



Akvaforsk
Genetics

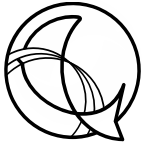
GENOME-WIDE ASSOCIATION STUDY FOR SURVIVAL TO *Streptococcus iniae* AND *S. agalactiae* IN Nile Tilapia (*Oreochromis niloticus*)

Sergio Vela Avitúa; Carlos Lozano; Rama Bangerá; Jose
Ospina; Morten Rye

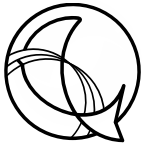
 A Benchmark
Company

ABCC
ASSOCIAÇÃO BRASILEIRA DE CRIADORES DE CAMARÃO

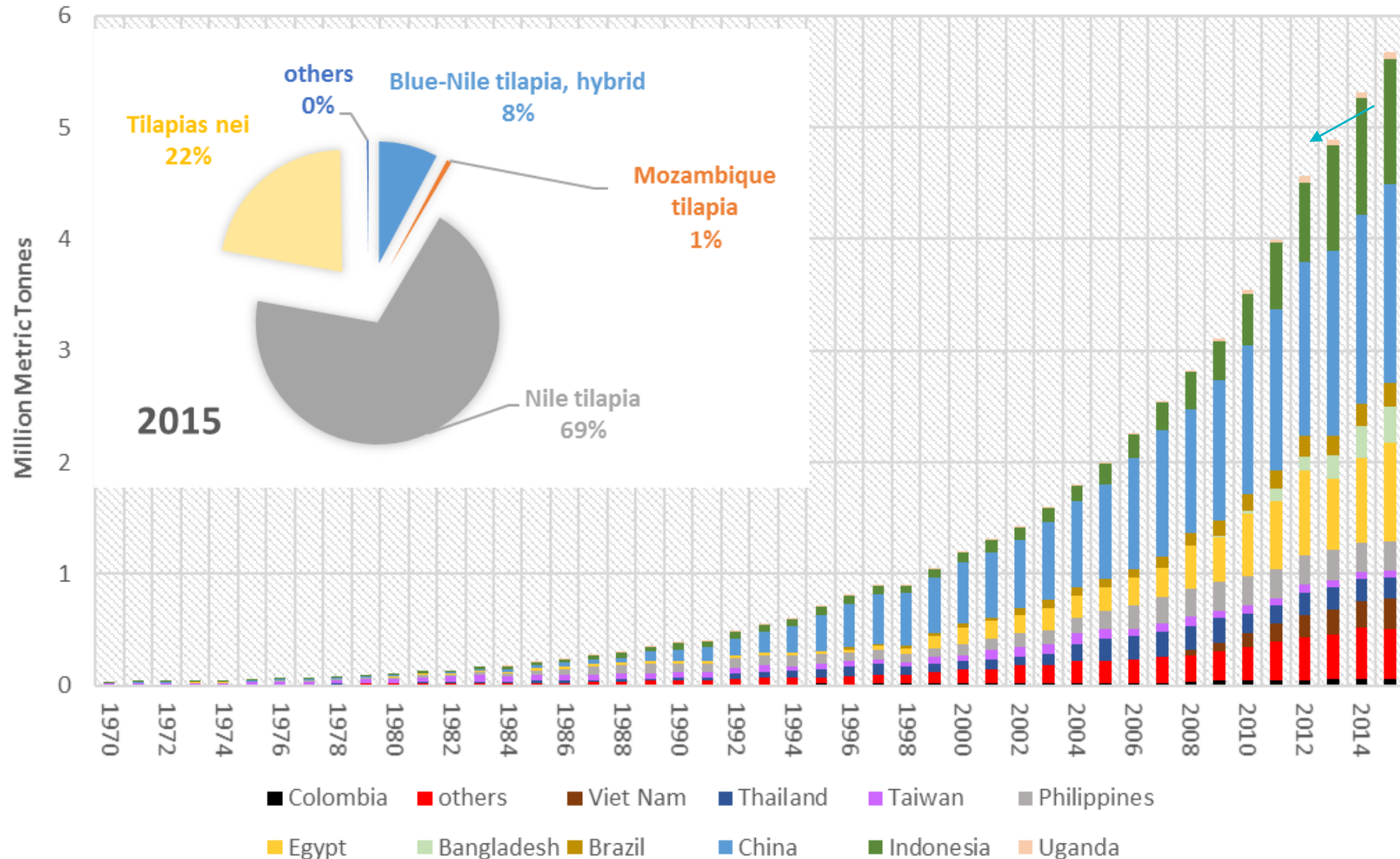




- **INTRODUCTION**
- MATERIAL AND METHODS
- RESULTS
- CONCLUSIONS



Tilapia aquaculture production by country

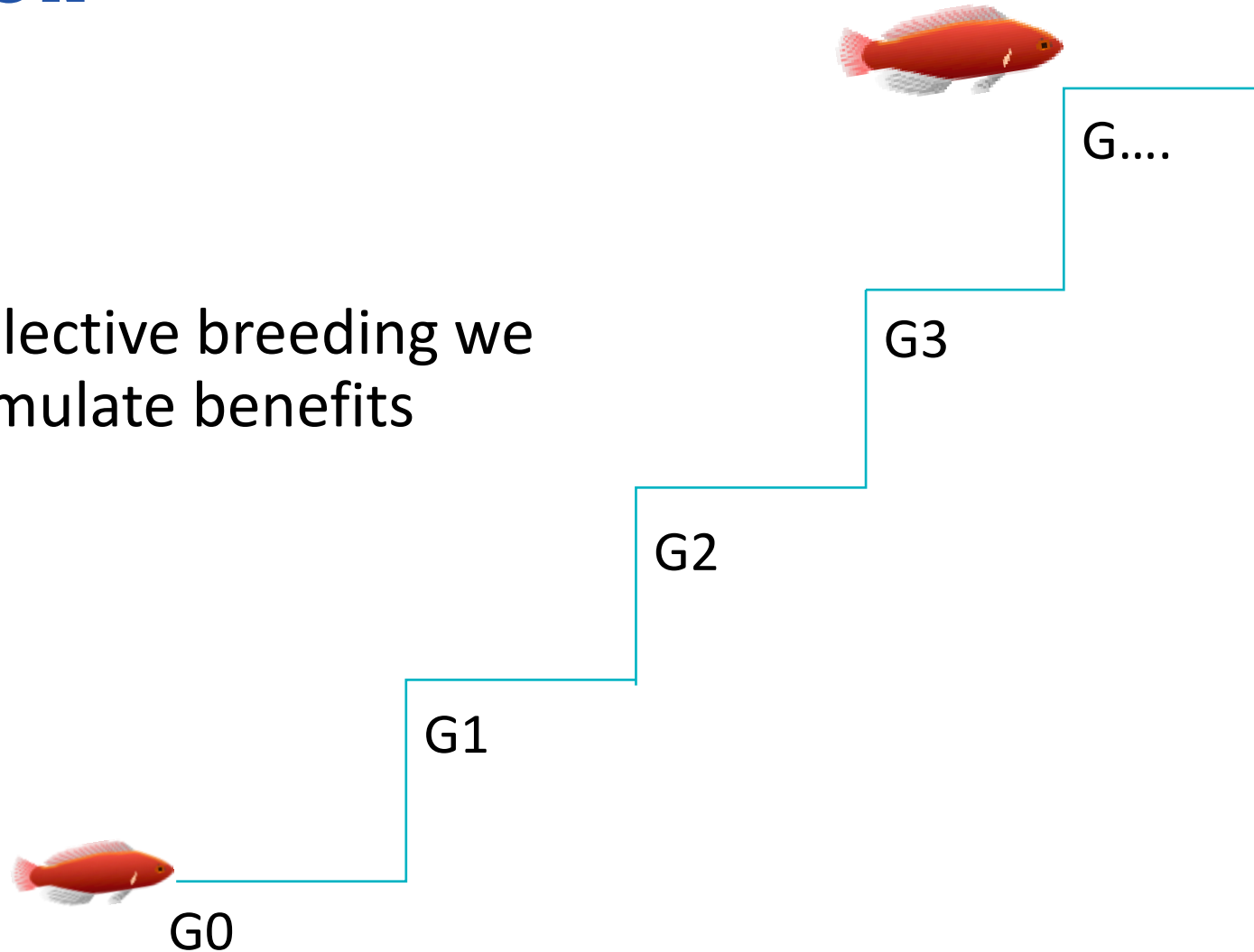


Based on FAO figis dataset (accessed 2017)



