

Supplemental Material

CBE—Life Sciences Education

Hiatt *et al.*

Appendix 1. Questions for Exploratory Surveys and Interviews

Surveys

1. Exploratory questions
2. Exploratory questions
3. Exploratory questions
4. Think aloud interview questions

Survey 1: Survey 1, MCU: 311 students from lower level classes on animal biology, plant biology, and introductory biology and upper level classes on evolution, stream invertebrates, anatomy and physiology, environmental toxicology, and science education research methods). PHS: four high school seniors enrolled in a primate biology class. Survey 2, RIU: 42 students in a senior-level evolution course.

Exploratory Assessment

What follows is a list of exploratory questions that will be used to develop a conceptual inventory of evolutionary developmental biology. Students should write brief written responses to each of the following questions. In a subset of cases, these written responses can be followed up with one-on-one interviews. Guidelines for what the administrator should be looking for plus suggestions for follow-up questions during the interviews are provided in brackets for each of the questions. Attached find an un-annotated set of questions that can be provided to the students.

Role of genes in development

1. You found a chicken egg, you hatched it out and observed that the chicken has a beak. Can you describe how your chicken's beak was formed?

[Here we are looking for mention of the following: genes (plural, i.e., not *gene*), *gene expression*, complex interactions during development.]

Ultimate causes

2. On the Galapagos there are birds (finches) with beaks of different shapes and sizes. Where did the different beak shapes in these species come from?

[Here we are looking for mention of the following: natural selection, genes or phenotype, drift, different food sources.]

Proximate causes

3. Say you collected eggs from two Galapagos finch species and hatched them out and they have different beak shapes. In these two birds what causes the different beak shapes?

[Here we are looking for mention of differences in gene expression, but also differences in the amino acid sequences of proteins (and hence changes in the function of those proteins) as potential explanations. If students simply say "Embryonic development was different", try to follow up with: "What about it was different?"

Why?". If students say "They have different genes", follow up with "How do you mean different? Do you mean one species has a gene that the other one does not or do you mean that the different birds have different versions of the same gene?" (i.e., different alleles).]

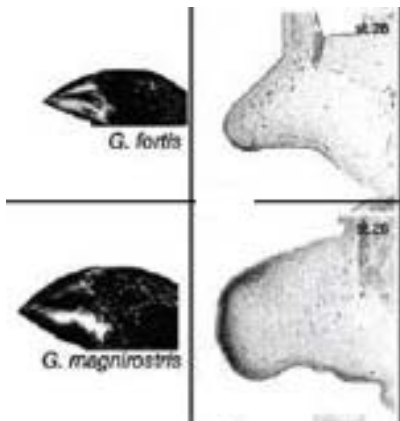
Change in gene expression as proximate cause

4. You have determined that a single gene is responsible for the difference in beak shape. [If students question how this was done, just tell them it was genetically mapped by doing crosses between the two species because they have fertile hybrids.] In your two individual birds you discover that the protein-coding regions of this gene are identical. Given this information, what do you think might cause the different beak shapes in these two birds?

[Here we are looking for the insight that, with this new information, it is now clear that differences in gene expression must be responsible. If students again suggest a different gene, remind them that we have already established that this gene was responsible.]

Molecular basis of change in gene expression

5. In your two birds, when you examine the embryonic tissue that gives rise to the beak, you discover that there are different levels of expression of the gene (amount of mRNA) you identified as responsible for differences in beak shape.



What might be causing these different levels of gene expression?

[Here we are looking for mention of cis-regulatory mutation(s) in enhancers (or the “promoter”) and/or epigenetic (methylation, etc). If students mention another gene again, remind them again that we've established that *this* gene is responsible. If they repeat that there are differences in the expression of this gene, ask them again what could be causing these differences.]

Synthesis of answers to questions 1-5

6. Now that you have all this information about genes and gene expression in your two birds, can you tell me again how these two birds have come to have such different beaks?

[Here we are looking for mention of the following: an initial mutation in an enhancer, the selection of the resulting phenotype (not the genotype directly) due to environmental challenges such as different food sources, and eventual fixation of the mutation causing a change in the beak shape distribution for the population. Watch for misconception that different food sources induced mutation (directed mutation).]

Deep Time

7. How long might it take for a population with similar beak shapes to change into a population with several different beaks?

Polymorphism versus polyphenism

8. As an assignment for your freshwater sciences class you have been collecting snails of the species *Physa gyrina* from different streams. You notice that their shells fall into two shapes, one thick-shelled and one thin-shelled. Imagine that you are holding these two types of snails, one of each type. How might you explain how it is that these two snails have such different shells?

[Here we are looking for mention of different genes/alleles or (possibly) environment. If student does not mention environmental influences, just proceed to next question.]

Correlation with possible environmental cue

9. You notice in comparing collections with your classmates that the snails of thicker shell occur in the streams with crayfish (and snails of thinner shell occur in streams without crayfish). How could crayfish be causing the differences in shell thickness?

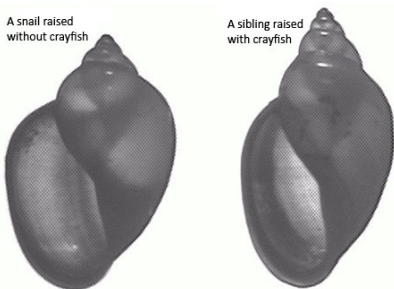
[Here we are looking for mention of local adaptation by natural selection and possibly environmental induction of greater thickness. If student doesn't mention latter, just proceed to next question.]

Suggestive evidence that thick shells are environmentally cued

10. Upon closer analysis, you discover that there are almost no genetic differences between the snail types. Given this information, what could be causing the difference in shell thickness?

[Here we are looking for mention of environmental induction of greater thickness. If student doesn't mention this, just proceed to next question.]

