GWAS analysis for resistance against enteric septicemia of catfish using the first-generation interspecific backcrosses

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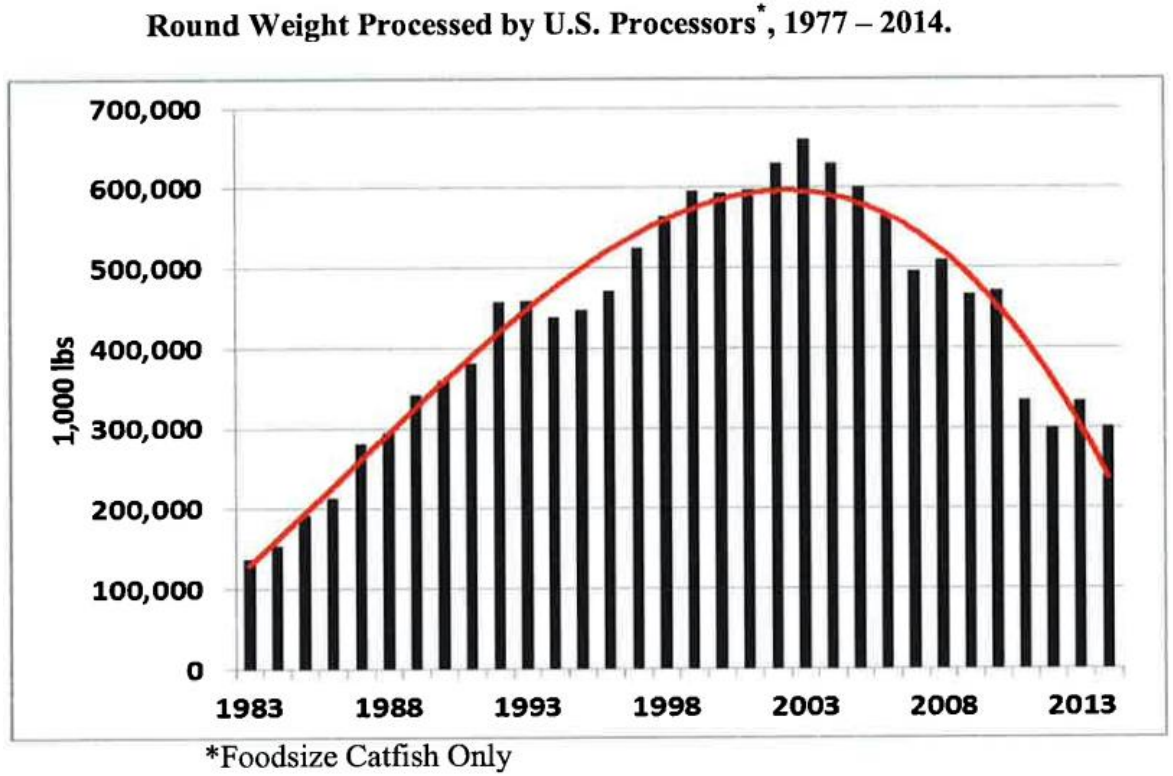
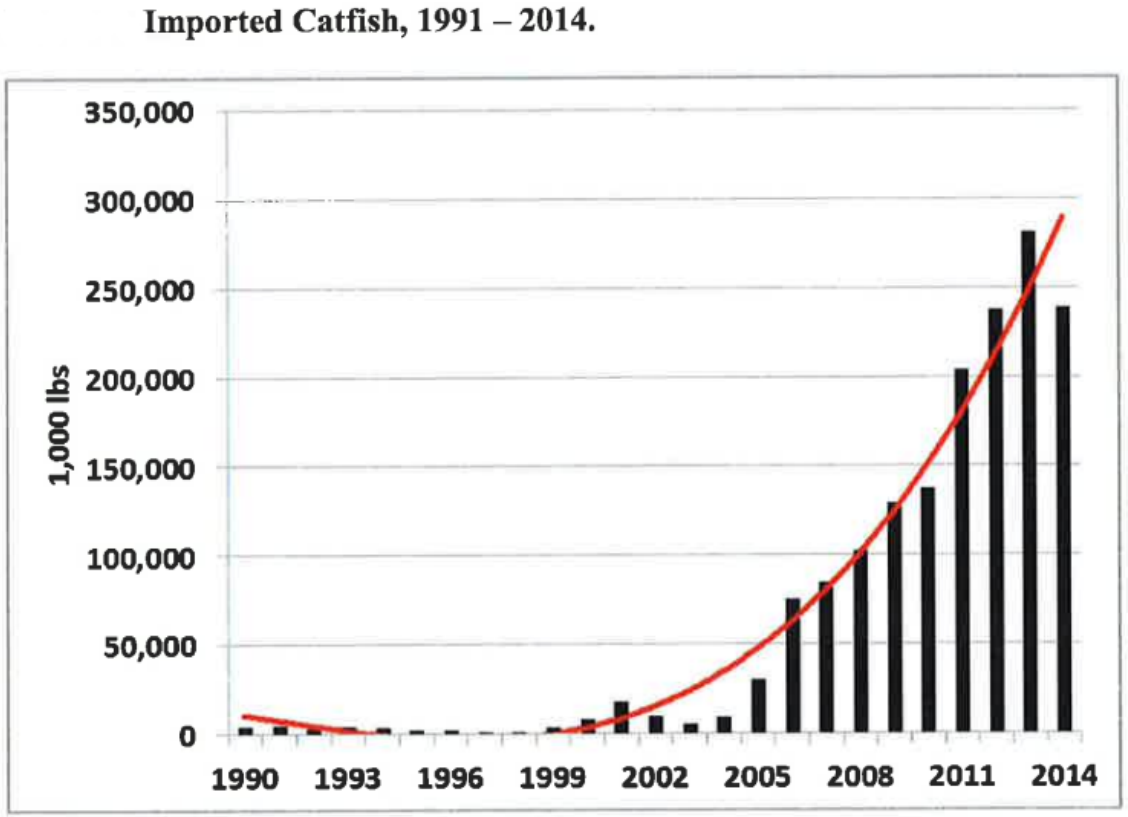
Introduction - Catfish

- Catfish can survive in a wide range of freshwater habitats such as lakes, rivers, and streams.
- Channel catfish, blue catfish, black bullhead, brown bullhead, flathead catfish, white catfish, yellow bullhead
- Catfish industry is the largest aquaculture industry in the United States, accounting for over 50% of all US aquaculture production.