ACCUMULATING BENEFITS BY SELECTION

- By selective breeding we accumulate benefits

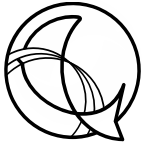




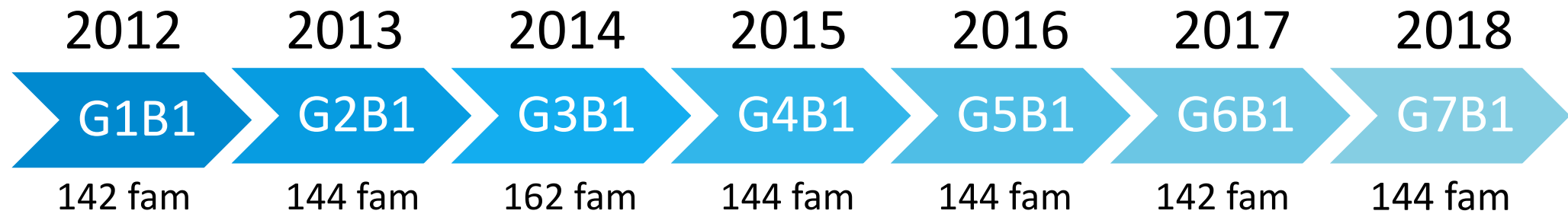
BASE POPULATION - SPRING GENETICS

- Origin :
 - Gift Project 1988-1997 (5 Generations of selection)
 - RIA 1 1997-2004 (4 Generations of selection)
 - Nicanor 2004-2009 (3 Generations of selection)
- To create the base population fry from Nicanor were introduced to Miami in 2010

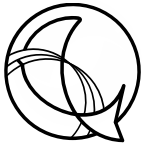




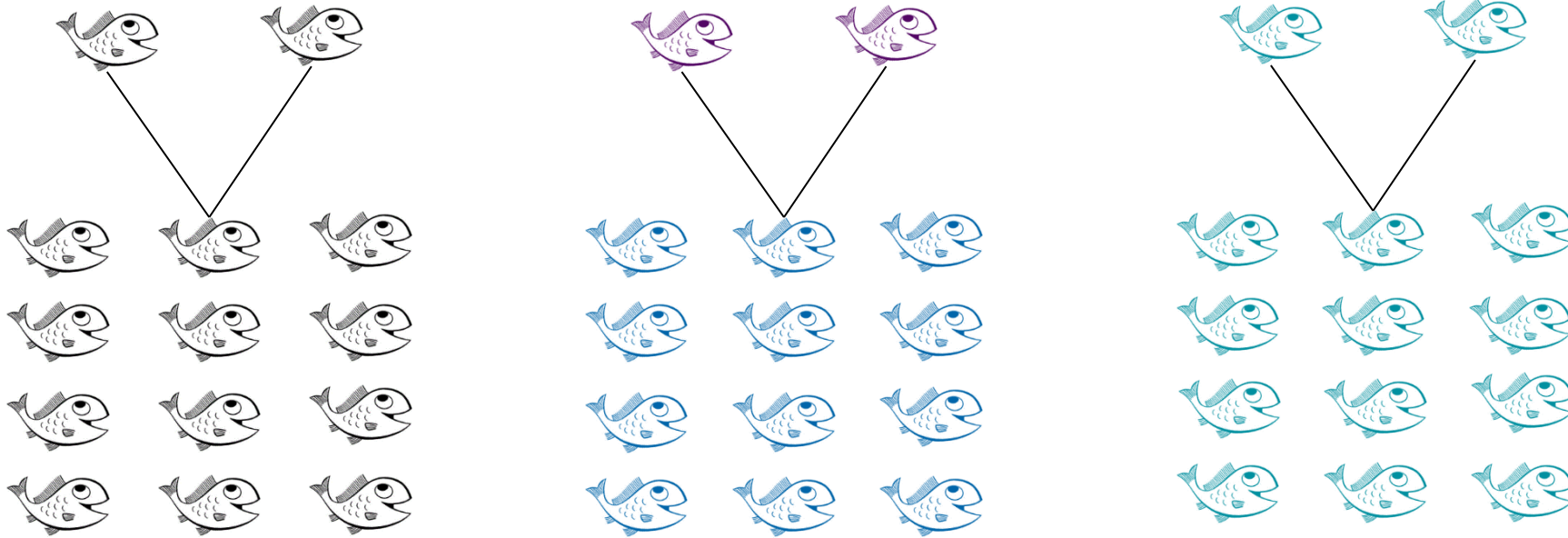
FAMILY PRODUCTION



Base population



FAMILY BASED BREEDING PROGRAMS



Select candidates based on the performance of their relatives
Test for different traits



