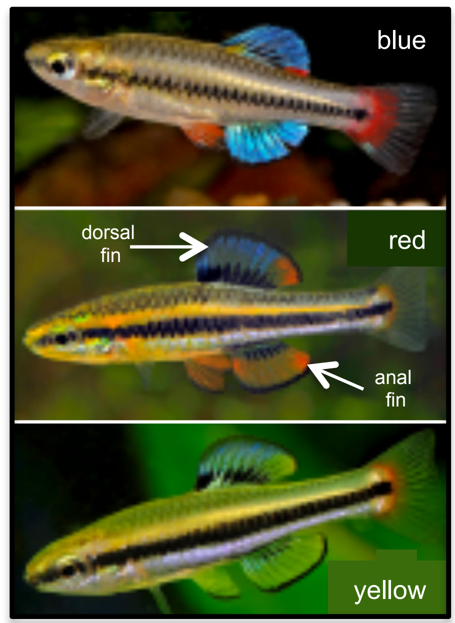
DATA Nugget

Why so blue? The determinants of color pattern in killifish, Part II
Featured scientist: Becky Fuller from The University of Illinois

In Part 1, you examined the effects of genetics and environment on anal fin color in male bluefin killifish. The data from Becky's experiment showed that both genetics and environment work together to determine whether male offspring had blue, yellow, or red anal fins. You will now examine how the father's genetics, specifically their fin color pattern, affects anal fin color in their sons. When we factor in the genetics of the father, and not just the population he came from, does this influence our interpretation of the data?

<u>Research Background</u>*: Effect of paternal genetics on male offspring anal fin color*

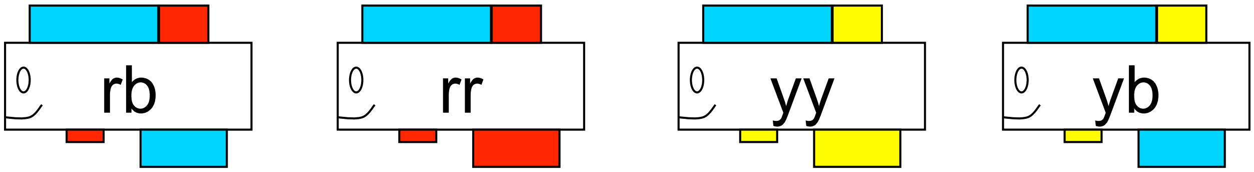
For her experiment, Becky collected male and female fish from both a swamp (26 Mile Bend) and a spring (Wakulla) population. Most of the males in the swamp have blue anal fins, but some have red or yellow. Most of the males from the spring have red or yellow anal fins, but some have blue. Becky decided to add data about the father's fin color pattern into her existing analysis from Part 1 to see how it affected her interpretation of the results.



The color polymorphism in bluefin killifish – males display anal fins in blue, red, or yellow. Photos by Tony Terceira.

In Part 1, Becky was looking at the genetics from the population level. Looking at the data this way, we saw parents from the 26 Mile Bend swamp population were more likely to have sons with blue anal fins than parents from the Wakulla spring. Parents from the 26 Mile Bend were also much more likely to have sons with higher levels of plasticity, meaning they responded more to the environment they were raised in. This means there was a big difference between the proportion sons with blue anal fins in the clear and brown water treatments.

Bringing in the color pattern of the fathers now allows Becky to look at the genetics from both the population and the individual level. From both the swamp and spring population, Becky collected males of all colors. Becky measured the color pattern of the fathers and recorded the color of their anal fins and the rear part of their dorsal fins. *She used males that were red on the rear portion of the dorsal fin with a blue anal fin (rb),* *males that were red on both fins (rr), males that were yellow on both fins (yy), and males that were yellow on the rear portion of the dorsal fin with blue a blue anal fin (yb).*



She randomly assigned half of each father's offspring to one of the water treatments, either clear or brown water. Once the sons developed their fin colors, she recorded the anal fin color. This experimental design allowed her to test whether sons responded differently to the treatment depending on the genetics of their father. She thought that <u>the anal fin color of the sons would be inherited genetically from the father, but would also respond plastically to the environment they were raised in.</u> She predicted fathers with blue anal fins would be more likely to have sons with blue anal fins, especially if they were raised in the brown water treatment. She also predicted that fathers with red and yellow anal fins could have sons with blue anal fins if they were raised in the brown water treatment, but not as many as the blue fathers.

