Marine Biological Laboratories
Workshop in Molecular Evolution

Adaptive protein evolution: Detecting changes in selection

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Phylogenetic approaches to the study of protein structure and function

- Ancestral protein reconstruction

- Computational analyses of selection (dN/dS)

-> Combining computational with experimental approaches allows us to test hypotheses of selection in protein evolution

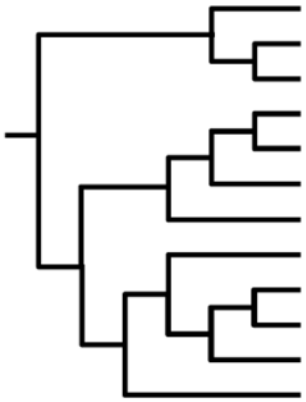
Codon models of evolution - Ratio of nonsynonymous/synonymous substitution rates

$\omega = dN/dS$

A measure of evolutionary constraint which can provide insight into the form and strength of selection on proteins.

- purifying selection (AA changes are purged) → $\omega < 1$
- neutral evolution → $\omega = 1$
- positive selection (AA changes are favoured) → $\omega > 1$

ADAPTIVE EVOLUTION:
Combining computational and experimental approaches to test hypotheses of selection in protein evolution



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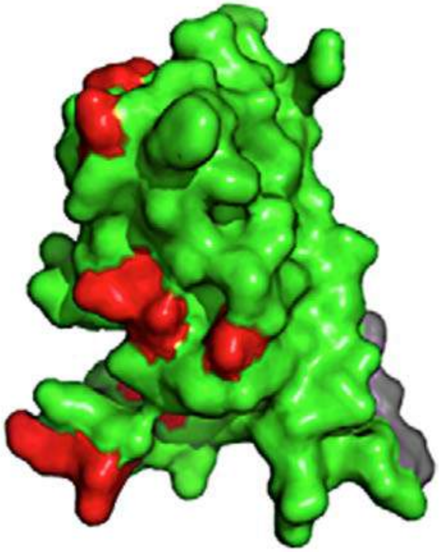
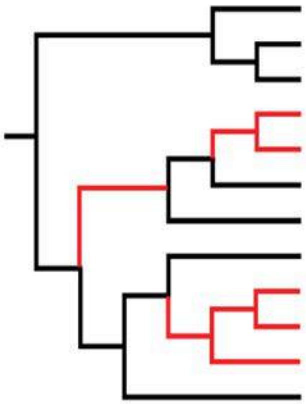


- Testing hypotheses of selection in a phylogenetic framework
- Identification of positively selected sites/regions
- Experimental studies of shifts in protein function

ADAPTIVE EVOLUTION:
Combining computational and experimental approaches to test hypotheses of selection in protein evolution



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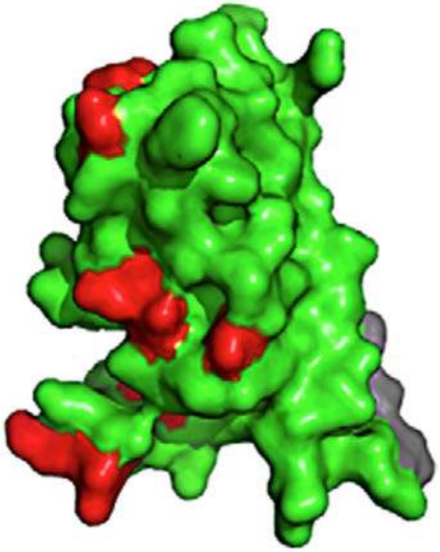

- Testing hypotheses of selection in a phylogenetic framework
- Identification of positively selected sites/regions
- Experimental studies of shifts in protein function

Testing hypotheses of selection in protein evolution: Combining computational and experimental approaches

1. Formulate hypothesis of selection acting on evolution of particular group of sequences
2. Collect target group of sequences
3. Test hypothesis of selection using codon models in a phylogenetic framework
4. Identify amino acid sites/regions that have been targets of selection
5. Formulate hypothesis of selection acting on particular aspect of protein function
6. Select proteins/mutants for experimental studies
7. Test hypothesis of selection using experiments investigating shifts in protein function

Testing hypotheses of selection in protein evolution: Combining computational and experimental approaches

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Testing hypotheses of adaptive evolution in protein function: Combining computational and experimental approaches

Computational analyses

- Statistical test for positive, or divergent selection in a phylogenetic context
- Identifies amino acid residues that may have been the targets of positive selection
- Does NOT prove adaptive evolution -> Need function

Experimental studies

- Empirical test for shifts in protein function as a consequence of substitutions at sites targeted by selection
- Does NOT prove anything about selection & adaptive evolution, need evolutionary context
- Ideally combined with ancestral reconstruction and mutagenesis studies

Testing hypotheses of adaptive evolution in protein function: Combining computational and experimental approaches

Computational analyses

- Statistical test for positive, or divergent selection in a phylogenetic context
- Identifies amino acid residues that may have been the targets of positive selection
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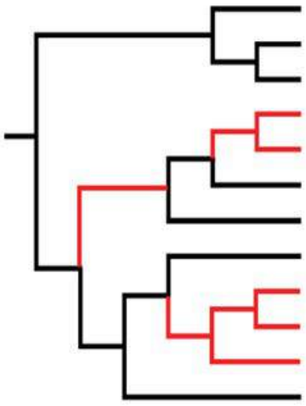
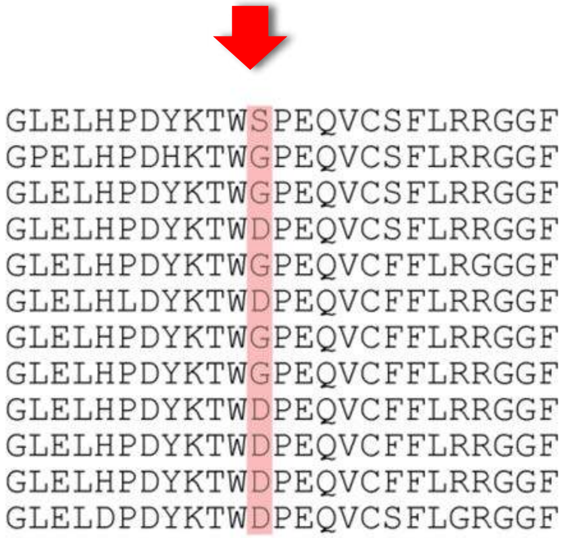
Experimental studies

- Empirical test for shifts in protein function as a consequence of substitutions at sites targeted by selection
- Ideally combined with ancestral reconstruction and mutagenesis studies

-> Taken together, computational and experimental approaches can be used to test for ADAPTIVE EVOLUTION in protein function as a consequence of natural selection!

Formulating specific and testable hypotheses of adaptive evolution


Pervasive vs. episodic selection



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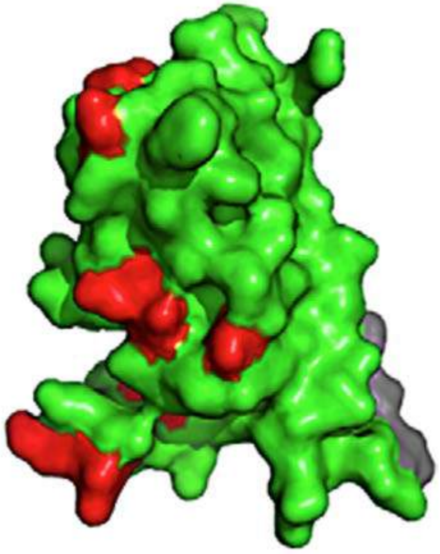
Sites targeted by selection


Effect on protein function

-> Answering the question, not only when/where but WHY did selection occur?

-> The role of a priori information in formulating hypotheses about function

Examples of positive selection on proteins: Experimental models

Host-viral arms races

- Primate intracellular immune response TRIM5a, protein kinase R (Sawyer et al. 2005, Elde et al. 2009)
- Potato virus Y (Moury & Simon 2011)
- Neutrophil-mediated host response to infection (Loughran et al. 2012)

Sperm-egg recognition proteins

- Abalone lysin, egg coat (Aagaard et al. 2013)

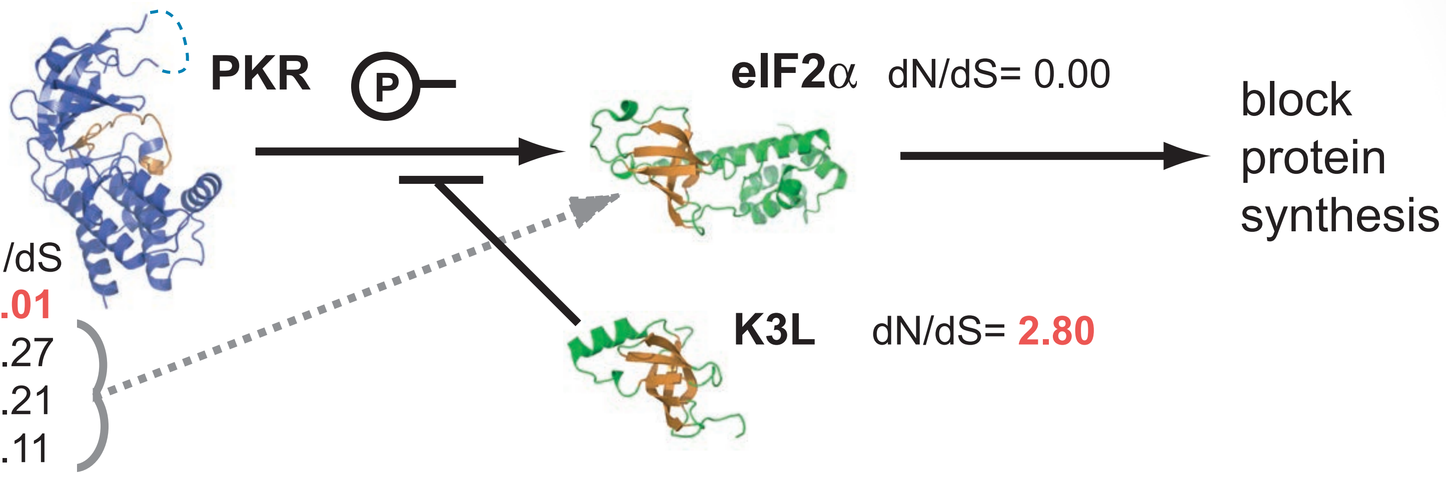
Sensory proteins

- Visual pigments in whales, fishes, birds, bats (Dungan & Chang 2017, Hauser et al. 2017, Nynatten et al 2021)

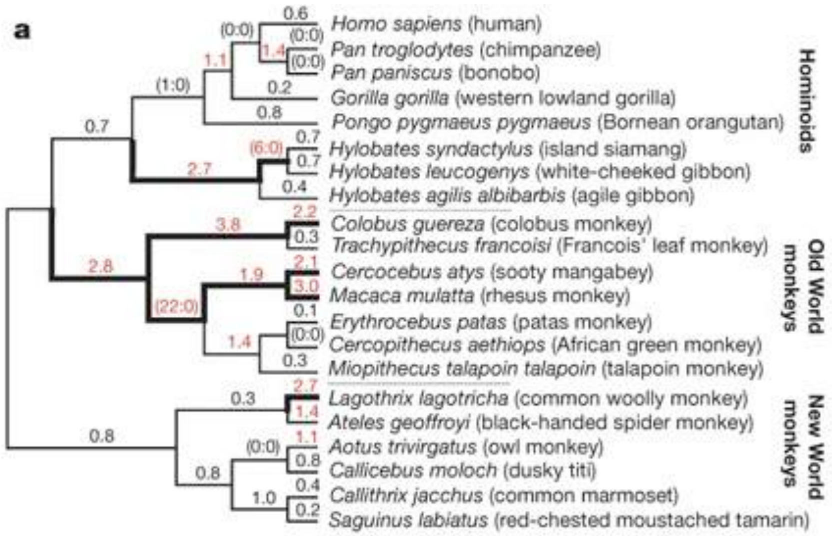
Transcription factors in development

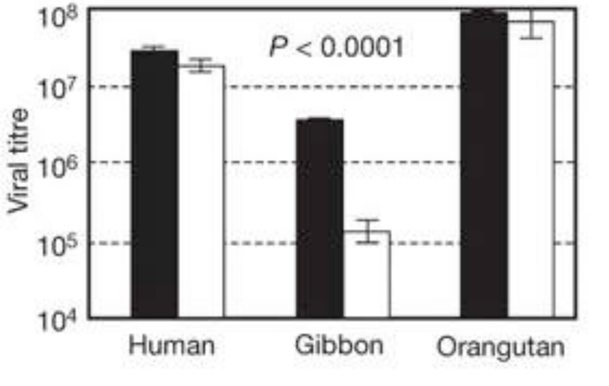
- Stem cell pluripotency (Baker et al. 2016)

Primate protein kinase R locked in arms race with viral protein mimic K3L



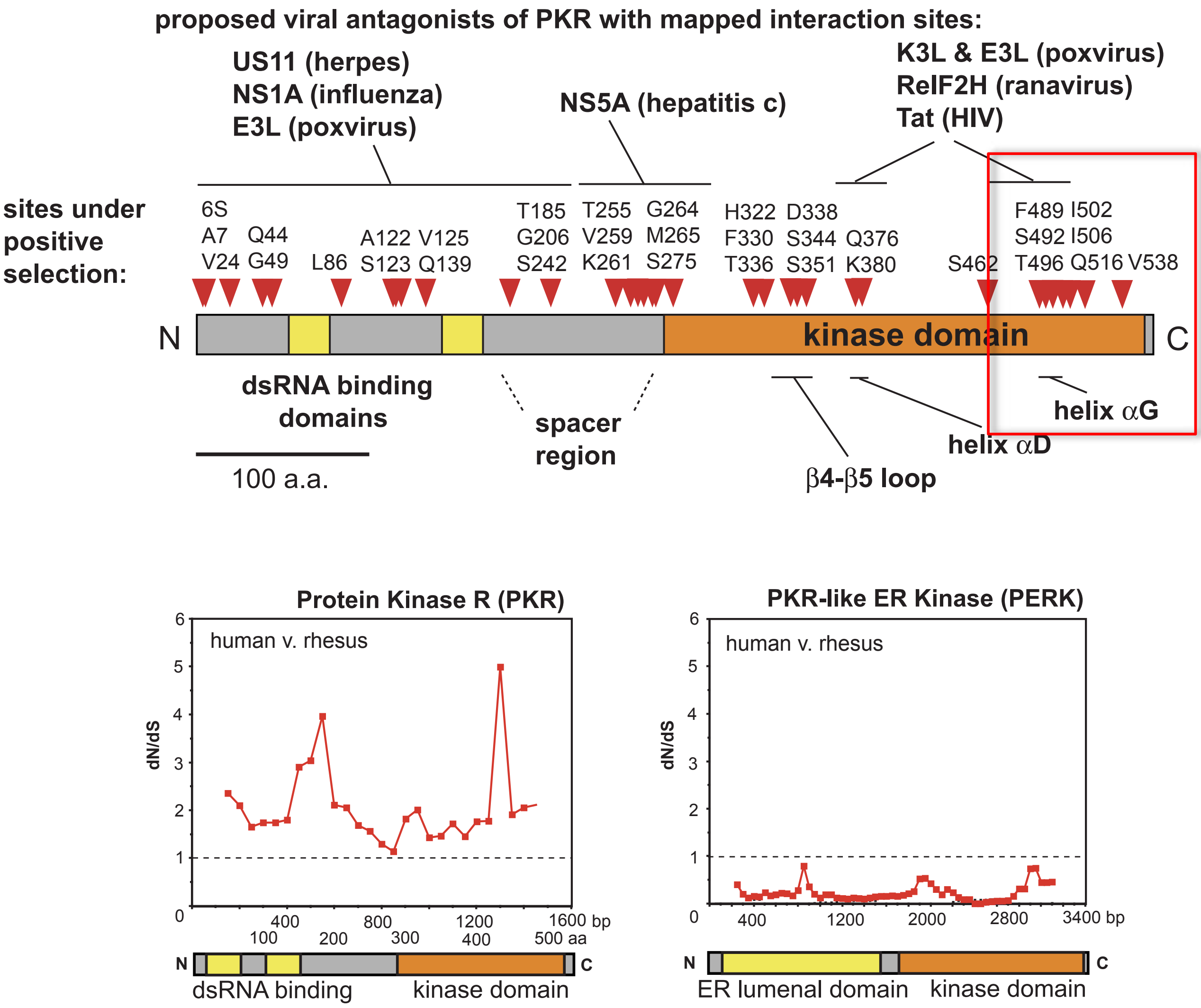
	dN/dS
PKR	2.01
HRI	0.27
PERK	0.21
GCN2	0.11