FAMILY PRODUCTION



1 couple per tank.
Natural mating

Collect eggs



Incubation



Transfer to nursery



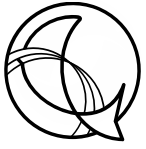
Separate rearing
units



Rear until pit-tagging



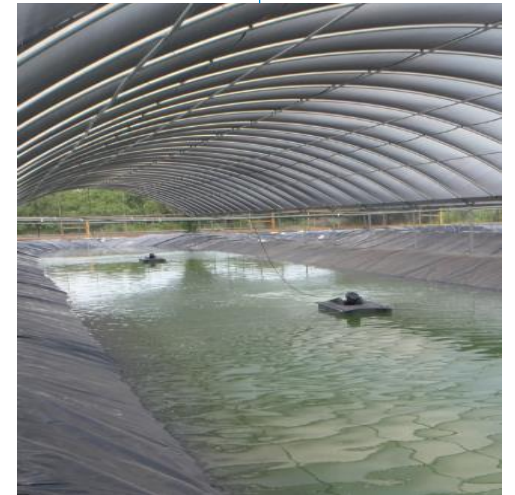
Photos : Hideyoshi Segovia

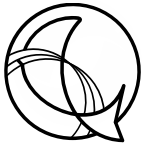


**Tagging
Tissue sampling**

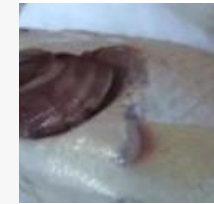
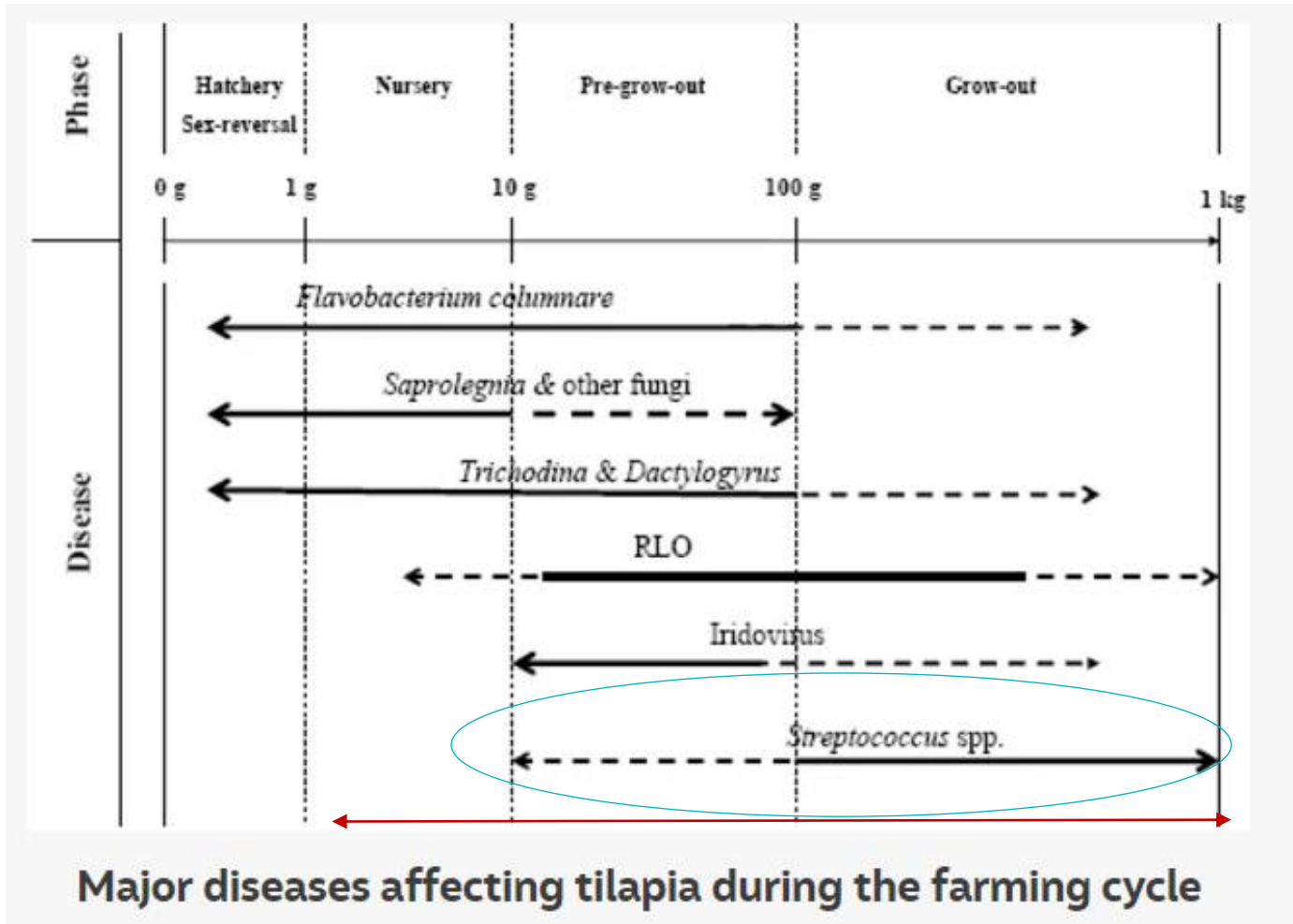
Disease ch-test

Growth

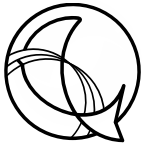




DISEASES



TLV



STREPTOCOCCUS DISTRIBUTION



	Global prevalence (as % of total streptococcal isolations)*
<i>S. agalactiae</i> Biotype 1	26
<i>S. agalactiae</i> Biotype 2	56
<i>S. iniae</i>	18

* Data generated by Intervet/Schering-Plough Animal Health, Singapore

From Sheehan et al 2009, Streptococcosis in tilapia: A more complex problem than expected?



Figure 1. Global distribution of *S. iniae*, *S. agalactiae* Biotype 1 and *S. agalactiae* Biotype 2 in tilapia and the countries where these pathogens have been identified and associated with disease.

-  *S. agalactiae* Biotype 2 (and *S. iniae*)
-  *S. agalactiae* Biotype 1 (and *S. iniae*)
-  *S. agalactiae* Biotype 1 and 2 (and *S. iniae*)

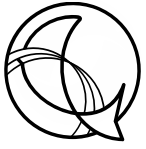


<i>S. agalactiae</i> vaccine strain	<i>S. agalactiae</i> challenge strain	Treatment group	% mortality	RPP*
<i>S. agalactiae</i> Biotype 1	<i>S. agalactiae</i> Biotype 1	VACCINATES	7	93%
		CONTROL	93	
	<i>S. agalactiae</i> Biotype 2	VACCINATES	87	0
		CONTROL	80	

Table 2. Sa1 vaccines do not protect against Sa2 challenge. * RPP (relative percent protection) includes mortality and recovery of challenge organism from surviving fish at the end of the observation period. RPP is calculated as $RPP = (1 - (\text{infection in vaccinates} / \text{infection in controls})) \times 100$.

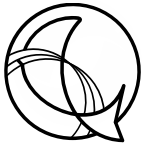
<i>S. agalactiae</i> vaccine strain	<i>S. agalactiae</i> challenge strain	Treatment group	% mortality	RPP*
<i>S. agalactiae</i> Biotype 2	<i>S. agalactiae</i> Biotype 1	VACCINATES	53	0
		CONTROL	53	
	<i>S. agalactiae</i> Biotype 2	VACCINATES	13	80%
		CONTROL	67	

Table 3. Sa2 vaccines do not protect against Sa1 challenge. * RPP (relative percent protection) includes mortality and recovery of challenge organism from surviving fish at the end of the observation period. RPP is calculated as $RPP = (1 - (\text{infection in vaccinates} / \text{infection in controls})) \times 100$.



STREPTOCOCCUS IMPORTANCE

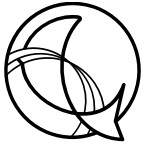
- “*Streptococcus agalactiae* and *Streptococcus iniae* appear to be the principal agents of streptococcosis in tilapia.”
- “*Streptococcus agalactiae* have distinct clusters (Biotypes)”
- “Immunity to *S. agalactiae* is biotype-specific”