<u>*Scientific Question*</u>: Is male anal fin color determined by genetics, environment, or both?

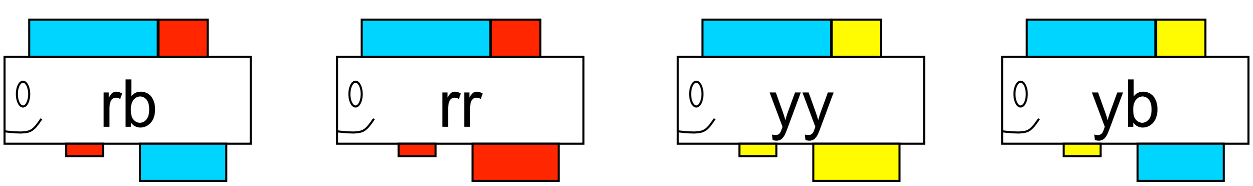
<u>*What is the hypothesis?*</u> Find the hypothesis in the Research Background and underline it. A hypothesis is a proposed explanation for an observation, which can then be tested with experimentation or other types of studies.

Becky in the field, with her colleague Katy, collecting fish in Wakulla Spring.

Scientific Data:

Use this information to complete the table below, and use the data to answer the scientific question:

Father #	Father's Color Pattern	Father's Anal Fin Color	Parent Population	Water Treatment	Total # of Sons	Total # of Sons with Blue Anal Fins	Proportion Sons w/ Blue Anal Fins
1	rb	blue	Wakulla	clear	10	0	0
2	rb	blue	Wakulla	clear	33	1	0.03
3	rr	red	Wakulla	clear	32	0	0
4	rr	red	Wakulla	clear	26	0	0
5	yb	blue	Wakulla	clear	15	0	0
6	yb	blue	Wakulla	clear	25	0	0
7	yy	yellow	Wakulla	clear	28	0	0
8	yy	yellow	Wakulla	clear	17	0	0
9	yy	yellow	Wakulla	clear	11	0	0
1	rb	blue	Wakulla	brown	16	0	0
2	rb	blue	Wakulla	brown	23	0	0
3	rr	red	Wakulla	brown	26	0	0
4	rr	red	Wakulla	brown	31	2	0.06
5	yb	blue	Wakulla	brown	31	1	0.03
6	yb	blue	Wakulla	brown	23	1	0.04
7	yy	yellow	Wakulla	brown	42	0	0
8	yy	yellow	Wakulla	brown	50	1	0.02
9	yy	yellow	Wakulla	brown	14	0	0
10	rb	blue	26 Mile Bend	clear	29	1	0.03
11	rb	blue	26 Mile Bend	clear	21	0	0
12	rr	red	26 Mile Bend	clear	17	1	0.06
13	rr	red	26 Mile Bend	clear	16	0	0
14	yb	blue	26 Mile Bend	clear	31	1	0.03
15	yb	blue	26 Mile Bend	clear	34	6	0.18
16	yy	yellow	26 Mile Bend	clear	14	0	0
17	yy	yellow	26 Mile Bend	clear	31	0	0
10	rb	blue	26 Mile Bend	brown	38	11	0.29
11	rb	blue	26 Mile Bend	brown	6	3	0.50
12	rr	red	26 Mile Bend	brown	24	2	0.08
13	rr	red	26 Mile Bend	brown	17	3	0.18
14	yb	blue	26 Mile Bend	brown	20	5	0.25
15	yb	blue	26 Mile Bend	brown	28	5	0.18
16	yy	yellow	26 Mile Bend	brown	25	0	0
17	yy	yellow	26 Mile Bend	brown	42	3	0.07