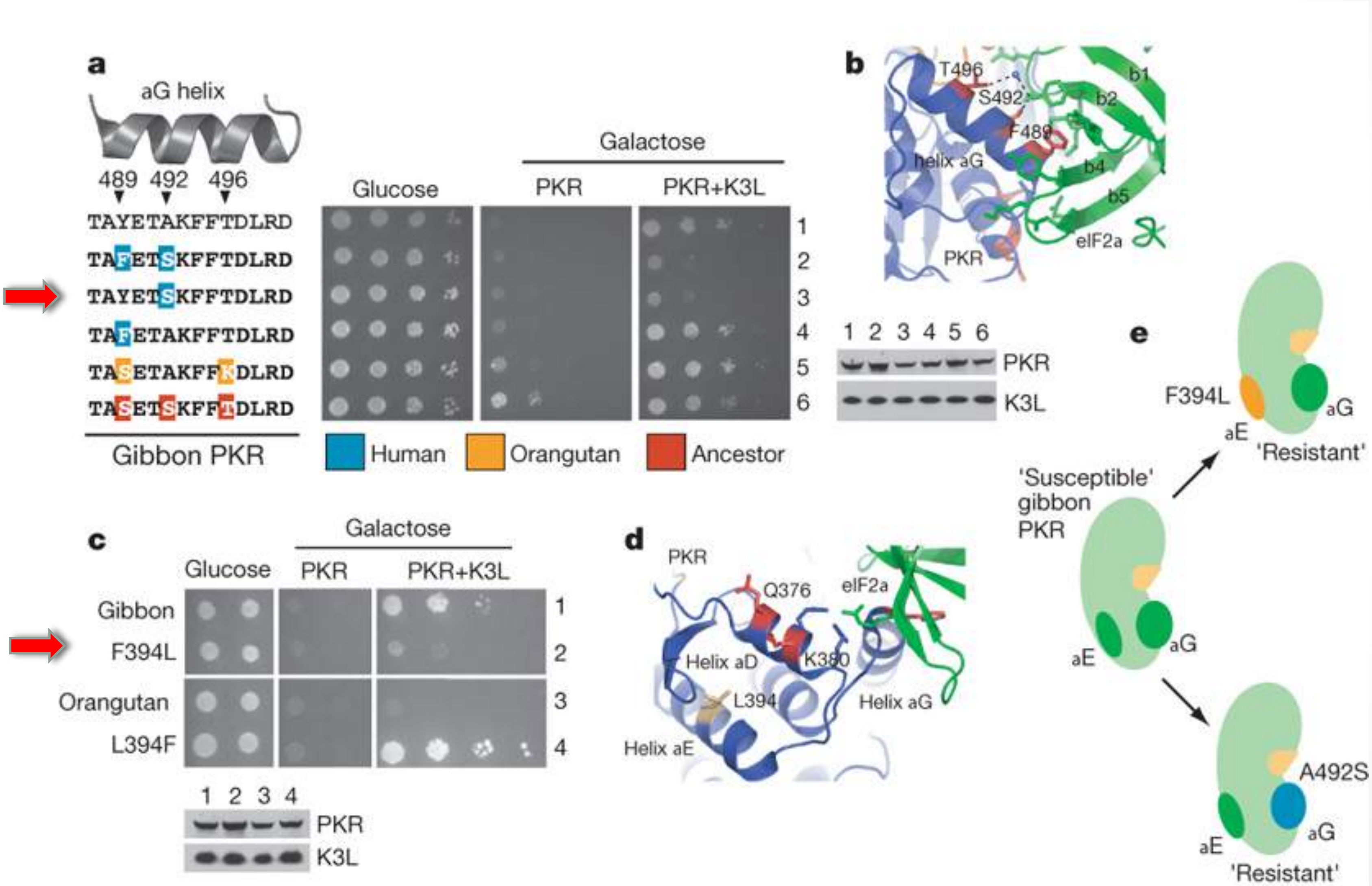
Elde et al. Nature 2009

Positive selection has shaped PKR in primate evolution



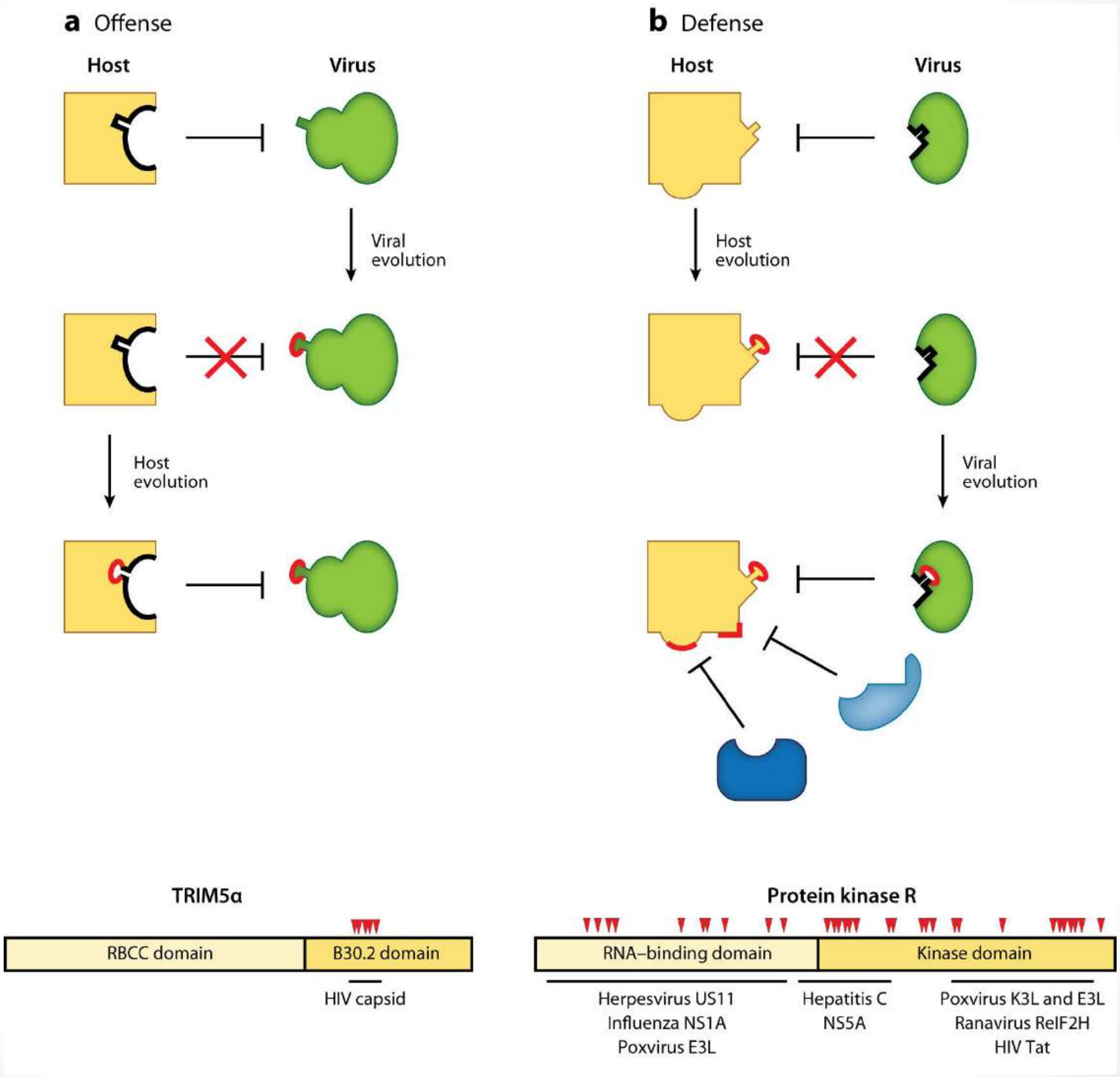
Elde et al. Nature 2009

Positively selected sites on distinct surfaces of the PKR kinase domain are crucial to K3L resistance



Elde et al. Nature 2009

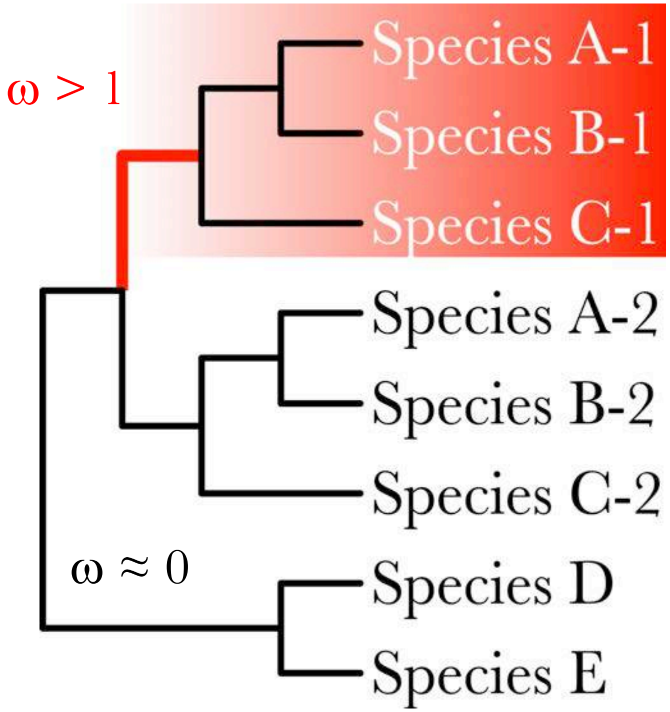
Adaptive protein evolution

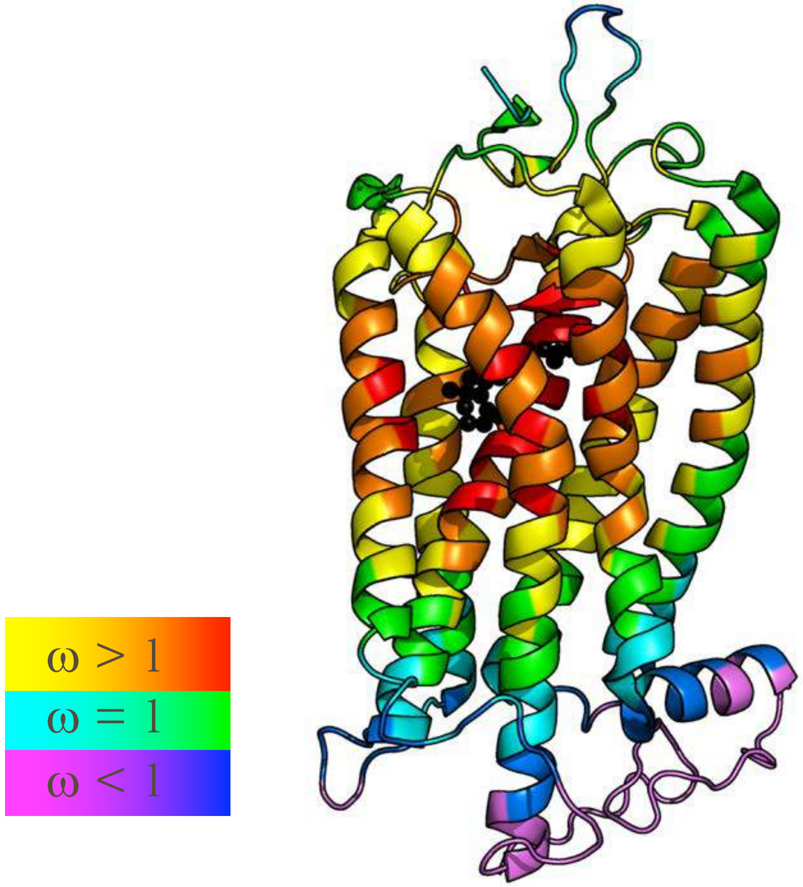


Beyond random sites models: Clade models

The form and strength of selection may vary across both the phylogeny and the protein.

Codon models can incorporate variation across sites and lineages, and can be used to detect positive and divergent selection.





Bielawski & Yang (2004)

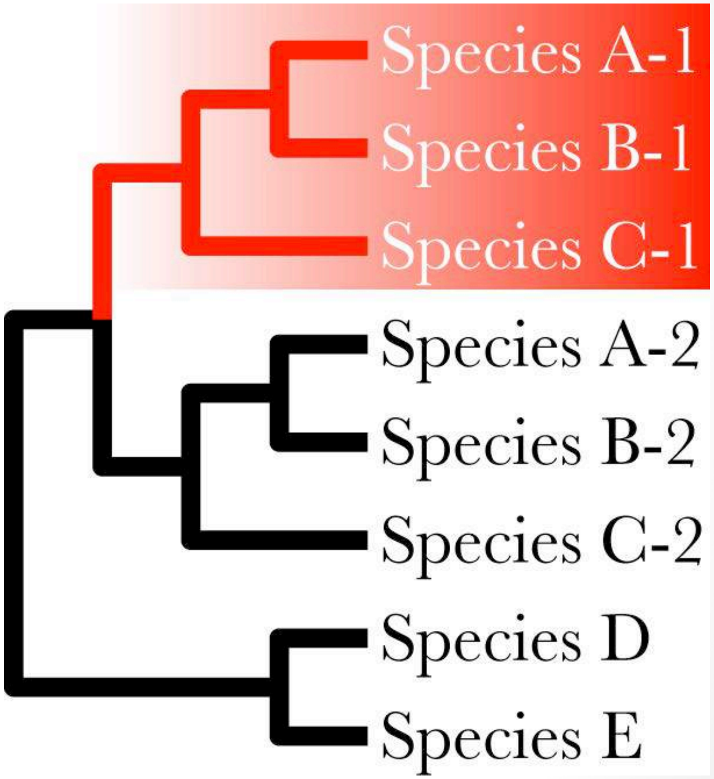
Codon models of divergent selection: Clade model C (CmC)

CmC assumes sites evolve in three ways:

1. purifying selection ($0 < \omega_0 < 1$) constrains evolution at some sites, operating consistently across the tree.

2. some sites evolve free of constraint (neutrally: $\omega_1 = 1$) across the entire tree.

3. the remaining sites evolve divergently ($\omega_2 \neq \omega_3$) between particular sections of the tree.



Bielawski & Yang (2004)

Codon model tests of divergent selection

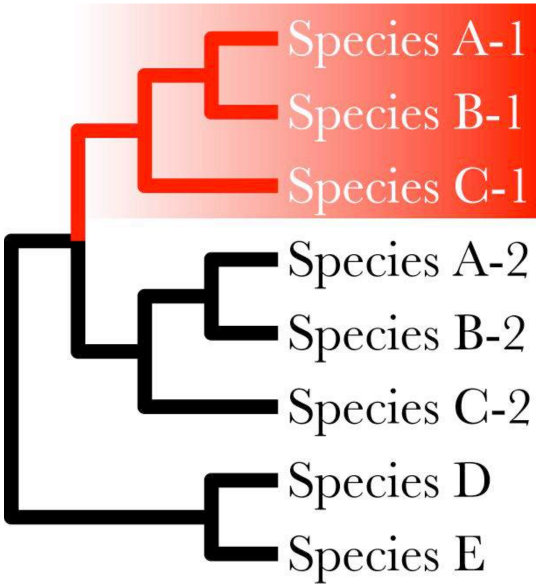
CmC assumes sites evolve in three ways:

1. <u>always purifying selection</u>
   ($0 < \omega_0 < 1$)

2. <u>always neutral</u>
   ($\omega_1 = 1$)

3. <u>divergent</u>
   ($\omega_2 \neq \omega_3$)

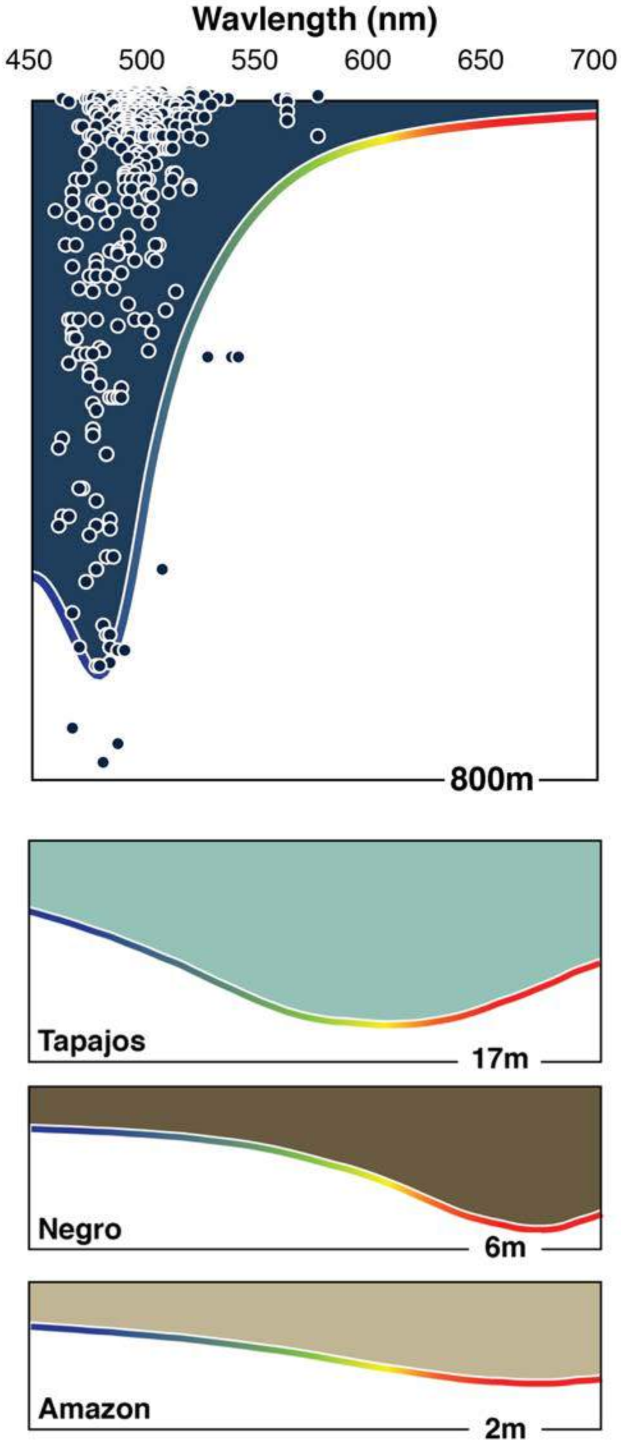
3 (alt). <u>no divergence</u>
   ($\omega_2 > 0$)



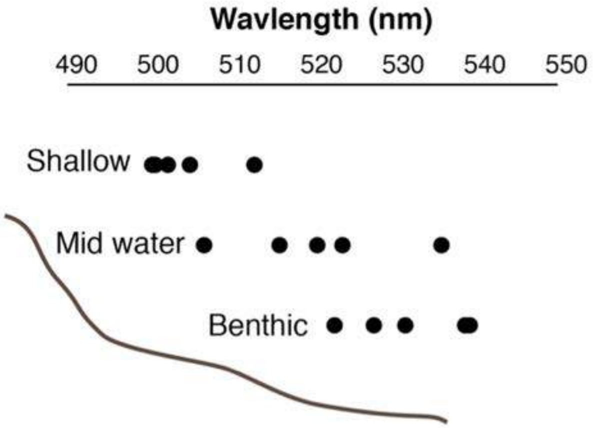
An improved null model for testing for divergent selection, M2a_rel model (Weadick & Chang 2012).