Introduction - Catfish



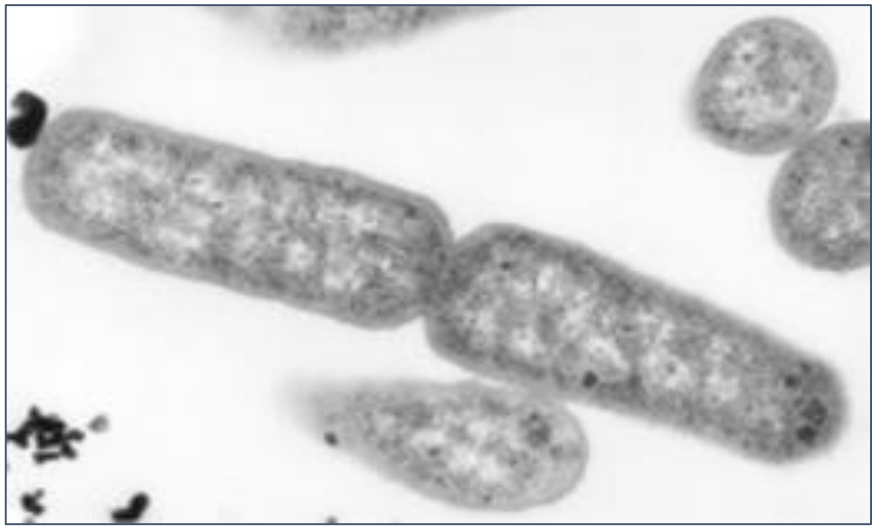
- increased feed and fuel costs
- international competition
- devastating diseases



Source: http://www.agecon.msstate.edu/whatwedo/budgets/docs/catfish2014.pdf

Introduction - ESC

- **Wide distribution**: in all catfish producing areas in the world, and in the US, mostly Mississippi, Alabama, Arkansas, and Louisiana
- **Huge losses**: $40-60 million annually



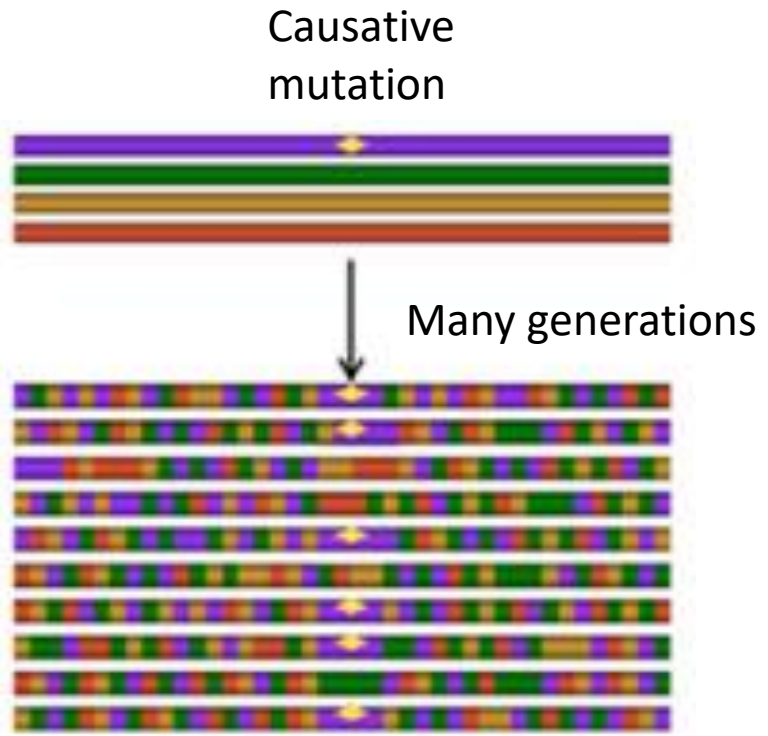
Transmission electron micrograph
Edwardsiella ictaluri

Symptoms including hole in the head

Introduction - GWAS

- GWAS can link genotype and phenotype, which examines a genome-wide set of genetic variants in individuals to find variants that associate with a phenotype of interest.

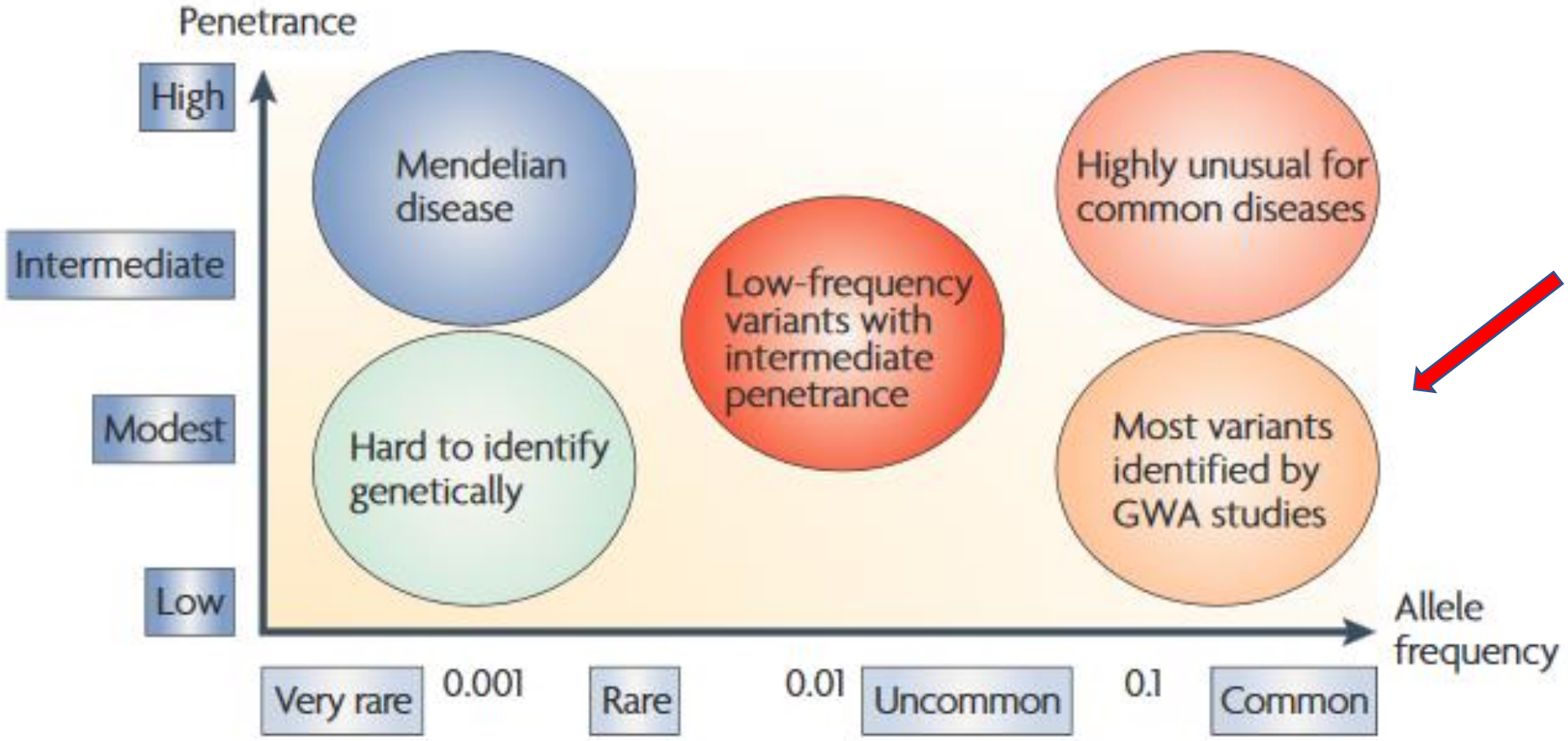
- GWAS is based upon the principle of linkage disequilibrium (LD) which is the nonrandom association between alleles at different loci.



