

The See-thru guppy: a transparent fish model

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Abstract. The guppy has the major classes of chromatophores found in teleosts: xantho-erythrophores, iridophores and melanophores. These color cells render the adult body essentially opaque. A transparent guppy was developed, the See-thru guppy, combining alleles that genetically remove the major classes of guppy color cells: xantho-erythrophores, iridophores, melanophores and leucophores. The See-thru guppy may have interesting applications for scientists already using the guppy as a model organism. In our own research into the inheritance of color patterns in the guppy, the See-thru guppy may be useful for exploring the specificity of mutations to color cell types.

Key Words: transparent guppy, Albino Blau, Glass Belly Panda.

Resumen. Guppy tienen las mayores clases de cromatóforos conocidas por los teleósteos: xantó-eritróforos, iridóforos y melanóforos. Estas células pigmentarias dan a la cuerpo de pescados adultos un carácter opaco. En el presente papel se describe el modo por la creación a una línea transparente de guppy llamado See-thru, por medio de la combinación de los genes alelos que eliminan genéticamente las principales células pigmentarias: las xantó-eritróforos, iridóforos, melanóforos y leucóforos. La línea de guppy See-thru puede tener aplicaciones científicas más interesantes, útil por los investigadores que utilizan el guppy como un organismo modelo. Las nuestras recercas sobre la transmisión de los tipos del color a la guppy, la línea obtenida pueden ser útil por la exploración la especificidad de los mutaciones a la nivel del tipos de células pigmentarias.

Palabras clave: guppy transparente, Albino Blau, Glass Belly Panda.

Rezumat. Guppy deține clasele majore de cromatofori cunoscute la teleostei: xanto-eritrofore, iridiofore și melanofoare. Aceste celule pigmentare conferă corpului peștilor adulți un caracter opac. În prezenta lucrare se descrie modul de creare a unei linii de guppy transparent, denumit See-thru, prin combinarea alelelor care elimină genetic celulele pigmentare principale: xanto-eritroforele, iridioforele, melanoforele și leucoforele. Linia de guppy See-thru poate avea aplicații științifice interesante, utile cercetătorilor care folosesc peștele guppy ca organism model. În ceea ce privește cercetările proprii asupra transmiterii tiparelor coloristice la guppy, linia creată ar putea fi utilă în vederea explorării specificității mutațiilor la nivelul tipurilor de celule pigmentare.

Cuvinte cheie: guppy transparent, Albino Blau, Glass Belly Panda.

Introduction. The guppy (*Poecilia reticulata*) has been of considerable interest to science because of the color polymorphism of the male and the consequent usefulness of the guppy as a model organism in the study of evolutionary ecology (Magurran 2005). There have been linkage maps constructed for guppy genes, but little work has been done on the molecular or cellular basis for guppy patterns. Much of the research using guppy color polymorphism as the visible expression of evolutionary change is based on studies made by Øjvind Winge in the 1920s and 1930s, most notably his eighteen genes paper (Winge 1927).

Extant studies of guppy chromatophores at the cellular level include a paper on the golden and blond mutations by Goodrich et al (1944) which studied mutant and wild type melanophores on the cellular level. In 1976 a study of the two structural chromatophores in the guppy, the iridophore and leucophore, were made by Ikuo Takeuchi (Takeuchi 1976) using electron microscopy. There were three papers on melanophores in 1978 and 1979 by P. L. Nayudu and C. R. Hunter, including a paper on the response of melanophores to melatonin (Nayudu & Hunter 1979). There was a study of guppy iridophores in 1982 (Gundersen & Rivera 1982).

Recently there have been some signs of a new interest in the molecular basis for guppy patterns. A study was published on 2005 that linked a number of phenotypic traits

to molecular markers (Watanabe et al 2005). In December 2008, the journal *Zebrafish* published a special pigment biology issue which included a study by scientists at the Max Planck Institute for Developmental Biology on the molecular and genetic basis for guppy color polymorphism (Tripathi et al 2008).

The zebrafish *Danio rerio* (Hamilton) has been the model organism of choice in developmental studies because of its high fecundity, external fertilization, *ex utero* development, optical clarity, and advancing genomic resources (Pickart et al 2004). In particular the stripe pattern on males and females has proven to be an easily manipulated system for studying the developmental genetic bases for the evolution of adult form in vertebrates (Parichy 2001). The guppy shares many of these advantages, except fertilization is internal and the guppy embryo develops inside the female.

More recently a transparent zebrafish "Casper" was developed for *in vivo* transplantation analysis (White et al 2008). The transparent zebrafish allows researchers to study the development of normal and cancer stem cells in live adult fish, previously only possible in embryogenesis because of the opacity of adult fish.

Previous to the creation of transparent zebrafish, a transparent medaka (*Oryzias latipes*, Chen, Uwa & Chu) was developed in Japan (Wakamatsu et al 2001). The transparent medaka, like the transparent zebrafish, is "see-through" by virtue of the fact the main classes of color cells are genetically removed by a combination of recessive alleles. The transparency of the skin allows internal organs to be viewed by the naked eye or with a simple stereoscopic microscope. The process of oocyte development in the female medaka can be observed. And noninvasive studies of morphological and molecular events in internal organs can be observed throughout juvenile and adult life. Transparent fish are valuable model organisms that have a great deal of relevance to the study of organ development and disease in humans.

