Metamorphosis—Triggers of Transformation

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This curriculum unit is recommended for:
IB or AP Biology classes

Keywords: metamorphosis, apoptosis, endocrine, evolution, environment

Teaching Standards: See Appendix 1 for teaching standards addressed in this unit.

Synopsis: In this unit you will find a summary of some of the latest research on the evolution and the mechanisms of metamorphosis. This information provided me with multiple ways to include metamorphosis in my IB Biology class. This unit incorporates a review of gene regulation, cell differentiation, and development—which are taught during the first year of the course—in order to prepare the students for their IB exams. I use examples of the causes of metamorphosis to review these concepts during new lessons on evolution, hormones, ecology, and animal behavior. Students don't always make the connection between the effects of hormones and environmental factors and the actions of genes, or how changes in DNA can affect the evolution of a species. With this background knowledge, students will design experiments testing the effects of some environmental factor on the transition from larva to pupa: crowding, temperature, food, moisture, or chemicals in the environment such as BPA. Students will also do an activity involving modeling of hormones and cell communication.

I plan to teach this unit during the coming year in to 30 students in IB Biology classes

Metamorphosis—Triggers of Transformation

Connie Scercy Wood

Introduction

The transformation of a caterpillar into a butterfly, or tadpole into a frog, seems magical. However there has to be a reason for the changes. DNA must be behind the changes, as well as hormones and environmental factors. Cells that were necessary in one stage are no longer needed and die as an organism enters a new stage. What determines which cells die and when?

I teach IB Biology III, the second year of a two year biology course in the International Baccalaureate Program. The students in the program are seniors and the class is generally racially diverse. About 70% of the students in our school are in the free or reduced lunch program. In IB Biology III the students have to design and carry out their own experiments. They often use mealworms and conduct behavior experiments. Mealworms are easy to work with and fairly inexpensive. These are not actually "worms", but the larval stage of the darkling beetle. We discuss how the larvae go through various changes that lead to the pupal stage and then to the adult beetle stage, and how those changes might affect the response to the stimuli during the experiment. If the larva is approaching the pupal stage, might its responses be slowing down as its body slows down? How might that affect the data being collected during the lab? So we know that the changes are taking place, but what I do not know is how to explain to the students what is going on inside the organism that leads to the drastic morphological changes as it progresses to the pupal stage. Most of the information available in textbooks and online does not go into the physiological basis for the changes—it simply describes the stages and if at all detailed, only estimates the length of those stages. There has to be significant genetic and hormonal activity, as well as apoptosis—programmed cell death—occurring inside the mealworm. And these changes have to have effects on the responses of the larvae to various stimuli.

This unit will incorporate a review of gene regulation, cell differentiation, and development—which are taught during the first year of the course—in order to prepare the students for their IB exams. I will use examples of the causes of metamorphosis to review these concepts during new lessons on evolution, hormones, and animal behavior. Students don't always make the connection between the effects of hormones and environmental factors and the actions of genes, or how changes in DNA can affect the evolution of a species. With this background knowledge, students will design experiments testing the effects of some environmental factor on the transition from larva to pupa: crowding, temperature, food, moisture, or chemicals in the environment such as

BPA. Students will also do an activity involving modeling of hormones and cell communication.

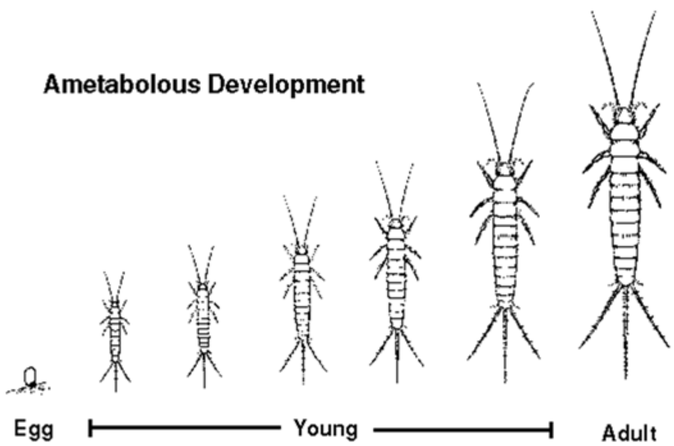
Background Information

Types of Metamorphosis

Metamorphosis involves changes that most animals undergo in their development from an egg to an adult form. Many invertebrates go through metamorphosis, including mollusks, crustaceans, echinoderms, cnidarians, and of course arthropods such as insects. The most well-known vertebrate metamorphosis is that of frogs. However, if you consider that metamorphosis is a change from a juvenile form to an adult form, triggered by hormones, then you could say that even humans go through a kind of metamorphosis as children develop into adults. The focus of this unit will be on insect metamorphosis.

There are three kinds of metamorphosis in insects, but a common theme to all is the transformation from a non-reproductive to a reproductive form. Ametabolous metamorphosis describes animals that do not change outwardly in appearance from the larval stage to the adult form, except in size and ability to reproduce.[1] Even after they reach sexual maturity, they continue to grow and molt—not so for the other types of metamorphosis.[2] An example is the silverfish (Figure 1).

