Environmental effects of light and predation on coloration in wild guppies

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ABSTRACT

A key goal in evolutionary biology is to understand the maintenance of trait variation in nature. This paper explores the ecology, trait variation, and possible adaptation of Trinidad guppies introduced to novel environments. Here we study two important environmental factors leading to changes in color polymorphisms of wild populations, which enable the guppy to survive and thrive under abrupt environmental change. Guppies were initially taken from one high-predation environment and introduced into two geographically isolated low-predation environments, one of which had its canopy trimmed to increase more light. In low predation environments, the expected lifespan increases dramatically, but so does population density, causing a reduction in food availability. The introduced guppies were photographed and measured bimonthly for twelve months for any changes in coloration from their ancestral measurements. Results show that little changes in coloration have occurred since the introduction, but fine scale measurements indicate that cyclical changes in coloration may occur as guppies initially adapt to abrupt changes in their environment. More time may be needed until we can see significant phenotypic and genetic changes in these wild streams and our future work will examine this. The merits of this study so far include a better understanding of both how trait polymorphisms can shift or be maintained in nature under environmental change, a topic quite important in the conservation of rare and endangered species.

Keywords: Environmental pressures, sexual selection, fitness, evolution, trait polymorphisms, coloration

MENTOR

David Reznick

Professor of Biology

Professor Reznick is an evolutionary biologist who has specialized in the empirical study of adaptation. He is the lead principal investigator of the Guppy Project, in which he oversees the execution of the introduction experiments, participation in the evaluation of the population dynamics of the introduced populations of guppies, the comparative and experimental study of life history evolution in Rivulus and laboratory evaluations of the evolution of life history traits.

Michelle Yoakim

Department of Biology

Michelle Yoakim is a graduating senior majoring in Biology. Michelle has held leadership positions in various UCR organizations including the Mini Medical School and the Student Run Health Clinic. Michelle joined Dr. Reznick's Evolutionary Biology lab as a second year because of an interest in the field of genetics. During her time in Dr. Reznick's lab she worked under the mentorship of graduate student Swanne Gordon. Over the past four years Michelle has positively contributed to the evolutoinary biology and natural selection projects in Dr. Reznick's lab through her meticulous analysis of the distribution of color in the captured guppies from the Caribbean Island of Trinidad. Michelle was awarded a UCR Undergraduate Research Grant to support her research and presented her results at the UCR symposium. Michelle thanks her mentors, Dr. Reznick and Swanne Gordon for their continued support throughout her research career. Michelle plans to continue her education and pursue a career in medicine.

Introduction:

It is well understood that phenotypes depend on the intricate interactions between genotype and environment (1). In this same respect, a central goal of current evolutionary biology research is to understand the origin and persistence of trait polymorphism in nature, or the presence of more than one genetic morph in the same population of a given species (2). Coloration is one type of well-studied trait that is often polymorphic. Such variation is widespread in nature, and has been studied in mammals, birds, reptiles, amphibians, and even fish (3). However, we still do not know why certain color polymorphisms evolve and/or are maintained in the wild. Our specific project aims to use a novel introduction of wild Trinidadian guppies (*Poecilia reticulata*) to examine the environmental factors leading to changes in color polymorphisms, which enable the guppy to survive under abrupt environmental change. This topic is important to many different fields because our rapidly changing environment (much of which is human-induced) forces organisms to adapt to changes in their environment or risk extinction. Polymorphism is closely related to adaptation because having more than one morph increases genetic variation and enables the species to survive a wider array of environmental conditions (4). Morphs can then be maintained by processes such as shifts in opposing selection causing one form to have higher fitness than the other(s). For example, in the banded snail (*Cepaea nemoralis*) shell color polymorphisms are related to seasonal differences in predation pressure, and to the tendency of predators to form search images for the most common morph, which gives an advantage to rare phenotypes (5). Morphs better able to camouflage themselves from bird predators or rare morphs can survive and reproduce (5). Having more morphs ensures the species survival as the environment and predators change during the season.