(Zhu et al. 2008)

Introduction - GWAS

Common disease/common variant hypothesis



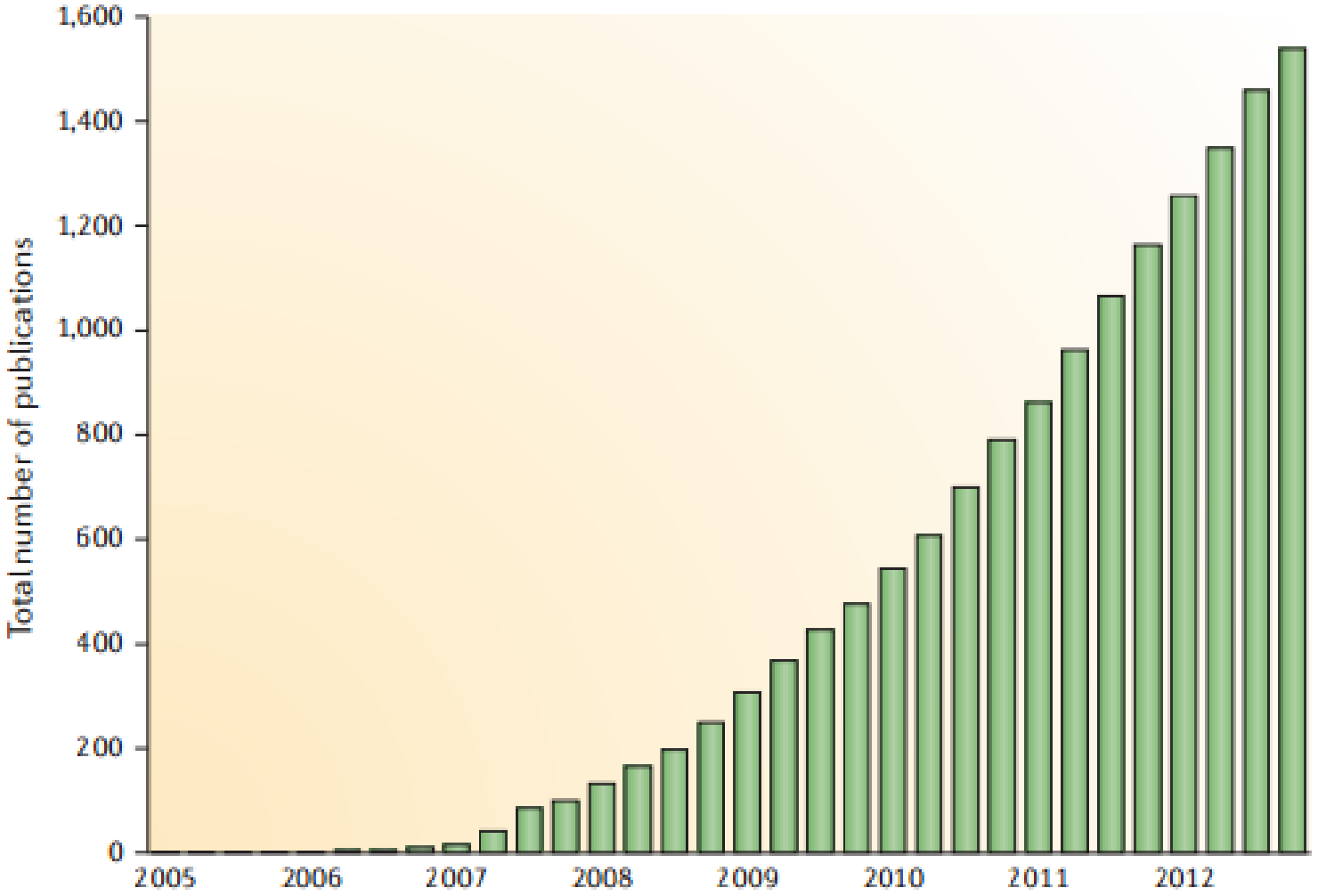
McCarthy et al. 2008

Introduction - GWAS

The first successful GWAS was reported in 2005. (Klein et al.)

