

QTL/GWAS/RAD all the acronyms of high- throughput genetic mapping

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Learning Objectives:

Explain the basic principles of genetic mapping

Understand the differences between types of genetic mapping
(GWAS/QTL)

Understand the power of reduced-representation sequencing

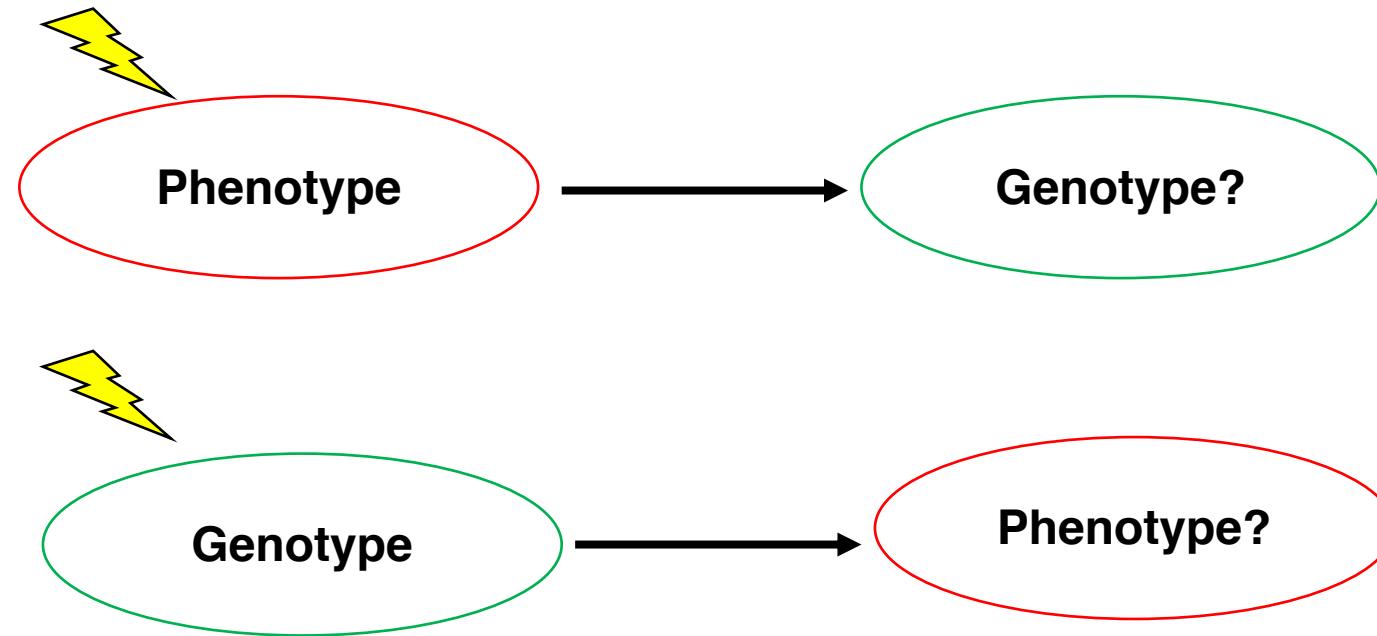
Describe how RAD-sequencing provides a “snapshot” of the genome

Become super excited to apply these techniques to stickleback
mitochondria

From last time: Forward and Reverse mutagenesis screens

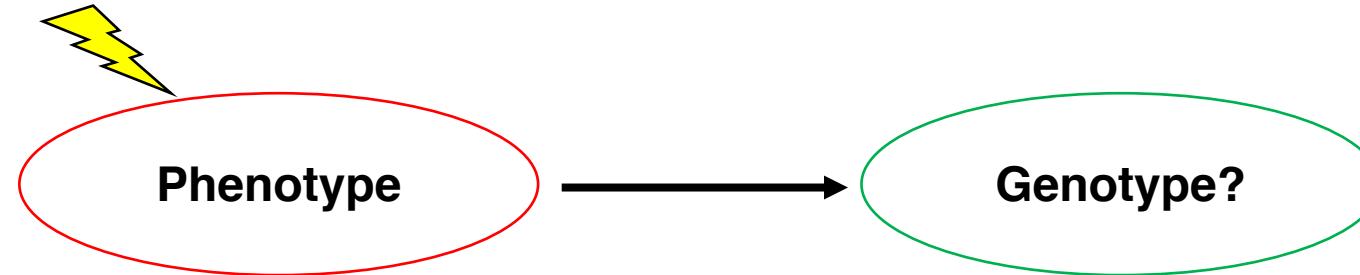
Forward Genetic
Mutant

Reverse Genetic
Mutant



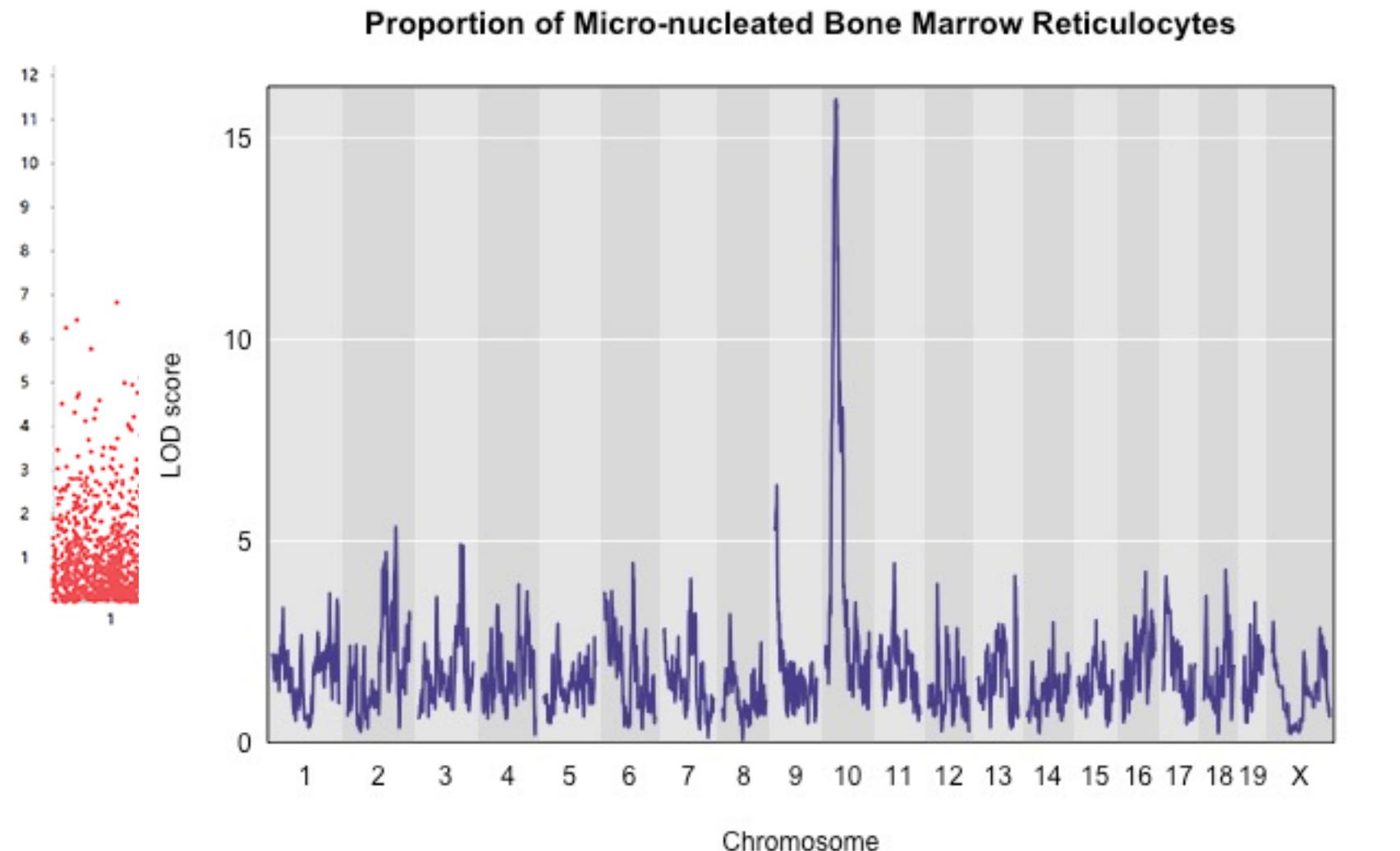
Traditional laboratory mutant screens fall into two categories: Forward and Reverse