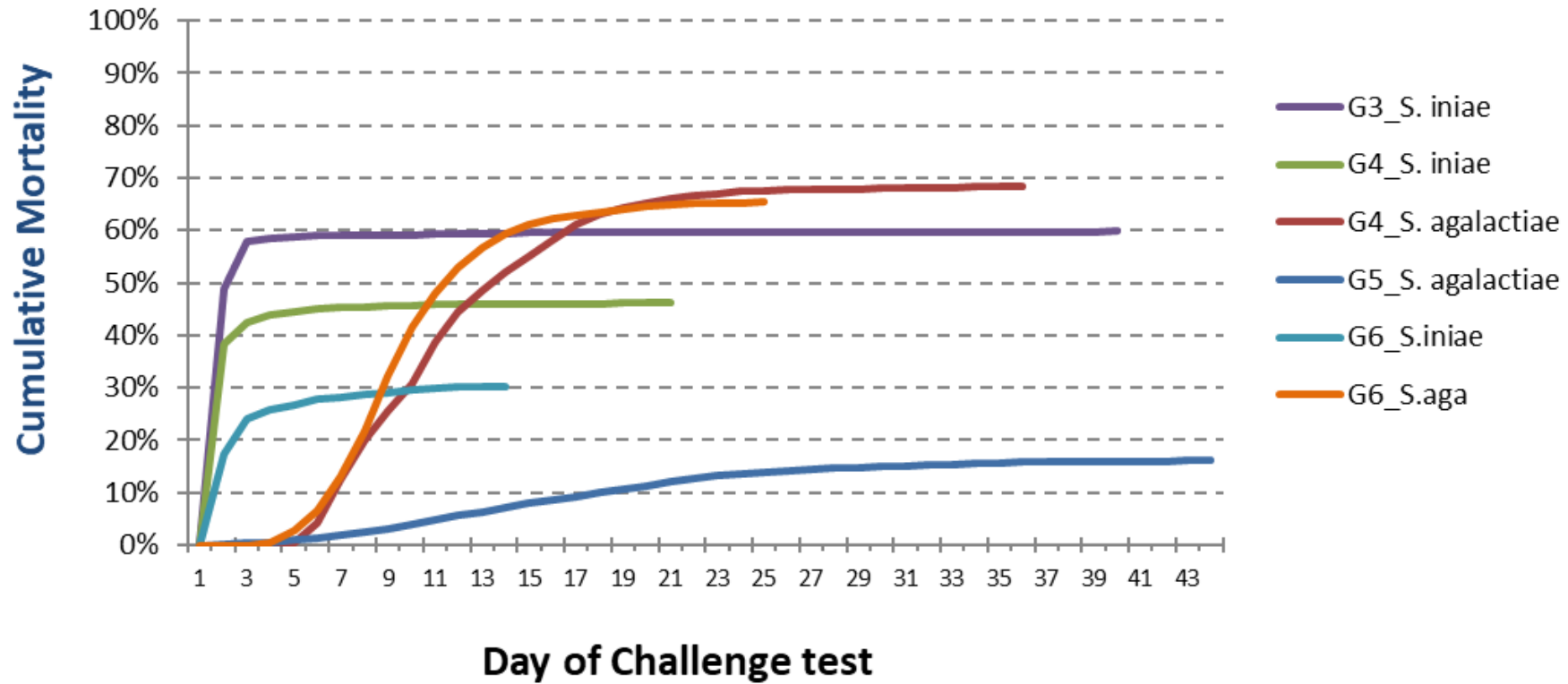
STREP. CH-TESTS

Generation	Method	Fish/fam	Weight(g)
G3B1	S. iniae (cohab)	10	179
G3B1	S. iniae	10	180
G4B1	S. agalactiae	15	179
G4B1	S. Iniae	20	29
G5B1	S. agalactiae	20	65
G6B1	S. iniae	25	-
G6B1	S. agalactiae	20	-

Agalactiae Biotype 2 used.



DATA FROM ALL GENERATIONS

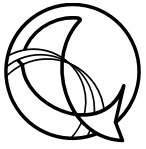


Cumulative mortality all generations



GENETIC PARAMETERS

Gen	Trait	$h^2 \pm SE$	$c^2 \pm SE$
3	Survival <i>S. iniae</i> (G3, G4)	0.41 ± 0.13	0.01 ± 0.05
4		0.53 ± 0.12	0.03 ± 0.04
All		0.49 ± 0.08	0.02 ± 0.02
4	Survival <i>S. agalactiae</i> (G4, G5)	0.36 ± 0.11	0.01 ± 0.04
5		0.12 ± 0.04	0.02 ± 0.02
All		0.16 ± 0.03	0.05 ± 0.01



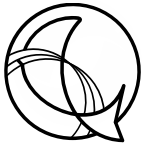
ASSORTATIVE MATING GROUPS *S. iniae*

Assortative mating groups	Number of families	avg_ <i>S. inea</i> G3B1 index parents*	Survival to inea in G4B1		
			Average**	Min	Max
none	132	101.6	54 %	0 %	100 %
yes_high	6	121.9	88 %	60 %	100 %
yes_low	6	80.2	10 %	0 %	42 %
Grand Total	144				

* Index (mean=100, sd=10). Avg=(sire index +dam index)/2

** mean family survival to *S. inea* during challenge test.

Both G3B1 and G4B1 challenge tests were done with injection



ASSORTATIVE MATING GROUPS *S. agalactiae*

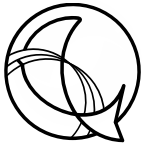
Assortative mating groups	Number of families	avg_ <i>S. aga.</i> G4B1 index parents*	Survival to <i>agalactiae</i> in G5B1		
			Average**	Min	Max
none	132	103.8	85 %	30 %	100 %
yes_high	6	122.8	98 %	95 %	100 %
yes_low	6	83.7	61 %	40 %	75 %
Grand Total	144				

* Index (mean=100, sd=10). Avg=(sire index +dam index)/2

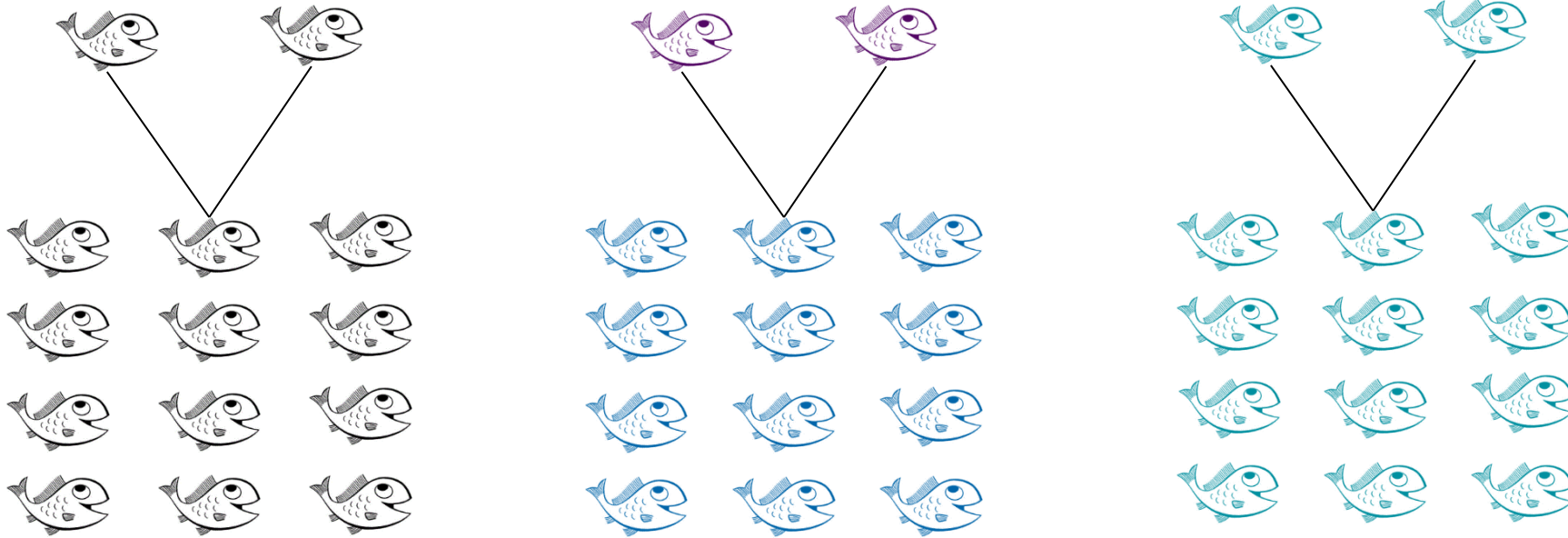
** mean family survival to *S. agalactiae natural* during challenge test.

