Biol 2202: Genetics Laboratory

Independent Project

2013

Maxwell Helmberger, Ramsey Hass,

Nick Motzko, Charles Schueller

**Mendelian Genetics and Transgene Inheritance**

A **transgene** is a gene inserted into an organism’s genome from another source, usually another organism. The transgenes you will be working with are modified versions of the Green Fluorescent Protein (GFP) gene, found in luminescent jellyfish and inserted into the genomes of zebrafish (*Danio rerio)*. There are five fluorescent protein transgenes; GloRFP, GloYFP, GloGFP, GloBFP, and GloPFP, which create red, yellow, green, blue, and purple pigments, respectively, in the muscle tissue of the fish. The color of a non-transgenic, or “wild-type” zebrafish is dull gray. In this assignment, you will examine and learn how transgenes are passed down from parents to offspring in zebrafish and how those transgenes express themselves in an organism.

In normal Mendelian genetics, an organism will have two copies of each gene (one from its mother and one from its father) at a **locus** (the name for a particular location in the genome). The forms of the genes present (called alleles) will result in a different **phenotype**, or set of physical characteristics, in the organism. Alleles can be dominant or recessive. A dominant allele will mask the recessive one if both are present in the same organism.

Transgenes, however, behave differently from normal genes. Transgenes are artificially added into an organism’s genome by scientists, and so there are not always two copies. In the transgenic strains of zebrafish called GloFish, a transgene-free locus is written as Glo-/Glo- in an organism’s genotype. A locus containing one transgene is simply written as GloXFP/Glo-, and one with two transgenes is written as GloXFP/GloXFP, where X represents the color of the created protein. The phenotype of a GloXFP/Glo- fish and a GloXFP/GloXFP fish will be the same. When multiple GloXFP transgenes are present in the same organism, each is on its own locus. The genotype of a fish homozygous for both RFP and YFP would be written as GloRFP/GloRFP;GloYFP/GloYFP. Note the alleles at the same locus are separated by an “/” and genes at different loci are separated by an “;”. The GloFish transgenes tested so far are incompletely dominant with one another. Therefore, the color of a fish homozygous for both GloRFP and GloYFP would be orange.

Scientists engineer organisms using transgenes in a variety of ways. Food crops can be altered to be more nutritious or to resist insect pests. Animals can be engineered to grow faster or produce more meat. Genetic engineering can also be used to prevent and treat certain diseases in humans. The transgenic strains of zebrafish called GloFish are sold as pets.

Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_



1a. The above data are the progeny resulting from a cross between two transgenic zebrafish. How many phenotypes do you observe?

b. How many fish of each phenotype do you observe?

2. Which of the previously described transgenes (GloRFP, GloYFP, GloBFP, GloGFP, and GloPFP) do you think could be present in these fish? Remember that multiple transgenes can be present in the same fish.

3. Based on this information, what colors (phenotypes) do you think the parents have?

4. Construct a Punnett Square for each of these crosses. Calculate the phenotypic ratios for each cross.

a. GloRFP/Glo-;Glo-/Glo- x Glo-/Glo-:GloBFP/Glo-

b. GloRFP/GloRFP;Glo-/Glo- x Glo-/Glo-;GloBFP/Glo-

c. Glo-/Glo-;GloPFP/GloPFP x GloRFP/GloRFP;Glo-/Glo-

5. Of the crosses in question 4, which one do you think resulted in the fish pictured above? Explain why you chose this answer.

6. As mentioned previously, scientists have used transgenes to modify many organisms besides zebrafish. Controversy has been raised over the ethics and safety of these genetically modified organisms. Do you think people should be altering the genomes of humans and other organisms? Talk to your neighbors and come up with one argument for and one argument against genetic engineering.

**Outline talk for teacher**

A **transgene** is a gene inserted into an organism’s genome from another source, usually another organism. The transgenes you will be working with contain sequences that encode proteins that are able to fluoresce. For example, the Green Fluorescent Protein (GFP) gene, used to make the green strain of GloFish, coms from jellyfish. These transgenes have been inserted into the genomes of zebrafish (*Danio rerio)*. There are five fluorescent protein transgenes; GloRFP, GloYFP, GloGFP, GloBFP, and GloPFP, which create red, yellow, green, blue, and purple pigments, respectively, in the muscle tissue of the fish. The color of a non-transgenic, or “wild-type” zebrafish is gray. In this assignment, you will examine and learn how transgenes are passed down from parents to offspring in zebrafish and how those transgenes express themselves in an organism.