Researchers who wish to use the guppy as a model organism for genetic, developmental or disease studies now have a transparent guppy as an option. Using three major recessive alleles affecting the three major classes of color cells, a transparent guppy "See-thru" was developed. The transparent guppy combines the albino (aa), Asian blau (rr) and Glass Belly (gbgb) mutations. These mutations genetically remove the melanophores, xantho-erythrophores and certain types of iridophores and leucophores from the guppy's skin (Figure 1).



Figure 1. Transparent female guppy. You can clearly see the embryo has developed to the stage where the female is about to give birth. The fry were born the next day

The See-thru guppy allows the researcher to study the main internal organs in live fish: heart, spleen, blood vessels, liver, gut, gonads, kidney, brain, spinal cord, lens, air bladder, and gills (Figures 2 and 3).



Figure 2. View of female from below



Figure 3. View of male from above

Materials and Methods. The guppies were bred in 21 liter tanks with manual water changes (30%) once a week. Tanks were painted black on the bottom and otherwise bare. Tanks were maintained at a constant temperature of 26°C. The light cycle was 14 hours daylight and ten hours night. The fish were fed *Artemia* and a commercial fish food flake (Omega One Natural Protein Formula, Sitka, Alaska). There was one feeding of flake food and two *Artemia* feedings per day.

Parental Strains. Two mutant parental strains were used to produce the progeny with pigment deficiencies.

1. Albino Blau. An Albino Blau strain from Luke Roebuck, an American guppy broker (<http://ppga.tripod.com/luke-sales.html>), was purchased. This strain combines the albino with Asian Blau mutations (Figure 4).



Figure 4. Luke Roebuck Albino Blau

The guppy albino mutation has been in the guppy hobby since the 1940s. It was first described by Dzwillo (Dzwillo 1959). Albinism is the result of the failure of melanin to be produced.

The Roebuck strain also has the Asian Blau mutation. This is a previously undescribed mutation that causes the failure of xanthophores and erythrophores to be expressed. Dzwillo describes a similar mutation called "blau" (Dzwillo 1959). Dzwillo's account of the blau mutation is somewhat ambiguous because he says it is a mutation affecting xanthophores and does not initially describe it as affecting erythrophores. But then he goes on to assign the gene symbol "r" to the mutation ("r" for red), adapted from a mutation of the medaka described by the scientist T. Aida, which is a mutation affecting both the yellow and red color cells. Dzwillo tells us that the blue base body color comes from the fact yellow color cells are missing in the skin, leaving the blue iridophores unfiltered by yellow pigment. (Hence the name. *Blau* is German for "blue.") Dzwillo describes the mutation as recessive.

The Asian Blau mutation when heterozygous suppresses the expression of erythrophores so it is dominant. When homozygous it suppresses expression of both erythrophores and xanthophores. For this reason I have given the genotype of a heterozygous Asian Blau mutant as Ab/-.

The albino and Asian blau genes together remove two main classes of guppy color cells, the xantho-erythrophores and melanophores.

2. Glass Belly Panda. The second strain used to create the See-Thru guppy was the Glass Belly Panda (Figure 5).



Figure 5. The Glass Belly Panda

The strain was sourced on the Aquabid site (www.aquabid.com) from a hobbyist in Taiwan, Chang Yi. The strain incorporates previously undescribed mutations, Moscow, Pink and Glass Belly. The relevant mutation is the Glass Belly mutation. The mutation gets its name from the fact the ventrum is rendered transparent by this mutation. The mutation appears to affect the leucophores, which are found in the skin covering the ventrum. It also appears to affect iridophores, notably in the eyes, but also in other areas of the body. It does not seem to affect blue iridophores in the fins.

There also exists in the hobby a strain that combines the glass belly mutation with albinism (Figure 6).



Figure 6. Albino Red Tail Glass Belly. Courtesy Andrew Lim

Notice that the eye is missing iridophores, as well as most of the body. The presence of yellow and red color in the body and the fins indicates that this fish does not have a mutation affecting this class of color cells. The glass belly mutation is autosomal recessive (unpublished data of Philip Shaddock).

Results

Generation of the transparent guppy. To produce a guppy with all three mutations (albino, Asian Blau, Glass Belly), male Roebuck Albino Blaus were placed in a breeding colony with virgin female Glass Belly Pandas. When the females matured and dropped, one was selected and removed to a drop tank. The cross resulted in a uniform phenotype for the F1 progeny, shown in Figure 7.



Figure 7. F1 male and female from the Albino Blau male X Glass Belly Panda female cross

The F1 drop was maintained in a closed colony. When the females from the F1 drop matured, four were randomly selected to produce the F2 generation. Analysis of the cross predicted that the incidence of a guppy with the genotype $aa\ Ab/-\ gbgb$ would be 1 in 16.

