

Evolution and Plasticity in Guppies: How Genes and Environment Shape Phenotypes

by

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Part I – Discovering Guppies

Trinidad is a tropical Caribbean island off the coast of Venezuela, best known for being the larger of the two islands that make up the country of Trinidad and Tobago. While exploring the numerous streams emerging from Trinidad's Northern Range Mountains, Professor John Endler observed diverse fish communities in the lowlands. These lowland stream communities were made up of many predatory species like the wolf fish and numerous types of cichlids, which were all preying on an abundant small fish. Endler noticed these small colorful fish occurred in the thousands, forming “shoals” or social groups, along the edges of the streams. The locals called them “millionfish” because of their abundance and the number of bright colors on their bodies, but we know them as guppies (Figure 1). The Trinidadian guppy, *Poecilia reticulata*, is usually less than two centimeters long, with females being larger than males. Males are brightly colored to attract females while females are drab, camouflaging into their environment. Males display their coloration in elaborate courtship displays to attract females. Guppies have internal fertilization, and females give birth to live young. As females age and grow, they are able to carry more babies and produce more offspring. Guppies in these environments grow quickly, mature early, and have many small babies so that they have some fitness before they are eaten by the larger predatory fish.

Endler then hiked upstream and climbed waterfalls expecting that these natural barriers prevented upstream fish movement. Instead he found many thriving guppy populations that lived without the large predatory fish. The only other fish living with guppies was the slightly larger killifish, which preys on juvenile guppies. He described these streams and the fish living there as “low predation” (Figure 2). He noticed that the guppies were very different from those that lived with many large predators in the downstream rivers, which he called “high predation” environments (Figure 3). The low predation guppies were larger, the males were more colorful, and their behavior differed; for example, the low predation males performed more courtship displays than high predation males. These differences seemed intuitive based on differences in the potential for predation in these environments. There is a risk of being spotted and eaten if a male performs flashy courtship displays in a stream with lots of predators. Similarly, higher



Figure 1. Trinidadian guppies in lab.



Figure 2. Low predation stream in Trinidad.



Figure 3. High predation stream in Trinidad.

mortality in high predation environments should favor individuals that grow quickly and become sexually mature at a young age (Reznick and Endler 1982).

Endler knew that different color patterns in guppies had a genetic basis because breeders had generated a diversity of fancy ornamental guppies that were sold in pet stores around the world. What Endler wanted to know was whether predators in nature could be driving the differences in color between high and low predation male guppies (Figure 4).

Endler designed one of the first experiments that illustrated the role of natural selection in generating the differences between low and high predation guppies (Endler, 1980). In 1976, Endler introduced guppies from a high predation location to a site above a waterfall where there were no fish except for the killifish. He came back two years later and examined the guppies at the experimental introduction site. He discovered that many traits had changed. Look at one of the figures from his paper and describe the changes he documented (Figure 5; based on Endler, 1980).