In normal Mendelian genetics, an organism will have two copies of each gene (one from its mother and one from its father) at a **locus** (the name for a particular location in the genome). The forms of the genes present (called alleles) will result in a different **phenotype**, or set of physical characteristics, in the organism. Alleles can be dominant or recessive. A dominant allele will mask the recessive one if both are present in the same organism.

Diagramming a basic Mendelian cross with a Punnett Square to refresh students would be a good idea here.

Transgenes, however, behave differently from normal genes. There does not have to be two copies. For GloFish, a transgene-free locus is written as Glo-/Glo- in an organism’s genotype. A locus containing one transgene (a fish hemizygous for the transgene) is simply written as GloXFP/Glo-, and one with two transgenes (a fish homozygous for the transgene) is written as GloXFP/GloXFP, where X represents the color of the created protein. Since presence of the transgene is dominant over absence of the transgene, the phenotype of a GloXFP/Glo- fish and a GloXFP/GloXFP fish will be the same. When multiple GloXFP transgenes are present in the same organism, each is at its own locus. The genotype of a fish homozygous for both RFP and YFP would be written as GloRFP/GloRFP;GloYFP/GloYFP. The color of such a fish would be orange.

It is vital to stress that Glo- is not the “recessive allele” of a GloXFP transgene, but the lack of any transgene at that point in the genome. You can refer to GloXFP/Glo- fish as **hemizygous** for that transgene (they may be hemizygous or homozygous for others, or they may only have that one transgene).

The semicolon separates the two different loci, while the forward slash separates the transgenes (or lack thereof) on each locus. When writing the genotype of a fish with multiple transgenes, order the colors RFP > YFP > GFP > BFP > PFP, like the colors of the rainbow. When crossing or directly comparing two differently colored fish, it is wise to include any “empty” loci in the genotype.

For example, GloYFP/Glo- is okay when describing a hemizygous yellow fish. If you were crossing it with a GloGFP/GloGFP fish, you would write both fish as GloYFP/Glo-;Glo-/Glo- and Glo-/Glo-;GloGFP/GloGFP.

It will be a good idea to demonstrate a Punnett Square involving transgenic fish for the students. A Punnett Square for transgenic zebrafish carrying two different transgenes is functionally the same as any two-point/dihybrid cross (such as green pea vs. yellow pea and round pea vs. wrinkled pea, a common example), though the way the genotype is written can confuse students.

As an example, consider a cross between a GloRFP/Glo-;GloYFP/Glo- fish and a GloRFP/Glo-;Glo-/Glo- fish. Each fish will transmit one transgene or transgene-free chromosome from each of its loci in its gametes.

The GloRFP/Glo-;GloYFP/Glo- fish will produce four possible gametes; GloRFP;GloYFP, GloRFP;Glo-, Glo-;GloYFP, and Glo-;Glo-.

The GloRFP/Glo-;Glo-/Glo- fish will produce two possible gametes, GloRFP;Glo- and Glo-;Glo-.

Students can visualize the possible gametes by drawing curves between the gene names like so:

Since some fish will produce more possible types of gametes than others, the Punnett “Square” may look more like a rectangle. This is acceptable. Making it a true square will only mean repeating some gametes.

Make sure students know how to calculate phenotypic ratios from a Punnett Square. The phenotypic ratio for the above cross is 3 orange: 3 red: 1 yellow: 1 wild-type.

Scientists engineer organisms using transgenes in a variety of ways. Food crops can be altered to be more nutritious or to resist insect pests. Animals can be engineered to grow faster or produce more meat. Genetic engineering can also be used to prevent and treat certain diseases in humans. Transgenic zebrafish branded as GloFish are sold as pets.

Answer Key

1a. The above data are the results of a cross between two transgenic zebrafish. How many phenotypes do you observe?

There are two phenotypes among the progeny, red and purple.

b. How many fish of each phenotype do you observe?

There are five red fish and four purple fish.

2. Which of the previously described transgenes (GloRFP, GloYFP, GloBFP, GloGFP, and GloPFP) do you think could be present in these fish? Remember that multiple transgenes can be present in the same fish.

GloRFP, GloBFP, and GloPFP could all be present (although GloPFP is not, as students will learn later)

3. Based on this information, what colors (phenotypes) do you think the parents are?

The parents in the cross are red and blue. Red and purple is also a plausible answer at this point in the exercise.