Forward Genetic
Mutant

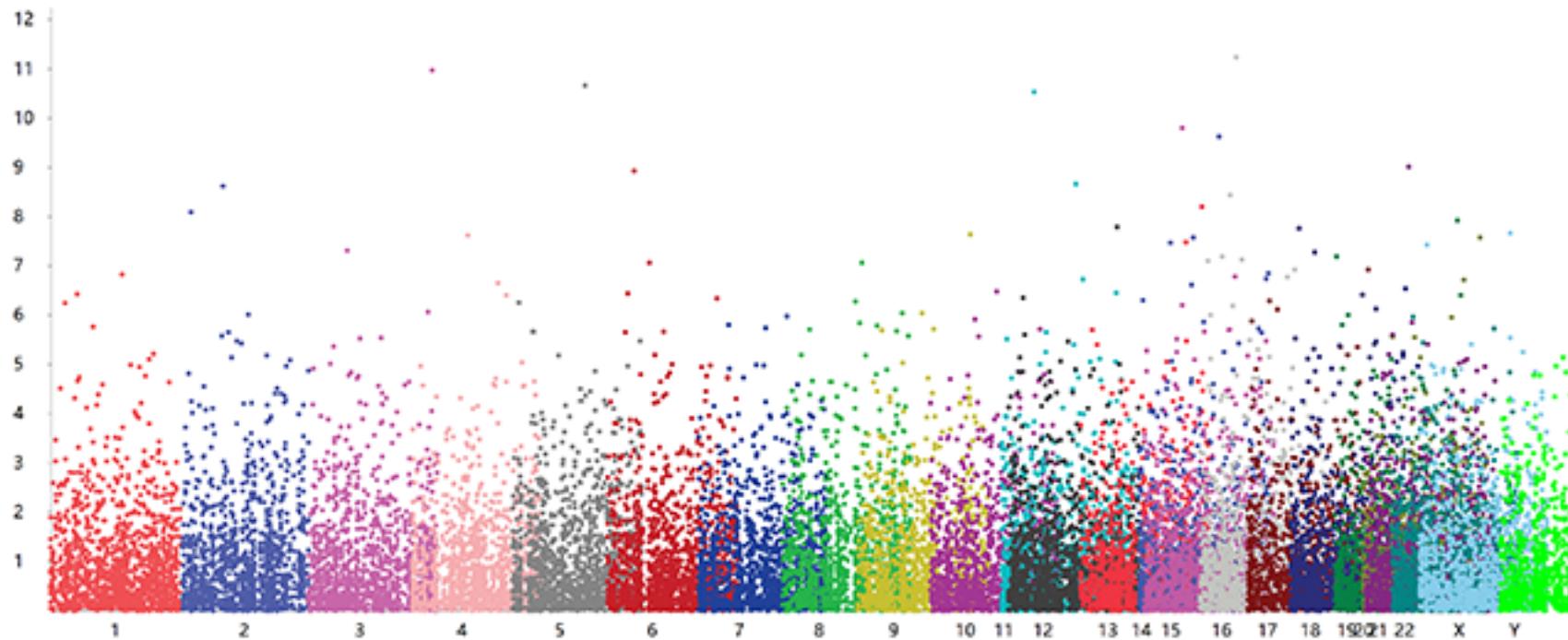


Goal: Identify the genotype underlying an observed phenotype

“Genetic Mapping” is a key component of forward mutagenesis screens

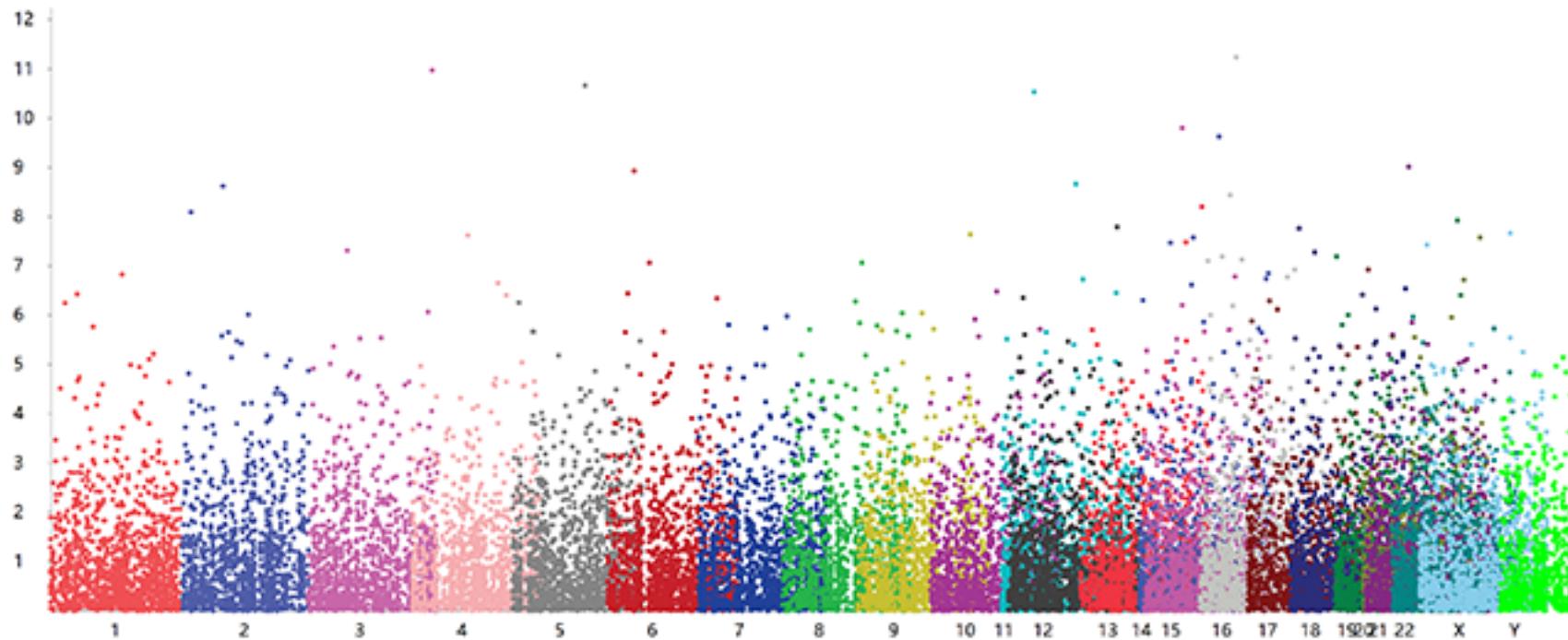


“Genetic Mapping” is a key component of forward mutagenesis screens



X-axis usually indicates the physical position of an allele/SNP/genetic marker in the genome

“Genetic Mapping” is a key component of forward mutagenesis screens



Y-axis indicates the strength of the association of the genotype with the phenotype

Genetic Mapping can be performed many ways.

Quantitative Trait Locus (QTL) Mapping

Genome Wide Association Studies (GWAS)

Does anyone know the difference between them?

Terrible hint: The names aren't actually useful

Genetic Mapping can be performed many ways.

Quantitative Trait Locus (QTL) Mapping

Genome Wide Association Studies (GWAS)