M2a_rel retains the third site class, but simply estimates a single ω ratio that applies to all branches of the phylogeny.

Vision in aquatic environments



- Marine environments become increasingly blue with depth
- Deep-sea fishes have blue-shifted Rhodopsin
- Rivers are **red-shifted** and dimmer than marine

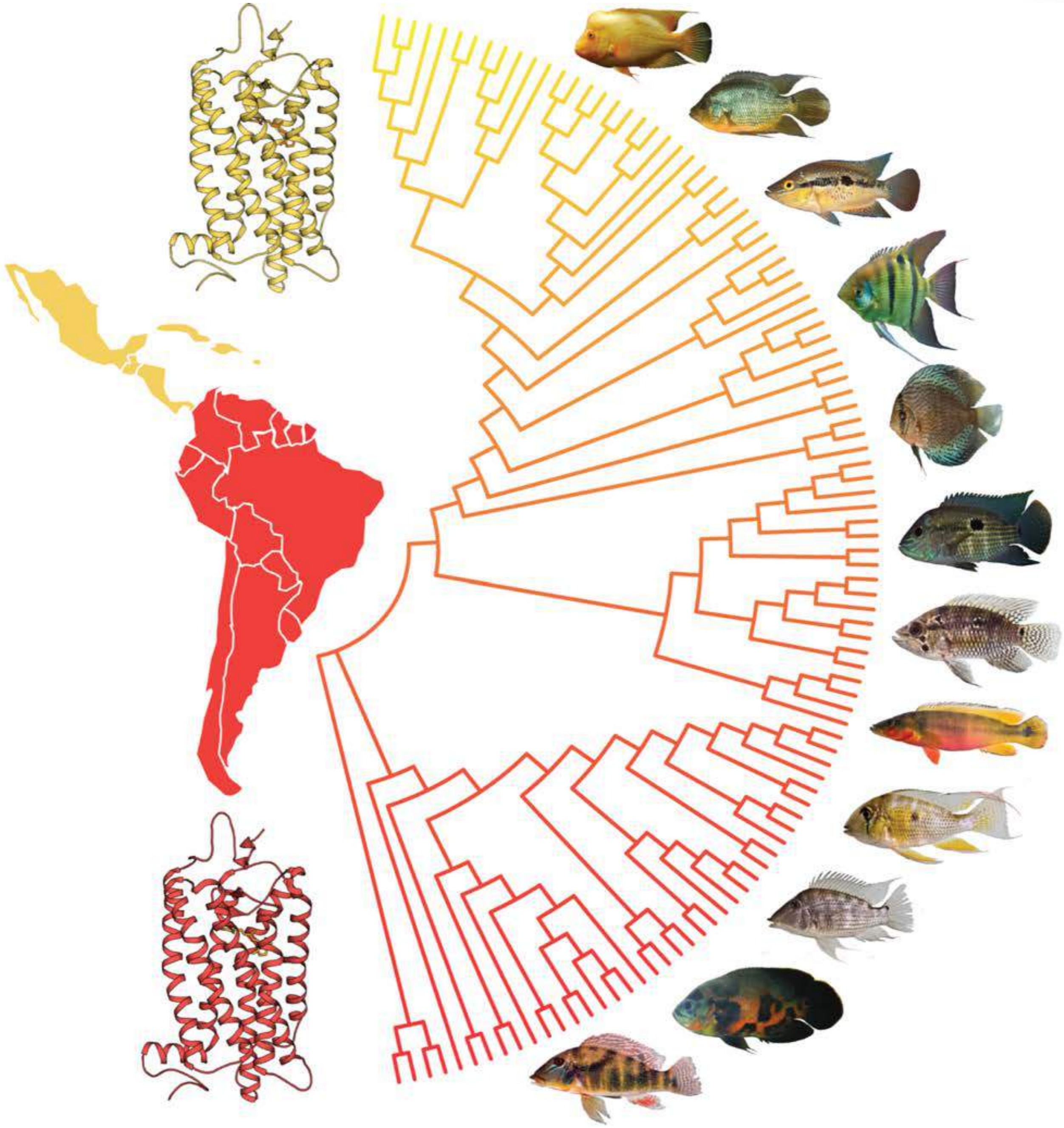


Freshwater fishes have **red-shifted** rhodopsin pigments

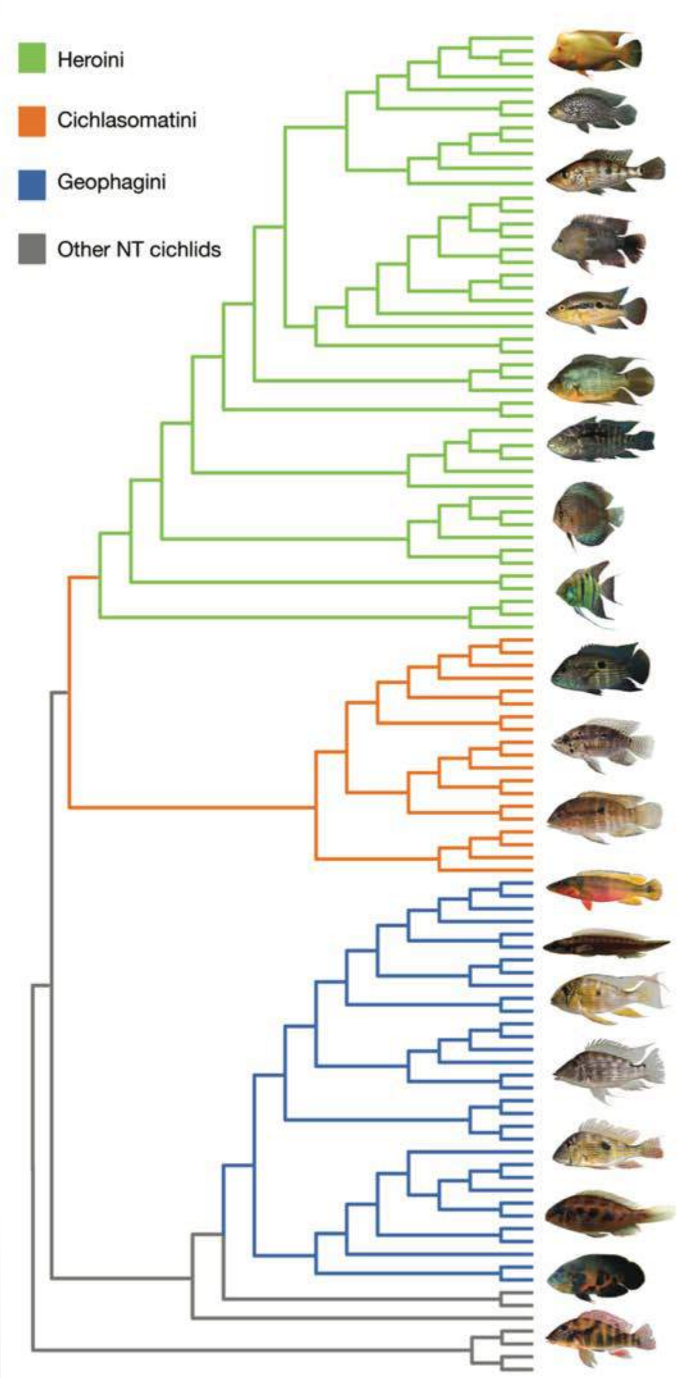
Molecular evolution of rhodopsin in Neotropical cichlids

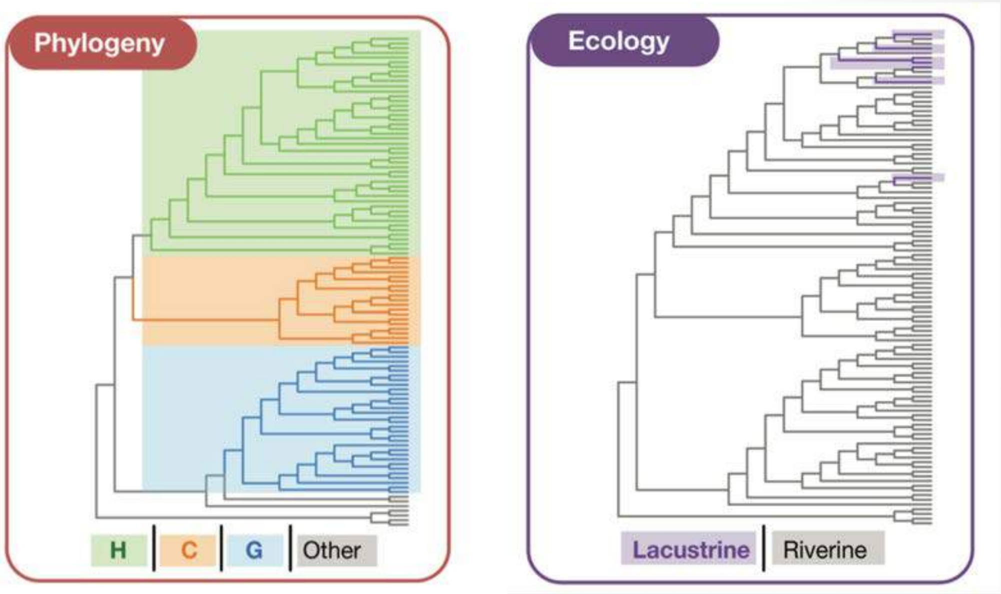
Frances Hauser

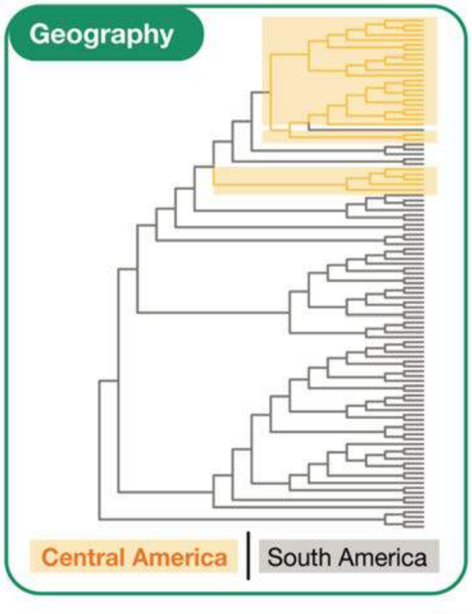
Hernan Lopez-Fernandez, ROM



Clade models used to test different hypotheses of Neotropical cichlid rhodopsin evolutionary divergence



