The genetic basis of red and blue coloration in *Betta Splendens*

Lab Meeting
February 07, 2020

Debbie Leung
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Background

Why study color in *Betta splendens*?

- Ornamental *Betta splendens* display a wide variety of colors

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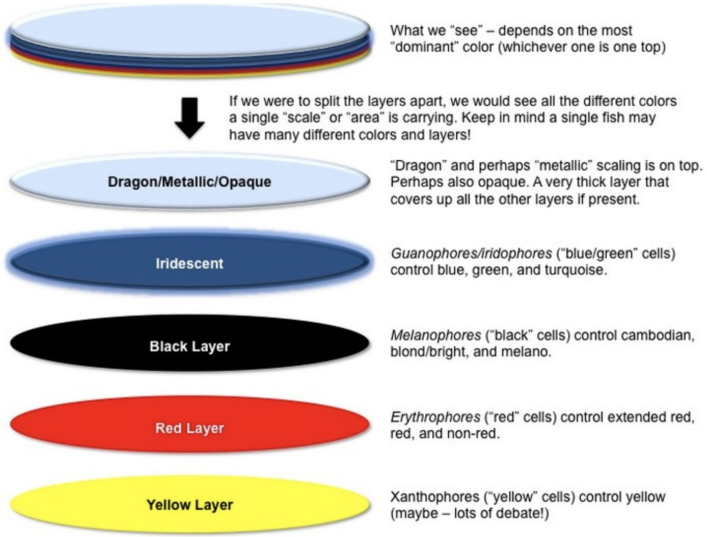
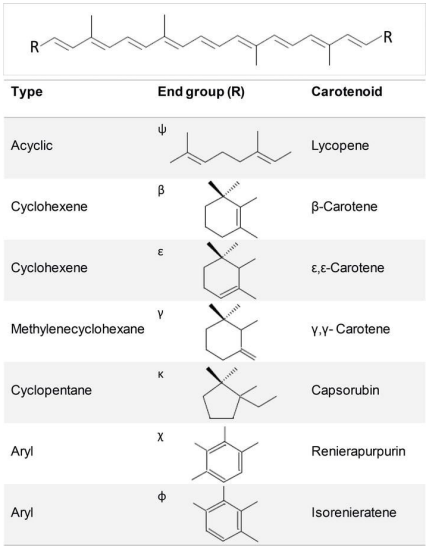
- Ornamental *Betta splendens* display a wide variety of colors, but what underlies these colors is unknown



From ingloriousbettas



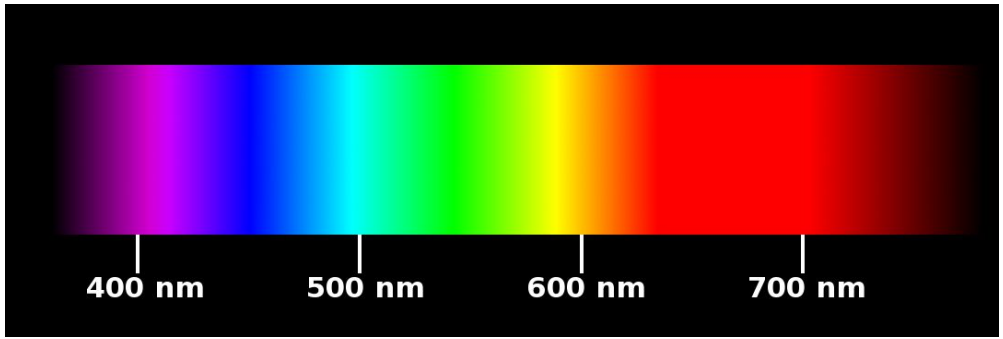
Type	End group (R)	Carotenoid
Acyclic	ψ	Lycopene
Cyclohexene	β	β-Carotene
Cyclohexene	ε	ε,ε-Carotene
Methylenecyclohexane	γ	γ,γ- Carotene
Cyclopentane	κ	Capsorubin
Aryl	χ	Renierapurpurin
Aryl	φ	Isorenieratene

From intechopen

Background

Why study color in *Betta splendens*?

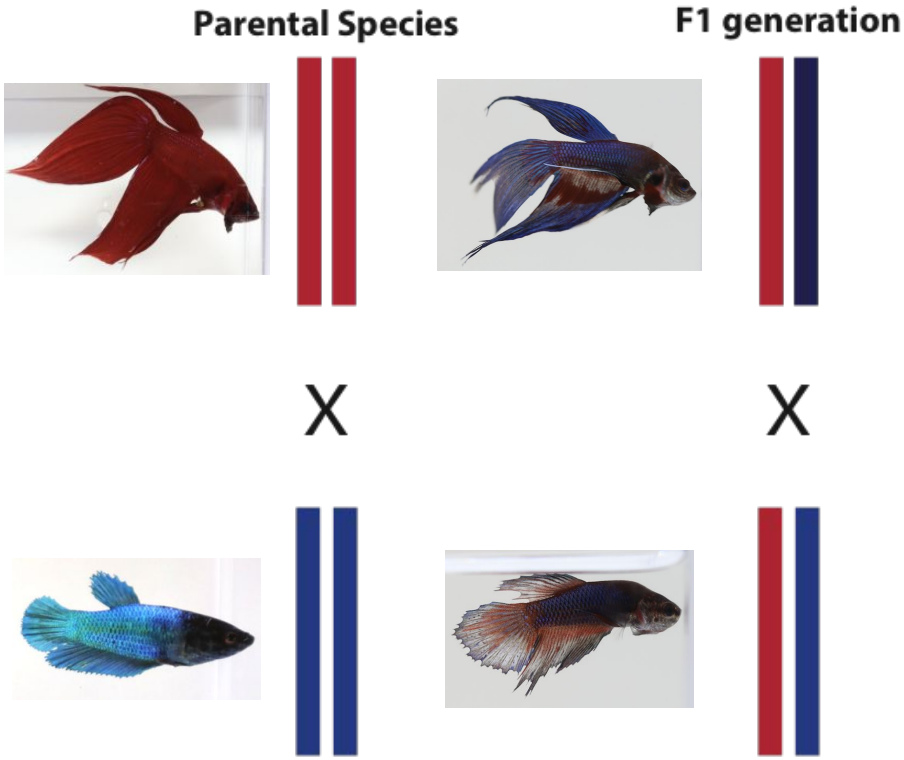
- Ornamental *Betta splendens* display a wide variety of colors, but what underlies these colors is unknown