In guppies a variety of color morphs are maintained by a tradeoff between natural and sexual selection. Female preference for more colorful males has been shown to cause the evolution of extreme male secondary sexual characteristics including male coloration and sexual display, however strong predation cause this to be less pronounced in certain environments (6). In order to study the effects of predation intensity on coloration in guppy populations, John Endler conducted both lab and field experiments. In his laboratory experiment, he constructed ten ponds in a greenhouse to accurately mimic natural stream sediments inhabited with predators of guppies (7). Endler conducted his field experiment in Trinidad, and transferred guppies from a high-predation stream to a tributary that had neither predators nor guppies (7). In the laboratory, he constructed artificial streams that either did or did not contain predators. Results of all these experiments showed that guppies introduced to streams that experienced little to no predation expressed a steady increase in the number of color spots over time, especially in orange spots which have been showed to be preferred by females. However, in response to the addition of predators (*C. alta*), there was a rapid decrease in the total spot number, primarily in the loss of orange, blue, and iridescent spots. These experiments in the greenhouse, undisturbed streams, and the field yielded parallel results due to a shift in the balance between sexual selection and predator avoidance as guppies are placed in one environment over another (7).

Other studies have been consistent with Endler's, showing traits other than color that also rapidly change when guppies are subject to abrupt changes in their environment. These traits include body size, life history traits, and other sexual characteristics and are mainly related to shifts in mortality rates (7, 8, 9). Comparative mortality rates are higher in areas of high- rather than low-predation (8, 5). Since their predators prey on larger fish, guppies mature and reproduce at earlier ages where they co-exist with them rather than in low-predation areas (5). However, upon introduction to novel low-predation environments guppies quickly adapt and become bigger with later ages of maturation (9).

In this study we aim to take the previous research one-step further by focusing on the finer scale ecological changes that lead to rapid shifts in body coloration in guppies introduced from one predation regime to the next. We also examine how changes in light canopy and not just predation may affect coloration over time. We do this by studying the bimonthly phenotypic change in male coloration in guppies introduced from one high-predation environment into two low-predation environments (one of which has its canopy trimmed to increase more light). As stated previously females, which typically have no body coloration, in low predation environments generally prefer greater male body coloration causing mainly high sexual selection pressures in those environments, whereas natural selection mainly via predation leads to reduced coloration in high predation environments (6). Hence, we expect that as the introduced guppies adapt to their new environments the frequency of color morphs with more elaborate coloration will increase in both sites.

High-predation guppy environments typically have more open canopies and hence more light when compared to low-predation sites (10). Light availability can affect the perception of color by predators as well as mates and hence may have an effect on how changes in coloration occur. Streams with more light also have larger amounts of unicellular algae. These algae are a major source of carotenoids, a known limited environmental resource in the diet of guppies that brightens the color saturation or chroma of the orange and yellow spots (11). However, we do not know the effect of more light on the size of color spots, which is what we measure here, but we assume it will also increase.

Guppies are ideal to study the evolution and maintenance of trait polymorphisms for various reasons. First, they represent one of the most complex male color polymorphisms known in nature (9). Second, guppies can be divided into two basic populations: high predation guppies coexist with predators which have a big effect on their phenotype causing the males to be smaller and have less colorful forms, whereas low predation guppies live in the more upstream regions of rivers above barrier waterfalls, which block the presence of strong predators causing the males to be bigger and more colorful as seen in Figure 1 (9). In both groups however, a variety of color forms exist which have a strong genetic and environmental basis.

This project was connected to a broader research project done by D.N. Reznick in March 2009 in which a guppy population from the high-predation environment of the Guanapo River on the island of Trinidad was introduced above a barrier waterfall into two low-predation tributaries in the same river (Taylor and Caigual streams). One low-predation introduction stream (Taylor) was further manipulated by trimming the forest canopy above the stream to increase natural light and make them more akin to natural high-predation sites. We first examined if there have been morphological and hence, given past results, likely heritable changes in coloration in the Taylor and Caigual. As body coloration seen by predators or females is related to body size we also measured changes in body area of the guppies as they adapt to their new predator-free environments. Second, we examined if the increased light manipulation in the Taylor has affected changes of coloration in spot size.