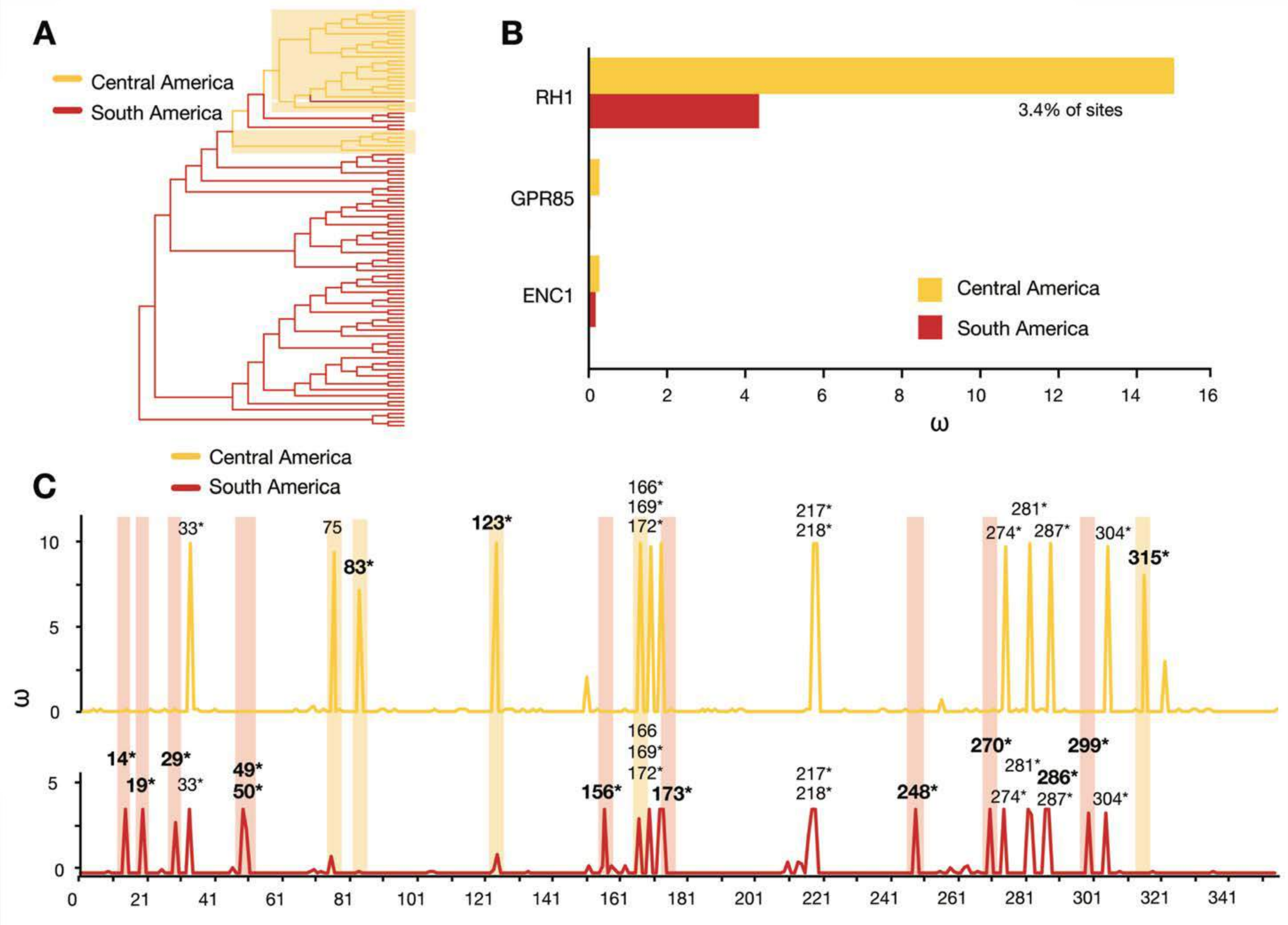




Hauser et al. 2017
MBE

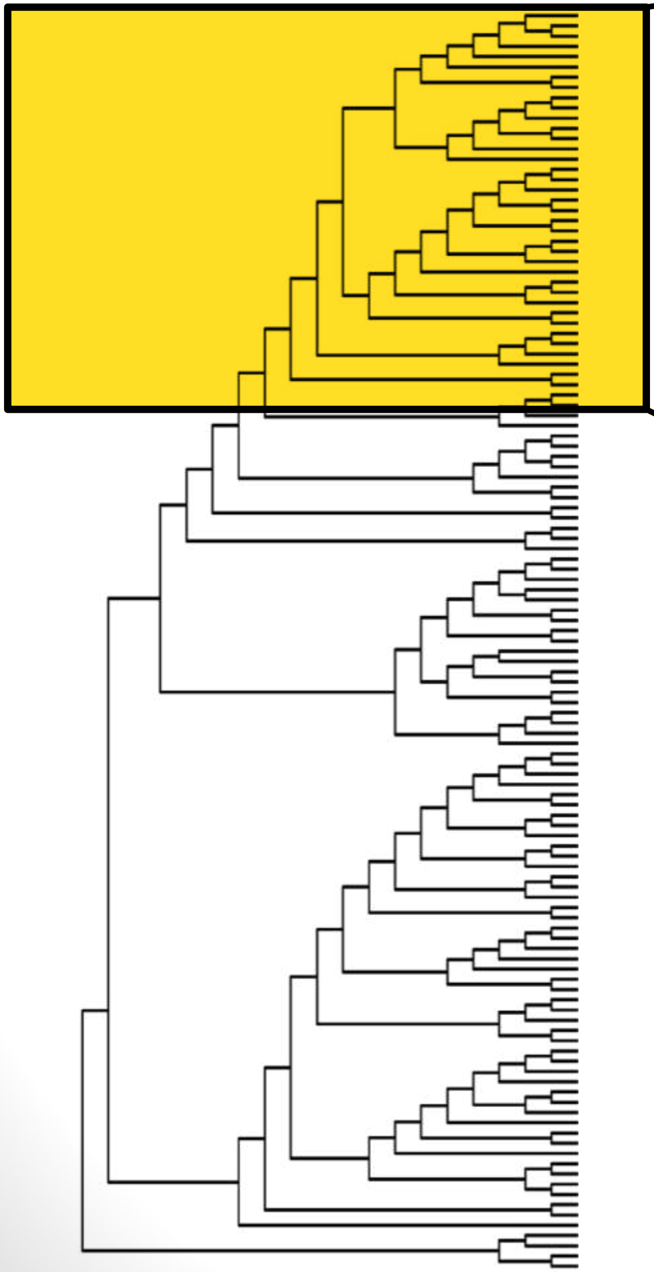
Model (foreground clade)	**np**	**lnL**	**k**	**AIC**	**ΔAIC**		**Parameters**			**Null**	**LRT**	**df**	***P***
M2a_rel	210	-5909.85	3.12	12239.70	17.46	**p:**	0.868	0.091	0.041	M1a	250.160	2	**0.0000**
						w:	0.009	1	5.470				
C+H+G	213	-5904.67	3.06	12235.34	13.10	**site**	**0**	**1**	**2**	M2a_rel	10.360	3	0.0157
						proportion	0.868	0.097	0.034				
						background	0.009	1	3.979				
						Cichlasomatini	0.009	1	6.357				
						Heroini	0.009	1	8.710				
						Geophagini	0.009	1	4.100				
Central America (clade)	211	-5900.12	2.41	12222.24	7.34	**site**	**0**	**1**	2	M2a_rel	19.464	1	**0.0000**
						proportion	0.868	0.097	0.034				
						background	0.009	1	4.476				
						foreground	0.009	1	11.660				
Cichlasomatini	211	-5909.84	3.12	12241.68	19.44	**site**	**0**	**1**	2	M2a_rel	0.020	1	0.8875
						proportion	0.868	0.091	0.040				
						background	0.009	1	5.430				
						foreground	0.009	1	5.710				
Heroini	211	-5906.21	3.09	12234.42	12.18	**site**	**0**	**1**	2	M2a_rel	7.280	1	**0.0070**
						proportion	0.868	0.095	0.037				
						background	0.009	1	4.630				
						foreground	0.009	1	8.020				
Geophagini	211	-5907.70	3.10	12237.40	15.16	**site**	**0**	**1**	**2**	M2a_rel	4.300	1	**0.0381**
						proportion	0.868	0.094	0.038				
						background	0.009	1	6.520				
						branch	0.009	1	4.200				
Central America	**211**	**-5896.45**	**3.07**	**12214.90**	**0.00**	**site**	**0**	**1**	**2**	**M2a_rel**	**26.800**	**1**	**0.0000**
						proportion	**0.868**	**0.097**	**3.440**				
						background	**0.009**	**1**	**4.500**				
						Central America	**0.009**	**1**	**14.800**				
Lake-dwelling	211	-5909.02	3.11	12240.03	25.13	**site**	**0**	**1**	**2**	M2a_rel	1.670	1	**0.1963**
						proportion	0.868	0.092	0.040				
						background	0.009	1	5.410				
						foreground	0.009	1	10.250				

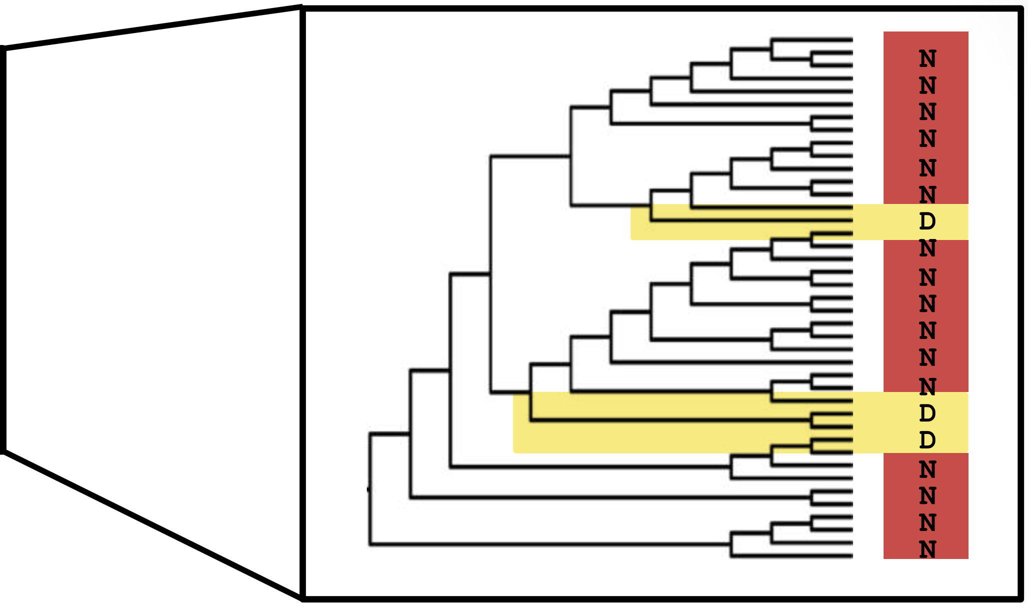
Accelerated rhodopsin divergence during invasion of Central America



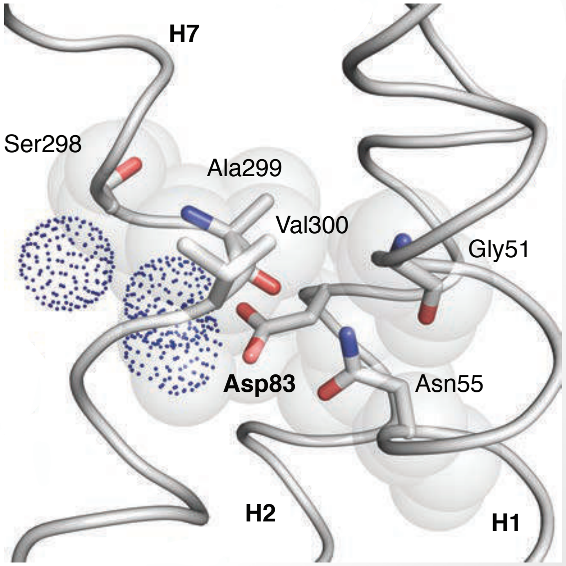
Hauser et al. 2017 *MBE*

Rhodopsin site 83 identified as under positive selection and undergoes independent transitions in Central American cichlids





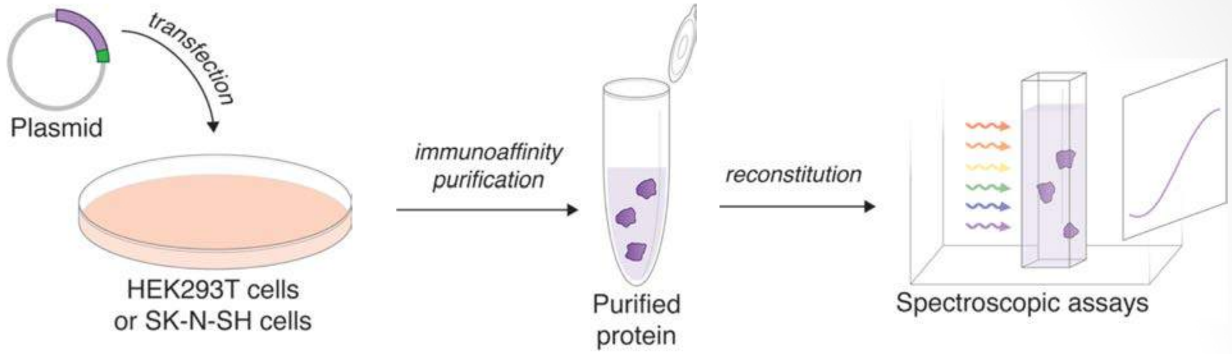
Independent N83D substitutions in Central American cichlid species

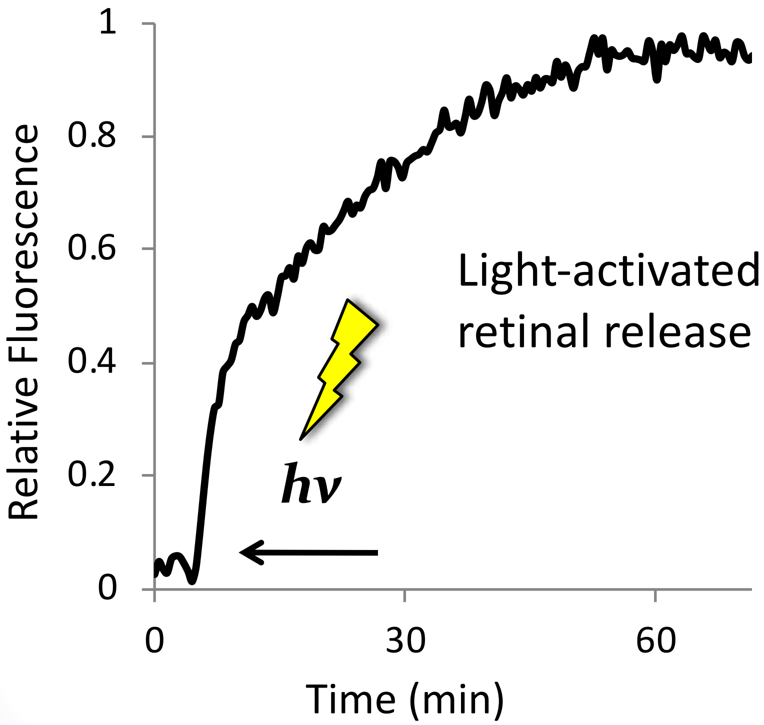


Hauser et al. 2017 *MBE*

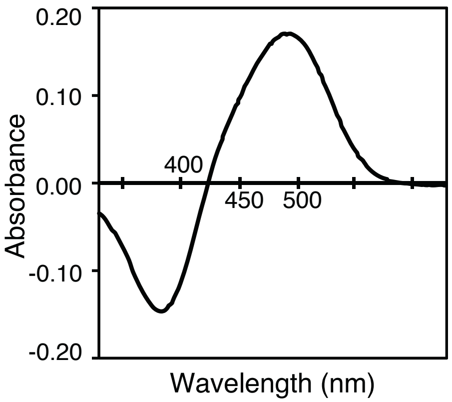
Spectroscopic assays of rhodopsin function