G4B1 S. aga. challenge test was done with injection

G5B1 S. aga. challenge test was a natural outbreak



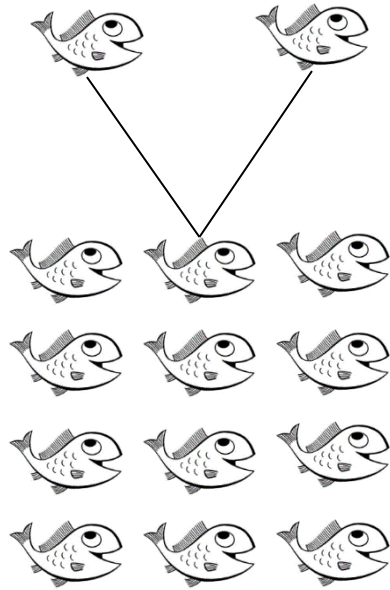
FAMILY BASED BREEDING PROGRAMS



Select candidates based on the performance of their relatives
Test for different traits



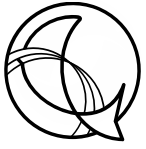
BREEDING PROGRAMS USING GENOMIC INFORMATION



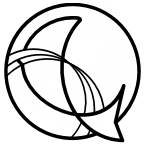
Individual genetic values are calculated for the candidates

Marker effects are calculated

Test for different traits
+
Genotypes



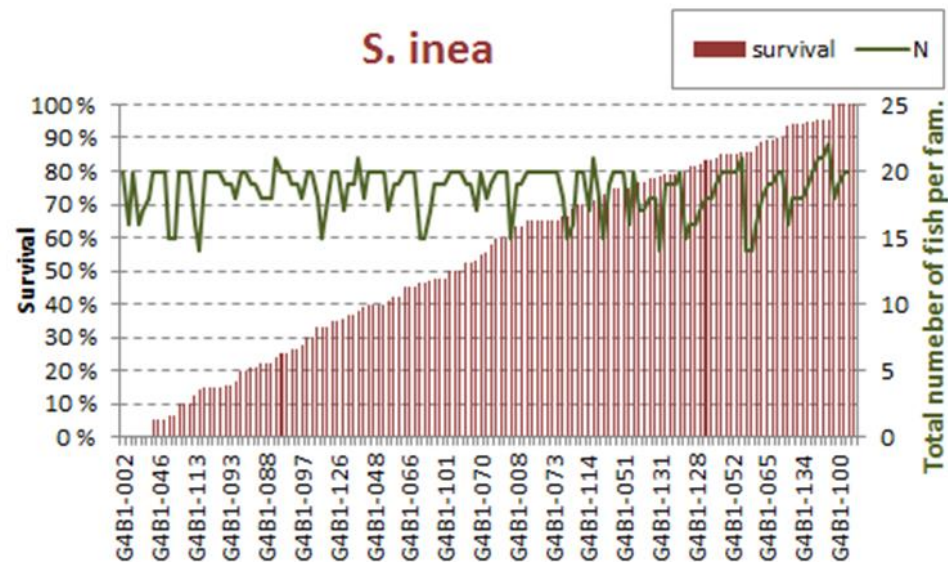
- INTRODUCTION
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CHALLENGE TEST

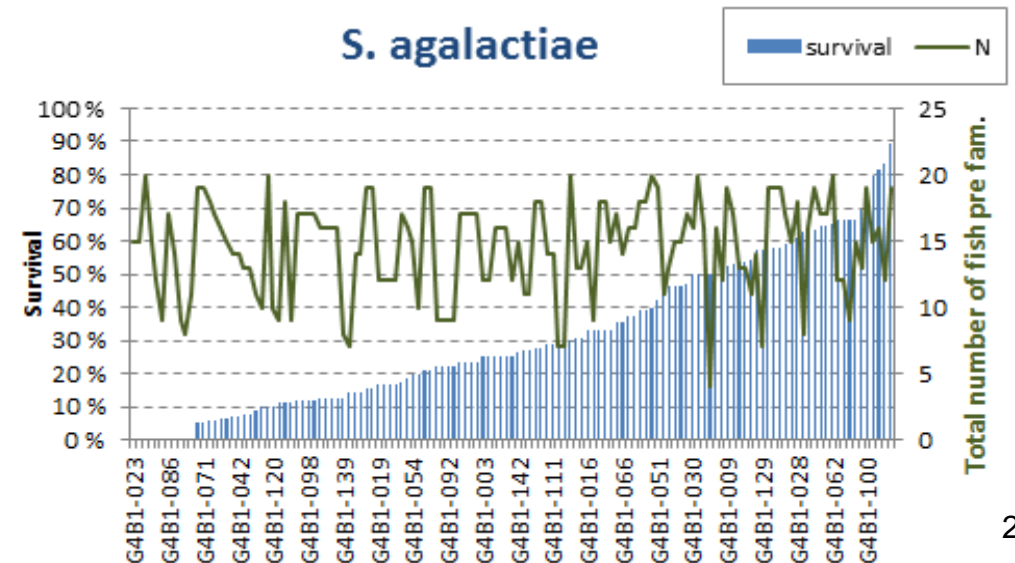
S. iniae

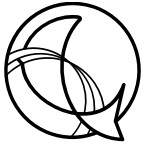
- 2686 Fish (20/family) were individually inoculated with *S. iniae* via IP
- Mortalities recorded for 21 days post infection
- High variation in mean family survival



S. agalactiae

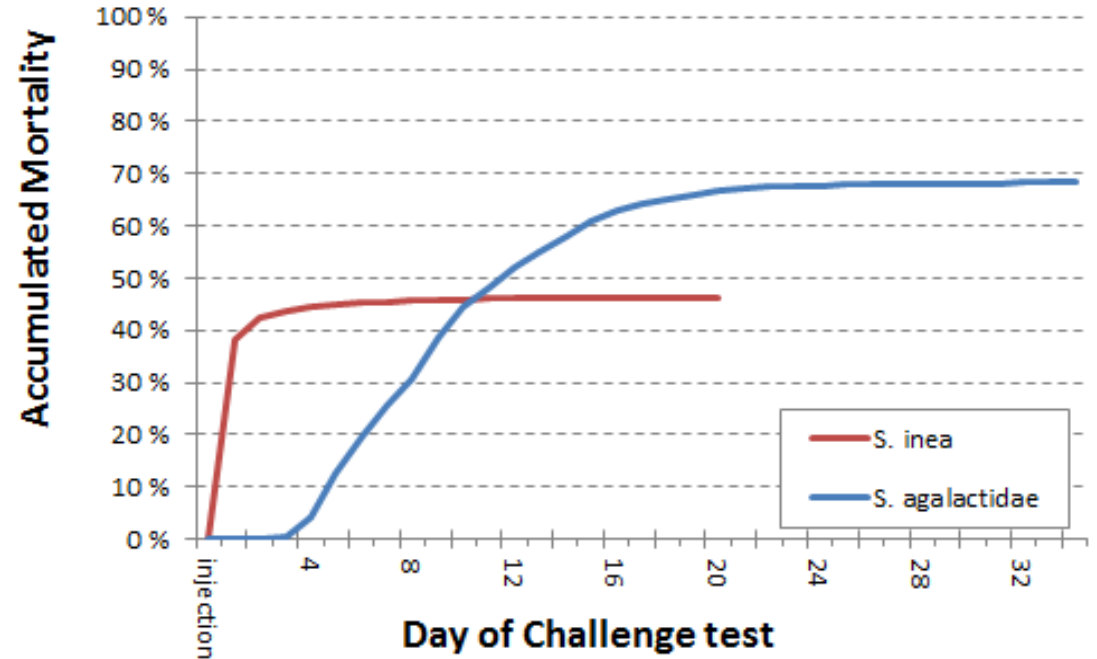
- 1912 Fish (15/family) were individually inoculated with *S. iniae* via IM
- Mortalities recorded for 34 days post infection
- High variation in mean family survival





CHALLENGE TEST

- *S. inea* mortalities mostly happened on day one and two, almost flatening at day 12
- *S. agalactiae* mortalities started on day 3 and occurred on a longer period of time than *S. inea*
- Mortality curves similar to previous years
- Flin clips of all fish in Ch-test preserved in 95% ethanol and stored at -20°C