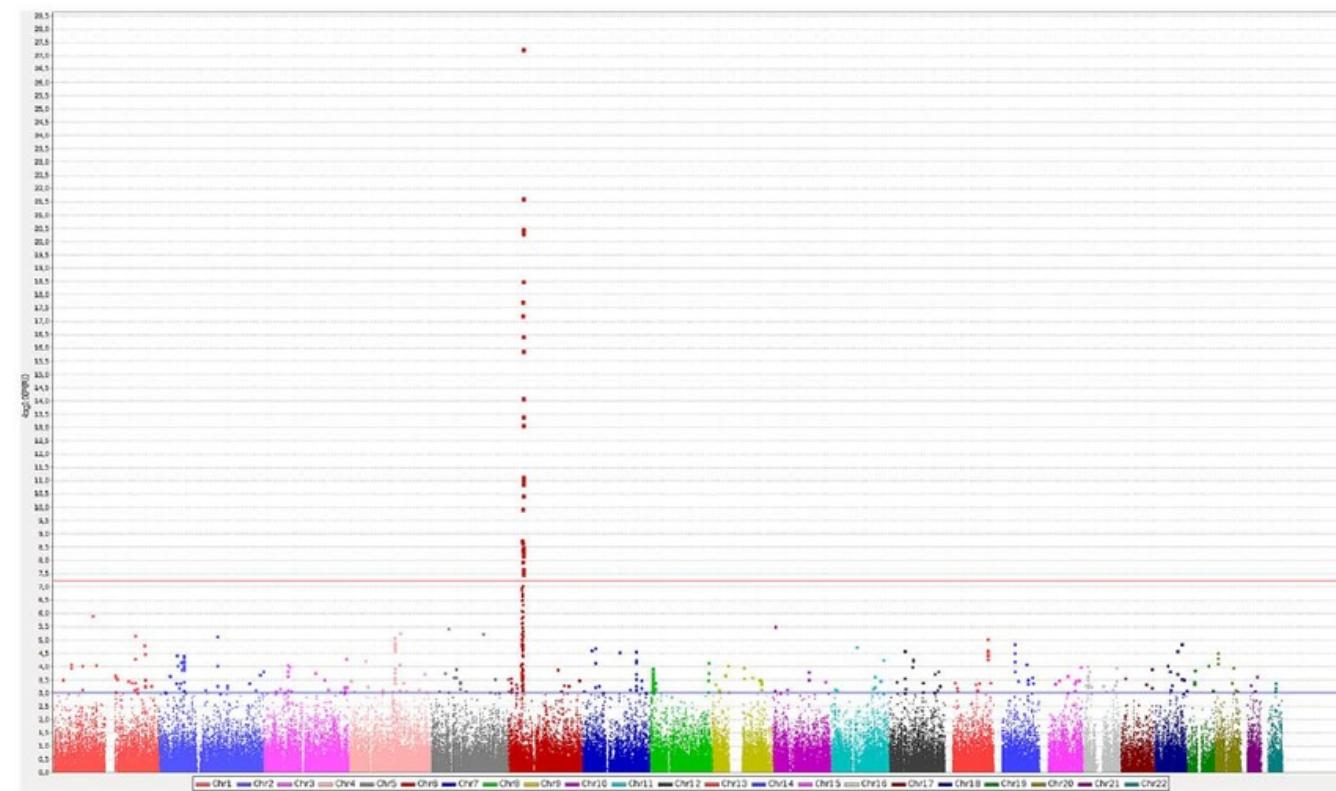
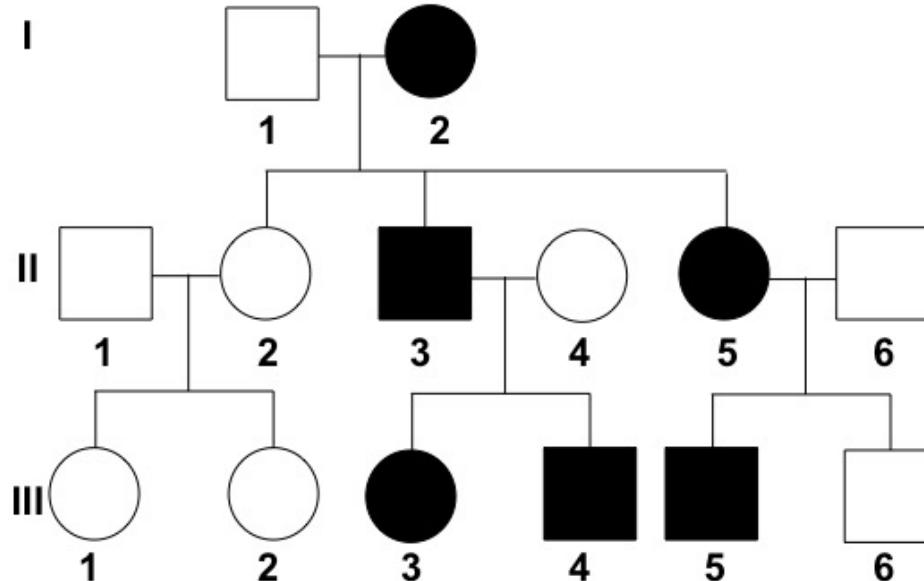
Genome Wide Association Studies don't always use whole genomes

Quantitative Trait Locus Mapping doesn't always involve quantitative traits

(we will talk about these things later)

A simplified view of GWAS

Pedigree of a single family with a disease -> sequence genomes -> find mutation only present in diseased individuals



GWAS is useful but not without problems

Linkage disequilibrium

Large regions of the genome may be “linked” meaning they are inherited in blocks

You are unable to disentangle associations of specific SNPs (single nucleotide polymorphisms) from larger linkage blocks

Population structure

You often need a lot of individuals

You may not know the relatedness of the individuals AND some individuals may be more related than others

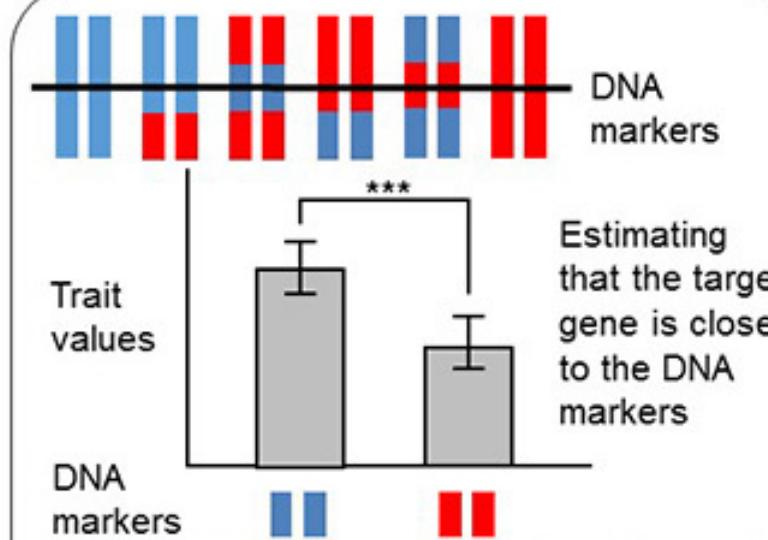
Genetic Mapping can be performed many ways.

Quantitative Trait Locus (QTL) Mapping

Genome Wide Association Studies (GWAS)

QTL analysis

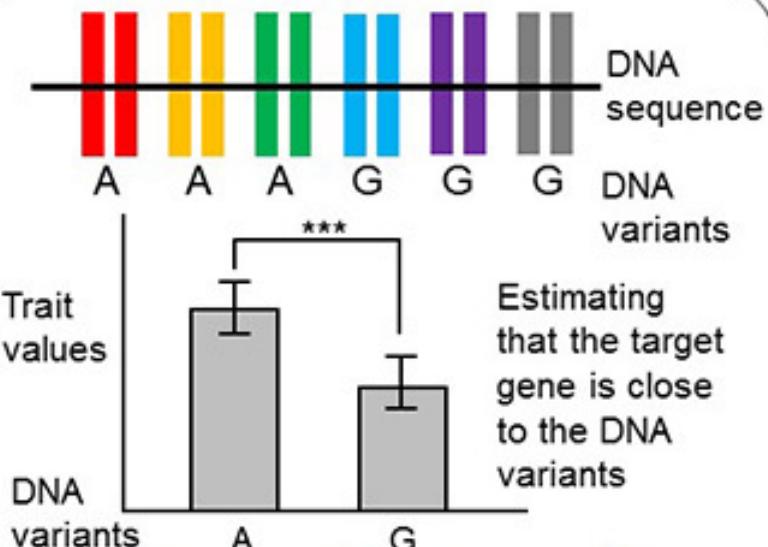
Crossed population
(F1 intercross for example)



Issues: only the 2 parents are analyzed, time and resources required to produce materials

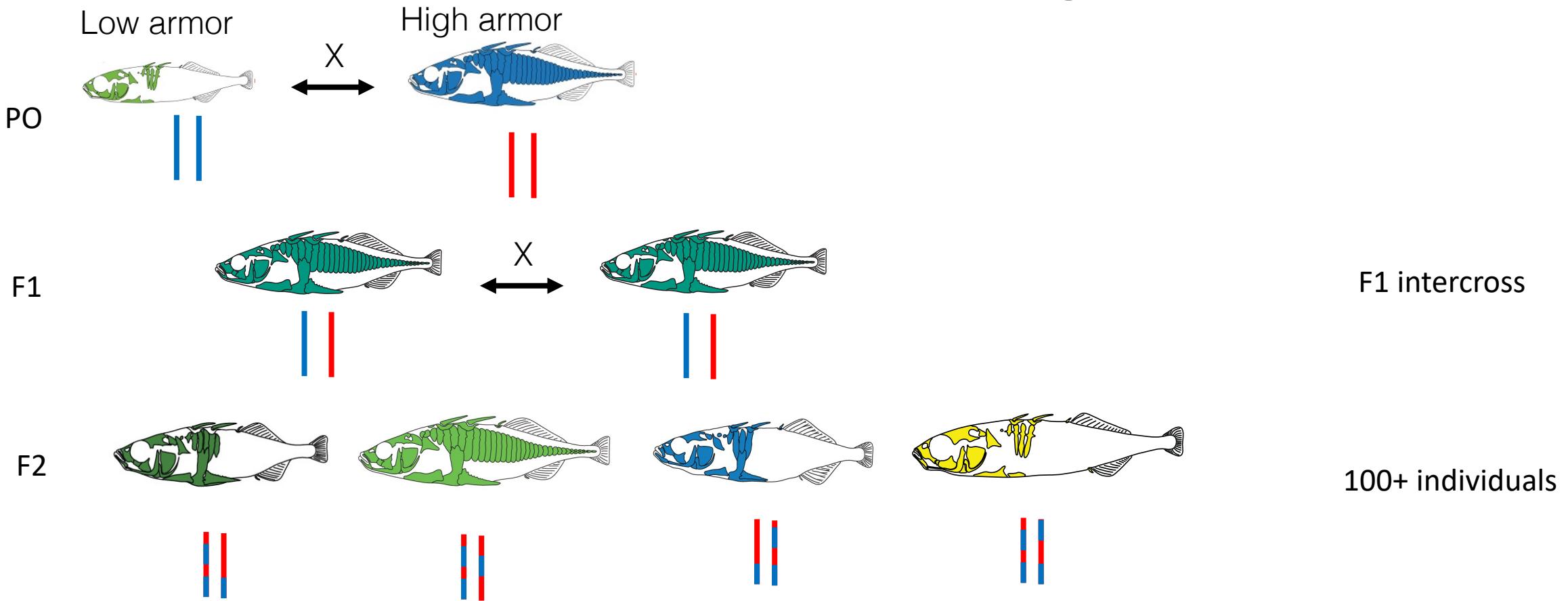
GWAS analysis

An interbreeding population of individuals of varying relatedness



Issues: Owing to population structure and linkage disequilibrium, it is difficult to identify genes