In vitro expression
& purification

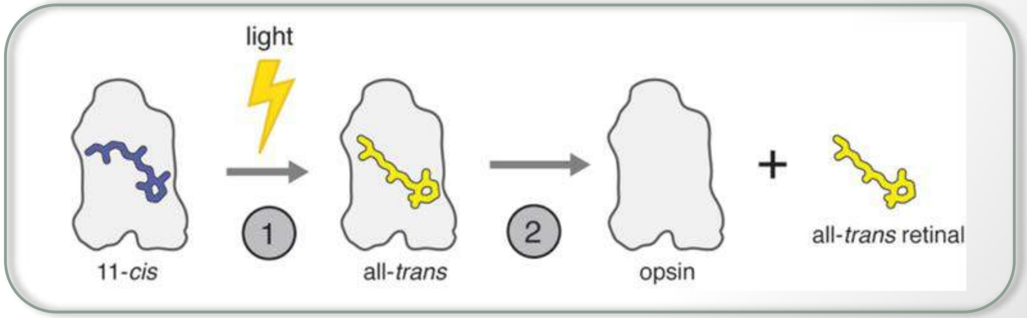




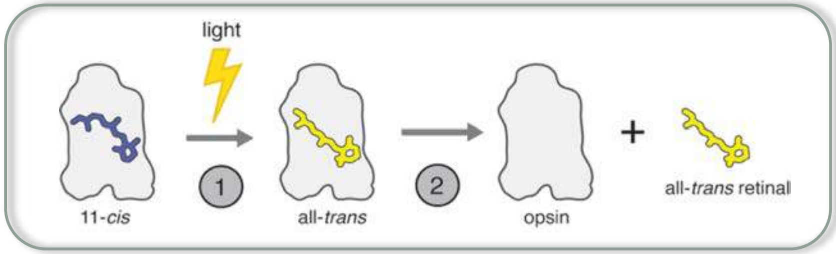
Light-activated
retinal release

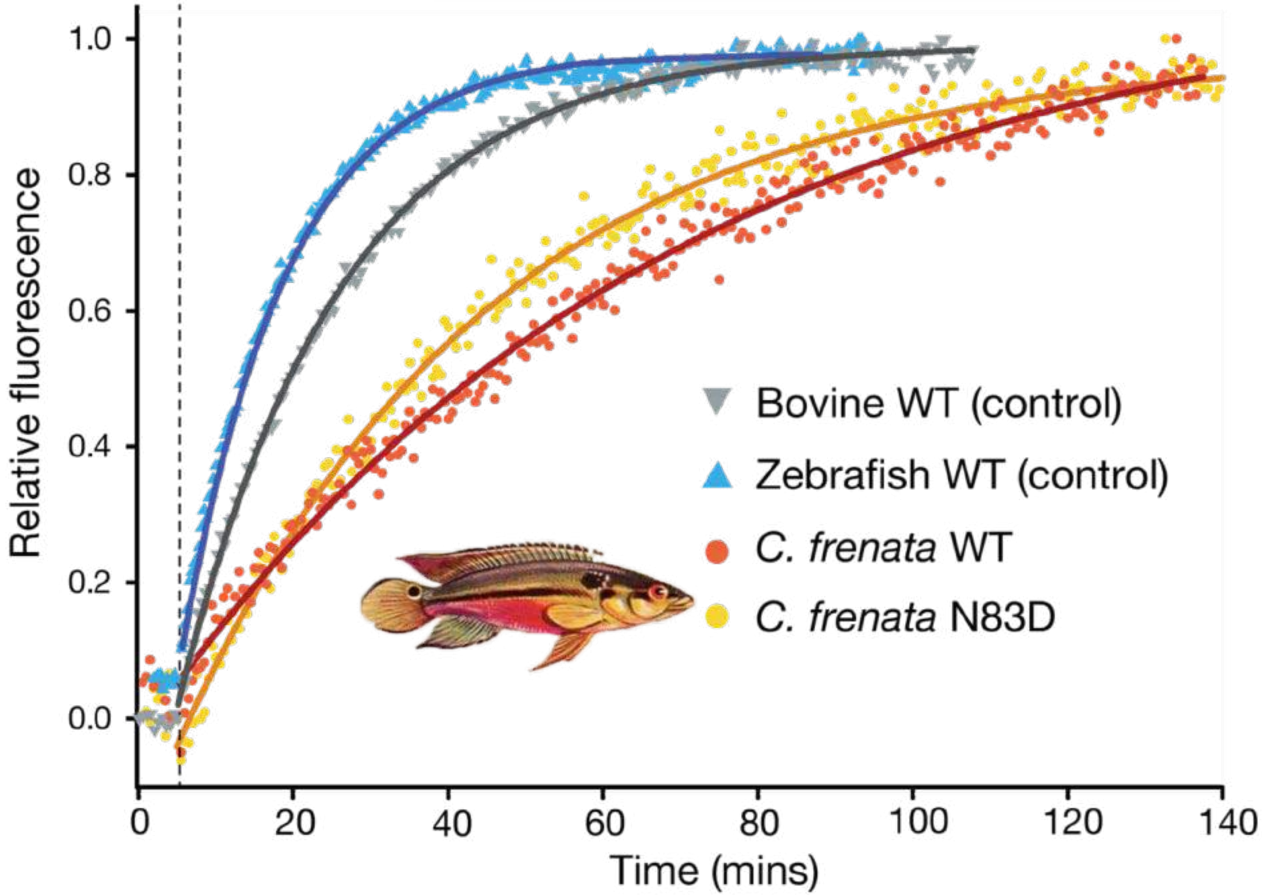


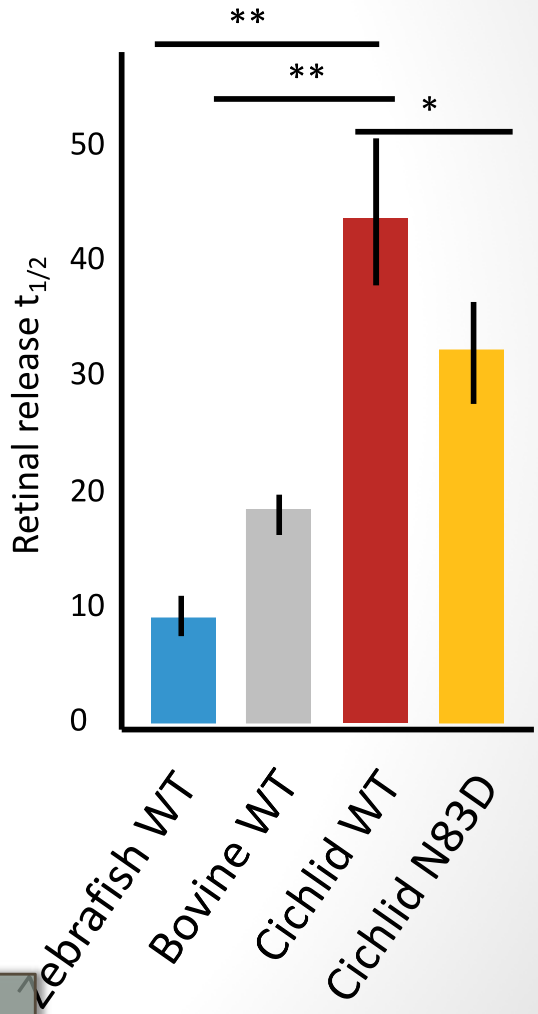
Dark-light
difference
spectrum



The N83D mutation produces a significant shift in rhodopsin function



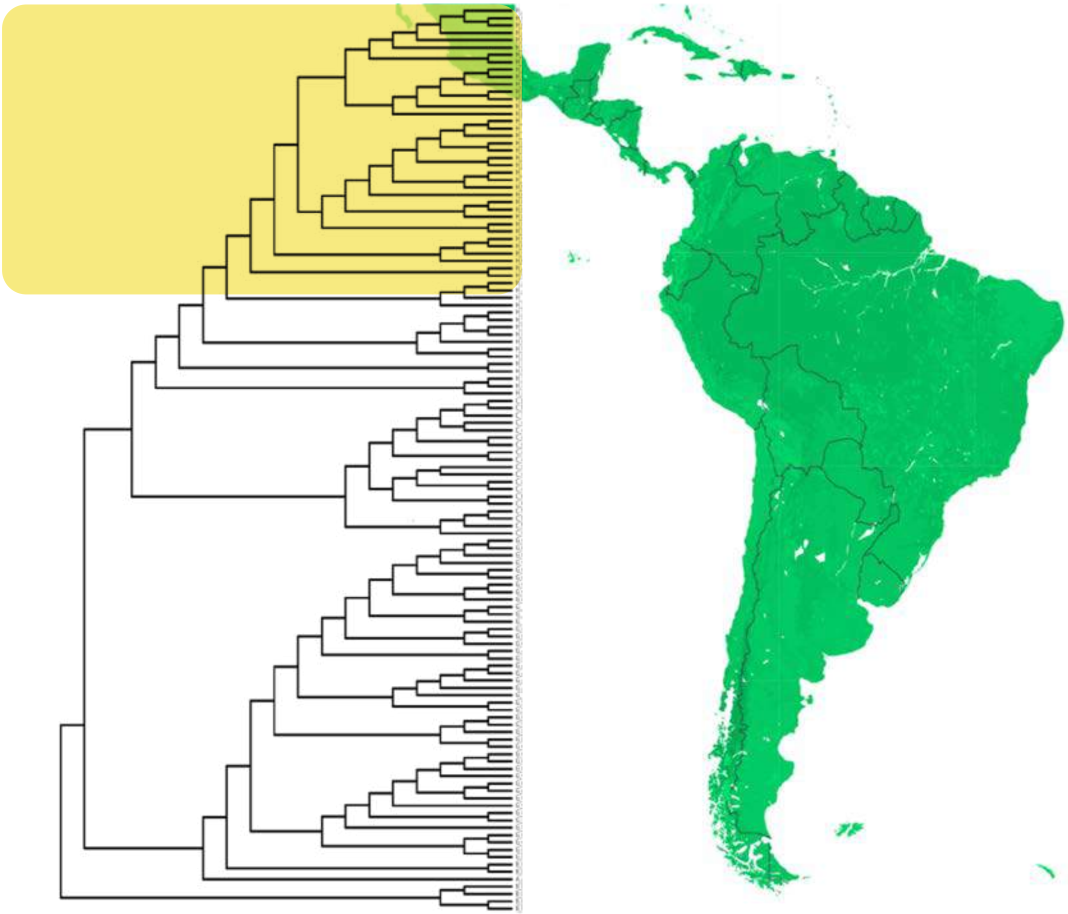




N83D in Central American fishes mediates faster kinetics

Hauser et al. 2017 *MBE*

Accelerated rhodopsin divergence during invasion of Central America



🟧 Central America

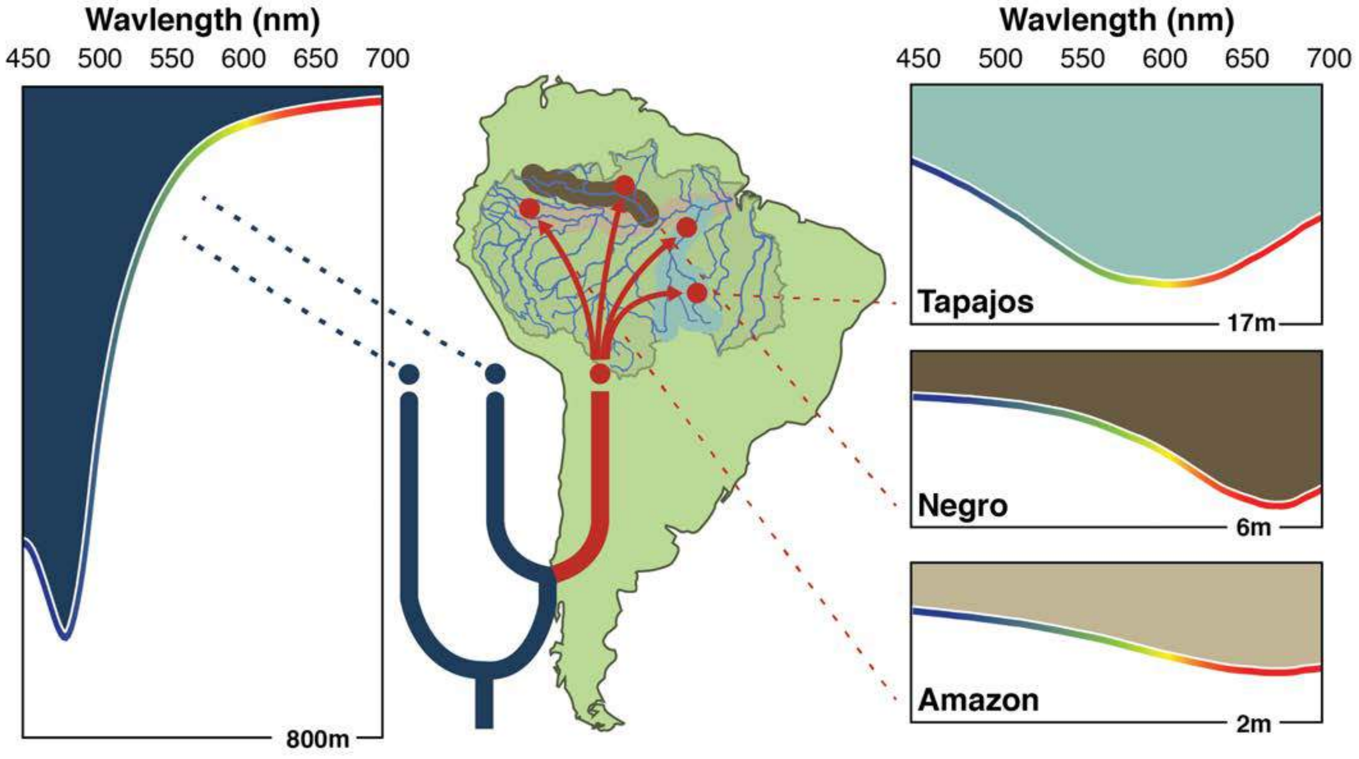
Combination of computational and functional studies suggest **adaptation** to increased light levels in clear streams of Central America

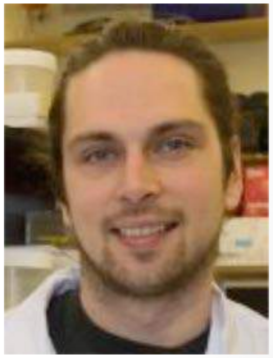
	Positive selection in RH1 3.4% of sites
Background (South America)	ω = 4.5
Foreground (Central America)	ω = **14.0**

Hauser et al. 2017 *MBE*

Rhodopsin evolution in marine-derived Amazonian anchovies

Hypothesis: Adaptive evolution in rhodopsin gene of freshwater anchovies





Alex Van Nynatten

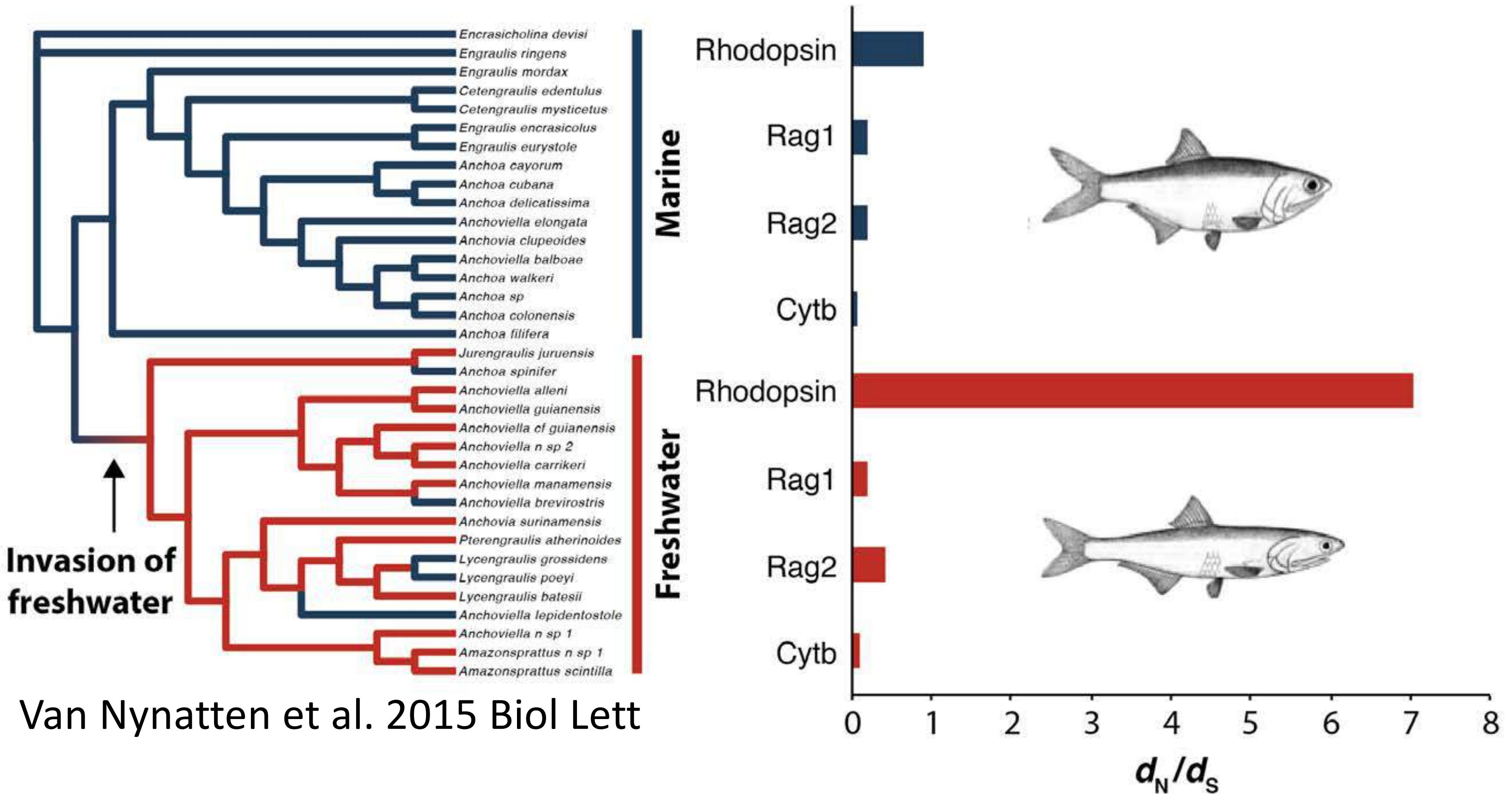
Nathan Lovejoy
U of Toronto

- Closely related marine and freshwater clades
- **Ancestrally marine**

Van Nynatten et al. 2015 Biol Lett

Rhodopsin evolution in marine-derived Amazonian anchovies

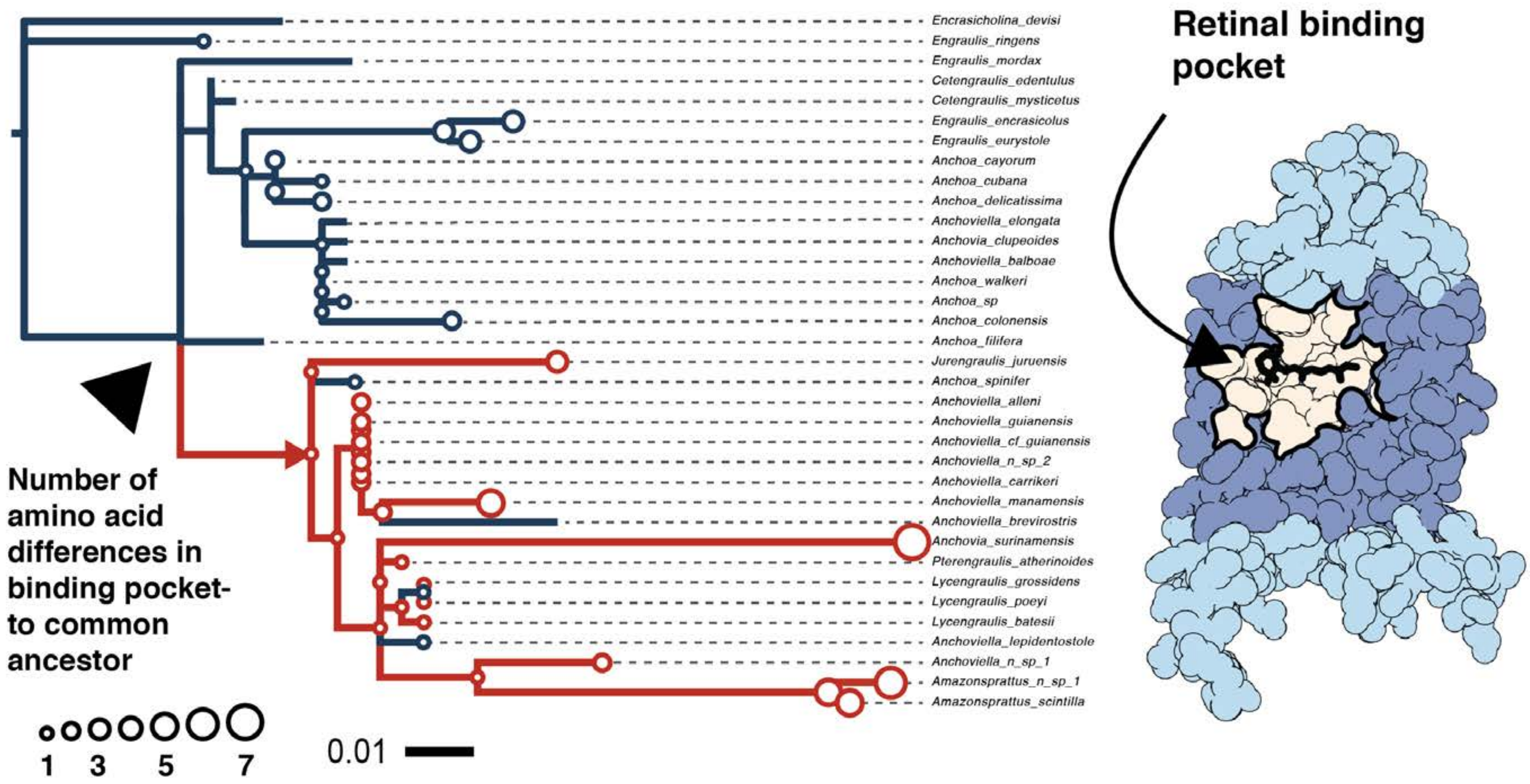
Hypothesis: Adaptive evolution in rhodopsin gene of freshwater anchovies



Van Nynatten et al. 2015 Biol Lett

- Increased dN/dS (CmC) in rhodopsin of the freshwater clade
- No difference in non-visual control genes