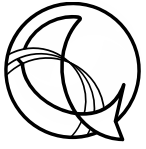




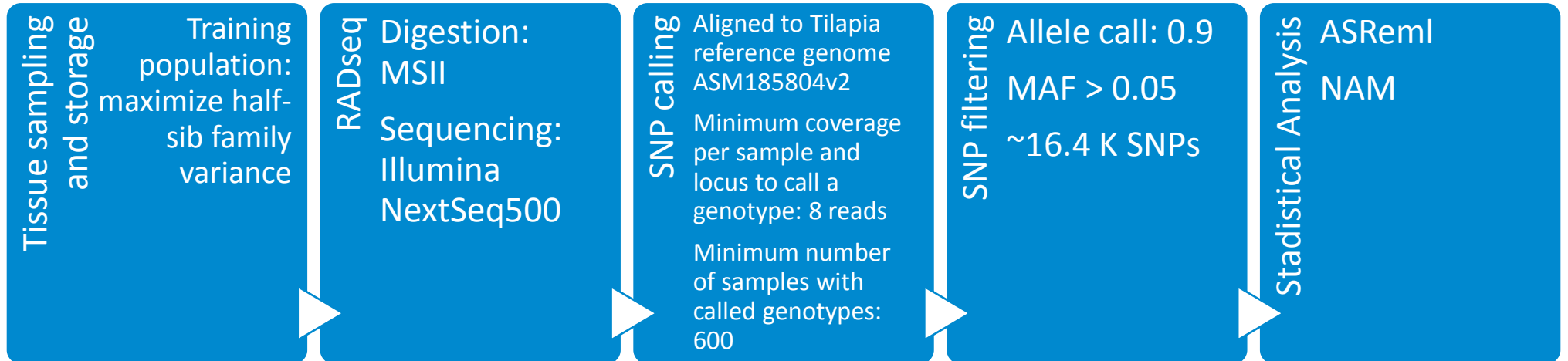
TRAINING DATA

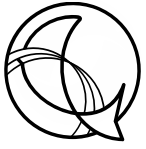
	<i>S. iniae</i>	<i>S. agalactiae</i>
Number of families	39	45
Total number of samples	321	414
Mean individuals/family	8.5	9.2
Mean survival (sd)	0.52 (0.5)	0.33 (0.47)





DATA PROCESSING





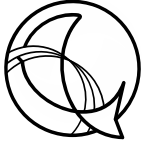
GWAS STATISTICAL MODEL

$$y = \mu + Xb + Za + e$$

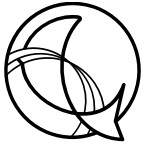
Where:

- y : the vector of phenotypic records
- μ : the overall mean
- b : unknown random allele substitution effect of the evaluated SNP
- a : random additive genetic effect $a \sim N(0, A\sigma_a^2)$ and A is the additive relationship matrix

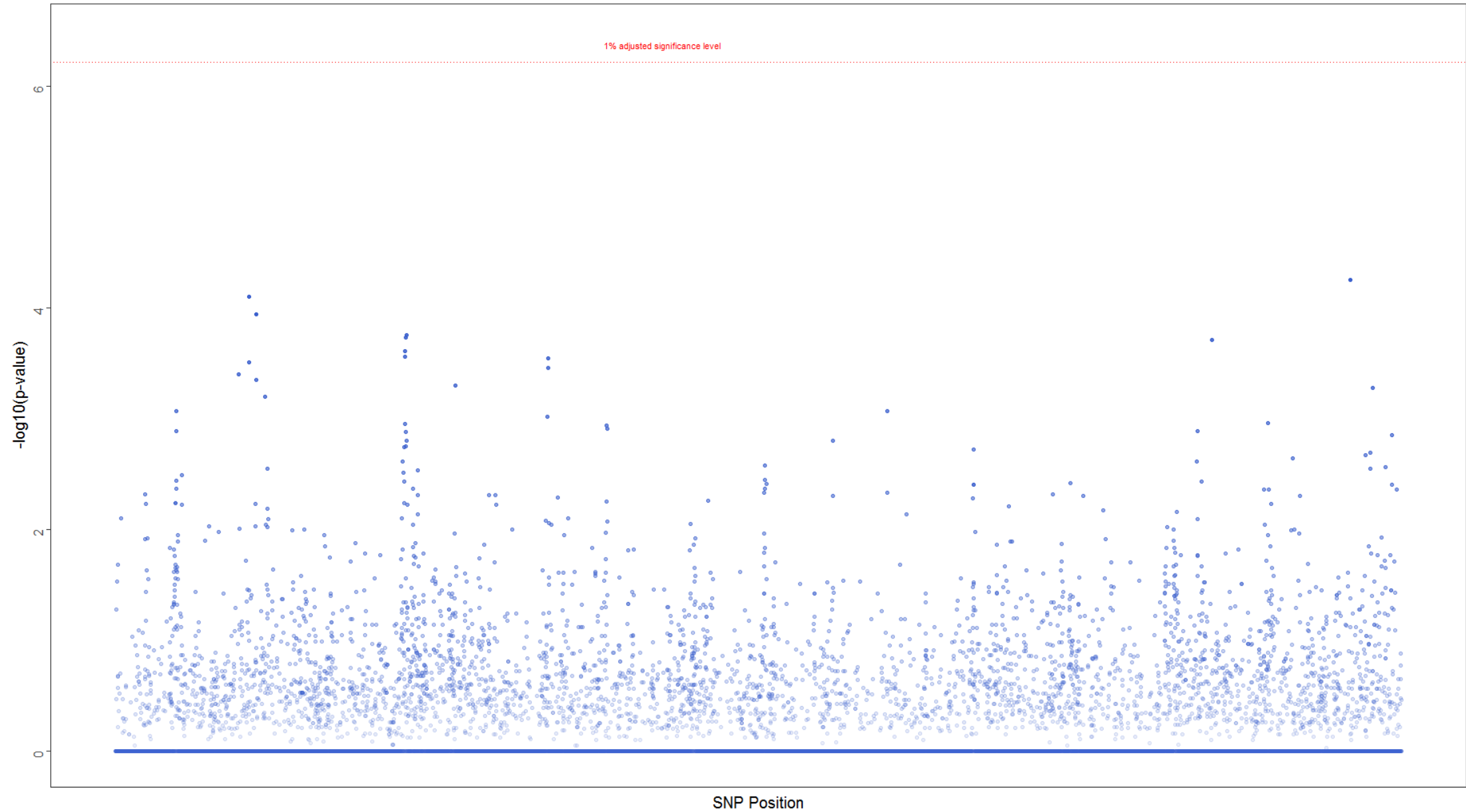
Significance of SNP was tested as LRT (*likelihood ratio test statistics*) comparing the model without fitting the current SNP.