QTL mapping can be used to map complex traits without concerns about populations structure because all individuals are siblings

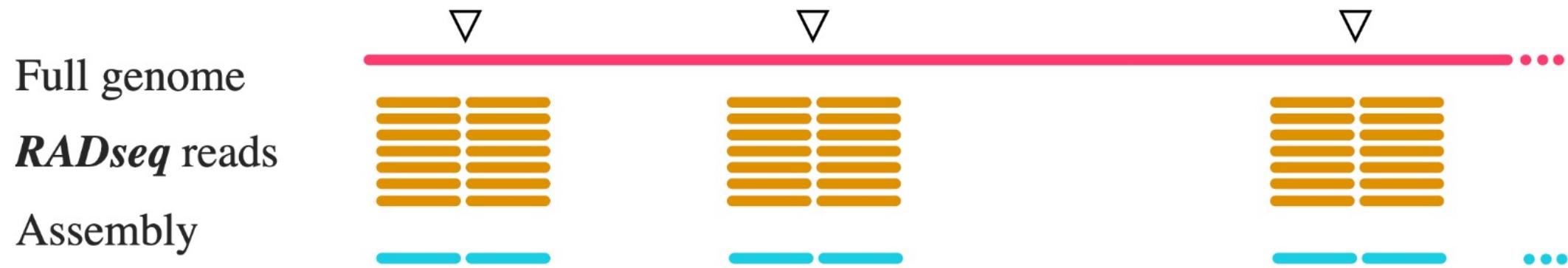
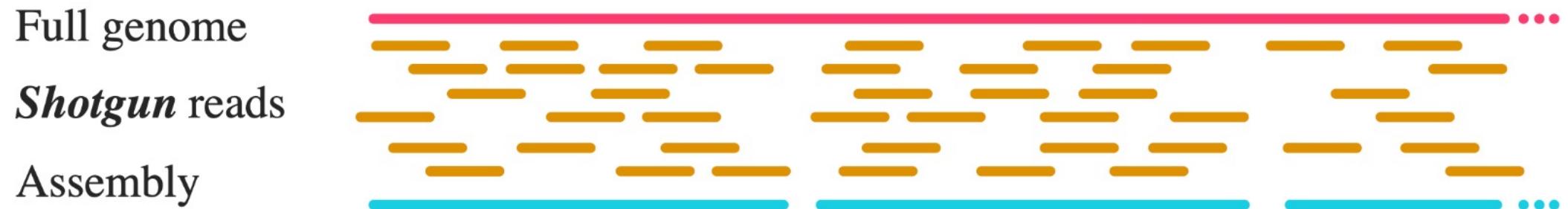


Sequencing 100s of genomes is expensive!!!!

We can use reduced representation sequencing to gain a "snapshot" of the genome and pool 100s of individuals into a single lane of sequencing

RAD-seq (Restriction-site Associated DNA) is a type of reduced representation sequencing developed by Eric Johnson and Bill Cresko at the University of Oregon

RAD-seq provides information about the DNA surrounding a selected restriction site across the genome



Let's talk through an example from stickleback (a not-so simple case)

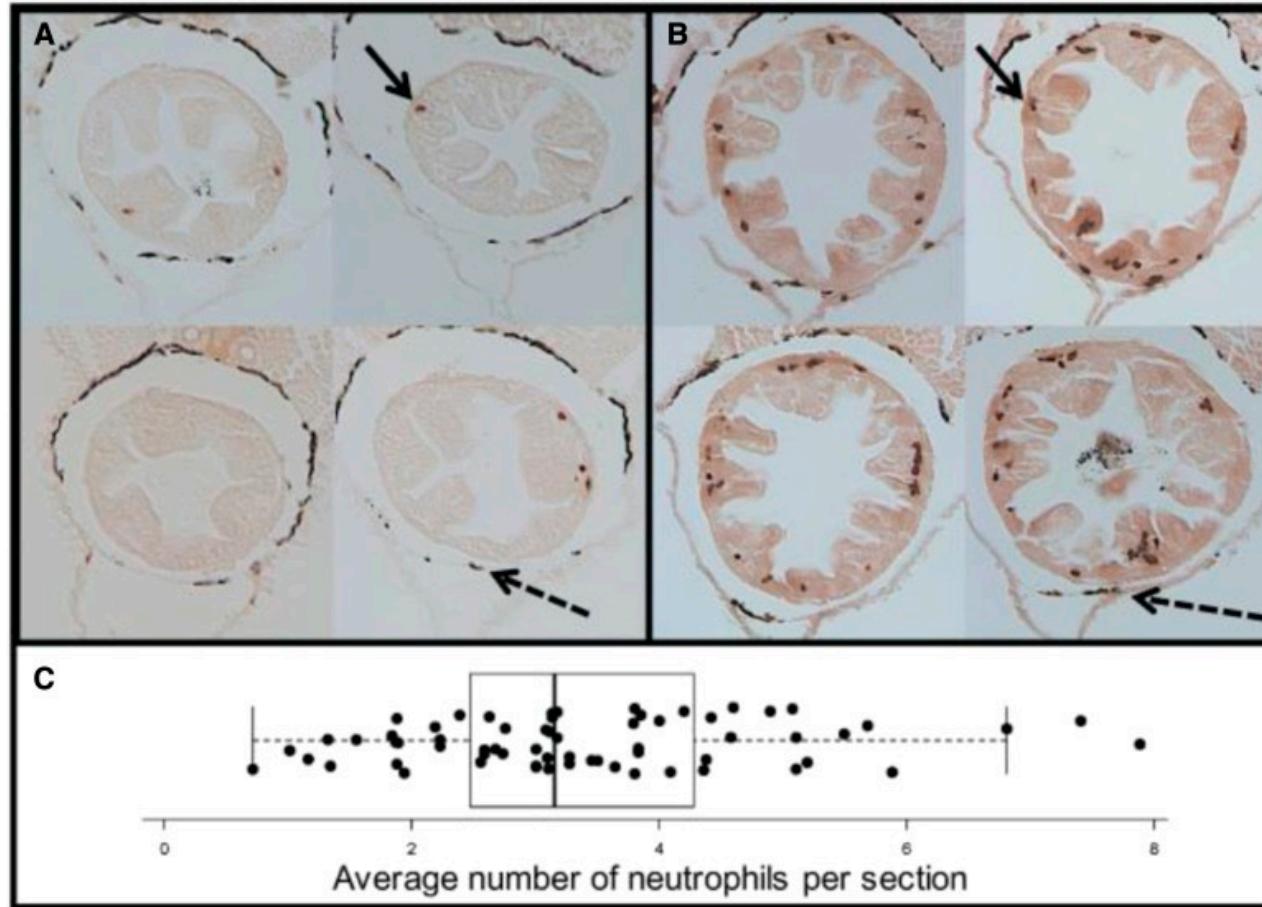
› G3 (Bethesda). 2020 Feb 6;10(2):613-622. doi: 10.1534/g3.119.400685.