96 cases
50 controls

Found a common intron variant associated with age-related macular degeneration



Pace of GWAS publications since 2005
(Manolio 2013)

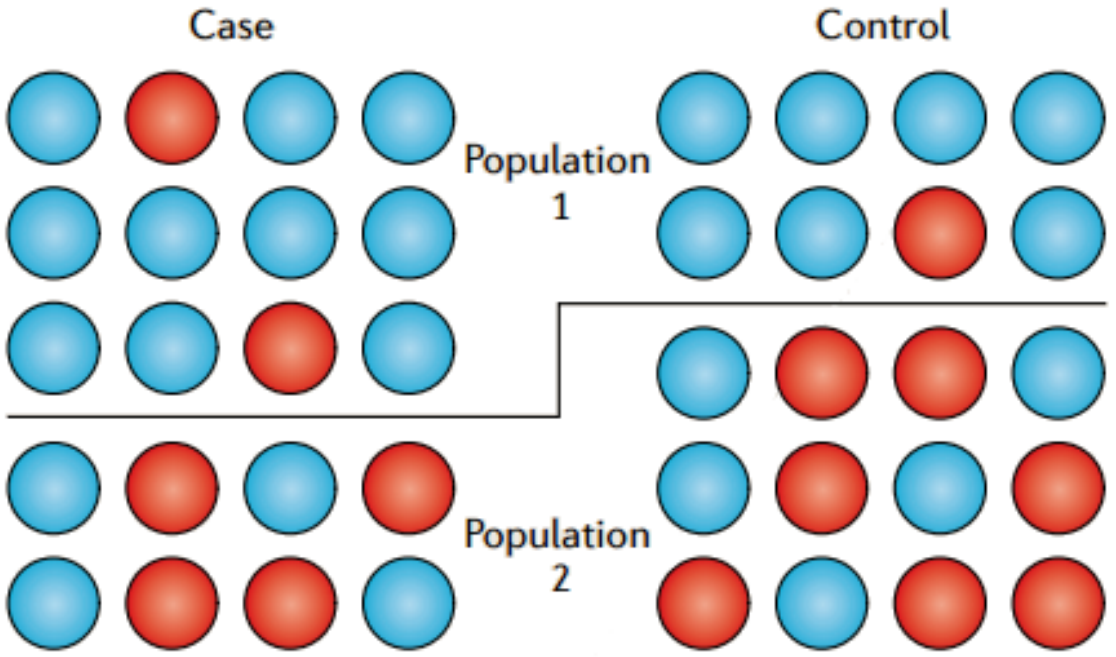
Introduction - GWAS

Advantages:

- The association mapping has high resolution.
- No pedigree information required

Disadvantages:

- Expensive
- Genotyping error
- Susceptible to population stratification



Balding 2006

Objectives

- Identification of quantitative trait locus (QTL) and SNPs associated with ESC resistance at species level

- Identification of potential candidate genes and pathways controlling ESC resistance

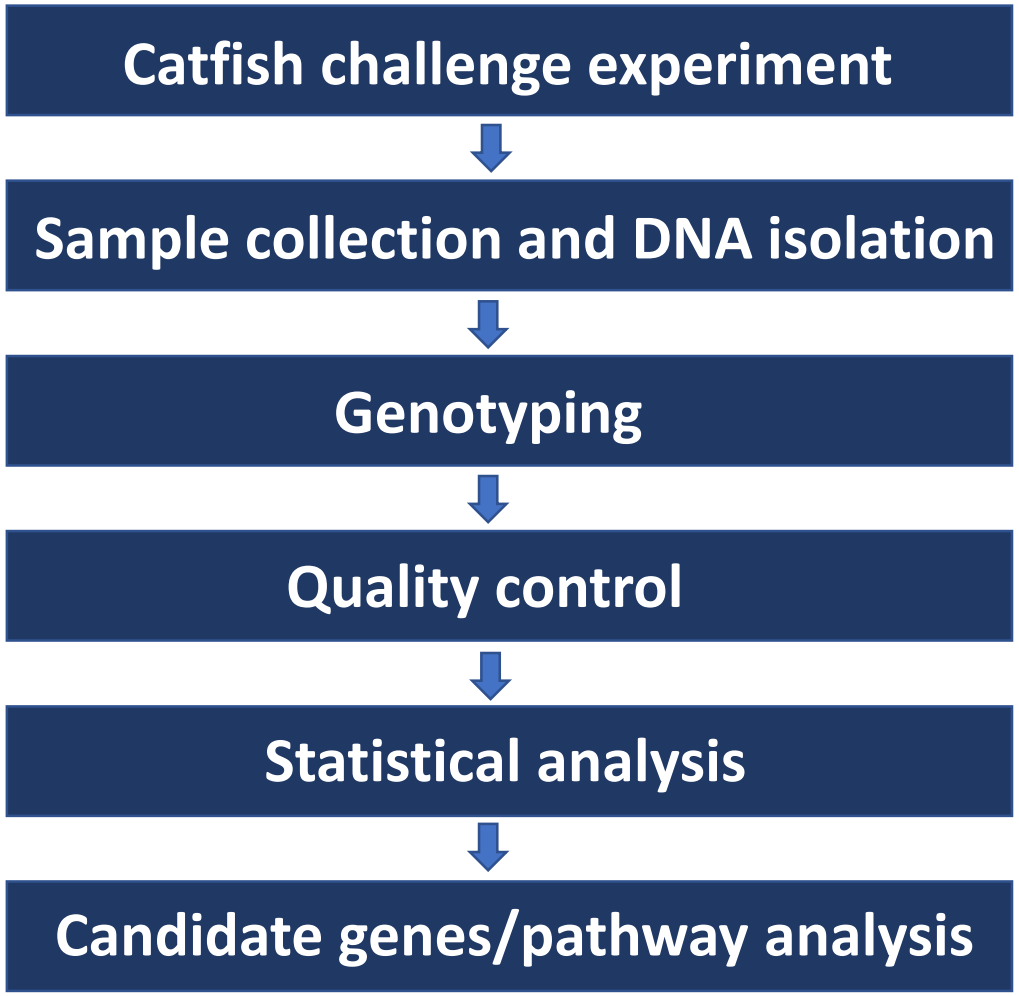
Resources Required for GWAS in this study

- ✓ Catfish population
- ✓ Development of the catfish 690K SNP array (Zeng et al., 2017)
- ✓ Powerful statistical tools: SVS software packages, PLINK, etc.