Confirmation that thick shells are environmentally cued



11. You decide to do an experiment and bring snail egg cases of *Physa gyrina* from the stream with no crayfish into your lab and place them into aquaria, some with crayfish and some without crayfish. You find that the snails in the presence of crayfish all develop with thicker shells while all of the snails without crayfish develop with thin shells. Explain these results.

[Here we are looking for a clear statement that the greater thickness is environmentally induced.]

Evolution of plasticity

12. Upon looking at the streams more closely, you notice that another closely related species, *Physa aplexa*, is also present in your streams, both with and without crayfish, but only exhibits thin shells. What is the difference between these two species?

How did this difference between the species arise?

[Here we are looking for a statement that the plastic response (in *Physa gyrina*) evolved in response to predation by crayfish. Follow up this answer by pointing that only heritable differences can be selected and that the student already noted that is an environmentally induced response. Ask the student, then, how this could have evolved? Here we are looking for mention that it is genetic variation underlying the response that has been selected.]

Conditions under which plasticity evolves

13. If crayfish had **always** been present (in high density and all seasons) in the past, would you have expected the same ability to develop thin and thick shells to evolve?

[Here we are looking for the idea that if crayfish had been constantly present the plastic response is less likely to have evolved; rather, simply evolving thick shells would have been more likely.]

Speciation

14. Assume you have two closely related fly species, Species A and Species B. You are bit surprised that when you force individuals from these two fly species to mate with each other, you in fact get both male and female progeny. On closer inspection you discover that the males, however, are sterile. Can you speculate as to why these hybrid males are sterile?



Species A



Species B

[Looking for notions of genetic incompatibility.]

You proceed to study these two species intensively. In one experiment, you discover that one copy of a single gene from Species B, when inserted into the genome of a male of Species A, causes this male to be sterile. Of the following, where might you expect this gene to be expressed?

- A. The developing heart
- B. in all cells
- C. in the developing testes
- D. in sensory cells of the antennae
- E. in the cells that produce male-specific pigmentation

Can you briefly describe why you chose this answer?

What are your thoughts about the role this gene (and its protein product) might play?

[Looking for explanation of choice c, namely that one would expect the gene to be expressed in the testes because it probably plays a critical role in reproductive development.]

Survey 2

Survey 2, RIU: 42 students in a senior-level evolution course.

Evo-Devo Survey

User ID: _____

Thank you for agreeing to participate in this study. Please answer the following questions about evo-devo concepts to the best of your ability. Please provide a detailed explanation and answer to each of the questions and avoid short responses.

Insects such as the fruit fly *Drosophila* possess three pairs of legs while other arthropods (e.g., *Artemia*, brine shrimp) can possess many more pairs of legs. In all arthropods, including insects, Dll is a regulatory gene that is required for leg formation.



Drosophila



Artemia

1. Provide an explanation for why *Drosophila* have fewer legs than *Artemia*.

Ubx is a gene that determines the features present in different segments along the body. In insects Ubx is expressed during embryonic development in the abdomen (the posterior portion of the body where there are no legs), but not in the thorax (the more anterior portion of the body that possesses legs) (see Figure 1).

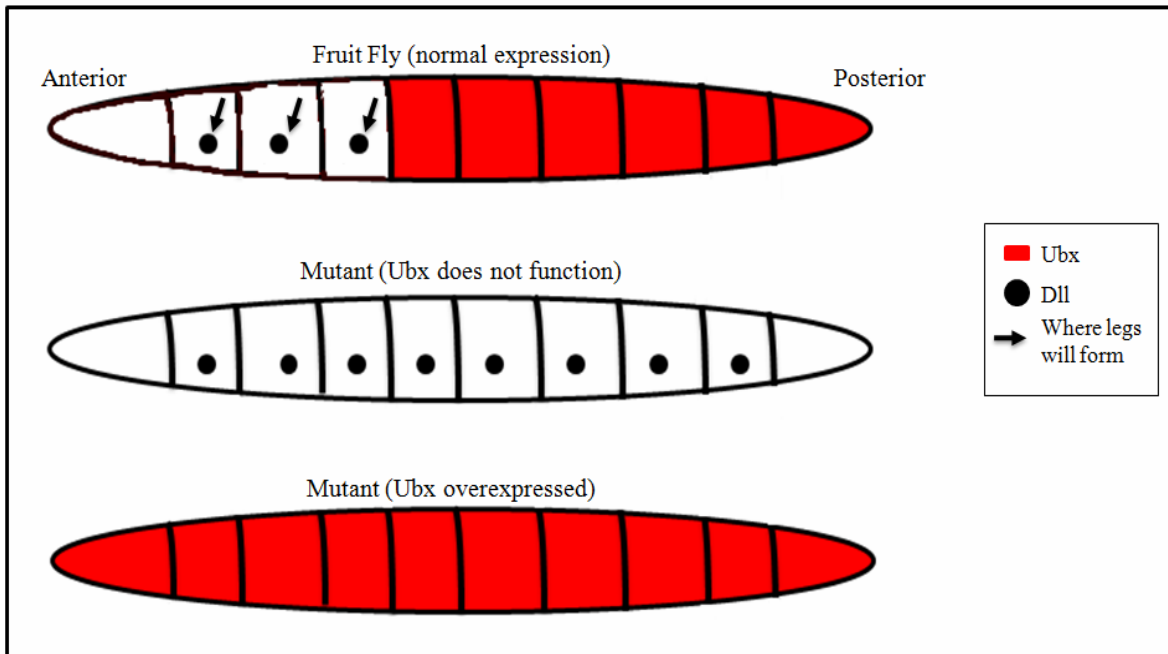


Figure 1. Normal expression of Ubx and Dll in the *Drosophila* embryo and in mutant embryos where Ubx is either not functional and is not expressed or is expressed all over the embryo. The views are only of the left side of the embryo.