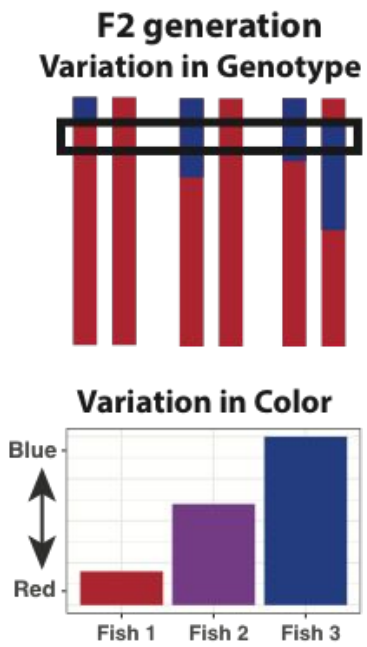


From wtamu

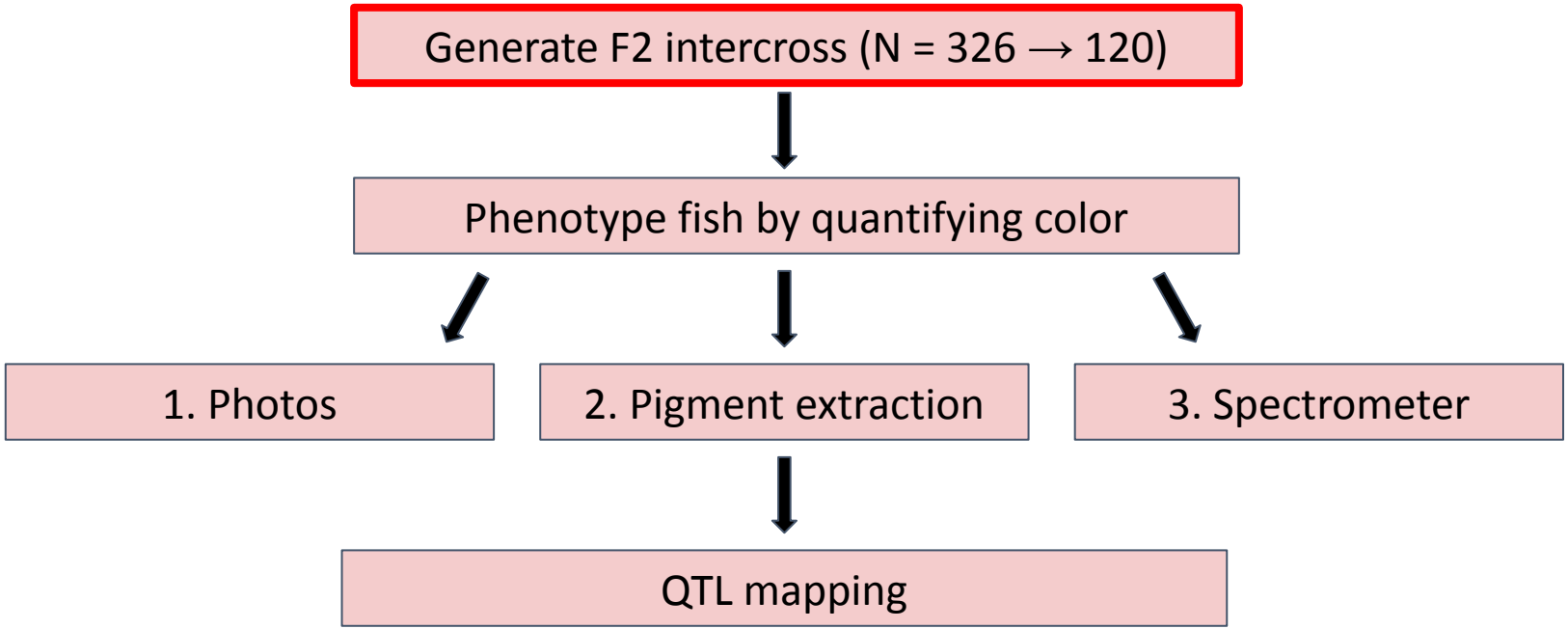
We are investigating the underlying genetic basis of red-blue coloration in Betta splendens