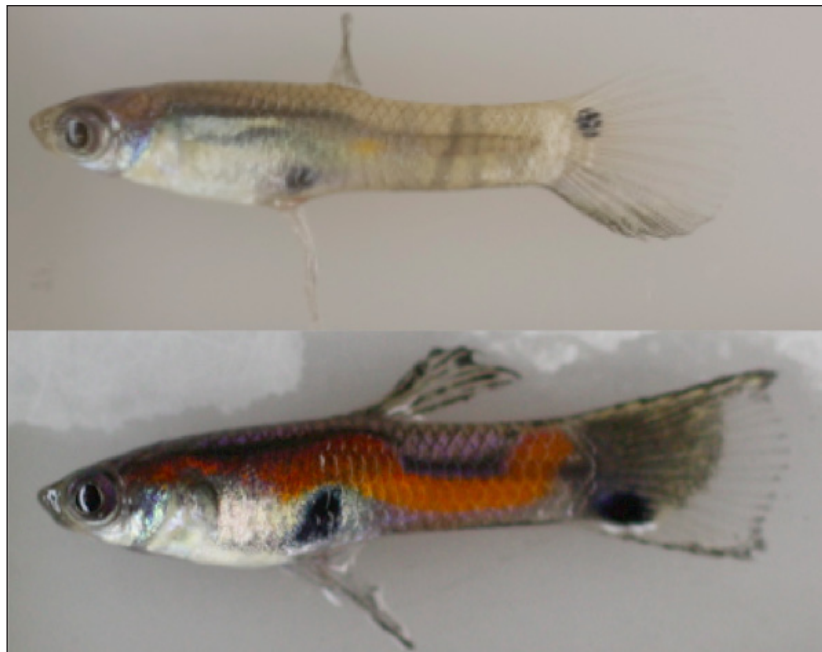


Figure 4. Males from high (top) and low (bottom) predation streams.

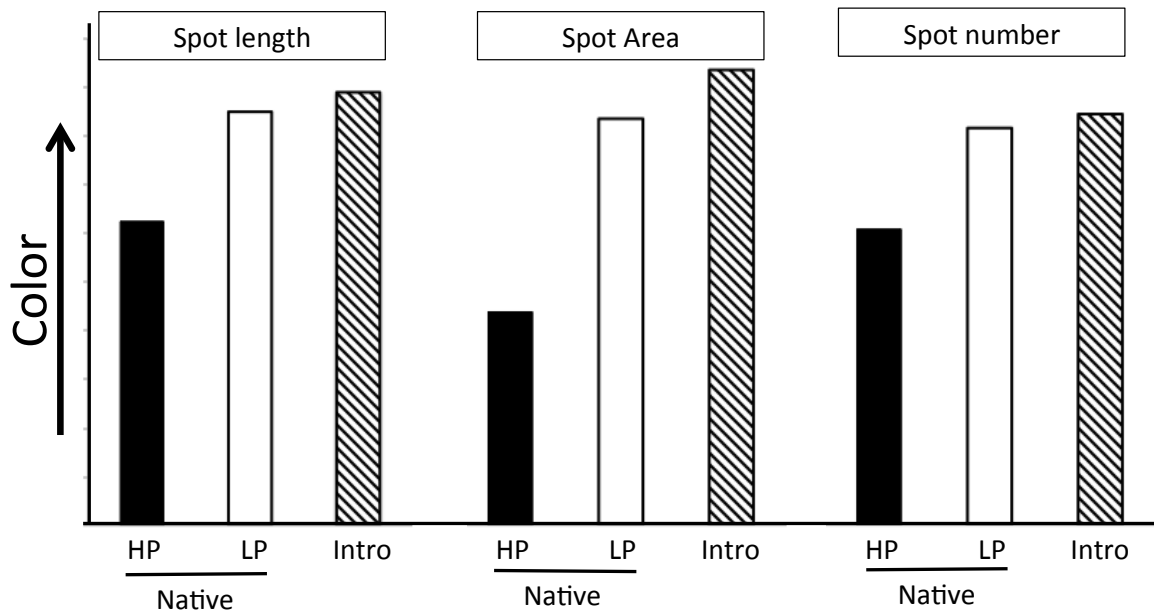


Figure 5. Results from a field experiment comparing a native high predation (HP) source population and a native low predation (LP) reference population to a population that was introduced (Intro) from a HP stream to a LP stream two years before. *Source:* adapted from Endler (1980).

Questions

1. What hypothesis was Endler testing with this experiment? What did he expect to find if his hypothesis was supported?
2. For each color trait, is the introduced population more similar to the high predation source population or the low predation reference population?
3. Based on this figure, what can you conclude about the rate of evolution of color patterns in guppies? (Remember that Endler returned to the streams two years after the introduction.)
4. Is it possible that evolution explains the differences between the three populations? Why or why not? Include a definition of evolution in your answer. Describe the selective force that is likely driving the changes. (Remember that there are no longer major predators on adult guppies in “Intro.”)
5. Finally, please take a moment to reflect on the ethics of Endler’s experiment in which he transferred fish to a previously uninhabited stream. If you were on the board that granted permission for the research, would you have approved this experiment? Why or why not?