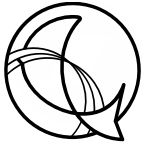


- INTRODUCTION
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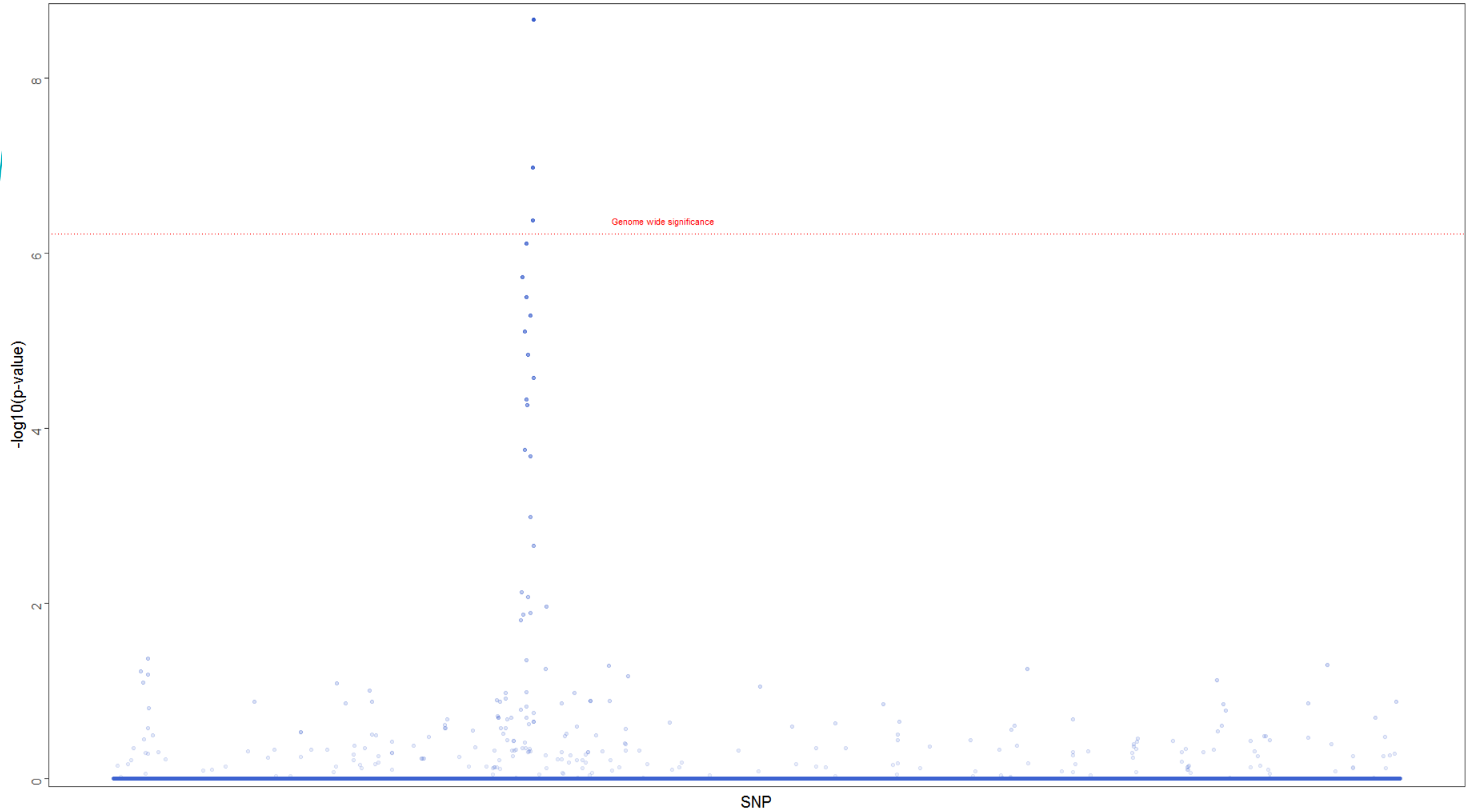


Manhattan plot of GWAS for *Streptococcus agalactiae* survival





Manhattan plot of GWAS for *Streptococcus iniae* survival

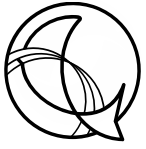




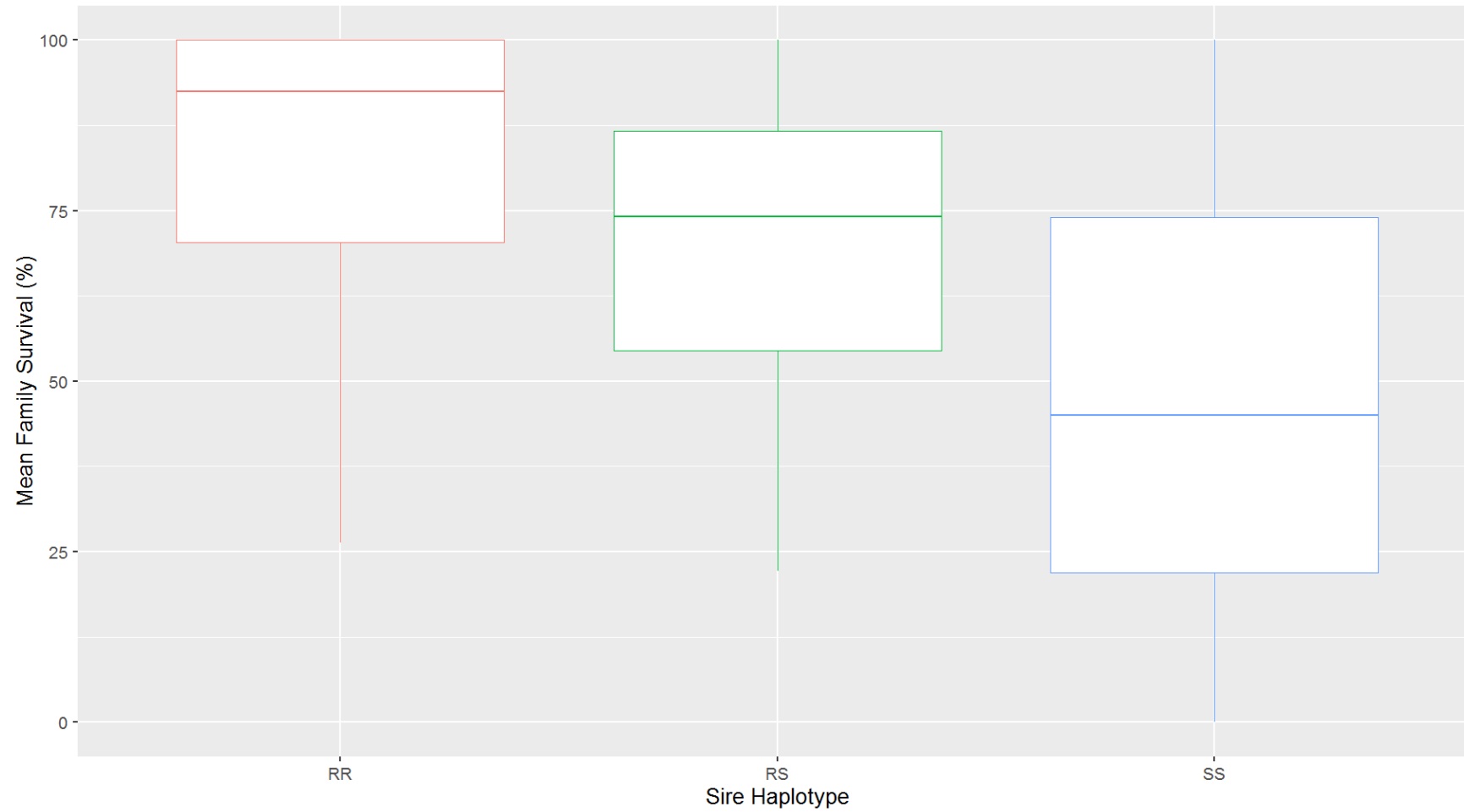
MARKER EFFECTS

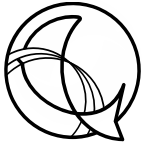
Id of the SNP	σ_p^2	σ_{SNP}^2	σ_a^2	Genetic variance of the SNP*
TN-QTL-SI-1	0.292	0.234	0.068	0.07
TN-QTL-SI-2	0.294	0.259	0.077	0.12
TN-QTL-SI-3	0.297	0.271	0.080	0.12

*Genetic variance explained by a SNP was estimated as $\sigma_{SNP}^2 = 2pqa^2$ where p and q are allele frequencies and a the estimated allele substitution effect.