Methodology: F2 Intercross for QTL Mapping

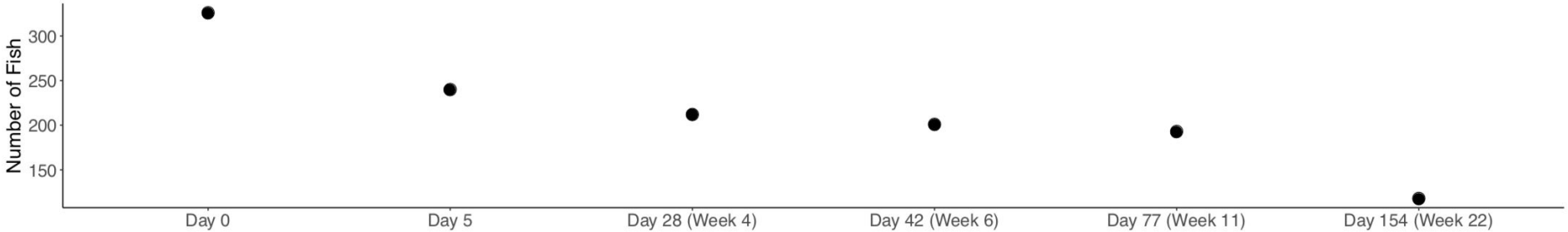




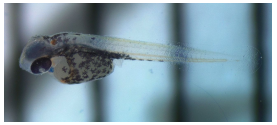
Experimental Workflow



Generating F2 Intercross



Spawn:
326 Fry
Split into 2x 400mL tanks with ⅛ dilution of rotifer w. fishwater



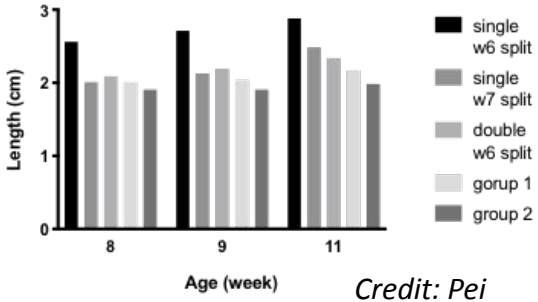
First split:
240 Fry
30 fish per 400mL tank

Second split:
212 Fish
8-9 fish per 400mL tank

Third split:
201 Fish
6 fish per 400mL tank (groups)
8 single-housed fish
6 double-housed fish

11th week:
193 Fish

22nd week:
118 Fish

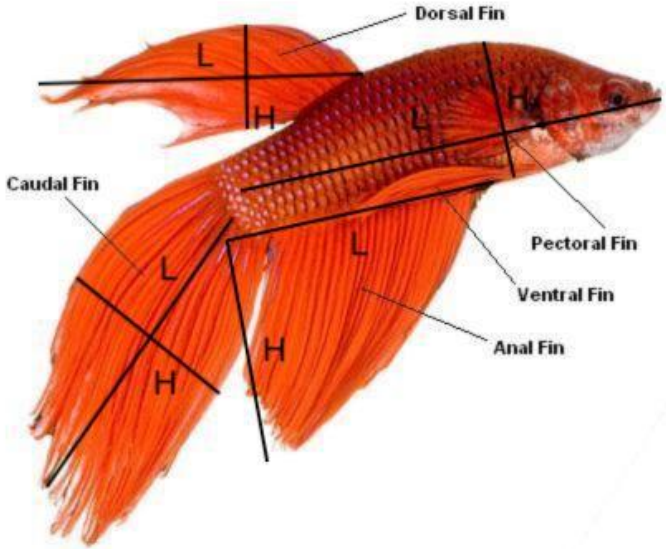


Credit: Pei

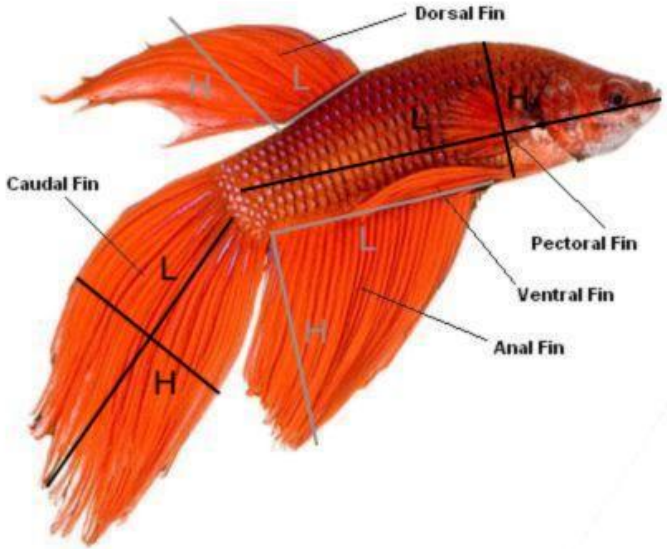
~36% survival rate as of February 2, 2020