Part II – Guppy Diets

Following Endler's work, scientists continued to study the evolution of color patterns in guppies. One important discovery was that females are particularly attracted to orange body and tail coloration on male guppies (Endler and Houde, 1995). Several years later, Professor Greg Grether was especially interested in how orange color was obtained by males. Like earlier scientists, Grether saw that color patterns differed from stream to stream, but he noted differences among streams that could not be explained by predators since all had only one minor predator, the killifish. One thing that was different in these streams was the amount of sunlight that made it through the rainforest canopy. This in turn affected the amount of algae available for the guppies to eat, specifically a unicellular algae that contains carotenoid compounds (Grether et al., 2000). (The word "carotene" comes from carrots, which get their orange color from these compounds.) To better understand how color on the body might relate to the amount of carotenoid-rich unicellular algae they consumed, Grether manipulated the amount of carotenoids in the diet of guppies. Once he was able to determine the sex when they were about six weeks old, Grether separated brothers into different tanks and reared them on three different diets: high levels, low levels, and trace levels of carotenoids (Grether, 2000). Use Figure 6 to answer the questions below.

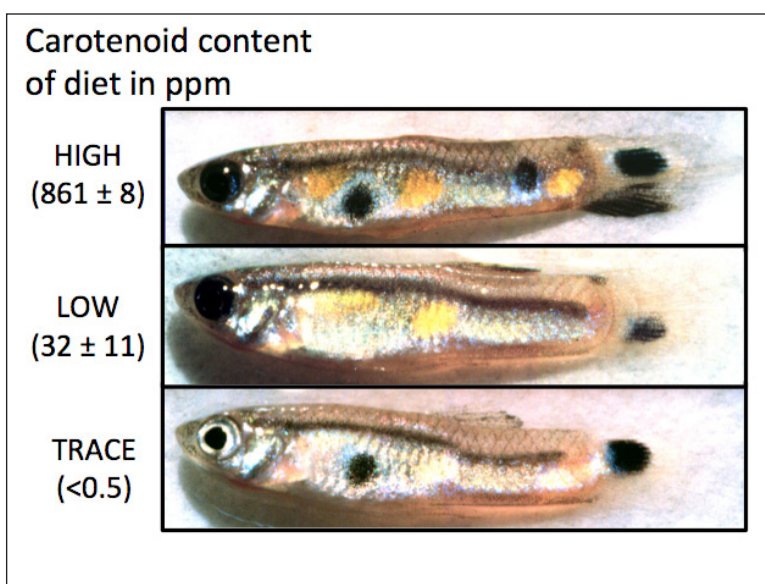


Figure 6. Full siblings raised on a diet with varying levels of carotenoids.
Credit: Photos by Greg Grether, adapted from Grether 2000; used with permission.

Questions

1. What hypothesis was Grether testing with this experiment? What did he expect to find if his hypothesis was supported?
2. Why did Grether use brothers in the three treatments instead of unrelated guppies?
3. What differences do you observe among the three brothers?
4. How might you explain the difference in color between the brothers?
5. Is this an example of evolution? Why or why not? If not, what is it?
6. Please answer Question 4 from Part I again: *Is it possible that evolution explains the differences between the three populations? Why or why not? Include a definition of evolution in your answer. Describe the selective force that is likely driving the changes.*

Part III – Guppies in the Laboratory

Because of the work of Endler, Grether, and other scientists, guppies quickly became a model system for studying how the environment can drive evolutionary change across generations and plastic changes within the lifetime of an individual. Many traits, including coloration, were found to evolve rapidly in response to changes in selection pressures, like predation risk. But the environment experienced during ontogeny (or development) can alter an individual's traits, like the example of plasticity in orange coloration that we saw earlier.