The females dropped between 13 and 17 fry. From the four drops only four guppies were collected that had the transparent phenotype: heterozygous or homozygous Asian Blau, albino with a transparent ventrum. There were three females and one male (Figure 8).

The male is an albino with a transparent ventrum, missing iridophores in the body and eyes and having no red color, but he does appear to have some yellow color. This suggests he has the genotype $aa\ Abab\ gbgb$.

The females appeared to be white in color, which would indicate they are homozygous for the Asian Blau gene.



Figure 8. Transparent F2 male



Figure 9. Two F2 males, showing a grey phenotype with (above) and without (below) the Asian Blau allele. Neither is homozygous for the Glass Belly allele

Siblings were colored as expected. The red colored guppy in Figure 9 shows the wild type F2 male that segregated out, showing that the original parental stock had a lot of erythrophore color. The F2 male below him shows no red but some yellow, indicating he

is probably heterozygous for the Asian Blau mutation. Figure 10 shows an albino F2 male with no red and no transparent ventrum. Finally, Figure 11 shows a female that has the transparent ventrum but is not albino.



Figure 10: Albino F2 male heterozygous for the Asian Blau mutation



Figure 11. F2 grey female with a transparent ventrum

Discussion. A transparent guppy can be useful in laboratories that already use guppies as a model organism in the study of evolutionary ecology, diseases and abnormal development of internal organs and in the study of vertebrate developmental expression patterns.

The new See-thru strain is smaller in body size than its parental strains. It is robust, very active in the tanks. The strain appears to be fertile. And the male persistently chases the females. We have collected two drops from the females. The F3 generation are 100% See-thru phenotype. It is too early to tell if there are homozygous Asian Blau males.

In our own work See-thru facilitates the study of the specificity of guppy mutations with regard to the color cells they affect. For example, the blue iridophores seen in the F2 See-thru male's caudal fin (Figure 8) are unaffected by the Glass Belly

mutation, while the iridophores in the body and eyes do fail to develop. Yet the Glass Belly Panda parental strain male shows iridophores in the dorsal area at the front of the body (Figure 5). These are absent in the See-Thru male (Figure 8). The See-thru strain will be useful in exploring the Asian Blau mutation and other color cell specific mutations further.

References

- Aquabid, 2009 <http://www.aquabid.com> (last view: 15.02.2009).
- Dzwillo M., 1959 [Genetic research on some domesticated strains of *Lebistes reticulatus* (Peters)]. Mitt Hamburg Zool Mus Inst 57:143–186.
- Gundersen R. E., Rivera E. R., 1982 An ultrastructural study of the development of the dermal iridophores and structural pigmentation in *Poecilia reticulata* (Peters). Journal of Morphology 172(3):349–359.
- Goodrich H. B., Josephson N. D., Trinkaus J. P., Slate J. M., 1944 The cellular expression and genetics of two new genes in *Lebistes reticulatus*. Genetics 29(6):584–592.
- Magurran A. E., 2005 Evolutionary Ecology: The Trinidadian Guppy (Oxford Series in Ecology and Evolution). Oxford University Press.
- Nayudu P. L., Hunter C. R., 1979. Cytological aspects and differential response to melatonin of melanophore based color mutants in the guppy. Copeia 2:232–242.
- Parichy D. M., 2001 Zebrafish hybrids suggest genetic mechanisms for pigment pattern diversification in *Danio*. Development Genes and Evolution 211(7):319–328.
- Pickart M. A., Sivasubbu S., Nielsen A. L., Shriram S., King R. A., Ekker S. C., 2004 Functional genomics tools for the analysis of zebrafish pigment. Pigment Cell Research 17:461–470.
- Roebuck L., 2009 <http://ppga.tripod.com/lukeales.html> (last view: 15.02.2009)
- Takeuchi I. K., 1976 Electron microscopy of two types of reflecting chromatophores (iridophores and leucophores) in the guppy, *Lebistes reticulatus* Peters. Cell and Tissue Research 173(1):17–27.
- Tripathi N., Hoffmann M., Dreyer C., 2008 Natural variation of male ornamental traits of the guppy, *Poecilia reticulata*. Zebrafish 5(4):265–278.
- Wakamatsu Y., Pristiyazhnyuk S., Kinoshita M., Tanaka M., Ozato K., 2001 The see-through medaka: A fish model that is transparent throughout life. Proc Natl Acad Sci USA 98(18):10046–10050.
- Watanabe T., Yoshida M., Nakajima M., Taniguchi N., 2005 Isolation and characterization of 43 microsatellite DNA markers for guppy (*Poecilia reticulata*). Molecular Ecology Notes 3(4):487–490.
- Winge Ø., 1927 The location of eighteen genes in *Lebistes reticulatus*. J Genet 18:1–42.
- White R. M., Sessa A., Burke C., Bowman T., LeBlanc J., Ceol C., Bourque C., Dovey M., Goessling W., Burns C. E., Zon L. I., 2008 Transparent adult zebrafish as a tool for in vivo transplantation analysis. Cell Stem Cell 2(2):183–189.

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