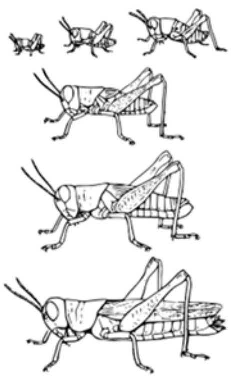
Figure 1—Ametabolous Development



http://www.cals.ncsu.edu/course/ent525/trans.html

Hemimetabolous metamorphosis is commonly known as incomplete or simple metamorphosis. In this kind of development, the animal goes through several stages called instars. Instars are distinct periods of development usually ending with molting of the exoskeleton as the organism grows. The last instar will result in much greater changes as the juvenile becomes an adult.[3] In hemimetabolous metamorphosis, the juvenile instars are called nymphs, which look similar to the adult form but may be lacking some key characteristics of the adult, such as wings and cannot yet reproduce (Figure 2). Grasshoppers and cockroaches are examples of insects that develop this way.[4]

Figure 2—Hemimetabolous Development

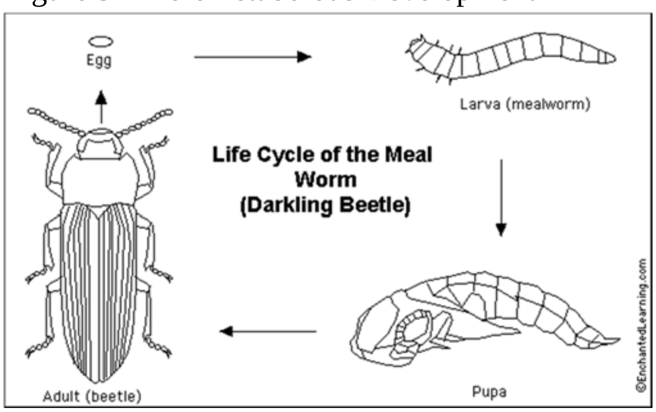


From Insects, their way and means of living, R.E.Snodgrass.

http://en.wikipedia.org/wiki/Metamorphosis

In insects that go through holometabolous development, or complete metamorphosis, the changes from the larval stage to the adult are drastic (Figure 3). The animal develops from an egg to a larva to a pupa to an adult. The larvae go through several instars as they feed and grow. After the final instar, the larva enters the pupal stage, a non-feeding stage during which the larval tissues are almost completely replaced by adult tissues, transforming the animal.[5] Adults will no longer molt and will have different habitats than the larvae.[6]

Figure 3—Holometabolous Development



http://www.enchantedlearning.com/subjects/insects/beetles/mealworm/mealwormlifecycle.shtml

It appears that holometabolous development evolved last. The fossil record shows early insects were ametabolous and the young looked basically like miniature adults. Later, hemimetabolous species evolved, but insect nymphs were competing for the same resources and habitats as the adults who were trying to reproduce. Under the competitive exclusion principle proposed by Georgii Gause, two species occupying the same niche, utilizing the same resources, will be in competition with each other. The outcome of this competition will be the extinction of one of them or resource partitioning. In this case the competition is between members of the same species, but at different stages of development. Natural selection would have favored changes in the species that resulted in resource partitioning between the larval and adult forms of the insects. The evolution of holometabolous development, about 280 million years ago, favored immature forms that consumed different foods or lived in different habitats than the adults and was a remedy to this competition. Subsequently, there was an explosion of holometabolous insect species and today 88% of all insect genera are holometabolous, compared to only 50% two hundred and fifty million years ago.[7,8]

Evolution of Metamorphosis in Insects

How did this change in mode of development come about? From ametabolous development to hemimetabolous development to holometabolous development. James Truman and Lynn Riddiford proposed a scenario based on the research that has been done on the causes of metamorphosis in their article, “Endocrine Insights Into the Evolution of Metamorphosis in Insects”.[9]

First, the larval stage. An embryo develops inside the egg, nourished by a yolk. If it stays there long enough it will develop until, when it comes out, it is like a miniature adult which continues to grow in size until it becomes the reproductive adult. This would be ametabolous development. If it came out earlier, it would be a nymph--which would then continue to grow, going through several instars and developing adult structures like wings and reproductive organs—hemimetabolous development. The level of development that the organism has reached by the time it comes out of the egg can vary. In some arthropods, the emerging instar is often more of a pronymph—a non-feeding nymph stage. The pronymph that emerges from the egg of silverfish has these characteristics.[10] In some hemimetabolous insects, cockroaches, lice and true bugs, the pronymph stage is passed inside the egg and what emerges from the egg is the first stage nymph. In others, like grasshoppers, the emerging insect is a pronymph that cannot walk, but may have some limited locomotion. The grasshopper pronymph can dig in the soil. Once it molts to the first instar, it can walk, but can’t dig. Dragonflies lay their eggs on the underside of leaves near water. When the pronymph hatches, if it falls on ground instead of water, it can move in a hopping motion to get to the water, where it continues development through nymphal stages which do not hop, but walk. The idea is that the larva stage evolved from the pronymph stage.[11]

Evidence for the larval stage being a form of the pronymph stage comes from studies of the nervous system of arthropods. In insects, this nervous system consists of a brain and ventral nerve cord with paired ganglia—concentrated areas of sensory and motor nervous tissue—located in segments of the insect. The nervous system begins forming from neuroblasts, basically nervous system stem cells. These cells give rise to neurons in the segmental ganglion and the cells are very similar in all insects. In ametabolous and hemimetabolous organisms the neurons are completely formed at hatching—so they form during the part of embryogenesis that correlates to the pronymph stage. However, in holometabolous insects, the neuroblasts begin to form neurons during embryogenesis, but then stop and do not continue until the larval stage. The numbers and types of neurons present in the larvae of insects is more like that of the late embryo stage of a grasshopper.[12]

The peripheral sensory system also shows a similar pattern of development. Proprioceptive neurons (neurons that detect muscle movement and body position) are fully formed at the beginning of the pronymph stage of grasshoppers. These same proprioceptors are also present by the beginning of the larval stage of insects.

