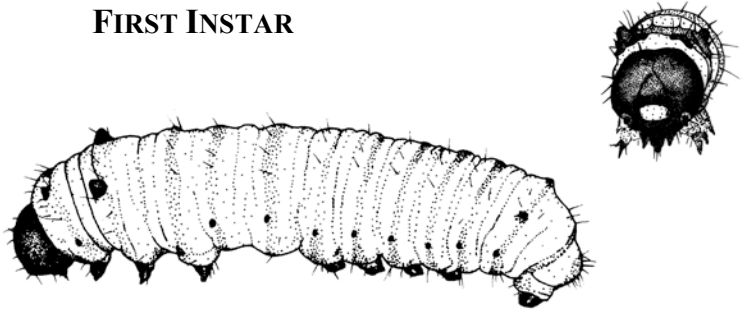


Figure 7. Body and head of first instar (x20)

Appearance: A newly-hatched monarch larva is pale green or grayish-white, shiny and almost translucent. It has no stripes or other markings. The head looks black, with lighter spots around the antennae and below the mouthparts, and may be wider than the body. There is a pair of dark triangular patches between the head and front tentacles which contain setae, or hairs. The body is covered with sparse setae. Older first instar larvae have dark stripes on a greenish background.

After hatching, the larva eats its eggshell (chorion). It then eats clusters of fine hairs on the bottom of the milkweed leaf before starting in on the leaf itself. It feeds in a circular motion, often leaving a characteristic, arc-shaped hole in the leaf. First (and second) instar larvae often respond to disturbance by dropping off the leaf on a silk thread, and hang suspended in the air.

SECOND INSTAR

Body Length: 6 mm to 9 mm

Body Width: 1 to 2 mm

Front Tentacles: 0.3 mm

Back Tentacles: Small knobs

Head Capsule: 0.8 mm diameter

Figure 8. Body and head of second instar (x12.5)

Appearance: Second instar larvae have a clear pattern of black (or dark brown), yellow and white bands, and the body no longer looks transparent and shiny. An excellent characteristic to use in distinguishing first and second instar larvae is a yellow triangle on the head and two sets of yellow bands around this central triangle. The triangular spots behind the head do not have the long setae present in the spots on the first instar larvae. The setae on the body are more abundant, and look shorter and more stubble-like than those on first instar larvae.

THIRD INSTAR

Body Length: 10 to 14 mm

Body Width: 2 to 3.5 mm

Front Tentacles: 1.7 mm

Back tentacles: 0.9 mm

Head Capsule: 1.5 mm in diameter

Figure 9. Body and head of third instar (x6)

Appearance: The black and yellow bands on the abdomen of a third instar larva are darker and more distinct than those of the second instar, but the bands on the thorax are still indistinct. The triangular patches behind the head are gone, and have become thin lines that extend below the spiracle. The yellow triangle on the head is larger, and the yellow stripes are more visible. The first set of thoracic legs are smaller than the other two, and are closer to the head.

Third instar larvae usually feed using a distinct cutting motion on leaf edges. Unlike first and second instar larvae, third (and later) instars respond to disturbance by dropping off the leaf and curling into a tight ball. Monarch biologist Fred Urquhart called this behavior "playing possum."