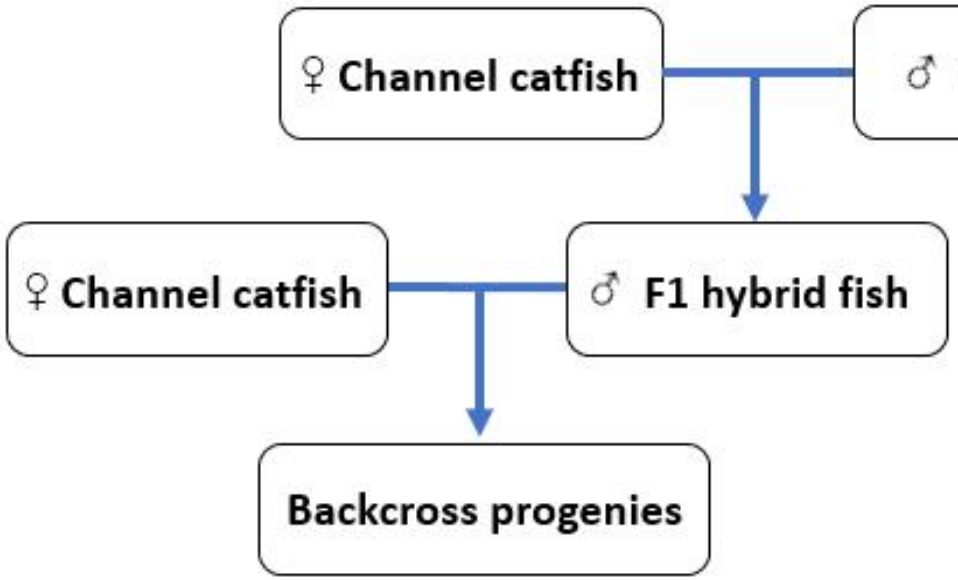
GOLDEN HELIX SNP & VARIATION SUITE

plink...

Flowchart


Catfish challenge experiment
Sample collection and DNA isolation
Genotyping
Quality control
Statistical analysis
Candidate genes/pathway analysis

Experiment design



Selective genotyping
288 phenotypic extremes in three 690k catfish SNP arrays

Family ID	Dam	Sire	Sample number	Susceptible sample number	Resistant sample number
1	Channel 1	Hybrid 1	71	36	35
2	Channel 2	Hybrid 2	70	34	36
3	Channel 3	Hybrid 3	70	36	34
4	Channel 4	Hybrid 4	77	36	41

Quality control and LD pruning

- Sample quality control

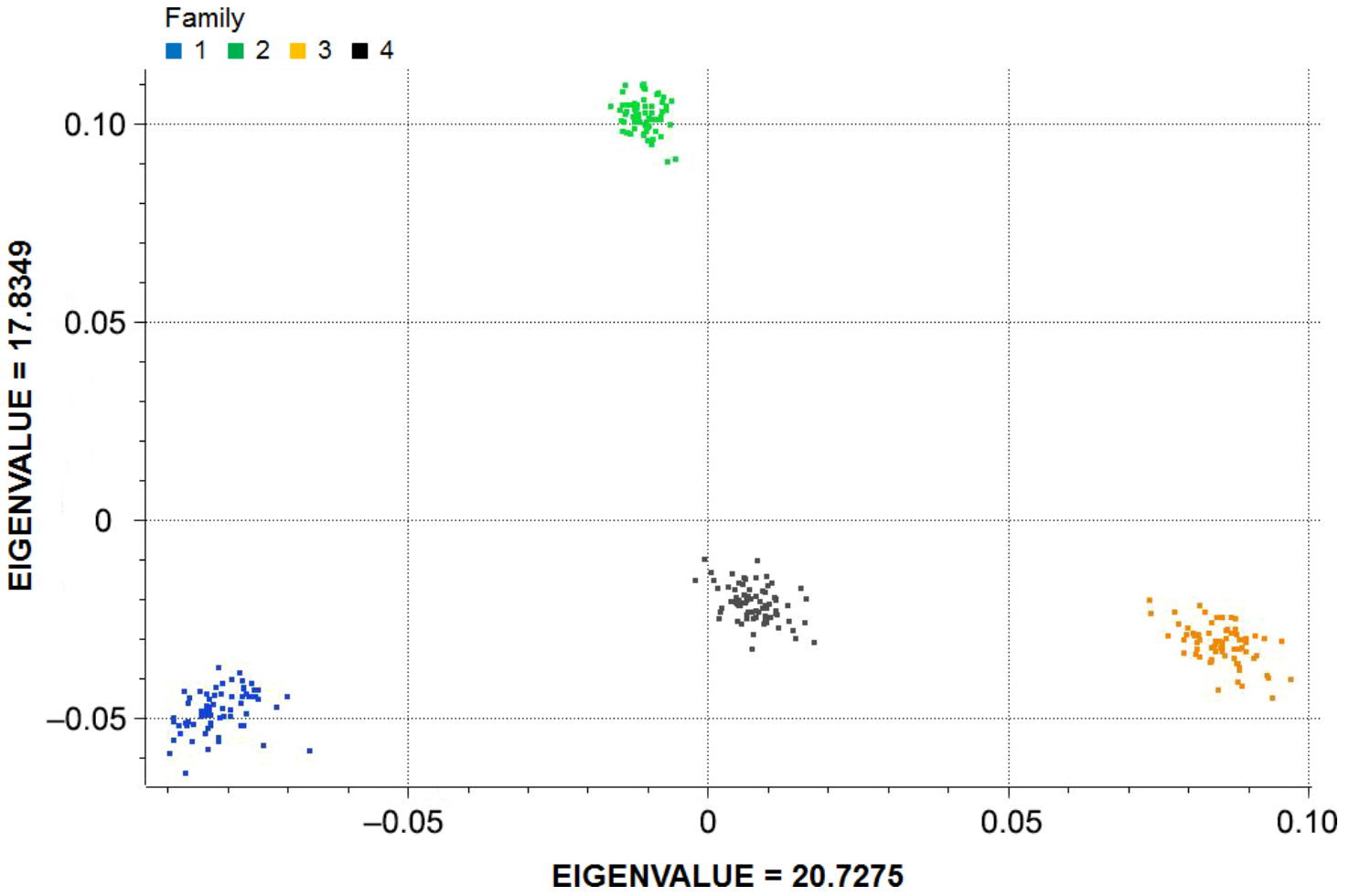
  No sample with genotype missingness > 5%
- SNP quality control

  Excluded SNPs with a minor allele frequency (MAF) < 0.05 or a call rate < 95%

- LD pruning was conducted to achieve a set of independent SNPs and LD blocks.
- LD pruning is a good practice prior to IBS analysis and PCA analysis which may be biased by large blocks of redundant SNPs.

PCA analysis

- Each dot represents one individual.
- Each family was grouped into a separate cluster.



Statistical analysis (EMMAX and QFAM)

- EMMAX (Efficient Mixed-Model Association eXpedited) analyses

$$Y = X\beta + Zu + e$$

Where **Y** is the vector of phenotype; **β** is the coefficient vector of fixed effects including first three principle components and fish body weight; **u** is the vector of the random effect, *Var*(u) = $G\sigma_g^2$, where σ_g^2 is the additive genetic variance and G is the genomic kinship matrix using the IBS; **e** is the vector of random residuals; **X** is the matrix of fixed effects and **Z** is the matrix of random additive genetic effects.