Amino acid substitutions in anchovy rhodopsin

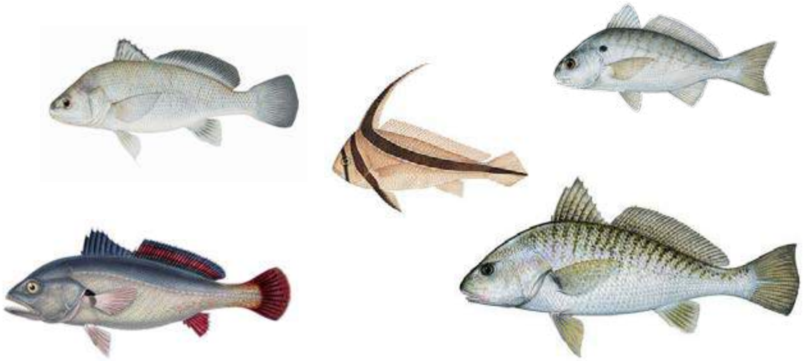


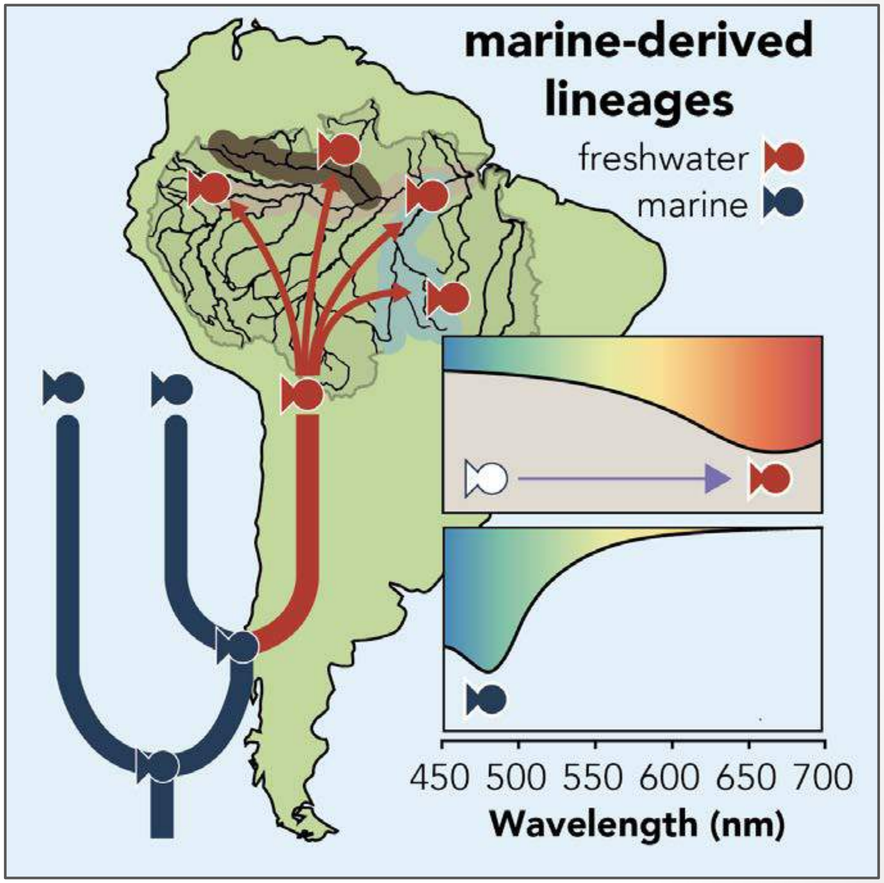
- Longer branch lengths in freshwater clade when scaled by number of amino acid substitutions in rhodopsin
- More remodelled chromophore binding pocket in freshwater clade

Larger circles at nodes represent more amino acid differences in retinal binding pocket residues with common ancestor (arrowhead)

Drum and croakers (family: Sciaenidae)

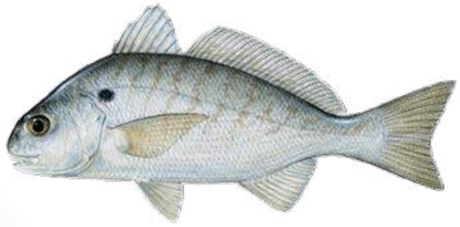
- Miocene marine incursion events resulted in dramatic radiation
- Diverse morphologies, adaptations to dim light

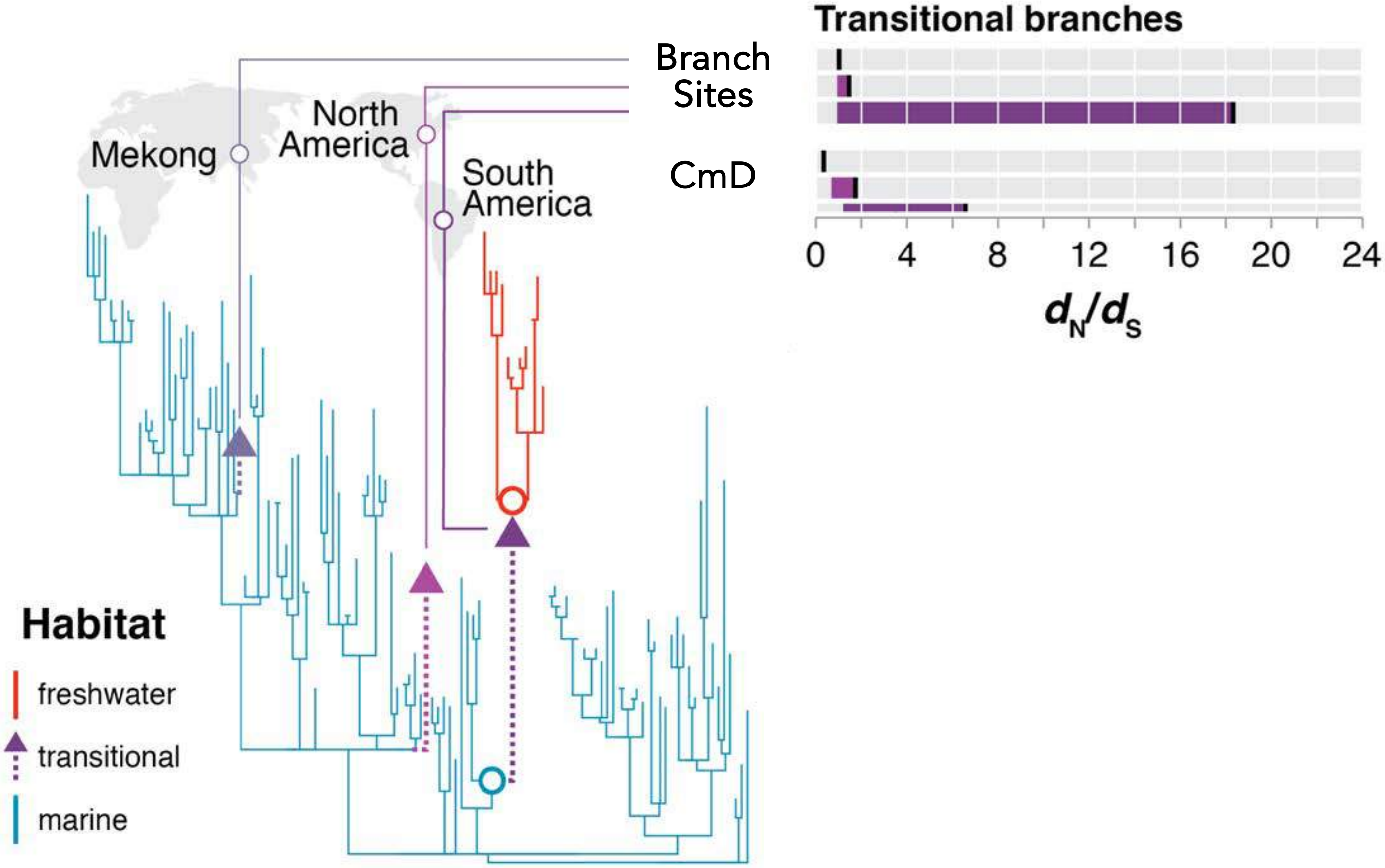




Positive selection in rhodopsin during marine to freshwater transition in South America

- Significantly higher rates of evolution on transitional branch
- Not on other transitional branches





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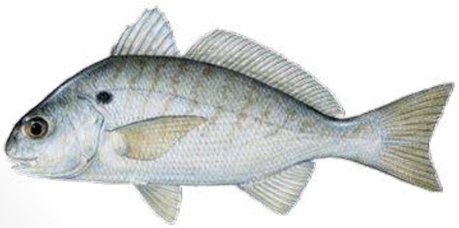
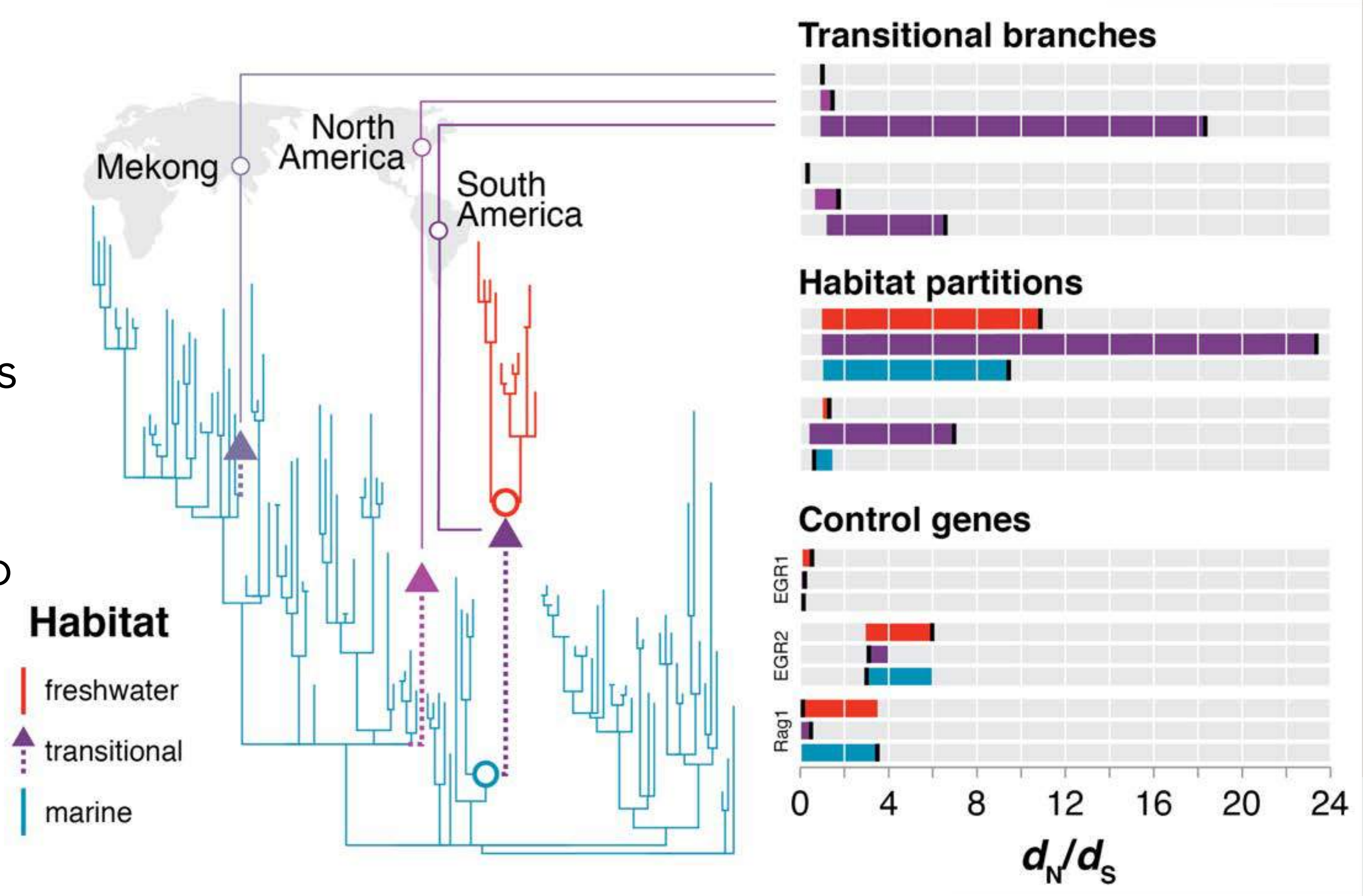
Positive selection on all ecological partitions, but highest on transitional branch

- Much higher d_N/d_S on the transitional branch than marine or freshwater clades
- No difference in control genes – seems specific to rhodopsin





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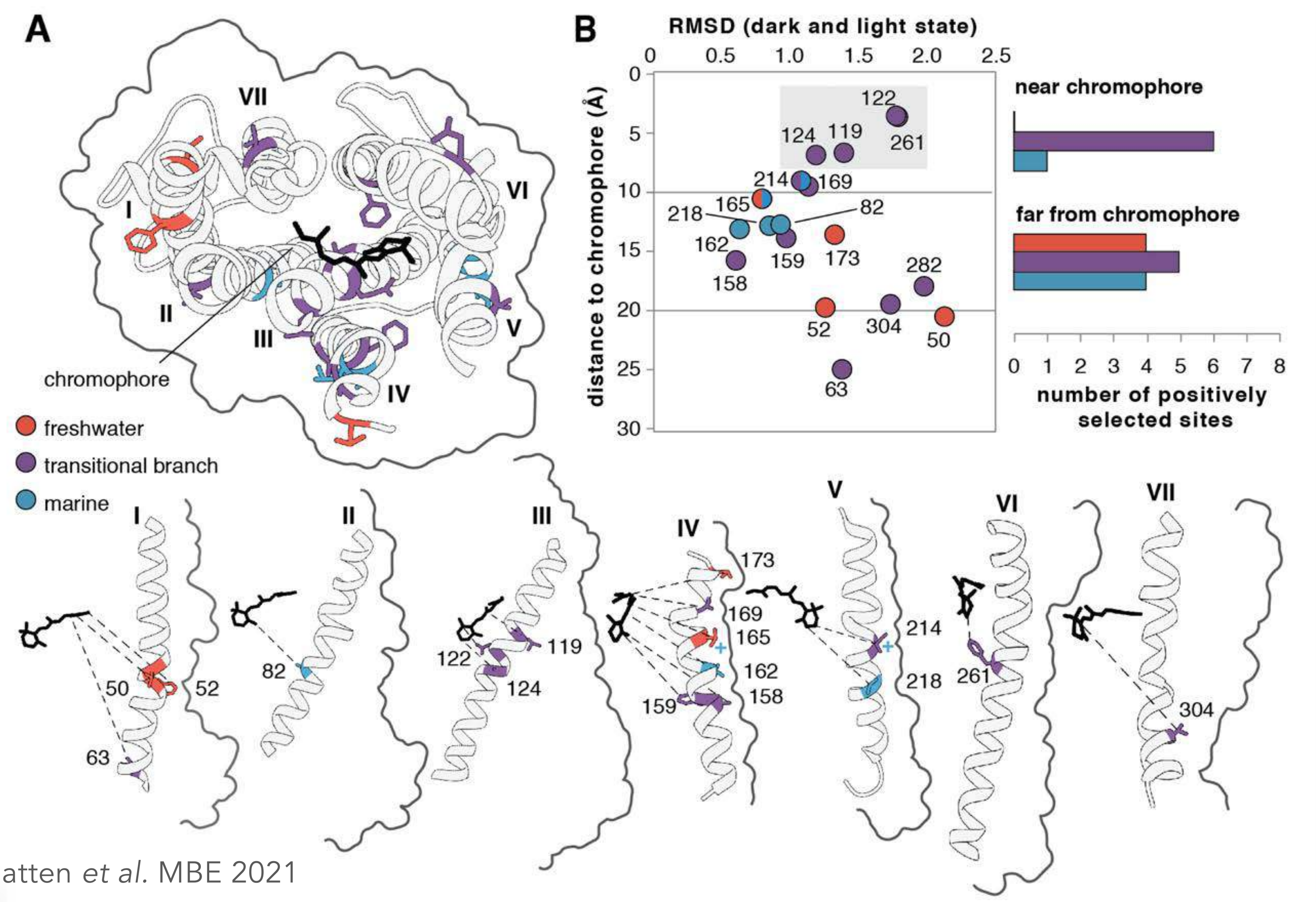
Positively selected sites tend to be in the binding site on the transitional branch