QTL Mapping of Intestinal Neutrophil Variation in Threespine Stickleback Reveals Possible Gene Targets Connecting Intestinal Inflammation and Systemic Health

Emily A Beck ¹, Mark C Currey ¹, Clayton M Small ¹, William A Cresko ²

We knew Oceanic and Freshwater stickleback exhibit different levels of intestinal inflammation (neutrophil abundance)

We generated an F2 Mapping family and measured intestinal neutrophil density



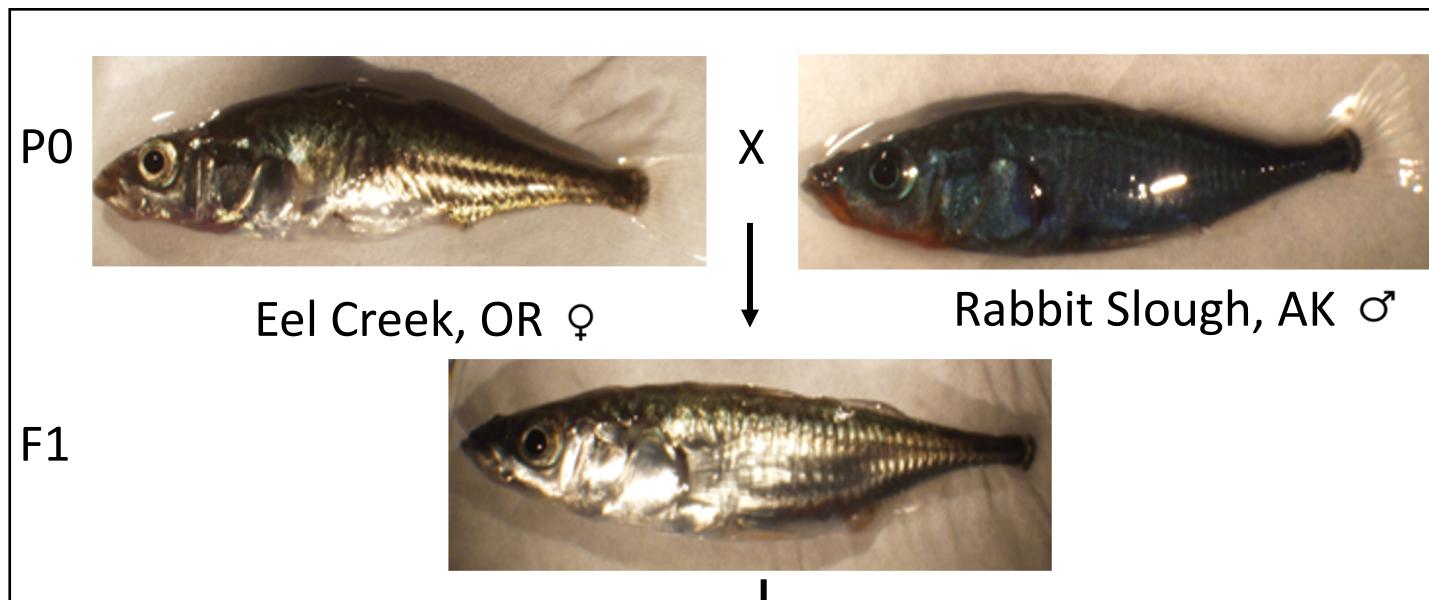
Group	Ensembl ID	Gene	Distance from SNP ^a (bp)	Immune Pathways	Disease Associations
LG3	ENSGACG00000014756	<i>f3</i>	37,324	Coagulation Cascade	—
LG3	ENSGACG00000015273	<i>map2k2b</i>	84,711	MAPK/ERK	—
LG3	ENSGACG00000015282	<i>tcf3a</i>	58,000	MAPK/ERK	—
LG3	ENSGACG00000015301	<i>unc13a</i>	15,909	—	ALS
LG3	ENSGACG00000015386	<i>epha4b</i>	25,437	MAPK/ERK	—
LG3	ENSGACG00000015719	<i>cldn18</i>	10,244	Tight Junctions	—
LG3	ENSGACG00000016028	<i>ripk2</i>	6,127	NFkB	—
LG3	ENSGACG00000016066	<i>tblxr1b</i>	16,774	—	Autism
LG3	ENSGACG00000016121	<i>rgs1</i>	1,892	—	Celiac Disease
LG3	ENSGACG00000016159	<i>pik3R3</i>	63,634	MAPK/ERK	—
LG3	ENSGACG00000016189	<i>scp2a</i>	0	—	Wheat Allergy
LG3	ENSGACG00000016212	<i>angptl3</i>	5,349	MAPK/ERK	—
LG3	ENSGACG00000016323	<i>c8b</i>	972	Complement Cascade	—
LG3	ENSGACG00000016338	<i>c8a</i>	0	Complement Cascade	—

“Chance favors the prepared mind”
– Louis Pasteur

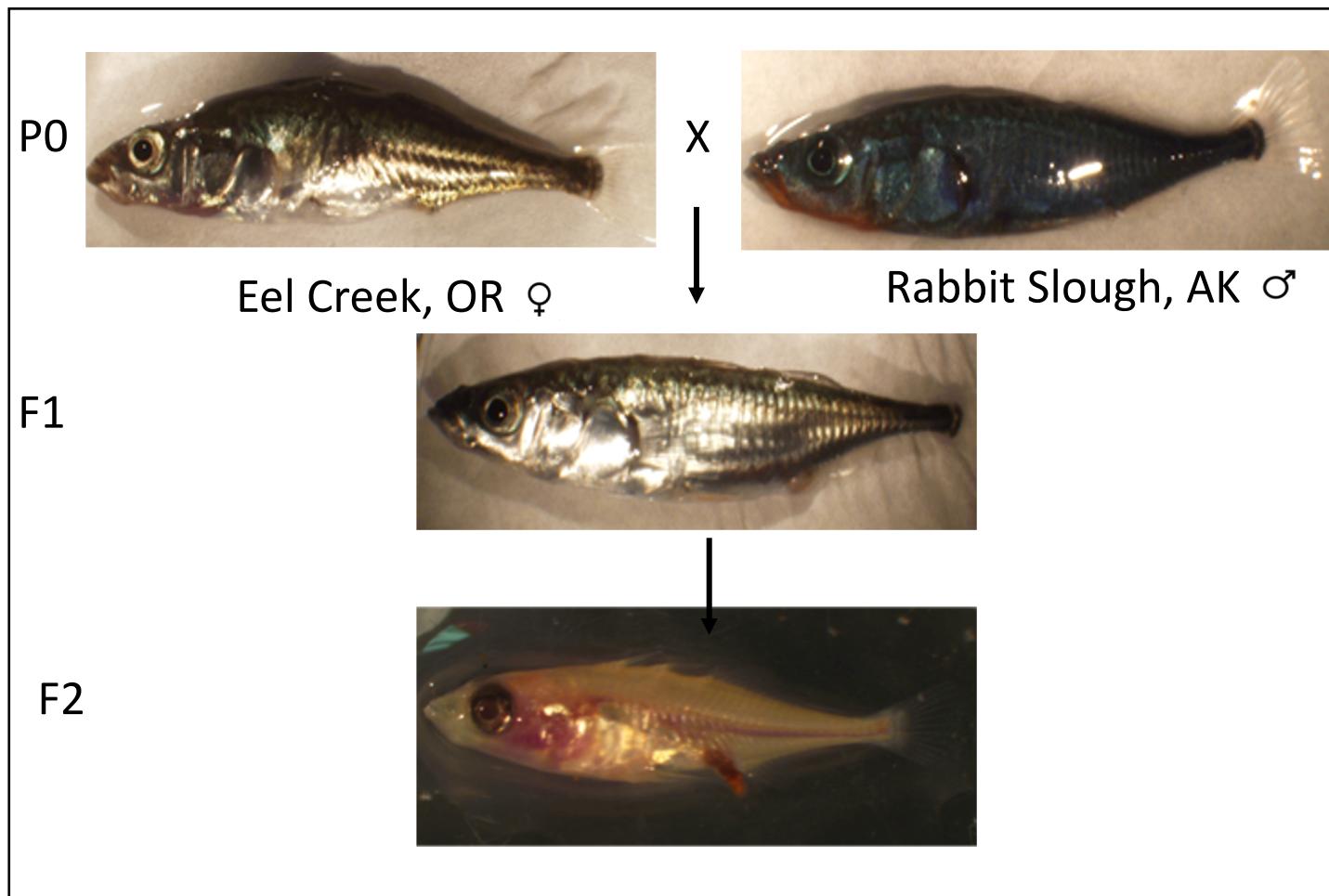
OR

The project I probably should not have started

I was making some mapping families...



I was making some mapping families...



Parents and F1s appeared wildtype

♂

♀



F1: ~30 fish 0/30 mutant

F2: 226 fish 57/226 mutant

25.2%

(pause for gasps from the crowd)

Think back to early days of genetics

Could this be a single gene???

R – dominant wildtype allele

r – recessive mutant allele

	R	r
R	RR Normal; Not a Carrier	Rr Carrier
r	Rr Carrier	rr Has Disease

Thought Experiment!

- (1) Start with the parents
- (2) Both parents were wildtype so they could be RR or Rr
- (3) If both were Rr 25% of my F1s would have been mutants
- (4) Both Parent NOT heterozygous Rr
- (5) If one parent is RR and the other is Rr all F1s would be wildtype
- (6) Ok...possible
- (7) If parents are RR x Rr; 50% of F1s would be carriers
- (8) If I selected two F1 hets (Rr) to make my F2 family, 25% of my F2 offspring would be mutant!!!!
- (9) What is my next experiment?

Parents and F1s appeared wildtype

♂

♀



F1: ~30 fish 0/30 mutant

F2: 226 fish 57/226 mutant

25.2%

What happens in the F3 generation?