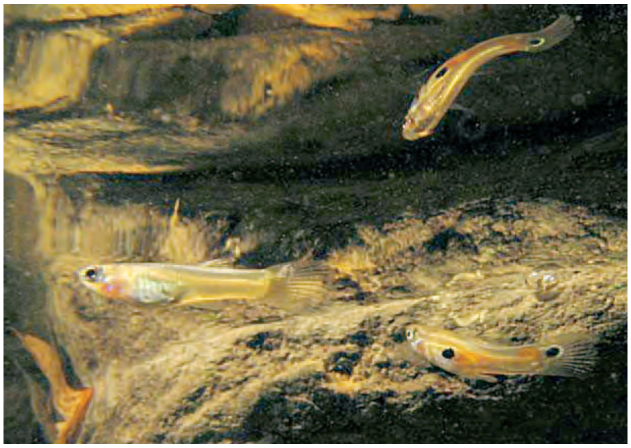

Figure 1: *Pictures of guppies in their natural environment in Trinidad. Males are smaller and colorful whereas females are larger and color-free as seen in the leftmost guppy in the left picture. Male Poecilia exhibit their array of color (seen in the picture to the right) by performing an S-shaped posture termed a "sigmoid display" (12) in order to attract females. Paul Bentzen©*

MATERIALS AND METHODS:

Morphological changes in coloration in Taylor and Caigual (introduced 2009)

Monthly pictures of all individually marked fish first introduced into both research streams have been taken since introduction in Trinidad. The pictures were sent to Riverside where we were able to analyze color variation between the ancestral Guanapo high-predation fish and the two introduced low-predation populations with the program ImageJ. The photos were analyzed bimonthly for body area and coloration (the area of each of the black and orange color spots) for one full year post-introduction. We needed to adjust for the effects of body size (so spots are not larger simply because male size is larger) so to obtain relative color area we divided the total area of each color group by body area. Three different people performed pictures measurements and the differences in their coloration measurements were compared using an ANOVA. Since significant differences in the measurement of "fuzzy" black color spots were found between the measurements we only used "distinct" black spots in all subsequent analyses.

Effect of light treatment in the introduced streams

Here we used the ImageJ measurements from both introductions streams for the first time to track fine scale measurements in the evolution of spot coloration under two ecological manipulations in guppies (predation and canopy openness). As stated previously, increasing the light in the two manipulated streams will increase algal productivity, the main source of guppies' dietary carotenoids, in those streams. Studies have shown that increased carotenoids in guppies' diets can affect the brightness of guppies' attractive yellow and orange spots (13) and that female preference for these spots can drive the rapid evolution and maintenance of color morphs with large amounts of these colors. Based on these findings we expected there to also be a higher frequency of morphs with brighter and larger spot colors in the Taylor stream (the introduced stream with the canopy manipulation) than the Caigual stream (natural low-predation introduced stream).

Statistical Analysis

To compare the beginning and end points of the introduction as done in the previous guppy transplants for body area simple ANOVA's (Analysis of Variance) were used to examine differences between the ancestral fish introduced into either the closed or open canopy introduction sites. Phenotypic divergence in male coloration across the twelve months from the wild data were analyzed using LMEs (Linear Mixed Effect Models, Rstudio v2.15) which allows a repeated-measures analysis with color relative area (black and orange separately) as the response variable, and month as a discrete explanatory variable. Separate analyses were done for both streams in order to see the effect of the light treatment.

RESULTS:

Body Area

Body area increased significantly (Figure 2) as expected in the manipulated open canopy Taylor introduction stream as guppies adapt to the new low-predation environment (ANOVA $F_{1,143}$= 9.312, p=0.0027). However, surprisingly, body area significantly decreased in the natural low-predation Caigual introduction site (ANOVA $F_{1,91}$= 10.03, p=0.0021). There were no significant differences between the initial two sets of Guanapo high-predation fish introduced into each low-predation site.

Table 1: Table of body area and standard deviation at month 0 and months 12 showing change between ancestral and derived fish in body area in each introduction stream.