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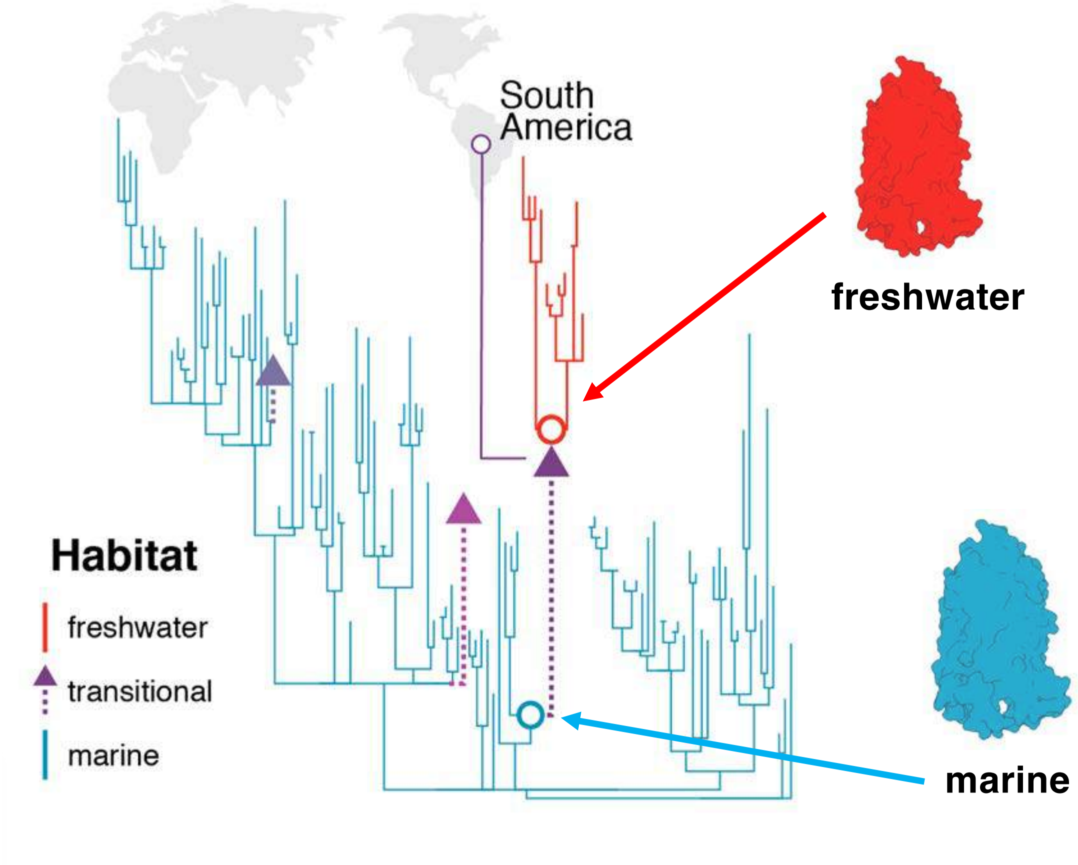
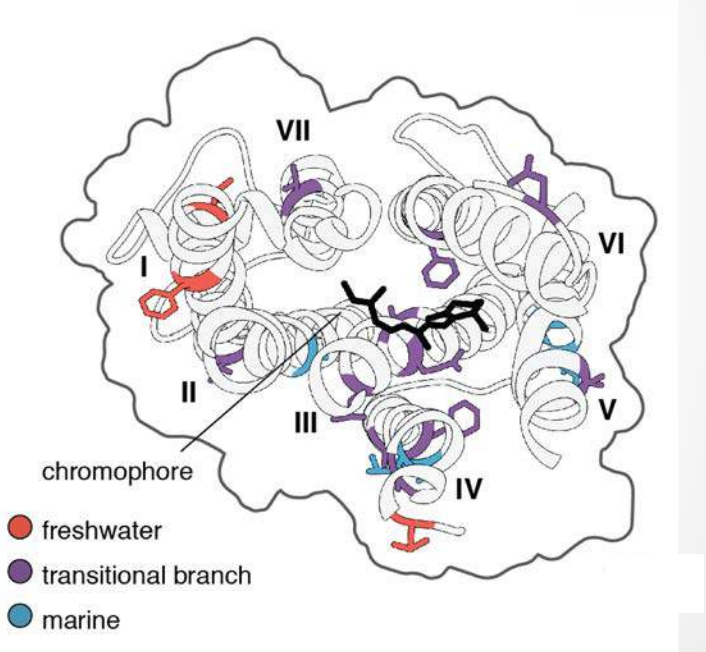
Using ancestral reconstructions to test evolutionary hypotheses of adaptation



Positively selected sites



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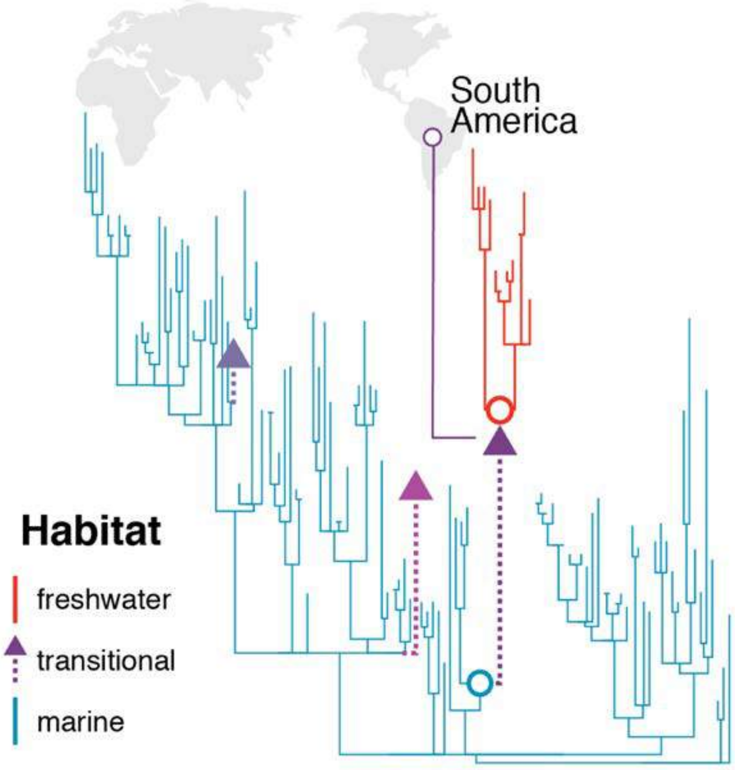
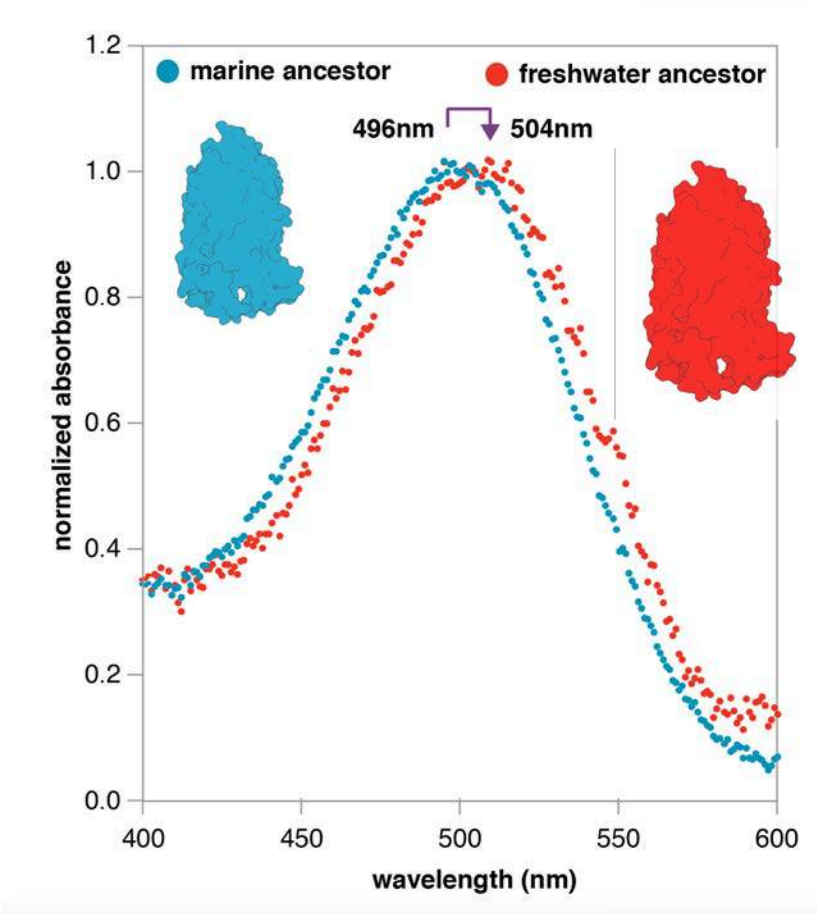
Freshwater rhodopsin has red-shifted spectral sensitivity





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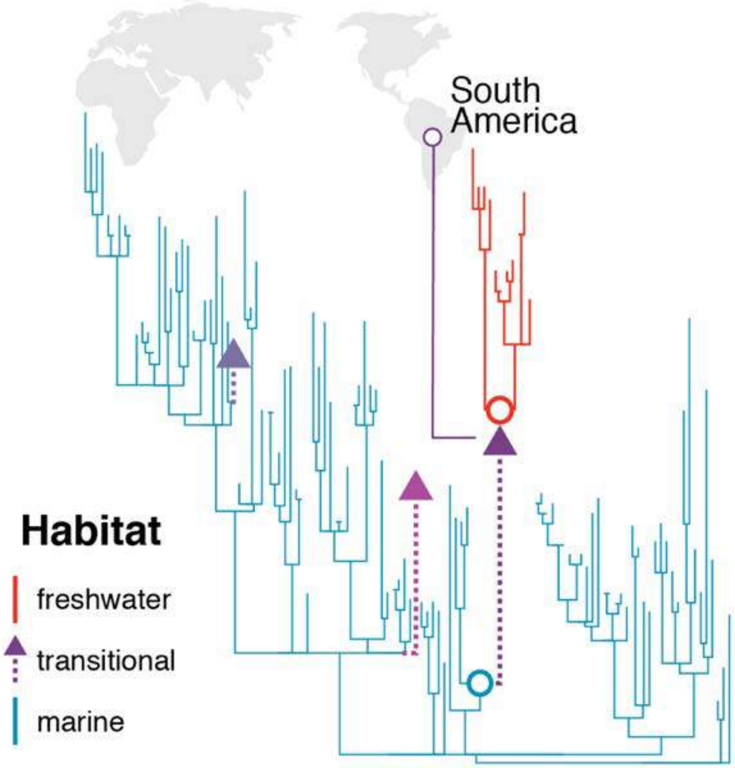
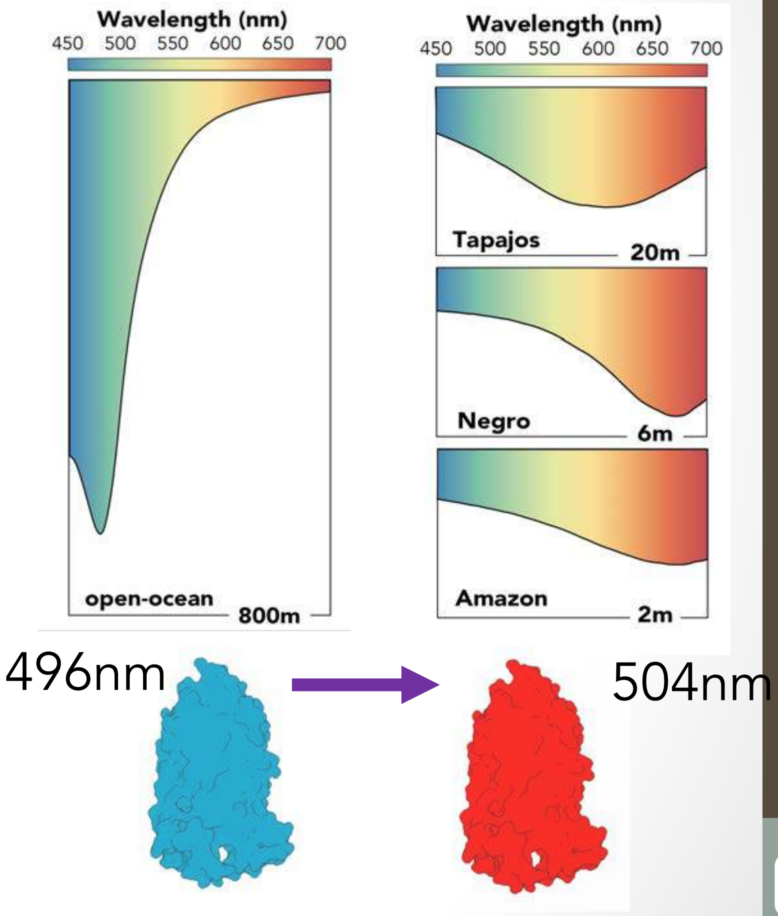
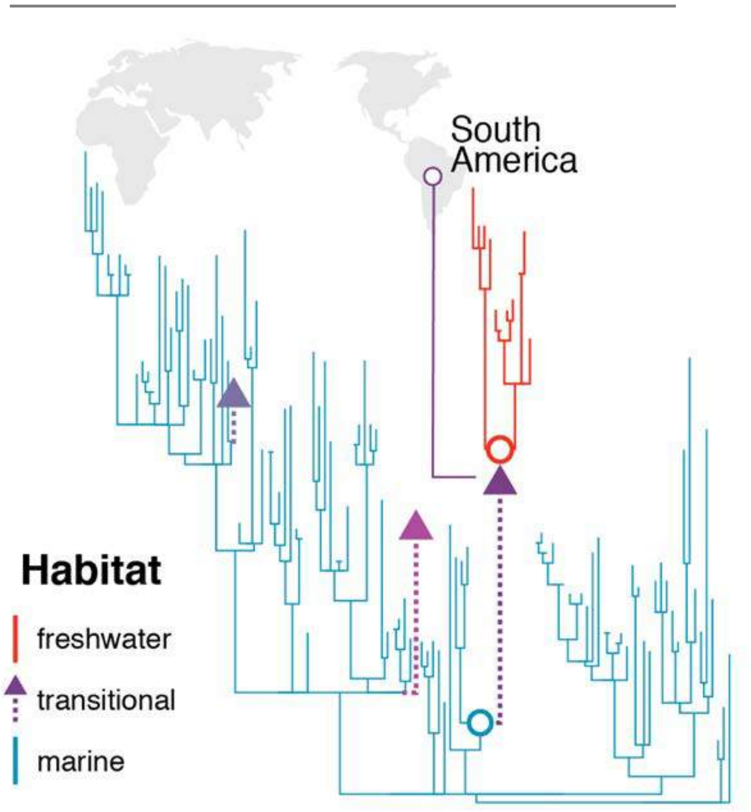
Freshwater croaker rhodopsin matches Amazonian underwater environment





Measuring dark adaptation in rhodopsin variants



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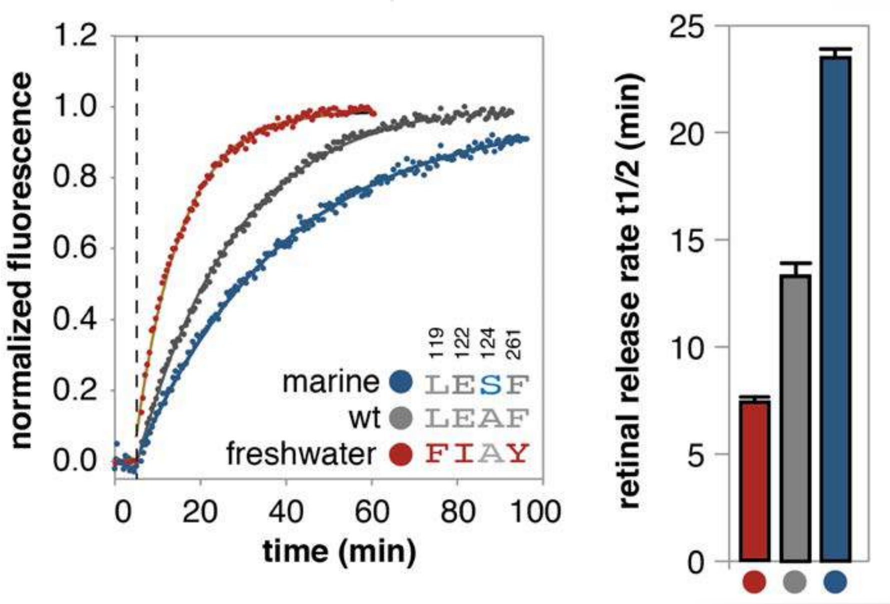
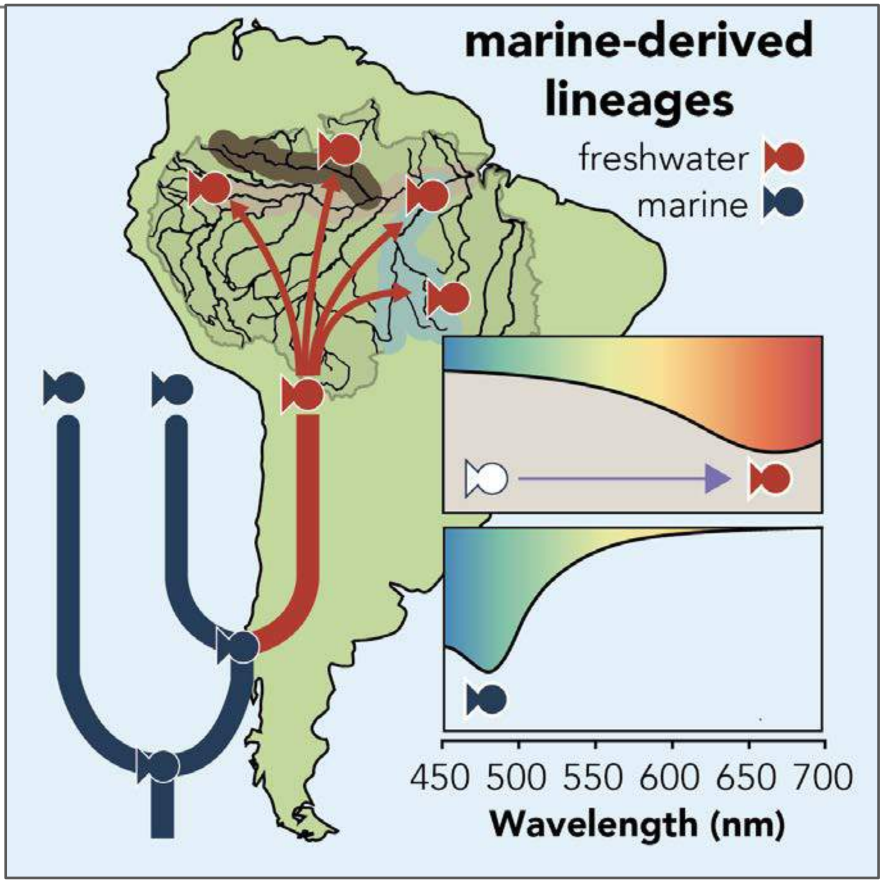
- Marine vs. freshwater variants show differences in retinal release rate
- Freshwater variant has faster kinetics, similar to cone opsins