Generating F2 Intercross

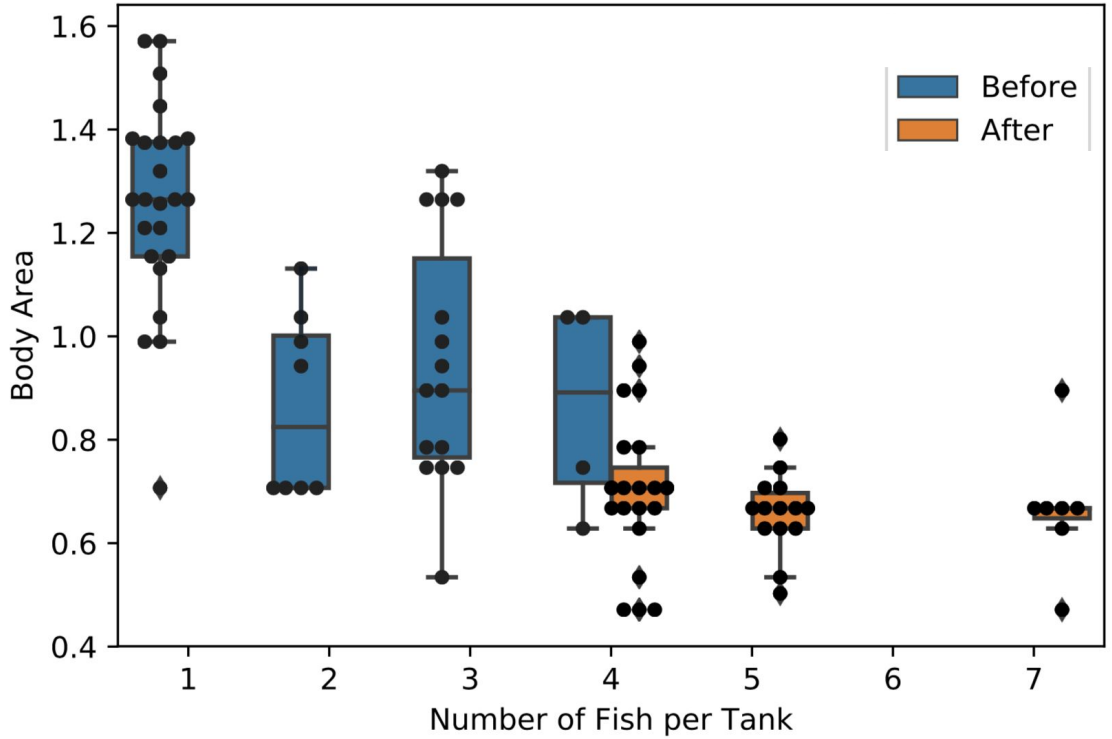
Current measurements



Improvements

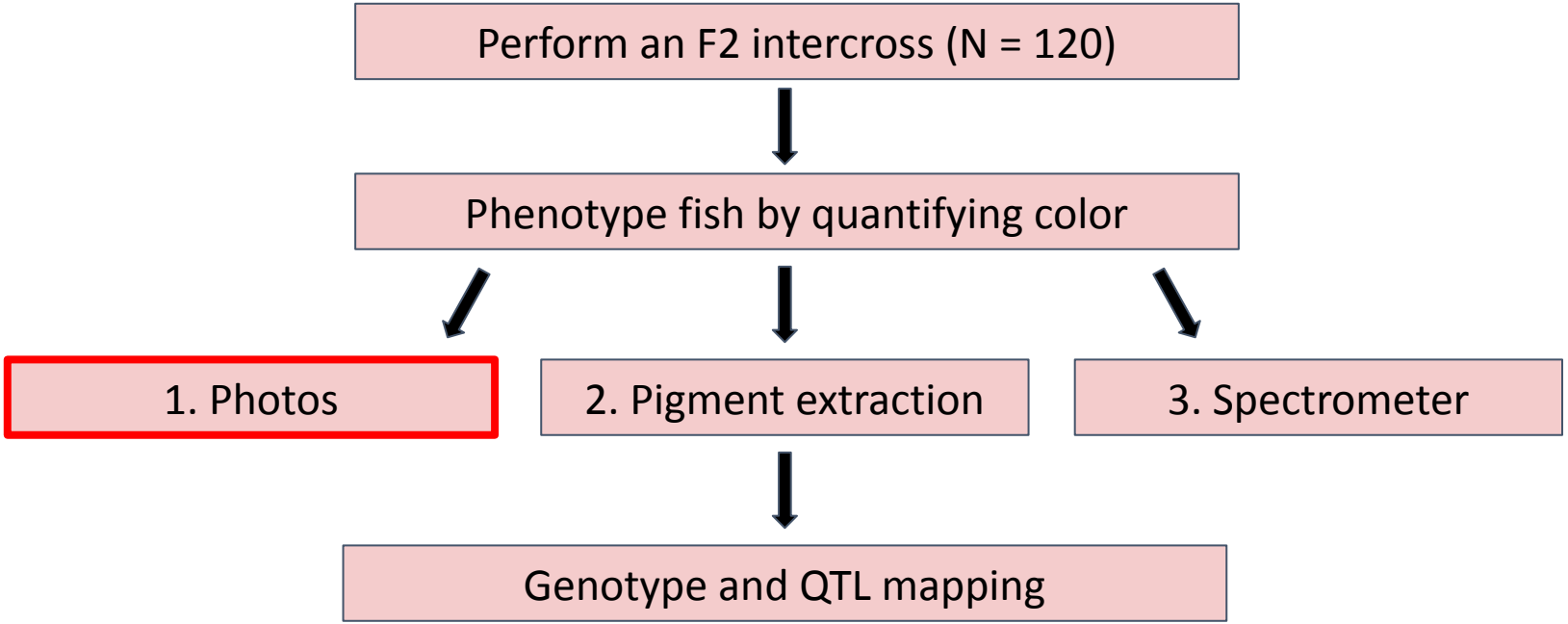


Generating F2 Intercross: Body Size



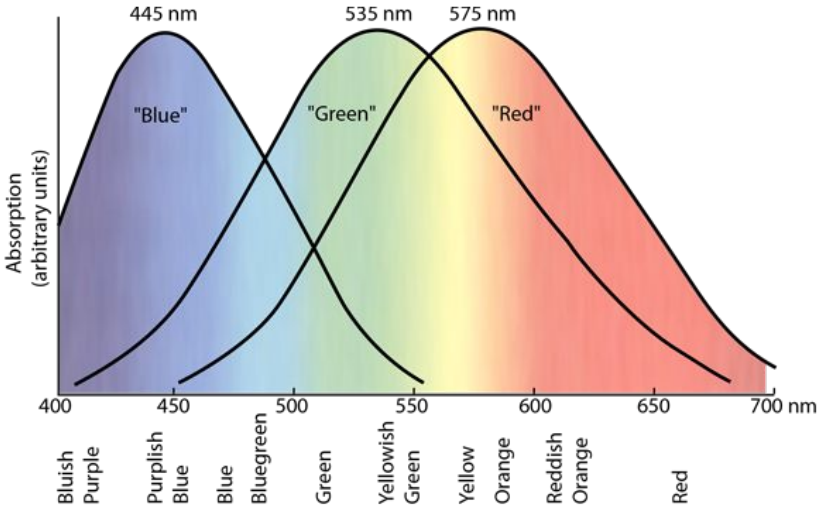
'After' measurements were taken 2 weeks after trip