Dll, on the other hand, is expressed in three pairs of spots in the thorax, corresponding to the three pairs of legs, but not in the abdomen. In a mutant of the fruit fly *Drosophila* in which Ubx does not function, scientists have discovered that Dll is misexpressed in pairs of spots along the entire abdomen. Conversely, in mutants in which Ubx is expressed all over the embryo, Dll is no longer expressed (see Figure 1).

2. Provide a possible explanation for why insects do not possess abdominal legs but crustaceans do.

Interestingly, when scientists investigated Ubx expression in crustaceans such as *Artemia* they found that crustaceans DO possess Ubx and it IS expressed in the abdomen. Scientists have also been able to express the *Artemia* version of Ubx throughout the *Drosophila* embryo, as they were able to do with the *Drosophila* version of the gene (see Figure 2).

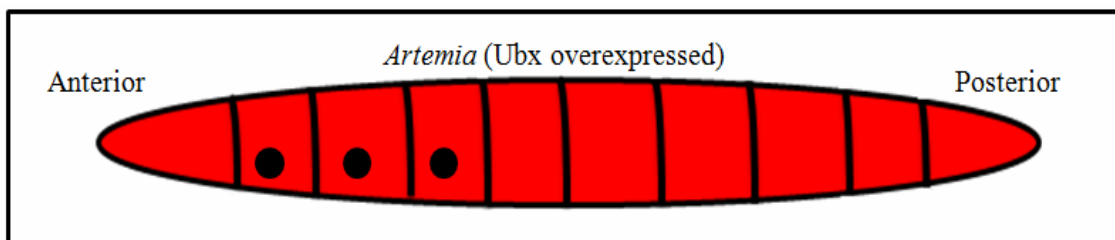


Figure 2. *Artemia* Ubx expression in a *Drosophila* embryo.

3. Given this additional information, can you think of another possible mechanism by which insects lost their legs?

Observe the figure below (Figure 3). All of the mice are genetically identical. The agouti gene is expressed at normal levels in the dark brown mice and the gene is over-expressed in the yellow mice. The Agouti gene results in yellow coloration and obesity. Considering this information, please answer the following questions in 100 words or more.



Figure 3. Agouti mice.

4. How is it possible for the mice to be genetically identical yet vary in phenotype?
5. How can changes in parental diet during gestation or development influence the resulting phenotype of the offspring?
6. The mottled mice vary in the amount of Agouti they express, with increased yellow pigmentation indicating an increase in Agouti expression. How could changes in the amount of gene expression result in novel phenotypes (new characteristics and forms)? Provide an example other than the one provided or illustrate if possible.
7. Many of the genes that specify the formation of the eyes, the heart, the nervous system and the anterior-posterior body axis all seem to be the same throughout the animal kingdom, despite the enormous diversity of these structures. **Explain** how it is possible for a limited number of genes to result in such a large number of phenotypes?

3. **Birds have necks of very different lengths. The long-necked birds have more neck vertebrae. Almost all mammals on the other hand, have only 7 neck vertebrae (even giraffes!). What is a most plausible explanation for this lack of variation in mammals?**

Observe the image below. All of the mice are genetically identical. The agouti gene is expressed at normal levels in the dark brown mice and the gene is over-expressed in the yellow mice. The Agouti gene results in yellow coloration and obesity. Considering this information, please answer the following questions.



Image from Morgan et al 1999

5. **How is it possible for the mice to be genetically identical yet vary in phenotype?**

6. **How can changes in parental diet during gestation or development influence the resulting phenotype of the offspring?**

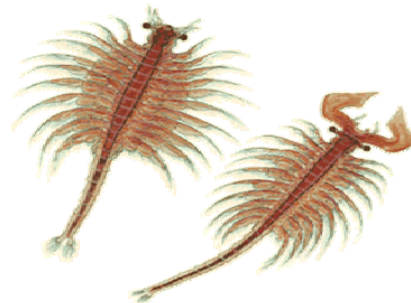
7. The mottled mice vary in the amount of Agouti they express, with increased yellow pigmentation indicating increase in Agouti expression. How could changes in the amount of gene expression result in novel phenotypes (new characteristics and forms)? Provide an example other than the one provided or illustrate if possible.

8. Many of the genes that specify the formation of the eyes, the heart, the nervous system and the anterior-posterior body axis all seem to be the same throughout the animal kingdom, despite the enormous diversity of these structures. Explain how it is possible for a limited number of genes to result in such a large number of phenotypes?

I. Insects such as *Drosophila* possess three pairs of legs while other arthropods (e.g., *Artemia*, brine shrimp) can possess many more pairs of legs. In all arthropods, including insects, Dll is a regulatory gene that is required for leg formation.