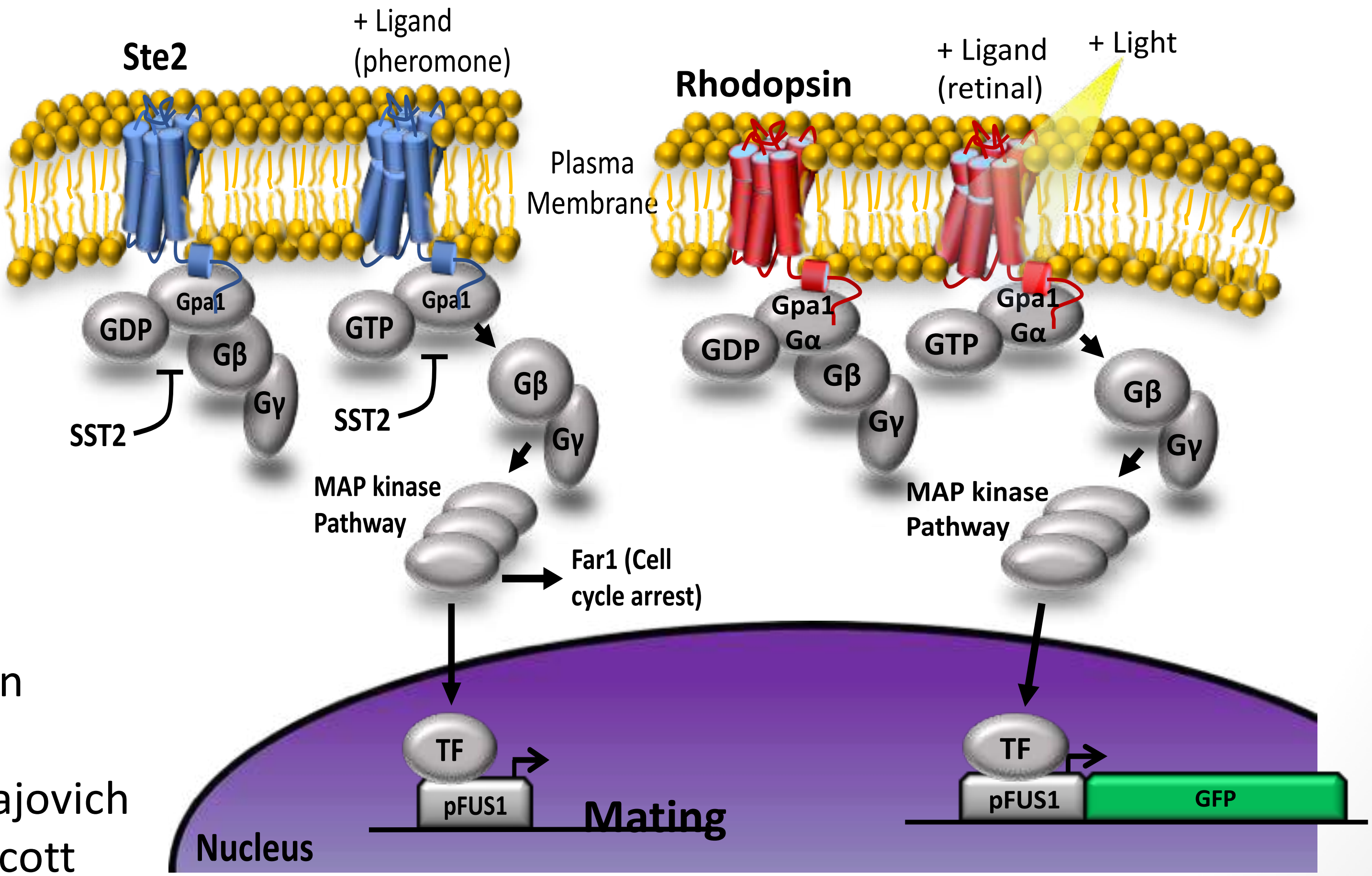
Ecological significance of faster dark adaptation in freshwater environments



High dynamic range of light intensities in freshwater necessitates rapid dark adaptation?

Evolution of deep diving cetaceans Dungan & Chang PNAS 2022

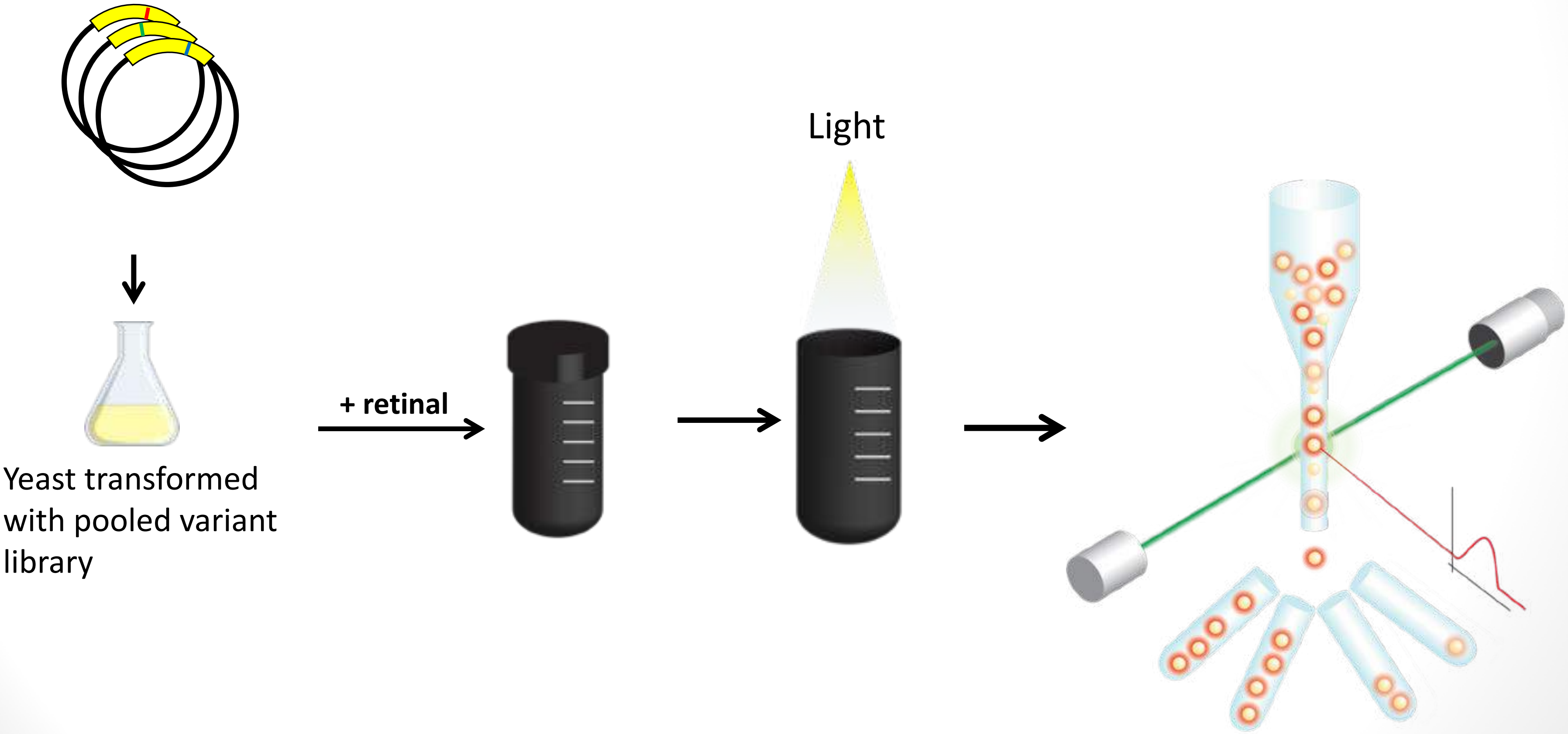
Future directions in molecular evolution: Deep scanning mutagenesis to investigate evolutionary processes



Steven Chen
Jing Liu
Sergio Peisajovich
Benjamin Scott

High-throughput rhodopsin variant libraries

Steven Chen
Benjamin Scott



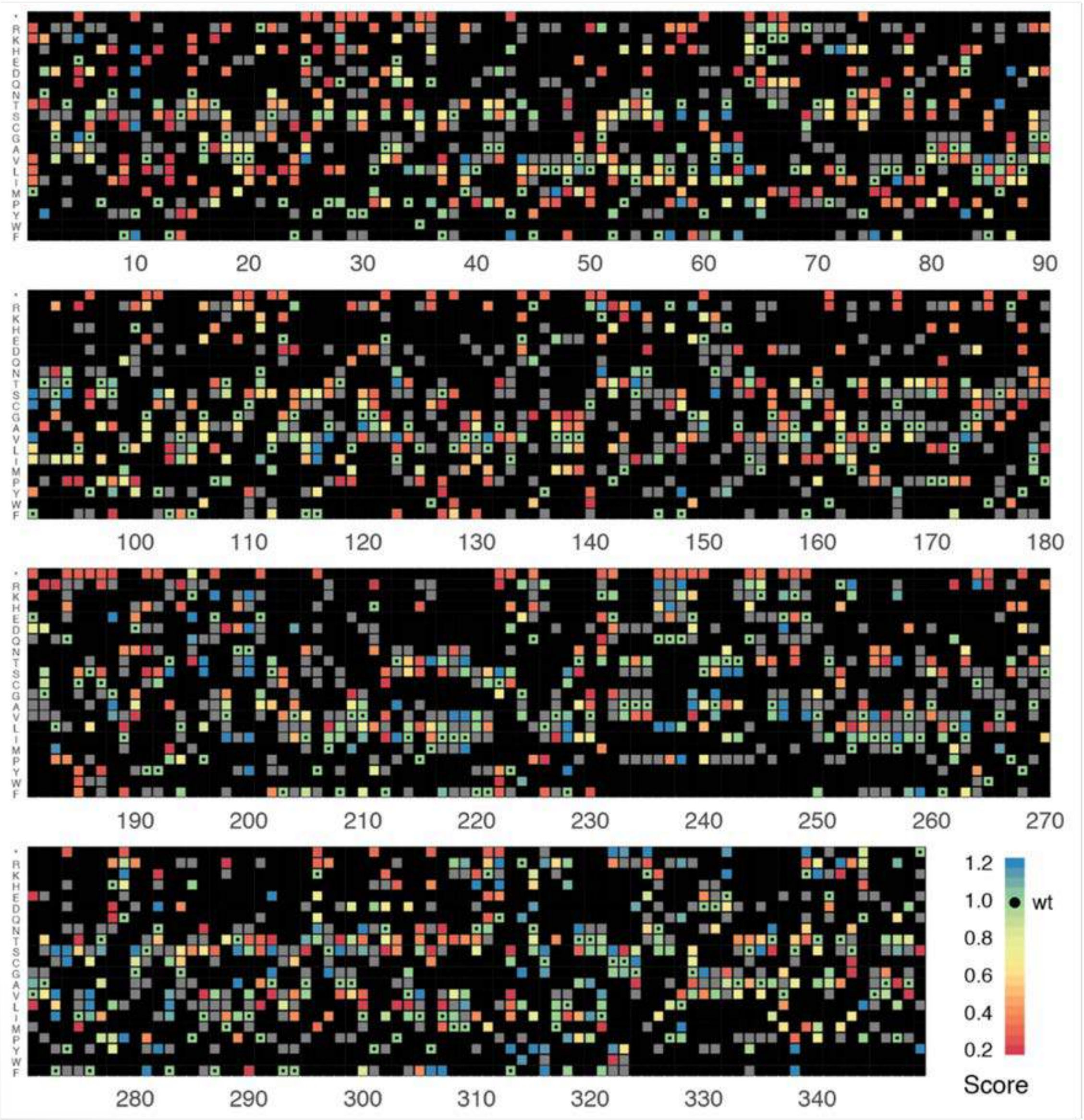
Random mutagenesis
optimized for 1 mutation/gene

Yeast transformed
with pooled variant
library

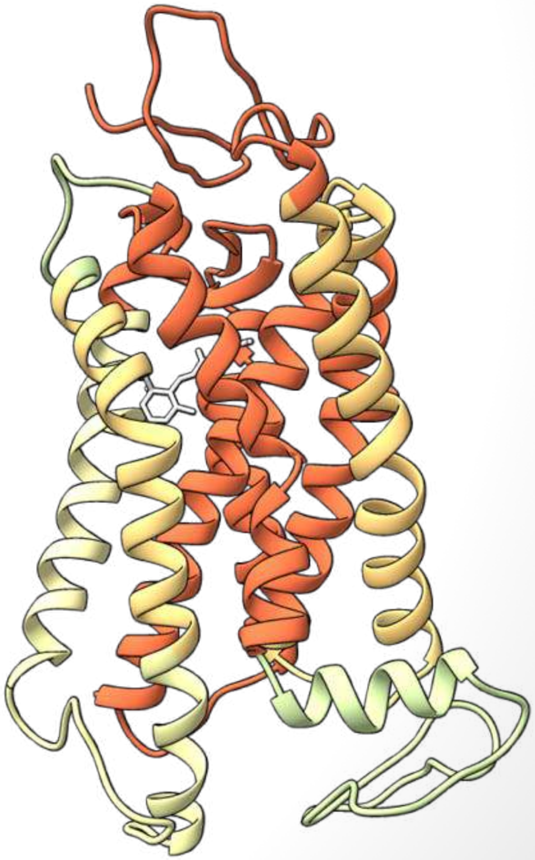
+ retinal

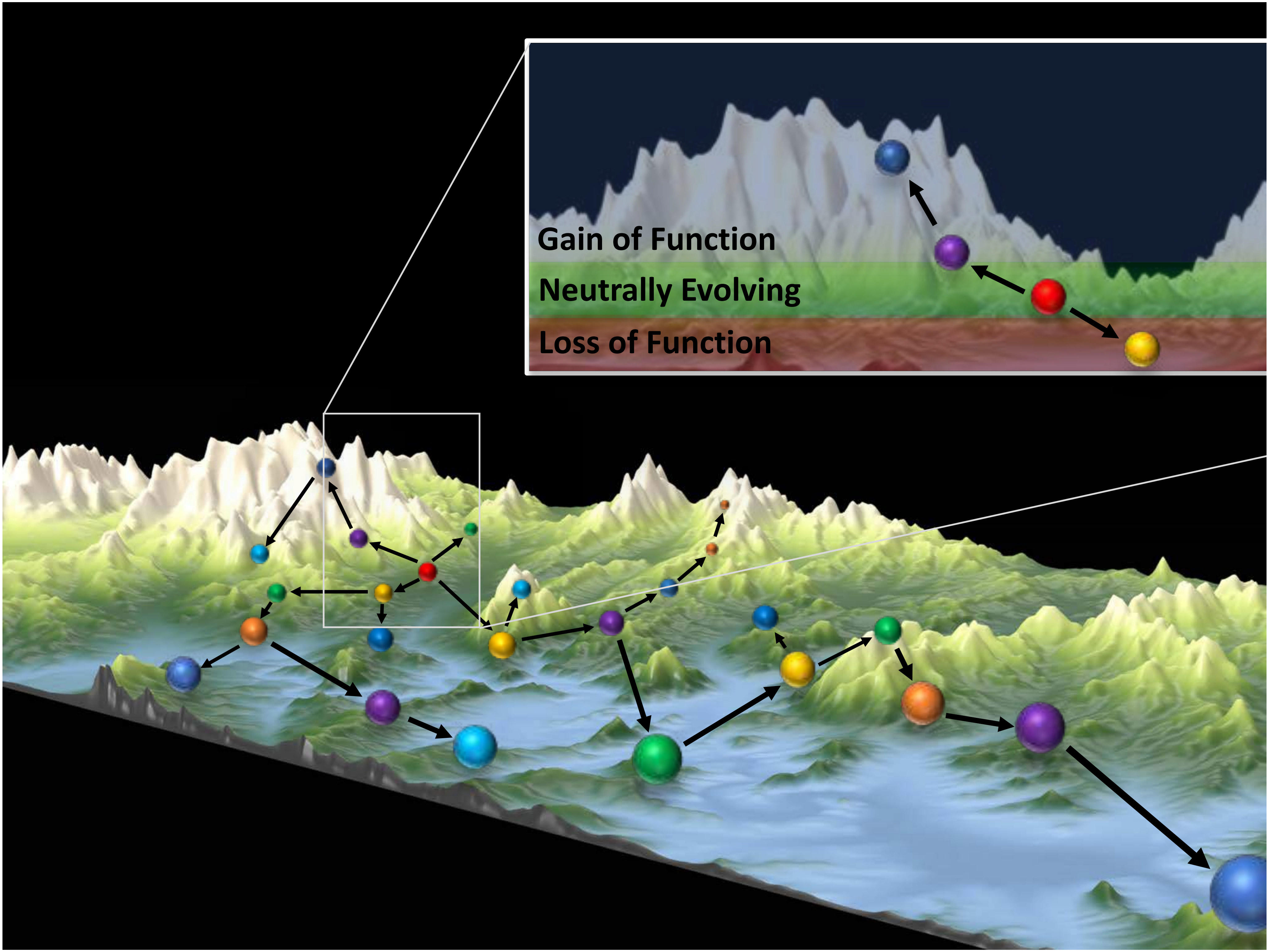
Light

High-throughput rhodopsin variant libraries



Steven Chen
Benjamin Scott




Gain of Function
Neutrally Evolving
Loss of Function