If the pronymph stage occurs inside the egg, a pronymphal cuticle will form after the body surface is complete. In holometabolous development the first cuticle is that of the first instar larva.[13]

So the development of the nervous system, sensory neurons and the first cuticle all provide support for the hypothesis that the larval stage evolved from the pronymph stage. But the pronymph does not feed in most hemimetabolous insects, and the larva of holometabolous insects does. If some pronymphs did not absorb all of the yolk while in the egg, that would have been a trait that was not advantageous. But if some of them evolved the ability to somehow eat the unabsorbed yolk, this might have been the origins of an early feeding stage.[14] Some hemimetabolous insects lay their eggs in the soil, or in plant material and the pronymphs have the ability to move in order to escape. If the stage was extended so that the pronymph was also feeding in the areas where it emerged, that would give it an advantage if it was using resources not available to, or not used by the nymphal or adult stages. Better resources would favor an extended pronymphal stage and both locomotor and feeding adaptations.[15] The pronymph/larva would become more and more like today's caterpillar.[16] The nymphs however would still be competing with the adults, so there would be selection for an extended pronymphal/larval stage in which most of the feeding was done and less or no feeding in the nymphal stages. The number of nymphal instars in hemimetabolous insects today varies with the amount of growth that occurs. If the amount of feeding and growth increased in the pronymphal/larval stage, then the nymphal stages might have become reduced to a nonfeeding stage— later evolving into the pupal stage.[17]

One of the big factors in the metamorphosis of insects are the imaginal discs. These discs are clusters of special epithelial cells that are mostly inactive in the larvae, but as metamorphosis begins and larval tissues start to degenerate, they divide to replace larval structures and tissues with the adult forms, using the degenerating larval tissues as their building material and energy source.[18,19] The discs can develop in two ways—they are either already differentiated as adult cells and inactive throughout the larval stage or they may be active during the larval stage as epidermal cells involved in cuticle development, that then start differentiating into adult cells at the onset of metamorphosis and the pupal stage. It is the latter that is thought to have evolved first. In insects today that use this method, there is a longer prepupal growth period because these cells have to switch from their larval functions to forming the imaginal discs which will form adult structures and tissues. Over time, as imaginal discs formed earlier in the larval stage, the larval stage became shorter, resulting in a shorter life cycle. However, there may be different imaginal discs within the same insect that originate in each of the two manners—indicating that selection for the early origination of the imaginal discs was specific to certain organs.[20] Mealworm larvae have wing imaginal discs that use the delayed method. They start out as epidermal cells that make cuticle, then after the last larval instar, they divide, detach from the cuticle and form imaginal discs between the epidermis and the cuticle.[21]

Hormones

Hormones play a key role in metamorphosis. The two most important hormones are ecdysones, steroid molecules that control molting, and juvenile hormone, organic sesquiterpene molecules which maintain the larva in the juvenile stage. Juvenile hormone (JH) is only found in insects, and in all types—from ametabolous to holometabolous forms.[22, 23] It is secreted by the corpora allata—endocrine glands near the brain of insects. Neuropeptides released by the brain either inhibit or stimulate the corpora allata to release JH. Juvenile hormone gets its name from the fact that when present, it inhibits metamorphosis and retains the insect in its juvenile form.[24] However, during the development of the embryo in the egg, JH levels are typically low. High levels of juvenile hormone might help explain the evolution of the pronymph into the larva. Experimental results from a temporary increase of JH during embryogenesis results in the early development of nymphal tissues in hemimetabolous embryos. Such a response could have led to early development of structures for survival outside the egg in the larval form of holometabolous insects if JH levels were increased. In at least one order of holometabolous insects, JH levels have indeed been shown to be high in later stages of embryogenesis. Later in development, high levels of juvenile hormone would allow for an extended period as a larva, as the hormone inhibited the maturation of the larva into the adult form.[25]

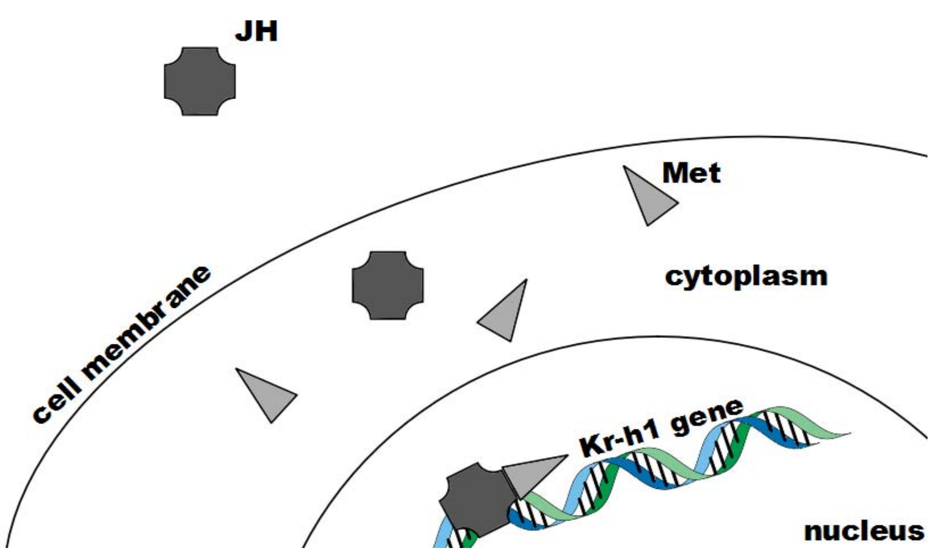
In ametabolous insects juvenile hormone is high in the first instars, then declines to allow for the growth of integumental scales—possibly an evolutionary precursor to the decrease in JH which allows for the larva to adult changes that occur in hemimetabolous and holometabolous insects.[26]