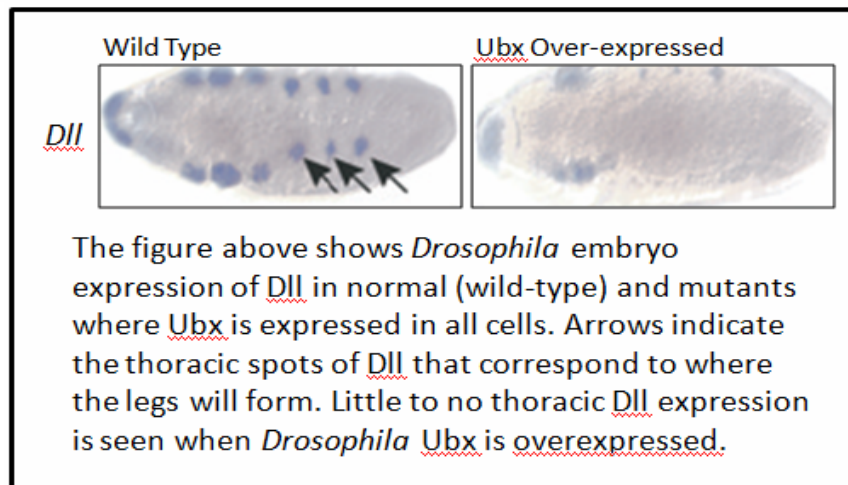
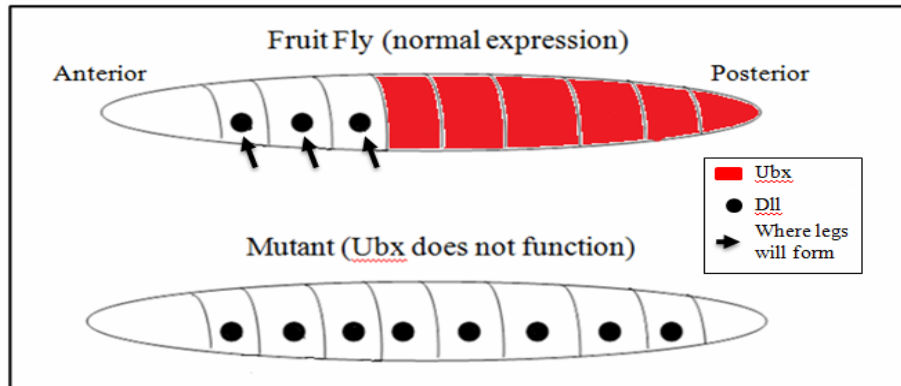
Drosophila



Artemia


9. Provide an explanation for why *Drosophila* have fewer legs than *Artemia*.

II. *Ubx* is a gene that determines the features present in different segments along the body. In insects *Ubx* is expressed or turned on during embryonic development in the abdomen (the posterior portion of the body where there are no legs), but not in the thorax (the more anterior portion of the body that possesses legs). *Dll*, on the other hand, is expressed in three pairs of spots in the thorax, corresponding to the three pairs of legs, but not in the abdomen. In a mutant of the fruit fly *Drosophila* in which *Ubx* does not function, scientists have discovered that *Dll* is misexpressed in pairs of spots along the entire abdomen.



10. Provide a possible mechanism for why insects do not possess abdominal legs.

Artemia Ubx Over-expressed in *Drosophila* embryo

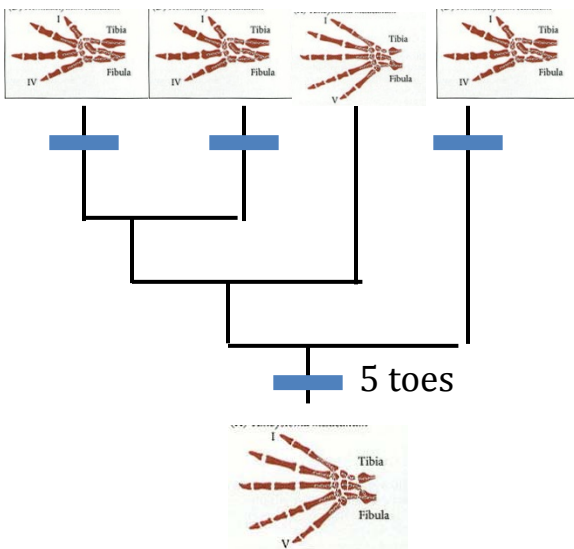


The figure shows Dll expression in a *Drosophila* embryo in which Artemia Ubx has been over-expressed. Thoracic spots of Dll are still present.

11. Given the additional information above, why, then, do crustaceans possess abdominal legs?

Interestingly, when scientists investigated Ubx expression in crustaceans they found that Ubx is expressed in the abdomen

12. Given this new information, can you think of another possible mechanism by which insects lost their legs?



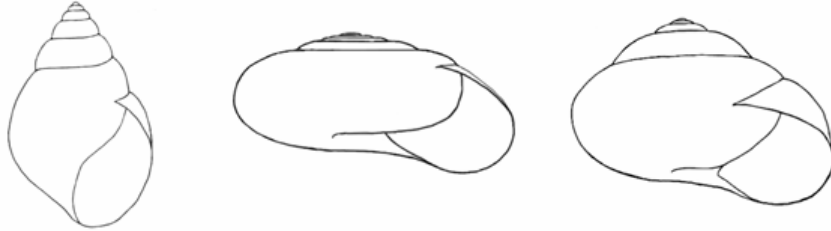
13. The common ancestor of the salamanders had 5 toes on each foot. Many species of salamanders have only 4 toes, and in each case they've lost the "thumb". What is a plausible explanation for this?

Survey 4. Think Aloud Interviews.

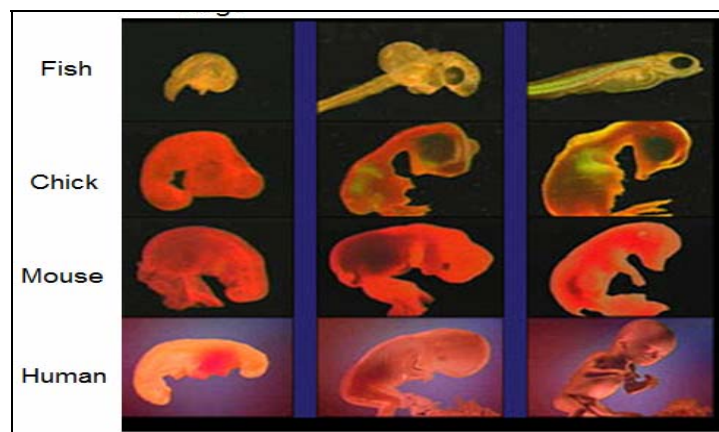
Evo-Devo Survey – Interviews, Subset 1

Script: Thank you for participating in our research project. I will be asking you several questions related to common biological concepts. As you contemplate each question we may ask you to describe your thought process and/or ask additional questions about your response. We may ask you to describe and explain your answer multiple times.