FOURTH INSTAR

Body Length: 13 to 25 mm

Body Width: 2.5 to 5 mm

Front Tentacles: 5 mm

Back Tentacles: 2 mm

Head Capsule: 2.2 mm in diameter

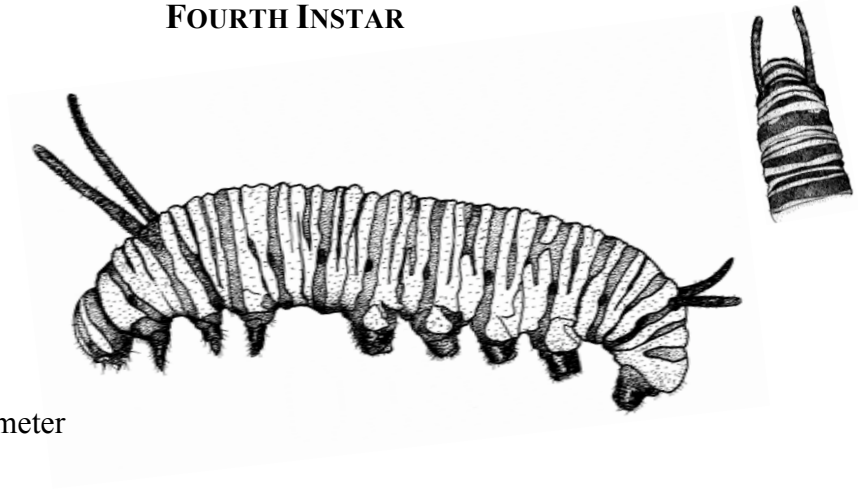


Figure 10. Body and head of fourth instar (x5)

Appearance: There is a distinct banding pattern on the thorax which is not present in the third instar larvae. The first pair of legs is even closer to the head, and there are white spots on the prolegs that were less conspicuous in the third instar.

FIFTH INSTAR

Body Length: 25 to 45 mm

Body Width: 5 to 8 mm

Front Tentacles: 11 mm

Back Tentacles: 4 mm

Head Capsule: 3.5 mm in diameter

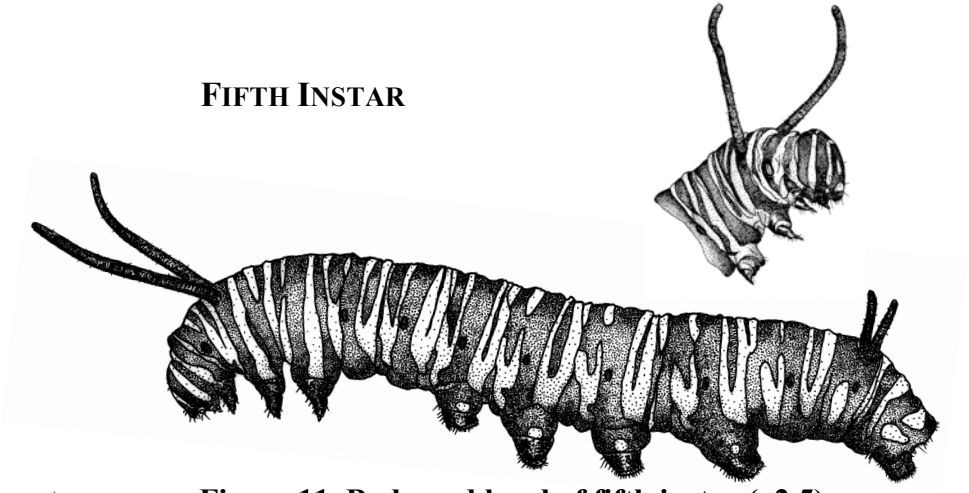


Figure 11. Body and head of fifth instar (x2.5)

Appearance: The body pattern and colors are even more vivid that they were in the fourth instar, and the black bands look wider and almost velvety. The front legs look much smaller than the other two pairs, and are even closer to the head. There are distinct white dots on the prolegs, and the body looks quite plump, especially just prior to pupating.

Fifth instar monarch larvae often chew a shallow notch in the petiole of the leaf they are eating, which causes the leaf to fall into a vertical position. They move much farther and faster than other instars, and are often found far from milkweed plants as they seek a site for pupating.

Journey North Data Entry

1. Enter the number of monarch butterfly observed. _____
2. Comments. Here's what information is needed.
    a. Where are you? (Park, Schoolyard Habitat, Backyard, Walking Home)

    b. What time is it?

    c. What's the weather like? (Cloudy, Full Sun, In the high 90's)

3. Optional: If you have access, take a photo and use the photo editor to add the date and time.
4. What is the date for your observation? _____
5. What is the location of your sighting?
    a. Country: _____
    b. State: _____
    c. City: _____
    d. Latitude: _____ (round to the nearest hundredth)
    e. Longitude: _____ (round to the nearest hundredth)

    To find your current latitude and longitude use your phone's compass or go to http://mynasadata.larc.nasa.gov/latitudelongitude-finder/ and insert your school's full address, including zip code.
6. What is your first name? _____
7. What is your last name? _____
8. If instructed, go to the Journey North app, login and enter and submit your data.