If there are multiple genes underlying the phenotype I will likely see a mixture of mutants/wildtype F3s

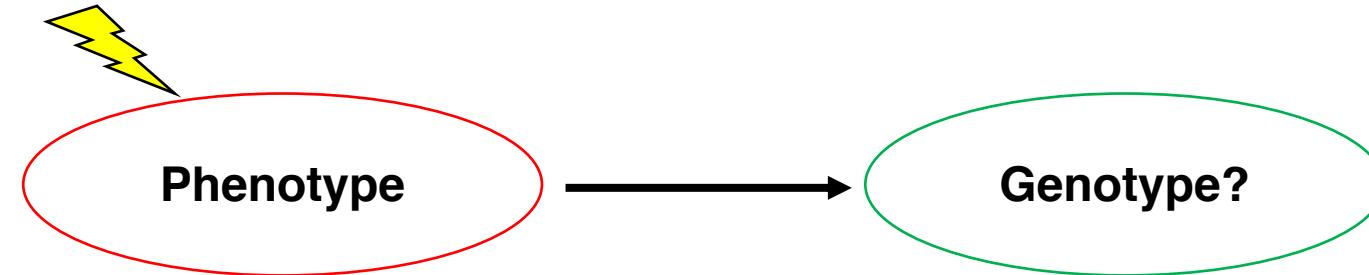
If it is a single gene $rr \times rr$ 100% of F3s will be mutant

F3: 100% mutant

F4: 100% mutant

What is the gene?

Forward Genetic
Mutant



We already have a mapping family! Let's do RAD-seq and QTL mapping!

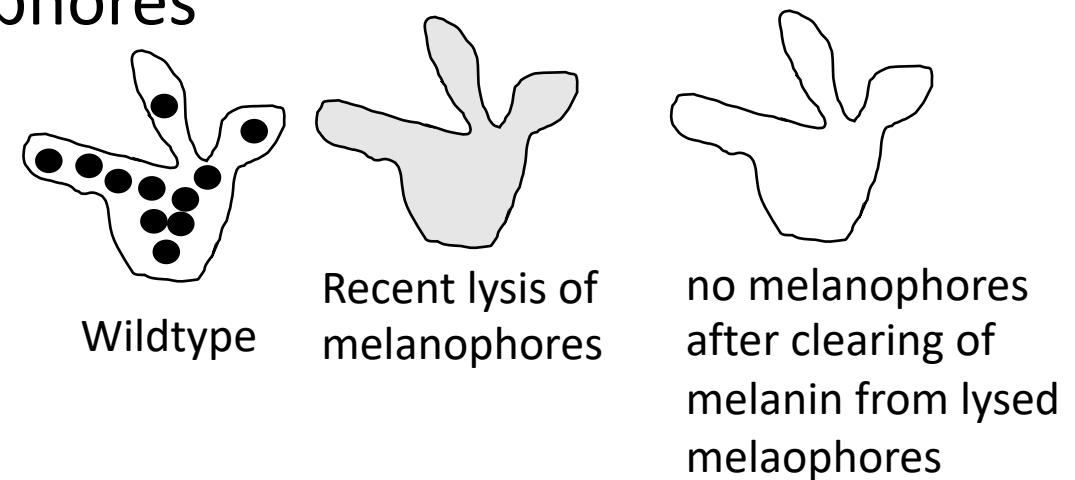
But this isn't a complex quantitative trait!
So what?!

Darn you linkage!!!!



190 F2s + 2 parents
30,595 SNPs

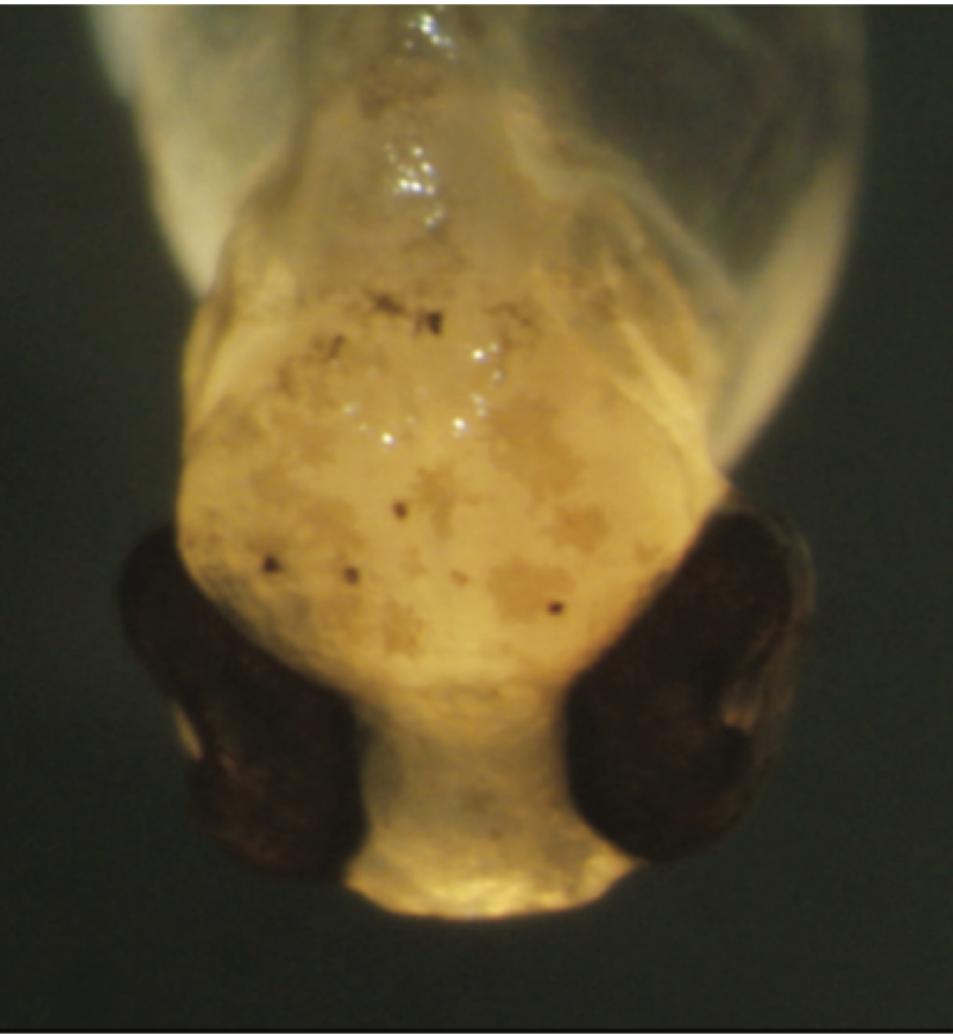
Appears to be a problem with endosome trafficking leading to death of lysosome-related organelles including melanophores



If endosome trafficking is responsible, melanocytes should be wildtype but devoid of melanophores



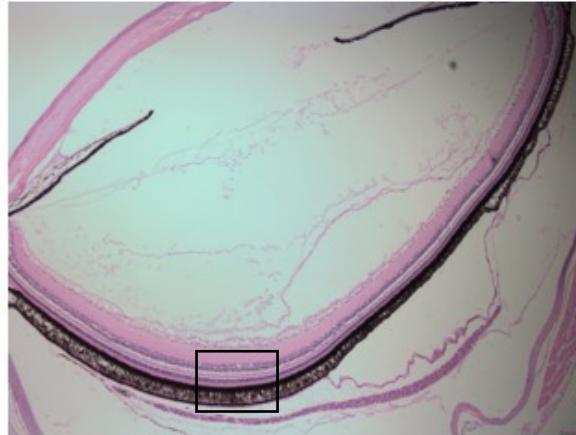
wildtype



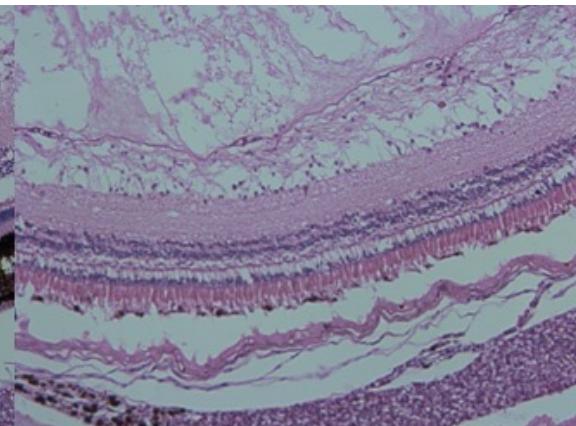
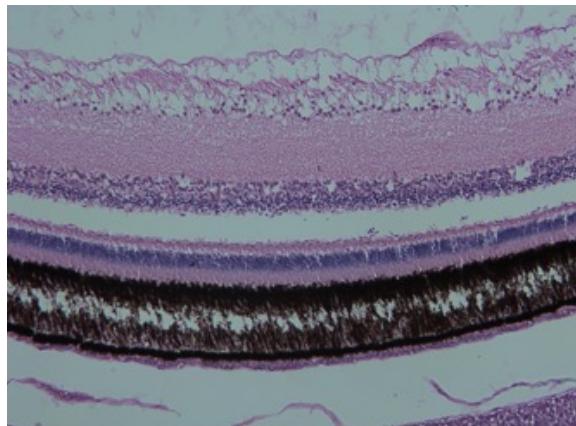
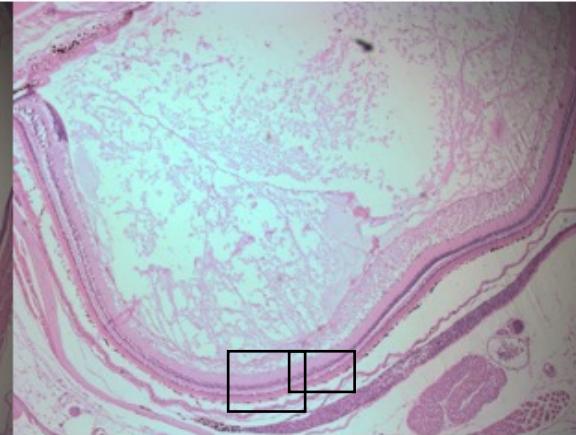
mutant

