



Shifting cichlid color vision:

Tbx2a acts in *trans* on LWS opsin expression

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Background

Why Study Cichlid Color Vision?

There is a strong co-evolution between behavioral ecology and visual tuning

Visual tuning requires expressing multiple genes that act together as a network

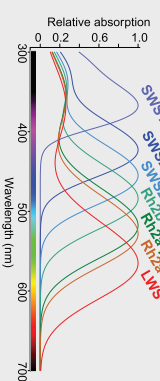
Convergent evolution is rampant in behavioral ecology but do networks evolve to converge on the same sets of genes or generate novel gene networks?

Color Vision is Mediated by Opsins

• Opsin gene sequence plays a primary role in tuning the wavelength of light to which cone cells are sensitive

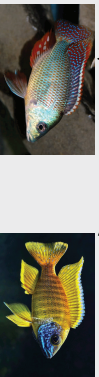
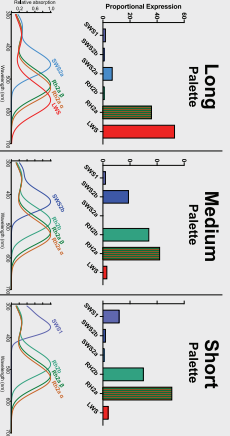
• Humans have 3 opsins, but cichlids have 7 opsins!

Cichlid Opsin Repertoire



Cichlids Tune Color Vision Via Expression

• Species differ in which opsins they express, and species generally express certain sets of opsins together



Tramtichromis intermedius
• Expression of the LWS opsin is controlled in *trans* (O'Quin et al 2012)

Aulonocara baenschi

What are the trans acting genetic mechanisms regulating LWS opsin expression?

eQTL for LWS Opsin Expression

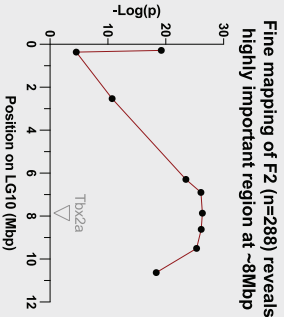
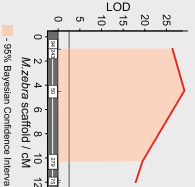
eQTL mapping of LWS opsin expression from a cross between:

Tramtichromis intermedius & *Aulonocara baenschi*

High LWS expression

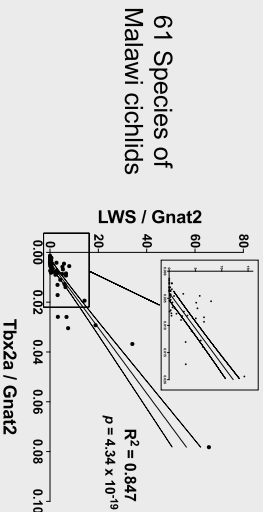
No LWS expression

Broad map reveals high LOD on LG 10
(O'Quin et al 2012)



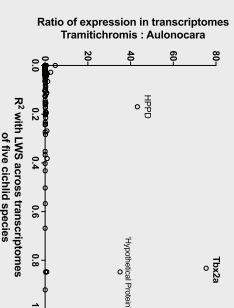
Tbx2a & LWS Expression

Correlation between expression of Tbx2a and LWS across species & across F2 individuals from TA cross



Retinal Transcriptomes

- 4 individuals - *Aulonocara*
- 2 individuals - *Tramtichromis*
- 5 additional cichlid species:
 - 2 'Short palette'
 - 3 'Long palette'



• *Tbx2a* is both **highly correlated with LWS across species and differentially expressed between T. intermedius and A. baenschi**

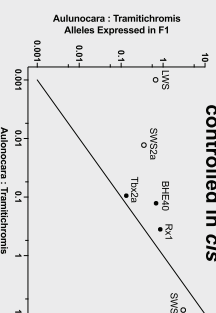
- Tbx2 is known to play a role in Rh2 opsin expression in zebrafish (Alvarez Delfin 2009)

Genomes

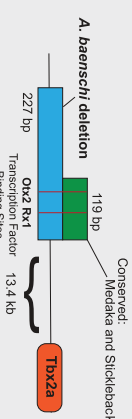
Genome Sequencing:

- Genomes were generated from two individuals of *Tramtichromis intermedius* and two *Aulonocara baenschi*
- LWS region is highly conserved between *T. intermedius* and *A. baenschi*
- JASPAR identified 2 Tbx2 binding sites between LWS and the next gene upstream with scores of 93% & 96%

Allele specific expression shows Tbx2a is controlled in cis



A. baenschi specific deletion upstream of Tbx2a



- Region is not deleted in 14 other cichlid species representing all palettes

Conclusions

Tbx2a acts in trans on LWS opsin expression

Tbx2 is a transcription factor known to act in cone cells
eQTL reveals highly significant effects of region on LG10 centered around Tbx2a

Expression of Tbx2a is positively correlated with LWS expression both across species, and within a cross between Long and Medium palette species

There are highly probable Tbx2 binding sites upstream of LWS in conserved regions

Expression of Tbx2a may differ between T. intermedius and A. baenschi due to 227bp deletion that occurs 13.4 kb upstream of Tbx2a in A. baenschi

This deletion was not observed in the other 14 cichlid species, some of which do express LWS.

This suggests cichlids may be converging upon similar visual system through different changes to the gene expression network

Future Directions

Generating CRISPR knockouts

Currently we are knocking out Tbx2a in Tilapia (a Long Palette species) to show Tbx2a is a transcription factor for LWS

Currently cutting out the 119 bp region upstream of Tbx2a to show that loss of this region is what drives low Tbx2a expression in *A. baenschi*

Expanding the understanding of the expression network

We're working to resolve the expression network underlying opsin genes in cichlids to determine whether networks evolve to converge on the same sets of genes or generate novel gene networks