Experimental Workflow

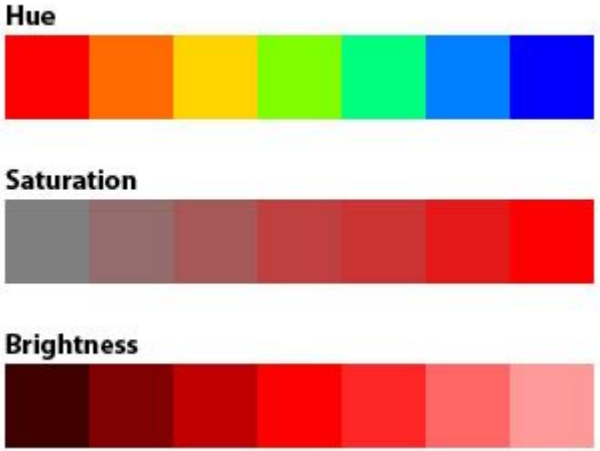


1. Photos

- Why: Get the relative RGB value for hue, saturation and brightness



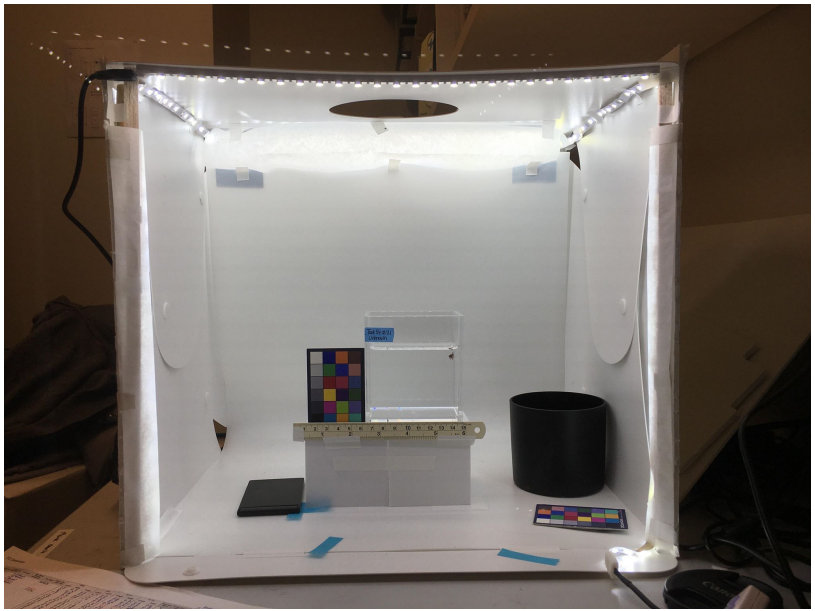
From Hyperphysics



From Kristoffer Helander

1. Photos

- Experimental Setup

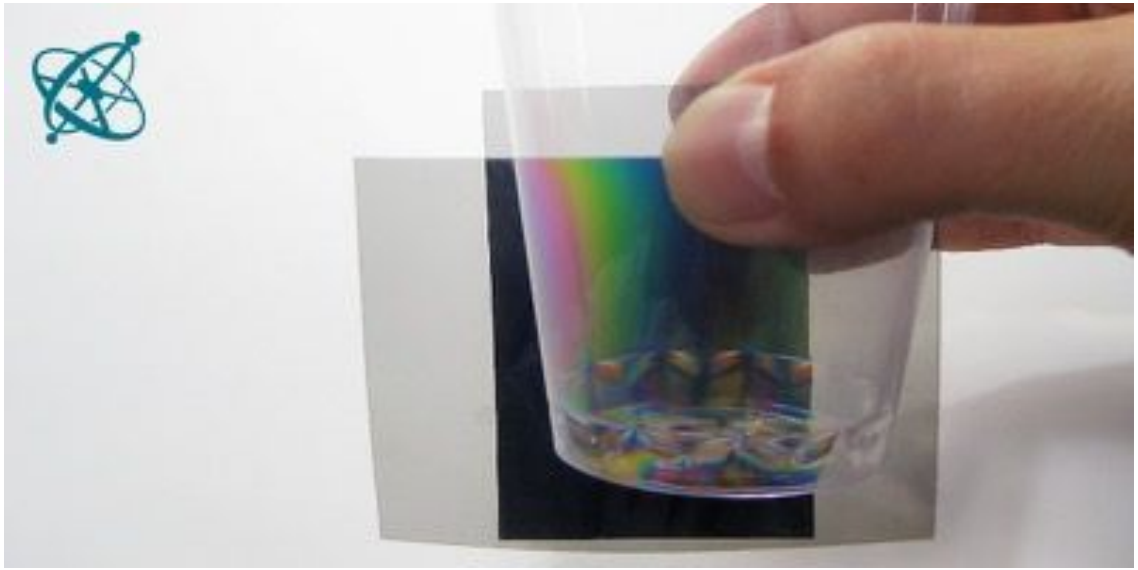


Materials:

- LED lights
- Camera
- Photo tank with fish
- White balance (calibration)
- Color matrix (calibration)

1. Photos

- Polarized light gives refraction due to plastic tension



1. Photos

- Polarized light gives refraction due to plastic tension