By Adulthood the pigment in the eye is gone as well

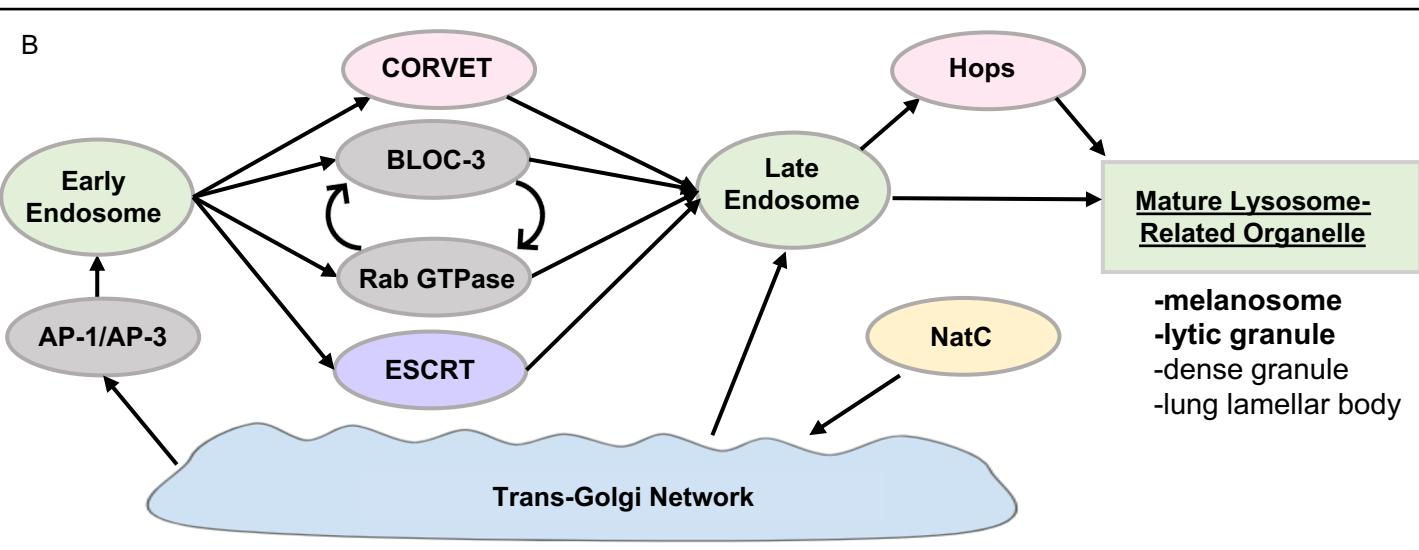
Wildtype



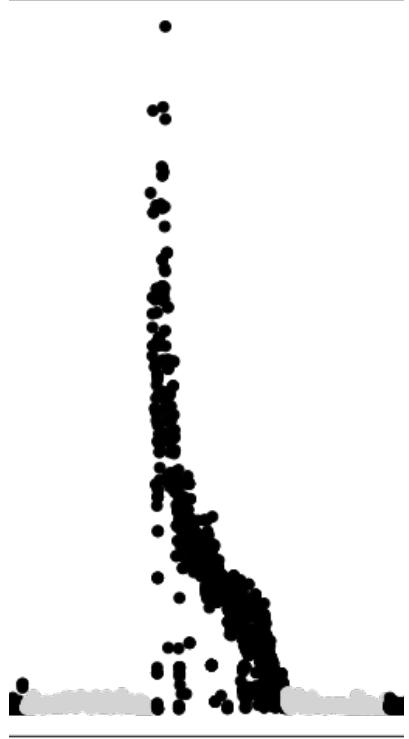
Mutant



But what is the gene?!?!?!



SNX18 AP-1 complex top 3 SNPs as boundaries



Gene Name	Genomic Position	Endosome-Lysosome Transport
GOLPH3	groupXIII:1,388,883-1,391,798	Rab Associated Protein (Rab GTPases)
VPS37B	groupXIII:2,950,458-2,959,985	Endosomal Sorting Complexes (ESCRT-I)
AP3S1	groupXIII:4,627,086-4,635,678	Adaptor Complex Protein (AP-3)
NAA35	groupXIII:7,397,696-7,405,305	N-acetyl Transferase Complex C (NatC)
RILPL1	groupXIII:8,293,913-8,298,787	Rab Associated Protein (Rab GTPases)
VPS33A	groupXIII:12,875,214-12,880,198	Tethering Complex (CORVET/Hops)
MVB12BB	groupXIII:13,673,988-13,697,933	Endosomal Sorting Complexes (ESCRT-I)
CHMP7	groupXIII:14,423,967-14,427,196	Endosomal Sorting Complexes (ESCRT-III)
HPS4	groupXIII:14,717,817-14,721,920	Biogenesis Lysosome-related Organelle Complex (BLOC-3)
AP3M2	groupXIII:15,975,063-15,980,229	Adaptor Complex Protein (AP-3)
STAMBP	groupXIII:18,916,597-18,920,696	STAM binding protein (Other)

Currently sanger sequencing endosome trafficking genes in this region (there are 11 genes candidates STAY TUNED)

SNX18 regulates ATG9A trafficking from recycling endosomes by recruiting Dynamin-2

Kristiane Søreng,¹ Michael J Munson,¹ Christopher A Lamb,² Gunnveig T Bjørndal,¹ Serhiy Pankiv,¹ Sven R Carlsson,³ Sharon A Tooze,² and Anne Simonsen¹

Autophagic Punctum

SNX18 tubulates recycling endosomes for autophagosome biogenesis

Helene Knævelsrud, Sven R Carlsson & Anne Simonsen 

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 Download citation  <https://doi.org/10.4161/auto.26124>

[J Cell Biol.](#) 2009 Oct 19; 187(2): 247–264.

PMCID: PMC2768840

doi: [10.1083/jcb.200907122](https://doi.org/10.1083/jcb.200907122)

PMID: 19841138

Article

AP-1 and KIF13A coordinate endosomal sorting and positioning during melanosome biogenesis

Cédric Delevoye,^{1,4} Ilse Hurbain,^{1,2,4} Danièle Tenza,^{1,2,4} Jean-Baptiste Sibarita,^{2,4} Stéphanie Uzan-Gafso,^{3,4} Hiroshi Ohno,⁵ Willie J.C. Geerts,⁶ Arie J. Verkleij,⁶ Jean Salamero,^{2,3,4} Michael S. Marks,⁷ and Graça Raposo^{1,2,4}

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/5441946>

SNX18 is an SNX9 paralog that acts as a membrane tubulator in AP-1-positive endosomal trafficking

Article in [Journal of Cell Science](#) · June 2008

DOI: 10.1242/jcs.028530 · Source: PubMed

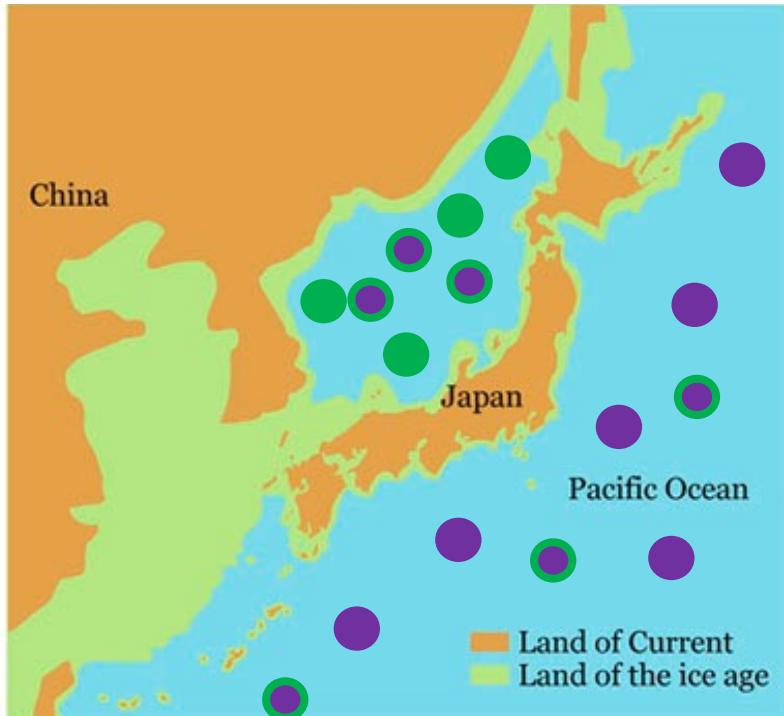
 Free Access

SNX-BAR-Mediated Endosome Tubulation is Co-ordinated with Endosome Maturation

Jan R.T. van Weering, Paul Verkade, Peter J. Cullen 

First published: 04 October 2011 | <https://doi.org/10.1111/j.1600-0854.2011.01297.x> | Citations: 101

What does this have to do with stickleback mitochondria?



