The Case of the Threespine Stickleback
A Model of Macroevolution
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Adapted for ENSI site by Larry Flammer

Synopsis
Students begin by seeking to answer a question: “Why have some freshwater populations of threespine stickleback fish lost their pelvic spines and body armor?” Data and analysis take them into some applied genetics and the Evo-Devo work on regulatory DNA, in which mutations can change where and when a gene is expressed, producing major changes in specific morphology (without fatal effects) on which natural selection can act. This reveals a likely pathway for new species to form in a relatively short time (macroevolution) without the heavy risk that a mutation in a protein-producing gene might bring. Applications to other species are also explored.

Principal Concepts
1. Major changes can be triggered relatively safely during development by mutations in regulatory DNA, unlike changes in the mRNA-coding regions of a gene. This is probably a major factor in the evolutionary changes processed through natural selection.

Associated Concepts
1. Traits subject to evolution must have a hereditary component.
2. Evidence of the present can reveal events of the past.
3. Most traits exist for the benefit of the individual rather than for the good of the species.
4. Traits are usually favored by natural selection only when they result in more reproductively successful offspring.

Assessable Objectives Students will...
1. Recognize a 9:3:3:1 ratio of combination of two traits in a generation as the results of a simple Mendelian dihybrid cross with two traits, each expressing in two forms, and each controlled by single genes with two alleles each, and with each gene showing dominance (two simple Mendelian traits inherited together).
2. Understand how a specific mRNA-binding dye can reveal the expression of a specific gene in a cell or group of cells.
3. Recognize how natural selection can explain different features of a species being expressed in different populations.
4. Recognize what atavisms are, and how regulatory DNA changes can explain the existence of atavisms.
5. Recognize how major evolutionary changes can occur relatively safely through the influence of regulatory DNA.

Materials
Copy of Teacher Instructions (PDF version of this page)
Key to Question Responses (3 pages, included with Teacher Instructions)

Time
About one 50 minute period (involves reading, discussing, answering questions; could go an additional period).

Student Handouts
The Case of the Three-Spine Stickleback (information packet: 11 pages)
Responses to Questions (questions repeated with spaces to answer: 3 pages)
Teaching Strategies & Preparation

**Prerequisites:** Students should have studied Mendelian genetics, including work with dihybrid crosses with two simple Mendelian traits showing dominance. It’s also helpful if students have already been introduced to natural selection and/or evolution, and possibly some discussion of microevolution vs macroevolution.

**Additional Resource:** The HHMI–Evolution DVD includes useful video clips and animations that could be used with this lesson. Each clip is about 2-3 minutes long. See review and source of this DVD at http://www.indiana.edu/~ensiweb/hhmievo.html

**Animations:**
- Stickleback CT Scan
- Pitx1 Expression (excellent demo of how gene regulators work)

**Video Clips:** (from Lecture 2: Natural Selection of Sticklebacks)
- Stickleback – Jeopardy
- Stickleback Environment (changes in glacial retreat in North America)
- Fossil Record of Stickleback Evolution (excellent, showing changes in the fossil record of sticklebacks over 25,000 years, going backwards from about 10,000 years ago)

**Glossary** (Some terms students should know (or learn in this lesson):
- **Microevolution**: descent with modification; the process by which species change over time as they interact with their environment, producing changes in gene frequencies.
- **Macroevolution**: the process by which all the groups of organisms have been produced, from different species to all the higher taxa. This could be by accumulated microevolution over long periods of time, or by major changes in morphology that are genetically-caused and can lead to new species in a relatively short time.
- **Morphological** (morphology): having to do with form or shape.
- **Precludes**: prevents or excludes
- **Atavisms**: structures that sometimes appear in an individual, revealing the presence of usually-hidden or suppressed genes that were expressed in an ancestral form, e.g., the occasional pelvic flipper on a dolphin, or an extra toe on a horse.

**STRATEGY:** Have students working in teams of 2-4, with one copy of the 11-page Information packet, and a copy of the 3-page Responses packet for each team. One student reads information to the team. When they get to an item to discuss and respond, they do that. Have students rotate turns reading and responding. If you prefer silent reading, you will need enough copies of the information packet for one per student. Note that the teacher “key to preferred responses” is only to serve as a guide to the teacher, never as “THE correct answers.” Students will present a variety of responses, but should at least begin to approach those “preferred responses.” If they’re way off target, you should use “guiding questions” to move the discussion toward more productive understanding along the lines of those “preferred responses.”

**Alternative Strategy:** Separate the Information Packet into 4 portions, and hand them out separately, so that students work on one section at a time, and can’t peek forward. For example, hand out pp. 1-2, then p. 3, then pp. 4-6, then pp. 7-11. This way, students can work with this as a true mystery case, and it is more like a scientific inquiry. (Thanks to beta-tester Kathy Van Hoeck for this suggestion. It worked well for her.)

**Procedures**
Introduce the Stickleback to the class. An excellent way to do this is to get the HHMI DVD or VHS tape (free), and show the clips suggested above. Alternatively, project the pictures from the Information packet, read the first two pages, and let them begin working at the top of page 3. Walk around, try to keep all teams at about the same stage, so you can engage all teams in discussion for some degree of informal formative assessment, clarifying and focusing as needed.
Assessment
See Assessable Objectives for focus of assessment questions.