Corey Handelsman was interested in studying both evolution and plasticity in the guppy system. Instead of focusing on color, Handelsman looked at life history traits, or traits associated with the timing of important events in an organism's lifetime. Examples of life history traits include the age and size when animals become sexually mature or the number and size of offspring produced. Investment in these traits typically involves a trade-off. For example, males may become sexually mature sooner so that they have more opportunities to mate, but they would then have a smaller body size. And they may not have as much success finding mates since females prefer to mate with larger males. Conversely, a male might mature later, missing mating opportunities, but be larger and more desirable to females.

Handelsman was interested in understanding how life history traits in guppies differed depending on predation, so he chose a river in Trinidad that had distinct high and low predation populations. Because of research done by other scientists, he knew that some fish from the high predation population swam upstream and founded the low predation population many years ago. So, even though they were closely related to the high predation population, the low predation population had been evolving without adult predation for many generations. He collected males and females from the two locations to bring back to the laboratory. He then kept guppies from both populations under identical laboratory conditions for several generations (and randomly bred them) to try to eliminate any influence of the environment from which they were collected. We call this a common garden experiment because the environment is held constant even though the animals or plants were collected from different environments. With this common garden design, any differences in phenotype that Handelsman detected between the two populations of lab-born fish could be attributed to underlying genetic differences.



Figure 7. Pike cichlid predator. Credit: Eva Fischer, with permission.



Figure 8. Recirculating system housing guppies at Colorado State University.

But Handelsman also wanted to quantify the effect that the presence of predators in the rearing environment had on the phenotype (phenotypic plasticity). For this, he needed to simulate predation risk without actually letting the experimental guppies get eaten. So Handelsman placed a cichlid predator (Figure 7) in the large tank that fed water into all of the guppy tanks (Figure 8). The cichlid was fed a live guppy each day, which released chemical distress cues from specialized cells in the guppy's skin. The guppies in these tanks smelled these distress cues as well as chemical cues from the cichlid predator. Handelsman divided each family so that one brother was raised in normal water and another brother was exposed to these chemical predator cues for their entire lives (Handelsman et al., 2013). Using this design, Handelsman could compare brothers reared in the two different environments to illustrate phenotypic

plasticity. Since the genetic background was basically held constant (by using siblings), Handelsman could attribute any phenotypic differences to the effect of the rearing environment.

The life history trait that Handelsman chose to study was growth rate. He expected that fish from the high predation population would have a faster growth rate. When predators target adults, predation acts as a selective force that favors individuals that are able to grow quickly, mature, and reproduce before being eaten by a predator. Predation on juveniles from cannibalism and killifish, which is more common in low predation environments, favors production of large babies. But in low predation environments, Handelsman hypothesized that female mate choice would be the strongest selective force. He knew that females prefer to mate with the largest and brightest males, so he expected slower growth but larger and more colorful males once they reached maturity. He measured the males for mass at two time points and divided the difference by the elapsed time to calculate growth in milligrams per day. He could then calculate the mean for each population in each environment and plot a reaction norm (Handelsman et al., 2013). A reaction norm, or norm of reaction, is a graph that shows phenotypes in different environments when the genetic background is held constant (by using brothers or clones; see Figure 9 for an example).

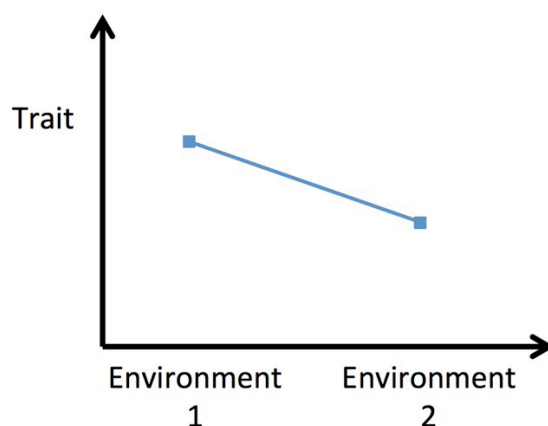


Figure 9. Standard layout to use in graphing reaction norms.