In hemimetabolous insects, at the beginning of the last nymphal instar, Juvenile Hormone levels drop and ecdysone triggers the onset of the adult stage. In holometabolous insects, Juvenile Hormone levels drop during the last larval instar; there is a small increase in ecdysone, which triggers the larva to stop eating, to spin a cocoon, and for pupal tissues to develop. There is then a second release of ecdysone in the presence of Juvenile Hormone, which causes the pupal cuticle to form. This reappearance of Juvenile Hormone is similar to its reappearance in the embryogenesis of more primal arthropods. In them, Juvenile Hormone disappears; appendages form; then Juvenile Hormone reappears for nymphal development. Then a third release of ecdysone commits the tissues to differentiation into adult tissues. So in the case of holometabolous insects, the last release of Juvenile Hormone in the larval stage, like the last release in hemimetabolous insects at the end of the nymphal stages, triggers the insect to begin its transformation into an adult during the pupal stage.[27]

Both ecdysones and JH may be released in inactive forms and activated or kept inactive in the hemolymph (blood) or at target tissues. The amounts of active and inactive products and the timing of their appearance can affect development of the insect.[28]

Both JH and ecdysone hormones ultimately affect gene expression by activating or inhibiting transcription factors that induce or inhibit genes. This is still a very active area of research. In holometabolous insects, JH binds to a receptor protein called Methoprene-tolerant (Met) which regulates a transcription factor gene—the Kruppel-homolog 1 (Kr-h1) gene (Figure 4). The Kr-h1protein acts as a transcription factor that suppresses the ecdysone-activated BR-C gene—a master switch for metamorphosis. Loss of Kr-h1 has been shown to result in early development of the pupa.[29]

Figure 4—JH and the Met nuclear receptor.



When JH disappears during the last larval instar, the Kr-h1 gene is down-regulated, the pupa forms and adult development begins, as the BR-C gene is no longer being suppressed.[30] The BR-C gene results in four different transcription factor proteins (perhaps due to transcriptional processing). These different transcripts are induced by ecdysone at different points in time and are tissue specific in their effects. In mutants lacking the BR-C gene, development of the larvae occurs, but there is no metamorphosis.[31] In hemimetabolous insects BR-C gene activity remains fairly constant throughout all the nymphal stages and results in gradual wing development and progression to the adult stage. JH seems to have little effect on the gene. In holometabolous insects, the BR-C gene is only active in the last instar and pupal stages, when JH levels have dropped, and wing development and progression to adulthood happen rapidly over a much shorter time. This could be another example of how an earlier mechanism for development could be slightly altered to result in holometabolous development.[32]

JH levels also decrease when the larva reaches critical weight during the last instar.[33] Drops in JH levels can be partly attributed to JH specific hydrolytic enzymes in the hemolymph. When needed it is protected from the enzymes by JH-binding proteins. [34]

Juvenile Hormone is also involved in yolk deposition during the formation of eggs, sperm development in males, and in diapause—keeping the insect in the larval or pupal stage during the winter. JH may also have a role in the caste system of social insects such as bees, not only in the determination of whether a bee is a worker or a queen, but also what tasks the workers do over the course of their lifetime. But that's another story.[35]

Ecdysone is produced when the brain releases neuropeptides which travel to the corpora cardia, a gland near the brain which is also involved in control of the heart. This gland then secretes prothoracicotropic hormone or PTTH which travels through the blood and stimulates the prothoracic glands to produce pre-ecdysone. Pre-ecdysone is further modified in the hemolymph, or at the target cells, into ecdysone, a steroid hormone.[36]

Levels of ecdysone increase near the end of each instar resulting in molting. A corresponding increase in JH keeps the organism in the juvenile stage, until the last instar when JH levels drop. Ecdysone levels then drop until the next instar nears its end. The molting process begins with the process of apolysis when ecdysone triggers the old cuticle to begin separation from the epidermal cells. A molting fluid containing inactive digestive enzymes is secreted between the epidermal cells and the old cuticle. The cells then begin dividing, wrinkling up in the space created by the separation of the cuticle. The new epidermal cells begin secreting a new cuticle layer underneath the old one, starting with a layer called cuticulin which will protect the cells from the enzymes in the molting fluid. Enzymes in the molting fluid become activated and begin the breakdown of the old cuticle. The body swells, splits the old exoskeleton and it is sloughed off in the process known as ecdysis. The new exoskeleton hardens and darkens and is complete within one to two days.[37]

In the last larval instar of holometabolous insects, when JH levels drop, there is a peak in ecdysone which results in the cessation of feeding and an increase in wandering. This allows the larva to find a suitable place for pupation and metamorphosis. It also triggers epidermal cells to form a pupal cuticle. A similar but smaller increase in ecdysone at the same point in the development of hemimetabolous insects occurs, which may set the stage for development into the adult. The increase in ecdysone at this point in the development of the holometabolous insects may have been an essential step in the evolution of the pupal stage..[38] The receptor for ecdysone is a nuclear hormone receptor made of two proteins—EcR and ultraspiracle protein (USP). Nuclear hormone receptors are receptors that may be found in the cytoplasm, are activated by signal molecules such as hormones, and then travel to the nucleus where they often act as transcription factors, turning genes on and off. When ecdysone binds to this receptor in the cytoplasm of the cell, the receptor is activated to become a transcription factor that activates the BR-C gene which then activates a whole series of other transcription factors that regulate many genes involved in molting and metamorphosis. Expression of the genes varies with ecdysone and JH levels, the stage of development, and the target cells.[39] Ecdysone regulates many other processes in the development of holometabolous insects including the growth of imaginal discs, and apoptosis during metamorphosis. It also is a factor in determining the social structure on honeybee colonies. The larvae which are destined to become queens experience higher levels of ecdysone than those destined to become workers. In worker bees, ecdysone levels increase over the course of their lifetime and seem to correspond to changes in their roles as nursery workers, honey makers, and foragers. It works in conjunction with JH to coordinate the expression of the hundreds of transcription factors and genes necessary to carry out the complex process of metamorphosis.[40]