- QFAM (Family based association test for quantitative traits)

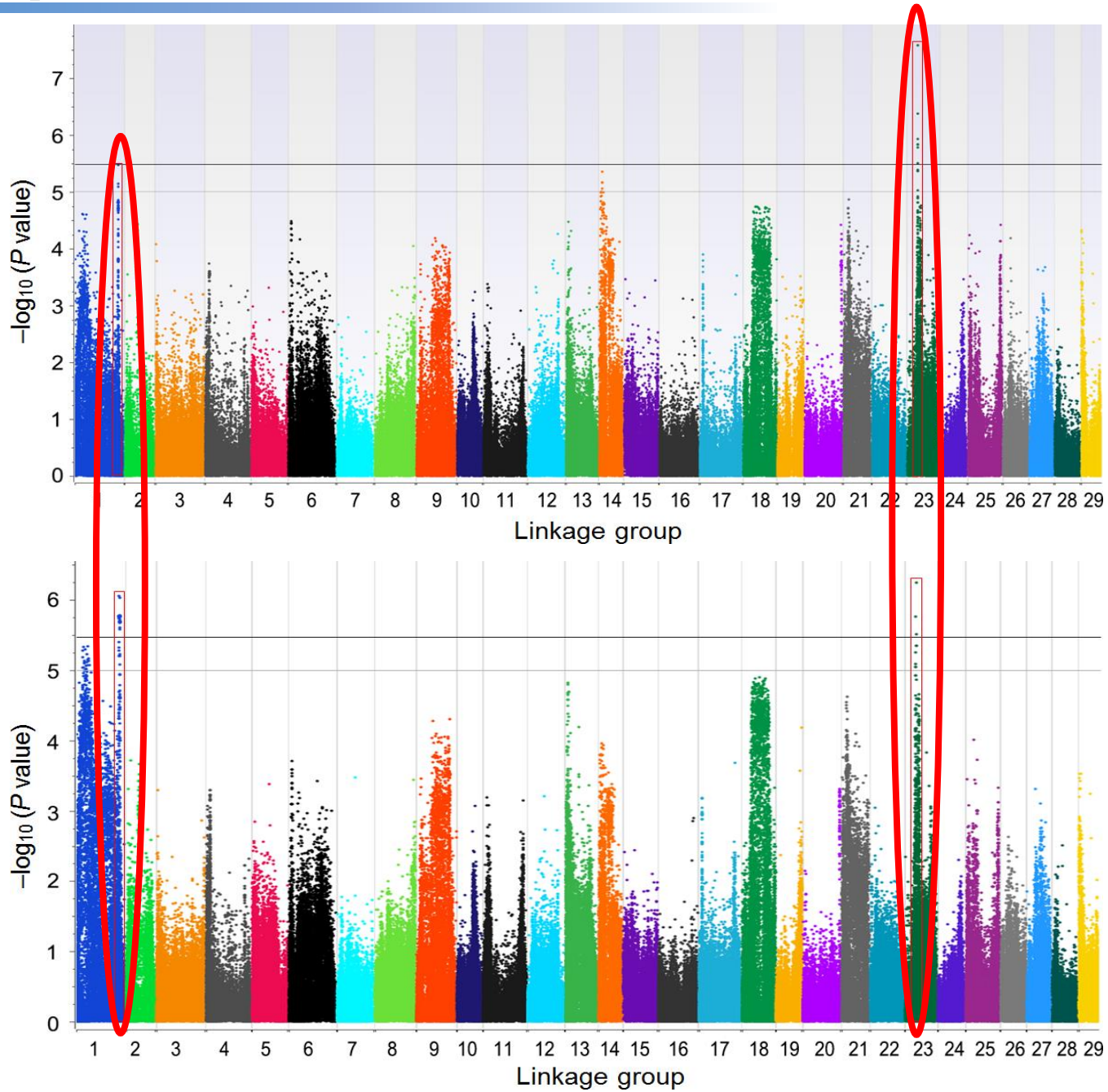
$$\hat{y}_{ij} = \mu + \beta_b b_i + \beta_w w_{ij}$$

QFAM partitions the genotypes into between-family (b) and within-family (w) components. The within-family analysis used in this study is robust to population stratification, which assesses transmission of alleles within a family, but without making use of allelic association observed across families.

Results - Manhattan plot

EMMAX result

QFAM result



Results - SNP

Examination of the associated SNPs reveals superior blue catfish alleles responsible for strong resistance against ESC.