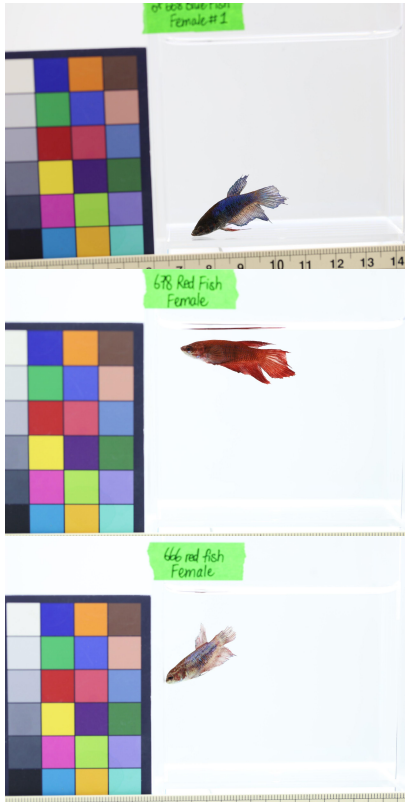
Without polarizing lens

0° polarizing lens

90° polarizing lens

1. Photos

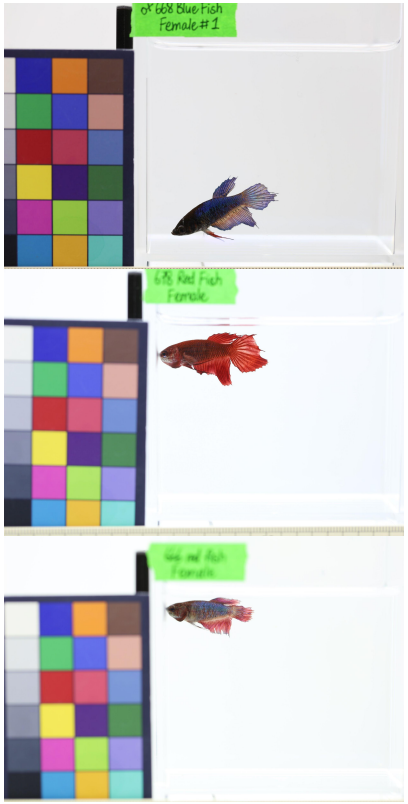
Before Mirror



Blue

Red

Red Blue



After Mirror

1. Photos

Before Mirror

1. Photos

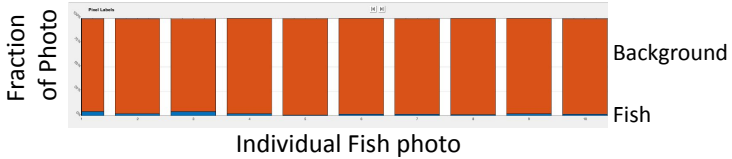
Through Matlab...

1. Label Photos

(Fish vs Background)

2. Calculate Weights

based on label proportions

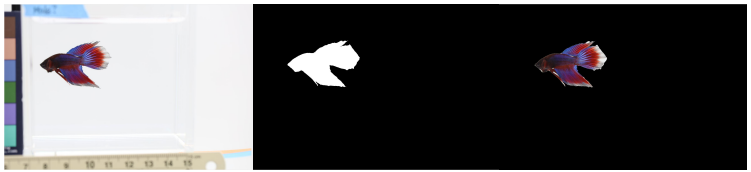


3. Train Network

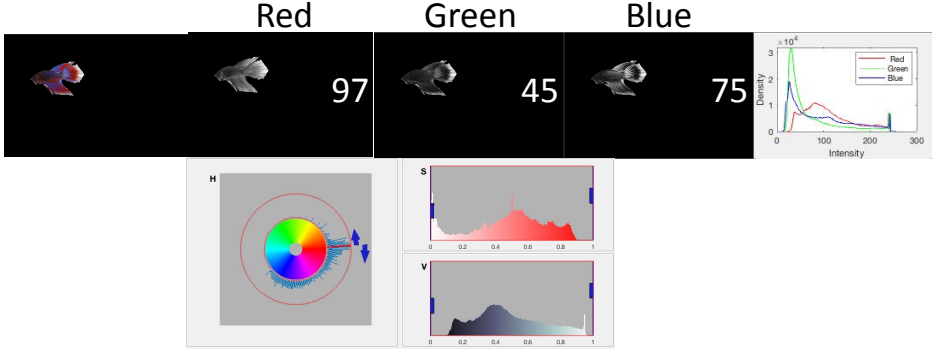
Split data:

1. training set (60%)
2. validation set (40%)

4. Mask out any non-Fish pixels



5. Calculate RGB and HSV Values



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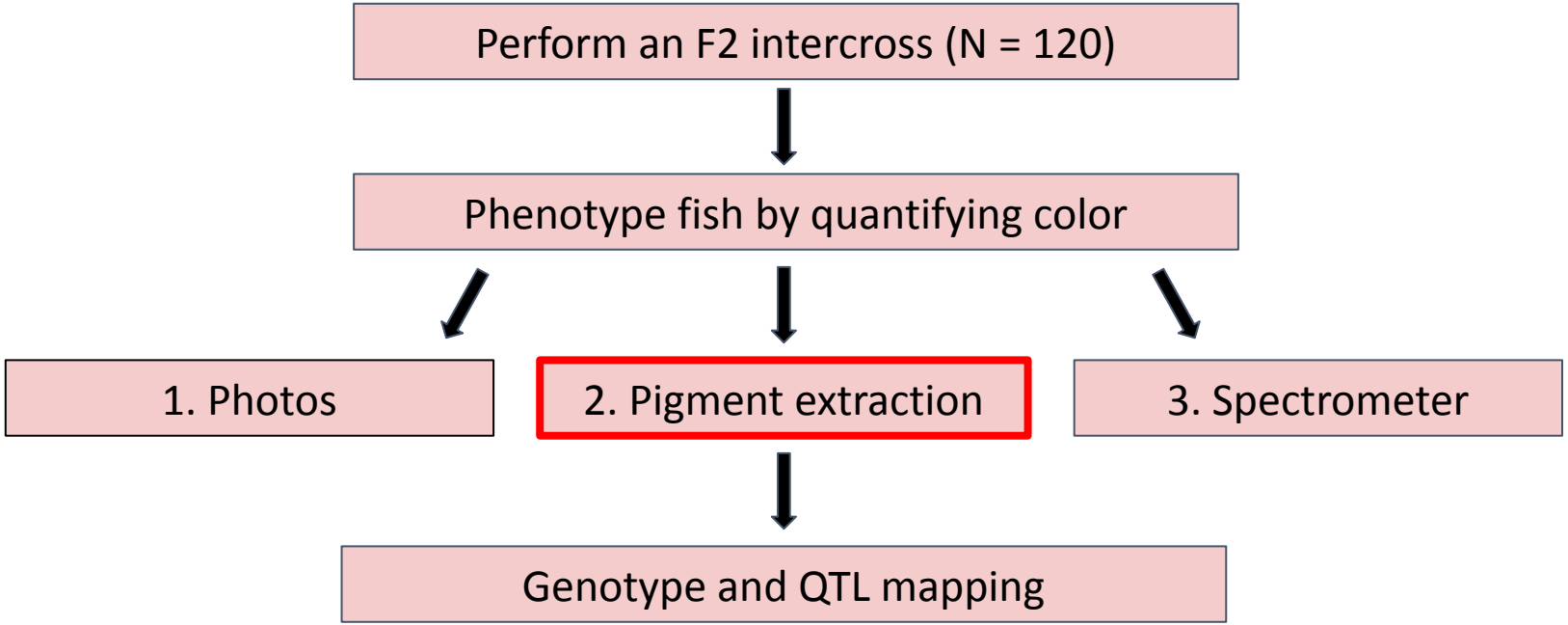
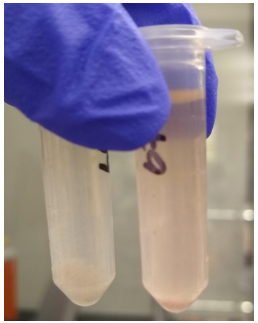


2. Pigment Extraction

- Redness is associated with the abundance of pteridines and carotenoids

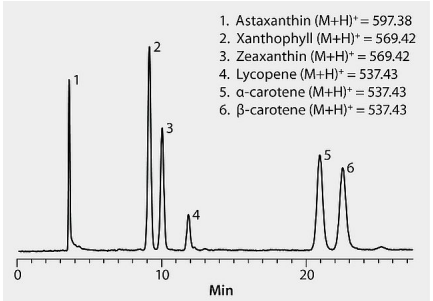
- Provide a quantitative measurement of carotenoids (redness)