Parent Population	Water Treatment	Father's Color Pattern	Average Proportion Sons w/ Blue Anal Fins	Standard Deviation (SD)	Sample Size (N)	Standard Error (SE)
Wakulla	clear	rb	0.015	0.021	2	0.015
Wakulla	clear	rr	0	0	2	0
Wakulla	clear	yb	0	0	2	0
Wakulla	clear	yy	0	0	3	0
Wakulla	brown	rb	0	0	2	0
Wakulla	brown	rr	0.032	0.046	2	0.032
Wakulla	brown	yb	0.038	0.008	2	0.006
Wakulla	brown	yy	0.007	0.012	3	0.007

Parent Population	Water Treatment	Father's Color Pattern	Average Proportion Sons w/ Blue Anal Fins	Standard Deviation (SD)	Sample Size (N)	Standard Error (SE)
26 Mile Bend	clear	rb	0.017	0.024	2	0.017
26 Mile Bend	clear	rr	0.029	0.042	2	0.029
26 Mile Bend	clear	yb	0.104	0.102	2	0.072
26 Mile Bend	clear	yy	0	0	2	0
26 Mile Bend	brown	rb	0.395	0.149	2	0.105
26 Mile Bend	brown	rr	0.130	0.066	2	0.047
26 Mile Bend	brown	yb	0.214	0.051	2	0.036
26 Mile Bend	brown	yy	0.036	0.051	2	0.036

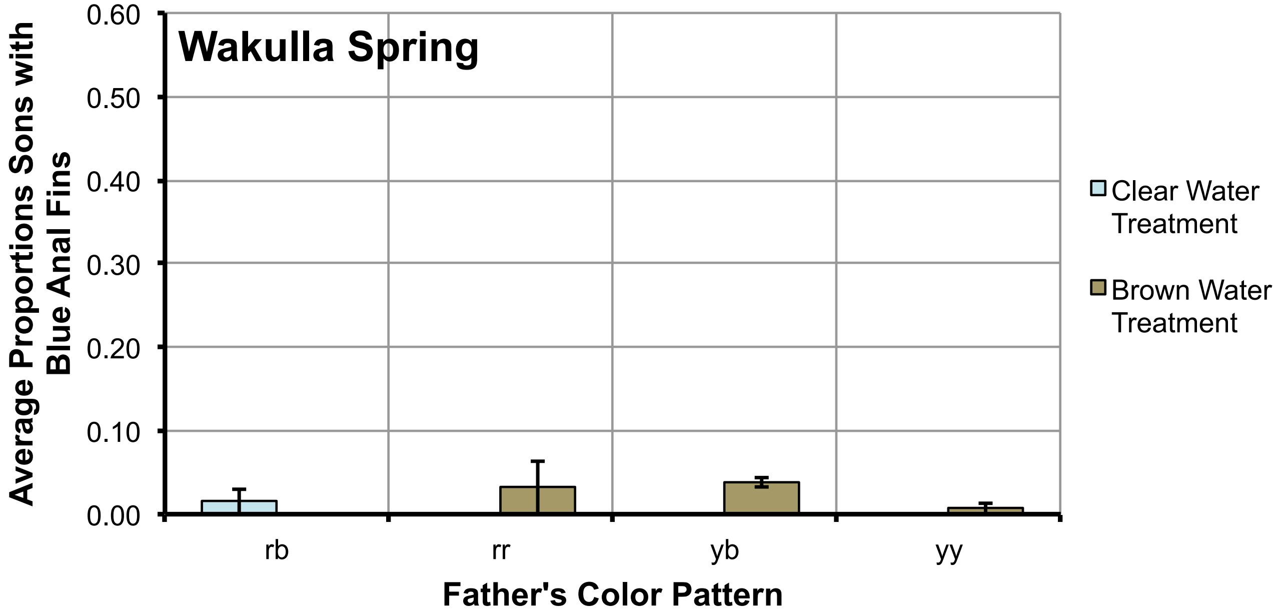
**Standard deviation (SD) tells us about the amount of variation in the data. A large SD means there is a lot of variation around the mean, while a small SD means the data points all fall very close to the mean. Standard error (SE) is SD divided by the square root of the sample size (N), and tells us how confident we are in our estimate of the mean. A large SE means we are not very confident, while a small SE means we are more confident.*