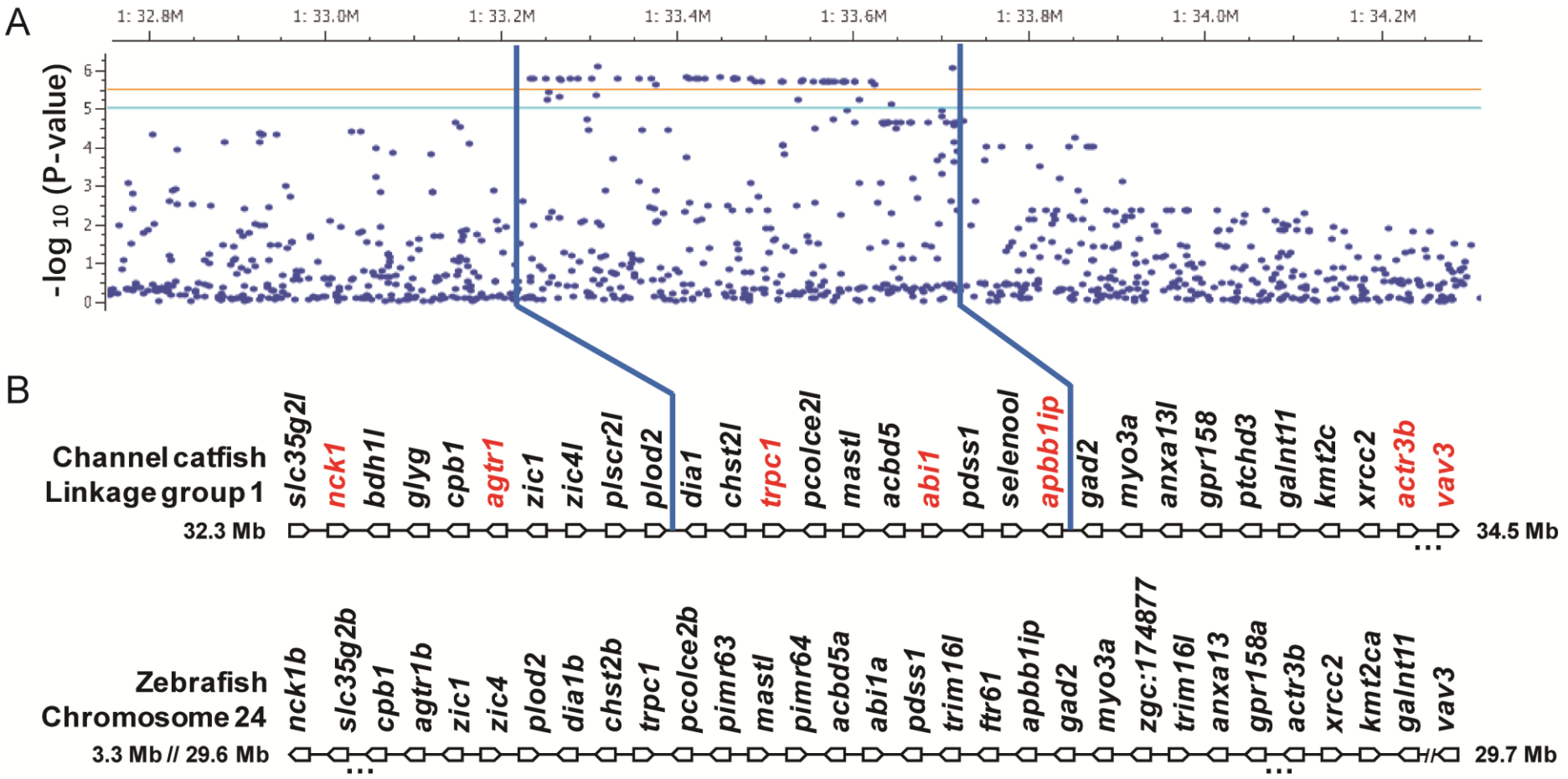
1. the interspecific SNPs on LG1

Example SNP
Channel catfish:
ATATTTATGCAGAAAACAACAAAGCAGAAGTCCTGCCCAAGATGACATTCAGCTTTACTTCTCACTAACCA
Blue catfish:
ATATTTATGCAGAAAACAACAAAGCAGAAGTCCTGACCAAGATGACATTCAGCTTTACTTCTCACTAACCA

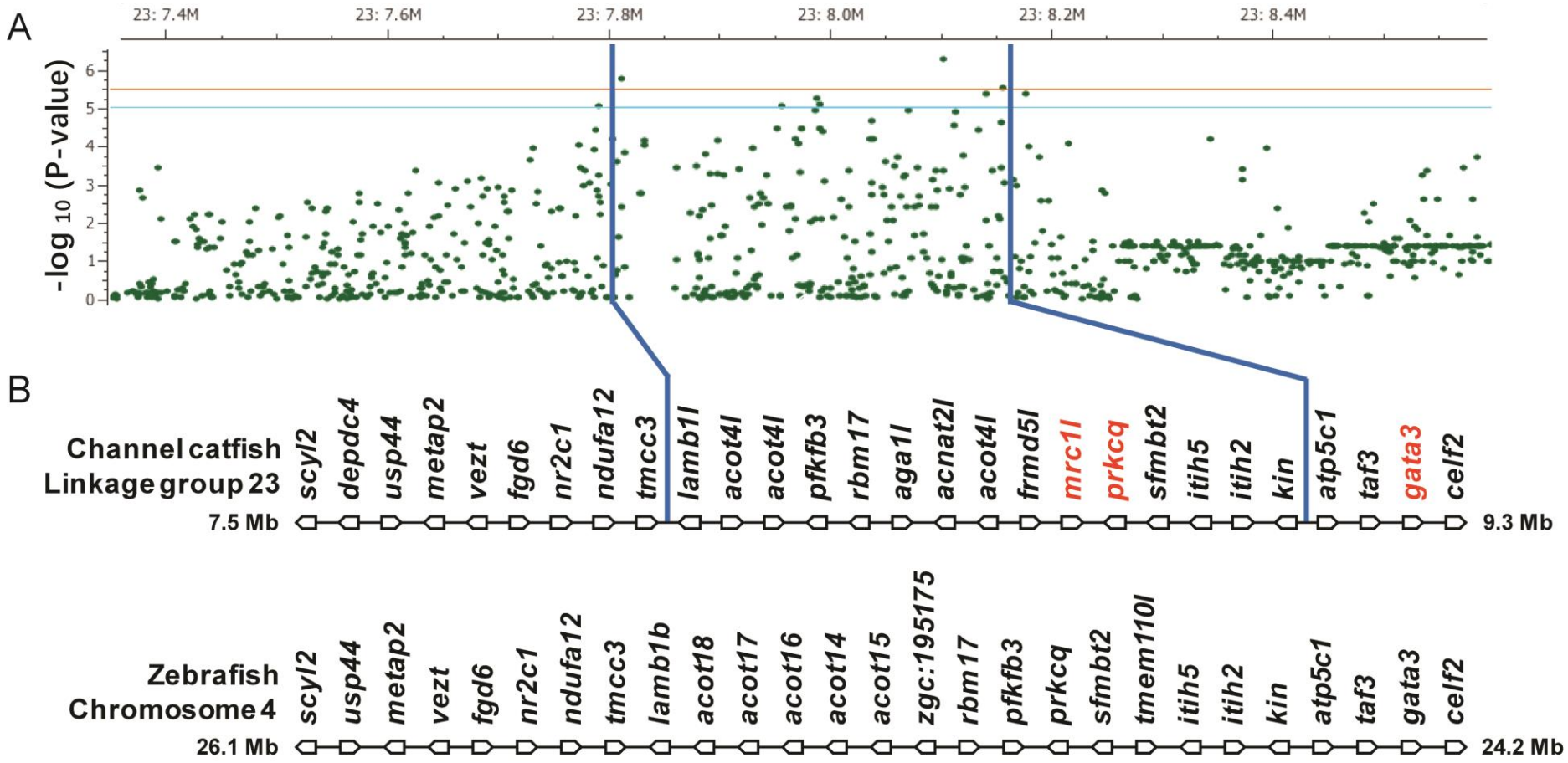
2. channel catfish-specific SNPs on LG23