1. Here is picture of snail shells with different shapes.

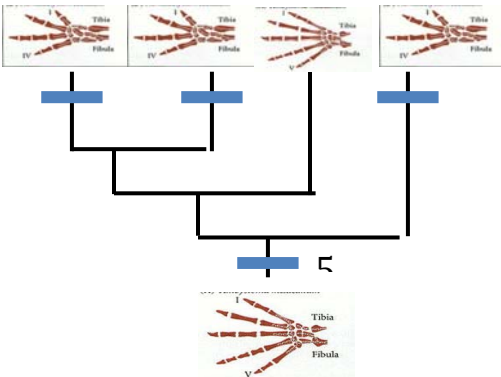


- a. What makes these shells possess different shapes? (*Concept 1.a*--looking for proximate mech)
 - b. Why do we have so many different types of snails? (*Concept 1.b*) looking for ultimate)
2. What determines the physical shape of an *organism*? (Ask to define if they use the following terms: gene, allele, expression, regulation, mutation)(*Concept 1.d and 1.f*--looking for developmental mechanisms such as cell division and growth, and all the things that regulate these processes)
 3. Show picture of embryo in several different stages.



- a. What is similar about the embryos in this picture? What is different? (*Concept 3.i and/or 2*--species develop similar structures overall, but details differ)

- b. What causes the embryos of different species to differ in physical appearance over the course of their development? (*Concept 1.d and 3.a*--looking for differences in development controlled by differences in regulatory genes)
 - c. Why do the embryos of many different species appear to develop in similar ways? (*Concept 2.a and 2.c*--looking for shared developmental processes due to shared ancestry)
 - d. What could happen if there was a change in the way an embryo developed? (*Concept 1.b, 3.a, 3.g*--looking for change in the phenotype or developmental outcome)
 - e. Can you describe an example of this kind of change? (*Concept 1.c, 3.a, 3.f, and 3.i*)
 - f. What role might genes play in determining the physical shape of an *embryo*? (Ask to define if they use the following terms: gene, allele, expression, regulation, mutation) (*Concept 1.c*, looking for developmental mechanisms such as cell division and growth, and all the things that regulate these processes)
 - g. How do new physical features come about? (looking for any of the following: a gene that regulates development mutates and this variant becomes fixed in a population due to the increased fitness it conveys to the individual possessing the mutation in virtue of the resulting change in phenotype)
4. Birds have necks of very different lengths. The long-necked birds have more neck vertebrae. Almost all mammals on the other hand, have only 7 neck vertebrae (even giraffes!). What is a most plausible explanation for this lack of variation in mammals?



5. The common ancestor of the salamanders had 5 toes on each foot. Many species of salamanders have only 4 toes, and in each case they've lost the "thumb". What is the most plausible explanation for this?
6. Is there any way a structure can stay the same without natural selection?

Evo-Devo Survey – Interviews, Subset 2

Script: Thank you for participating in our research project. I will be asking you several questions related to common biological concepts. As you contemplate each question we may ask you to describe your thought process and/or ask additional questions about your response. We may ask you to describe and explain your answer multiple times.

1. The following picture shows mice that are genetically identical (like identical sextuplets that are the result of one fertilized egg splitting into six identical cells). [If respondents define and use appropriate definition of gene and protein, continue on to the following questions]

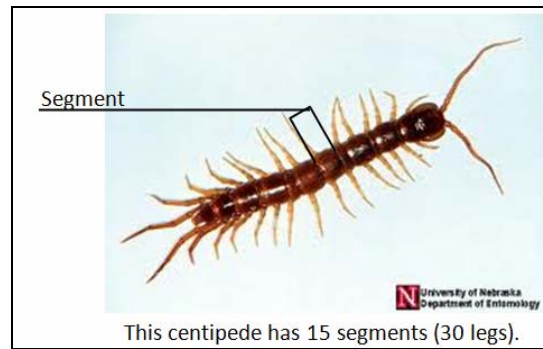


- a. How it is possible for these mice to look so different? (looking for environmental effects)
- b. What if I told you that in the lighter colored mice there was a greater amount of substance produced called AG? How is it possible to have different amounts of AG made when the genes are the same? (looking for environmental effects on gene regulation)
- c. Scientists think these differences in color might result from the type of food a mother eats while pregnant. They test several different types of food and find the following:

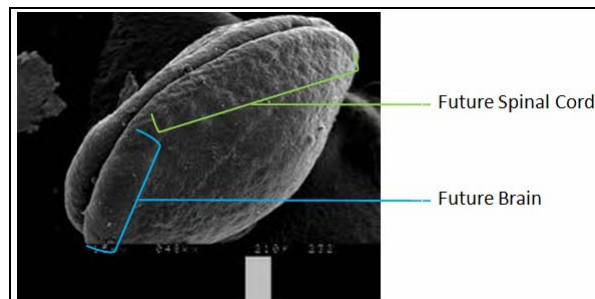
Mother's Diet	Coat Color of Offspring	AG production in offspring
100% Corn	Dark brown	none
50/50 Corn and soybean mix	Spotted, brown and yellow	Slight increase
100% Soybean	Yellow	High increase

What do these data indicate? (soybeans increase AG production leading to yellow coloration)

2. All centipedes have an odd number of leg-bearing segments. Centipedes vary in the number of leg-bearing segments, from as few as 5 to as many as 125, but none possess an even number.
 - a. How might you explain this fact?
 - b. If student provides a NS answer that even numbers are less fit or maladaptive, provide with following scenario: Scientists conduct an experiment where they physically cut the legs off of centipedes so that there are an even number of leg pairs (e.g.: 100 legs, 96 legs, etc) and find there is no effect on how well they survive and reproduce in the wild. Then, why are there no centipedes with an even number of leg pairs found in the wild? [Bias—alternative to Salamander question] Show centipede image and point out segment.
 - c. What would an experimental biologist do get an even number of leg-bearing segments?