Questions

- Using Figure 9 as a guide, sketch a graph of your expectations. You should draw one line for the high predation (HP) guppies and one for the low predation (LP) guppies. Please state your reasoning. Remember that Handelsman expected the high predation guppies to have a faster growth rate, but you can disagree with him as long as you state your reasoning.

Download the Excel file [guppy_genes.xlsx](#) that contains raw data from Handelsman et al. (2013). Use the data from the sheet titled “Graph 1” to calculate the four means that you will need to graph the reaction norms for the two populations (high predation = HP, low predation = LP). Make a graph using the “marked line” chart function. Use the graph to answer Questions 2–7.

- What does the slope of each line tell you?
- What can you say about the differences and similarities between the slopes in your figure?
- What does the difference in elevation between the two lines tell you about the difference between these populations?
- Look at the points on the figure that correspond to the normal environment for each population (for example high predation guppies normally experience predator cues in the rearing environment). What can you say about these two points? How would you expect growth rates to compare in the wild between these two populations of guppies?

6. What can you say about the plasticity in these populations? Do you think selection can act on plasticity, and can it evolve?
7. What data would you like to collect to help you decide whether plasticity has changed in response to selection?



Next, Handelsman wanted to look at the variation among families within each population. To do this, he plotted the individual reaction norms for each family. Use the same approach as before to make a graph for the low predation families (data in Excel sheet called “Graph 2”) and one for the high predation families (data in Excel sheet called “Graph 3”). You will again use the “marked line” chart and will probably have to switch the x- and y-axes by right clicking on the chart and selecting “select data” followed by “switch row/column.”

Questions

8. What does the slope of each line tell you?
9. What does the average elevation, or mean of both points of a given line, tell you?
10. What can you say about the differences and similarities among the slopes in your figure? Compare the slopes between low predation and high predation.
11. What does this tell you about the underlying genetic variation for plasticity in these populations?

Part IV – Tilapia Story

Hopefully, you now have a better understanding of the difference between evolution and plasticity, but you may be wondering why this distinction is so important. The following is an example of a human induced environmental change that is currently occurring.

Tilapia farming is growing and promises to be economically important for the still developing country of Trinidad and Tobago (Article 1). But fish escape these farms, often in large numbers, and will have an impact on the native fish species (Article 2). Read the two following articles and answer the questions further below.

Article 1: A brief press release from the Ministry of Food Production: available at <http://www.agriculture.gov.tt/main-media/latest-news/press-releases/322-new-hope-for-the-tilapia-industry.html>.

New Hope for the Tilapia Industry

8th May, 2013: Senator the Honourable Devant Maharaj, Minister of Food Production, hosted a media briefing today, in collaboration with the Seafood Industry Development Company (SIDC) and the Sugarcane Feeds Centre (SFC) to inform the public of the recent initiatives taken to improve Tilapia and by extension the fisheries industry. Speaking on behalf of the SIDC and SFC were Dr. Doon Ramsaroop, CEO, SIDC and Mr. Suresh Benny, Director SFC.

The SIDC shed light on their acquisition of high quality tilapia feed from GISIS in Ecuador and gave their assurance that this feed shall be available at a low cost to the farmer on a consistent basis. Reducing the cost of production which is significantly impacted by the cost of feed (an estimated 60–65% of production costs are feed costs) is one of the many efforts being made to increase tilapia production locally. At present it is cheaper to purchase imported filets than those produced locally. For local production of tilapia to grow it has to be sold at competitive yet affordable prices.