There are many regions of mitochondrial admixture

Mitotypes are segregating within the population.

Can we map nuclear loci that are disproportionately present with one mitotype vs another?

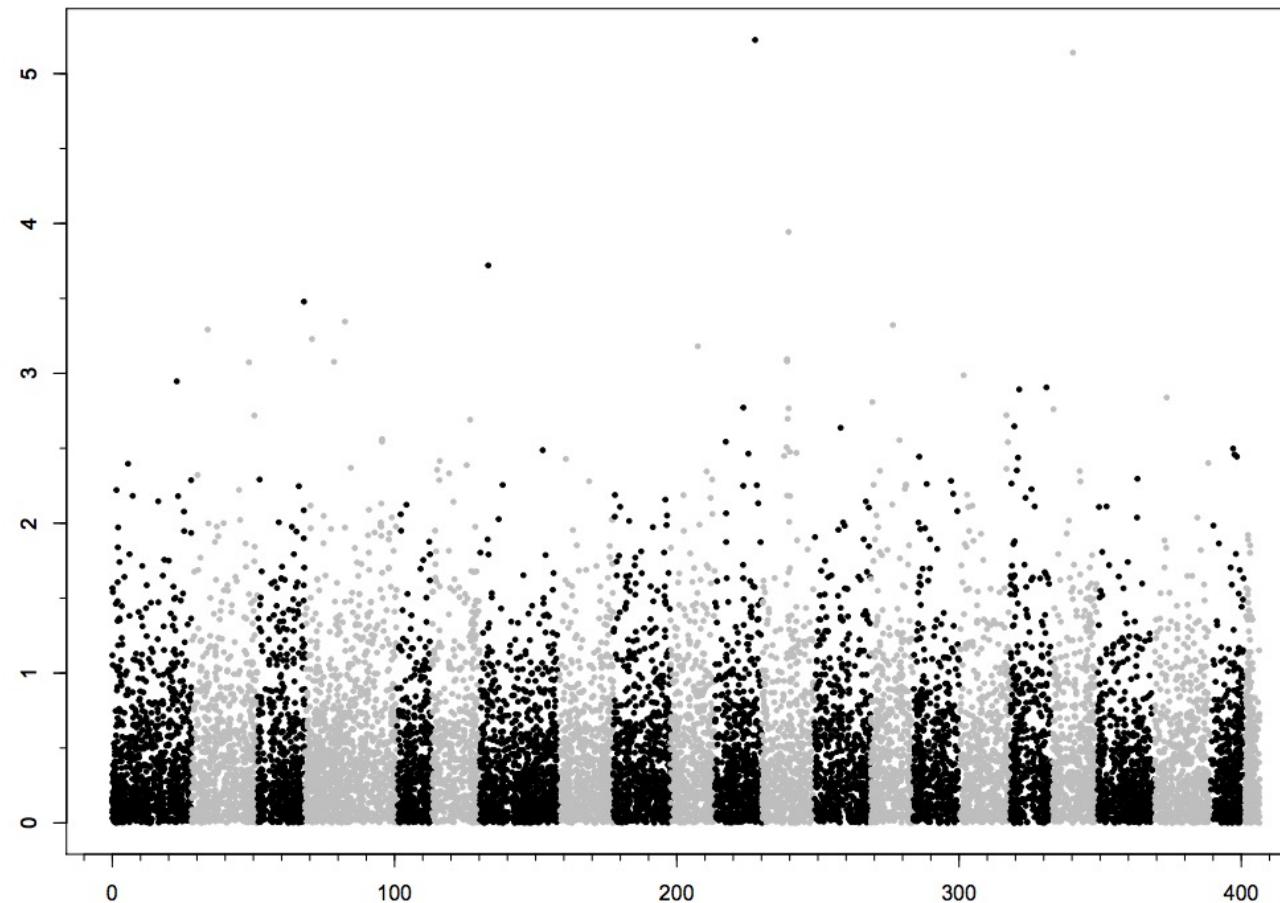
Should we use GWAS or QTL Mapping?

Why not both??

GWAS using RAD-seq from Middleton Island (admixture of TNP and ENA fish)

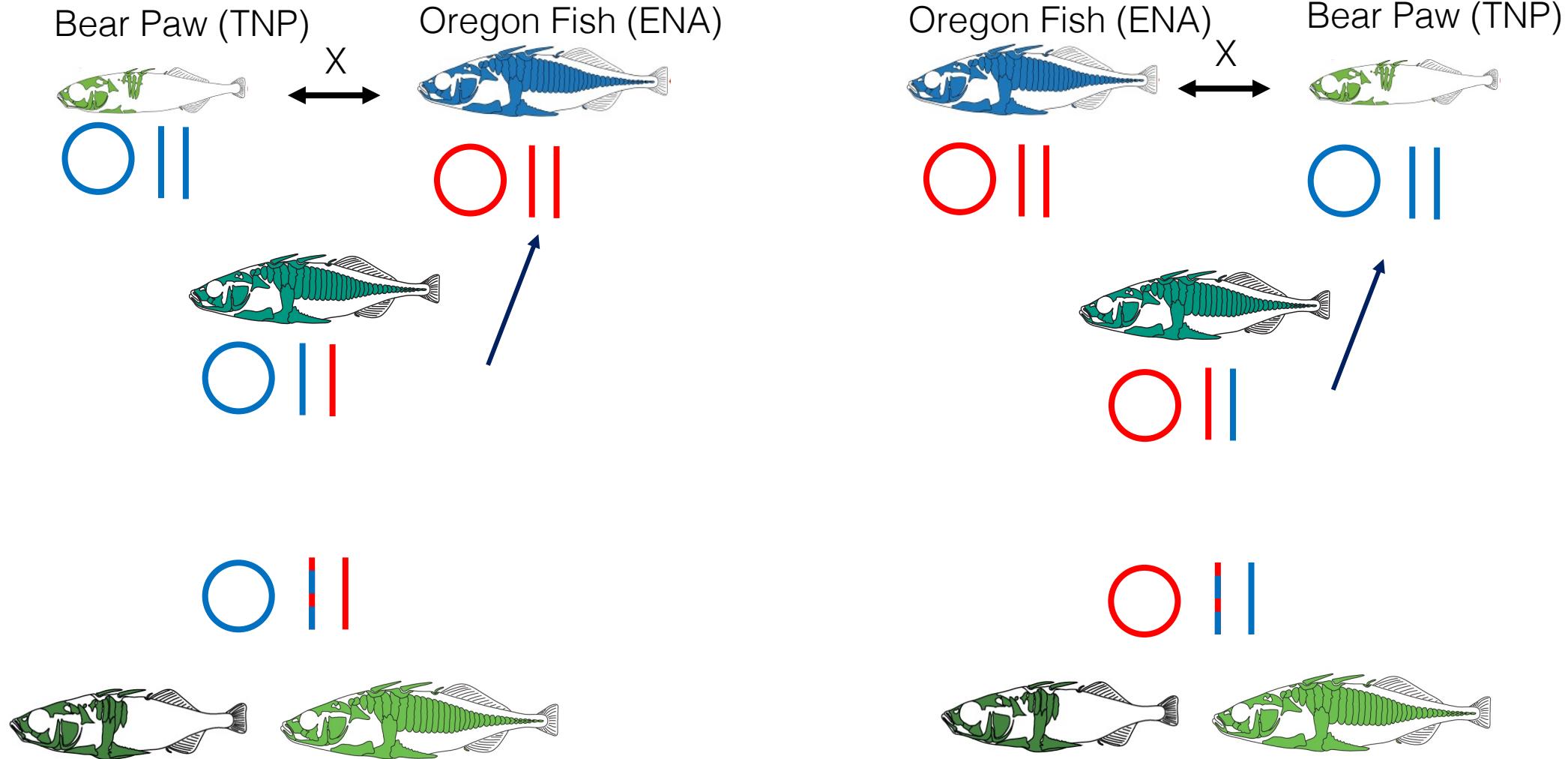
We now have RAD-seq data for a population that is 80% ENA and 20% TNP mitotype. All fish have been mitotyped.

Less than 100 individuals



Can we create another type of QTL Mapping Family

Best option for incompatibility : F1 backcross



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Caitlin Smith

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