4. Construct a Punnett Square for each of these crosses. Calculate the phenotypic ratios for each cross.

a. GloRFP/Glo-;Glo-/Glo- x Glo-/Glo-:GloBFP/Glo-

b. GloRFP/GloRFP;Glo-/Glo- x Glo-/Glo-;GloBFP/Glo-

c. Glo-/Glo-;GloPFP/GloPFP x GloRFP/GloRFP;Glo-/Glo-

a.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | GloRFP;Glo- | GloRFP;Glo- | Glo-;Glo- | Glo-;Glo- |
| Glo-;GloBFP | GloRFP/Glo-;GloBFP/Glo- | GloRFP/Glo-;GloBFP/Glo- | Glo-/Glo-;GloBFP/Glo- | Glo-/Glo-;GloBFP/Glo- |
| Glo-;Glo- | GloRFP/Glo-;Glo-/Glo- | GloRFP/Glo-;Glo-/Glo- | Glo-/Glo-;Glo-/Glo- | Glo-/Glo-;Glo-/Glo- |
| Glo-;GloBFP | GloRFP/Glo-;GloBFP/Glo- | GloRFP/Glo-;GloBFP/Glo- | Glo-/Glo-;GloBFP/Glo- | Glo-/Glo-;GloBFP/Glo- |
| Glo-;Glo- | GloRFP/Glo-;Glo-/Glo- | GloRFP/Glo-;Glo-/Glo- | Glo-/Glo-;Glo-/Glo- | Glo-/Glo-;Glo-/Glo- |

Condensing the Punnett Square by eliminating the repeat columns/rows is acceptable. The condensed square would like look this.

|  |  |  |
| --- | --- | --- |
|  | GloRFP;Glo- | Glo-;Glo- |
| Glo-;GloBFP | GloRFP/Glo-;GloBFP/Glo- | Glo-/Glo-;GloBFP/Glo- |
| Glo-;Glo- | GloRFP/Glo-;Glo-/Glo- | Glo-/Glo-;Glo-/Glo- |

Phenotypic ratio is – 1 Purple: 1 Red: 1 Blue: 1 Wild-Type/Gray

b.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | GloRFP;Glo- | GloRFP;Glo- | GloRFP;Glo- | GloRFP;Glo- |
| Glo-;GloBFP | GloRFP/Glo-;GloBFP/Glo- | GloRFP/Glo-;GloBFP/Glo- | GloRFP/Glo-;GloBFP/Glo- | GloRFP/Glo-;GloBFP/Glo- |
| Glo-;Glo- | GloRFP/Glo-;Glo-/Glo- | GloRFP/Glo-;Glo-/Glo- | GloRFP/Glo-;Glo-/Glo- | GloRFP/Glo-;Glo-/Glo- |
| Glo-;GloBFP | GloRFP/Glo-;GloBFP/Glo- | GloRFP/Glo-;GloBFP/Glo- | GloRFP/Glo-;GloBFP/Glo- | GloRFP/Glo-;GloBFP/Glo- |
| Glo-;Glo- | GloRFP/Glo-;Glo-/Glo- | GloRFP/Glo-;Glo-/Glo- | GloRFP/Glo-;Glo-/Glo- | GloRFP/Glo-;Glo-/Glo- |

The phenotypic ratio is 1 Purple: 1 Red.

c.

|  |  |  |
| --- | --- | --- |
|  | Glo-;GloPFP | Glo-;GloPFP |
| GloRFP;Glo- | GloRFP/Glo-;GloPFP/Glo- | GloRFP/Glo-;GloPFP/Glo- |
| GloRFP;Glo- | GloRFP/Glo-;GloPFP/Glo- | GloRFP/Glo-;GloPFP/Glo- |

The phenotypic ratio is 100% Red-Purple (all of the fish will be the same color).

5. Of the crosses on the previous page, which one do you think resulted in the fish pictured above? Explain your answer.

Cross B is the cross that produced the pictured fish. It is the only cross that resulted in only red and purple (in this case RFPxBFP results in the purple color, not PFP) fish; in other words, Cross B’s phenotypic ratio most closely matched the above data.

6. As mentioned previously, scientists have used transgenes to modify many organisms besides zebrafish. Controversy has been raised over the ethics and safety of these genetically modified organisms. Do you think people should be altering the genomes of humans and other organisms? Talk to your neighbors and come up with one argument for and one argument against genetic engineering.

There are many possible answers for this. You may want to allow students to access the Internet to help find answers to this question.

Examples of arguments for:

- Genetically modified crops can produce bigger yields for people in developing countries.

- Genetic engineering could cure debilitating genetic diseases in humans

Examples of arguments against:

- In humans, could lead to genetic inequality between the rich and poor.

- Genetically modified crops can be dangerous if we don’t properly determine their safety.