Tilapia is now one of the world's fastest growing aquaculture products. Thus, the SFC, whose broad objective is the development of sustainable, intensive production systems for livestock and aquaculture with emphasis on environmental conservation and management, undertakes research and demonstration work on the development tilapia production systems and the information is made available to the farming community. Additionally, at SFC live and processed fish are sold for consumption and fingerling (young fish) are produced for sale to farmers and the general public.

Upon Minister Maharaj's initiative the Ministry provided promotional support for the sale of tilapia fish during the last week of the Lenten period. The fish sold during the Lenten period was partially supplied by local farmers. During that period a record two (2) tonnes of tilapia of which fillet was much more in demand than the processed whole fish. Minister Maharaj noted that this is the growing trend and emphasizes the need for dedicated processing facilities which the Ministry is already seeking to address. In the tilapia industry opportunities for investment, foreign exchange earnings and employment can be capitalized upon. This industry could eventually contribute significantly to the country's protein requirements as well as food security and ultimately to its GDP.

Article 2: A journal article reviewing the impacts on native fish populations when tilapia escape from fisheries. The abstract is reprinted below. In addition, please read the introduction (p. 464) and the section titled “Tilapia introductions: a summary of potential impacts” (pp. 467–468) of:

Canonico, G.C., A. Arthington, J.K. McCrary, and M.L. Thieme. 2005. The effects of introduced tilapias on native biodiversity. *Aquatic Conservation: Marine and Freshwater Ecosystems* 15:463–483. [Note: a subscription to this journal may be required to access the complete article; check with your instructor.]

ABSTRACT: The common name “tilapia” refers to a group of tropical freshwater fish in the family Cichlidae (Oreochromis, Tilapia, and Sarotherodon spp.) that are indigenous to Africa and the southwestern Middle East. Since the 1930s, tilapias have been intentionally dispersed worldwide for the biological control of aquatic weeds and insects, as baitfish for certain capture fisheries, for aquaria, and as a food fish. They have most recently been promoted as an important source of protein that could provide food security for developing countries without the environmental problems associated with terrestrial agriculture. In addition, market demand for tilapia in developed countries such as the United States is growing rapidly.

Tilapias are well-suited to aquaculture because they are highly prolific and tolerant to a range of environmental conditions. They have come to be known as the “aquatic chicken” because of their potential as an affordable, high-yield source of protein that can be easily raised in a range of environments — from subsistence or “backyard” units to intensive fish hatcheries. In some countries, particularly in Asia, nearly all of the introduced tilapias produced are consumed domestically; tilapias have contributed to basic food security for such societies.

This review indicates that tilapia species are highly invasive and exist under feral conditions in every nation in which they have been cultured or introduced. Thus, the authors have concluded that, despite potential or observed benefits to human society, tilapia aquaculture and open-water introductions cannot continue unchecked without further exacerbating damage to native fish species and biodiversity. Recommendations include restricting tilapia culture to carefully managed, contained ponds, although exclusion is preferred when it is feasible. Research into culture of indigenous species is also recommended.

Questions

1. What are some ways that the escaped tilapia might affect guppies directly? What about indirect effects?
2. Do you think high predation or low predation populations would be better able to respond to invasive tilapia?
3. Does your answer to Question 2 change if you use the graphs that you made to answer the question? Please mention all three graphs in your answer.
4. Some have argued that detailed information about animal behavior is critical in predicting how species will respond to human-induced rapid environmental changes (Sih et al., 2011). They suggest that an estimate of the genetic variation in a population is not enough to predict whether or not that population can respond rapidly enough to avoid extinction. Assuming this is true, what other pieces of information would be useful in predicting how guppies will respond to tilapia? Please propose three questions.
5. If guppies form the foundation of the freshwater food web in Trinidad and Tobago and are important grazers of algae, how might their decline affect the ecosystem?
6. Pretend that you are asked to speak to the president of Trinidad and Tobago about the pros and cons of tilapia farming in the country. Based on the two articles that you read, what would you say to the president?



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