3. The following embryo is undergoing a stage of development called neurulation. This is the first step to forming the spinal cord and brain.



- a. This process is the same in all animals with a spine (vertebrates). Given that changes in genetic material are always occurring, why has there been so little change in this process over time? [developmental constraint or, alternatively, “selection” acting on the developing embryo, independent of environmental interactions; in the end point is that *most changes* would result in embryo not be viable]
 - b. What would happen if there was a change in this process? [inviability or, rarely, novelty]
4. Many of the genes that specify the formation of the eyes, the heart, the nervous system and the anterior-posterior body axis all seem to be the same throughout the animal kingdom, despite the enormous diversity of these structures. Explain how it is possible for a limited number of genes to result in such a large number of phenotypes?
5. Is there any way a structure can stay the same without natural selection?

Evo-Devo Survey – Interviews, Subset 3

Script: Thank you for participating in our research project. I will be asking you several questions related to common biological concepts. As you contemplate each question we may ask you to describe your thought process and/or ask additional questions about your response. We may ask you to describe and explain your answer multiple times.

Insects such as *Drosophila* have three pairs of legs while other arthropods (e.g., *Artemia*, brine shrimp) have many more pairs of legs. In all arthropods, including insects, Dll is a gene that is required for leg formation.



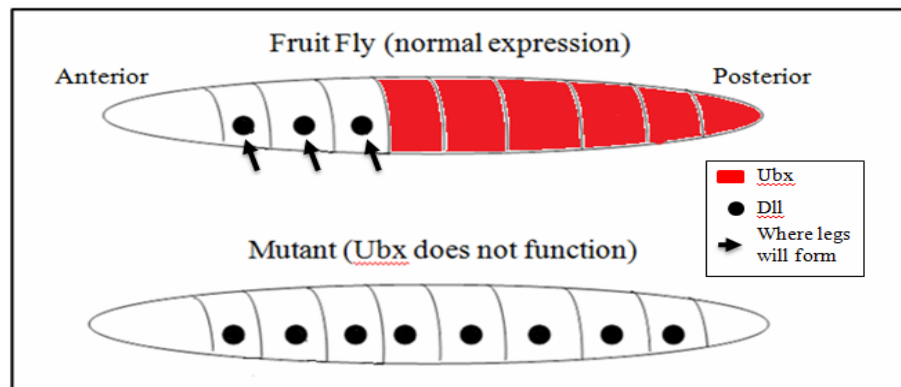
Drosophila

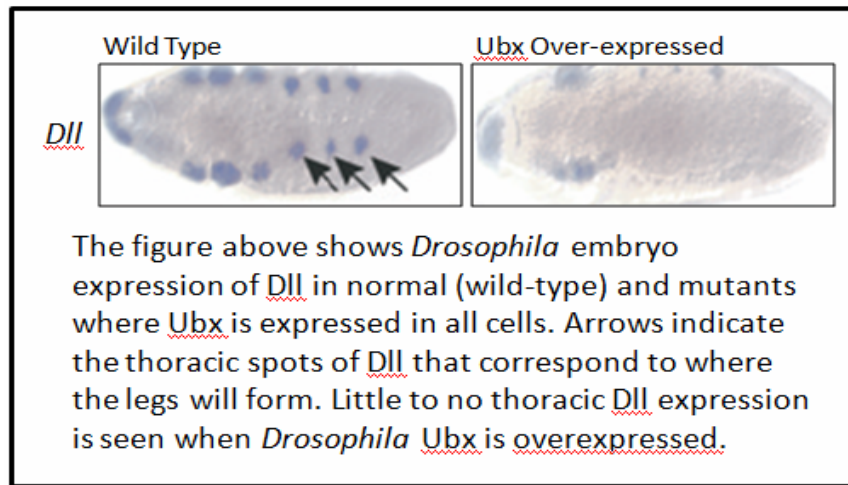


Artemia

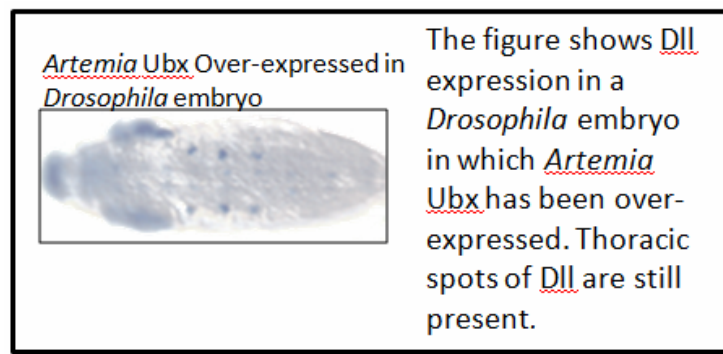
14. Provide an explanation for why *Drosophila* have fewer legs than *Artemia*.

Ubx is a gene that determines the features present in different segments along the body. In insects Ubx is expressed or turned on during embryonic development in the abdomen (the posterior portion of the body where there are no legs), but not in the thorax (the more anterior portion of the body that possesses legs). Dll, on the other hand, is expressed in three pairs of spots in the thorax, corresponding to the three pairs of legs, but not in the abdomen. In a mutant of the fruit fly *Drosophila* in which Ubx does not function, scientists have discovered that Dll is misexpressed in pairs of spots along the entire abdomen.