Apoptosis

When the larva transforms into the adult during the pupal stage, there is much more happening than just cell differentiation. In fact, most of the larval tissues are completely replaced by new adult cells from the imaginal discs. Apoptosis is programmed cell death, in which specifically targeted cells are marked for destruction to make way for new cells. Pulses of the hormone ecdysone trigger the beginning of the end for many of the larval cells. Studies of *Drosophila* have uncovered some of the mechanisms in this process. In studies of the apoptosis of the larval salivary glands of *Drosophila*, there are proteases called caspases which degrade cellular proteins and activate other proteins of apoptosis when they themselves are activated.[41] There are also several *Drosophila* inhibitors of apoptosis (*diap*) genes which code for proteins that bind to the caspases, inhibiting them from destroying the cell. To get apoptosis started, three genes—*head involution defective* (*hid*), *grim*,and reaper (*rpr*) activate the caspases by producing proteins which block the *diap* protiens from binding to the caspases. These genes, as well as the caspase gene, are induced at the beginning of and midway through the pupal stage, by ecdysone when it binds to receptor proteins that regulate the BR-C gene or directly to the E93 gene.[42] The way that ecdysone creates different effects in different cells at different times is like having one conductor who directs musicians who are playing different instruments, and different songs, and at different times.

Environmental Effects on Metamorphosis

Insect metamorphosis can be affected by temperature, humidity, day length, and food and water supply. If food and water are not adequate, larvae will not grow as well and the adult they become will not be as large or as fertile. Increasing temperatures due to climate change may actually benefit some stages of development, but may hurt others. For example, a study of the Bog Fritillary butterfly showed that while egg, pupal, and adult stages benefited from warmer temperatures, overwintering larvae were more at risk due to disease and fungal infection from warmer winters. This resulted in the prediction that the overall population of the butterflies would decrease with climate change[43]Pheromones given off by other insects can affect metamorphosis. When “Superworms”, the larvae of *Zophobia moris*, are confined together, they will not progress to the pupal stage. Diapause can result if conditions are not right, resulting in the delay of metamorphosis.

Endocrine Disrupters

Endocrine disrupters are natural and man-made chemicals that have the ability to interfere with the normal functioning of hormones because they either mimic their shape and bind to a hormone receptor molecule (for example, an estrogen-mimic), or interfere with how hormones function (for example, a thyroid hormone antagonist). These can lead to depression of hormone-mediated pathways, or activation at the wrong time. Any body function that involves hormones can be affected but the early stages of development, when hormone levels are critical are the most sensitive to the effects of endocrine disrupters. Metamorphosis is a delicate dance of hormones whose amounts and arrival times are crucial for the development of the organism. Sources of endocrine disruptors include pesticides such as DDT, dioxins, pharmaceuticals, and Bisphenol-A (BPA).[44]

Juvenoids, such as methoprene, are chemical mimics of the Juvenile Hormone, a sesquiterpine. Juvenoids have been used as pesticides on crops and to control mosquitoes, causing disruption in the development of insect pests, but with the advantage of being safe for vertebrates. They work as insecticides by disrupting the normal balance of JH in the life cycle, causing the insects to develop abnormally. They do not accumulate in soil or water, but can however affect the life cycles of non-pest insects.[45]

Strategies and Lesson Plans

I teach the second year of the IB Biology course, during which we cover evolution and classification, ecology and body systems. I plan to incorporate the metamorphosis of insects as part of each of these units, using the topic to help students make connections between these seemingly disparate subjects. To do so, I will have to switch the sequence in which I usually teach them so I can introduce the process of metamorphosis in each topic in a manner that does not require total understanding of everything about it in the beginning.

Classification Lesson

I will begin the year with a unit on classification. This is a brief unit in the IB curriculum, but students are introduced to the characteristics of the phylum Arthropoda. I will have them review the basics of the life cycles at this time. Most will be familiar with complete and incomplete metamorphosis, but may not have learned about ametabolous development. Students will create posters of the three types of life cycles and Venn diagrams to describe how the three types of development compare.

Endocrine System Lesson

I will teach a short unit on the endocrine system. The hormones involved in metamorphosis—ecdysone and Juvenile Hormone—will be used as examples. To help students understand how these molecules can trigger the changes seen in the insects as they go through metamorphosis, as well as how hormones work in their own bodies, students will create models. Nova has a lesson plan on the effects of hormones on obesity. A link to this activity can be found in the teacher resources section of this unit. It includes a short video about a hormone that was discovered that causes obesity and a student guide for making simple models of various cell receptors for hormones. At the end, students are supposed to design their own model, and I will have them make and demonstrate a model for Juvenile Hormone and ecdysone to show how these work in insects. Figure 4 shows how JH activates the nuclear receptor Met to regulate a transcription factor gene. Ecdysone works in a similar way.

Materials for the Nova activity and the Ecdysone/JH models

String, Signs for the leptin feedback cycle

For each group—4 twist ties, a clear soda bottle, a funnel to fit the bottle, 20 ml vegetable oil, water, 2 pipe cleaners, 1 ziplock bag, 8 cotton swabs, a styrofoam ball, small ball of clay, 10-15 toothpicks

Additional materials for the model of Juvenile Hormone and ecdysone—variety of odd shaped candies or Styrofoam balls, clay or play dough, beads, poster board, dry pasta, zip lock bags, string, etc.