	Taylor Month 0	Caigual Month 0	Taylor Month 12	Caigual Month 12
Mean Body Area (cm^2)	0.54	0.55	0.61	0.50
Standard Deviation	0.01	0.01	0.01	0.01

Morphological changes in coloration in Taylor and Caigual (introduced 2009)

No significant changes in coloration were found in both the Taylor and Caigual when both introduction streams were analyzed together across all months, and overall there was a non-gradual trend. There were also no significant differences between the ancestral fish introduced into either introduction site. For these reason, here we only provide the figures for the separate analyses of each introduction stream for easier visibility of the results (Figure 3). Fish in the closed canopy Caigual site did not significantly change in orange (p=0.6989) or black (p= 0.5857) coloration over time. Fish in the open canopy Taylor site also did not significantly change in orange (p= 0.2635) or black (p= 0.1362) coloration over time. There was, however, a significant spike in orange coloration in month six corresponding to the harshest month of the wet season in both streams**.**

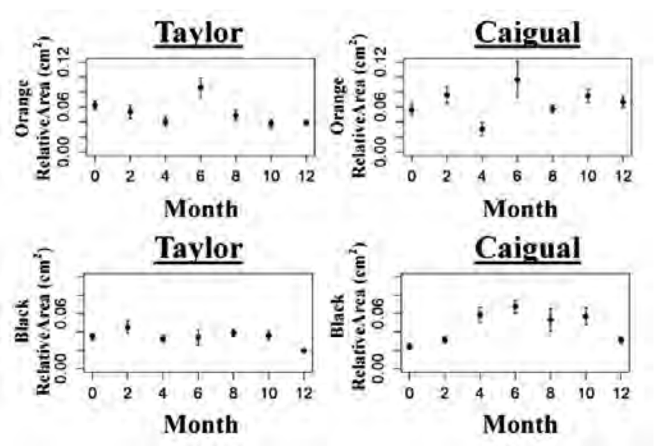

Figure 2: *Graphs of phenotypic changes of male coloration across months 0 through 12 using introduction stream data and showing change between ancestral and derived fish in body area. Figure 2 shows the bi-monthly changes in relative areas of orange and black coloration for both introduction streams. Lines represent standard errors.*

Effect of light treatment in the introduced streams

We found no effect of light treatment i.e., differences in relative area spot coloration between the Taylor and Caigual introduction sites. The only difference between the two streams was found in body area, which indirectly may have an effect on sizes of spots and hence how females or potential predators perceive color.

DISCUSSION:

The maintenance of color polymorphisms in the wild is an important topic in terms of the ability of certain organisms to survive in changing environments. Here we used an introduction experiment to study how different ecological factors can affect adaptation to novel environments of a polymorphic trait in guppies. Our results show that guppies introduced from one high-predation environment into two low-predation environments; one with canopy trimmed and one left natural, can have rapid and complex changes in color in just twelve short months. We now discuss our results and implications for these findings in greater detail.

Within both the Taylor and Caigual river environments there was a significant change in body area. We had expected, based on multiple previous experiments in this system (8, 5, 14, 15), that body size would increase as predation pressure was relaxed in the new introduced environments. Interestingly however, we only found an increase in the Taylor stream and not the Caigual where the guppies are actually becoming smaller as they adapt to their new environment. This decrease in size in the Caigual cannot be due to the light manipulation since it is actually the Taylor stream that had the canopy trimmed. It is possible however that the increased productivity, and hence increase food, due to the open canopy has caused the rapid jump in body area in only twelve months, or three guppy generations.

In terms of coloration we also found no significant changes in either size of orange or black spots as guppies adapt to the novel low-predation introduction sites. Female guppies prefer coloration especially orange spots which are a sign of male quality, and black spots which may be a color contrast making the guppy seem more bright (6). In high-predation environments however, there is a trade-off in that too elaborately colored guppies are more easily preyed upon. Hence we had expected that as the guppies are introduced from a high-predation environment into the low-predation introduction sites that both color groups would increase in size. This did not happen and black spots remained the same in both the Taylor and Caigual introduction sites. In orange coloration, it seemed to be a seasonal effect in that the only significant increase seemed to have been at month six, corresponding to height of the wet season. There was no difference in coloration between the light manipulated Taylor and natural Caigual environments, which tells us that there was no light effect on the size of color spots. It is possible that even though the size of color spots did not change that the brightness or hue did, however we did not measure those in this experiment.