2. Provide a possible mechanism for why insects do not possess abdominal legs.



3. Given the additional information above, why, then, do crustaceans possess abdominal legs?

Interestingly, when scientists investigated Ubx expression in crustaceans they found that Ubx is expressed in the abdomen

4. Given this new information, can you think of another possible mechanism by which insects lost their legs?
5. Is there any way a structure can stay the same without natural selection?

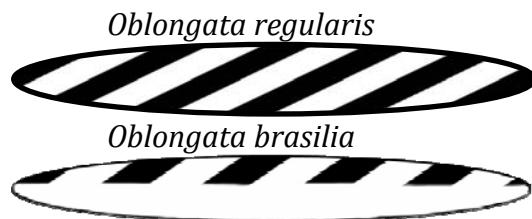
Evo-Devo Survey – Interviews, Subset 4

RIU: 14 students who identified as biology majors.

Script: Thank you for participating in our research project. I will be asking you several questions related to common biological concepts. As you contemplate each question we may ask you to describe your thought process and/or ask additional questions about your response. We may ask you to describe and explain your answer multiple times.

In the following questions (*Oblongata* and *Danio* questions) if students are not able to define gene, allele, genome, and protein appropriately, then use alternative terminology.

1. The gene (alt: genetic material) *Diaganum* is required for diagonal stripes to form along worms belonging to the genus *Oblongata*. While traveling through Brasil, you come across a kind of *Oblongata* that only has diagonal stripes on its back and no stripes on the belly.



- a. Explain what change occurred that might have resulted in this new pattern. (If answer is mutation, ask to describe what kind of mutation, mutation in what gene?)

When you return from your trip you decided to see what other patterns of *Oblongata* exist around the world. You find the following patterns:

i.



ii.



iii.



iv.



v.



- b. Explain how these changes in pattern may have occurred. (ask to define any terminology or jargon used).

- c. Is Diaganum the only gene (alt: genetic material) responsible for the patterns you observe? Could more than one gene be responsible for these patterns? If so, how? [most traits are polygenic, there is also gene interaction]
 - d. Is the gene Diaganum found in all *Oblongata* cells? (If yes, stop. If no, ask which cells)
 - i. If yes, ask: If found all cells, then what makes each cell different?
(looking for distinction between gene itself (found in all cells) and gene product/mRNA/protein (found in only some cells))
 - e. Do you think the Diaganum gene could be responsible for more than just stripe formation?
 - f. The Diaganum gene is not always on. When and where do you think Diaganum is not on? (Does student assume this is occurring during development?) [looking for temporal and spatial dynamics of gene expression]
 - g. What do you think is making the Diaganum gene turn off?
 - h. What would happen to the physical pattern of an *Oblongata* if Diaganum were turned on longer? Not turned on at all? What would cause a gene (alt: genetic material) to change in that way? [Temporal]
 - i. What are some other ways the pattern might be controlled?
 - j. You notice some *Oblongata* worms have thicker stripes and some have thinner stripes. What might have caused this to happen? [Spatial]
2. You decided that you want to look at how Diaganum gene functions in other organisms. You find that Diaganum plays a role in a large number of patterns seen in butterflies, fishes, and many mammals, like bears.
- a. How can a single gene (alt: element of genetic material) be responsible for so many different color patterns in so many different species? (looking for developmental differences in expression and developmental differences downstream of Diaganum—that is, targets might be different in different species)
 - b. When comparing the products of the Diaganum gene of worms and bears, there have been very few changes. Why would these products stay unchanged for so long?
 - c. What do you think would happen if the Diaganum gene did not work?
 - d. Some say that new forms evolve as the result of new tools (new genes, new pathways, etc.). Others claim new forms evolve as the result of using old tools in new ways. Which of these descriptions best depicts the role Diaganum has played in the evolution of pigment pattern? Can you think of an example to explain the difference between truly novel traits and tinkering with the old?
3. Is there any way a structure can stay the same without natural selection?

4. There is a species of fish that have recently populated a cave. These fish have lost their vision (development of their eyes) much quicker than would be predicted if it were by random mutations occurring in the absence of the selection for vision. In fact, it seems that these fish are better at sensing the water because of enhanced nostrils, since it helps the fish to survive and multiply.
 - a. Explain why you think the cave fish lost their vision. (positive selection for increased olfactory senses on a pleiotropic gene resulting in loss of eyes)
 - b. Why do fish that do not live in caves not have enhanced nostrils? (maintenance selection for vision greater than increased olfactory or no positive selection for the trait)

Evo-Devo Survey – Interviews, Subset 5

Script: Thank you for participating in our research project. I will be asking you several questions related to common biological concepts. As you contemplate each question we may ask you to describe your thought process and/or ask additional questions about your response. We may ask you to describe and explain your answer multiple times.

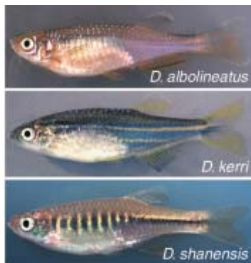
In the following questions (Oblongata and Danio questions) if students are not able to define gene, allele, genome, and protein appropriately, then use alternative terminology.

1. The gene Puma is required for stripe formation in Zebrafish, genus *Danio*. There are several different pigments (colored cells) that make up the patterns that are observed. The following picture shows two kinds of Zebrafish found in Asia.



- a. Explain what change occurred that might have resulted in this new pattern. (If answer is mutation, ask to describe what kind of mutation, mutation in what gene?)

Here are several other kinds of Zebrafish found in Asia:



- b. Explain how these changes in pattern may have occurred. (ask to define any terminology or jargon used).

Scientists have conducted several experiments to alter the pattern. They find that when they remove the gene (alt: genetic material) Puma, the cells do not make a striped pattern instead produce a random pattern.