Evolution Lesson

The next unit will be on evolution. After an introduction on natural selection and how it works to change populations, students will do a simulation activity of the peppered moth's directional selection. There is a very good simulation at http://www.techapps.net/interactives/pepperMoths.swf Students often don't get the point that there has to be variation in the population for natural selection to act on. Where does that variation come from? So we will review DNA mutations and recombinations, and how they create new traits in population. The evolution of metamorphosis depended on mutations that shifted the timing of developmental stages. I will use insects again as an example to discuss how the process of metamorphosis could have evolved from ametabolous to holometabolous development using an activity in which students capture food for their insect instars. Students will receive cutout pictures of either hemimetabolous or holometabolous insects or insects that have a non-feeding pronymph stage. First they will put their pictures in order, starting with the egg. Some students will be given only adult versions of the insects to use in the simulation. Several types of food for the insects will be provided—Skittles, M&Ms and marshmallows. The hemimetabolous insects will all eat the same type of food—Skittles--and sit at the same table. But holometabolous larvae will eat M&Ms while the adults eat marshmallows at a different table. I will declare that the eggs have hatched and that each insect, adult or larva must eat at least two food items—except for the non-feeding pronymphs! The amount of candy should be calculated so that at least one person does not get their full allotment in the hemimetabolous group and is declared dead. This will demonstrate competition between the adults and the juveniles in insects that go on with incomplete metamorphosis. "Molting" then occurs to complete the first instar and then another round of feeding takes place. This activity will continue until the last instar. At that point, the larvae in the holometabolous group will become pupae and will no longer eat until they emerge as adults. What should occur at the end is that some of the hemimetabolous insects will have "died" from the intraspecific competition and most of the holometabolous insects will have survived. The class will then discuss the evolutionary ramifications of this demonstration and I will share with them the fact that today more than 88% of insects go through complete metamorphosis.[46]

Materials Needed

Laminated enlarged pictures of the stages of metamorphosis—the images at the beginning of this unit will work well. About one set for every two students and extra pictures of the adult stages.

3 kinds of candy—M&Ms, Skittles, marshmallows

Ecology Lesson

The third unit will be the ecology unit. I will again bring in insects as we discuss food chains and communities (how are insects important to an ecosystem?), intraspecific competition and competitive exclusion (what might happen if larvae and adults were sharing the same resources?), niche (how are the niches of holometabolous larvae and adults different?) and the effects of environmental factors on the distribution of plants and animals (how might insects be affected by climate change?) As we cover the flow of energy in ecosystems we will also look at how r and K selected species utilize that energy, using insects as an example of r-selection. At this point, I will have students compare the advantages and disadvantages of complete and incomplete metamorphosis in terms of the amount of intraspecific competition and the energy sources used with each strategy. When we look at human impacts on the environment, I will introduce the topic of endocrine disruptors and we will use insects as an example of how these chemicals might affect not only the insects, but how that would affect the entire food web in a community. Students will each research one source of endocrine disruptors and describe how it gets into the environment, what its short and long term effects might be and how its use could be avoided.

Animal Behavior Lesson

The final unit is on animal behavior. In the past I have had students conduct experiments using superworms, *Zophobia moris,* and their kinetic response to various stimuli such as light, temperature, pH, etc. Superworms are available from most pet stores which sell them as food for reptiles. Students have to come up with a research question, design and carry out the experiment, and analyze the results, all on their own. With the background they will now have on the life cycle of these insects, I want students to take into account the stage of the life cycle the superworms are at when the experiments are done and assess whether it has an effect. Because I do not raise the larvae myself, we will not know what instar they are in when the experiments are done. Students could get an estimate by continuing to observe the superworms they used in the experiment and record when they molt or enter the pupal stage. Superworms will not progress through metamorphosis if they are kept together, but stay in the larval stage. The plastic tubes used to culture fruit flies would make good individual housing for the superworm larvae if food and a source of moisture are also added.

Students will also have the option to design an experiment to test the effects of an environmental factor on the progression of the superworms through their lifecycle—using temperature, spacing, BPA, food, water, or some other factor. To test the effects of BPA, students could use the strips of thermal receipt paper which contain BPA that many stores use, wrapping it around the container or making bedding out of it.

Materials Needed

Superworms—Students need to have enough to collect plenty of data with. Fortunately they are not very expensive. I like to have at least 10 per student.

Food for the superworms—bran meal is best, but you can use plain uncooked oatmeal.

Water source—Pieces of apple, even just apple cores work well. Cotton balls soaked in water. Do not give them a dish of water, they will crawl in and drown.

A variety of containers for students to select from to perform their experiments. These worms can crawl and climb pretty well so you want something with high sides or a top. Large petri dishes with lids work well depending on what the student wants to accomplish.

Plastic spoons—there will be some who don't want to touch the worms!

Lamps, heat sources, ice or a fridge, thermal receipt paper—students should also come up with materials they can bring in to use as their independent variable.

Having learned so much about their life cycle and its evolutionary history, I hope that students will have a greater appreciation and understanding for what is going on inside these insects and that the understanding will result in a more thoughtful and meaningful lab experience.

Appendix 1: Implementing Teaching Standards

Standards for IB Biology Higher Level

2.1.8 Explain that cells in multicellular organisms differentiate to carry out specialized functions by expressing some of their genes but not others.—This unit will address this standard directly as students learn about how hormones will affect genes in certain cells but only at certain times in the development of insects.

2.1.9 State that stem cells retain the capacity to divide and have the ability to differentiate along different pathways.—The imaginal discs provide a very good example of stem cells that are retained in the larvae of insects until they are activated to begin dividing and forming adult cells.

5.4.7 Explain how natural selection leads to evolution—Students will consider how natural selection has resulted in the evolution of complete metamorphosis.

5.4.8 Explain two examples of evolution in response to environmental change; one must be antibiotic resistance.—the example of insect metamorphosis and how it is affected by endocrine disruptors provides students with another example they can use for a response to environmental change.

6.5.7 State that the endocrine system consists of glands that release hormones that are transported in the blood.—Students will learn about the glands and hormones involved in insect metamorphosis and well as some in the human body that affect obesity.