Extensions & Variations
Try the “Blocks & Screws” lesson (http://www.indiana.edu/~ensiweb/lessons/bl%26scr.html). This novel exercise directs attention to how evolution consists of the gain and loss of expressed traits, as naturally selected, while their genetic basis may still be retained, resulting in atavisms appearing from time to time.

See other lessons for teaching about macroevolution at http://www.indiana.edu/~ensiweb/lessons/sp.evid.html
And check out the links from our EvoDevo page at http://www.indiana.edu/~ensiweb/macro.evodevo.html

References:

Carroll, S.B., Grenier and Weatherbee. 2005, From DNA to Diversity. Figure 4.6, page 112. Blackwell Publishing, and Howard Hughes Medical Institute DVD. 2006. Evolution - constant change and common threads


Szeto et al., 1999, Genes and Development. 13:484-494

US Geological Survey (manatee photo)

Skulls Unlimited Newsletter, 2006 (www.skullsunlimited.com) (manatee skeleton)


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RESPONSES TO QUESTIONS

PAGE 2: Are Pelvic Spines and Body Armor Inherited?

As we stated above, in order for particular traits to be affected by natural selection, they must vary within populations and they must be inherited. So what about pelvic spines and body armor? We know that these traits vary between marine and some freshwater populations, but are these traits inherited? Perhaps simply living in certain kinds of freshwater causes the loss of these traits in a reversible manner. How could we test this? What could you do to that might tell you if pelvic spines and/or body armor are inherited? Discuss your ideas with your classmates and record your best ideas below.

Various responses possible, e.g.,

Compare DNA in fish from the two populations: Do we find more differences than we do within those populations?

Mate marine fish with freshwater fish, then cross their hybrid progeny with each other; if most (3/4) show armor and few (1/4) show no armor in this F2 generation, suggesting that armor is a simple mendelian dominant trait. Likewise for pelvic spines. Followed together (two traits), should get 9:3:3:1 ratio in the F2.

PAGE 3:

Based upon what you have learned about the rules of inheritance, what can you conclude about the alleles that control pelvic spines and body armor in threespine sticklebacks? Write your answer in the space below. Using letters to represent the alleles (i.e., different forms) of the genes that control pelvic spines and body armor, diagram what happened in this breeding test.

Approx. 9:3:3:1 ratio of phenotype combinations is strong indicator that these traits are hereditary.

Using A for armor allele, and P for pelvic spines, cross could be diagramed as: (could show in Punnett square)

\[
\begin{array}{c}
AA PP \times aa pp \\
\downarrow \\
all Aa Pp \\
\end{array}
\]

F1 generation (all doubly heterozygous - hybrids)

Based upon your answer to the question on the previous page, perhaps you can make another prediction. What do you think would happen if the fish from the first (F₁) generation were artificially bred with each other? Write down your prediction here.

Most of their offspring would have armor and spines, some with armor and not spines, some with spines and no armor, and a few with no armor and no spines (9:3:3:1 ratio)

PAGE 4:

Based upon these results, what would you conclude about the pattern of inheritance of these two traits (i.e., pelvic spines and body armor) in threespine sticklebacks? Write your answer in the space below. Again, using letters to represent different alleles of the genes controlling pelvic spines and body armor, diagram what happened when members of the F₁ generation were bred with each other.

Armor and Pelvic Spines are both dominant traits, controlled by two separate genes, each with two alleles.

\[
\begin{array}{c}
Aa Pp \times Aa Pp \\
\downarrow \\
9/16 Aa Pp : 3/16 Aa pp : 3/16 aa Pp : 1/16 aa pp \\
\end{array}
\]

F2 generation (9:3:3:1 ratio)

(9 armor - spine : 3 armor - no-spine : 3 no-armor - spine ; 1 no-armor and no-spine)

(Continue with Part 2 on page 5 in the Information Packet)
Here is what they did. Recall that the DNA code of genes is translated into mRNA in places where those genes are activated. These days, scientists are able to detect the presence of particular mRNA by binding these mRNAs to a blue dye that will be visible in the tissues only where the specific mRNA molecules are being made. The scientists did an experiment where the pattern of Pitx1 mRNA production was compared between marine and freshwater stickback embryos. Discuss this experiment with your classmates and write down what you think probably happened. Do this below.

Embryos of marine sticklebacks showed the dye color for Pitx1 in the regions where pelvic spines would form, while embryos of freshwater stickbacks did not.

So what can you infer about where the coding region of the Pitx1 gene is being transcribed into mRNA in these embryos? Write your answer below and explain why you said what you did. Did your prediction match the results of the experiment. Explain.

The Pitx1 coding region is not being transcribed into mRNA in the pelvic spine region of freshwater stickbacks. If it was, we should see the blue dye in that region. However, it IS being transcribed in the "nose" and jaw, as shown by the blue dye in those regions.

(Prediction responses may vary as to whether results of experiment matched student predictions - either they did, or didn't, and student should explain why.)