0.3 x 0.2 cm



Blue Fish

Red Fish

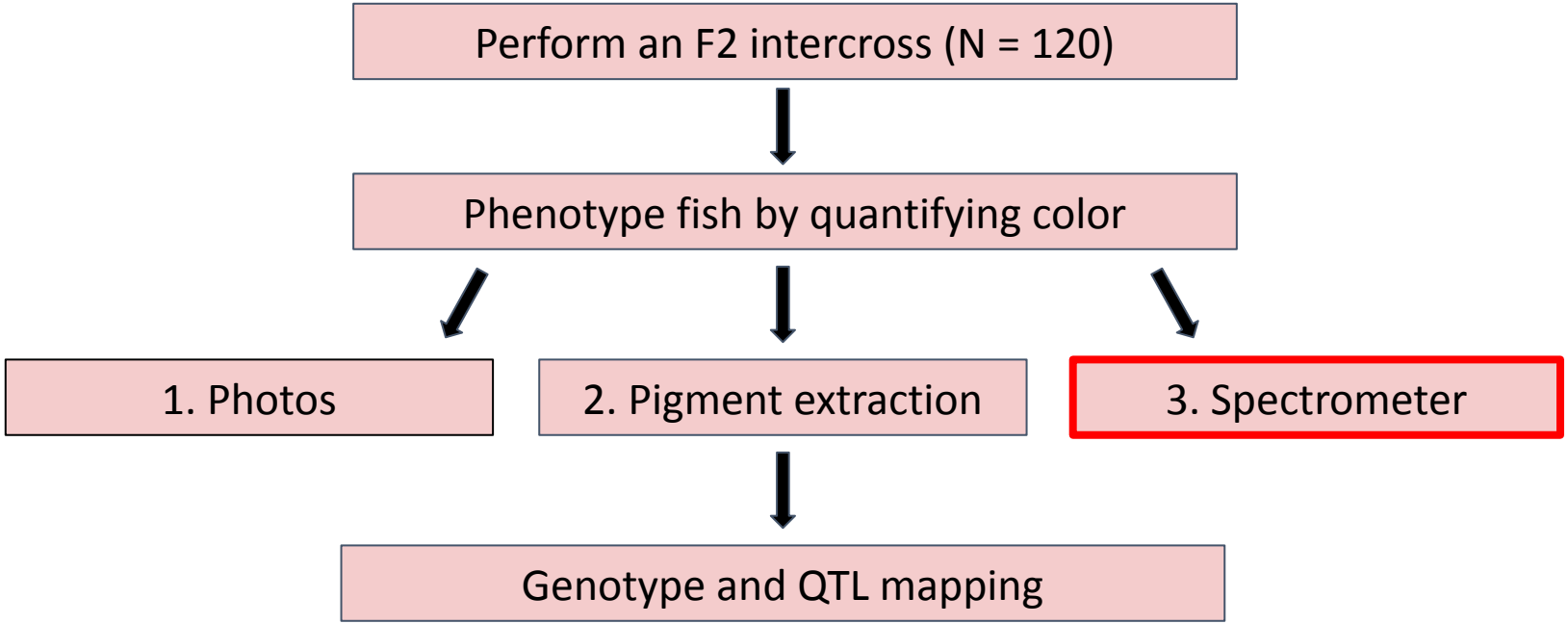


HPLC +
UV-Vis Spectrophotometer

2. Pigment Extraction

- Protocol:
  1. Ground thawed tissue in 2 mL methyl tertiary-butyl ether (MTBE) for 2 minutes.
  2. Combine it with the 2 mL extract in a 9 mL falcon tube; rinse eppendorf with 1mL of MTBE.
  3. Add 2 mL of 1% NH_4OH to the tube
  4. Vortex the tube for 1 minute
  5. Centrifuge the tube for 5 minutes at 3000 rpm
  6. Carotenoids will be partitioned into the top (MTBE) layer and pteridines into the bottom (NH_4OH) layer

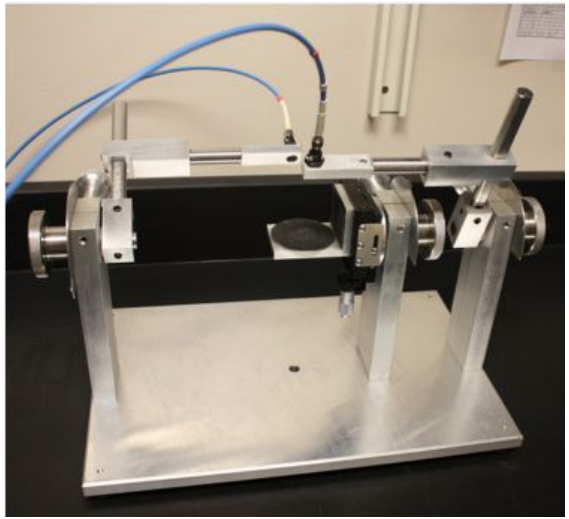
Experimental Workflow



3. Spectrometer

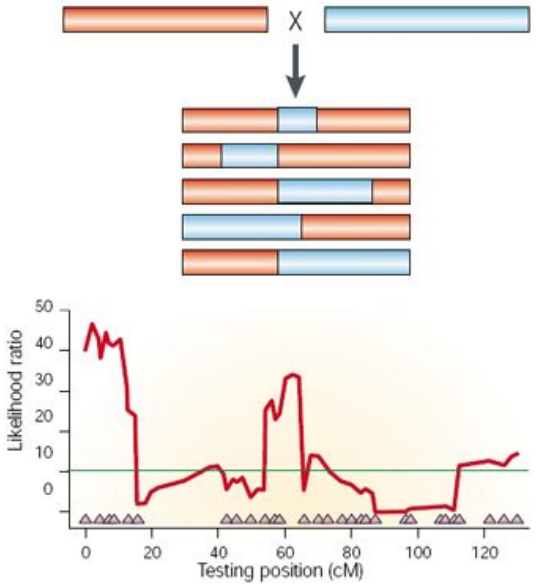
- Blueness is likely from iridescence and is structural
- Quantify iridescence (blueness)

Reflection probe and Light probe



Future Directions

- Continue quantifying color of the F2s through photos
- Start pigment extraction
- Start spectrometer
- Genotyping/Sequencing
- QTL Mapping



Thank You!

Bendesky Lab members, especially

- Young Mi
- Pei
- Madison
- Hiroki
- Claire
- Amy