E.1.4 Explain how animal responses can be affected by natural selection, using two examples.—Students will design experiments to test the effects of an environmental condition on the behavior of superworm larvae.

E.3.2 Analyse data from invertebrate behavior experiments in terms of the effect on chances of survival and reproduction—Students will analyse the data from the experiments they perform.

G.5.1 Distinguish between r-strategies and K-strategies—Students will use insects and their method of reproduction as an example of r-strategy.

G5.2 Discuss the environmental conditions that favor either r-strategies or K-strategies—Students will use insects as an example of an r-strategy organism and how it is affect by environmental conditions.

Bibliography

Aldaz, Silvia, and Luis M. Escudero. "Quick Guide--Imaginal Discs." Current Biology.

Accessed October 3, 2014. http://www.sciencedirect.com/science/article/pii/S0960982210002915.

Buszczak, Michael, and William A Segraves. "Insect Metamorphosis: Out with the Old, in with the New." *Current Biology*, 2000, R830-833. Accessed November 30, 2014. http://www.cell.com/current-biology/abstract/S0960-9822(00)00792-2?cc=y.

Jabr, Ferris. "How Did Insect Metamorphosis Evolve?" Scientific American Global RSS. August 10, 2012. Accessed June 13, 2014. http://www.scientificamerican.com/article/insect-metamorphosis-evolution/.

Konopova, Barbora, Vlastimil Smykal, and Marek Jindra. "Common and Distinct Roles of Juvenile Hormone Signaling Genes in Metamorphosis of Holometabolous and Hemimetabolous Insects." PLOS ONE:. December 8, 2011. Accessed November 29, 2014. http://www.plosone.org/article/info:doi/10.1371/journal.pone.0028728#s1.

Meyer, John R. "Hormonal Control of Molting & Metamorphosis." NC State University General Entomology. January 1, 2005. Accessed August 15, 2014. http://www.cals.ncsu.edu/course/ent425/tutorial/endocrine2.html.

Mitra, Anirudda. "Cinderella's Shoes--how and Why Insects Remodel Their Bodies between Life Cycles." *Current Science* 104, no. 8 (2013): 1028-036. Accessed January 1, 2014. http://www.academia.edu/3224811/Cinderella_s_new_shoes_how_and_why_insects_remodel_their_bodies_between_life_stages.

Nemec, Vaclav. "Juvenoids: From Basic Research to Practical Use. A Short Review." *Bulletin of Insectology* 48 (1994): 67-74. Accessed November 30, 2014. http://www.bulletinofinsectology.org/pdfarticles/vol48-1994-067-074nemec.pdf.

Pechenik, Jan A. "The Arthropods." In *Biology of the Invertebrates*. 5th ed. Boston: McGraw-Hill, Higher Education, 2005.

"The Butterfly Effect: How a Little (Climate) Change Can Lead to Chaos." Conservation Biology News. September 4, 2012. Accessed November 30, 2014. https://conservationbiologynews.wordpress.com/2012/09/04/the-butterfly-effect-how-a-little-climate-change-can-lead-to-chaos/.

Truman, James W., and Lynn M. Riddiford. "Endocrine Insights Into the Evolution of Metamorphosis in Insects." *Annual Review of Entomology* 47 (2002): 467-500. 2002. Accessed June 15, 2014. http://web.neurobio.arizona.edu/gronenberg/nrsc581/neuromodulation/endocrineevolution.pdf.

"What Are Endocrine Disruptors?| Endocrine Disruptor Screening Program | US EPA." EPA. August 11, 2011. Accessed November 30, 2014. http://www.epa.gov/endo/pubs/edspoverview/whatare.htm.

Teacher Resources

"How Reception Works in Cell Signaling PowerPoint Templates." How Reception

Works in Cell Signaling PowerPoint Templates. https://www.boundless.com/physiology/textbooks/boundless-anatomy-and-physiology-textbook/cellular-structure-and-function-3/how-reception-works-in-cell-signaling-43/how-reception-works-in-cell-signaling-43-powerpoint-templates/. –contains very helpful diagrams, textbook readings and quizzes on cell signaling.

Meyer, John R. "THE TRANSFORMERS." The Transformers. December 30, 2013. http://www.cals.ncsu.edu/course/ent525/trans.html.-- source of pictures for types of metamorphosis.

http://www.pbs.org/wgbh/nova/education/activities/3313_03_nsn.html --**Nova activity** on Obesity and hormones using models.

https://www.youtube.com/watch?v=TCeYRayiybQ&app=desktop Fly life cycle video.

http://www.wormman.com/breeding_superworms.cfm --Good info on breeding superworms

http://www.westknollfarm.com/Meal-Worms.html --More info on raising mealworms

https://www.youtube.com/watch?v=ex-jbQtClPM --video showing materials used to breed superworms.

http://www.sciencedirect.com/science/article/pii/S0960982210002915 --article on imaginal discs.

http://www.nature.com/nrg/journal/v7/n12/box/nrg1989_BX4.html --has good graph of insect hormones changes

http://www.mun.ca/biology/desmid/brian/BIOL3530/DEVO_13/ch13f17.jpg –excellent image of molting process

http://www.mun.ca/biology/desmid/brian/BIOL3530/DEVO_13/ch13f18.jpg --good image of hormones and metamorphosis

http://www.biographix.cz/wp-content/uploads/2014/04/Bombyx_Bugs_72dpi.jpg --image of hormones and metamorphosis

https://www.boundless.com/physiology/textbooks/boundless-anatomy-and-physiology-textbook/cellular-structure-and-function-3/how-reception-works-in-cell-signaling-43/how-reception-works-in-cell-signaling-43-powerpoint-templates/ --Boundless.com provides sharable, editable PowerPoints and resources on all kinds of topics for free if

you sign up. This one is on cell signaling and helped me with images and a PowerPoint to help students understand how hormones lead to expression of genes.