Our results did not provide a strong relationship between an environment i.e. canopy cover manipulation and expression of color polymorphism and guppy body area, and this could be due to multiple reasons. First, Trinidad undergoes a strong wet and dry season every year. The wet season (October) is associated with massive floods result in an increase in mortality of guppies. Additionally, the strong shift in coloration at month six reveals what a potentially important factor (wet season) in the evolution of coloration in wild guppies. Second, we have only analyzed one year of our data. It is possible that not enough time has passed for us to see a strong effect in either color change or a light effect. Lastly, both the Caigual and the Taylor are plagued by parasites which can affect both growth and coloration, potentially leading to the death of guppies expressing color, as mortality engendered by parasites is not dependent on color polymorphisms. Future work will focus on continuing to follow coloration and the light effect in these streams, studying the role of parasites in hindering the evolution of coloration, and finally performing a common garden experiment to see if any genetic and hence heritable changes have occurred in the introduction sites.

Our results are vital in the understanding of color polymorphisms and in the organism's ability to adapt to changes in their environment. These ideas better enable us to follow the diversity of life due to these adaptations over time (16).

REFERENCES

Magellan, K. Magurran, A.E. 2009. The Effect of Social Environment During Ontogeny on Life History Expression in the Guppy Poecilia reticulata. Journal of Fish Biology. Vol. 74. Issue 10. 2329-2337.

Fitzpatrick, J. L. et al. 2008. Female promiscuity promotes the evolution of faster sperm in cichlid fishes. PNAS. Vol. 106 no. 4.

McKinnon, J. S. Pierotti, M. E. 2010. Colour Polymorphism and Correlated Characters: Genetic Mechanisms and Evolution. Molecular Ecology. Vol. 19. Issue 23. 5101-5125.

Badyaev A. 2005. Stress-induced Variation in Evolution: from Behavioural Plasticity to Genetic Assimilation. Proceedings of the Royal Society.

Reznick, D. Ghalambor, C. Crooks, K. 2008. Experimental Studies of Evolution in Guppies: A Model for Understanding the Evolutionary Consequences of Predator Removal in Natural Communities. Molecular Ecology. 97-107.

Houde, A. E. Sex, Color, and Mate Choice in Guppies. Princeton University Press. 1997.

Endler, J. 1980. Natural Selection on Color Patterns in Poecilia reticulata. Evolution. Vol. 34. No. 1. 76-91.

Gordon, S. P., D. N. Reznick, M. T. Kinnison, M. Bryant, D. J. Weese, K. Räsänen, N. P. Millar, and A. P. Hendry. 2009. Adaptive changes in life history and survival following a new guppy introduction. American Naturalist 174: 34-45

Reznick, D. Buckwalter, G. Groff, J. Elder, D. 2001. The evolution of senescence in natural populations of guppies (Poecilia reticulata): A Comparative Approach. Experimental Gerontology. Vol. 36. Issues 4-6.791-812.

Grether, G. Hudon, J. Endler, J. 2001. Carotenoid Limitation and Mate Preference Evolution: A Test of the Indicator Hypothesis in Guppies (Poecilia Reticulata). Evolution. 1712-1724.

Grether, G. 2005. Environmental Change, Phenotypic Plasticity, and Genetic Compensation. American Society of Naturalists. Vol 166. No. 4. E115-E123.

Liley, N. R. 1966. Ethological Isolating Mechanisms in Four Sympatric Species of Poecilia Fishes. Behaviour (Suppl. XIII). 13, 1–197.

Grether, G. 2000. Carotenoid Limitation and Mate Preference Evolution: A Test of the Indicator Hypothesis in Guppies (Poecilia Reticulata). Evolution. 1712-1724.

Reznick, D. Butler, M. Rodd, H. Ross, P. 1996. Life-History Evolution in Guppies (Poecilia Reticulata) 6. Differential Mortality as a Mechanism for Natural Selection. Evolution. Vol. 50. No. 4. 1651-1660.

Sheppard, P. 1953. Polymorphism, Linkage and the Blood Groups. The American Naturalist. Vol. 87. No. 836. 283-294.

Futuyma, D. 2009. Natural Selection and Adaptation. Evolution. Second Edition.