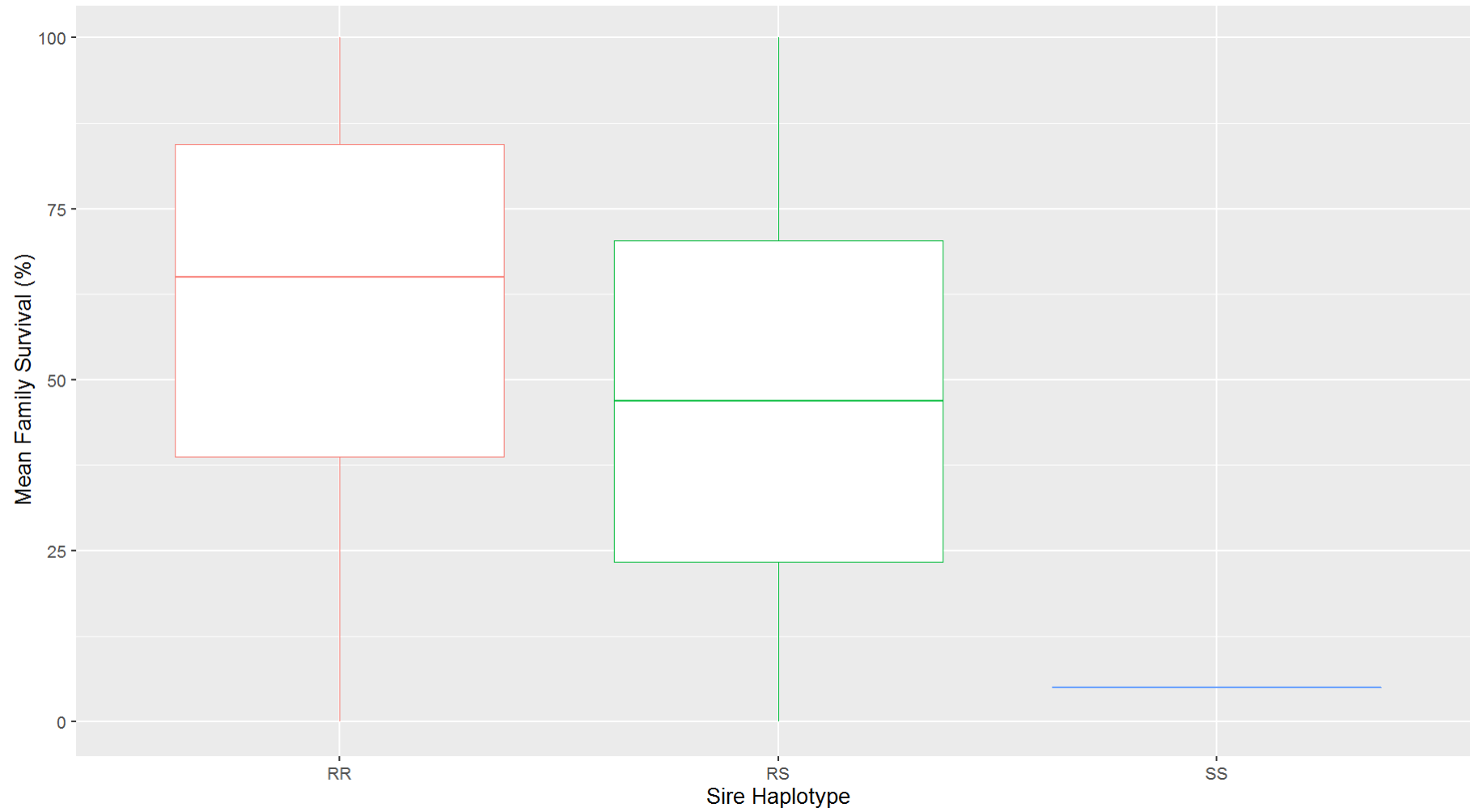


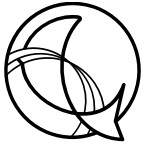
TN-QTL-SI-1



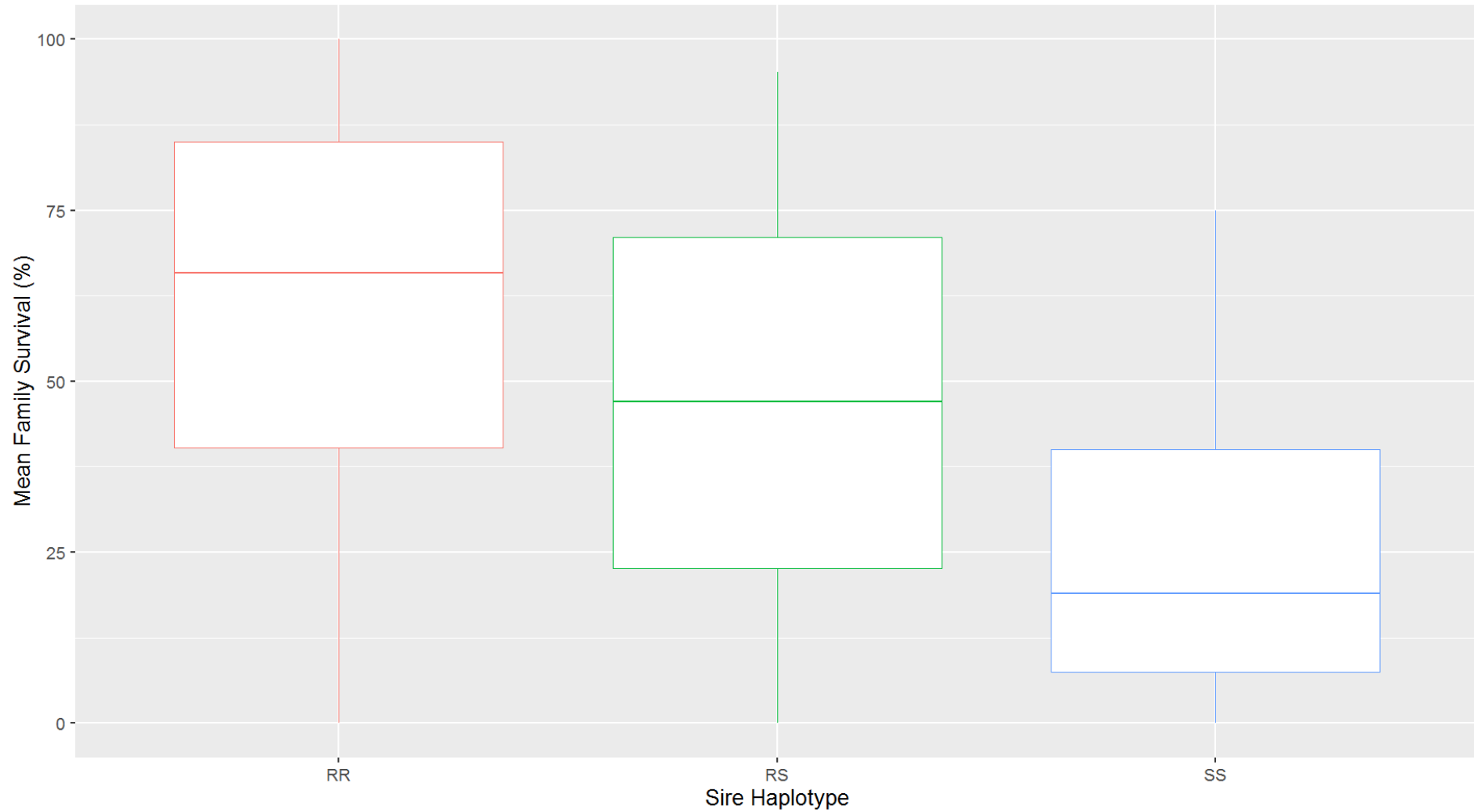


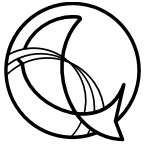
TN-QTL-SI-2





TN-QTL-SI-3

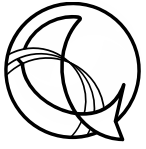




CONCLUSIONS

S. AGALACTIAE

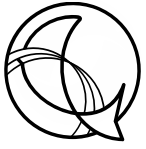
- In our study, no areas of significant effect were found for survival to *S. agalactiae*
- Given results from current and previous studies, it is likely that survival to *S. agalactiae* is a polygenic trait
- In order to perform within family selection, the implementation of genomic selection for this disease, further research on the method more suitable according to the genetic architecture of the trait



CONCLUSIONS

S. iniae

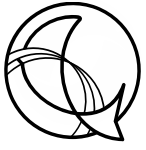
- An area of significant effect were found for survival to *S. iniae* in the studied population where a clear peak in one single area of the genome is observed
- Average family survival by haplotype showed that parents with favorable alleles produce families with higher average survival than parents homozygote to unfavorable allele
- Implementation of marker assisted selection to produce families with higher survival to the disease is possible using the identified markers.



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THANK YOU

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