Boundless.com –Check this out! Free online textbooks for teachers of AP and IB level classes. You can assign reading, quizzes and there are editable PowerPoints you can use in class.

http://kidshealth.org/classroom/9to12/body/systems/endocrine.pdf --lesson plans and activities on the endocrine system.

http://highered.mheducation.com/sites/9834092339/student_view0/chapter46/intracellular_receptor_model.html --short animation of intracellular receptors and hormones. With quiz.

http://www.vivo.colostate.edu/hbooks/pathphys/endocrine/index.html --informational resource for teachers and students on the endocrine system and how it works.

http://www.niehs.nih.gov/health/assets/docs_a_e/ehp_student_edition_lesson_are_edcs_blurring_issues_of_gender_508.pdf --article analysis activity on endocrine disruptors.

http://entomology.unl.edu/k12/mealworms/development.htm --example of mealworm experiment with temperature

http://www.oardc.ohio-state.edu/gk12/images/Climate_Change_&_Insect_Developent.pdf --lesson plan for testing the effects of temperature on insect metamorphosis—focus on climate change.

http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0068107 --experiment to see if food or temperature made a difference when insects exposed to a pesticide

Notes

[1] Meyer, John R. "THE TRANSFORMERS." The Transformers. December 30, 2013. Accessed October 5, 2014. http://www.cals.ncsu.edu/course/ent525/trans.html.
[2] Mitra, Anirudda. "Cinderella's Shoes--how and Why Insects Remodel Their Bodies between Life Cycles." *Current Science* 104, no. 8 (2013): 1028-036. Accessed January 1, 2014.
http://www.academia.edu/3224811/Cinderella_s_new_shoes_how_and_why_insects_remodel_their_bodies_between_life_stages. p1028
[3] Ibid p1029.
[4] Meyer.
[5] Ibid.
[6] Mitra. p1029.
[7] Pechenik, Jan A. "The Arthropods." In *Biology of the Invertebrates*. 5th ed. Boston: McGraw-Hill, Higher Education, 2005.p377.
[8] Truman, James W., and Lynn M. Riddiford. "Endocrine Insights Into the Evolution of Metamorphosis in Insects." *Annual Review of Entomology* 47 (2002): 467-500. 2002. Accessed June 15, 2014. p 469.
http://web.neurobio.arizona.edu/gronenberg/nrsc581/neuromodulation/endocrineevolution.pdf.
[9] Ibid.
[10] Ibid p. 469-470.
[11] Ibid p.472.
[12] Ibid p.473.
[13] Ibid.
[14] Jabr, Ferris. "How Did Insect Metamorphosis Evolve?" Scientific American Global RSS. August 10, 2012. Accessed June 13, 2014.
http://www.scientificamerican.com/article/insect-metamorphosis-evolution/.
[15] Truman and Riddiford. p.473.
[16] Jabr.
[17] Truman and Riddiford. p.474-475.
[18]Aldaz, Silvia, and Luis M. Escudero. "Quick Guide--Imaginal Discs." Current Biology. Accessed October 3, 2014.
http://www.sciencedirect.com/science/article/pii/S0960982210002915.
[19] Jabr.
[20] Truman and Riddiford. p.476.
[21] Ibid p. 475.
[22] Ibid, p480-481.
[23] Mitra, p.1030.
[24] Ibid.
[25] Truman and Riddiford, p.483.
[26] Mitra, p.1030.
[27] Truman and Riddiford, p.487.

[28] Ibid, p.480-481
[29] Konopova, Barbora, Vlastimil Smykal, and Marek Jindra. "Common and Distinct Roles of Juvenile Hormone Signaling Genes in Metamorphosis of Holometabolous and Hemimetabolous Insects." PLOS ONE: December 8, 2011. p.1. Accessed November 29, 2014. http://www.plosone.org/article/info:doi/10.1371/journal.pone.0028728#s1.
[30] Ibid p.1-2.
[31] Truman and Riddiford, p. 491.
[32] Mitra, p.1034.
[33] Ibid, p1032.
[34] Ibid, p.1031.
[35] Pechenik, p 380.
[36] Meyer, John R. "Hormonal Control of Molting & Metamorphosis." NC State University General Entomology. January 1, 2005. Accessed August 15, 2014. http://www.cals.ncsu.edu/course/ent425/tutorial/endocrine2.html.
[37] Ibid.
[38] Mitra, p. 1032.
[39] Ibid, p. 1031-1032.
[40] Ibid, p.1032.
[41] Buszczak, Michael, and William A Segraves. "Insect Metamorphosis: Out with the Old, in with the New." *Current Biology*, 2000, R830-833. Accessed November 30, 2014. http://www.cell.com/current-biology/abstract/S0960-9822(00)00792-2?cc=y.
[42] Ibid.
[43] "The Butterfly Effect: How a Little (Climate) Change Can Lead to Chaos." Conservation Biology News. September 4, 2012. Accessed November 30, 2014. https://conservationbiologynews.wordpress.com/2012/09/04/the-butterfly-effect-how-a-little-climate-change-can-lead-to-chaos/.
[44] "What Are Endocrine Disruptors?| Endocrine Disruptor Screening Program | US EPA." EPA. August 11, 2011. Accessed November 30, 2014. http://www.epa.gov/endo/pubs/edspoverview/whatare.htm.
[45] Nemec, Vaclav. "Juvenoids: From Basic Research to Practical Use. A Short Review." *Bulletin of Insectology* 48 (1994): 67-74. Accessed November 30, 2014. P.70-72. http://www.bulletinofinsectology.org/pdfarticles/vol48-1994-067-074nemec.pdf.
[46] Truman and Riddiford, p 469.