The diagram in Figure 7 shows the working hypothesis that the scientists investigating Pitx1 expression in sticklebacks have proposed to explain their results. Although they have not yet identified the specific mutation that causes the transcription of Pitx1 mRNA to be “turned off” in the pelvis, it seems very likely that it is located in the piece of “regulatory” DNA segment that normally activates Pitx1 transcription in the pelvis (i.e., the green circle in Figure 7). The “regulatory” pieces that control transcription of the gene in the olfactory region (i.e., “nose”) and jaw are unaffected. How do we know this?

The dye color still appears in the olfactory and jaw regions of embryos of both marine and freshwater stickbacks.

Most of you would agree that if a human were to be born without a pelvis, this would represent a drastic (and detrimental) change. Natural selection would certainly not preserve this variation (why not?). Yet, in threespine sticklebacks, selection has clearly favored the elimination of the pelvis in a very “short” time (i.e., no more than 12,000 years). Why? Although we don’t know for sure, apparently fish that normally prey on sticklebacks are absent from some freshwater lakes; and, conversely, in those lakes, large predatory insect larvae that can grab the spines of sticklebacks (and eat them) are often present. How might such observations explain the absence of pelvic spines in terms of Darwin’s theory of natural selection? Write your explanation below and continue on the back of this page if necessary.

Why not? Without a pelvis, we couldn’t stand and reproduction would be rather challenging! In terms of natural selection, apparently pelvic spines are a deterrent to predators of marine fish, therefore they contribute to their survival in the marine environment, but those predators are not in freshwater environment, so spines would be of no advantage. Furthermore, spines contribute to predator success (predatory insect larvae) in freshwater, so presence of spines would be strongly selected against in freshwater, resulting in their elimination.
PAGE 8 (Continued):
Another feature of evolutionary theory is that variation in populations ultimately arises from gene mutation followed by selection for a particular mutation. In this example, scientists think they have identified both the type of mutation involved and its approximate location in the DNA. In this case, a presumably small genetic change (mutation in a piece of regulatory DNA) results in a large-scale morphological change (the loss of the pelvis). So is this microevolution or macroevolution? Write a short paragraph giving your answer and justifying your position.

[student could answer with either Microevolution, or Macroevolution, but must explain why; for example:]

Macroevolution: a small change in the regulatory DNA causes a large change in the morphology (no pelvis develops), without affecting other features. This can then be selected for or against, resulting in a new species in a fairly short period of time (the marine and freshwater populations no longer mate). OR

Microevolution: this single mutation could well be part of a series of changes that could accumulate to produce a new species, but their eggs and sperm can still produce viable offspring, so it's just a variant of one species.

PAGE 9:
As you can see from looking carefully at Figure 8, mice whose Pitx1 gene has been “knocked out” not only fail to survive, but show multiple defects in the head, jaw, pelvis, and hind limbs. Yet, threespine sticklebacks that have completely lost the pelvis show no defects. How can you explain this difference? How would the diagram of the Pitx1 gene that is shown in Figure 7 for marine and freshwater sticklebacks look if it was showing the gene in a Pitx1 “knockout” mouse? Discuss this with your classmates and summarize your ideas in the space below. Your instructor will discuss this with you. Understanding the difference between these two cases is critical to understanding how evolution does (and doesn’t) work.

In the “knockout” mouse, the Pitx1 coding region was damaged (segment deleted), not the regulator regions, so none of the features requiring the Pitx1 protein would develop normally. The diagram would show three normal and functional regulator regions, but the Pitx1 coding region would be shown as “mutated.”

Multi-effect genes (like Pitx1) can tolerate mutations in the regulatory region more easily than in the coding region, where mutations could be more widely disruptive. In general, regulatory mutations can produce profound but specific changes, but they may be better tolerated (due to their limited effect on specific tissues) so the change survives, subject to current selective pressures.

PAGE 10:
Now, based upon what you have learned, you should be able to make a reasonable (and perhaps testable) hypothesis about the genetic basis of limb loss and pelvic reduction in manatees and how this condition might have evolved. Again, using the type of diagram shown in Figure 7, what would you hypothesize that appearance of the Pitx1 gene complex would be in manatees? Show the diagram below and explain your reasoning.

In manatees, increased dependence on aquatic life (and less dependence on terrestrial life) would favor mutations in the regulatory area for Pitx1 that was normally activated in the pelvic region in early manatees. It would look like the second diagram in Figure 7.

PAGE 11:
In at least some species that have lost their hind limbs, rare individuals occur in whom the lost structure re-appears. These “re-appearances” of ancestral structures are called atavisms. Figure 10 shows an atavistic hind limb in a dolphin that was caught by Japanese researchers in 2006. Given what you have learned about the Pitx1 gene, what might be the genetic basis of this atavism? Use another diagram of the Pitx1 gene complex to illustrate your hypothesis. [See first diagram in Figure 7]

Either the pelvis-regulatory region back-mutated to sufficiently functional condition to allow Pitx1 production in the pelvic region, or some other factor stimulated some Pitx1 production in that region, perhaps exposure of mother to environmental chemicals, (pesticides, hormones, wastes) during embryonic development.