- c. Do you think Puma is the only gene responsible for the patterns you observe? Could more than one gene (alt: genetic material) be responsible for these patterns? If so, how? [polygenic trait, also gene interaction]
- d. Is the Puma gene found in all Zebrafish cells? (If yes, stop. If no, ask which cells)
 - i. If yes, ask: If found all cells, then what makes each cell different?
(looking for distinction between gene itself (found in all cells) and gene product/mRNA/protein (found in only some cells))
- e. Do you think the Puma gene could be responsible for more than just stripe formation?

- f. The Puma gene is not always on. When and where do you think the Puma gene is turned on? (Does student assume this is occurring during development?) [looking for temporal and spatial dynamics of gene expression]
 - g. What do you think is making the Puma gene turn off?
 - h. What would happen to the physical pattern of a Zebrafish if Puma were turned on longer? Not turned on at all? What would cause a gene (alt: genetic material) to change in such a way? [Temporal]
 - i. What are some other ways the pattern might be controlled?
 - j. You notice some Zebrafish have thicker stripes and some have thinner stripes. What might have caused this to happen? [Spatial]
2. You decided that you want to look at how the Puma gene functions in other organisms. You find that Puma plays an important role in a large number of patterns seen in butterflies, fishes, and many mammals, like mice.
- a. How can a single gene (alt: genetic material) be responsible for so many different color patterns in so many different species?
 - b. When comparing the products of the Puma gene in Zebrafish and mice, there have been very few changes. Why would these products stay unchanged for so long?
 - c. What do you think would happen if the Puma gene did not work?
 - d. Some say that new forms evolve as the result of new tools (new genes, new pathways, etc.). Others claim new forms evolve as the result of using old tools in new ways. Which of these descriptions best depicts the role Puma has played in the evolution of pigment pattern? Can you think of an example to explain the difference between truly novel traits and tinkering with the old?
3. Notochords form in many different species during development. In some species without skeletons, notochords serve an important function as stiff rod down the body axis. In vertebrates, notochords do not exist in adults. Interestingly, all vertebrates develop and possess a notochord as embryos but they do not serve any function.
- a. Why have vertebrates retained notochords as embryos?
 - b. Is there any way a structure can stay the same without natural selection?

Evo-Devo Survey – Interviews, Subset 6

Script: Thank you for participating in our research project. I will be asking you several questions related to common biological concepts. As you contemplate each question we may ask you to describe your thought process and/or ask additional questions about your response. We may ask you to describe and explain your answer multiple times.

1. You found a chicken egg, you hatched it out and observed that the chicken has a beak. Can you describe how your chicken's beak was formed?

[Here we are looking for mention of the following: genes (plural, i.e., not gene), gene *expression*, complex interactions during development.]

Ultimate causes

2. On a volcanic island in the Pacific you find sparrows with beaks of different shapes and sizes. Where did the different beak shapes in these species come from?

[Here we are looking for mention of the following: natural selection, genes or phenotype, drift, different food sources.]

Proximate causes

3. Say you collected eggs from two sparrow species and hatched them out and they have different beak shapes. In these two birds what causes the different beak shapes?

[Here we are looking for mention of differences in gene expression, but also differences in the amino acid sequences of proteins (and hence changes in the function of those proteins) as potential explanations. If students simply say "Embryonic development was different", try to follow up with: "What about it was different? Why?". If students say "They have different genes", follow up with "How do you mean different? Do you mean one species has a gene that the other one does not or do you mean that the different birds have different versions of the same gene?" (i.e., different alleles).]

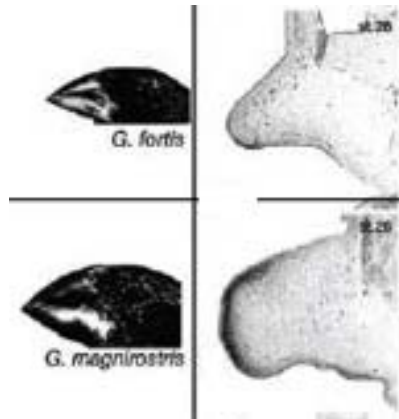
Change in gene expression as proximate cause

4. You have determined that a single gene is responsible for the difference in beak shape. [If students question how this was done, just tell them it was genetically mapped by doing crosses between the two species because they have fertile hybrids.] In your two individual birds you discover that the protein-coding regions of this gene are identical. Given this information, what do you think might cause the different beak shapes in these two birds?

[Here we are looking for the insight that, with this new information, it is now clear that differences in gene expression must be responsible. If students again suggest a different gene, remind them that we have already established that this gene was responsible.]

Molecular basis of change in gene expression

5. In your two birds, when you examine the embryonic tissue that gives rise to the beak, you discover that there are differences in the amount of products produced by the gene you identified as responsible for differences in beak shape.



What might be causing these different levels of gene expression?

[Here we are looking for mention of cis-regulatory mutation(s) in enhancers (or the “promoter”) and/or epigenetic (methylation, etc). If students mention another gene again, remind them again that we've established that *this* gene is responsible. If they repeat that there are differences in the expression of this gene, ask them again what could be causing these differences.]

Synthesis of answers to questions 1-5

6. Now that you have all this information about genes and gene expression in your two birds, can you tell me again how these two birds have come to have such different beaks?

[Here we are looking for mention of the following: an initial mutation in an enhancer, the selection of the resulting phenotype (not the genotype directly) due to environmental challenges such as different food sources, and eventual fixation of the mutation causing a change in the beak shape distribution for the population. Watch for misconception that different food sources induced mutation (directed mutation).]

Deep Time

7. How long might it take for a population with similar beak shapes to change into a population with several different beaks?
8. Is there any way a structure can stay the same without natural selection?