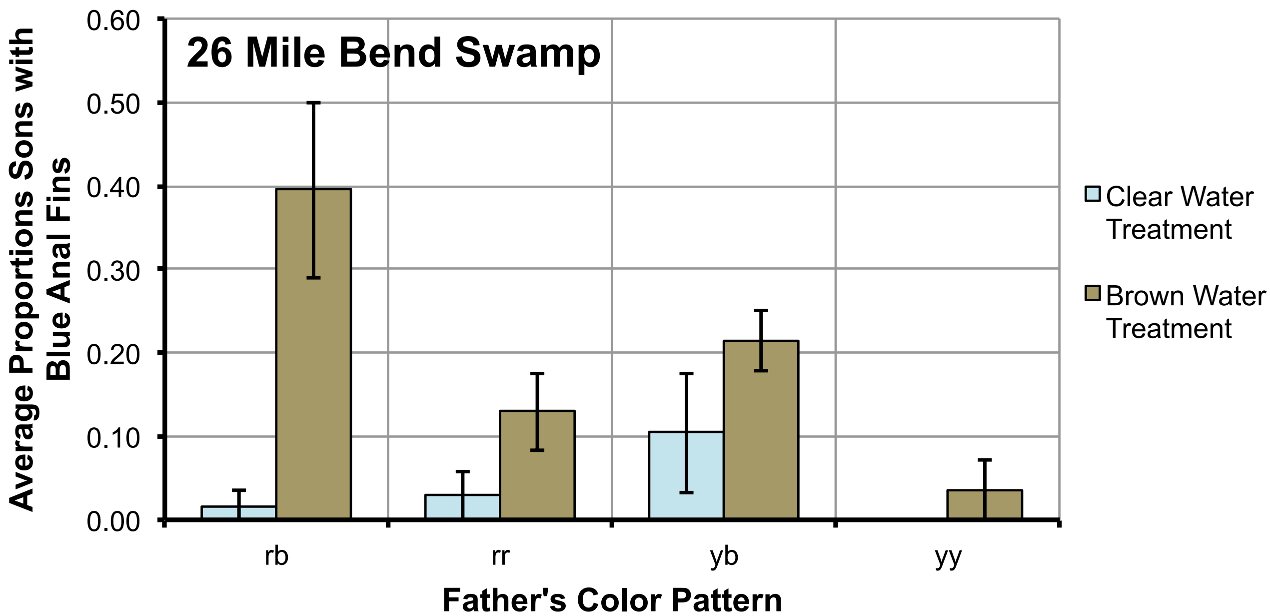
What data will you graph to answer the question?

Independent variables: Parent Population (Wakulla, 26 Mile Bend), Father's Color Pattern (rb, rr, yb, yy), and Water Treatment (clear, brown)

Dependent variable: Average proportion of sons with blue fins

Draw your graphs below: Identify any changes, trends, or differences you see in your graph. Draw arrows pointing out what you see, and write one sentence describing what you see next to each arrow.





Interpret the data:

Make a claim that answers the scientific question.

Male anal fin color is determined by genetics (population-level and paternal) and the environment. Paternal fin color pattern had an effect on the proportion of sons that had blue anal fins, but this pattern was more pronounced in the swamp (26 Mile Bend) than in the spring habitat (Wakulla).

What evidence was used to write your claim? Reference specific parts of the tables or graphs.

Male offspring that had fathers with yellow anal fins (yy) were very unlikely to have blue fins in any of the treatments(Range yy: 0-3.6%). Males with red anal fins (rr) had slightly higher proportions of sons with blue anal fins (range rr: 0-13%). Males that had blue anal fins were more likely to have offspring with blue anal fins in most cases (Range rb: 0-39.5%, range yb: 0-21.4%). In some cases, they still had low proportions of sons with blue anal fins (rb: 26 Mile Bend, clear water treatment 1.7%) relative to other treatments with parents from the same location.