Example SNP
Channel catfish:
CACATACAACAGAGATAAAACAAATGAGCTTTTACAGATGGGTATATAACACAGGCATGGGCTATGGAGCC
Channel catfish:
CACATACAACAGAGATAAAACAAATGAGCTTTTACGGATGGGTATATAACACAGGCATGGGCTATGGAGCC

Results - Gene



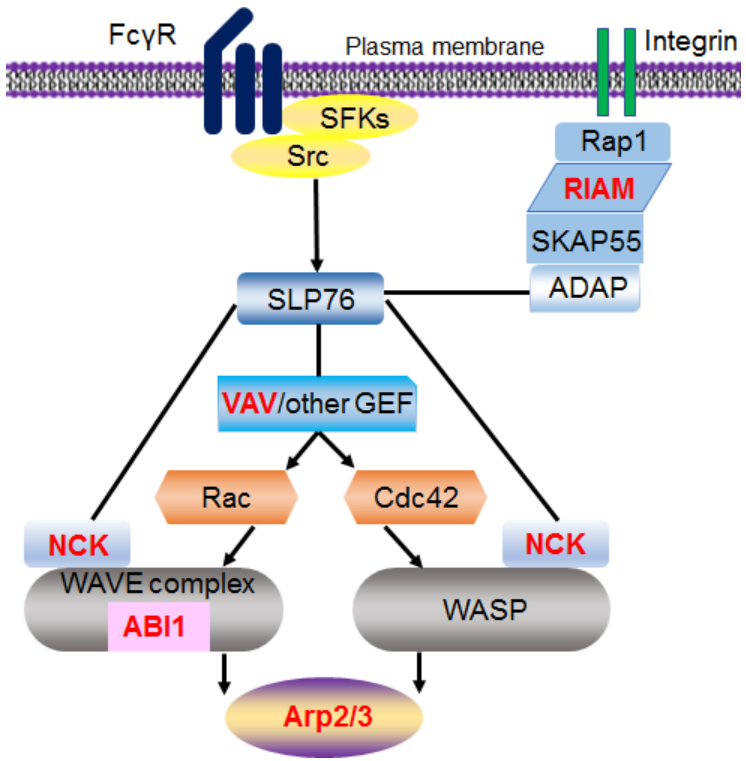
Results - Gene



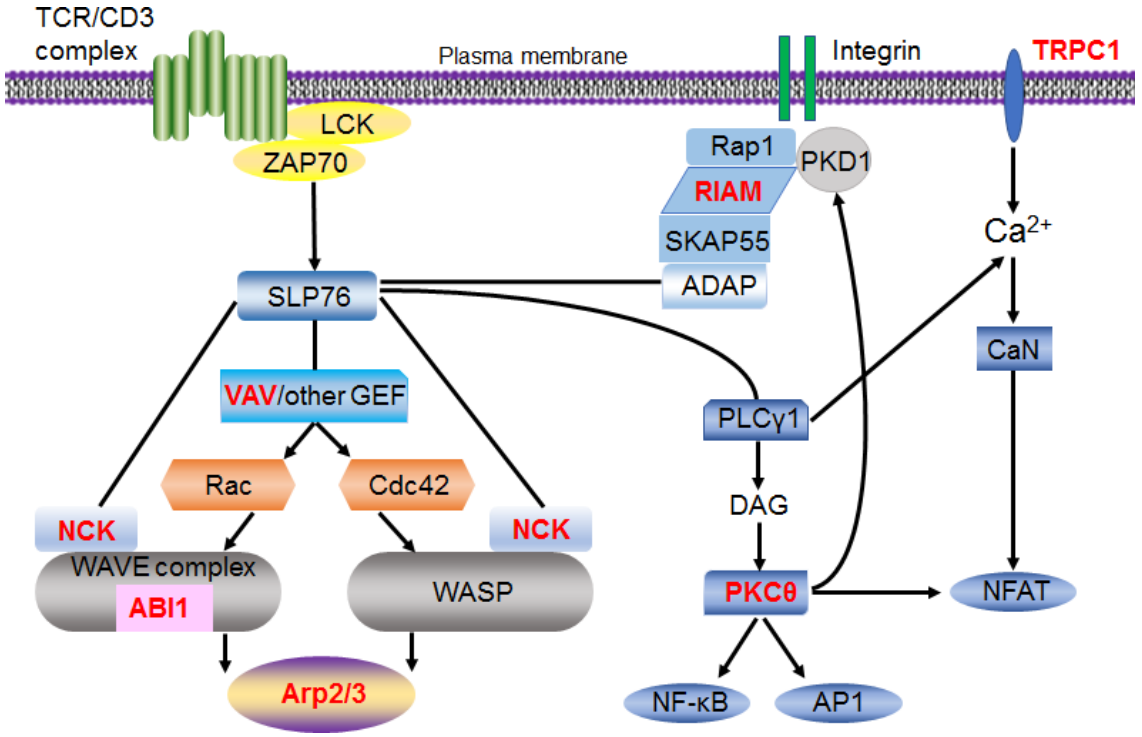
Results - Function

Linkage group	Gene	Location (bp)	Function
1	*nck1*	32,352,283-32,413,183	actin filament organization Phagocytosis T cell activation B cell receptor signaling
	agtr1	32,480,471-32,494,698	regulation of inflammatory response
	trpc1	33,472,942-33,504,891	calcium ion transport B cell receptor signaling
	abi1	33,560,300-33,609,297	actin polymerization or depolymerization Phagocytosis
	apbb1ip	33,632,904-33,677,852	T cell activation
	actr3b	34,277,661-34,297,129	actin nucleation Phagocytosis
	vav3	34,542,282-34,629,025	Phagocytosis B cell receptor signaling
23	*mrc1l*	7,943,812-7,946,175	cellular response to lipopolysaccharide endocytosis T cell activation
	prkcq	7,954,997-7,964,953	inflammatory response T cell activation
	gata3	8,239,408-8,259,340	inflammatory response T cell differentiation humoral immune response

Results - Involved pathway



Phagocytosis



T-cell activation

Conclusions

1. Two significantly associated QTL for ESC resistance were identified on LG1 and LG23.
2. The significant QTL on LG1 is consistent with the finding of previous studies (Zhou et al. 2017), reflecting the power of GWAS.
3. Examination of the associated SNPs revealed superior blue catfish alleles responsible for strong resistance against ESC.
4. The candidate genes were found to be involved in the pathways of phagocytosis and T-cell activation.
5. The positionally related immune genes were functionally related in similar pathways.

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Q and A