Fathers from the Wakulla spring that had rr, yy, and yb did not produce any blue offspring in the clear water treatment. There was only one rb father that had one son (out of 33) with a blue anal fin. While fathers from Wakulla spring produced slightly more blue sons in the brown water treatment (5 out of 256), overall we see that fathers from Wakulla had very few sons with blue fins. This pattern was consistent across father’s fin color pattern.

Explain your reasoning and why the evidence supports your claim. Connect the data back to what you learned about the effect of paternal fin color pattern on male offspring anal fin color.

We saw in Part 1 that the anal fin color of the sons was determined by the population of origin of the parents were from (genetics), the water treatment (environment), and the interaction between the two (GxE). Here, we see that the fin color pattern of the father also had an effect on the proportion of sons that had blue anal fins, and that this effect depends on both the population of the parents and the water treatment.

Fathers from Wakulla had very few sons with blue fins. These low numbers were consistent across the brown and clear water treatment, and the fin color patterns of the fathers. For rr, yy, and yb fathers, there were only blue sons in the brown water treatment. For rb fathers there was only one blue son, and he was in the clear water treatment.

Fathers from 26 Mile Bend had more blue sons overall, and this effect depended on the water treatment and the fin color pattern of the father. Sons raised in the brown water treatment were much more likely to have blue anal fins than were sons raised in the clear water treatment. This effect was most pronounced for sons of fathers with the rb color pattern, meaning that these sons inherited a greater ability to respond to the environment from their fathers.

Teacher Note: Again we see evidence for a gene by environment (GxE) interaction in the data, but this time at the level of the genetics of the father. It will be a challenge for students to identify this interaction on their own, so you might want to pause here to have a classroom discussion. Ask students to look at their graphs to see if they find evidence that the effects of the water treatments differ for sons with fathers of different color patterns. Looking at the graph for fathers from the 26 Mile Bend Swamp population, we see that sons from rb fathers responded more to the water treatment than did other sons. Stated in another way, we see that the treatments had a stronger effect on the sons of rb fathers than it did for the other fathers. We do not see this pattern in the Wakulla Spring population. Therefore, the expression of the anal fin color trait (phenotype) depends on the population of the parents, the color pattern of the father, the environment that the sons experience, and interaction between all three independent variables!

Did the data support Becky's hypothesis? Use evidence to explain why or why not. If you feel the data was inconclusive, explain why.

Yes, the data supports Becky's hypothesis. The data elaborate on the previous dataset where we saw that all three types of variation (genetic, environmental, and an interaction between genetics and environment) affect the presence of blue anal fin color in nature. In this dataset, we see that it is not only the genetics of the population that matter, but also that the genetics of the father affects anal fin color of the sons. This suggests that at least some part the trait is inherited from the father. Sons of 26 Mile Bend fathers with the rb color pattern also responded to the water treatment to a greater degree, compared to sons with fathers of other color patterns. This suggests that traits for phenotypic plasticity are also inherited from the father.

<u>Your next steps as a scientist</u>: Science is an ongoing process. What new question do you think should be investigated? What future data should be collected to answer your question?

`See following Teacher Note.`

Teacher Note: In this dataset, we focus on the fin color patterns of the fathers and how this affects the anal fin color of the sons. We were unable to look at how the genetic color patterns of the mother fish affect the offspring because they have fins that have no color. The females still have genes for fin color pattern, but they do not express this trait. For her experiments, Becky was able to choose fathers non-randomly by color pattern, but the mothers are chosen randomly in respect to fin color pattern because Becky could not see it. In the experiment described in Part 1 and 2 of this Data Nugget, the genetics of the mother were taken into account at the population genetic level (because fathers were mated with mothers from their own population), but not at the individual genetic level (because Becky did not know the color pattern of the mothers). This may have contributed to some of the variation (seen as large error bars on the graphs) in the data.

Becky wants to figure out how the mother's genes contribute to the expression of fin color pattern in her sons. In a future experiment, Becky will use a cool trick. She is able to treat the mothers with testosterone, which causes them to express the genes for fin color pattern. She can then set up an experiment where she can control for the fin color pattern of both the fathers and mothers.